Uses of Ethanolic Coconut Husk Extract for Surimi Gel Strengthening and Enhancement of Oxidative Stability of Emulsion Surimi Gel

Natchaphol Buamard

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Food Science and Technology

Prince of Songkla University

2018

Uses of Ethanolic Coconut Husk Extract for Surimi Gel Strengthening and Enhancement of Oxidative Stability of Emulsion Surimi Gel

Natchaphol Buamard

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Food Science and Technology
Prince of Songkla University
2018

Thesis Title	Uses of Ethanolic Coconut Husk Extract for Surimi Gel Strengthening and Enhancement of Oxidative Stability of Emulsion Surimi Gel
Author	Mr. Natchaphol Buamard
Major Program	Food Science and Technology

Major Advisor

…….……....
(Prof. Dr. Soottawat Benjakul)

Examining Committee:

….……...…….....Chairperson
(Asst. Prof. Dr. Manee Vittayanont)

…….…….....…...Committee
(Prof. Dr. Soottawat Benjakul)

…….…….....Committee
(Asst. Prof. Dr. Saowakon Wattanachant)

…….…….....Committee
(Assoc. Prof. Dr. Jirawat Yongsawatdigul)

The Graduate School, Prince of Songkla University, has approved this thesis as partial fulfillment of the requirements for the Doctor of Philosophy Degree in Food Science and Technology.

.....
(Prof. Dr. Damrongsak Faroongsarng)
Dean of Graduate School

This is to certify that the work here submitted is the result of the candidate's own investigations. Due acknowledgement has been made of any assistance received.

……..Signature
(Prof. Dr. Soottawat Benjakul)
Major Advisor

……..…Signature
(Mr. Natchaphol Buamard)
Candidate

I hereby certify that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any degree.

……Signature
(Mr. Natchaphol Buamard)
Candidate

ชื่อวิทยานิพนธ์ การใช้สารสกัดเอทานอลจากกาบมะพร้าวสำหรับการปรับปรุงความแข็งแรงเจลซูริมิและการเพิ่มความคงตัวต่อปฏิกิริยาออกซิเดชันของอิมัลชันซูริมิเจล

ผู้เขียน นายณัชพล บัวมาศ

สาขาวิชา วิทยาศาสตร์และเทคโนโลยีอาหาร

ปีการศึกษา 2561

บทคัดย่อ

การศึกษาผลของสารสกัดเอทานอลจากกาบมะพร้าวเตรียมโดยใช้เอทานอลที่ระดับความเข้มข้นร้อยละ 60 (E60) และ 80 (E80) ซึ่งมีปริมาณฟีนอลิกรวมเท่ากับ 463.74 และ 453.93 มก. แทนนินต่อกรัมตามลำดับ ต่อสมบัติเจลซูริมิจากปลาหลังเขียว พบว่าเจลที่เติม E60 หรือ E80 มีค่าแรงเจาะทะลุสูงขึ้นเมื่อเติมสารสกัดที่ระดับเพิ่มขึ้นและเจลมีค่าแรงเจาะทะลุสูงสุดเมื่อเติม E60 ร้อยละ 0.125 หรือ E80 ร้อยละ 0.075 (P<0.05) ทั้ง E60 และ E80 ไม่ส่งผลเสียต่อคุณลักษณะทางประสาทสัมผัสของเจลซูริมิ อย่างไรก็ตามการเติม E60 และ E80 ทำให้ค่าความขาวของเจลซูริมิลดลง ไม่พบแถบของโปรตีนไมโอซินเส้นหนักทั้งในเจลที่เติม E60 และ E80 การเติม E60 และ E80 สามารถลดการย่อยสลายตัวเองของเจลซูริมิและเพิ่มการเชื่อมประสานของโปรตีนในระหว่างการให้ความร้อนโดยแสดงจากการเพิ่มขึ้นของค่าโมดูลัสสะสม (G′) เจลซูริมิที่เติมสารสกัดกาบมะพร้าวมีโครงข่ายที่เชื่อมประสานกันมากขึ้นและมีโครงสร้างทางจุลภาคที่เป็นระเบียบและหนาแน่นกว่าชุดควบคุม

เมื่อศึกษาผลของสารสกัดเอทานอลจากกาบมะพร้าวที่เตรียมโดยเอทานอลความเข้มข้นร้อยละ 60 (E60) หรือ ECHE ที่ระดับต่าง ๆ (ร้อยละ 0-0.03 ของปริมาณโปรตีน) ต่อการจับรวมตัวของแอกโตไมโอซินธรรมชาติ (NAM) ที่เหนี่ยวนำด้วยความร้อน พบว่าระหว่างการให้ความร้อนจาก 20 ถึง 90 องศาเซลเซียส สารละลาย NAM มีค่าความขุ่น ปริมาณไฮโดรโฟบิกพื้นผิว และปริมาณพันธะไดซัลไฟด์สูงขึ้น การจับรวมตัวเพิ่มมากขึ้นเมื่อปริมาณ ECHE เพิ่มขึ้น ในขณะที่การละลายของโปรตีนและปริมาณหมู่ซัลไฮดริลลดลง (P<0.05) อีกทั้งกิจกรรมของ Ca^{2+}-ATPase มีปริมาณลดลงอย่างเห็นได้ชัดในสารละลาย NAM ที่เติม ECHE เมื่อให้ความร้อนที่ 40 องศาเซลเซียส ค่าศักย์ซีต้าแสดงให้เห็นว่าสารละลาย NAM ที่เติม ECHE มีประจุลบที่ต่ำลงเมื่อความเข้มข้นของ ECHE เพิ่มขึ้น (P<0.05) การเชื่อมประสานของสายโปรตีนเกิดมากขึ้นระหว่างการให้ความร้อนในสภาวะที่มี ECHE ดังแสดงจากการเพิ่มขึ้นของค่า G′ ขนาดอนุภาค และลักษณะโครงสร้างทางจุลภาค

เมื่อเติม ECHE (ร้อยละ 0-0.25 ของปริมาณโปรตีน) ในซูริมิจากปลาหลังเขียวที่มีการเซ็ตตัวต่ำ (เติม EGTA) พบว่าค่าแรงเจาะทะลุของเจลซูริมิเพิ่มขึ้นเมื่อระดับของ ECHE เพิ่มขึ้นจนถึงระดับร้อยละ 0.15 (P<0.05) ค่าความขาวของเจลมีค่าลดลงเมื่อเติม ECHE ส่วนแถบโปรตีนของเจลลดลงที่ทุกระดับของ ECHE ดังนั้นการเติม ECHE สามารถเพิ่มการเชื่อมประสานของโปรตีนระหว่างการเซ็ตตัว (40 องศาเซลเซียส นาน 30 นาที) โดยเฉพาะอย่างยิ่งเมื่อระดับของ ECHE เพิ่มขึ้นซึ่งบ่งชี้ได้จากการเพิ่มขึ้นของ G′ เจลที่เติม ECHE ในระดับร้อยละ 0.15 ซึ่งมีการเชื่อมประสานของโครงข่ายโปรตีนที่เป็นระเบียบและหนาแน่นกว่าชุดควบคุมที่ไม่มีการเติม ECHE

จากการศึกษาบทบาทของ ECHE (ร้อยละ 0-0.10 ของปริมาณโปรตีน) ต่อสมบัติเจลซูริมิปลาหลังเขียวที่เตรียมภายใต้สภาวะการเกิดเจลต่าง ๆ ได้แก่ การใช้ความดันที่ 300 MPa นาน 30 นาที (HP) และการใช้ HP ร่วมกับการให้ความร้อน (90 องศาเซลเซียส นาน 20 นาที) (HP/H) พบว่าที่ระดับ ECHE เดียวกัน เจล HP/H มีค่าแรงเจาะทะลุสูงกว่าเจล HP (P<0.05) การใช้ ECHE จนถึงระดับร้อยละ 0.075 สามารถเพิ่มค่าแรงเจาะทะลุและความสามารถในการอุ้มน้ำของเจลที่เตรียมได้จากทั้งสองวิธี เจล HP/H ที่เติม ECHE มีการย่อยสลายตัวเองของเจลซูริมิลดลง การเติม ECHE ร้อยละ 0.075 ในเจล HP/H ส่งผลให้เจลมีโครงข่ายที่เชื่อมประสานมากกว่าเจล HP หรือเจลที่ผ่านการให้ความร้อนสองขั้นตอน

จากการวิเคราะห์องค์ประกอบของ ECHE พบคอนเดนส์แทนนินเป็นองค์ประกอบหลักและมีกรดฟีนอลิกอิสระ ได้แก่ กรดแทนนิกและคาเทชินเป็นหลัก กิจกรรมการต้านอนุมูลอิสระในระบบหลอดทดลองของ ECHE ที่ระดับต่าง ๆ (50-200 มก./ล.) เพิ่มขึ้นเมื่อระดับของ ECHE เพิ่มขึ้น (P<0.05) กิจกรรมการจับอนุมูลอิสระ DPPH และการจับโลหะลดลงร้อยละ 50 เมื่อให้ความร้อนที่อุณหภูมิสูงกว่า 90 องศาเซลเซียส นานกว่า 60 นาที (P<0.05) ผลของ ECHE (200 และ 400 มก./ล.) ต่อการเกิดลิพิดออกซิเดชันของอิมัลชันน้ำมันกุ้งที่เก็บรักษาที่อุณหภูมิ 30 องศาเซลเซียส เป็นระยะเวลา 12 วัน พบว่าลิพิดออกซิเดชันของอิมัลชันที่เติม ECHE ลดลงบ่งชี้ได้จากค่า conjugated diene (CD), thiobarbituric acid-reactive substances (TBARS) และ ρ-anisidine (AnV) ที่ต่ำกว่าชุดควบคุม ปริมาณกรดอีโคซะเพนตะอีโนอิก (EPA) และกรดโดโคซะเฮกซะอีโนอิก (DHA) คงเหลือมากกว่าในอิมัลชันที่มีการเติม ECHE (200 มก./ล.) เมื่อสิ้นสุดระยะเวลาการเก็บรักษา

เมื่อเติม ECHE (ร้อยละ 0-0.25) ในซูริมิเจลที่เติมน้ำมันปลากะพงที่ผ่านการพรีอิมัลซิฟายด์ด้วยโปรตีนสกัดจากถั่วเหลือง พบว่า เจลที่เติมน้ำมันปลากะพงพรีอิมัลซิฟายด์ในสภาวะที่มี ECHE ที่ระดับร้อยละ 0.20-0.25 ซึ่งมีขนาดเส้นผ่านศูนย์กลางของอิมัลชัน (d_{43}) เฉลี่ยเท่ากับ 17.18-33.01 ไมโครเมตร มีค่าแรงเจาะทะลุสูงสุดและมีค่าความแข็งและค่าการยึดเกาะภายในที่เพิ่มขึ้น (P<0.05) ค่าความขาวของเจลซูริมิลดลงเมื่อระดับของ ECHE เพิ่มขึ้น (P<0.05) เมื่อเก็บรักษาที่

อุณหภูมิ 4 องศาเซลเซียสนาน 10 วัน ลิพิดออกซิเดชันของเจลซูริมิลดลงเมื่อเติม ECHE ที่ระดับสูงขึ้นดังแสดงจากค่า PV และ TBARS ที่ลดลง (P<0.05) อย่างไรก็ตาม การเติม ECHE ไม่มีผลต่อปริมาณจุลินทรีย์ทั้งหมดและแบคทีเรียที่เจริญได้ที่อุณหภูมิต่ำในเจลซูริมิ

ดังนั้นสารสกัดเอทานอลจากกาบมะพร้าวสามารถใช้เป็นสารเชื่อมประสานโปรตีนที่มีฤทธิ์ต้านอนุมูลอิสระซึ่งสามารถใช้ในการเพิ่มสมบัติการเกิดเจลรวมทั้งเพิ่มความคงตัวต่อปฏิกิริยาออกซิเดชันในซูริมิหรือผลิตภัณฑ์อื่น

Thesis Title	Uses of Ethanolic Coconut Husk Extract for Surimi Gel Strengthening and Enhancement of Oxidative Stability of Emulsion Surimi Gel
Author	Mr. Natchaphol Buamard
Major Program	Food Science and Technology
Academic Year	2018

ABSTRACT

Effects of coconut husk ethanolic extracts on gelling properties of surimi from sardine (*Sardinella albella*) were investigated. Extracts prepared using 60% ethanol (E60) and 80% ethanol (E80) with total phenolic content of 463.74 and 453.93 mg tannin/g, respectively, were incorporated into surimi gel. Gels added with E60 or E80 had the increases in breaking force as the levels increased and the highest breaking force was observed when added with 0.125% E60 and 0.075% E80 ($P<0.05$). Both E60 and E80 had no detrimental effect on sensory attributes of surimi gel. However, slight decrease in whiteness was found in gel added with the coconut husk extracts. Disappearance of major myosin heavy chain took place when incorporated with both E60 and E80, regardless of levels used. Lower autolysis of surimi gel was found in the presence of both extracts. Addition of E60 and E80 could increase the cross-linking of proteins during heating as indicated by the higher G'. Surimi gel added with coconut extracts had highly interconnected network and their microstructure was finer and denser network than that of the control.

Heat-induced aggregation of natural actomyosin (NAM) extracted from sardine muscle was studied in the presence of E60, named as ECHE, at various levels (0-0.03%, based on protein content). During heating from 20 to 90 °C, NAM solution showed an increase in turbidity, surface hydrophobicity and disulfide bond contents. Aggregation was more pronounced as ECHE concentration increased ($P<0.05$), while protein solubility and total sulfhydryl group were decreased ($P<0.05$). Furthermore, the Ca^{2+}-ATPase activity of NAM was noticeably decreased in the presence of ECHE during heating up to 40 °C. Zeta potential analysis revealed that NAM added with ECHE became less negatively charged when the concentrations of ECHE increased

(P<0.05). Cross-linking of protein strands was enhanced in the presence of ECHE during heating as evidenced by the higher G', particle size as well as microstructure.

When ECHE at various levels (0-0.25%, based on protein content) was added into low setting surimi prepared from sardine (containing EGTA), breaking force of gels increased as the levels of ECHE increased up to 0.15% (P<0.05). The decrease in whiteness was found in gel added with ECHE. MHC band intensity was decreased, regardless of ECHE concentrations. Addition of ECHE could therefore increase the cross-linking of proteins during setting (40 °C, 30 min), especially with increasing concentrations, as indicated by the increased G'. Gel added with 0.15% ECHE had highly interconnected network with finer and denser structure than the control gel (without ECHE).

Impact of ECHE at various levels (0–0.10%, based on protein content) on gel properties of sardine surimi under different gelling conditions including pressurization at 300 MPa, 30 min (HP); and pressurization, followed by heating (90 °C, 20 min) (HP/H) were investigated. At the same level of ECHE, HP/H gel had the higher breaking force (P<0.05) than HP counterpart. The increases in breaking force and water holding capacity were observed as the levels of ECHE were increased up to 0.075% for all gels, regardless of gelling processes used (P<0.05). Lower autolysis of surimi gel was also found in HP/H gel in the presence of ECHE. With addition of ECHE at a concentration of 0.075%, HP/H gel had a network with higher connectivity than HP gel and traditional two-step heated gel.

ECHE was characterized and condensed tannin was found as an abundant compound in ECHE. The major free phenolics in ECHE were tannic acid and catechins. Antioxidative activities of ECHE at different levels (50-200 mg/L) tested by all *in vitro* assays increased as its concentration increased (P<0.05). DPPH radical scavenging activity and metal chelating activity were decreased up to 50% when heated at temperature higher than 90 °C for longer than 60 min (P<0.05). Impact of ECHE (200 and 400 mg/L) on lipid oxidation of shrimp oil-in-water emulsion was monitored throughout 12 days of storage at 30 °C. Lipid oxidation of emulsion added with ECHE was retarded as evidenced by the lower conjugated diene (CD), thiobarbituric acid-reactive substances (TBARS) value and ρ-anisidine value (AnV) than those of the

control. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were more retained in the sample added with ECHE (200 mg/L) at the end of storage ($P<0.05$).

When ECHE at various levels (0-0.25%) was incorporated into sardine surimi gel containing pre-emulsified seabass oil, the addition of seabass oil pre-emulsified with soy protein isolate in the presence of ECHE at levels of 0.20-0.25%, which had the average major mean diameter (d_{43}) of 17.18-33.01 μm, yielded surimi gel with the highest breaking force. The resulting gels also had the increases in hardness and cohesiveness ($P<0.05$). Decrease in whiteness was found in surimi gel added with ECHE, especially with increasing ECHE levels ($P<0.05$). During storage at 4 °C for 10 days, lipid oxidation of pre-emulsified gel as determined by peroxide value (PV) and TBARS was lowered as the levels of ECHE increased ($P<0.05$). Nevertheless, addition of ECHE did not affect total viable count and psychrophilic bacterial count in surimi gels.

Therefore, ECHE could be a potential antioxidative protein cross-linker for enhancement of gel formation and oxidative stability for surimi or other products.

ACKNOWLEDGEMENT

I would like to express my deepest appreciation and sincere gratitude to my advisor, Prof. Dr. Soottawat Benjakul of the Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, for his kindness, guidance and assistance during my study since the first day of being his student. Not only the academic knowledge dedicate to me, but also invaluable suggestion and lessons for my personal life have been kindly provided. His determination and perseverance to train me to be a good researcher with responsibility, vigilance and honesty are always acknowledged.

I would like to express my profound gratitude to Prof. Dr. Kunihiko Konno of the Faculty of Fisheries Sciences, Hokkaido University, Japan for his warm welcome, kindness, generous contribution and unconditional help, particularly during my 3 month-stay in Hakodate, Japan.

I am grateful to my examining committee, Asst. Prof. Dr. Manee Vittayanont and Asst. Prof. Dr. Saowakon Wattanachant of the Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University and Assoc. Prof. Dr. Jirawat Yongsawatdigul of the School of Food Technology, Institute of Agricultural Technology, Suranaree University of Technology for the valuable time devoted to my thesis and their kindness, comments and helpful suggestion.

I would like to express my deepest appreciation to my beloved family for the great encouragement and support. Finally, my deep gratitude is dedicated to my dear friends and colleagues of Fish Chemistry and Biochemistry laboratory (2205) members who shared the hard time with me during my study and gave me their help.

This study could not be succeeded without the financial support from the Prince of Songkla University Ph.D. Scholarship and a scholarship for an Overseas Thesis Research from Graduate School, Prince of Songkla University. The Thailand Research Fund (TRF) Distinguished Research Professor Grant was also acknowledged for the financial support.

Natchaphol Buamard

CONTENTS

Page

Abstract (Thai)……... v

Abstract (English)……... viii

Acknowledgment …….. xi

Contents…… xii

List of Tables……. xviii

List of Figures…….……...… xx

Chapter

1. Introduction and review of literature………..…….. 1

1.1 Introduction……... 1

1.2 Review of Literature……. 3

1.2.1 Coconut and coconut husk……...…… 3

1.2.2 Chemical composition of coconut husk…….……...…...…… 3

1.2.2.1 Tannins……..……... 4

1.2.2.2 Lignins……...…...……... 6

1.2.3 Surimi and gelling property...…….. 7

1.2.3.1 Surimi gelation…….....…… 7

1.2.3.2 Thermal induced gelation……...…… 8

1.2.3.3 High pressure induced gelation…….…..…..….. 8

1.2.4 Quality of surimi gel as affected by endogenous enzyme……... 10

1.2.4.1 Setting phenomenon…...…… 10

1.2.4.2 Softening phenomenon…..…….…… 12

1.2.5 Improvement of surimi gel property using plant phenolic……. 13

1.2.5.1 Cross-linking activity of protein as affected by phenolic compounds……... 14

1.2.5.2 Oxidation of phenolic compounds……... 15

1.2.6 Emulsion gel……... 16

1.2.7 Pre-emulsification for emulsion gel……... 18

CONTENTS (Continued)

Chapter	Page
1.2.8 Use of antioxidants from plants in fish and fish products……..	19
1.2.8.1 Extraction of plant phenolics…..…….	20
1.2.8.2 Applications of plant phenolics in fish and fish products…	21
1.3 References……..……..	26
1.4 Objectives…...……..	47
2. Improvement of gel properties of sardine (*Sardinella albella*) surimi using coconut husk extracts……..……..…….	48
2.1 Abstract…….	48
2.2 Introduction……..	48
2.3 Materials and methods……..	50
2.4 Results and discussion……..	55
2.4.1 Total phenolic and tannin contents of coconut husk……	55
2.4.2 Effect of coconut husk extracts at different levels on properties of surimi gel……..	56
2.4.2.1 Breaking force and deformation……	56
2.4.2.2 Expressible moisture content…….	58
2.4.2.3 Whiteness……...	60
2.4.2.4 TCA-soluble peptide content…….	60
2.4.2.5 Protein patterns……..	61
2.4.3 Characteristics of surimi gel added with coconut husk extract at the selected levels……..…….	63
2.4.3.1 Textural properties…..…….…….…….	63
2.4.3.2 Dynamic rheological properties…….	64
2.4.3.3 Microstructures……..……	65
2.4.3.4 Likeness score……	67
2.5 Conclusion…….……..…….	68
2.6 References……	68

CONTENTS (Continued)

Chapter **Page**

3. Cross-linking activity of ethanolic coconut husk extract toward sardine (*Sardinella albella*) muscle proteins..... 74

3.1 Abstract..... 74

3.2 Introduction..... 74

3.3 Materials and methods..... 76

3.4 Results and discussion..... 80

3.4.1 Total phenolic content and composition of ECHE..... 80

3.4.2 Heat-induced aggregation and physicochemical properties of NAM as affected by ECHE at different levels..... 80

3.4.2.1 Protein aggregation 80

3.4.2.2 Protein solubility..... 81

3.4.2.3 Surface hydrophobicity..... 82

3.4.2.4 Total sulfhydryl and disulfide bond contents..... 84

3.4.2.5 Ca^{2+}-ATPase..... 86

3.4.3 Changes in dynamic rheology, charge, size and microstructure of NAM as affected by ethanolic coconut husk extract 87

3.4.3.1 Dynamic rheological properties..... 87

3.4.3.2 Zeta potential and particle size..... 88

3.4.3.3 Microstructure..... 89

3.5 Conclusion..... 91

3.6 References..... 91

4. Improvement of gel quality of sardine surimi with low setting phenomenon by ethanolic coconut husk extract..... 97

4.1 Abstract..... 97

4.2 Introduction..... 97

4.3 Materials and methods..... 99

4.4 Result and discussion..... 103

4.4.1 Effect of ECHE on gel properties of low setting surimi..... 103

CONTENTS (Continued)

Chapter	Page
4.4.1.1 Breaking force and deformation…….……..	103
4.4.1.2 Textural profile analysis……	105
4.4.1.3 Expressible moisture content…….	107
4.4.1.4 Whiteness……...	107
4.4.1.5 TCA-soluble peptide content…….	108
4.4.1.6 Protein patterns……..	109
4.4.1.7 Microstructures……	110
4.4.2 Impact of ECHE on dynamic rheological properties of low setting surimi paste during setting……...	111
4.5 Conclusion…….....	113
4.6 References…..…….....	113
5. Combination effect of high pressure treatment and ethanolic extract from coconut husk on gel properties of sardine surimi……..……...	118
5.1 Abstract…….....	118
5.2 Introduction……...	118
5.3 Materials and methods.…….....	120
5.4 Results and discussion.…….	125
5.4.1 Effect of ECHE at different levels on properties of high pressure treated surimi gels…….…….……..	125
5.4.1.1 Breaking force and deformation...……...….……..	125
5.4.1.2 Texture profiles…….…….……..	128
5.4.1.3 Expressible moisture content…. ……..…….……..	129
5.4.1.4 Whiteness……..…. ……..…….……..	130
5.4.1.5 TCA-soluble peptide content…. ……..…….……..	131
5.4.2 Characteristics of high pressure treated surimi gel added with ECHE at the selected level ……..…..……..	132
5.4.2.1 Protein patterns…….	132
5.4.2.2 Solubility……..	133

CONTENTS (Continued)

Chapter **Page**

5.4.2.3 Microstructures……..……..…….. 135

5.6 Conclusion…..……...…….. 137

5.7 References…..……...……..... 137

6. Ethanolic coconut husk extract: *In vitro* antioxidative activity and effect on oxidative stability of shrimp oil emulsion ……..…….... 141

6.1 Abstract……..... 141

6.2 Introduction……... 141

6.3 Materials and methods.……..... 143

6.4 Results and discussion.……. 150

6.4.1 Condensed tannin content in ECHE…….…….. 150

6.4.2 Phenolic compounds in ECHE.....……...…..…….. 150

6.4.3 *In vitro* antioxidative activities of ECHE.…….…….. 151

6.4.4 Oxygen radical absorbance capacity of ECHE…… 154

6.4.5 Thermal stability of ECHE……..……..…….. 155

6.4.6 Oxidative stability of shrimp oil-in-water emulsion containing ECHE……..……. 157

6.4.6.1 Peroxide value (PV)……..…..…...…….. 157

6.4.6.2 Conjugated diene (CD)…… 157

6.4.6.3 Thiobarbituric acid reactive substances (TBARS) …….. 158

6.4.6.4 ρ-Anisidine value (AnV)…….. 158

6.4.6.5 Fatty acid profiles……. 161

6.5 Conclusion…… 163

6.6 References……. 163

7. Effect of ethanolic coconut husk extract and pre-emulsification on properties and stability of surimi gel fortified with seabass oil during refrigerated storage……...…….. 168

7.1 Abstract……..... 168

CONTENTS (Continued)

Chapter	Page
7.2 Introduction…….…….....……...……	168
7.3 Materials and methods……..	170
7.4 Results and discussion…….…….....…….…...…..	175
7.4.1 Characteristics of pre-emulsified seabass oil…….....……...…..	175
7.4.2 Effect of ECHE on gel properties of surimi gel fortified with pre-emulsified seabass oil during refrigerated storage……..…….	177
7.4.2.1 Breaking force and deformation ……..	177
7.4.2.2 Textural properties……	179
7.4.2.3 Whiteness…….	182
7.4.3 Oxidative stability…….	183
7.4.4 Microbiological quality…….	185
7.5 Conclusion.……..	188
7.6 References…….	188
8. Conclusion and suggestion……..	192
8.1 Conclusion…...…….....	192
8.2 Suggestion.……...	193
Vitae……..	194

LIST OF TABLES

Table		Page
1.	Chemical composition of husk from coconut fruit with the age of 11 months…….. ……...…	4
2.	Phenolic antioxidants from different natural sources and their uses in various food systems……..……...	23
3.	Expressible moisture content and whiteness of gels from sardine surimi added without and with E60 and E80 at different levels…..….	59
4.	Textural properties of gels from sardine surimi added without and with E60 and E80 at different levels……	63
5.	Likeness score of gels from sardine surimi added without and with E60 and E80 at different levels……..	65
6.	Ca^{2+}-ATPase activity of NAM solution (1 mg/mL) added without and with ECHE at different levels after heating from 20 to 60 °C……..	87
7.	Zeta potential and particle size of NAM solutions (1 mg/mL) added without and with ECHE at different levels after heating to 90 °C……..	89
8.	Textural profiles of low setting sardine surimi gel as affected by ECHE at different levels……...	106
9.	Expressible moisture content, whiteness and TCA-soluble peptide content of low setting sardine surimi as affected by ECHE at different levels…….	108
10.	Breaking force, deformation, expressible moisture content, whiteness and TCA-soluble peptide contents of pressurized surimi gel without and with subsequent heating in the absence or presence of ECHE at different levels……..	127
11.	Textural properties of pressurized surimi gel without and with subsequent heating in the absence and presence of ECHE at different levels…….	129
12.	Solubility of gels from sardine surimi prepared by different gelation processes without and with ECHE at a level of 0.075%.....	135
13.	Phenolic compounds in ECHE…….	151

LIST OF TABLES (Continued)

Table		Page
14.	Fatty acid profiles of lipid extracted from shrimp oil in-water emulsion added with and without ECHE at day 0 and 12 of storage at 30 °C…….	162
15.	Textural profiles of surimi gels fortified with pre-emulsified seabass oil in the presence or absence of ECHE at different levels during the refrigerated storage at 4 °C for 10 days…….	181
16.	Whiteness of surimi gels fortified with pre-emulsified seabass oil in the presence or absence of ECHE at different levels during refrigerated storage at 4 °C for 10 days. ……..	183
17.	Total viable count and psychrophilic bacterial count of surimi gels fortified with pre-emulsified seabass oil in the presence or absence of ECHE at different levels before and after storage at 4 °C for 10 days…	187

LIST OF FIGURES

Figure		Page
1.	Cross section of a coconut…….……...…	3
2.	Chemical structure of tannic acid……	5
3.	Three fundamental monomers in lignin……..	6
4.	Comparative schematics of protein surimi with and without high pressure processing…….	10
5.	Transglutaminase mediated protein cross-linking. Transamidation between protein-bound Gln and Lys residues leads to the formation of ε-(γ-glutamyl) lysine peptide bonds…….	11
6.	Protein cross-linking induced by phenolic compounds……	15
7.	Protein cross-linking induced by oxidized phenolic compounds…….	16
8.	Scheme demonstrating the detrimental effects of lipid and protein oxidation and preventive role of polyphenols…….	21
9.	Breaking force and deformation of gels from sardine surimi without and with E60 and E80 at different levels…….	58
10.	TCA-soluble peptide content of gels from sardine surimi without and with E60 or E80 at different levels……..	61
11.	SDS-PAGE patterns of protein in gels from sardine surimi added with E60 and E80 at different levels……	62
12.	Elastic modulus of sardine surimi gel without and with E60 and E80 at different levels during heating from 10 to 90 °C…….	65
13.	Electron microscopic images of surimi gel added with coconut husk extracts prepared using E60 and E80 at different levels……..	66
14.	Turbidity and solubility of NAM solution (1 mg/mL) added without and with ECHE at different levels during heating from 20 to 90 °C…….....	82

LIST OF FIGURES (Continued)

Figure		Page
15.	Surface hydrophobicity of NAM solution (1 mg/mL) added without and with ECHE at different levels during heating from 20 to 90 °C……...…….	84
16.	Total sulfhydryl group and disulfide bond content of NAM solution (1 mg/mL) added without and with ECHE at different levels during heating from 20 to 90 °C……	85
17.	Elastic modulus of NAM solution (20 mg/mL) added without and with ECHE at different levels during heating from 20 to 90 °C…….	88
18.	Transmission electron micrograph of fresh NAM solution and NAM solutions added without or with 0.01% or 0.03% of ECHE.....…..	90
19.	Breaking force and deformation of low setting sardine surimi gel as affected by ECHE at different levels……..	105
20.	Protein patterns of low setting sardine surimi gel as affected by ECHE at different levels…….	110
21.	Electron microscopic images of low setting surimi gel added with ECHE at different levels …….……	111
22.	Storage modulus of low setting sardine surimi paste as affected by ECHE at different levels…….	113
23.	SDS-PAGE patterns of gels from sardine surimi prepared by different gelation processes without and with 0.075% ECHE…….....	133
24.	Electron microscopic images of gels from sardine surimi prepared by different gelation processes without and with 0.075% ECHE ....…	136
25.	ABTS radical scavenging activity, DPPH radical scavenging activity, ferric reducing antioxidant power, chelating activity on ferrous ion, hydrogen peroxide scavenging activity and singlet oxygen scavenging activity of ECHE at different levels……	153

LIST OF FIGURES (Continued)

Figure		Page
26.	Fluorescence decay curve of fluorescein in the presence of ECHE and Trolox……..	154
27.	DPPH radical scavenging activity, ferric reducing antioxidant power and chelating activity of ECHE after heating at different temperatures for various times……	156
28.	Effect of α-tocopherol, ascorbic acid and ECHE at different concentrations on the formation of lipid oxidation products in shrimp oil-in-water emulsion stored at 30 °C for a period of 12 days……	160
29.	Particle size distribution and microstructures of seabass oil pre-emulsified using soy protein isolate in the absence and presence of ECHE at different levels. ……	176
30.	Changes in breaking force and deformation of surimi gels fortified with pre-emulsified seabass oil in the absence and presence of ECHE at different levels during the refrigerated storage at 4 °C for 10 days...	179
31.	Changes in PV and TBARS of surimi gels fortified with pre-emulsified seabass oil in the absence and presence of ECHE at different levels during the refrigerated storage at 4 °C for 10 days…	185

CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

1.1 Introduction

Coconut is abundant in coastal areas of tropical countries including Thailand. During processing, the husk of coconut is removed and the millions of tons of husk are generated each year (Panyakaew and Fotios, 2011). Its husk is composed mainly of lignin and cellulose, which are similar to those found in wood (Rodrigues *et al.*, 2008; Van Dam *et al*., 2004). Phenolic compounds namely 4-hydroxybenzoic acid (4-HBA), ferulic acid as well as tannic acid were found in coconut husk (Dey *et al*., 2003; Rodrigues and Pinto, 2007). Several phenolic compounds have been known to possess antioxidant (Maqsood et al., 2012), antimicrobial and antiviral (Carvalho *et al*., 2013; Hossain *et al*., 2014; Ramadan *et al.,* 2015) and anticancer activities (Gawlik-Dziki *et al*., 2012). Nowadays, the natural phenolic compounds have gained increasing interest as the additives in foods because of their safety and abundance.

Surimi, the washed fish mince, has gained popularity as a raw material for preparing several products with elastic texture (Mansfield, 2003). Generally, lean fish are commonly used for production of surimi since they yield surimi with white color and better gel property as compared to dark fleshed fish. Due to the insufficient lean fish, pelagic dark-fleshed fish such as sardine (*Sardinella albella*) and mackerel (*Rastrelliger kanagurta*), have been used as an alternative raw material for surimi production (Chaijan *et al*., 2004; Arfat and Benjakul, 2012). Although those dark-fleshed fish contain high level of ω-3 polyunsaturated fatty acids, which have been considered beneficial to human health, their high lipid, myoglobin and sarcoplasmic protein content contribute to the difficulties in making high-quality surimi (Riediger *et al*., 2009; Chaijan *et al*., 2010). During processing, storage or transportation, these dark fleshed fish are susceptible to lipid oxidation, which is associated with the rancidity and loss in nutritive value (López-de-Dicastillo *et al*., 2012). Dark fleshed fish also contain high level of proteases, particularly heat-activated

proteases, which show an adverse effect on gel formation (Hu *et al*., 2010). To tackle such a drawback, several additives have been used widely. Protein additives such as egg white, whey protein concentrate, soy protein isolate, etc. are commonly used for improvement of surimi gel (Rawdkuen and Benjakul, 2008; Hunt *et al*., 2009; Jafarpour *et al*., 2012). However, some proteins may cause the allergy to consumers (Leduc *et al*., 1999). Additionally, cross-linking enzyme, especially microbial transglutaminase (MTGase) has been employed to increase gel strength of surimi, particularly those with low setting phenomenon (Sato *et al*., 2007; Cardoso *et al*., 2009). However, the cost of enzyme is still of major concern for industry. Plant phenolics can be used as protein cross-linker, which is able to strengthen the gel of proteins, especially surimi. Balange and Benjakul (2009) reported that the use of phenolic compounds, especially tannic acid, in the oxidized form, effectively increased gel strength of surimi from mackerel. Additionally, the kiam wood extract also exhibited the gel strengthening effect in surimi from mackerel. Kaewdang and Benjakul (2015) reported that the extracts from coconut husk improved gel strength of gelatin from yellowfin tuna swim bladder. Nevertheless, the excessive concentration of the extracts induced the coagulation of those proteins, in which the ordered structure could not be formed. Therefore, the phenolic extract from the cheap and abundant source such as coconut husk can serve as a new protein cross-linker, which can be used for surimi.

Fish and fish products are susceptible to spoilage, associated with the short shelf-life. Due to the high content of polyunsaturated fatty acid (PUFA), they are also prone to lipid oxidation, thereby limiting the shelf-life (Wu and Mao, 2009; Takeungwongtrakul and Benjakul, 2013). PUFA as well as astaxanthin, especially from shellfish leftover, have gained increasing interest. Nevertheless, the product supplemented with shrimp oil is more likely susceptible to oxidation, which causes the off-odor/flavor in the products. To conquer the obstacle governed by lipid oxidation, antioxidants are of the choices to retard the loss in quality and acceptability. Additionally, the awareness of using synthetic additive can be minimized. Furthermore, new natural additive from coconut husk can be gained and the underutilized coconut husk is better exploited.

1.2 Review of literature

1.2.1 Coconut and coconut husk

Coconuts (*Cocos nucifera* Linn.) are abundant in coastal areas of tropical countries and widely applied in many products, both food and non-foods. Young coconut is a normally consumed as refreshment, consisting of soft white meat and sweet white transparent aroma juice (Terdwongworakul *et al*., 2009). For a mature coconut, it consists of the copra or white meat (28 wt. %), which is surrounded by a protective shell (12 wt. %) and thick husk (35 wt. %) as shown in Fig.1 (Van Dam *et al*., 2004).

The coconut husk is remained in large quantities after coconut production. The husk comprises 30% fiber and 70% pith. Lignin and phenolics are found at high amount with the range of 45-48%, in both fiber and pith (Bilba *et al*., 2007). The husks have been used for the production of paper pulps, which have high lignin and cellulose contents. The material can be characterized by high toughness and durability due to its high lignin content, compared to other natural fiber (Silva *et al*., 2000).

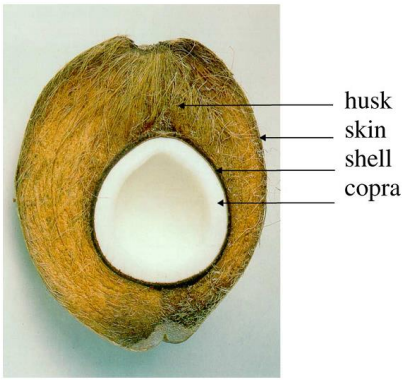


Figure 1. Cross section of a coconut
Source: Van Dam *et al*. (2004)

1.2.2 Chemical composition of coconut husk

The coconut husk compositions are influenced by the maturity of the fruits and also processing conditions. The chemical composition of coconut husk is shown in Table 1.

Table 1. Chemical composition of husk from coconut fruit with the age of 11 months

Composition	% (wet basis)
Moisture	10.66 ± 0.05
Ash	0.95 ± 0.01
Protein	0.9 ± 0.2
Acid soluble lignin	1.61 ± 0.07
Acid insoluble lignin	33 ± 3
Sugar	
Glucan	15 ± 2
Xylan	19 ± 2
Galactan	0.09 ± 0.02
Arabinan	0.27 ± 0.02
Mannan	0.020 ± 0.003

Source: Prado *et al*. (2014)

1.2.2.1 Tannins

Tannins and its derivatives such as gallic acid and ellagic acid are commonly found in higher herbaceous and woody plants (Labieniec and Gabryelak, 2006). Tannins, also called tannic acid, have a structure consisting of a central glucose and 10 galloyl groups. Tannic acid is a water-soluble polyphenol containing sugar esters, mainly glucose and phenol carboxylic acids, such as gallic acid or its stable dilactone ellagic acid (Fig. 2). Hydrolyzable tannins such as tannic acid and epigallocatechin gallate have been reported to have antioxidant, antimicrobial and antiviral activities (Akiyama *et al*., 2001; Rodrigues *et al*., 2008). The antioxidant activity of tannic acid has been previously attributed to its capacity to form a complex with iron ions and reduce Fe(III) to Fe(II) (Andrade Jr *et al.,* 2006). As an antioxidant compounds, tannic acid was also shown to prevent lipid oxidation, via suppressing hydroxyl radical formation. Maqsood and Benjakul (2010) reported that tannic acid exhibited higher antioxidative activity assayed by DPPH and ABTS radical scavenging activity, FRAP than cathechin, caffeic acid and ferulic acid. Compared to butylated hydroxyanisole (BHA), α-tocopherol and trolox, Gülçin *et al*.

(2010) found that addition of tannic acid into linoleic acid emulsion up to 15 µg/mL could show higher inhibition toward lipid oxidation (97.7%). Tannic acid also prevented lipid oxidation of fish oil emulsion and fish mince at the concentration of 100 mg/L and 100 mg/kg, respectively (Maqsood and Benjakul, 2010). Thiansilakul *et al*. (2012) also reported that tannic acid at 200 mg/kg could retard the oxidation of myoglobin, thereby maintaining the redness of bighead carp flesh during the extended storage.

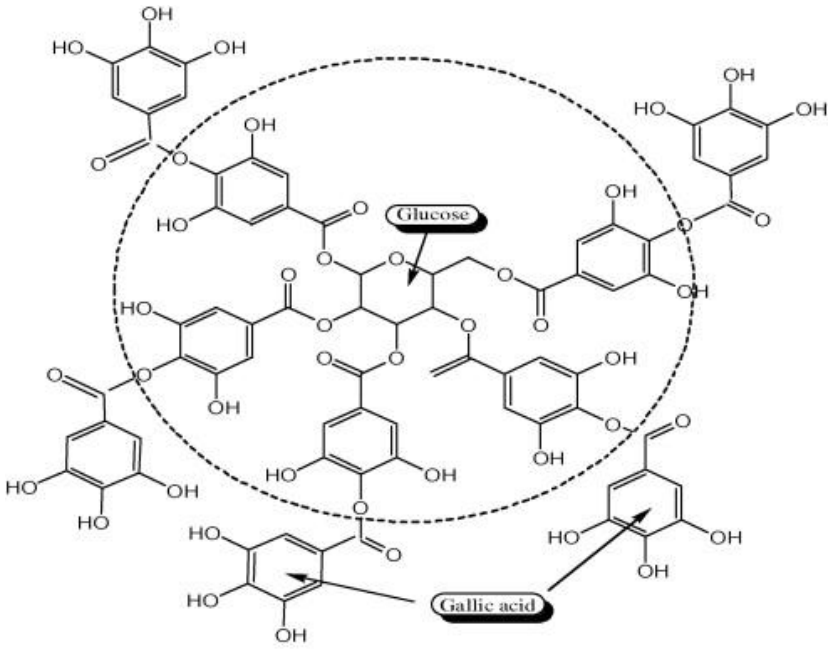


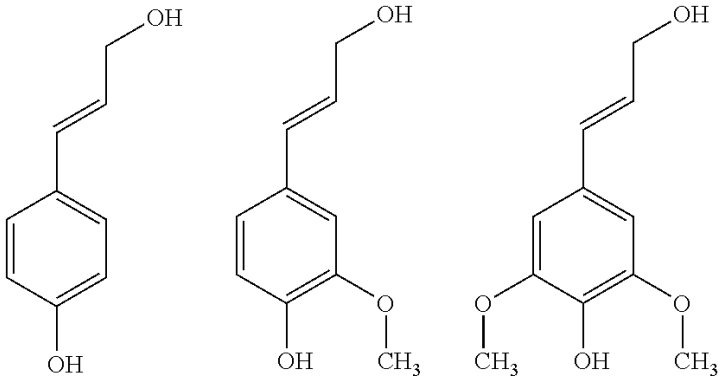
Figure 2 Chemical structure of tannic acid. The shade circle highlights pentagalloylglucose and the core structure of tannic acid.

Source: Gülçin *et al*. (2010)

As a food additive, a safe dosage of tannic acid ranges from 10 to 400 µg, depending on the kind of food to which it is added (Chen and Chung, 2000). The consumption of polyphenol-rich fruits, vegetables and beverages, such as tea and red wine, containing tannic acid, has been linked with inhibitory and preventive effects in various human cancers and cardiovascular diseases. Moreover, tannic acid inhibited skin, lung and forestomach tumors induced by polycyclic aromatic hydrocarbon carcinogens and *N*-methyl-*N*-nitrosourea in mice (Andrade *et al*., 2005).

1.2.2.2 Lignins

Lignin is another major component of plant material and the most abundant form of aromatic carbon in the biosphere (Monties and Fukushi, 2001). Lignin is a complex and high-molecular-mass phenolic compound (600-1000 kDa) that is generated through radical polymerization of the component phenolics, particularly coniferyl alcohol, *ρ*-coumaryl alcohol, sinapyl alcohol (Fig. 3) and other phenolic acids (Ralph *et al.*, 2004). Lignin is varied in structures, depending on the degree of polymerization of those phenolics.



(a) (b) (c)

Figure 3. Three fundamental monomers in lignin: *ρ*-coumaryl alcohol (a), coniferyl alcohol (b) and sinapyl alcohol (c).

Source: Ralph *et al*. (2004)

Lignin is generally composed of several phenolic groups with different substituents in the position 2-, 4- and 6- (Poletto and Zattera, 2013). Due to varying special functional groups, such as phenolic hydroxyl, alcohol hydroxyl and carboxyl groups, etc., lignin can be used for the production of materials with unique properties, e.g., dispersants, absorbents, resin, surfactants, etc. (Gosselink, 2004).

Lignin possessing antioxidative activity has been used in fields of cosmetics, medicals and pharmaceuticals (Ugartondo *et al*., 2008). Arshanitsa *et al*. (2013) reported that lignin extracted using methanol from wheat straw showed radical scavenging activity, including ABTS radicals, DPPH radical and superoxide anion-radical scavenging

activities. García *et al*. (2011) found that lignin extract using ethanol (60% v/v) showed similar ABTS radical scavenging activity to that of natural antioxidants such as gallic acid and catechin, and also a commercial Trolox.

However, lignin or other phenolic antioxidants used in food products must be safe for consumption. Compared to (-)-Epicatechin, the lignins are less or equally cytotoxic than epicatechin after 48 and 72 h of exposure. Comparing two cell lines, the human keratinocytes were more affected after 24 h exposure than that of mouse fibroblast (Ugartondo *et al*., 2006; Ugartondo *et al*., 2008). Nevertheless, the effective concentration of lignin is much smaller than its cytotoxic concentration (from 5 to 10-fold lower). Therefore, those compounds thus exhibited antioxidant activity at non-cytotoxic concentrations.

1.2.3 Surimi and gelling property

Surimi is refined fish protein concentrate manufactured by repeated washing of fish mince with chilled water until most of the water-soluble protein is removed (Park and Morrissey, 2000). Generally, lean fish have been widely used for surimi production because of their high gel forming ability and white color (Jiang *et al*., 2000; Hsu *et al*., 2002). Due to the insufficient fish resources, dark fleshed fish such as sardine and mackerel have been used as an alternative raw material for surimi production instead of lean fish (Chaijan *et al*., 2004). However, it shows higher lipid and sarcoplasmic protein contents as well as proteolytic activity (Chaijan *et al*., 2010). The autolysis of true sardine and Indian anchovy was caused by both acidic and alkaline heat stable proteases (Sirigan *et al*., 2006; Klomklao *et al*., 2008). Poor gel properties are gained when surimi is produced from dark fleshed species. Sarcoplasmic proteins at high amount contributed to the decrease in breaking force of surimi gels (Lee *et al*., 2011; Hemung and Chin, 2013).

1.2.3.1 Surimi gelation

Gelation is an aggregation of proteins, in which a three-dimensional network with water trapped inside can be formed (Chanasttru *et al*., 2007). When myofibrillar proteins are solubilized by salt and then unfolded, the network is formed and

it is stabilized by intermolecular bonds including ionic linkages, hydrophobic interactions, covalent bonds and hydrogen bonds (Lanier *et al.*, 2005). Myosin is the most important component for gel formation in fish gel products. Gels prepared from myosin alone have higher gel strength and elasticity than those prepared from natural actomyosin (NAM) (Ikeuchi *et al*., 1992).

1.2.3.2 Thermal induced gelation

The heat induced denaturation and aggregation of muscle proteins, which consists of myosin, actin, tropomyosin and troponin, result in the formation of 3-dimensional network of surimi gel (Zhang *et al*., 2013). Dynamic viscoelastic behavior analysis revealed that the gelation of NAM from haddock occurred in two stages: at temperature range of 36-45 °C and 59-90 °C (Leelapongwattana *et al*., 2008), while surimi from Alaska pollock showed the gelation at temperature range of 37-40 °C and 47-75 °C (Yin and Park, 2014). Zhu *et al.* (2015) reported that the incubation temperature used for gel formation affected surimi gel from yellowtail seabream. The gel incubated at 40 °C, followed by cooking at 90 °C (suwari) showed higher gel strength than that incubated at 67 °C, followed by 90 °C (modori). The first stage of gelation was due to myofibrillar proteins in surimi, which undergo aggregation or entanglement. The second stage was attributed to the formation of irreversible gel network (Rawdkuen *et al*., 2007). Sano *et al*. (1990) revealed that the heat-induced gelation of surimi consists of two reactions: (1) aggregation of the globular head segments of the myosin molecules, which is associated with the oxidation of sulfhydryl groups and (2) network formation caused by the unfolding of the helical tail segment. Different fish species showed varying gel forming ability and cross-linking activity of MHC (Chanarat *et al*., 2012).

1.2.3.3 High pressure induced gelation

High pressure processing (HPP) has become increasing interest for food industry (Considine *et al*., 2008). HPP has been used for destruction of microorganisms, activation and inactivation of enzymes, and formation of protein gels (Simonin *et al*., 2012; Reyes *et al*., 2015). HPP has been used in the food industry in Japan, mainly for

pasterurisation and processing of jams, jellies as well as surimi gels (Yaldagard *et al*., 2008). For surimi industry, the use of HPP can improve gelation of surimi and fish mince by inducing protein aggregation. HPP has been known to destabilize non-covalent protein-protein interactions and promote the dissociation of oligomeric proteins, the formation of more complex systems and the unfolding and breakdown of other components (Rivalain *et al*., 2010). It has been reported that hydrogen bonds will be formed when a level of pressure below 150 MPa was applied. When pressures were higher than 200 MPa, ionic interactions and hydrophobic interactions became predominant (Yaldagard *et al*., 2008). Herranz *et al*. (2013) reported that gels from low quality frying fish surimi pressurized at 80 MPa were more flexible and elastic. The use of HPP at 200 MPa on frozen frying fish yielded more elasticity suwari gel as evidenced by the increased storage modulus (G´) (Moreno *et al*., 2015). Liang *et al*. (2017) found that when bighead carp surimi was subjected to high pressure treatment at 500 MPa for 30 min, the resulting gel had the highest gel strength and springiness, compared to the traditional two-step heated gels.

Prior to heat-induced gelation, both HPP and TGase can be used for enhancing gel strength and deformability of surimi, in which protein polymerization was induced and a denser fibrous structure was developed (Zhu *et al*., 2014). Cando *et al*. (2015) reported that the mechanical properties of surimi gel with low salt content (0.3%) from Alaska pollock were improved by the application of HPP at level of 300 MPa, followed by heat treatment. In the presence of 0.3 and 3% of salt, HPP treatment induces protein unfolding of Alaska pollock surimi. Hydrophobic residues and sulfhydryl groups were exposed, followed by protein aggregation during heat-induced gelation. These results in increased hydrophobic interaction and disulfide bond formation, which stabilized the water/protein system. At low salt content, surimi without HPP treatment showed less protein denaturation, thereby hindering the formation of ordered protein network (Fig. 4). The denaturation of these proteins was in accordance with Cando *et al*. (2014) who documented that HPP at higher levels induced protein denaturation in isolated hake myofibrils with a reduction of α-helix together but an increase in β-structure as monitored by FTIR. At level of 500 MPa, myosin was completely denatured as indicated by the

absence of T_{peak} in DSC thermogram. High pressure pretreatment of surimi increased the availability of sulfhydryl groups, resulting in the increased formation of disulfide bonds during heating (Moreno *et al*., 2015). Pressure induces the formation of heat labile hydrogen-bonded structures, while heat treatment gives rise to structures that are primarily stabilized by disulfide bonds and hydrophobic interactions (Angsupanich *et al*., 1999).

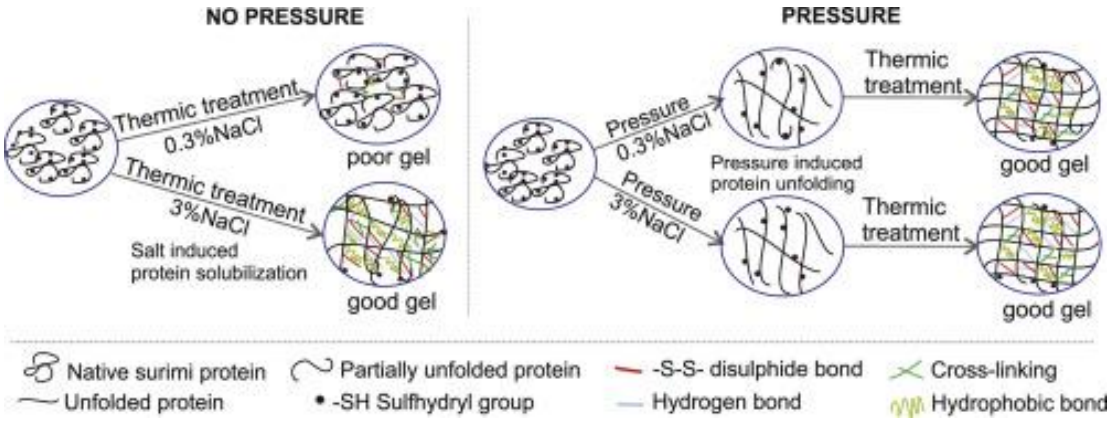


Figure 4. Comparative schematics of protein surimi with and without high pressure processing

Source: Cando *et al*. (2015)

1.2.4 Quality of surimi gel as affected by endogenous enzymes

1.2.4.1 Setting phenomenon

Setting is a phenomenon explaining the increased textural properties of surimi gels after pre-incubation at temperature between 0 and 40 °C. Setting has been maximized to strengthen cooked gels as this pre-incubation period allows endogenous transglutaminase (TGase) to form covalent glutamyl-lysine crosslinks between proteins (Lanier, 2000). The setting response relates to the habitat temperature of fish species and it affects the gelation behavior. Setting of Alaska pollock surimi from Seattle, USA., was done either at 5 °C (low temperature) or 25 °C (medium temperature) (Zhang, *et al*., 2013; Zhu *et al*., 2014). High temperature (40 °C) has been applied for setting of surimi from tropical or warm water fish species such as threadfin bream, Indian mackerel, sardine, yellow stripe travelly, goatfish and lizardfish (Arfat and Benjakul, 2012; Chanarat *et al*.,

2012; Tadpitchayangkoon *et al*., 2012). Gelation of fish paste has been reported to have a close relationship with the endogenous TGase activity via the formation of cross-links between myosin heavy chains (Benjakul *et al*., 2003). These enzymes induce the formation of ε-(γ-glutamyl) lysine cross-link in the protein via acyl transfer between the ε-amino groups of a lysine residue and γ-amide group of a glutamine residue (Chanarat *et al*., 2012) (Fig. 5). Partially purified TGase from different fish species, namely bigeye snapper, Indian oil sardine, tilapia and common carp had molecular weights range from 73 to 95 kDa. Moreover, the specific activity and the optimum temperature is also affected by fish species (Binsi and Shamasundar, 2012).

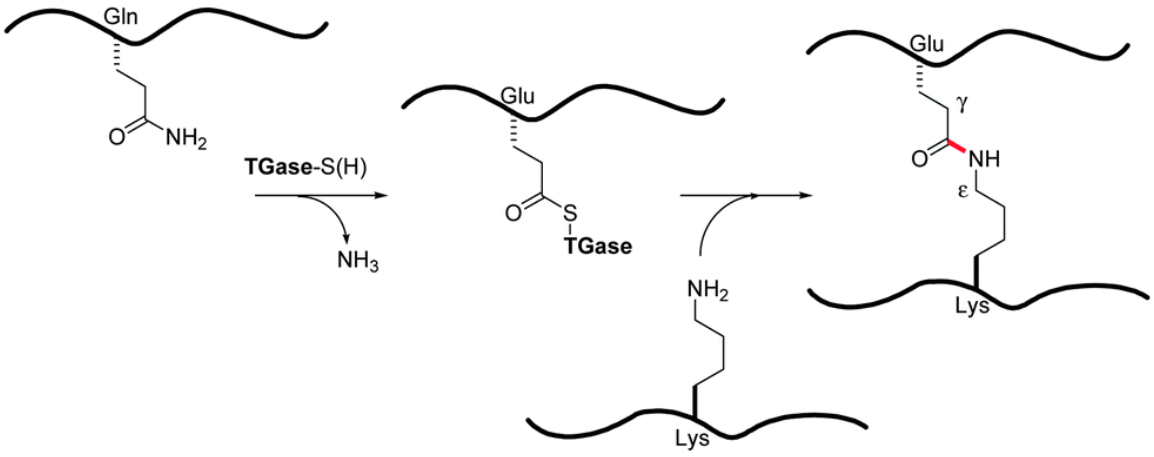


Figure 5. Transglutaminase (TGase) mediated protein cross-linking. Transamidation between protein-bound Gln and Lys residues leads to the formation of ε-(γ-glutamyl) lysine peptide bonds.

Source: Oteng-Pabi *et al*. (2014)

Covalent ε-(-γ-glutamyl) lysine cross-liking between myosin heavy chains is catalyzed by TGase at low temperatures (Kütemeyer *et al*., 2005). Thus, setting at low temperature improves the gel strength of surimi products via enhancing protein cross-linking. The subsequent heating process at high-temperature aims to inactivate heat-stable protease and induce the complete gelation (Benjakul *et al*., 2004b). TGase from fish has been found to be Ca^{2+} dependent enzyme but differ in the requirement of Ca^{2+} ion, depending on fish species. The addition of calcium compound to surimi enhanced TGase-

mediated setting, resulting in stronger gels (Benjakul *et al*., 2010; Yin *et al*., 2014). Different fish species possessing varying levels of TGase render surimi gels with different properties (Asagami *et al*., 1995; Ramírez *et al*., 2000). However, some chemicals also affected the setting phenomenon of surimi gel. Kataoka *et* al. (1998) reported that when ethylene glycol bis(β-aminoethylether)-*N,N,-N',-N'*-tetraacetic acid (EGTA) was added into pollock surimi paste with a concentration range of 0-10 mM, gel strength of the resulting gels was decreased with a dose-dependent manner. Addition of EGTA at a concentration of 10 mmol per kg surimi also decreased gel strength of walleye pollock surimi. This result was supported by the presence of myosin heavy chain band retained in the SDS-PAGE pattern, which indicated the inhibition of cross-linking of MHC by TGase through chelation of calcium ions (Hossain *et al*., 2001; Yongsawatdigul *et al*., 2002). Owing to the low level of endogenous TGase in some fish species, microbial TGase (MTGase) has been introduced for improvement of surimi gel quality (Chanarat *et al*., 2012). The property of surimi gel from Indian mackerel and threadfin bream was improved by addition of MTGase (Chanarat and Benjakul, 2013; Kaewudom *et al*., 2013)

1.2.4.2 Softening phenomenon

Gel softening termed “modori” generally occurs when gel is heated at 40 to 60 °C for a long period. As a result, an irreversible proteolytic degradation of myofibrillar proteins, especially myosin heavy chains, take place, resulting in disintegration of gel structure. This process causes the negative effects on the quality of surimi products and reduces their commercial value (Hu *et al*., 2010). Modori is mainly due to the autolysis caused by sarcoplasmic proteases, especially serine- and cysteine-type proteases, which are activated at postmortem pH (Hu *et al*., 2012). Although sarcoplasmic proteins are water-soluble, some of them are retained after washing process (Hossain *et al*., 2004). Higher proteolytic activity, caused by cathepsin L, is found at temperature above 50 °C and leads to the rapid and severe degradation of myofibrillar proteins, especially myosin (Kudre *et al*., 2013). Among the numerous proteases present in muscle, cysteine endoproteases cause the most adverse effects on texture, owing to their thermostability and ability to cleave internal peptide bonds, resulting in shorter peptide chains. Softening of

threadfin bream is due to a cysteine protease, which has the maximum autolytic activity at 50-60 °C (Oujifard *et al*., 2012). When Pacific whiting surimi was incubated at 55 °C, most of MHC was degraded when the incubation time increased, which was in accordance with the lower breaking force or gel strength of the resulting surimi gel (Rawdkuen *et al*., 2007). To tackle the excessive autolytic activity of surimi, numerous food-grade protease inhibitors, which are capable of limiting the modori phenomenon and improving gelling properties, have been widely used in surimi (Rawdkuen *et al*., 2007; Campo-Deaño and Tovar, 2009; Fowler and Park, 2015). The most frequently used protease inhibitors are egg white, whey protein concentrate and soy protein isolate (Campo-Deaño and Tovar, 2009; Ouijifard *et al*., 2012; Kudre *et al*., 2013). However, some undesirable odor, flavor and color have been reported in surimi gel with the addition of those inhibitors. Recently, Fowler and Park (2015) reported that the addition of salmon plasma protein significantly improved gel strength of Pacific whiting surimi subjected to the two steps ohmic heating, ohmically heating to 60 °C and holding for 30 min followed by ohmically heating to 90 °C. Furthermore, Klomklao *et al*. (2015) reported that protease inhibitor from tuna roe effectively improved the gel strength of surimi from bigeye snapper via inhibiting proteolysis. Protease inhibitor purified from adzuki bean seed (0.5-3%) was also reported as a potential trypsin inhibitor as indicated by the lowered TCA soluble peptide content in threadfin bream muscle and increased breaking force of threadfin bream surimi gel (Klomklao and Benjakul, 2015). Two proteinase inhibitors, which were purified from common carp sarcoplasmic protein, were identified as inhibitor I and II and exhibited molecular mass of 47 and 52 kDa, respectively. Those inhibitors were alpha-1-proteinase inhibitor and effectively reduced autolytic degradation of bigeye snapper surimi (Siriangkanakun *et al*., 2016).

1.2.5 Improvement of surimi gel property using plant phenolics

Protein modification via cross-linking is of the approach to obtain desirable characteristics in the food, particularly jelly product with unique textural properties such as surimi gel. Various cross-linkers have been used in protein-based foods to improve their functional properties. However, some protein cross-linkers are toxic or allergy and cannot

be use in foods. Therefore, natural protein cross-linkers, especially those from plant polyphenolics, have been paid more interest due to their safety and efficacy in food systems (Maqsood *et al*., 2013).

1.2.5.1 Cross-linking activity of protein as affected by phenolic compounds

Phenolic compounds containing hydroxyl group and phenol ring, which are polar and hydrophobic portion, respectively. The phenolic group is an excellent hydrogen donor that forms hydrogen bond with carbonyl group of the protein (Fig. 6). For phenolic compounds with high protein affinity, they must be small enough to penetrate inter-fibrillar regions of protein molecules, but large enough to crosslink peptide chains more than one point (Mulaudzi *et al*., 2012). Phenolics may interact with proteins via reversible or irreversible interactions. For reversible interaction, hydrogen bonding, hydrophobic interaction and van der Waals forces are involved, whereas irreversible interaction of protein-phenolics is related to covalent bond. (Ozdal *et al*., 2013). Recently, there is some information on the utilization of plant extract as the cross-linking agents in food proteins, particularly surimi (Balange and Benjakul, 2009a; Shitole *et al*., 2014a). The phenolics were extracted from water seaweed and incorporated to strengthen surimi gel from lesser sardine and mackerel at different levels (0.5-2.5%). It was found that surimi gel from lesser sardine and mackerel added with 2.0% water seaweed extract (WSE), which had 16.24 mg tannin/g dry seaweed powder, could increase gel strength by 76.27 and 32.45%, compared with the gel without WSE, respectively (Shitole *et al*., 2014a, b). Majumdar *et al*. (2015) reported that addition of phenolics extracted from garlic using water at 1% into Thai pangas surimi gel resulted in the stronger protein network formation and higher water holding capacity, compared with the gel without the garlic extract. Moreover, gel properties of sardine surimi gel can be improved by the mixture of tannic acid (1%) and squid ink tyrosinase (500 U/g protein) (Vate and Benjakul, 2015). However, the higher amount of extract used could lower gel strength, mainly caused by the excessive cross-linking and unconformational alignment of protein network (Shitole *et al*., 2014a).

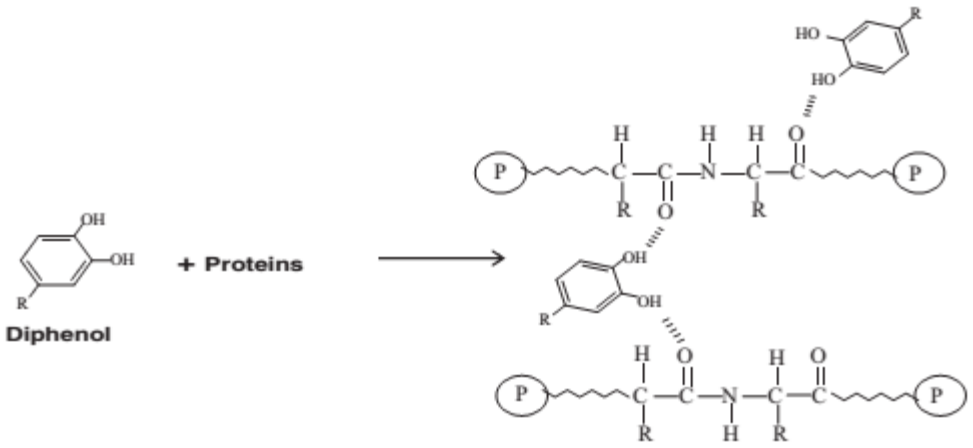


Figure 6. Protein cross-linking induced by phenolic compounds.
Source: Maqsood *et al*. (2013)

1.2.5.2 Oxidation of phenolic compounds

Alkaline solution has been introduced to oxidize phenols in the presence of oxygen. Polyphenols can be oxidized by molecular oxygen at alkaline pH to quinones, which react with side chain amino groups of peptides, leading to the formation of protein cross-links (Heck *et al*., 2013). These reactive quinones also can irreversibly react with the sulfhydryl and/or amino groups of proteins. Moreover, quinones can undergo condensation reactions, resulting in the formation of high molecular weight pigments with brown color named as tannins. Tannins are highly reactive and can readily interact with SH and amino groups of proteins (Charlton *et al*., 2002). The formation of rigid molecular structures by reaction of ortho-quinones with proteins is shown in Fig.7. Balange and Benjakul (2009a, b) reported that the oxidized tannic acid, which was bubbled at pH 8 for 1 h, induced protein cross-linking in surimi from bigeye snapper and mackerel, resulting in the increased gel strength and sensory property. The increase in gel strength was also found when oxidized kiam wood was applied to mackerel surimi (Balange and Benjakul, 2011). Temdee and Benjakul (2015) also reported that the oxidized form of wood extracts, kiam and cashew bark extracts, prepared using laccase at 20 U/mL showed the cross-linking activity as evidenced by the decreases in free amino group contents of gelatin. Gel network with

increased protein cross-linking had the thick strands with small voids. The use of oxidized phenolic compounds can be a new protein cross-linker for improving gel properties of surimi both, lean and dark-fleshed fish. Basically, it has no negative effect on the whiteness of the surimi gel from dark flesh fish (Balange and Benjakul, 2009a).

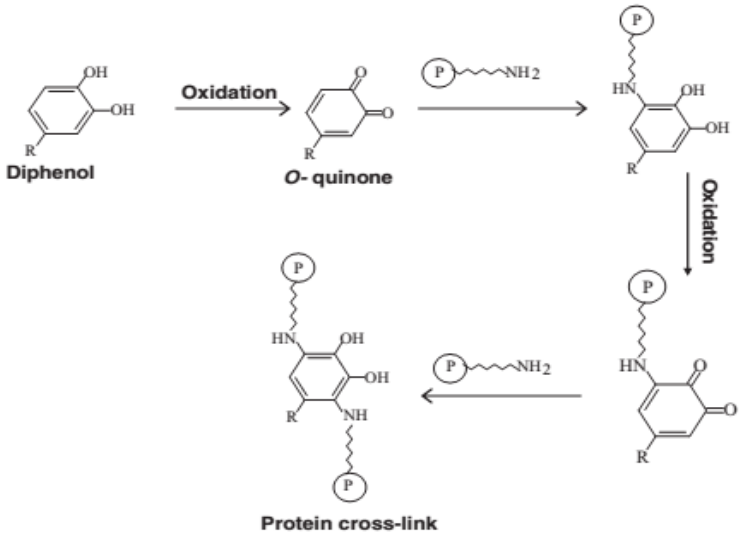


Figure 7. Protein cross-linking induced by oxidized phenolic compounds.
Source: Maqsood *et al*. (2013)

1.2.6 Emulsion gel

Emulsion gel, such as frankfurter, is commercially prepared by poultry, pork, beef or fish emulsified with fat from several sources. During comminution, muscle proteins are disrupted and the size of fatty tissue is continuously reduced. In the presence of salt, myofibrillar proteins can be soluble and migrate to the fat droplet surface, concentrate and form protein matrix at the interface of fat and water (Youssuf and Barbut, 2010).

To increase the nutritive value and improve textural property of frankfurter or other emulsion gel products, several fats and/or oils rich in unsaturated fatty acids have been supplemented such as olive oil, canola oil, linseed oil as well as marine oil from fish, shrimp or algae (Jiménez-Colmenero, 2007; Takeungwongtrakul and Benjakul, 2013). The

fortification of PUFA in freshwater fish emulsion sausage was also done by Panpipat and Yongsawatdigul (2008). The refined tuna oil was added into emulsion sausages prepared from African walking catfish and rohu at three levels (2, 6 and 10%) and stored at 4 °C for 4 weeks. The sausages fortified with tuna oil showed high level of *n*-3 (EPA and DHA), but lower level of *n*-6 fatty acids. However, fortification of refined tuna oil at the levels used had no effect on textural properties of fish sausages. Debusca *et al*. (2013) reported that the fortification of surimi with n-3 rich oils at a level of 10% directly could improve the rheological and textural characteristics of surimi gel from Alaska pollock. These oils had been reported to act as a gel filling in the protein matrix. Hence, fortification of surimi with PUFA rich oil is useful in developing surimi products with nutritional benefits.

Emulsion stability is a key factor determining the quality of sausage. To enhance the stability, several and combined preparation methods of emulsion have been reported. Different oil-in-water emulsions, including sodium caseinate (SC), soy protein isolate (SPI), sodium caseinate and TGase (SC+TG) and sodium caseinate, TGase and meat slurry (SC+TG+MS) were tested for stabilization efficiency. Every protein presented potential fat and water binding properties, since there was no noticeable release of exudate during heating and after 3 days of chilled storage, which reflected high thermal emulsion stability and creaming stability, respectively (Delgado-Pando *et al*., 2010). The stabilizing effect of protein, including SC, SPI and TG+MS, in frankfurters were confirmed by Jiménez-Colmenero *et al*. (2010). The use of those proteins yielded the sausage with good water and fat binding properties, greater hardness, cohesiveness and chewiness but poorer adhesiveness than control. Delgado-Pando *et al*. (2011) reported that substitution of pork backfat using oil-in-water emulsion (olive, linseed and fish oil mixed with caseinates) in low-fat frankfurters showed slight increases in lipid oxidation and microorganism count during chilled storage at 2 °C for 41 days. Moreover, the sausage added with emulsified fish oil mixed with TGase had the highest oxidation. This was possibly because TGase had ability to interfere the antioxidative activity of caseinates in the system, limiting their ability to inhibit lipid oxidation. Thus, antioxidants could be used to hinder these drawbacks.

The uses of phenolic acid and plant extract can retard lipid oxidation in emulsion gel systems. Maqsood *et al*. (2013) reported that the addition of tannic acid (0.02 and 0.04%) and ethanolic kiam wood extract (0.08%) effectively lowered lipid oxidation in striped catfish sausage during the refrigerated storage of 20 days. Moreover, samples added with 0.04% tannic acid yielded more compact structure with no visible voids. Rosemary leaf extract (0.5-2%) was also decreased lipid oxidation during chilled storage with a dose-dependent manner. Apple phenolics at an optimum level (0.5 g/kg in total fat) was an effective antioxidant in Chinese-style sausage than BHT (0.15 g/kg in total fat) during 120 days of storage (Yu *et al*., 2015). Recently, Rysman *et al.* (2016) also reported that emulsion sausage added with 3% apple phenolics had lower TBARS value, compared with the sample added with 0.05% sodium ascorbate, which is commonly employed in meat products.

1.2.7 Pre-emulsification for emulsion gel

Generally, it has been considered difficult to produce surimi containing high contents of oil, as fish protein and oil are not compatible. This results in the difficulty in mixing to obtain uniform and stable emulsion. Shabanpour *et al*. (2015) reported that addition of bulk oil could interfere the network formation of myofibril proteins, resulting in surimi gel with poor quality.

To conquer the aforementioned problem, pre-emulsification is a process of preparing an emulsion stabilized with an emulsifier, which is typically a protein of a non-meat origin such as soy protein (Kang *et al*., 2016). Pre-emulsification is generally used when incorporating fats that are difficult to stabilize. The process can improve fat binding ability, enhance physical stability, and is usually easier to disperse fat or oil into a water-based system such as a meat batter (Jiménez-Colmenero, 2007). It was noted that the addition of pre-emulsified oil could yield the frankfurter sausage with a higher β-sheet and a lower α-helices content. Moreover, tyrosine residues were exposed and the formation of hydrophobic interactions were more pronounced (Kang *et al*., 2016). Cáceres *et al*. (2008) reported that pre-emulsification of fish oil (1-6%) with caseinates could be an alternative

approach to enrich PUFA into emulsion gels. The bologna sausage added with pre-emulsified fish oil yielded the higher hardness and shear stress when fish oil level increased up to 6% in the final product, compared with the sausage without the emulsified oil added. Based on scanning electron microscopic study, a more compact protein network was found when the high levels of the pre-emulsified oil were added. Cheetangdee (2017) reported that the addition of pre-emulsified soybean oil using fish protein isolate or sodium caseinate as an emulsifier to substitute porcine fat at 25-45% substitution level into sausage increased the water holding capacity of the sausage, compared to that without pre-emulsified soybean oil or soybean oil in its native form. Thus, the emulsification process with animal or vegetable oil resulted in meat batters having improved stability; smaller particles of fat globules, better distribution of fat globules in protein matrix, larger coverage of protein film packaged fat globule, leading to better immobility of moisture and fat during the heating process (Zhuang *et al*., 2016).

1.2.8 Use of antioxidants from plants in fish and fish products

Fish and fish products, such as surimi and fish emulsion sausage, has been popular due to its acceptable taste and high nutrition value (Jayathilakan *et al*., 2012). Recently, the fortification of *n*-3 PUFA has been gained more interests to improve nutritional and textural properties of fish products (Oliveira *et al*., 2013). However, the major problem in fat containing foods or food supplemented with marine fish oil is their high susceptibility to lipid oxidation (Hughes *et al*., 2012). Lipid oxidation is a principal cause of quality deterioration in muscle foods, especially in fish containing high amount of PUFA (Jittrepotch *et al*., 2006). Thus, the use of antioxidants is necessary.

Many synthetic antioxidants such as BHA, BHT, THBQ and PG have been widely used in different food products (Lorenzo *et al*., 2013). BHT and α-tocopherol have been reported to be effective in retarding the oxidative deterioration in complex food system such as fish muscle and fish oil from Kilka fish (Bagheri and Sahari, 2013). However, their negative effects on health have been considered. To conquer the problem associated with lipid oxidation and health-risk from the use of synthetic antioxidant, plant

phenolics have been applied into marine oils, meat, fish and other food model system, especially fat/oil containing foods (Espinosa *et al*., 2015).

1.2.8.1 Extraction of plant phenolics

Yields, phenolic contents as well as antioxidant activities of the plant extracts is governed by extraction solvents and their concentrations (Turan, 2014). The herbal plant extracts (including rosemary, thyme, sage and bay) prepared using different solvents including methanol, ethanol and acetone were compared. Methanolic extracts had the highest total phenolic content (78.4-177.4 mg gallic acid equivalent/g extracts) and DPPH radical scavenging activities. Moreover, antioxidant activity of all plant extracts, which was monitored by conjugated diene formation was above 89.7% at the concentration between 250-2,000 ppm in linoleic acid emulsion system during 16 h of incubation at 37 °C. Petroleum ether extract of *Dorystoechas hastata* L. had carnosic acid and carnosol contents (531.3 and 389.9 mg/g extract, respectively) (Erkan *et al.*, 2011). The extract showed the higher antioxidative potential based on DPPH and ABTS radical scavenging activities and TBARS assays during incubation in linoleic acid at 37 °C for 123 h, compared with methanol and water extracts. Date seed extracts (DSE) using ethanol and acetone at various concentrations (0, 20, 40, 60, 80 and 100%) as extracting media had different yield, total phenolic contents and total flavonoid contents. The use of ethanol at 60% and acetone at 80% showed the highest extract yield, total phenolic and flavonoid contents (Maqsood *et al*., 2015). These results were related with the high ABTS and DPPH radical scavenging activities and ferric reducing antioxidant power. Furthermore, the lipid oxidation in mackerel mince was delayed as affected by ethanolic and acetonic date extracts at level of 100 and 200 ppm with a concentration dependent manner during 12 days of iced storage. Therefore, extraction solvents and their concentrations affect yields, phenolics and flavonoids contents as well as antioxidant activities, mainly due to varying polarity (Do *et al*., 2014). Moreover, the residual of extraction solvents is needed to be considered as consumer's health-risk.

1.2.8.2 Applications of plant phenolics in fish and fish products

Plant phenolics or extracts can be used as natural antioxidants. Antioxidant action is dependent on the ability of phenolics to scavenge free radicals and/or chelate prooxidative metal ions (Fig. 8). Plant phenolics have been employed in various food systems (Table 2) (Končić *et al*, 2011; Adjimani and Asare, 2015). Phenolic compounds act as antioxidants due to their capacity of transferring single-electron and/or hydrogen-atom to free radicals, and also due to their ability to bind potentially prooxidant metal ions, resulting in a stable phenoxyl radical (Craft *et al*., 2012). It was reported that polyphenols bind ion via the ortho-dihydroxy (catechol) or trihydroxy-benzene (galloyl) group as well as the carbonyl group on C_3 carbon and hydroxyl group on C_4 carbon of flavonoids (Petry *et al*., 2010).

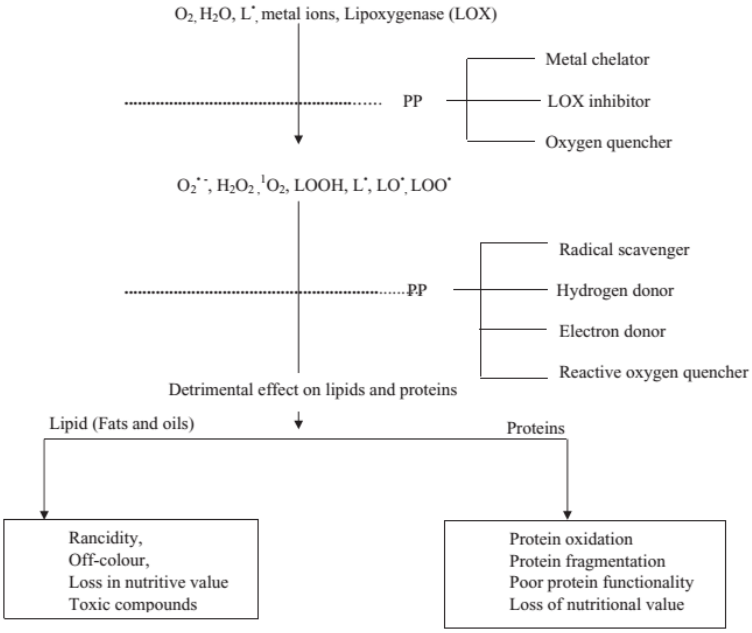


Figure 8. Scheme demonstrating the detrimental effects of lipid and protein oxidation and preventive role of polyphenols (PP).

Source: Maqsood *et al.* (2013)

Extracts from rosemary and green tea, which are rich in phenolic compounds, have been added to Bologna type sausages with the final concentrations of

500 and 400 ppm of total phenolic compounds, respectively. Lipid oxidation during modified atmosphere packaging (MAP) storage (30% CO_2, 1% O_2 and 69% N_2) at -80 °C for 4 weeks was monitored (Jongberg *et al*., 2013). As compared to the control, TBARS values were reduced by 80 or 73% in sausages added with green tea and rosemary extract, respectively. Amongst leaves, stems and flowers of three aromatic herbs, thyme, rosemary and lavender, the extracts from rosemary leaves and thyme leaves at a concentration of 100 ppm exhibited higher antioxidant activity than rosemary stems and thyme flowers in oil-in-water emulsion as indicated by the lower peroxide value (PV) during 42 days of storage at 33 °C (Gallego *et al*., 2013). Espinosa *et al*. (2015) reported that 11 phenolic compounds extracted from red propolis, including vanillic acid, caffeic acid, *trans*-cinnamic acid, 2,4-dihydroxycinnamic acid, *p*-coumaric acid, quercetin, *trans*-ferulic acid, *trans*,*trans*-farnesol, rutin, gallic acid or sinapic acid, showed antioxidant activity at a concentration of 200 ppm in emulsion fortified with *n*-3 fatty acid. Higher efficacy in retardation of lipid oxidation was found for sinapic acid and rutin, compared to others.

Grape seed extract (GSE) showed a potential antioxidative activity in dry fermented sausage during ripening. After 48 days of ripening process at 12 °C, TBARS value of the sausage added with GSE (1,000 mg/kg) and BHT (200 mg/kg) were 0.23 and 0.26 mg malondialdehyde (MDA)/kg, respectively (Lorenzo *et al.*, 2013). Apart from the seed, phenolic compounds were also extracted from grape dietary fibre (GDF) and added to horse mackerel mince at various concentrations (0, 2 and 4%). After the storage at -20 °C, grape fibre extract could retard lipid oxidation during 3 months of storage in a dose-dependent manner (Sánchez-Alonso *et al*., 2007). Methanolic anise seed extract was found to be a source of phenolic compounds (42.09 mg/g extract), mainly flavonoids (28.08 mg/g extract) and phenolic acids (14.01 mg/g extract). The high amount of phenolics contributed to potential antioxidative activities. EC_{50} value used for inhibition of lipid peroxidation and β-carotene bleaching, the reducing power and DPPH radical scavenging activity were 132, 530, 464 and 687 μg/mL, respectively (Martins *et al*., 2016). Therefore, the different plants and parts varied in phenolics and antioxidant capacity (Bhandari and Kwak, 2015).

Table 2. Phenolic antioxidants from different natural sources and their uses in various food systems

Sources	Active compounds	Food systems	References
Apple pomace	Chlorogenic acid, phlorizin and phloretin	Chinese sausage	Yu *et al*. (2015)
Cocoa	Quercetin-3-O-galactoside, quercetin-3-O-arabinoside, procyanidin and epicatechin gallate	Pork sausage	Ribas-Agusti *et al*. (2014)
Clove bud	Eugenol and caryphyllene	Silver carp fillets	Shi *et al*. (2014)
Date seed	*p*-hydroxybenzoic, protocatechuic and *m*-coumaric	Mackerel mince	Maqsood *et al*. (2015)
Grape seed	Gallic acid, epicatechin epigallocatechin, catechin and procyanidin	Silver carp fillets	Shi *et al*. (2014), Ribas-Agusti *et al*. (2014)
		Mackerel mince	Özen and Soyer (2018)
Grape dietary fiber/pomace	Gallic acid, catechin, cyanidin 3-*O*-glucoside, quinic acid, syringic acid and vanillic acid	Horse mackerel mince	Sánchez-Alonso *et al*. (2007)
		Raw and cooked chicken hamburger	Sáyago-Ayerdi *et al*. (2009)
		Fish oil, fish oil-in-water emulsion and mackerel mince	Pazos *et al*. (2005)
Green tea	Catechin	Tench fillets	Gai *et al*. (2014)

Table 2. Phenolic antioxidants from different natural sources and their uses in various food systems (cont.)

Sources	Active compounds	Food systems	References
Kiam wood	Tannic acid	Asian seabass mince	Maqsood and Benjakul (2013)
Mastic fruit	Caffeic acid, rutin, quercetin and vanillin	Pork sausage	Botsaris *et al*. (2015)
Moringa leaf	Tetradecanoic acid, hexadecenoic acid, phytol, 1,5-cyclodecadiene	Ground raw beef	Falowo *et al*. (2016)
Pomegranate	Anthocyanin	Mackerel mince	Özen and Soyer (2018)
Potato peel	Quinic acid, chlorogenicacid, caffeic acid and methyl caffeate	Horse mackerel mince	Farvin *et al*. (2012), Wu *et al*. (2012)
Rapeseed	Sinapic acid, *ρ*-hydroxybenzoic, vanillic, gentisic, caffeic, and chlorogenic acids	Mayonnaise dressing	Kim and Lee (2017)
Red propolis	Vanillic acid, caffeic acid, trans-cinnamic acid, *p*-coumaric acid, quercetin, rutin and gallic acid	Oil-in-water emulsion from echium oil	Espinosa *et al*. (2015)
Rosemary	Rosmarinic acid, caffeic acid, carnosol and carnosic acid	Sardine mince	Serdaroglu and Felekoglu (2005)
		Soy milk enriched with fish oil	Qiu *et al*. (2018)

Table 2. Phenolic antioxidants from different natural sources and their uses in various food systems (cont.)

Sources	Active compounds	Food systems	References
Stevia, bertoni	Vanillic acid 4-O-β-d-glucopyranoside, protocatechuic acid, caffeic acid, chlorogenic acid and cryptochlorogenic acid	Fish oil	Yu *et al*. (2017)
Strawberry	Hydroxycinnamic acids, cyanidin-3-glucoside and pelargonidin-3-rutinoside	Oil-in-water emulsion	Goulas and Manganaris (2011)
Tea	Catechin, epicatechin, epicatechin gallate, epigallocatechin gallate	Fresh shrimp	Sae-leaw *et al*. (2017)

1.3 References

Adjimani, J. P. and Asare, P. 2015. Antioxidant and free radical scavenging activity of iron chelators. Toxicol. Rep. 2: 721-728.

Akiyama, H., Fujii, K., Yamasaki, O., Oono, T. and Iwatsuki, K. 2001. Antibacterial action of several tannins against *Staphylococcus aureus*. J. Antimicrob. Chemother. 48: 487-491.

Andrade Jr., R. G., Dalvi, L. T., Silva, J. M. C., Lopes, G. K. B., Alonso, A. and Hermes-Lima, M. 2005. The antioxidant effect of tannic acid on the in vitro copper-mediated formation of free radicals. Arch. Biochem. Biophys. 437: 1-9.

Andrade Jr., R. G., Ginani, J. S., Lopes, G. K. B., Dutra, F., Alonso, A. and Hermes-Lima, M. 2006. Tannic acid inhibits *in vitro* iron-dependent free radical formation. Biochimie. 88: 1287-1296.

Arfat, Y. A. and Benjakul, S. 2012. Gelling characteristics of surimi from yellow stripe trevally (*Selaroides leptolepis*). Int. Aquat. Res. doi: 10.1186/2008-6970-4-5.

Asagami, T., Ogiwara, M., Wakameda, A. and Noguchi, S. F. 1995. Effect of microbial transglutaminase on the quality of frozen surimi made from various kinds of fish species. Fish. Sci. 61: 267-272.

Aungsupanich, K., Edde, M. and Ledward, D. A. 1999. Effects of high pressure on the myofibrillar proteins of cod and turkey muscle. J. Agric. Food Chem. 47: 92-99.

Balange, A. and Benjakul, S. 2009a. Enhancement of gel strength of bigeye snapper (*Priacanthus tayenus*) surimi using oxidised phenolic compounds. Food Chem. 113: 61-70.

Balange, A. and Benjakul, S. 2009b. Effect of oxidised phenolic compounds on the gel property of mackerel (*Rastrelliger kanagurta*) surimi. LWT-Food Sci. Technol. 42: 1059-1064.

Bao, H. N. D. and Ohshima, T. 2014. Strategies to minimize lipid oxidation of aquatic food products post-harvest. *In* Antioxidants and Functional Components in Aquatic Foods (Kristinsson, H. G. ed.). p. 95-125. Wiley-Blackwell, UK.

Benjakul, S., Chantarasuwan, C. and Visessanguan, W. 2003. Effect of medium temperature setting on gelling characteristics of surimi from some tropical fish. Food Chem. 82: 567-574.

Benjakul, S., Visessanguan, W. and Chantarasuwan, C. 2004a. Cross-linking activity of sarcoplasmic fraction of bigeye snapper (*Priacanthus tayenus*) muscle. LWT-Food Sci. Technol. 37: 79-85.

Benjakul, S., Visessanguan, W. and Chantarasuwan, C. 2004b. Effect of high temperature setting on gelling characteristics of suimi from some tropical fish. Int. J. Food Sci. Technol. 39: 671-680.

Benjakul, S., Yarnpakdee, S., Visessanguan, W. and Phatcharat, S. 2010. Combination effects of whey protein concentrate and calcium chloride on the properties of goatfish surimi gel. J. Texture Stud. 41: 341-357.

Bhandari, S. R. and Kwak, J.-H. 2015. Chemical composition and antioxidant activity in different tissues of *Brassica* vegetables. Molecules. 20: 1228-1243.

Bilba, K., Arsene, M.-A. and Ouensanga, A. 2007. Study of banana and coconut fibers: Botanical composition, thermal degradation and textural observations. Biores. Technol. 98: 58-68.

Binsi, P. K. and Shamasudar, B. A. 2012. Purification and characterisation of transglutaminase from four fish species: Effect of added transglutaminase on the viscoelastic behaviour of fish mince. Food Chem: 132: 1922-1929.

Bond, J. S. and Butler, P. E. 1987. Intracellular proteases. Ann. Rev. Biochem. 56: 333.

Botsaris, G., Orphanides, A., Yiannakou, E., Gekas, V. and Goulas, V. 2015. Antioxidant and antimicrobial effects of *Pistacia lenticus* L. extracts in pork sausages. Food Technol. Biotechnol. 53: 472-478.

Boyer, R. F. and McCleary, C. J. 1987. Superoxide ion as a primary reductant in ascorbate-mediated ferritin iron release. Free Rad. Bio. Med. 3: 389-395.

Cáceres, E., García, M. L. and Selgas, M. D. 2008. Effect of pre-emulsified fish oil – as source of PUFA *n*−3 – on microstructure and sensory properties of *mortadella*, a Spanish bologna-type sausage. Meat Sci. 80: 183-193.

Cando, D., Moreno, H. M., Tovar, C. A., Herranz, B. and Borderias, J. 2014. Effect of high pressure and/or temperature over gelation of isolated hake myofibrils. Food Bioprocess Technol. 7: 3197-3207.

Cando, D., Herranz, B., Borderias, A. J. and Moreno, H. M. 2015. Effect of high pressure on reduced sodium chloride surimi gels. Food Hydrocolloids. 51: 176-187.

Campo-Deaño, L. and Tovar, C. 2009. The effect of egg albumen on the viscoelasticity of crab sticks made from Alaska pollock and Pacific whiting surimi. Food Hydrocolloids. 23: 1641-1646.

Cao, M.-J., Wu, L.-L., Hara, K., Weng, L. and Su, W.-J. 2005. Purification and characterization of a myofibril-bound serine proteinase from the skeletal muscle of silver carp. J. Food Biochem. 29: 533-546.

Cardoso, C., Mendes, R., Vaz-Pires, P. and Nunes, M. L. 2009. Effect of dietary fibre and MTGase on the quality of mackerel surimi gels. J. Sci. Food Agric. 89: 1648-1658.

Carvalho, O. V., Botelho, C. V., Ferreira, C. G. T., Ferreira, H. C. C., Santos, M. R., Diaz, M. A. N., Oliveira, T. T., Soares-Martins, J. A. P., Almeida, M. R. and Júnior, A. S. 2013. *In vitro* inhibition of canine distemper virus by flavonoids and phenolic acids: Implications of structural differences for antiviral design. Res. Vet. Sci. 95: 717-724.

Chaijan, M., Benjakul, S., Visessanguan, W. and Faustman, C. 2004. Characteristics and gel properties of muscles from sardine (*Sardinella gibbosa*) and mackerel (*Rastrelliger kanagurta*) caught in Thailand. Food Res. Int. 37:1021–1030.

Chaijan, M., Panpipat, W. and Benjakul, S. 2010. Physicochemical properties and gel-forming ability of surimi from three species of mackerel caught in Southern Thailand. Food Chem. 121: 85-92.

Chan, W. K. M., Faustman, C., Yin, M.C. and Decker, E. 1997. Lipid oxidation induced by oxymyoglobin with involvement of H_2O_2. Meat Sci. 42: 181-190.

Chanarat, S. and Benjakul, S. 2013. Impact of microbial transglutaminase on gelling properties of Indian mackerel fish protein isolates. Food Chem: 136: 929-937.

Chanarat, S., Benjakul, S. and H-Kittikun, A. 2012. Comparative study on protein cross-linking and gel enhancing effect of microbial transglutaminase on surimi from different fish. J. Sci. Food Agric. 92: 844-852.

Chanasattru, W., Decker, E. A. and McClements, J. 2007. Modulation of thermal stability and heat-induced gelation of β-lactoglobulin by high glycerol and sorbitol levels. Food Chem. 103: 512-520.

Chandrasekara, A. and Shahidi, F. 2011. Inhibitory act ivies of soluble and bound millet seed phenolics and free radicals and reactive oxygen species. J. Agric. Food Chem. 59: 428-436.

Charlton, A. J., Baxter, N. J., Khan, M. L., Moir, A. J. G., Haslam, E. and Davis, A. P. 2002. Polyphenol/peptide binding and precipitation. J. Agric. Food Chem. 50: 1593-1601.

Cheetangdee, N. 2017. Effect of partial replacement of porcine fat with pre-emulsified soybean oil using fish protein isolate as emulsifier on characteristic of sausage. J. Food Sci. Technol. 54: 1901-1909.

Chen, S. C. and Chung, K. T. 2000. Mutagenicity and antimutagenicity studies of tannic acid and its related compounds. Food Chem. Toxicol. 38: 1-5.

Considine, K. M., Kelly, A. L., Fitzgerald, G. F., Hill, C. and Sleator, R. D. 2008. High-pressure processing – effects on microbial food safety and food quality. FEMS Microbiol. Lett. 281: 1-9.

Craft, B., Kerrihard, A., Amarowicz, R. and Pegg, R. 2012. Phenol-based antioxidants and the *in vitro* methods used for their assessment. Compr. Rev. Food Sci. Food Saf. 11: 148-173.

Debusca, A., Tahergorabi, R., Beamer, S. K., Partington, S. and Jaczynski, J. 2013. Interactions of dietary fibre and omega-3-rich oil with protein in surimi gels developed with salt substitute. Food Chem. 201-208.

Delgado-Pando, G., Cofrades, S., Ruiz-Capillas, C., Solas, M. T. and Jiménez-Colmenero, F. 2010. Healthier lipid combination oil-in-water emulsions prepared with various protein systems: an approach for development of functional meat products. Eur. J. Lipid Sci. Technol. 112: 791-801.

Delgado-Pando, G., Cofrades, S., Ruiz-Capillas, C., Solas, M. T., Triki, M. and Jiménez-Colmenero, F. 2011. Low-fat frankfurters formulated with a healthier lipid combination as functional ingredient: Microstructure, lipid oxidation, nitrite content, microbiological changes and biogenic amine formation. Meat Sci. 89: 65-71.

Dey, G., Sachan, A., Ghosh, S. and Mitra, A. 2003. Detection of major phenolic acids from dried mesocarpic husk of mature coconut by thin layer chromatography. Ind. Crop Prod. 18: 171-176.

Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S. and Ju, Y-.H. 2014. Effect of extraction solvent on total phenolic content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. J. Food Drug Anal. 22: 296-302.

Erkan, N., Akgonen, S., Ovat, S., Goksel, G. and Ayranci, E. 2011. Phenolic compounds profile and antioxidant activity of *Dorystoechas hastata* L. Boiss et Heldr. Food Res. Int. 44: 3013-3020.

Espinosa, R. R., Inchingolo, R., Alencar, S. M., Rodriguez-Estrada, M. T. and Castro, I. A. 2015. Antioxidant activity of phenolic compounds added to a functional emulsion containing omega-3 fatty acids and plant sterol esters. Food Chem. 182: 95-104.

Falowo, A. B., Muchenje, V., Hugo, A., Aiyegoro, O. A. and Fayemi, P. O. 2016. Antioxidant activities of *Moringa oleifera* L. and *Bidens pilosa* L. leaf extracts and their effects on oxidative stability of ground raw beef during refrigeration storage. CYTA-J. Food. 15: 249-256.

Farvin, K. H. S., Grejsen, H. D. and Jacobsen, C. 2012. Potato peel extract as a natural antioxidant in chilled storage of minced horse mackerel (*Trachurus trachurus*): Effect on lipid and protein oxidation. Food Chem. 131: 843-851.

Field, J. M., Rodger, G. W., Hunter, J. C., Serafina-Fracassini, A. and Spina, M. 1978. Isolation of elastin from bovine auricular cartilage. Arch. Biochem. Biophys. 191: 705-713.

Fowler, M. R. and Park, J. W. 2015. Effect of salmon plasma protein on Pacific whiting surimi gelation under various ohmic heating conditions. LWT-Food Sci. Technol. 61: 309-315.

Fu, X., Xu, S. and Wang, Z. 2009. Kinetics of lipid oxidation and off-odor formation in silver carp mince: The effect of lipoxygenase and hemoglobin. Food Res. Int. 42: 85-90.

Gai, F., Gasco, L., Ortoffi, M., Gonzáles-Rodríguez, Á. and Parisa, G. 2014. Effects of green tea natural extract on quality parameters and lipid oxidation during storage of tench (*Tinca tinca*) fillets. J. Appl. Ichthyol. 30: 64-71.

Gallego, M. G., Gotrdon, M. H., Segovia, F. J., Skowyra, M. and Almajno, M. P. Antioxidant properties of three aromatic herbs (rosemary, thyme and lavender) in oil-in-water emulsions. J. Am. Oil Chem. Soc. 90: 1559-1568.

García, A., Amendola, D., González, M., Spigno, G. and Labidi, J. 2011. Lignin as natural radical scavenger. Study of the antioxidant capacity of apple tree pruning lignin obtained by different methods. Chem. Eng. Trans. 24: 925-930.

Garry, D., Meeson, A., Yan, Z. and William, R. 2000. Life without myoglobin. Cell. Mol. Life Sci. 57: 896-898.

Gawlik-Dziki, U., Jeżyna, M., Świeca, M., Dziki, D., Baraniak, B. and Czyż, J. 2012. Effect of bioaccessibility of phenolic compounds on *in vitro* anticancer activity of broccoli sprouts. Food Res. Int. 49: 469-476.

Goulas, V. and Manganaris, G. A. 2011. The effect of postharvest ripening on strawberry bioactive composition and antioxidant potential. J. Sci. Food Agric. 91: 1907-1914.

Gülçin, İ., Huyut, Z., Elmastaş, M. and Aboul-Enein, H. Y. 2010. Radical scavenging and antioxidant activity of tannic acid. Arabian. J. Chem. 3: 43-53.

Hall, G. M. and Ahmad, N. H. 1997. Surimi and fish-mince products, *In* Fish Processing Technology (Hall, G. M., ed.). p. 74-92. Chapman and Hall. New York.

Heck, T., Faccio, G., Richter, M. and Thöny-Meyer, L. 2013. Enzymatic-catalyzed protein crosslinking. Appl. Microbiol. Biotechnol. 97: 461-475.

Hemung, B.-O. and Chin, K. B. 2013. Effects of fish sarcoplasmic proteins on the properties of myofibrillar protein gels mediated by microbial transglutaminase. LWT-Food Sci. Technol. 53: 184-190.

Hernández-Brionesa, A., Velázqueza, G., Vázquezc, M. and Ramírez, J. A. 2009. Effects of adding fish gelatin on Alaska pollock surimi gels. Food Hydrocolloids. 23: 2446-2449.

Herranz, B., Tovar, C. A., Borderias, J. and Moreno, H. M. 2013. Effect of high-pressure and/or microbial transglutaminase on physicochemical, rheological and microstructural properties of frying fish surimi. Innov. Food Sci. Emerg. 20: 24-33.

Hossain, M. I., Itoh, Y., Morioka, K. and Obatake, A. 2001. Inhibiting effect of polymerization and degradation of myosin heavy chain during preheating at 30 ^{o}C and 50 ^{o}C on the gel-forming ability of walleye pollack surimi. Fish. Sci. 67: 718-725.

Hossain, M. A., Kamal, M. M., Shikha, F. H. and Hoque, M. S. 2004. Effect of washing and salt concentration on the gel forming ability of two tropical fish species. Int. J. Agric. Biol. 6: 762-766.

Hossain, M. A., Kalbani, M. S. A. A., Farsi, S. A. J. A., Weli, A. M. and Al-Riyami, Q. 2014. Comparative study of total phenolics, flavonoids contents and evaluation of antioxidant and antimicrobial activities of different polarities fruits crude extracts of *Datura metel* L. Asian Pac. J. Trop. Dis. 4: 378-383.

Hsu, C.-K. and Chiang, B.-H. 2002. Effects of water, oil, starch, calcium carbonate and titanium dioxide on the colour and texture of threadfin bream and hairtail surimi gels. Int. J. Food Sci. Technol. 37: 387-393.

Hu,Y., Ji, R., Jiang, H., Zhang, J., Chen, J. and Ye, X. 2012. Participation of cathepsin L in modori phenomenon in carp (*Cyprinus carpio*) surimi gel. Food Chem. 134: 2014-2020.

Hu, Y., Morioka, K. and Itoh, Y. 2010. Participation of cysteine protease cathepsin L in the gel disintegration of red bulleye (*Priacanthus macracanthus*) surimi gel paste. J. Sci. Food Agric. 90: 370-375.

Hughes, B. H., Perkins, L. B., Calder, B. L. and Skonberg, D. I. 2012. Fish oil fortification of soft goat cheese. J. Food Sci. 77: S128-S133.

Hunt, A., Park, J. W. and Handa, A. 2009. Effect of various types of egg white on characteristics and gelation of fish myofibrillar proteins. J. Food Sci. 74: C683-692.

Ikeuchi, Y., Tanji, H., Kim, K. and Suzuki, A. 1992. Mechanism of heat-induced gelation of pressurized actomyosin: Pressure-induced changes in actin and myosin in actomyosin. J. Agric. Food Chem. 40: 1756-1761.

Jafarpour, A., Hajiduon, H.-A. and Rez aie, M. A comparative study on effect of egg white, soy protein isolate and potato starch on functional properties of common carp (*Cyprinus carpio*) surimi gel. Food Process. Technol. doi: 10.4172/2157-7110.1000190.

Jan, S., Khan, M. R., Rashid, U. and Bokhari, J. 2013. Assessment of antioxidant potential, total phenolics and flavonoids of different solvent fractions of *Monotheca buxifolia* fruit. Osong Public Health Res. Perspect. 4: 246-254.

Jayathilakan, K., Sultana, K., Radhakrishna, K. and Bawa, A. S. 2012. Utilization of byproducts and waste materials from meat, poultry and fish processing industries: a review. J. Food Sci. Technol. 49: 278-293.

Jiang, S.-T., Hsieh, J.-F., Ho, M.-L. and Chung, Y.-C. 2000. Microbial transglutaminase affects gel properties of golden threadfin bream and pollack surimi. J. Food Sci. 65: 694-699.

Jiménez-Colmenero, F. 2007. Healthier lipid formulation approaches in meat-based functional foods. Technological options for replacement of meat fats by non-meat fats. Trends Food Sci. Technol. 18: 567-578.

Jiménez-Colmenero, F., Herrero, A., Pintado, T., Solas, M. T. and Ruiz-Capillas, C. 2010. Influence of emulsified olive oil stabilizing system used for pork backfat replacement in frankfurters. Food Res. Int. 43: 2068-2076.

Jittrepotch, R., Ushio, H. and Oshima, T. 2006. Effects of EDTA and a combined use of nitrite and ascorbate on lipid oxidation in cooked Japanese sardine (*Sardinops melanosticus*) during refrigerated storage. Food Chem. 99: 70-82.

Jose, M., Cyriac, M. B., Pai, V., Varghese, I. and Shantaram, M. 2014. Antimicrobial properties of *Cocos nucifera* (coconut) husk: An extrapolation to oral health. J. Nat. Bol. Med. 5: 359-364.

Jridi, M., Siala, R., Fakhfakh, N., Ayadi, M. A., Elhatmi, M., Taktak, M. A., Nasri, M. and Zouari, N. 2015. Effect of rosemary leaves and essential oil on turkey sausage quality. Acta Aliment. 44: 534-541.

Kaewdang, O. and Benjakul, S. 2015. Effect of ethanolic extract of coconut husk on gel properties of gelatin from swim bladder of yellowfin tuna. LWT-Food Sci. Technol. 62: 955-961.

Kaewudom, P., Benjakul, S. and Kijroongrojana, K. 2013. Properties of surimi gel as influenced by fish gelatin and microbial transglutaminase. Food Biosci. 1: 39-47.

Kang, Z. L., Chen, F. S. and Ma, H. J. 2016. Effect of pre-emulsified soy oil with soy protein isolate in frankfurters: A physical-chemical and Raman spectroscopy study. LWT-Food Sci. Technol. 74: 465-471.

Kataoka, J., Ishizaki, S. and Tanaka, M. 1998. Effects of chitosan on gelling properties of low quality surimi. J. Muscle Food. 9: 209-220.

Kim, Y. S. and Lee, J. H. 2017. Effects of hydrolyzed rapeseed cake extract on the quality characteristics of mayonnaise dressing. J. Food Sci. 82: 2847-2856.

Klomklao, S. and Benjakul, S. 2015. Effect of trypsin inhibitor in adzuki bean (*vigna angularis*) on proteolysis and gel properties of threadfin bream (*Nemipterus bleekeri*). LWT-Food Sci. Technol. 63: 906-911.

Klomklao, S., Benjakul, S., Kishimura, H., Osako, K. and Simpson, B. K. 2015. Trypsin inhibitor from yellowfin tuna (*Thunnus albacores*) roe: Effects on gel properties of

surimi from bigeye snapper (*Priacanthus macracanthus*). LWT-Food Sci. Technol. 65: 122-127.

Klomklao, S., Kishimura, H. and Benjakul, S. 2008. Endogenous proteinases in true sardine (*Sardinops melanostictus*). Food Chem. 107: 213-220.

Končić, M. Z., Barbarić, M., Perković, I. and Zorc, B. 2011. Antiradical, chelating and antioxidant activities of hydroxamic acids and hydroxyureas. Molecules. 16: 6232-6242.

Kudre, T., Benjakul, S. and Kishimura, H. 2013. Effects of protein isolates from black bean and mungbean on proteolysis and gel properties of surimi from sardine (*Sardinella albella*). LWT-Food Sci. Technol. 50: 511-518.

Kütemeyer, C., Froeck, M., Werlein, H.-D. and Watkinson, B. M. 2005. The influence of salts and temperature on enzymatic activity of microbial transglutaminase. Food Control. 16: 735-737.

Labieniec, M. and Gabryelak, T. 2006. Oxidatively modified proteins and DNA in digestive gland cells of the fresh-water mussel *Unio tumidus* in the presence of tannic acid and its derivatives. Mutat. Res. 603: 48-55.

Lanier, T. C. 2000. Surimi gelation chemistry. *In* Surimi and Surimi Seafood. (Park, J. W. ed.). p. 237-266. Taylor and Francis Group. Boca Raton, FL.

Leduc, V., Demeulemester, C., Guizard, C., Guern, L. L., Polack, B. and Peltre, G. 1999. Immunochemical detection of egg-white antigens and allergens in meat products. Allergy. 54: 464-472.

Lee, E.-J., Kim, Y.-H., Lee N.-H., Hong, S.I., Yamamoto, K. and Kim, Y.-J. 2011. The role of sarcoplasmic protein in hydrostatic pressure-induced myofibrillar protein denaturation. Meat Sci. 87. 219-222.

Lee, J., Koo, N. and Min, D. B. 2004. Reactive oxygen species, aging, and antioxidative nutraceuticals. Compr. Rev. Food Sci. Food Saf. 3: 21-33.

Leelapongwattana, K., Bejakul, S., Vissessangan, W. and Howell, N. K. 2008. Raman spectroscopic analysis and rheological measurements on natural actomyosin from haddock (*Melanogrammus aeglefinus*) during refrigerated (4 °C) and frozen (−10 °C) storage in the presence of trimethylamine-N-oxide demethylase from kidney of lizardfish (*Saurida tumbil*). Food Chem. 106. 1253-1263.

Liang, Y., Guo, B., Zhou, A., Xiao, S. and Liu, X. 2017. Effect of high pressure treatment on gel characteristics and gel formation mechanism of bighead carp (*Aristichthys nobilis*) surimi gels. J. Food Proc. Preserv. 41: e13155.

López-de-Dicastillo, C., Gómez-Estaca, J., Catalá, R., Gavara, R. and Hernández-Muñoz, P. 2012. Active antioxidant packaging films: Development and effect on lipid stability of brined sardines. Food Chem. 131: 1376-1384.

Lorenzo, J. M., González-Rodrígueza, R. M., Sánchezb, M., Amadoc, I. R. and Francoa, D. 2013. Effects of natural (grape seed and chestnut extract) and synthetic antioxidants (buthylatedhydroxytoluene, BHT) on the physical, chemical, microbiological and sensory characteristics of dry cured sausage “chorizo”. Food Res. Int. 54: 611-620.

Majumdar, R. K., Saha, A., Dhar, B., Maurya, P. K., Roy, D., Shitole, S. and Balange, A. K. 2015. Effect of garlic extract on physical, oxidative and microbial changes during refrigerated storage of restructured product from Thai pangas (*Pangasianodon hypothalamus*) surimi. J. Food Sci. Technol. doi: 10.1007/s13197-015-1952-7.

Mansfield, B. 2003. Fish, factory trawlers, and imitation crab: the nature of quality in the seafood industry. J. Rural Stud. 19: 9-21.

Maqsood, S. and Benjakul, S. 2010a. Comparative studies of four different phenolic compounds on *in vitro* antioxidative activity and the preventive effect on lipid oxidation of fish oil emulsion and fish mince. Food Chem. 119: 123-132.

Maqsood, S. and Benjakul, S. 2010b. Synergistics effect of tannic acid and modified atmosphere packaging on the preventon of lipid oxidation and quality losses of refrigerated striped catfish slices. Food Chem. 121: 29-38.

Maqsood, S. and Benjakul, S. 2013. Effect of kiam (*Cotylelobium lanceolatum* Craib) wood extract on the haemoglobin-mediated lipid oxidation of washed Asian sea bass mince. Food Bioprocess Technol. 6: 61-72.

Maqsood, S., Benjakul, S. and Balange, A. K. 2012. Effect of tannic acid and kiam wood extract on lipid oxidation and textural properties of fish emulsion sausages during refrigerated storage. Food Chem. 130: 408-416.

Maqsood, S., Benjakul, S. and Shahidi, F. 2013. Emerging role of phenolic compounds as natural food additives in fish and fish products. Crit. Rev. Food Sci. Nutr. 53: 162-179.

Maqsood, S., Kittiphattanabawon, P., Benjakul, S., Sumpavapol, P. and Abushelaibi, A. 2015. Antioxidant activity of date (*Phoenix dactylifera* var. Khalas) seed and it preventive effect on lipid oxidation in model systems. Int. Food Res. J. 22: 1180-1188.

Martin, N., Barros, L., Santos-Buelga, C. and Ferreira, I. C. F. R. 2016. Antioxidant potential of two Apiaceae plant extracts: A comparative study focused on the phenolic composition. Ind. Crop. Prod. 79: 188-194.

Moreno, H. M., Bargiela, V., Tovar, C. A., Canfo, D., Borderias, A. J. and Herranz, B. 2015. High pressure applied to frozen flying fish (*Parexocoetus brachyterus*) surimi: Effect on physicochemical and rheological properties of gels. Food Hydrocolloids. 48: 127-134.

Mulaudzi, R. B., Ndhlala, A. R., Kulkarni, M. G. and Staden, J. V. 2012. Pharmacological properties and protein binding capacity of phenolic extracts of some Venda medicinal plants used against cough and fever. J. Ethnopharmacol. 143: 185-193.

Oliveira, A. C. M., Himelbloom, B. H., Montazeri, N., Davenport, M., Biceroglu, H., Brenner, K. A., Thomas, S. R. and Crapo, C. A. 2013. Development and characterization of fish sausages supplemented with salmon oil. J. Food Process. Preserv. 38: 1641-1652.

Oteng-Pabi, S. K., Pardin, C., Stoica, M. and Keillor, J. W. 2014. Site-specific protein labelling and immobilization mediated by microbial transglutaminase. Chem. Commun. 50: 6604-6606.

Oujifard, A., Benjakul, S., Ahmad, M. and Seyfabadi, J. 2012. Effect of bambara groundnut protein isolate on autolysis and gel properties of surimi from threadfin bream (*Nemipterus bleekeri*). LWT-Food Sci. Technol. 47: 261-266.

Ozdal, T., Capanoglu, E. and Altay, F. 2013. A review on protein-phenolic interactions and associated changes. Food Res. Int. 51: 954-970.

Özen, B. Ö. and Soyer, A. 2018. Effect of plant extracts on lipid and protein oxidation of mackerel (*Scomber scombrus*) mince during frozen storage. J. Food Sci. Technol. 55: 120-127.

Panpipat, V. and Yongsawatdigul, J. 2008. Stability of potassium iodide and omega-3 fatty acids in fortified freshwater fish emulsion sausage. LWT-Food Sci. Technol. 41: 483-492.

Panyakaew, S. and Fotios, S. 2011. New thermal insulation boards made from coconut husk and bagasse. Energ. Buildings. 43: 1732-1739.

Park, J. W. and Morrissey, M. T. 2000. Manufacturing of surimi gel from light muscle fish. *In* Surimi and Surimi Seafood (Park, J. W. ed.). p. 23-58. Marcel Dekker Inc. New York.

Pazos, M., Gallardo, J. M., Torres, J. L. and Medina, I. 2005. Activity of grape poly phenols as inhibitors of the oxidation of fish lipids and frozen fish muscle. Food Chem. 92: 547-557.

Petry, N., Egli, I., Zeder, C., Walczk, T. and Hurrell, R. 2010. Polyphenols and phytic acid contribute to the low iron availability from common beans in young women. J. Nutr. 140: 1977-1982.

Poletto, M. and Zattera, A. J. 2013. Materials produced from plant biomass: Part III: Degradation kinetics and hydrogen bonding in lignin. Mater. Res. 16: 1065-1070.

Prado, J. M., Forster-Carneiro, T., Rostagno, M. A., Follegatti-Romero, L. A., Filho, F. M. and Meireles, A. A. 2014. Obtaining sugars from coconut husk, defatted grape seed, and pressed palm fiber by hydrolysis with subcritical water. J. Supercrit. Fluids. 89: 89-98.

Qiu, X., Jacobsen, C. and Moltke Sørensen, A. D. 2018. The effect of rosemary (*Rosmarinus officinalis* L.) extract on the oxidative stability of lipids in cow and soy milk enriched with fish oil. Food Chem. 263: 119-126.

Qui, C., Xia, W. and Jiang, Q. 2013. Effect of high hydrostatic pressure (HHP) on myofibril-bound serine proteinases and myofibrillar protein in silver carp (*Hypophthalmichthys molitrix*). Food Res. Int. 52: 199-205.

Ralph, J., Lundquist, K., Brunow, G., Lu, F., Kim, H., Schatz, P. F., Marita, J. M., Hitfield, R. D., Ralph, S. A., Christensen, J. H. and Boerjan, W. 2004. Lignins: Natural polymers from oxidative coupling of 4-hydroxyphenylpropanoids. Phytochem. Rev. 3: 29-60.

Ramadan, H., Min, B., Tiwari, A. K., Reddy, G., Adesiyun, A., Hinton Jr., A. and Abdela, W. 2015. Antibacterial activity of pomegranate, orange and lemon peel extracts against food-borne pathogens and spoilage bacteria *in vitro* and on poultry skin. Int. J. Poult. Sci. 14: 229-239.

Ramírez, J. A., Santos, I. A., Morales, O. G., Morrissey, M. T. and Vazquez, M. 2000. Application of microbial transglutaminase to improve mechanical properties of surimi from silver carp. Cienc. Technol. Aliment. 3: 21-28.

Rawdkuen, S. and Benjakul, S. 2008. Whey protein concentrate: Autolysis inhibition and effects on the gel properties of surimi prepared from tropical fish. Food Chem. 106: 1077-1084.

Rawdkuen, S., Benjakul, S., Visessanguan, W. and Lanier, T. C. 2007. Effect of cysteine proteinase inhibitor containing fraction from chicken plasma on autolysis and gelation of Pacific whiting surimi. Food Hydrocolloid. 21: 1209-1216.

Reyes, J. E., Tabilo-Munizaga, G., Perez-Won, M., Maluenda, D. and Roco, T. 2015. Effect of high hydrostatic pressure (HHP) treatments on microbiological shelf-life of chilled Chilean jack mackerel (*Trachurus murphyi*). Innov. Food Sci. Emerg. 29: 107-112.

Ribas-Agusti, A., Ribas-Agustí, M, Gratacós-Cubarsí, M., Sárraga, C., Guàrdia, M. D., García-Regueiro, J. A. and Castellari, M. 2014. Stability of phenolic compounds in dry fermented sausages added with cocoa and grape seed extracts. LWT-Food Sci. Technol. 57: 329-336.

Riediger, N. D., Othman, R. A., Suh, M. and Moghadasian, M. H. 2009. A systemic review of the roles of *n-3* fatty acids in health and disease. J. Am. Diet. Assoc. 109: 668-679.

Rivalain, N., Roquain, J. and Demazeau, G. 2010. Development of high hydrostatic pressure in biosciences: Pressure effect on biological structures and potential applications in biotechnologies. Biotechnol. Adv. 28: 659-672.

Rodrigues, S. and Pinto, G. A. S. 2007. Ultrasound extraction of phenolic compounds from coconut (*Cocos nucifera*) shell powder. J. Food Eng. 80: 869-872.

Rodrigues, S., Pinto, G. A. S. and Fernandes, F. A. N. 2008. Optimization of ultrasound extraction of phenolic compounds from coconut (*Cocos nucifera*) shell powder by response surface methodology. Ultrason. Sonochem. 15: 95-100.

Rysman, T., Van Hecke, T., De Smet, S. and Van Royen, G. 2016. Ascorbate and apple phenolics affect protein oxidation in emulsion-type sausages during storage and *in vitro* digestion. J. Agric. Food Chem. 64: 4131-4138.

Sae-leaw, T., Benjakul, S. and Simpson, B. K. 2017. Effect of catechin and its derivatives on inhibition of polyphenoloxidase and melanosis of Pacific white shrimp. J. Food Sci. Technol. 54: 1098-1107.

Sánchez-Alonso, I., Jiménez-Escrig, A., Saura-Calixto, F. and Borderias, A. J. 2007. Effect of grape antioxidant dietary fibre on the prevention of lipid oxidation in minced fish: Evaluation by different methodologies. Food Chem. 101: 372-378.

Sano, T., Noguchi, S. F., Matsumoto, J. J. and Tsuchiya, T. 1990. Effect of ionic strength on dynamic viscoelastic behavior of myosin during thermal gelation. J. Food Sci. 55: 51-54.

Sato, K., Tanaka, C., Kotaru, M., Yoshikawa, H., Kawabata, M. and Keuchi, T. 2007. Different arrangement of ε-(γ-Glutamyl)lysine cross-linking in Alaska pollock (*Theragra Chalcogramma*) surimi proteins by streptoverticillium and endogenous transglutaminases during suwari process. J. Food Biochem. 25: 397-409.

Sáyago-Ayerdi, S. G., Brenes, A. and Goñia, I. 2009. Effect of grape antioxidant dietary fiber on the lipid oxidation of raw and cooked chicken hamburgers. LWT-Food Sci. Technol. 42: 971-976.

Serdaroglu, M. and Felekoglu, E. 2005. Effect of using rosemary extract and onion juice on oxidative stability of sardine (*Sardina pilchardus*) mince. J. Food Qual. 28: 109-120.

Shabanpour, B., Etemadian, Y. and Taghipour, B. 2015. Physicochemical and rheological parameters changes for determining the quality of surimi and kamaboko produced by conventional, acid and alkaline solubilization process methods from common kilka (*Clupeonella cultriventris caspia*). Iran. J. Fish. Sci. 14: 826-845.

Shi, C., Cui, J., Yin, X., Luo, Y. and Zhou, Z. 2014. Grape seed and clove bud extracts as natural antioxidants in silver carp (*Hypophthalmichthys molitrix*) fillets during chilled storage: Effect on lipid and protein oxidation. Food Control. 40: 134-139.

Shitole, S. S. and Balange, A. K. 2014. Enhancement of gel strength of surimi from Japanese threadfin bream (*Nemipterus japonicus* Bloch, 1791) using seaweed extract. Fish. Technol. 51: 102-105.

Shitole, S. S., Balange, A. K. and Gangan, S. S. 2014a. Use of seaweed (*Sargassum tenerrimum*) extract as a gel enhancer for lesser sardine (*Sardinella brachiosoma*) surimi. Int. Aquat. Res. doi: 10.1007/s40071-014-0055-9.

Shitole, S. S., Balange, A. K., Gangan, S. S. and Maqsood, S. 2014b. Improvement in textural properties of mackerel (*Rastrelliger kanagurta*) surimi by using seaweed (*Sargassum tenerrimum*) extract as natural gel enhancer. J. Meat Sci. 10: 74-79.

Simonin, H., Duranton, F. and de Lamballerie, M. 2012. New insight into the high-pressure processing of meat and meat products. Comp. Rev. Food Sci. F. 11: 285-306.

Silva, G. G., Souza, D. A., Machado, J. C. and Hourston, D. J. 2000. Mechanical and thermal characterization of native Brazillian Coir fiber. J. Appl. Polym. Sci. 76: 1197-1206.

Siriangkanakun, S., Li-chan, E. C. Y. and Yongsawadigul, J. 2016. Identification and characterization of alpha-I-proteinase inhibitor from common carp sarcoplasmic proteins. Food Chem. 192: 1090-1097.

Sirigan, P., Raksakulthai, N. and Yongsawatdigul, J. 2006. Autolytic activity and biochemical characteristics of endogenous proteinases in Indian anchovy (*Stolephorus indicus*). Food Chem. 98: 678-684.

Su, H., Chen, W., Fu, S., Wu, C., Li, K., Huang, Z., Wu, T. and Li, J. 2014. Antimicrobial effect of bayberry leaf extract for the preservation of large yellow croaker (*Pseudosciaena crocea*). J. Sci. Food. Agric. 94: 935-942.

Sun, X. D. and Holley, R. A. 2011. Factors influencing gel formation by myofibrillar proteins in muscle foods. Compr. Rev. Food Sci. Food Safety. 10: 33-51.

Tadpitchayangkoon, P., Park, J. W. and Yongsawatdigul, J. 2012. Gelation characteristics of tropical surimi under water bath and ohmic heating. LWT-Food Sci. Technol. 46: 97-103.

Takeungwongtrakul, S. and Benjakul, S. 2013. Oxidative stability of shrimp oil-in-water emulsions as affected by antioxidant incorporation. Int. Aquat. Res. 5: doi: 10.1186/2008-6970-5-14.

Temdee, W. and Benjakul, S. 2015. Effect of phenolic compounds and bark/wood extacts oxidised by laccase on properties of cuttlefish (*Sepia pharaonis)* skin gelatin. Int. Food Res. J. 22: 246-253.

Terdwongworakul, A, Chaiyapong, S., Jarimopas, B. and Meeklangsaen, W. 2009. Physical properties of fresh young Thai coconut for maturity sorting. Biosyst. Eng. 103: 208-216.

Thiansilakul, Y., Benjakul, S., Grunwald, E. W. and Richard, M. P. 2012. Retardation of myoglobin and haemoglobin-mediated lipid oxidation in washed bighead carp by phenolic compounds. Food Chem. 134: 789-796.

Turan, S. 2014. Effects of some plant extracts on the oxidative stability of canola oil and its purified triacylglycerols. J. Food Qual. 37: 247-258.

Ugartondo, V., Mitjans, M., Lozano, C., Torres, J. L. and Vinardell, M. P. 2006. Comparative study of the cytotoxicity induced by antioxidant epicatechin conjugates obtained from grape. J. Agric. Food Chem. 54: 6945-6950.

Ugartondo, V., Mitjans, M. and Vinardell, M. P. 2008. Comparative antioxidant and cytotoxic effects of lignins from different souces. Biores. Technol. 99: 6683-6687.

Van Dam, J. E. G., Van Den Oever, M. J. A., Teunissen, W., Keijsers, E. R. P. and Peralta, A. G. 2004. Process for production of high density/high performance binderless

boards from whole coconut husk: Part 1: Lignin as intrinsic thermosetting binder resin. Ind. Crop Prod. 19: 207-216.

Vannabun, A., Ketnawa, S., Phongthai, S., Benjakul, S. and Rawdkuen, S. 2014. Characterization of acid and alkaline proteases from viscera of farmed giant catfish. Food Biosci. 6: 9-16.

Vate, N. K. and Benjakul, S. 2015. Effect of the mixtures of squid ink tyrosinase and tannic acid on properties of sardine surim gel. J. Food Sci. Technol. doi: 10.1007/s13197-015-1974-1.

Wu, H. C., Chen, H. M. and Shiau, C. Y. 2003. Free amino acid and peptide as related to antioxidant properties in protein hydrolysates of mackerel (*Scomber austriasicus*). Food Res. Int. 36: 949-957.

Wu, N., Fu, K., Fu, Y.-J., Zu, Y.-G., Chang, F.-R., Chen, Y-H., Liu, X.-L., Kong, Y., Liu, W. and Gu, C.-B. 2009. Antioxidant activities of extracts and main components of pigeonpea (*Cajanus cajan* L. Millsp.) leaves. Molecules. 14: 1032-1043.

Wu, S., Pan, S. and Wang, H. 2014. Effect of trehalose on *Lateolabrax japonicus* myofibrillar protein during frozen storage. Food Chem. 160: 281-285.

Wu, T. and Mao, L. 2009. Application of chitosan to maintain the quality of kamaboko gels made from grass carp (*Ctenopharyngodon idellus*) during storage. J. Food Process. Pres. 33: 218-230.

Wu, Z.-G., Xu, H.-Y., Ma, Q., Cao, Y., Ma, J.-N. and Ma, C.-M. 2012. Isolation, identification and quantification of unsaturated fatty acids, amides, phenolic compounds and glycoalkaloids from potato peel. Food Chem. 135: 2425-2429.

Yaldagard, M., Mortazavi, S. A. and Tabatabaie, F. 2008. The principles of ultra high pressure technology and its application in food processing/preservation: A review of microbiological and quality aspects. Afr. J. Biotechnol. 7: 2739-2767.

Yin, T. and Park, J. W. 2014. Effects of nano-scaled fish bone on the gelation properties of Alaska pollock surimi. Food Chem. 150: 463-468.

Yin, T., Reed, Z. H. and Park, J. W. 2014. Gelling properties of surimi as affected by the particle size of fish bone. LWT-Food Sci. Technol. 58: 412-416.

Yongsawatdigul, J., Worratao, A. and Park, J. W. 2002. Effect of endogenous transglutaminase on threadfin bream surimi gelation. J. Food Sci. 67: 3258-3263.

Yu, H., Yang, G., Sato, M., Yamaguchi, T., Nakano, T. and Xi, Y. 2017. Antioxidant activities of aqueous extract from *Stevia rebaudiana* stem waste to inhibit fish oil oxidation and identification of its phenolic compounds. Food Chem. 232: 379-386.

Yu, H., Qiu, C., Zhang, P., Ge, Q., Wu, M., Wu, J., Wang, M. and Wang, Z. 2015. Antioxidant effect of apple phenolic on lipid peroxidation in Chinese-style sausage. J. Food Sci. Technol. 52: 1032-1039.

Zhang, L., Xue, Y., Xu, J., Li, Z. and Xue, C. 2013. Effects of high-temperature treatment (⩾100 °C) on Alaska Pollock (*Theragra chalcogramma*) surimi gels. J. Food Eng. 115: 115-120.

Zhou, F., Zhao, M., Zhao, H., Sun, W. and Cui, C. 2014. Effects of oxidative modification on gel properties of isolated porcine myofibrillar protein by peroxyl radicals. Meat Sci. 96: 1432-1439.

Zhu, Z., Lanier, T. C., Farkas, B. E. and Li, B. 2014. Transglutaminase and high pressure effects on heat-induced gelation of Alaska pollock (*Theragra chalcogramma*) surimi. J. Food. Eng. 131: 154-160.

Zhu, J., Fan, D., Zhao, J., Zhang, H., Huang, J., Zhou, W., Zhang, W. and Chen, W. 2015. Enhancement of the gelation properties of surimi from yellowtail seabream (*Paragyrops edita, Sparidae*) with Chinese oak silkworm pupa, *Antheraea pernyi*. J. Food Sci. doi: 10.1111/1750-3841.13184.

1.4 Objectives

1. To extract phenolic compounds from coconut husk for using as gel strengthener in sardine surimi.
2. To investigate the cross-linking activity of ethanolic coconut husk extract toward sardine muscle protein.
3. To elucidate the gel strengthening effect of coconut husk extract in surimi with low setting phenomenon.
4. To investigate the effect of high pressure in combination with ethanolic coconut husk extract on gel properties of surimi.
5. To study *in vitro* antioxidative activity of ethanolic coconut husk extract.
6. To study the impact of ethanolic coconut husk extract on property and oxidative stability of emulsion surimi gel during refrigerated storage.

CHAPTER 2
IMPROVEMENT OF GEL PROPERTIES OF SARDINE (*SARDINELLA ALBELLA*) SURIMI USING COCONUT HUSK EXTRACTS

2.1 Abstract

Effects of coconut husk ethanolic extracts on gelling properties of surimi from sardine (*Sardinella albella*) were investigated. Extracts prepared using 60% ethanol (E60) and 80% ethanol (E80) with total phenolic content of 463.74 and 453.93 mg tannin/g were incorporated into surimi gel. Gels added with E60 or E80 had the increases in breaking force as the levels increased and the highest breaking force was observed when added with 0.125% E60 and 0.075% E80 ($P<0.05$). Both E60 and E80 had no detrimental effect on sensory attributes of surimi gel. However, slight decrease in whiteness was found in gel added with the coconut husk extracts. Electrophoretic studies showed that disappearance of major myosin heavy chain took place when incorporated with both E60 and E80, regardless of levels used. Lower autolysis of surimi gel was found in the presence of both extracts. Addition of E60 and E80 could increase the cross-linking of proteins during heating as indicated by the higher G'. Surimi gel added with coconut extracts had highly interconnected network and their microstructure was finer and denser network than that of the control. Thus, coconut husk extracts at an appropriate level could improve gel strength of sardine surimi with an increased acceptability.

2.2 Introduction

Coconuts are abundant in coastal areas of tropical countries including Thailand. During processing, the husk of coconut is removed and the millions of tons of husk are generated each year (Panyakaew and Fotios, 2011). The husk is mainly composed of lignin and cellulose, which are dietary fiber and recommended as GRAS (Woods and Gorbach, 2001). Additionally, husk is rich in phenolic compounds, namely 4-hydroxybenzoic acid (4-HBA), ferulic acid, tannic acid as well as lignin phenols such as vanillic acid, *ρ*-coumaric acid and syringic acid (Lobbes *et al*., 1999; Rodrigues and

Pinto, 2007). Phenolic compounds have been known to possess antioxidant (Maqsood *et al.*, 2012), antimicrobial and antiviral (Carvalho *et al*., 2013; Hossain *et al.*, 2014) and anticancer activities (Gawlik-Dziki, *et al*., 2012). Furthermore, the use of phenolic compounds has been extended as a protein cross-linking agent, particularly in the oxidized form (Balange and Benjakul, 2009a).

Surimi, the washed fish mince, has gained popularity as raw material for preparing several products with elastic texture (Mansfield, 2003). Generally, lean fish are commonly used for production of surimi since they yield surimi with white color and better gel property as compared to dark fleshed fish. Due to the insufficient lean fish, pelagic dark-fleshed fish such as sardine (*Sardinella albella*) and mackerel (*Rastrelliger kanagurta*), have been used as alternative raw material for surimi production (Chaijan *et al*., 2004; Arfat and Benjakul, 2012). Nonetheless, those pelagic dark-fleshed fish have high lipid, myoglobin and sarcoplasmic protein contents, which contribute to the difficulties in making high-quality surimi (Chaijan *et al.*, 2010). Dark-fleshed fish also contain high level of proteases, particularly heat-activated proteases, which show an adverse effect on gel formation (Hu *et al*., 2010). Those proteases have been reported to induce the degradation of myofibrillar proteins associated with gel weakening. These limit the use of dark-fleshed fish as raw material for production of surimi and other products.

To tackle such a drawback, various food-grade additives and cross-linking enzymes such as microbial transglutaminase has been used (Oujifard *et al*., 2012; Kaewudom *et al*., 2013). Balange and Benjakul (2009a, b) reported that oxidized phenolic compounds, such as oxidized tannic acid or ferulic acid, could increase breaking force and deformation of surimi from bigeye snapper and mackerel. However, the oxidation of phenolic compound from *ortho*-diphenol into ortho-quinone form, either enzymatically or by molecular oxygen, could negatively affect the quality of some food products by decreasing desirable aroma, and lowering antioxidant activities (Nikolantonaki *et al.*, 2014). Since phenolic compounds are rich in hydroxyl groups, surimi gel can be strengthened via hydrogen bond and other interactions (Ali, 2002). Extracts from coconut husk containing phenolic compounds could be used as natural protein cross-linker possessing antioxidant activity. The present study aimed to

investigate the effect of coconut husk extracts on the properties of surimi gel from sardine, an abundant dark fleshed-fish in the Southern Thailand.

2.3 Materials and methods

2.3.1 Chemicals

All chemicals were of analytical grade. Tannic acid, sodium dodecyl sulphate (SDS) and β-mercaptoethanol (*β*-ME) were obtained from Sigma (St. Louis, MO, USA). *N,N,N′,N′*-tetramethyl ethylene diamine (TEMED), acrylamide and bisacrylamide were purchased from Fluka (Buchs, Switzerland). Trichloroacetic acid, Folin–Ciocalteu's phenol reagent, acetic acid and tris (hydroxylmethyl) aminomethane were obtained from Merck (Darmstadt, Germany).

2.3.2 Materials

Husk of coconut (*Cocos nucifera* Linn.) fruit with the age of about 11 months was collected from the local market in Hat Yai, Songkhla Province, Thailand and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai, Thailand. Husk sample was prepared as per the method of Vazquez-Torres *et al.* (1992) with slight modifications. Husk was dried at 60 °C in the cabinet rotary dryer for 16 h and then defibered. Husk sample was then subjected to grinding using a mill (IKA Labortechnik colloid mill, Selangor, Malaysia). The prepared sample was then sieved with the aid of sieve shaker (Model EVJ1, Endecotts Ltd., London, UK) with a sieve size of 6 mm (Woven wire sieves, Endecotts Ltd., London, UK). The coarse form was further blended using a blender (Panasonic, Model MX-898N, Berkshire, UK) and finally sieved using a stainless-steel sieve of 80 mesh. The coconut husk powder obtained was further dried in a hot air oven (Memmert, Schwabach, Germany) at 105 °C overnight to obtain moisture content of 3%. The obtained powder was placed in a polyethylene bag, sealed and kept at room temperature until use.

Frozen surimi, AA grade, from sardine (*S. albella*) was purchased from Pacific Fish Processing Co., Ltd. (Songkhla, Thailand) and kept at -20 °C until use, but not longer than two months.

2.3.3 Preparation of coconut husk extract

The husk powder (10 g) was mixed with 350 mL of ethanol at concentrations of 40, 60, 80 and 100% (v/v). The extraction was performed at room temperature (28-30 °C) for 3 h by stirring the mixture continuously at low speed using a magnetic stirrer (IKA-Werke, Staufen, Germany). Thereafter, the mixtures were centrifuged at 5000 ×g for 30 min at room temperature using a RC-5B plus centrifuge (Beckman, JE-AVANTI, Fullerton, CA, USA) and the supernatant was then filtered through a Whatman filter paper No.1 (Whatman International Ltd., Maidstone, UK) (Jan *et al*., 2013). The filtrates were evaporated at 40 °C using an Eyela rotary evaporator (Tokyo Rikakikai, Co. Ltd., Tokyo, Japan) and purged with nitrogen gas to remove the residual ethanol. The extracts were then dried using a Scanvac Model Coolsafe 55 freeze dryer (Coolsafe, Lynge, Denmark) to obtain the dry extract. Dried extracts were powdered using a mortar and pestle. The powders prepared using 40, 60, 80 and 100% ethanol were referred to as E40, E60, E80 and E100, respectively. The powders were kept in an amber bottle and stored in a desiccator until use or analysis.

2.3.3.1 Total phenolic content

Total phenolic content of coconut husk extracts was determined using Folin-Ciocalteau Reagent (FCR) as described by Slinkard and Singleton (1997) with a slight modification. Coconut husk extract (100 µL) was mixed with 0.75 mL of FCR, which was prediluted 10-fold with distilled water. After 5 min, the reaction was added with 0.75 mL of 6% (v/v) sodium carbonate. The solution was mixed and allowed to stand for 1 h at room temperature. The absorbance at 760 nm was read using UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). Standard solutions of tannic acid (0-600 mg/L) were used for standard curve preparation. The phenolic content was expressed as mg tannic acid equivalents per g dry weight of coconut husk extract (Rodrigues and Pinto, 2007).

2.3.3.2 Tannin content

Tannin content of coconut husk extracts was determined by high performance liquid chromatography (HPLC) as per the method of Temdee and Benjakul (2014). The HPLC system was composed of an Agilent 1100 series HPLC (Alginet, Wilmington, DE, USA), quaternary pump with the seal wash option, degasser,

solvent, cabinet and preparative auto-sampler with thermostat equipped with a diode array detector. The separation was done by a Hypersil ODS C18 4.0 × 250 mm, 5 µm column (Cole-Parmer, London, UK). HPLC conditions were as follows: mobile phase: 0.4% formic acid:acetronitrile (85:15), flow rate: 0.8 mL/min, temperature: 25 °C. Detection was performed at 280 nm. The concentration of extracts was 25 mg/mL and the injection volume was 20 µL. Standard tannin was used for peak identification.

2.3.4 Preparation of surimi gel added with coconut husk extract

Frozen surimi, having 17.4% protein as determined by the method of AOAC (2000), was tempered in running water (25-30 °C) for 30 min until the core temperature reached 0-2 °C. The surimi was chopped into small pieces and mixed with 2.5% salt in a mixer (National Model MK-5080M, Selangor, Malaysia) for 2 min. During chopping, the temperature was maintained below 10 °C. E60 and E80 were firstly dissolved in cold distilled water. They were added into surimi paste to obtain different levels (0.025, 0.05, 0.075, 0.10, 0.125 and 0.15% of protein content). The moisture content of the surimi paste was adjusted to 80% with cold distilled water. Consequently, the mixture was chopped for another 3 min and the paste was stuffed into a polyvinylidene chloride casing with a diameter of 2.5 cm. Both ends were sealed tightly and subjected to the incubation at 40 °C for 30 min, followed by heating at 90 °C for 20 min. Subsequently, all gels were cooled in iced water for 30 min and stored at 4 °C for 18-24 h prior to analyses.

2.3.4.1 Breaking force and deformation

Breaking force (gel strength) and deformation (deformability) of surimi gels were determined using a texture analyzer (Model TA-XT2, Stable MicroSystems, Surrey, UK) as described by Benjakul *et al.* (2007). Five cylindrical samples (2.5 cm in height) were prepared and equilibrated at room temperature (28-30 °C) for 1 h before analyses. A spherical plunger (diameter 5 mm) was pressed into the cut surface of a gel sample perpendicularly at a constant depression speed (60 mm/min). The force to puncture into the gel (breaking force) and the distance at which the plunger punctured into the gel (deformation) were both recorded.

2.3.4.2 Expressible moisture content

Gel samples were measured for expressible moisture content according to the method of Benjakul *et al*. (2007). Cylindrical gel samples were cut into a thickness of 5 mm, weighed accurately (*X*) and placed between three pieces of Whatman filter paper No.1 (Whatman International Ltd., Maidstone, UK) at the bottom and two pieces on the top of the sample. A standard weight of 5 kg was placed on the top of the sample for 2 min. The sample was then removed from the papers and weighed again (*Y*). Expressible moisture content was calculated with the following equation and expressed as percentage of sample weight:

$$\text{Expressible moisture (\%)} = [(X\text{-}Y)/X] \times 100$$

2.3.4.3 Whiteness

Whiteness of gel samples was determined using a colorimeter (HunterLab, Colorflex, Hunter Associates Laboratory, VA, USA). CIE L^*, a^* and b^* values were measured and whiteness was then calculated using the following equation (Park, 1994):

$$\text{Whiteness} = 100\text{-}[(100\text{-}L^*)^2 + a^{*2} + b^{*2}]^{1/2}$$

where L^* is the lightness; a^* is the redness/greenness; and b^* is the yellowness/blueness.

2.3.4.4 TCA-soluble peptide content

TCA-soluble peptide content was determined as per the method of Morrissey *et al*. (1993). Gel sample (3 g) was homogenized with 27 mL of cold 5% TCA at a speed of 11,000 rpm for 2 min using a homogenizer (IKA Labortechnik, Selangor, Malaysia). The homogenate was allowed to store in ice for 1 h and centrifuged at 8,000 ×g for 10 min. TCA-soluble peptide content in the supernatant was measured according to the Lowry method (Lowry *et al.*, 1951) and expressed as µmol tyrosine equivalent/g sample.

2.3.4.5 SDS-polyacrylamide gel electrophoresis

Protein patterns of surimi gels were analyzed by SDS-PAGE under the reducing condition according to the method Laemmli (1970). To the finely chopped gel

samples, 27 mL of heated SDS solution (85 °C) were added. The mixture was then homogenized at a speed of 11,000 rpm for 2 min. The homogenate was incubated at 85 °C for 1 h to dissolve total proteins. The mixtures were centrifuged at 3,500 ×g for 20 min to remove undissolved matters. Protein concentration of the supernatant was determined by the Biuret method (Robinson and Hogden, 1940) using bovine serum albumin as standard. SDS-PAGE gel consisted of 10% running gel and 4% stacking gel. After separation, the proteins were stained with 0.02% (w/v) Coomassie Brilliant Blue R-250 in 50% (v/v) methanol and 7.5% (v/v) acetic acid and destained with 50% methanol and 7.5% (v/v) acetic acid, followed by 5% methanol (v/v) and 7.5% (v/v) acetic acid.

2.3.5 Characterization of surimi gel added with coconut husk extracts at the selected levels

Surimi gels containing the selected coconut husk extracts (E60 or E80) with the appropriate levels were characterized in comparison with the control gel.

2.3.5.1 Texture profile analysis

Gel samples were subjected to texture profile analysis (TPA) following the method of Kaewudom *et al*. (2013) with a slight modification. A texture analyzer (Model TA-XT2, Stable MicroSystems, Surrey, UK) with a cylinder probe (diameter 35 mm) was used for determination of hardness, springiness, cohesiveness, gumminess and chewiness.

2.3.5.2 Dynamic rheology

Surimi pastes containing E60 and E80 were prepared as previously described and were subjected to dynamic rheological measurements following the method of Rawdkuen et al. (2008) with a slight modification. A rheometer (HAAKE RheoStress1, ThermoFisher Scientific, Karlsruhe, Germany) with 35 mm, 4^{o} slope cone and plate geometry was used for monitoring the changes in storage or elastic modulus (G'). An oscillation of 1 Hz with 1% deformation was used for testing. This condition yielded a linear response in the viscoelastic region. The temperature sweep was recorded during heating up from 10 to 90 °C with heating rate of 1 °C/min. To minimize water evaporation of surimi pastes during measurement, silicon oil was applied to cover the samples.

2.3.5.3 Microstructure of surimi gel

Gel samples were examined for microstructure using a scanning electron microscope (SEM). The samples with a thickness of 2-3 mm were fixed with 2.5% (v/v) glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 3 h at room temperature. The samples were rinsed with distilled water. Fixed specimens were dehydrated in ethanol with serial concentrations of 25, 50, 70, 80, 90 and 100%. Samples were critical point dried using CO_2 as transition fluid. The prepared samples were mounted on a bronze stub and sputter-coated with gold. The specimens were visualized using SEM (Quanta 400, FEI, Eindhoven, the Netherlands).

2.3.5.4 Sensory evaluation

Gel samples were cut into bite-size (1 cm thick and 2.5 cm in diameter), equilibrated at room temperature (28-30 °C) for 30 min, coded with 3-digit random numbers and kept the gel samples in plastic box with the cover before the sensory evaluation. Eighty non-trained panelists (aged between 20 and 45), who were the students and staffs at Department of Food Technology and were accustomed to with surimi products, were asked to determine for color, taste, texture and overall liking of gel samples using 9-point hedonic scale (Meilgaard *et al*., 1999). Gel samples were served on the white paper dishes at room temperature under the fluorescent daylight-type illumination. Between samples, panelists were asked to rinse their mouth with distilled water at room temperature.

2.3.6 Statistical analysis

Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by the Duncan's multiple range test (Steel and Torrie, 1980). Statistical analysis was performed using SPSS for Windows (SPSS Inc., Chicago, IL, USA). Data with $P < 0.05$ were considered to be statistically significant.

2.4 Results and discussion

2.4.1 Total phenolic and tannin contents of coconut husk extracts

Different total phenolic contents were observed for coconut husk extracts prepared using ethanol at different concentrations. The highest total phenolic content was found in E60, followed by E80, E40 and E100 (463.74, 453.93, 387.95 and

255.87 mg TAE/g sample), respectively. It was noted that phenolics could be extracted to a higher content when ethanol concentration increased up to 60%. Nevertheless, the efficiency was lowered when ethanol concentration was above 60%. Kallel *et al*. (2014) found that 50% ethanol was appropriated for extraction of phenolic from garlic husk waste. Additionally, Nour *et al*. (2013) reported that the increase of ethanol concentration (40-96%) resulted in a greater amount of phenolics extracted from black currants. The result indicated that polarity of extracting media was the prime factor affecting the extraction of phenolic compounds from coconut husk.

E60 had the higher content of tannin (24.08 mg tannin/ g of dry extract) than others. E80, E40 and E100 showed the tannin content of 11.95, 9.69 and 5.27 mg tannin/ g of dry extract, respectively. Tannin contents in the extracts were generally in accordance with the total phenolic content. Woods and barks of different trees mainly contain tannins (Fradinho *et al*., 2002; Temdee and Benjakul, 2014). Apart from tannin, other components such as lignin, lignan, cellulose, flavonoids, phlobatannins and other polyphenol have been reported as the constituent in wood, husk, etc. (Bilba *et al.*, 2007; Balange and Benjakul, 2009a; Israel *et al.*, 2011).

2.4.2 Effect of coconut husk extracts at different levels on properties of surimi gel

2.4.2.1 Breaking force and deformation

Breaking force and deformation of surimi gel from sardine added without and with E60 or E80 at different levels (0.025-0.15%, based on protein) are shown in Fig. 9. For the gel incorporated with E60, breaking force increased with increasing levels of E60 added up to 0.125% ($P<0.05$). However, the decrease in breaking force was observed when E60 at higher level (0.15%) was added ($P<0.05$). For those containing E80, the increase in breaking force was obtained as E80 level increased up to 0.075% and the slight decrease in breaking force was found when E80 level increased ($P<0.05$). Incorporation of E80 at 0.125 and 0.15% resulted in the intensive coagulation of proteins during mixing and chopping. As a result, very poor gels were obtained in the presence of excessive amount of both extracts. Gels added with E60 showed the higher breaking force than those containing E80 and the control gel. Amongst all gel samples, that incorporated with 0.125% E60 had the highest

breaking force ($P<0.05$). This more likely resulted from the greater cross-linking induced by phenolics, particularly tannin. When the extracts were added into surimi gel at high levels, the excessive cross-linking might lead to the protein coagulation, in which the ordered and fine gel network could not be formed. At high level of extracts, self-aggregation of phenolic compounds plausibly led to the loss in protein cross-linking capability (Balange and Benjakul, 2009a). The results indicated that phenolic compounds in the coconut husk extract at the optimum condition were able to enhance gel strength of sardine surimi. The addition of E60 or E80 generally had no effect on deformation of resulting surimi gel ($P>0.05$). However, at 0.025 and 0.05%, the deformation of sample added with E60 was higher than that of gel containing E80 ($P<0.05$). Balange and Benjakul (2009b) reported that the incorporation of oxidized form 0.40% ferulic acid, 0.50% tannic acid, 0.50% cathechin and 0.10% caffeic acid could increase gel strength of mackerel surimi effectively. Enhanced protein cross-linking or interaction of myofibrillar proteins in film from bigeye snapper by phenolic compounds was also reported by Prodpran *et al*. (2012). In the present study, phenolics in reduced form were used as protein cross-linkers without prior oxidation. Interaction between hydroxyl group of polyphenol and hydrogen acceptor in protein molecules by hydrogen bonds, and also protein-polyphenol hydrophobic interactions more likely contributed to the increased gel strength (Rattaya *et al.*, 2009). Since the major bonds were more likely weak bonds, particularly hydrogen bonds, the deformation was not much affected by incorporation of coconut husk extracts. Nevertheless, some covalent bonds could be involved to some extent. Therefore, coconut husk extract, particularly E60, could be used as gel strengthener in surimi.

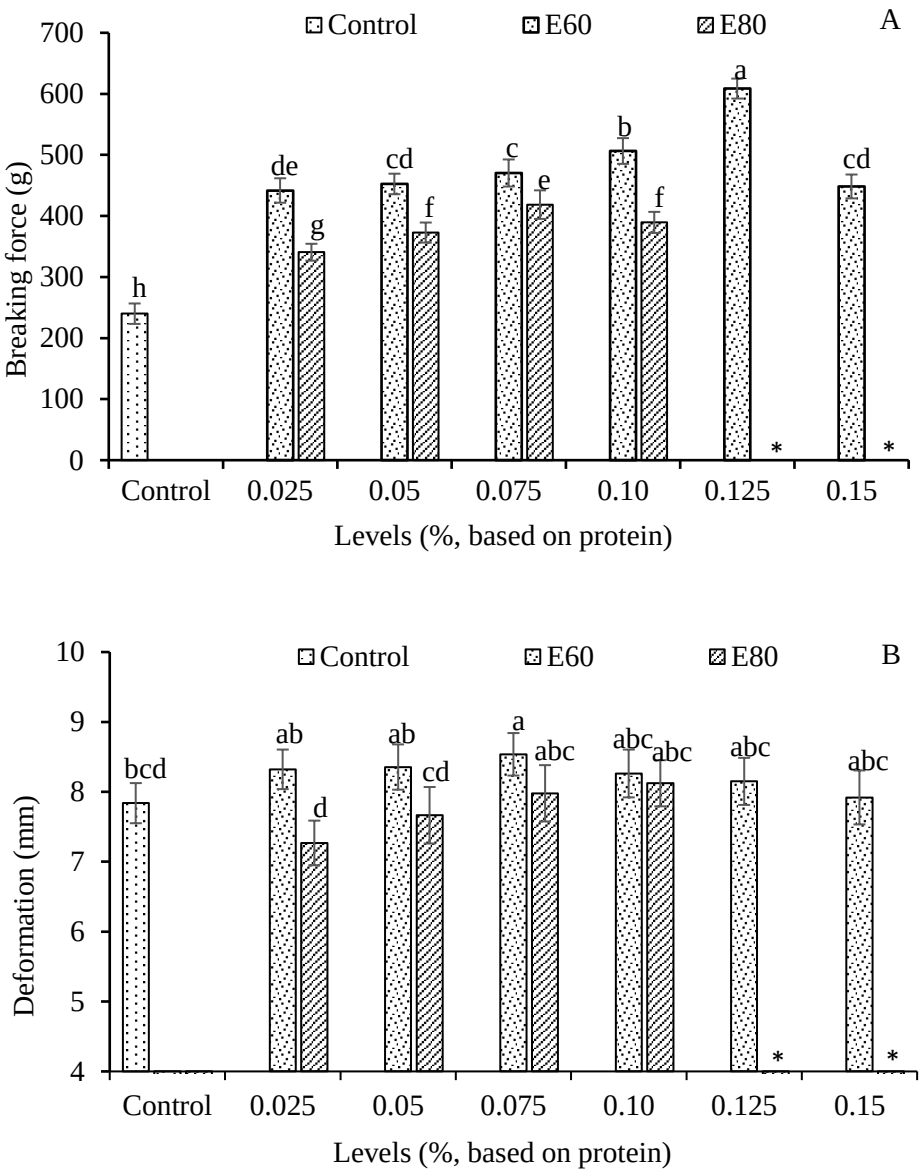


Figure 9. Breaking force (A) and deformation (B) of gels from sardine surimi without and with E60 and E80 at different levels. Bars represent the standard deviation (n=3). Different letters on the bars indicate significant differences (P<0.05). * Gel could not be formed due to the excessive coagulation.

2.4.2.2 Expressible moisture content

Expressible moisture content of gel from sardine surimi added with coconut husk extracts at different levels is shown in Table 6. Surimi gels showed the continuous decreases in expressible moisture content when the levels of E60 increased up to 0.10% (P<0.05). However, the marked increase in expressible moisture content

was noticeable when E60 at 0.125% was added (P<0.05). Although the addition of 0.125% E60 yielded the gel with the highest breaking force, it lowered water holding capacity of gels as evidenced by the increased expressible water content. Nevertheless, the expressible moisture content of gel added with 0.125% E60 was not different from the control gel (P>0.05). When the appropriate level of phenolic compound was added, the cross-linking of proteins could be enhanced, leading to the formation of stronger network with greater water holding capacity (Balange and Benjakul, 2009b). For gel added with E60 ranging from 0.025 to 0.1%, no marked differences in expressible moisture content were found. The excessive coagulation or precipitation of proteins could lead to lower water holding capacity when high level of the extracts or phenolic was added into surimi (Balange and Benjakul, 2009a; Arfat and Benjakul, 2013). Therefore, coconut husk extract also determined water holding capacity of surimi gel.

Table 3. Expressible moisture content and whiteness of gels from sardine surimi added without and with E60 and E80 at different levels.

Extracts	Level (%)	Expressible moisture content (%)	Whiteness
Control	-	10.05±0.39^{a}	69.22±0.18^{a}
E60	0.025	9.43±0.35^{b}	66.54±0.63^{d}
	0.050	9.33±0.26^{b}	66.14±0.63^{e}
	0.075	7.40±0.34^{c}	66.08±0.68^{e}
	0.100	6.40±0.28^{de}	66.03±0.68^{e}
	0.125	10.14±0.24^{a}	65.97±0.34^{e}
	0.150	7.25±0.28^{c}	65.64±0.54^{f}
E80	0.025	6.66±0.23^{d}	68.54±0.54^{b}
	0.050	6.04±0.43^{e}	68.29±0.54^{b}
	0.075	6.38±0.31^{de}	67.96±0.76^{c}
	0.100	7.49±0.17^{de}	67.77±0.19^{c}

Values are mean ± SD (n=3). Different superscripts in the same column denote the significant differences (P<0.05). Control: without extract.

2.4.2.3 Whiteness

Whiteness of all surimi gels added with E60 and E80 decreased when the levels increased ($P<0.05$) (Table 3). All gel samples showed the lowered whiteness than the control ($P<0.05$). At the same level of E60 or E80, the gels added with E60 had the lower whiteness than those incorporated with E80. Due to the higher content of phenolic compounds and tannin in E60, this might result in the darker color of gels containing E60, in comparison with those added with E80. Addition of oxidized phenolic compounds resulted in the decrease in whiteness of gel of bigeye snapper surimi (Balange and Benjakul, 2009a), fish emulsion sausage (Maqsood *et al*., 2012) and mayonnaise (Li *et al.*, 2014). Naturally, the plant phenolic compounds have dark color. As a consequence, the addition of those phenolics might cause the darkening of the final products. However, sardine has a high content of dark muscle and myoglobin (Kudre *et al*., 2013). Therefore, the addition of coconut husk extracts did not cause the severe discoloration of surimi gel from sardine.

2.4.2.4 TCA-soluble peptide content

TCA-soluble peptide content of gels from sardine surimi added with different levels of E60 and E80 is presented in Fig. 10. TCA-soluble peptide content indicates proteolytic degradation occurring during setting and gelation. The highest TCA-soluble peptide content was obtained in gel without the addition of coconut husk extract. The degradation mediated by endogenous proteases in surimi gels took place to a high extent in the absence of coconut husk extract (control). This result was related with the lowest breaking force in the control gel. Kudre *et al*. (2013) reported that proteolysis in surimi from sardine could be enhanced at 40 °C and reached the maximum at 65 °C. TCA-soluble peptide content of surimi gel decreased when the level of E60 or E80 increased ($P<0.05$). The results obtained suggested that E60 and E80 played a role in inhibition of muscle protein degradation in a dose-dependent fashion. When myofibrillar proteins were cross-linked, the cleavage sites for proteases were reduced, leading to more resistance to hydrolysis. In addition to hydrogen bond, hydrophobic interactions may occur between phenolic compounds and hydrophobic amino acids such as alanine, valine, isoleucine, leucine, methionine, phenylalanine, tyrosine, tryptophan, cysteine and glycine residues (Prigent *et al*., 2003). Furthermore,

the phenolics might interact directly with proteases, resulting in the loss in their activity. Surimi gel from bigeye snapper incorporated with phenolic compounds extracted from kiam wood had the decreased protein solubility associated with the enhanced protein cross-linking (Balange and Benjakul, 2011). Therefore, protein degradation in sardine surimi gels could be decreased by the addition of E60 or E80, especially at high levels.

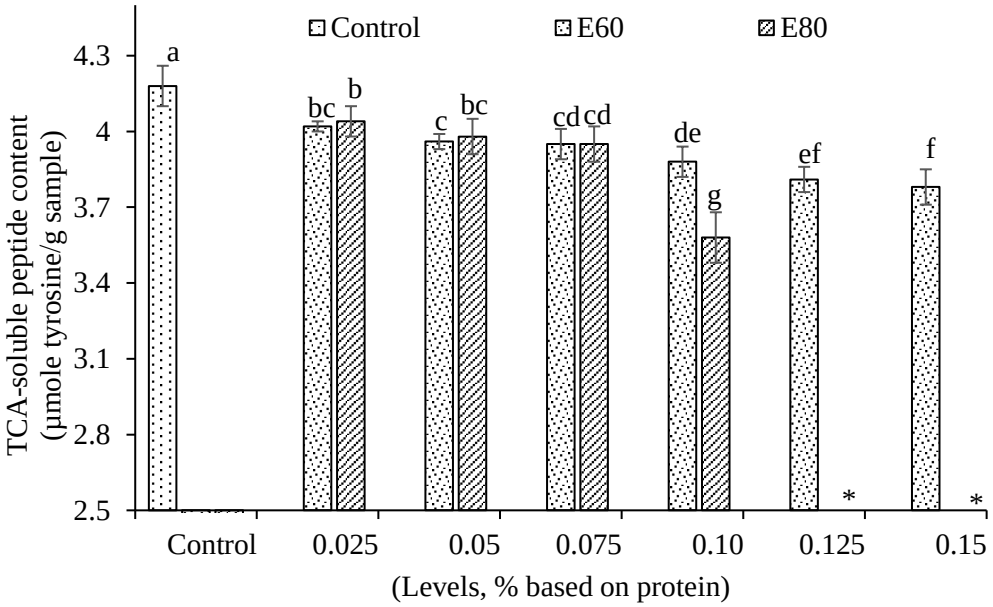


Figure 10. TCA-soluble peptide content of gels from sardine surimi without and with E60 or E80 at different levels. Bars represent the standard deviation (n=3). Different letters on the bars indicate significant differences (P<0.05). * Gel could not be formed due to the excessive coagulation.

2.4.2.5 Protein patterns

Protein patterns of surimi gels without and with the addition of E60 and E80 at various levels are depicted in Fig. 11. Surimi paste contained myosin heavy chain (MHC) and actin as the major proteins. In the control gel, MHC still remained to some extent. The disappearance was mostly mediated by endogenous transglutaminase (Kaewudom *et al.,* 2013). Nevertheless, MHC of gels with addition of coconut husk extracts completely disappeared, regardless of level used. In addition to weak bond, phenolics might undergo autoxidation during extraction or mixing with surimi paste. Those oxidized phenolic compounds, electrophilic in nature, could induce the

formation of non-disulfide covalent bonds between proteins (Benjakul and Vissessangan, 2003). Those cross-links induced by oxidized phenolic compounds via non-disulfide covalent bonds contributed partially to gel strength enhancement (Shitole *et al*., 2014). No changes in actin band intensity were found between the control gel and the gel with addition of E60 or E80 at all levels used. Actin was found to be unchanged in band intensity, suggesting that actin was not polymerized by transglutaminase or oxidized phenolics. Actin was also reported to be resistant to proteolysis (Balange and Benjakul, 2009a). Protein cross-linking mediated by phenolic in coconut husk extract in conjunction with endogenous transglutaminase contributed to the increased breaking force of surimi gels.

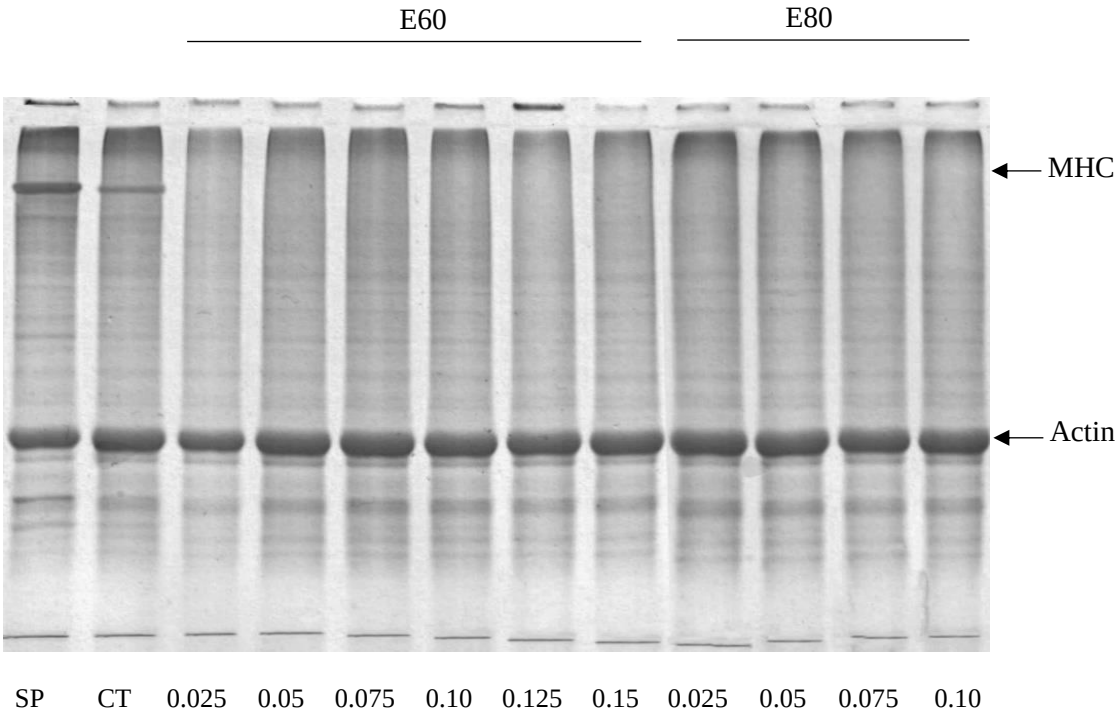


Figure 11. SDS-PAGE patterns of protein in gels from sardine surimi added with E60 and E80 at different levels. MHC, myosin heavy chain; SP, surimi paste; and CT, the control gel. Numbers denote the levels of coconut husk extract (%, based on protein).

2.4.3 Characteristics of surimi gel added with coconut husk extract at the selected levels

2.4.3.1 Textural properties

Textural properties of surimi gels added with coconut husk extracts, E60 at 0.1 and 0.125% as well as E80 at 0.05 and 0.075% are shown in Table 4. In comparison with the control gel, those incorporated with E60 or E80 showed the increases in hardness ($P<0.05$), representing the force required to compress sample to attain a given deformation, except the gel added with 0.05% E80, which had similar hardness to the control ($P>0.05$). Similar results were observed for gumminess, the energy required to breakdown a semi-solid food ready for swallowing and chewiness, the required energy to chew the sample to the point required for swallowing it. The highest hardness in gel added with 0.125% E60 was in agreement with the highest breaking force (Fig. 9). Nevertheless, there were no differences in springiness, elastic recovery that occurs when the compressive force is removed and cohesiveness, capability in breaking down the internal structure among all samples ($P<0.05$). In general, E60 increased hardness, gumminess and chewiness more effectively than E80, regardless of the levels. Phenolics in coconut husk extract with different types and forms, as influenced by extracting selecting media, could play a role in protein cross-linking in different fashions. This could lead to the different TPA characteristics of various gels.

Table 4. Textural properties of gels from sardine surimi added without and with E60 and E80 at different levels.

Extracts	Level (%)	Hardness (N)	Springiness (cm)	Cohesiveness (ratio)	Gumminess (N)	Chewiness (N.cm)
Control	-	121.34±0.88^{c}	0.94±0.01^{a}	0.78±0.03^{a}	93.39±0.43^{d}	91.42±0.58^{c}
E60	0.10	141.71±1.14^{a}	0.95±0.02^{a}	0.80±0.01^{a}	114.68±0.25^{a}	107.05±0.57^{a}
	0.125	143.21±1.27^{a}	0.95±0.01^{a}	0.77±0.01^{a}	112.71±0.86^{b}	106.17±0.52^{a}
E80	0.05	122.64±1.01^{c}	0.94±0.01^{a}	0.77±0.01^{a}	93.46±0.53^{d}	85.43±0.57^{d}
	0.075	129.93±0.81^{b}	0.95±0.01^{a}	0.79±0.00^{a}	102.95±0.90^{c}	97.40±0.53^{b}

Values are mean ± SD (n=3). Different superscripts in the same column denote the significant differences ($P<0.05$). Control: without extract.

2.4.3.2 Dynamic rheological properties

Changes in elastic modulus (G') of sardine surimi paste added with E60 at 0.1 and 0.125% as well as E80 at 0.05 and 0.075% during transition from sol to gel as a function of temperature are depicted in Fig. 12. The samples added with E60 or E80 showed the higher G' than the control (without E60 or E80). G', the stored energy of a viscoelastic material during the formation process, increased continuously and reached the highest value at approximately 35 °C in all samples. This indicated that the formation of protein network structure via hydrogen bonds between protein molecules (Zhang *et al.*, 2013). Thereafter, G' rapidly decreased and the lowest value was obtained at about 50 °C. Optimum temperature for endogenous proteolytic enzymes activity was in the range of 50-60 °C (Klomklao *et al*., 2008). Disaggregation of actin-myosin network structure more likely resulted in a decreased G' and the enhancement of protein mobility (Zhang *et al.,* 2015). G' was then increased again when heated up to 65 °C. This probably resulted from an increase in the number of cross-links between dissociated protein molecules and the denaturation of myosin heavy chain and actomyosin, leading to a formation of a thermo-irreversible gel network (Mleko and Foegedign, 2000). Unfolded proteins might favor the interaction aggregation via reactive groups or domains. Hydrophobic domains plausibly underwent interaction via hydrophobic-hydrophobic interaction (Benjakul *et al*., 2005), while sulfhydryl groups could be oxidized, in which disulfide bond could be formed. As a result, protein aggregation was enhanced. Thereafter, G' decreased again until the temperature reached 80 °C. This might be due to the rupture of hydrogen bonds during heating process (Zhang *et al.,* 2013). For the gel incorporated with E60, higher G' was found when E60 at a level of 0.125% was added, compared with gel added with 0.10% E60. For those containing E80, there was no difference in G' of samples containing 0.05 and 0.075% E80. The highest G' in the sample added with 0.125% E60 was in agreement with the highest breaking force (Fig. 9). It was noted that the higher G' was noticeable when E60 or E80 was added into paste before heating. This indicated the potential protein cross-linking activity of phenolics in the extracts. During heating, the denatured or unfolded muscle proteins were more polymerized by those phenolics in coconut husk extract, especially at high level.

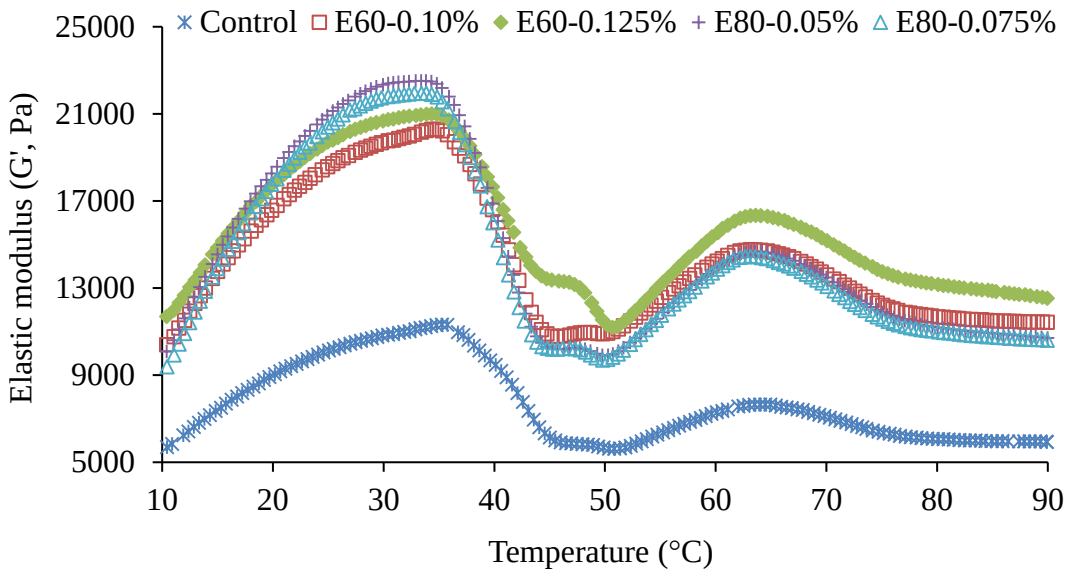


Figure 12. Elastic modulus (G') of sardine surimi gel without and with E60 and E80 at different levels during heating from 10 to 90 °C.

2.4.3.3 Microstructures

Microstructures of surimi gel added with E60 and E80 at various levels are illustrated in Fig. 5. The control gel (without E60 or E80 addition) had a coarser network with the larger void or cavities. Surimi gel network became finer and denser with the addition of coconut husk extracts, as compared with the control gel. The results suggested that phenolic compounds might induce the cross-linking of those myofibrillar proteins effectively (Shitole *et al*., 2014). Fine and ordered gel network more likely imbibed water. In the presence of E60 at a level of 0.125%, some particulates including coagulated or precipitated proteins, were formed within the ordered filamentous gel network. This might result in the low water holding capacity, as evidenced by the increased expressible moisture content. Thus, the addition of E60 and E80 into surimi gel affected the alignment of proteins within surimi gel networks.

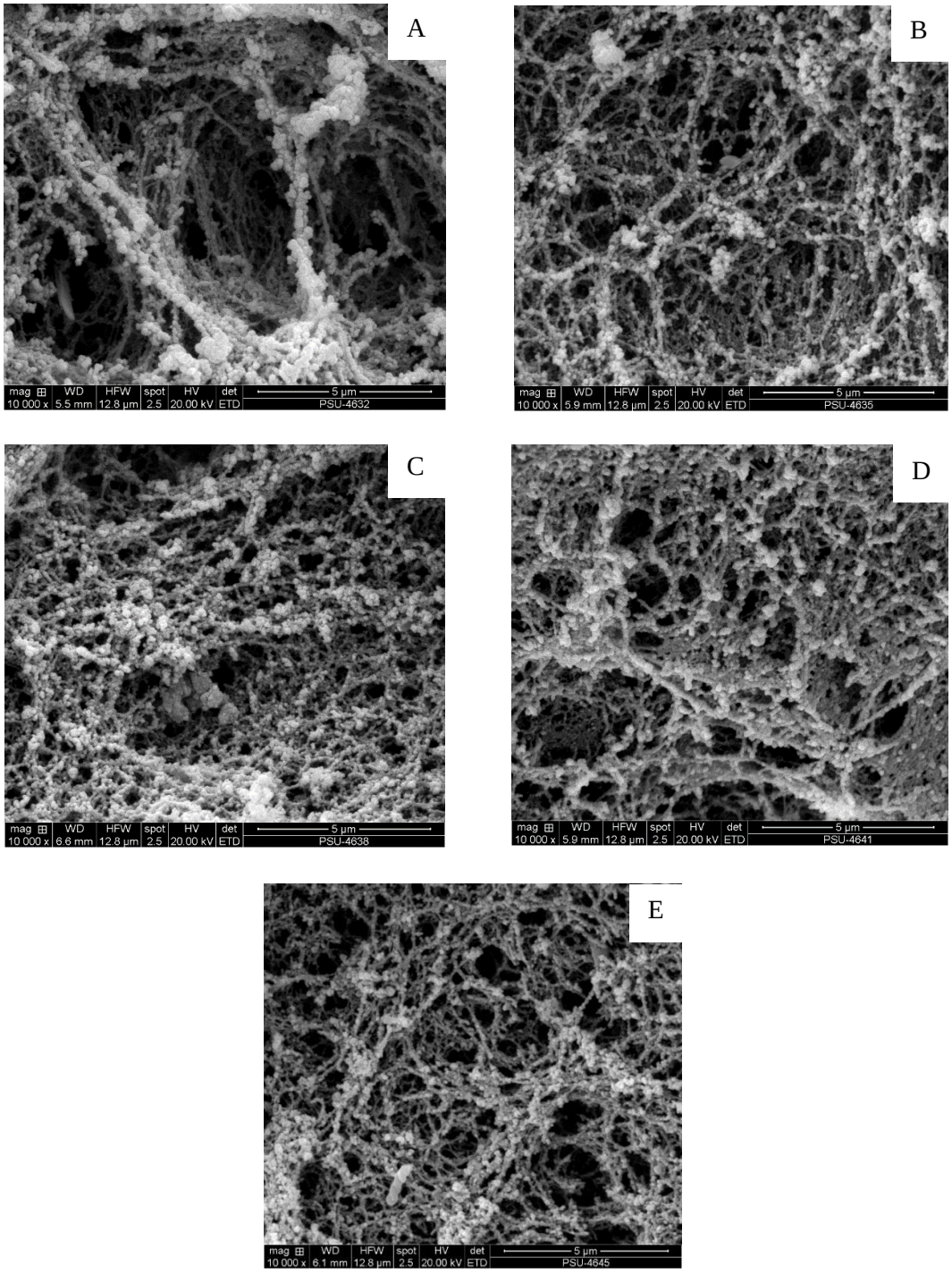


Figure 13. Electron microscopic images of surimi gel added with coconut husk extracts prepared using E60 and E80 at different levels (magnification: 10,000×): (A) surimi gel without coconut husk extract; (B) surimi gel added with 0.10% E60; (C) surimi gel added with 0.125% E60; (D) surimi gel added with 0.05% E80; and (E) surimi gel added with 0.075% E80.

2.4.3.4 Likeness score

Likeness score of gels from sardine surimi without and with E60 and E80 at various levels is shown in Table 5. Addition of E80 decreased color likeness of resulting gel, compared with the control. Gel added with 0.075% E80 had the lower taste likeness score than the control ($P<0.05$). It was noted that gel added with 0.125% E60 showed the higher texture and overall likeness score than others ($P<0.05$). This was coincidental with the increased gumminess and chewiness of gel added with 0.125% E60 (Table 4). Phenolic compounds play a role in the sensory attributes of many food products (O'Connell and Fox, 2001). There was no detrimental effect on the acceptability when chestnut and grape seed extracts, containing a high proportion of polyphenols, were added into dry cured sausages. Moreover, the incorporation of seaweed extract into lesser sardine surimi had no impact on sensory property. (Lorenzo *et al.*, 2013; Shitole *et al.*, 2014). Although the addition of coconut husk extracts lowered the acceptability of color and taste attributes of the resulting gels, the higher acceptability in texture attribute and overall liking were observed. Therefore, the results more likely reflected the texture attribute of surimi gel played an important role in sensory properties and increased the overall liking of sardine surimi gel.

Table 5. Likeness score of gels from sardine surimi added without and with E60 and E80 at different levels.

Extracts	Level (%)	Colour	Taste	Texture	Overall
Control	-	6.91 ± 1.09^{a}	6.94 ± 1.25^{a}	6.78 ± 1.30^{b}	6.69 ± 1.01^{b}
E60	0.10	6.45 ± 1.18^{ab}	6.78 ± 1.27^{ab}	6.96 ± 1.12^{ab}	6.92 ± 1.07^{ab}
	0.125	6.51 ± 1.47^{ab}	7.00 ± 1.29^{a}	7.10 ± 1.19^{a}	7.20 ± 1.03^{a}
E80	0.05	6.37 ± 1.16^{b}	6.71 ± 1.33^{ab}	6.73 ± 1.03^{b}	6.67 ± 1.09^{b}
	0.075	6.47 ± 1.13^{ab}	6.65 ± 1.27^{b}	6.94 ± 1.17^{ab}	6.69 ± 0.97^{b}

Values are mean ± SD (n=80). Different superscripts in the same column denote the significant differences ($P<0.05$). Control: without extract.

2.5 Conclusion

Surimi gel strengthening agent could be extracted from coconut husk using 60 or 80% ethanol. Addition of coconut husk extract yielded the surimi gel from sardine with the increased breaking force, textural and sensory characteristics. However, the addition of E60 or E80 caused a slight decrease in whiteness, particularly with increasing level. Therefore, the extract from coconut husk could be used as a natural additive to improve the gel properties of surimi manufactured from dark-fleshed fish.

2.6 References

Ali, H. 2002. Protein-Phenolic interaction in food. M.Sc. Dissertation, McGill University.

AOAC. 2000. Official Methods of Analysis. 18th ed. Association of Official Chemists. Washington D.C.

Arfat, Y. A. and Benjakul, S. 2013. Gel strengthening effect of zinc salts in surimi from yellow stripe trevally. Food Biosci. 3: 1-9.

Balange, A. and Benjakul, S. 2009a. Enhancement of gel strength of bigeye snapper (*Priacanthus tayenus*) surimi using oxidized phenolic compounds. Food Chem. 113: 61-70.

Balange, A. and Benjakul, S. 2009b. Effect of oxidised phenolic compounds on the gel property of mackerel (*Rastrelliger kanagurta*) surimi. LWT-Food Sci. Technol. 42: 1059-1064.

Balange, A. and Benjakul, S. 2011. Effect of kiam wood extract as influenced by pH during oxygenation on mackerel surimi gel. Food Biochem. 35: 574-595.

Benjakul, S. and Vissessanguan, V. 2003. Transglutaminase-mediated setting in bigeye snapper surimi. Food Res. Int. 36: 253-266.

Benjakul, S., Visessanguan, V. and Srivilai, C. 2007. Gel properties of bigeye snapper (*Priacanthus tayenus*) surimi as affected by setting and plasma proteins. J. Food Qual. 24: 453-471.

Benjakul, S., Visessanguan, V., Thongkaew, C. and Tanaka, M. 2005. Effect of frozen storage on chemical and gel-forming properties of fish commonly used for surimi production in Thailand. Food Hydrocolloid. 19: 197-207.

Benjakul, S., Visessangan, W., Tueksuban, J. and Tanaka, M. 2004. Effect of some protein additives on proteolysis and gel-forming ability of lizardfish (*Saurida tumbil*). Food Hydrocolloid. 18: 395-401.

Bilba, K., Arsene, M.-A. and Ouensanga, A. 2007. Study of banana and coconut fibers: Botanical composition, thermal degradation and textural observation. Biores. Technol. 98: 58-68.

Carvalho, O. V., Botelho, C. V., Ferreira, C. G. T., Ferreira, H. C. C., Santos, M. R., Diaz, M. A. N., Oliveira, T. T., Soares-Martins, J. A. P., Almeida, M. R. and Júnior, A. S. 2013. *In vitro* inhibition of canine distemper virus by flavonoids and phenolic acids: Implications of structural differences for antiviral design. Res. Vet. Sci. 95: 717-724.

Fradinho, D., Neto, C. P., Evtuguin, D., Jorge, F., Irle, M. and Gil, M. 2002. Chemical characterization of bark and of alkaline bark extracts from maritime pine grown in Portugal. Ind. Crops Prod. 16: 22-32.

Gawlik-Dziki, U., Jeżyna, M., Świeca, M., Dziki, D., Baraniak, B., and Czyż, J. 2012. Effect of bioaccessibility of phenolic compounds on *in vitro* anticancer activity of broccoli sprouts. Food Res. Int. 49: 469-476.

Hossain, M. A., Kalbani, M. S. A. A., Farsi, S. A. J. A., Weli, A. M. and Al-Riyami, Q. 2014. Comparative study of total phenolics, flavonoids contents and evaluation of antioxidant and antimicrobial activities of different polarities fruits crude extracts of *Datura metel* L. Asian Pac. J. Trop. Dis. 4: 378-383.

Hu, Y., Morioka, K. and Itoh, Y. 2010. Participation of cysteine protease cathepsin L in the gel disintegration of red bulleye (*Priacanthus macracanthus*) surimi gel paste. J. Sci. Food Agric. 90: 370-375.

Israel, A. U., Ogali, R. E., Akaranta, O. and Obot, I. B. 2011. Extraction and characterization of coconut (*Cocos nucifera* L.) coir dust. Songklanakarin J. Sci. Technol. 33: 717-724.

Jan, S., Khan, M. R., Rashid, U. and Bokhari, J. 2013. Assessment of antioxidant potential, total phenolics and flavonoids of different solvent fractions of *Monotheca buxifolia* fruit. Osong Public Health Res. Perspect. 4: 246-254.

Kaewudom, P., Benjakul, S. and Kijroongrojana, K. 2013. Properties of surimi gel as influenced by fish gelatin and microbial transglutaminase. Food Biosci. 1: 39-47.

Kallel, F., Driss, D., Chaari, F., Belghith, L., Bouaziz, F., Ghorbel, R. and Chaabouni, S. E. (2014). Garlic (*Allium ativum* L.) husk waste as a potential source of phenolic compounds: Influence of extracting solvents on its antimicrobial and antioxidant properties. Ind. Crops Prod. 62: 34-41.

Klomklao, S., Kishimura, S. and Benjakul, S. 2008. Endogenous proteinases in true sardine (*Sardinops melanostictus*). Food Chem. 107: 213-220.

Ko, W. C., Yu, C. C. and Hsu, K. C. 2007. Contribution of hydrophobicity, net charge, and sulphydryl groups of thermal properties of ovalbumin. LWT-Food Sci. Technol. 40: 1316-1320.

Kudre, T., Benjakul, S. and Kishimura, H. 2013. Effect of protein isolates from black bean and mungbean on proteolysis and gel properties of surimi from sardine (*Sardinella albella*). LWT-Food Sci. Technol. 50: 511-518.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of head of bacteriophage T4. Nature. 227: 680-685.

Li, C.-Y., Kim, H.-W., Li, H., Lee, D-.C. and Rhee, H.-I. 2014. Antioxidative effect of purple corn extracts during storage of mayonnaise. Food Chem. 152: 592-596.

Lobbes, J. M., Fitznar, H. P. and Kattner, G. 1999. High-performance liquid chromatography of lignin-derived phenols in environmental samples with diode array detection. Anal. Chem. 71: 3008-3012.

Lorenzo, J. M., González-Rodríguez, R. M., Sánchezb, M., Amado, I. R. and Franco, D. 2013. Effects of natural (grape seed and chestnut extract) and synthetic antioxidants (buthylatedhydroxytoluene, BHT) on the physical, chemical, microbiological and sensory characteristics of dry cured sausage "chorizo". Food Res. Int. 54: 611-620.

Lou, S.-N., Lin, Y.-S., Hsu, Y.-S., Chiu, E.-M. and Ho, C.-T. 2014. Soluble and insoluble phenolic compounds and antioxidant activity of immature calamodin affected by solvents and heat treatment. Food Chem. 161: 246-253.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. 193: 256-275.

Mansfield, B. 2003. Fish, factory trawlers, and imitation crab: the nature of quality in the seafood industry. J Rural Stud. 19: 9-21.

Maqsood, S., Benjakul, S. and Balange, A. K. 2012. Effect of tannic acid and kiam wood extract on lipid oxidation and textural properties of fish emulsion sausages during refrigerated storage. Food Chem. 130: 408-416.

Meilgaard, M., Civille, G. V. and Carr, B. T. 1999. Sensory Evaluation Techniques 3rd ed. CRC Press. Florida.

Mleoko, S. and Foegeding, E. A. 2000. pH induced aggregation and weak gel formation of whey protein polymers. J. Food Sci. 65: 139-143.

Morrisey, M. T., Wu, J. W., Lin, D. and An, H. 1993. Protease inhibitor effects on torsion measurement and autolysis of pacific whiting surimi. J. Food Sci. 58: 1050-1054.

Nikolantonaki, M., Magiatis, P. and Waterhouse, A. L. 2014. Measuring protection of aromatic wine thiols from oxidation by competitive reactions vs wine preservatives with ortho-quinones. Food Chem. 163: 61-67.

Nour, V., Stampar, F., Veberic, R. and Jakopic, J. 2013. Anthocyanins profile, total phenolics and antioxidant activity of black currant ethanolic extracts as influenced by genotype and ethanol concentration. Food Chem: 141: 961-966.

O'Connell, J. E. and Fox, P. F. 2001. Significance and applications of phenolic compounds in the production and quality of milk and dairy products. Int. Dairy J. 11: 103-120.

Oujifard, A., Benjakul, S. Ahmad, M. and Seyfabadi, J. 2012. Effect of bambara groundnut protein isolate on autolysis and gel properties of surimi from threadfin bream (*Nemipterus bleekeri*). LWT-Food Sci. Technol. 47: 261-266.

Park, J. W. 1994. Functional protein additives in surimi gels. J. Food Sci. 59: 525-527.

Prigent, S. V. E., Gruppen, H., Visser, A. J., Van Koningsveld, G. A., de Jong, G. A. H. and Voragen, A. G. J. 2003. Effects of non-covalent interactions with 5-*O* (*ortho*)-caffeoylquinic acid (chlorogenic acid) on the heat denaturation and solubility of globular proteins. J. Agric. Food Chem. 51: 5088-5095.

Prodpran, T., Benjakul, S. and Phatcharat, S. 2012. Effect of phenolic compounds on protein cross-linking and properties of film from fish myofibrillar protein. Int. J. Biol. Macromol. 51: 774-782.

Rattaya, S., Benjakul, S. and Prodpran, T. 2009. Properties of fish skin gelatin film incorporated with seaweed extract. J. Food Eng. 95: 151-157.

Rawdkuen, S., Benjakul, S., Vissessanguan, W. and Lanier, T. C. 2008. Rheological and textural properties of Pacific whiting surimi gels as influenced by chicken plasma. Int. J. Food Prop. 11: 820-832.

Robinson, H. W. and Hogden, C. G. 1940. The biuret reaction in the determination of serum proteins. I. A study of the conditions necessary for the production of a stable color which bears a quantitative relationship to the protein concentration. J. Biol. Chem. 135: 707-725.

Rodrigues, S. and Pinto, G. A. S. 2007. Ultrasound extraction of phenolic compounds from coconut (*Cocos nucifera*) shell powder. J. Food Eng. 80: 869-872.

Shitole, S. S., Balange, A. K. and Gangan, S. S. 2014. Use of seaweed (*Sargassum tenerrimum*) extract as a gel enhancer for lesser sardine (*Sardinella brachiosoma*) surimi. Int. Aquat. Res. 6: 55.

Slinkard, K. and Singleton, V. L. 1997. Total phenol analyses: Automation and comparison with manual methods. Am. J. Enol. Viticult. 28: 49-55.

Steel, R. G. D. and Torrie, J. H. 1980. Principles and Procedures of Statistics: A Biometrical Approach 2nd Ed. McGraw-Hill. New York.

Tabilo-Munizaga, G. and Barbosa-Canovas, G. 2005. Rheology for the food industry. J. Food Eng. 42: 66-71.

Temdee, W. and Benjakul, S. 2014. Effect of oxidized kiam wood and cashew bark extracts on gel properties of gelatin from cuttlefish skins. Food Biosci. 7: 95-104.

Vázquez-Torres, H., Canché-Escamilla, G. and Cruz-Ramos, C. A. 1992. Coconut husk lignin. II. Characterization by infrared and nuclear magnetic resonance spectroscopy. J. Appl. Polym. Sci. 45: 645-653.

Woods, M. N. and Gorbach, S. L. 2001. Influences of fiber on the ecology of the intestinal flora. In CRC Handbook of Dietary Fiber in Human Nutrition (Spiller, G. A., ed.). p.257-270. CRC Press. Florida.

Zhang, F., Fang, L., Wang, C., Shi, L., Chang, T., Yang, H. and Cui, M. 2013. Effects of starches on the textural, rheological, and color properties of surimi-beef gels with microbial transglutaminase. Meat Sci. 93: 533-537.

Zhang, T., Xue, Y., Li, Z., Wang, Y. and Xue, C. 2015. Effects of deacetylation of konjac glucomannan on Alaska pollock surimi gels subjected to high-temperature (120 °C) treatment. Food Hydrocolloid. 43: 125-131.

CHAPTER 3

CROSS-LINKING ACTIVITY OF ETHANOLIC COCONUT HUSK EXTRACT TOWARD SARDINE (*SARDINELLA ALBELLA*) MUSCLE PROTEINS

3.1 Abstract

Effects of ethanolic coconut husk extract (ECHE) at different levels (0-0.03%, based on protein content) on heat-induced aggregation of natural actomyosin (NAM) extracted from sardine (*Sardinella albella*) muscle were studied. During heating from 20 to 90 °C, NAM solution showed an increase in turbidity, surface hydrophobicity and disulfide bond contents. Aggregation was more pronounced as ECHE concentration increased ($P<0.05$). While protein solubility and total sulfhydryl group were decreased ($P<0.05$). Furthermore, the Ca^{2+}-ATPase activity of NAM was noticeable decreased in the present of ECHE during heating up to 40 °C. Zeta potential analysis revealed that NAM added with ECHE became less negatively charged when the concentrations of ECHE increased ($P<0.05$). Cross-linking of protein strands was enhanced in the presence of ECHE during heating as evidenced by the higher G', particle size as well as microstructure. Thus, ECHE could be served as cross-linking agent for fish muscle proteins.

3.2 Introduction

Fish muscle proteins have been known to possess several functional properties, particularly gelation. Myofibrillar proteins play an esteemed role in gelation. Thus, washing process is implemented to remove water soluble proteins, and simultaneously concentrate myofibrillar proteins. Surimi is the concentrated myofibrillar proteins, prepared from fish mince subjected washing process, in which undesirable components including sarcoplasmic proteins, lipids, and heme pigments are removed (Mansfield 2003). Generally, lean fish are commonly used for surimi production because of the good gel forming ability and whiteness. Nevertheless, some dark flesh fish such as sardine have been used for surimi production, in which particular gelly products are still requiring their type of surimi.

Sardine (*Sardinella albella*) is one of the abundant dark flesh fish species in Southern Thailand. This species has been used as the raw material for surimi production owing to its abundance. However, sardine and other dark fleshed fish have high lipid, myoglobin and sarcoplasmic protein contents, associated with the difficulties in making good-quality surimi (Chanarat *et al*. 2012). Additionally, surimi from dark-fleshed fish is vulnerable to protein degradation mediated by heat-activated proteases. Those proteases could induce the degradation of myofibrillar proteins, leading to the lower gel strength and poor water holding capacity (Buamard and Benjakul 2015; Kudre *et al*. 2013). Some protease inhibitor and protein cross-linking agents has been introduced to conquer the problem (Richards *et al*. 2014).

Coconuts are abundant in coastal areas of tropical countries including Thailand. The husk, which is the fibrous external portion of coconut fruits, is removed during processing and a large amount of husk is considered as a waste (Panyakaew and Fotios 2011). The husk is a major source of carbohydrate and phenolics, particularly tannic acid (Sueli and Gustavo 2007). Apart from tannic acid, the husk also contains other phenolic compounds such as hydroxybenzoic acid, ferulic acid, vanilic acid as well as syringic acid (Rodrigues *et al*. 2008).

The extract from coconut husk has been reported to enhance the properties of nanocomposite films prepared from tilapia skin gelatin (Nagarajan *et al*. 2015). Moreover, the use of coconut husk extract at an appropriate level improved gel property of gelatin from yellowfin tuna swim bladder (Kaewdang and Benjakul, 2015). Recently, Buamard and Benjakul (2015) have found that coconut husk extract could increase the gel strength of surimi gel from sardine. However, no information regarding physicochemical changes and heat-induced aggregation of fish myofibrillar proteins as induced by coconut husk extract exists. Therefore, the present study aimed to elucidate the effect of ethanolic coconut husk extract at different concentrations on the physicochemical properties and heat-induced aggregation of natural actomyosin from sardine (*S. albella*), a dark fleshed fish abundant in Thailand.

3.3 Materials and methods

3.3.1 Chemicals

All chemicals were of analytical grade. Adenosine-5'-triphosphate (ATP), 8-anilino-1-napthalenesulphonic acid (ANS), guanidine thiocyanate and Tris-maleate were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Potassium chloride, sodium chloride, calcium chloride, trichloroacetic acid and ammonium molybdate were obtained from Merck (Darmstadt, Germany). 5,5-Dithiobis (2-nitrobenzoic acid) (DTNB) was procured from Wako Pure Chemical Industries (Tokyo, Japan).

3.3.2 Collection and preparation of coconut husk

Husk of coconut (*Cocos nucifera* Linn.) fruit with the age about 11 months was collected from the local market in Hat Yai, Songkhla Province, Thailand and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai, Thailand. Husk sample was prepared as per the method of Vázquez-Torres *et al*. (1992) with slight modifications. The obtained powder was placed in a polyethylene bag, sealed and kept at room temperature until use.

3.3.3 Preparation of ethanolic coconut husk extract

Coconut husk powder was subjected to extraction according to the method of Buamard and Benjakul (2015) using 60% (v/v) ethanol. The powder named ‘ECHE’ was transferred into amber bottle and stored in a desiccator until use or analysis.

3.3.4 Total phenolic content and tannic acid content

Total phenolic content of ECHE was determined using Folin-Ciocalteau Reagent (FCR) as described by Slinkard and Singleton (1997) with a slight modification. The phenolic content was reported as mg tannic acid equivalents (TAE) per g dry weight of ECHE. The determination of tannic acid content was performed using high performance liquid chromatography (HPLC) as per the method of Temdee and Benjakul (2014). The HPLC system was included with an Agilent 1100 series HPLC (Alginet, Wilmington, DE, USA), quaternary pump with the seal wash option,

degasser, solvent, cabinet and preparative auto-sampler with thermostat equipped with a diode array detector. The separation was done by a Hypersil ODS C18 4.0 × 250 mm, 5 µm column (Cole-Parmer, London, UK). HPLC conditions were as follows: mobile phase: 0.4% formic acid:acetronitrile (85:15), flow rate: 0.8 mL/min, temperature: 25 °C. Detection was performed at 280 nm. The concentration of extracts was 25 mg/mL and the injection volume was 20 µL. Standard tannic acid was used for peak identification.

3.3.5 Collection and preparation of sardine flesh

Sardine with an average weight of 55-60 g were caught from Songkhla coast along the Gulf of Thailand during May and July, 2015. Fish were placed in ice with a fish/ice ratio 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai within 40 min. The fish were immediately headed, gutted and washed with water. The flesh was excised manually and then kept on ice until natural actomyosin (NAM) was extracted.

3.3.6 Preparation of natural actomyosin (NAM)

NAM was prepared as per the method of Arfat and Benjakul (2012) with a slight modification. NAM pellet was dissolved in chilled 0.45 M NaCl, pH 7.0 for 30 min at 4 °C and then centrifuged at 5,000 ×g for 20 min at 4 °C. The supernatant was collected and used as NAM.

3.3.7 Study on heat-induced aggregation of NAM as affected by ECHE at different concentrations

Sardine NAM was diluted to 1 mg/mL with chilled 0.45 M NaCl (pH 7.0). NAM solutions containing ECHE at different concentrations (0.01, 0.02 and 0.03%, based on protein content) were heated using a waterbath (WNB 14, Memmert, Schwabach, Germany) at a heating rate of 0.88 C/min from 20 to 90 °C. NAM without the extract was used as the control. The samples were taken every 10 °C of temperature increment. When the designated temperature was reached, the samples were cooled with iced water immediately. The obtained NAMs were then subjected to analyses.

3.3.7.1 Determination of protein aggregation

NAM solutions without and with ECHE at various concentrations were placed in the cuvette. The development of aggregation in NAM solution was monitored by the increase in absorbance at 660 nm (Benjakul *et al*. 2001) using a UV-visible spectrophotometer (UV-1601, Shimadzu, Kyoto, Japan).

3.3.7.2 Determination of protein solubility

NAM solution added without and with ECHE at different concentrations were centrifuged at 3,500 ×g for 20 min at 4 °C to remove the debris. The supernatant was determined for protein concentration by the Bradford method (Bradford 1976) using bovine serum albumin as a standard.

3.3.7.3 Determination of surface hydrophobicity

Surface hydrophobicity was determined as described by Balange and Benjakul (2010) using 8-anilo-1-napthalenesulphonic acid (ANS) as a probe. For each treatment, the initial slope of the plot between fluorescence intensity and protein concentration was referred to as S_0ANS.

3.3.7.4 Determination of total sulfhydryl groups and disulfide bond contents

Total sulfhydryl group content was determined following the method of Ellman (1959) as modified by Benjakul *et al*. (2001). The sulfhydryl group content was calculated using the extinction coefficient of 13,600/M/cm. The determination of disulfide bond content was performed following the method of Thannhauser *et al*. (1987). Disulfide bond content was calculated using the extinction coefficient of 13,900/M/cm.

3.3.7.5 Determination of Ca^{2+}-ATPase activity

Ca^{2+}-ATPase activity of NAM with different treatments was determined as per the method of Jiang *et al*. (1988) with a slight modification. After the reaction, the mixture was centrifuged at 3,500 ×g for 5 min and the inorganic phosphate released in the supernatant was determined by the method of Fiske and Subbarow (1925). Ca^{2+}-

ATPase activity was expressed as μmole of inorganic phosphate released/mg protein/min.

3.3.8 Study on dynamic rheology, charge, size and microstructure of NAM as affected by ECHE at different concentrations

3.3.8.1 Dynamic rheology

NAM solutions (20 mg/mL) containing ECHE at levels of 0-0.03% were prepared as previously described. The samples were subjected to dynamic rheological analysis following the method of Visessanguan *et al*. (2003) with a slight modification. A rheometer (HAAKE RheoStress1, ThermoFisher Scientific, Karlsruhe, Germany) equipped with 60 mm parallel plate and a gap of 1.0 mm was used for monitoring the changes in storage or elastic modulus (G'). An oscillation of 2.1 Hz with a maximum strain amplitude of 0.05 was used for testing. This condition yielded a linear response in the viscoelastic region. The temperature sweep was recorded during heating up from 20 to 90 °C with heating rate of 1 °C/min.

3.3.8.2 Zeta potential and particle size

The zeta (ζ) potential and particle size of NAM solutions (1 mg/mL) without and with ECHE at level of 0.01 and 0.03% were determined following the method of Arfat and Benjakul (2012).

3.3.8.3 Transmission electron microscopy

NAM solutions (1 mg/mL) without and with ECHE (0.01 and 0.03%) were prepared according to the method of Arfat and Benjakul (2012). The treated NAM was visualized using a JEOL JEM-2010 transmission electron microscope (JEOL Ltd., Tokyo, Japan) at magnification 60,000× with accelerating voltage of 160 kV.

3.3.9 Statistical analysis

The experiments were run in triplicate with three lots of sample. Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by the Duncan's multiple range test (Steel and Torrie 1980). Statistical analysis was performed using SPSS for Windows (SPSS Inc., Chicago, IL, USA). Data with $P<0.05$ were considered to be statistically significant.

3.4 Results and discussion

3.4.1 Total phenolic content and composition of ECHE

Total phenolic content of ECHE was 453 ± 6 mg TAE/g ECHE. Coconut husk could therefore be a potential source of phenolic compounds. Saha *et al.* (2013) reported that four wood extracts from padouk, tali, moabi and movingui had different total phenolic content (54-992 mg gallic acid equivalent/g of extract). These differences in total phenolic content could be from the variation in tree species, plant nutrition and environment (Chinnici *et al*. 2015).

ECHE had 28 mg tannic acid/g ECHE. Ethanolic kiam wood and cashew wood extracts consisted of tannic acid at levels of 193 and 75 mg/ g dry extract, respectively (Temdee and Benjakul 2014). Apart from tannic acid and other phenolic compounds such as lignin, 4-hydrobenzoic acid, *ρ*-coumaric acid and ferulic acid (Balange and Benjakul 2009), some aldehydes including protocatechucaldehyde, coniferaldehyde and sinapaldehyde are found in wood extracts (Alañón, *et al*. 2011). Thus, the result suggested that ECHE contained tannic acid, which was reported as protein cross-linker (Balange and Benjakul 2009).

3.4.2 Heat-induced aggregation and physicochemical properties of NAM as affected by ECHE at different concentrations

3.4.2.1 Protein aggregation

Protein aggregation of NAM in the absence and presence of ECHE at different levels was monitored during heating up to 90 °C as shown in Fig. 14A. During heating, a rapid increase in A_{660} representing the turbidity of all NAM samples was observed at 40 °C. However, negligible increase was noticeable when NAM samples were heated at 30 °C. When NAM solution was heated up to 40 °C, hydrophobic domains and reactive group were exposed to some degree and underwent aggregation readily. The instability of hydrogen bonds at higher temperature causes the unfolding of protein molecules and release the hydrophobic portions (Niwa 1992). Increased turbidity of NAM solution during heating was related with the formation of the protein aggregate via several bondings. It was noted that the higher turbidity development of NAM was found when ECHE was incorporated particularly at higher levels (P<0.05).

Phenolics in ECHE could play an important role in cross-linking of proteins via hydrogen bond with hydroxyl group and hydrophobic interaction with phenol ring of ECHE. In addition to weak bonds, phenolics might undergo autoxidation during extraction, mixing or heating of NAM solution. Those oxidized phenolic compounds, electrophilic in nature, could also induce the formation of non-disulfide covalent bonds between protein chains (Benjakul and Vissessangan 2003). Prodpran *et al*. (2012) found that phenolic compound could enhance protein cross-linking or interaction of myofibrillar proteins prepared from bigeye snapper. Interaction between hydroxyl group of polyphenol and hydrogen acceptor in protein molecules by hydrogen bonds as well as protein-polyphenol hydrophobic interaction as influenced by phenolic compounds was reported by Ozdal *et al*. (2013). Therefore, the aggregation of NAM from sardine was enhanced by the incorporation of ECHE.

3.4.2.2 Protein solubility

Solubility of NAM solutions in the absence or presence of ECHE at different levels during heating from 20 to 90 °C is shown in Fig. 14B. The solubility of all NAM solutions was sharply decreased at the temperature of 40 °C and continuously decreased at the lower rate. Regardless of the incubation temperatures, the control solution showed the highest protein solubility, followed by NAM added with ECHE at 0.01, 0.02 and 0.03%, respectively (P<0.05). Furthermore, there were differences in solubility of NAM solutions since the initial incubation (at 20 °C). This reflected the efficiency of ECHE to attach the muscle proteins with subsequent cross-linking as indicated by the decrease in solubility. The decrease in protein solubility coincided well with the increased turbidity (Fig. 14A). Balange and Benjakul (2010) reported that muscle proteins from sardine most likely underwent conformational changes to a higher extent at 40 °C. With the exposure of hydrophobic patches and reactive groups, as induced by heat, the aggregation by phenolics in ECHE could be enhanced in conjunction with other bonds. Several bonds are involved in gelation of fish proteins. Those include disulfide bond, hydrophobic interaction, etc. (Chaijan *et al*. 2010). This resulted in the decreased protein solubility. In the presence of ECHE, the loss in protein solubility was in dose-dependent manner. The result suggested that the loss in protein solubility was due to the combined effects between heating and ECHE incorporation.

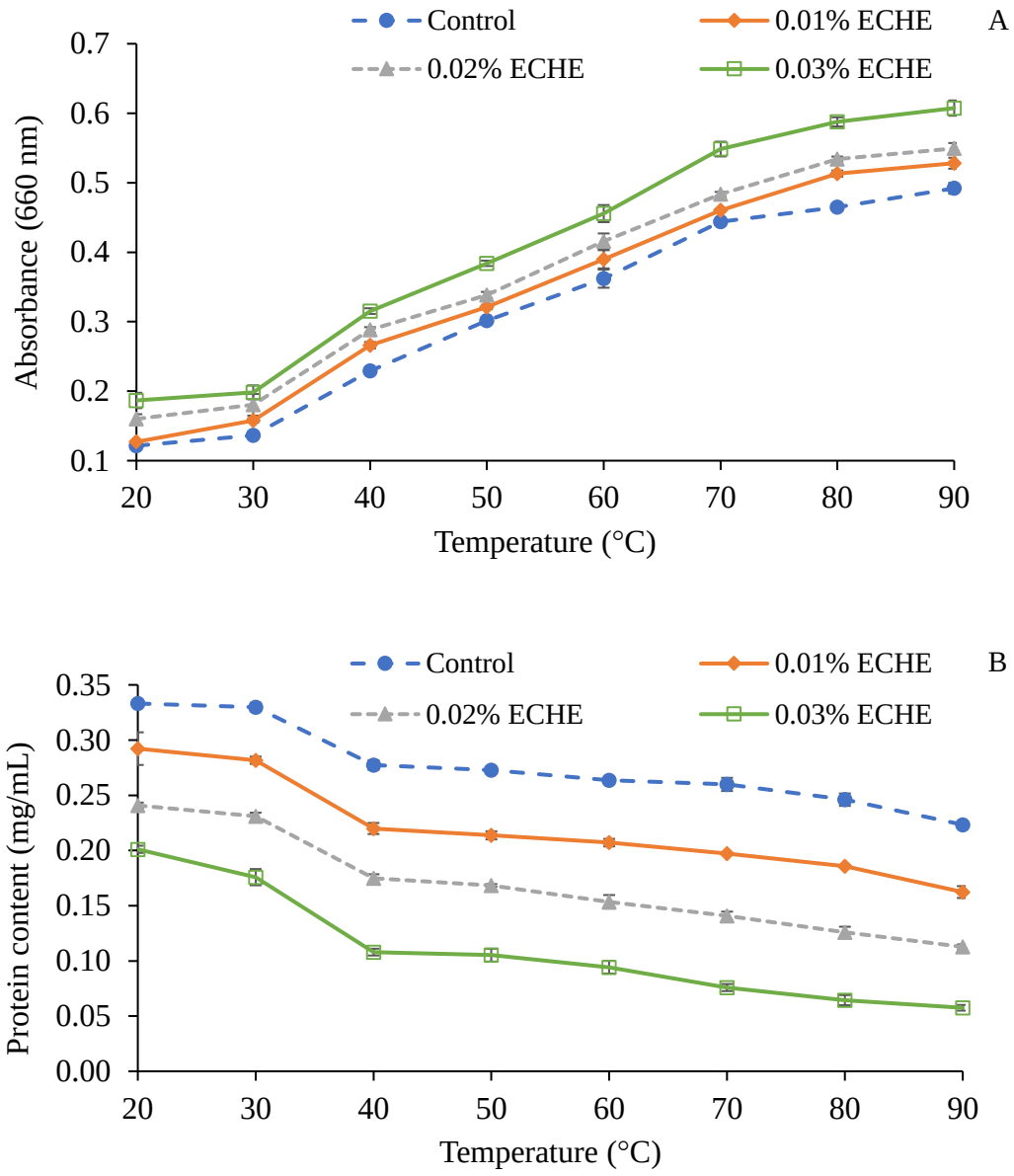


Figure 14. Turbidity (A) and solubility (B) of NAM solution (1 mg/mL) added without and with ECHE at different levels during heating from 20 to 90 °C. Heating rate was 0.88 °C /min. Bars represent the standard deviation (n=3).

3.4.2.3 Surface hydrophobicity

Surface hydrophobicity (S_0ANS) of NAM in the absence and presence of ECHE at various levels as affected by heating at different temperatures is shown in Fig. 15. S_0ANS of all NAM samples increased continuously when heated at temperature above 30 °C (P<0.05). The increase reached the maximum at 70 °C. Thereafter, the

slight decrease in S_0ANS was noticeable up to 90 °C. The increase in S_0ANS was more likely associated with the unfolding of proteins as induced by heat. The exposure of hydrophobic domains at high temperature was indicated by the increase S_0ANS (Wang *et al*. 2009). ANS, a fluorescence probe, has been used to bind amino acids containing aromatic ring, such as phenylalanine and tryptophan, and can be used to monitor the conformational changes occurring in the proteins (Benjakul *et al*. 1997). During heating at 80-90 °C, the exposed hydrophobic residues might undergo interaction via hydrophobic-hydrophobic interaction. This was evidenced by the lowered S_0ANS. The exposure of hydrophobic domains was a prerequisite for formation of large myosin aggregates via hydrophobic-hydrophobic interaction (Yarnpakdee *et al*. 2009). S_0ANS of NAM increased as the level of ECHE increased ($P<0.05$), regardless of heating temperatures. This result revealed that ECHE likely altered the conformation of NAM to some extent, as indicated by the increased surface hydrophobicity. When ECHE interacted or attached to protein molecules, it might induce the changes in protein conformation to some degrees, in which hydrophobic domains were exposed to aqueous phase. At the room temperature, NAM added with higher level of ECHE exhibited higher S_0ANS. The decrease in S_0ANS in sample added with ECHE heated at 80-90 °C could be due to self-aggregation of the extracts (Balange and Benjakul 2010), in conjunction with protein-protein interaction. The result suggested that ECHE was able to enhance the interconnection of muscle protein via hydrophobic interaction.

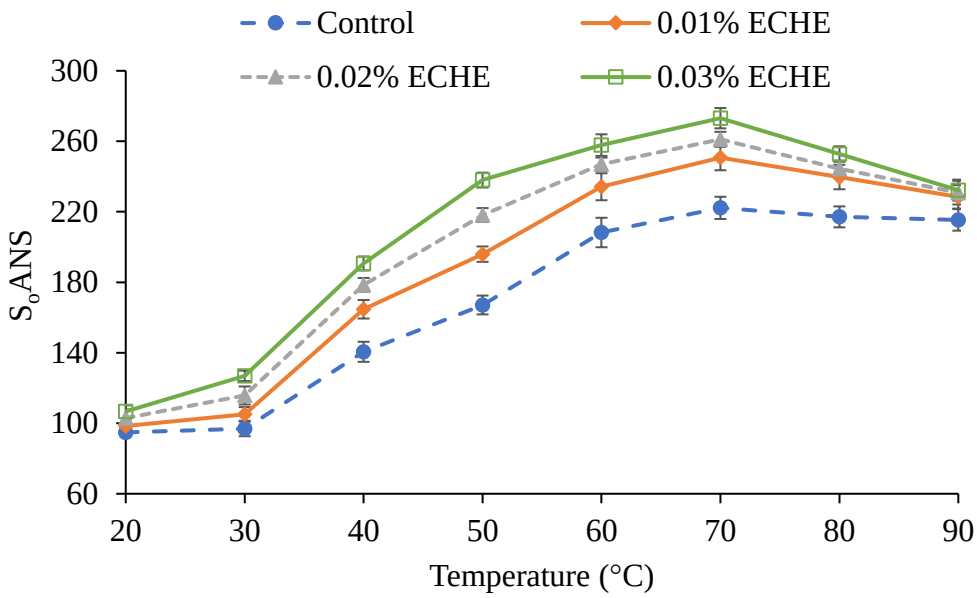


Figure 15. Surface hydrophobicity (S_0ANS) of NAM solution (1 mg/mL) added without and with ECHE at different levels during heating from 20 to 90 °C. Heating rate was 0.88 °C/min. Bars represent the standard deviation (*n*=3).

3.4.2.4 Total sulfhydryl group and disulfide bond contents

Total sulfhydryl (SH) group and disulfide bond contents of NAM without and with the addition of ECHE at different concentrations during heating from 20 to 90 °C are depicted in Fig. 16A and 16B, respectively. Total SH content of all samples decreased continuously after heating at 40 °C or above. Continuous decrease in total sulfhydryl group was found as the temperature increased. In the presence of ECHE, total SH content of NAM decreased in a dose dependent manner (P<0.05). NAM containing 0.03% ECHE showed the lowest total SH group content, especially after heating at 90 °C. The decreases in total SH content were in accordance with the increased disulfide bond formation (Fig. 16B), especially at temperature above 40 °C (P<0.05). An inter-molecular disulfide bond is formed by the oxidation of two cysteine molecules on neighboring proteins (Lanier, 2000). This indicated that sulfhydryl groups could be oxidized to disulfide bond when NAM was heated, especially at high temperature. Arfat and Benjakul (2012) reported that disulfide bond formation in yellow stripe travelly required the temperature above 35 °C, whereas oxidation of SH

groups in goatfish and tilapia NAM occurred at 40 and 50 °C, respectively (Yarnpakdee *et al*. 2009; Yongsawatdigul and Park 2003). ECHE might play a role in inducing the change of NAM conformation. This resulted in the exposure of SH group to a higher extent. SH groups were readily oxidized to disulfide bonds. The formation of disulfide bond correlated with the increased turbidity and the decreased solubility of NAM solution (Fig. 14). Apart from enhancement of hydrophobic interaction, ECHE, particularly at high concentrations, could induce disulfide bond formation of NAM from sardine muscle.

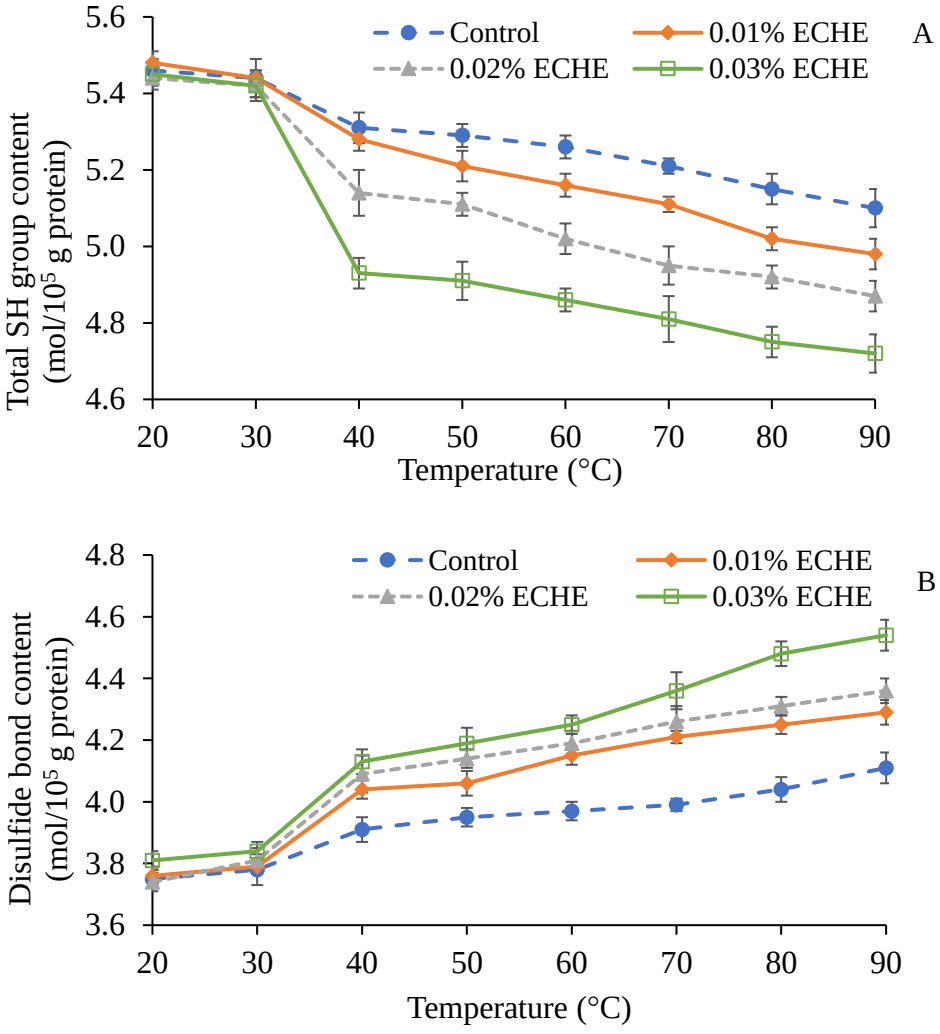


Figure 16. Total sulfhydryl group (A) and disulfide bond content (B) of NAM solution (1 mg/mL) added without and with ECHE at different levels during heating from 20 to 90 °C. Heating rate was 0.88 °C/min. Bars represent the standard deviation (*n*=3).

3.4.2.5 Ca^{2+}-ATPase activity

Changes in Ca^{2+}-ATPase activity of NAM during heating from 20 to 60 °C as influenced by ECHE at various concentrations are shown in Table 6. The Ca^{2+}-ATPase activity was decreased continuously in all samples as the temperature increased from 20 to 40 °C (P<0.05), regardless of ECHE levels added. Ca^{2+}-ATPase activity was not detectable when heated at temperature above 40 °C. Esturk and Park (2014) reported that the transition of myosin protein from fish generally occurred at temperature above 40 °C, but could vary, depending on fish species. Ca^{2+}-ATPase has been known not only as a good indicator of myosin denaturation but also a sensitive indicator of the aggregation of myosin molecules (Benjakul *et al*., 1997). At the same temperature for NAM heating, ECHE at higher concentrations resulted in the higher decrease in Ca^{2+}-ATPase (P<0.05). The results suggested that ECHE might induce the denaturation of Ca^{2+}-ATPase by altering of myosin conformation, particularly at myosin head portion. SH1 and SH2, SH groups located in the head portion, play an important role in the Ca^{2+}-ATPase activity (Bobkova *et al*. 1999). Since the oxidation of sulfhydryl group was enhanced by ECHE, this could be associated with the loss in Ca^{2+}-ATPase, particularly at head domain of myosin heavy chain. The decrease in Ca^{2+}-ATPase activity was in accordance with the increased surface hydrophobicity and total disulfide bond content (Fig. 15 and 16B). Additionally, the enhanced aggregation between myosin head induced by ECHE might hinder the active sites of Ca^{2+}-ATPase. Thus, ECHE directly affected the myosin heavy chain by inducing conformational changes. This could further favor the subsequent aggregation of proteins.

Table 6. Ca^{2+}-ATPase activity of NAM solution (1 mg/mL) added without and with ECHE at different levels after heating from 20 to 60 °C.

Temperature	ECHE level (%)			
(°C)	Control	0.01	0.02	0.03
20	0.15 ± 0.02 ^{Aa}	0.14 ± 0.02 ^{ABa}	0.13 ± 0.01 ^{ABa}	0.11 ± 0.01 ^{Ba}
30	0.10 ± 0.02 ^{Ab}	0.10 ± 0.01 ^{Ab}	0.10 ± 0.02 ^{Aa}	0.05 ± 0.01 ^{Bb}
40	0.08 ± 0.02 ^{Ac}	0.07 ± 0.01 ^{Ac}	0.05 ± 0.02 ^{Bb}	0.03 ± 0.01 ^{Cc}
50	ND	ND	ND	ND
60	ND	ND	ND	ND

Value are mean ± SD (n=3). ND: Not detectable. Different uppercase superscripts in the same row indicate significant differences (P<0.05). Different lowercase superscripts in the same column indicate significant differences (P<0.05).

3.4.3 Changes in dynamic rheology, charge, size and microstructure of NAM as affected by ethanolic coconut husk extract

3.4.3.1 Dynamic rheological properties

Elastic modulus (G') of NAM added without and with ECHE at various concentrations as a function of heating temperatures is shown in Fig. 17. G' value reflects the stored energy of a viscoelastic material during the formation process, representing the elastic characteristics of a sample (Tabilo-Munizaga and Barbosa-Cánovas 2005). The slight increase in G' of all NAM samples was observed at 40 °C, indicating the onset of gelation or the formation of an elastic protein network. The initial increase of G' could be related to interactions between protein strands (Zhang *et al*. 2013). For the control (without ECHE), the gradual increase in G' was observed but the marked increase was noticeable at temperature above 80 °C. This increase in G' was more likely related to the formation of several bondings, including hydrophobic-hydrophobic interaction, disulfide bonds (Fig 15 and 16B). When NAM was added with ECHE, a higher G' was found, compare with the control (without ECHE). The sharp increases in G' were observed at lower temperature when ECHE at higher levels was incorporated. The drastic increases were found at the temperature of 78, 68 and 65 °C for NAM samples added with 0.01, 0.02 and 0.03% ECHE, respectively. This result indicated that the higher aggregation induced by ECHE resulted in enhanced

interconnection between protein chains, which were associated with increased viscosity. At final heating (90 °C), NAM added with 0.03% ECHE exhibited the highest G', whilst the control NAM showed the lowest G'. Thus, ECHE was able to induce the network formation via protein cross-linking in a dose-dependent manner.

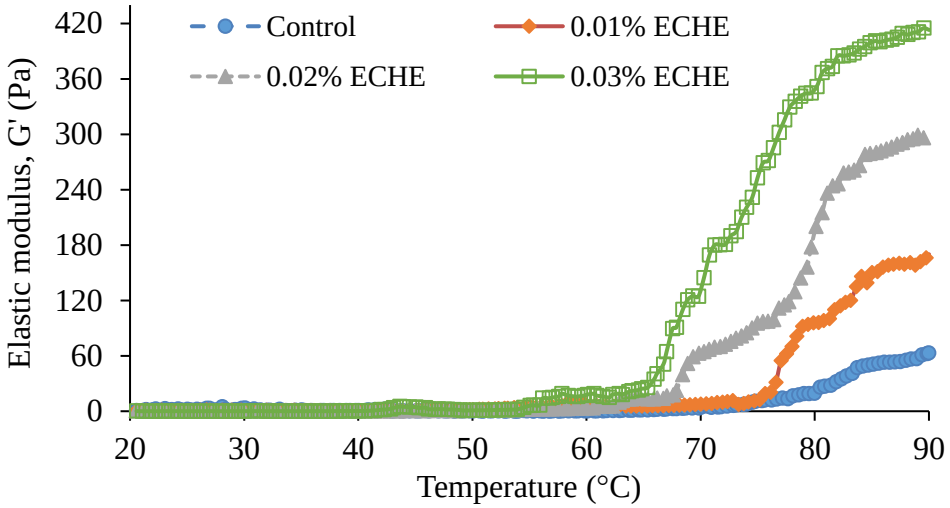


Figure 17. Elastic modulus (G') of NAM solution (20 mg/mL) added without and with ECHE at different levels during heating from 20 to 90 °C.

3.4.3.2 Zeta potential and particle size

Zeta potential (ζ) values of fresh NAM (unheated) and NAM added without and with ECHE after heating to 90 °C are shown in Table 7. The highest negative charge was found in fresh NAM, followed by NAM after heating without the extract and that incorporated with ECHE at 0.01 and 0.03%, respectively ($P<0.05$). At neutral pH, the negative charge in NAM solution was governed by acidic amino acids such as glutamic acid and aspartic acid, in which carboxyl groups were deprotonated (Binsi *et al*. 2006). When NAM was heated, the proteins underwent unfolding with subsequent aggregation as indicated by increased turbidity (Fig. 14A). Those negatively charged domains might be masked when the proteins were aggregated as shown by the lower negative charge. ECHE might induce the aggregation of the protein strands by several bondings such as hydrophobic interaction via phenol ring of phenolic compounds and by hydrogen bond from hydroxyl group of the phenolics (Partasarathi

et al. 2005; Prado *et al*. 2014). With increasing aggregation, negative charge of proteins, particularly at surface, could be decreased via protein-protein interaction.

The particle sizes of fresh NAM and NAM added without and with ECHE (0.01 and 0.03%) after heating to 90 °C are shown in Table 7. When fresh NAM was heated, the particle size of fresh NAM was increased from 729.52 to 948.93 nm. The larger particle was formed when ECHE was added into NAM solution. Heated NAM had particle size of 1,075.20 and 1,339.72 nm when ECHE at 0.01 and 0.03% was added, respectively ($P<0.05$). The increase in particle size of NAM coincided with the increase in turbidity (Fig. 14). The aggregation of the heated NAM molecules mostly determined size of particulates, and it was shown that ECHE effectively induced the aggregation of NAM. These results suggested that protein molecules underwent unfolding when sufficient heat was applied and ECHE simultaneously acted as cross-linker via those exposed reactive domains. Therefore, the aggregation was augmented by ECHE as indicated by the increase in particle size of the protein aggregate.

Table 7. Zeta potential and particle size of NAM solutions (1 mg/mL) added without and with ECHE at different levels after heating to 90 °C.

Treatment	Zeta potential (mV)	Particle size (nm)
Unheated NAM	-18.73 ± 0.60 ^{d}	729.52 ± 14.05 ^{d}
Heated NAM	-13.59 ± 0.87 ^{c}	948.93 ± 17.32 ^{c}
NAM + 0.01% ECHE	-5.82 ± 0.85 ^{b}	1,075.20 ± 19.08 ^{b}
NAM + 0.03% ECHE	-2.11 ± 0.71 ^{a}	1,339.72 ± 15.24 ^{a}

Values are mean ± SD (n=3). Different lowercase superscripts in the same column indicate significant differences ($P<0.05$).

3.4.3.3 Microstructures

Fresh NAM, the NAM solutions without and with ECHE at 0.01 and 0.03% after heating to 90 °C showed the differences in TEM microstructure as illustrated in Fig. 18. Fresh NAM showed the finer and continuous filament structure. When NAM was heated, a larger cluster with higher interconnection was observed. It was found that interconnection of protein network was not uniform in the control after heating (Fig 18B). Balange and Benjakul (2010) reported that the incubation at 40 °C

could give the sufficient energy to unfold protein strands, which allowed the extract or other substances to interact easily. When 0.01% ECHE was added, the interconnection between heated protein chains was enhanced and the larger strands were noticeable. Nevertheless, in the presence of ECHE at a level of 0.03% (Fig. 18D), the denser coagulates were formed. This indicated the effectiveness of ECHE as the protein cross-linker for NAM. Thus, the addition of ECHE could induce the aggregation of NAM, but the degree of aggregation varied, depending on the ECHE level used.

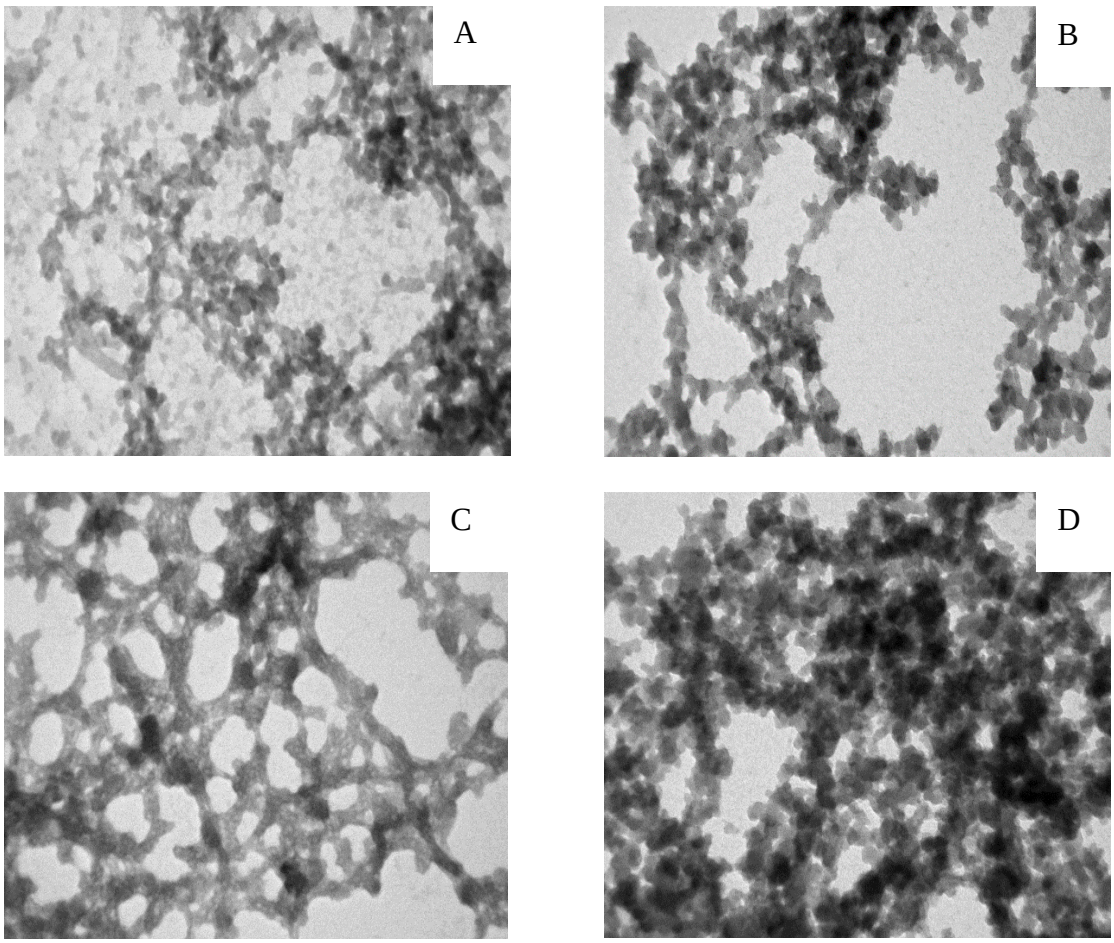


Figure 18. Transmission electron micrograph of fresh NAM solution (A) and NAM solutions added without (B) or with 0.01% (C) or 0.03% (D) of ECHE after heating at 0.88 °C/min. Magnification: 60,000×.

3.5 Conclusion

ECHE effectively induced the aggregation of NAM during heating as evidenced by the increased turbidity and larger particle size of agglomerated proteins. Protein aggregation was also enhanced via hydrophobic interactions and disulfide bond when ECHE was incorporated. Therefore, the extract from coconut husk could serve as a natural additive to foster the cross-linking of NAM, thereby strengthening gel produced from sardine muscle proteins.

3.6 References

Alañón, M. E., Castro-vázqueza, L., Díaz-marotob, M. C., Hermosín-gutiérrezb, I. and Gordon, M. H. 2011. Antioxidant capacity and phenolic composition of different woods used in cooperage. Food Chem. 129: 1584-1590.

Arfat, Y. A. and Benjakul, S. 2012. Impact of zinc salts on heat-induced aggregation of natural actomyosin from yellow stripe trevally. Food Chem. 135: 2721-2727.

Balange, A. and Benjakul, S. 2009. Enhancement of gel strength of bigeye snapper (*Priacanthus tayenus*) surimi using oxidized phenolic compounds. Food Chem. 113: 61-70.

Balange, A. K. and Benjakul, S. 2010. Cross-linking activity of oxidized tannic acid towards mackerel muscle proteins as affected by protein types and setting temperatures. Food Chem. 120: 268-277.

Benjakul, S., Seymour, T. A., Morrissey, M. T. and An, H. 1997. Physicochemical changes in Pacific whiting muscle proteins during iced storage. J. Food Sci. 62: 7729-733.

Benjakul, S. and Visessanguan, W. 2003. Transglutaminase-mediated setting in bigeye snapper surimi. Food Res. Int. 36: 253-266.

Benjakul, S., Vissessanguan, W., Ishizaki, S. and Tanaka, M. 2001. Differences in gelation characteristics of natural actomyosin from two species of bigeye

snapper, *Priacanthus tayenus* and *Priacanthus macracanthus*. J Food Sci. 66: 1311-1318.

Binsi, P. K., Shamasundar, B. A. and Dileep, A. O. 2006. Some physic-chemical, functional and rheological properties of actomyosin from green mussel (*Perna viridis*). Food Res. Int. 39: 992-1006.

Bobkova, E. A., Bobkov, A. A., Levisky, D. I. and Reisler, E. 1999. Effects of SH1 and SH2 Modifications on myosin similarities and differences. Biophys J. 76: 1001-1007.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254.

Buamard, N. and Benjakul, S. 2015. Improvement of gel properties of sardine (*Sardinella albella*) surimi using coconut husk extracts. Food Hydrocolloid. 51: 146-155.

Cetin-karaca, H. and Newman, M. C. 2015. Antimicrobial efficacy of plant phenolic compounds against *Salmonella* and *Escherichia coli*. Food Biosci. 11: 8-16.

Chaijan, M., Panpipat, W. and Benjakul, S. 2010. Physicochemical properties and gel forming ability of surimi from three species of mackerel caught in Southern Thailand. Food Chem. 121: 85-92.

Chanarat, S., Benjakul, S. and H-Kittikun, A. 2012. Comparative study on protein cross-linking and gel enhancing effect of microbial transglutaminase on surimi from different fish. J Sci. Food Agric. 92: 844-852.

Chinnici, F., Natali, N., Bellachioma, A., Versari, A. and Riponi, C. 2015. Changes in phenolic composition of red wines aged in cherry wood. LWT-Food Sci. Technol. 60: 977-984.

Ellman, G. L. 1959. Tissue sulfhydryl groups. Arch. Biochem. Biophys. 82: 70-77.

Esturk, O. and Park, J. W. 2014. Comparative study on degradation, aggregation and rheological properties of actomyosin from cold, temperate and warm water fish species. Turk. J. Fish. Aquat. Sci. 14: 67-75.

Fiske, C. H. and Subbarow, Y. 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66: 375-400.

Jan, S., Khan, M. R., Rashid, U. and Bokhari, J. 2013. Assessment of antioxidant potential, total phenolics and flavonoids of different solvent fractions of *Monotheca buxifolia* fruit. Osong Public Health Res. Perspect. 4: 246-254.

Jiang, S. Z., Hwang, D. C. and Chen, C.S. 1988. Denaturation and change in SH group of actomyosin from milkfish (*Chanos chanos*) during storage at -20 °C. J. Agric. Food Chem. 36: 433-437.

Kaewdang, O., Benjakul, S., Kaewmanee, T. and Kishimura, H. 2015. Effect of ethanolic extract of coconut husk on gel properties of gelatin from swim bladder of yellowfin tuna. LWT-Food Sci. Technol. 62: 955-961.

Koolen, H.H. F., Silva, F.M.A., Gozzo, F.C., Souza, A.Q.L. and Souza, A.D.L. 2013. Antioxidant, antimicrobial activities and characterization of phenolic compounds from buriti (*Mauritia flexuosa* L. f.) by UPLC-ESI-MS/MS. Food Res. Int. 51: 467-473.

Kudre, T., Benjakul, S. and Kishimura, H. 2013. Effects of protein isolates from black bean and mungbean on proteolysis and gel properties of surimi from sardine (*Sardinella albella*). LWT-Food Sci. Technol. 50: 511-518.

Lanier, T. C. 2000. Surimi gelation chemistry. In Surimi and Surimi Seafood (Park, J. W. ed.) p.237-265. Marcel Dekker. New York.

Mansfield, B. 2003. Fish, factory trawlers, and imitation crab: the nature of quality in the seafood industry. J. Rural Stud. 19: 9-21.

Martins, N., Barros, L., Santos-Buelga, C., Silva, S., Henriques, M. and Ferreira, I. C. F. R. 2015. Decoction, infusion and hydroalcoholic extract of cultivated thyme:

Antioxidant and antibacterial activities, and phenolic characterization. Food Chem. 167: 131-137.

Niwa, E. 1992. Chemistry of surimi gelation. In Surimi Technology (Lanier, T. C. and Lee, C. M., eds). p.389-428. Marcel Dekker. New York.

Ozdal, T., Capanoglu, E. and Altay, F. 2013. A review on protein–phenolic interactions and associated changes. Food Res. Int. 51: 954-970.

Panyakaew, S. and Fotios, S. 2011. New thermal insulation boards made from coconut husk and bagasse. Energ. Buildings. 43: 1732-1739.

Parthasarathi, R., Subramanian, V. and Sathyamurthy, N. 2005. Hydrogen bonding in phenol, water, and phenol-water clusters. J. Phys. Chem. A. 109: 843-850.

Prado, J. M., Forster-Carneiro, T., Rostagno, M. A., Follegatti-Romero, L. A., Maugeri, F. and Meireles, M. A. A. 2014. Obtaining sugars from coconut husk, defatted grape seed, and pressed palm fiber by hydrolysis with subcritical water. J. Supercrit. Fluids. 89: 89-98.

Prodpran, T., Benjakul, S. and Phatcharat, S. 2012. Effect of phenolic compounds on protein cross-linking and properties of film from fish myofibrillar protein. Int. J. Biol. Macromol. 51: 774-782.

Rawdkuen, S., Benjakul, S., Vissessanguan, W. and Lanier, T. C. 2008. Rheological and textural properties of Pacific whiting surimi gels as influenced by chicken plasma. Int. J. Food Prop. 11: 820-832.

Richards, M., Kock, H. L. D., Duodu, K. G. and Buys, E. M. 2014. The effect of legume protease inhibitors on native milk and bacterial proteases. LWT-Food Sci. Technol. 57: 628-633.

Rodrigues, S., Pinto, A. G. S. and Fernandes, F. A. N. 2008. Optimization of ultrasound extraction of phenolic compounds from coconut (*Cocos nucifera*) shell powder by response surface methodology. Ultrason. Sonochem. 15: 95-100.

Saha, J. B. T., Abia, D., Dumarçay, S., Ndikontar, M. K., Gérardin, P. and Noah, J. N. 2013. Antioxidant activities, total phenolic contents and chemical compositions

of extracts from four Cameroonian woods: Padouk (*Pterocarpus soyauxii* Taubb), tali (*Erythrophleum suaveolens*), moabi (*Baillonella toxisperma*), and movingui (*Distemonanthus benthamianus*). Ind. Crops Prod. 41: 71-77.

Steel, R. G. D. and Torrie, J. H. 1980. Principles and procedures of statistics. McGraw-Hill Book, New York.

Sueli, R. and Gustavo, A. S. P. 2007. Ultrasound extraction of phenolic compounds from coconut (*Cocos nucifera*) shell powder. J. Food Eng. 80: 869-872.

Tabilo-Munizaga, G. and Barbosa-Canovas, G. 2005. Rheology for the food industry. J. Food Eng. 42: 66-71.

Thannhauser, T. W., Konishi, Y. and Scheraga, H. A. 1987. Analysis of disulfide bonds in peptides and proteins. Method. Enzymol. 143: 155-161.

Vázquez-Torres, H., Canché-Escamilla, G. and Cruz-Ramos, C. A. 1992. Coconut husk lignin. II. Characterization by infrared and nuclear magnetic resonance spectroscopy. J. Appl. Polym. Sci. 45: 645-653.

Visessanguan, W., Benjakul, S. and Tanaka, M. 2003. Effect of microbial transglutaminase on rheological properties of oxidized and non-oxidized natural actomyosin from two species of bigeye snapper. J. Sci. Food Agric. 83: 105-112.

Wang, H., Pato, M., Pietrasik, Z. and Shand, P. 2009. Biochemical and physicochemical properties of thermally treated natural actomyosin extracted from normal and PSE pork *Longissimus* muscle. Food Chem. 113: 21-27.

Yarnpakdee, S., Benjakul, S., Visessanguan, W. and Kijroongrojana, K. 2009. Thermal properties and heat-induced aggregation of natural actomyosin extracted from goatfish (*Mulloidichtys martinicus*) muscle as influenced by iced storage. Food Hydrocolloids. 23: 1779-1784.

Yongsawatdigul, J. and Park, J. W. 2003. Thermal degradation and aggregation of threadfin bream actomyosin. Food Chem. 83: 409-416.

Zhang, F., Fang, L., Wang, C., Shi, L., Chang, T., Yang, H. and Cui, M. 2013. Effects of starches on the textural, rheological, and color properties of surimi-beef gels with microbial transglutaminase. Meat Sci. 93: 533-537.

Zhang, T., Xue, Y., Li, Z., Wang, Y. and Xue, C. 2015. Effects of deacetylation of konjac glucomannan on Alaska pollock surimi gels subjected to high-temperature (120 °C) treatment. Food Hydrocolloid. 43: 125-131.

CHAPTER 4

IMPROVEMENT OF GEL QUALITY OF SARDINE SURIMI WITH LOW SETTING PHENOMENON BY ETHANOLIC COCONUT HUSK EXTRACT

4.1 Abstract

Effects of ethanolic coconut husk extract (ECHE) at various levels (0-0.25%, based on protein content) on gel properties of low setting surimi prepared from sardine (*Sardinella albella*) were studied. Breaking force of gels containing EGTA increased as the levels of ECHE increased up to 0.15% ($P<0.05$) and decreased when ECHE was above 0.15%. The decrease in whiteness was found in gel added with ECHE. Protein patterns were not different between gels, regardless of ECHE levels. Lower autolysis of surimi gel was also found in the presence of ECHE. Addition of ECHE could increase the cross-linking of proteins during setting (40 °C, 30 min), especially with increasing levels, as indicated by the increased G'. Gel added with 0.15% ECHE had highly interconnected network with finer and denser structure than the control gel (without ECHE). Thus, an optimum level of ECHE could improve quality of sardine surimi with low setting phenomenon.

4.2 Introduction

Coconut husk, a fibrous external portion of the fruit of coconut, is generated during the fruit separation. Those husks are considered as underutilized resources (Panyakaew and Fotios, 2011). Various phenolic compounds have been found in coconut husk, namely 4-hydroxybenzoic acid, ferulic acid, tannic acid as well as lignin phenols such as vanillic acid, *ρ*-coumaric acid and syringic acid (Lobbes *et al.*, 1999; Rodrigues and Pinto, 2007). Phenolic compounds have been known to possess antioxidant (Maqsood *et al.*, 2012), antimicrobial and antiviral (Carvalho *et al.*, 2013; Hossain *et al.*, 2014) and anticancer activities (Gawlik-Dziki, *et al.*, 2012). Furthermore, phenolic compounds have been demonstrated as a protein cross-linking agent, which was able to strengthen the protein gels. (Balange and Benjakul, 2009; Temdee and Benjakul, 2014).

Surimi, water-washed fish flesh, has gained popularity as a raw material for preparing several products with elastic texture (Mansfield, 2003). Thermal aggregation of myosin is an important process for formation of elastic gels and largely affects consumer preference and acceptance of surimi-based products (Sakamoto *et al.*, 1988). Surimi gels from some fish species undergo thermal degradation by endogenous proteolytic enzymes, resulting in the weakening of surimi gel. Heat activated proteases have been reported to hydrolyse muscle proteins of Pacific whiting and arrowtooth flounder at the temperature of 55-65 °C (Seymour *et al*., 1994; Visessanguan *et al.*, 2003). Dark-fleshed fish, such as sardine and mackerel, also contain high level of proteases, which show an adverse effect on gel formation (Hu *et al.*, 2010). Apart from those proteases, some fish species have low content of endogenous transglutaminase (TGase) or calcium ion such as goatfish (Benajukl *et al.*, 2010), king weakfish (Kuhn *et al*., 2004) and hairtail (Hu *et al*., 2015). Those low setting surimi generally have the poor quality due to the low protein cross-linking mediated by endogenous TGase.

TGase is an enzyme catalysing acyl-transfer reaction, resulting in the formation of ε-(γ-glutamyl)lysine cross-links in muscle proteins. It contributes to the setting phenomenon during incubation of surimi at 4-40 °C (Benjakul and Visessanguan, 2003). Yongsawatdigul *et al.* (2002) reported that ethyleneglycol tetraacetic acid (EGTA) inhibited endogenous TGase activity via chelating Ca^{2+}, a divalent cation required for the activation of TGase. Addition of EGTA at a concentration of 10 mM into surimi from walleye pollack suppressed the gel formation as indicated by the decrease in breaking force and the retained MHC band (Banlue *et al.*, 2010). The use of potential protein cross-linker, especially from plant origin with safety could be a potential means to enhance the cross-linking of low setting surimi with negligible level of endogenous TGase. Recently, ethanolic coconut husk extract (ECHE) has been reported to strengthen the gel from sardine surimi prepared by two-steps heating (Buamard and Benjakul, 2015). Nevertheless, no information regarding the use of ECHE in the low setting surimi (surimi with poor setting ability) has been reported. Therefore, this study aimed to investigate the effect of ECHE at different levels on the properties of surimi gel from sardine added with EGTA to inactivate the endogenous TGase, mimicking the low setting surimi.

4.3 Materials and methods

4.3.1 Chemicals

All chemicals were of analytical grade. Sodium dodecyl sulphate (SDS) and *β*-mercaptoethanol (*β*-ME) were obtained from Sigma (St. Louis, MO, USA). *N,N,N′,N′*-tetramethyl ethylene diamine (TEMED), acrylamide and bisacrylamide were purchased from Fluka (Buchs, Switzerland). Trichloroacetic acid, Folin–Ciocalteu's phenol reagent, acetic acid and tris (hydroxylmethyl) aminomethane were purchased from Merck (Darmstadt, Germany). EGTA (*O,O'*-Bis(2-aminoethyl)ethyleneglycol-*N,N,N′,N′*-tetraacetic acid) was procured from Dojindo (Kumamoto, Japan).

4.3.2 Preparation of coconut husk

Husk of coconut (*Cocos nucifera* Linn.) fruit with the age of about 11 months was collected from the local market in Hat Yai, Songkhla Province, Thailand and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai, Thailand. Husk sample was prepared as per the method of Vázquez-Torres *et al*. (1992) with slight modifications. The obtained powder (80 mesh) was placed in a polyethylene bag, sealed and kept at room temperature until use.

4.3.3 Preparation of ethanolic coconut husk extract

Coconut husk powder was subjected to extraction according to the method of Buamard and Benjakul (2015). The husk powder (10 g) was mixed with 350 mL of 60% ethanol. The extraction was performed at room temperature (25-28 °C) for 3 h by stirring the mixture continuously at the low speed using a magnetic stirrer (IKA-Werke, Staufen, Germany). Thereafter, the mixture was centrifuged at 5,000 ×g for 30 min at room temperature using a RC-5B plus centrifuge (Beckman, JE-AVANTI, Fullerton, CA, USA) and the supernatant was then filtered through a Whatman filter paper No.1 (Whatman International Ltd., Maidstone, UK). The filtrate was evaporated at 40 °C using an Eyela rotary evaporator (Tokyo Rikakikai, Co. Ltd., Tokyo, Japan) and purged with nitrogen gas to remove the residual ethanol. Ethanolic coconut husk extract (ECHE) was subsequently dried using a Scanvac Model Coolsafe 55 freeze dryer (Coolsafe, Lynge, Denmark) to obtain the dry extract. Dried extract was powdered using a mortar and pestle. The ECHE powder was transferred into amber

bottle and stored in a desiccator until use. ECHE powder had phenolic content of 432.28 mg tannic acid equivalent/g as determined by Folin-Ciocalteu reagent (Slinkard and Singleton, 1997).

4.3.4 Preparation of surimi gel added with ECHE

Frozen surimi, AA grade, from sardine (*S. albella*) was purchased from Pacific Fish Processing Co., Ltd. (Songkhla, Thailand) and kept at -20 °C until use, but not longer than two months. Surimi was tempered in running water (25-28 °C) until the core temperature reached 0-2 °C. The surimi was chopped into small pieces and mixed with 2.5% salt in a mixer (National Model MK-5080M, Selangor, Malaysia) at a speed of 2,200 rpm for 1 min. During chopping, the temperature was maintained below 10 °C. EGTA (0.57%) was used to chelate native calcium ion in surimi so that endogenous TGase activity is suppressed. Prior to addition, either EGTA or ECHE were dissolved in cold distilled water and pHs of solutions were adjusted to 7. ECHE was added into surimi paste to obtain different levels (0.05, 0.10, 0.15, 0.20 and 0.25% of protein content). The moisture content of surimi paste was adjusted to 80% with cold distilled water. Subsequently, the mixture was chopped for 30 s, followed by 10 s of a rest interval for a total time of 3 min to avoid heat generated. The paste was stuffed into a polyvinylidene chloride casing with a diameter of 2.5 cm. Both ends were sealed tightly and subjected to the incubation at 40 °C for 30 min, followed by heating at 90 °C for 20 min. Thereafter, all gels were cooled in iced water for 30 min and stored at 4 °C for 18-24 h prior to analyses. For the positive control gel, it was prepared as previously described, except EGTA and ECHE was excluded.

4.3.5 Analyses

4.3.5.1 Breaking force and deformation

Breaking force and deformation of surimi gels were determined using a texture analyzer (Model TA-XT2, Stable MicroSystems, Surrey, UK) as described by Benjakul *et al.* (2007). Five cylindrical samples (2.5 cm in height) were prepared and equilibrated at room temperature (25-28 °C) for 1 h before analyses. A spherical plunger (diameter 5 mm) was pressed into the cut surface of a gel sample perpendicularly at a constant depression speed (60 mm/min). The force to puncture into the gel (breaking

force) and the distance at which the plunger punctured into the gel (deformation) were both recorded.

4.3.5.2 Texture profile

Gel samples were subjected to texture profile analysis (TPA) following the method of Kaewudom *et al*. (2013) with a slight modification. A texture analyzer (Model TA-XT2, Stable MicroSystems, Surrey, UK) with a cylinder probe (diameter 35 mm) was used for determination of hardness, springiness, cohesiveness, gumminess and chewiness.

4.3.5.3 Expressible moisture content

Expressible moisture content of surimi gel was measured according to the method of Benjakul *et al*. (2007). Cylindrical gel samples were cut into a thickness of 5 mm, weighed accurately (X) and placed between three pieces of Whatman filter paper No.1 (Whatman International Ltd., Maidstone, UK) at the bottom and two pieces on the top of the sample. A standard weight of 5 kg was placed on the top of the sample for 2 min. The sample was then removed from the papers and weighed again (Y). Expressible moisture content was calculated with the following equation and expressed as percentage of sample weight:

$$\text{Expressible moisture (\%)} = [(X\text{-}Y)/X] \times 100$$

4.3.5.4 Whiteness

Whiteness of gel samples was determined using a colorimeter (HunterLab, Colorflex, Hunter Associates Laboratory, VA, USA). CIE L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness) values were measured and whiteness was then calculated using the following equation (Park, 1994):

$$\text{Whiteness} = 100\text{-}[(100\text{-}L^*)^2 + a^{*2} + b^{*2}]^{1/2}$$

4.3.5.5 TCA-soluble peptide content

TCA-soluble peptide content was determined as per the method of Morrissey *et al.* (1993). Gel sample (3 g) was homogenized with 27 mL of cold 5%

TCA at a speed of 11,000 rpm for 2 min using a homogenizer (IKA Labortechnik, Selangor, Malaysia). The homogenate was allowed to store in ice for 1 h and centrifuged at 8,000 ×g for 10 min. TCA-soluble peptide content in the supernatant was determined according to the Lowry method (Lowry *et al.*, 1951) and expressed as µmole tyrosine equivalent/g sample.

4.3.5.6 SDS-polyacrylamide gel electrophoresis

Protein patterns of surimi gels were analyzed by SDS-PAGE under the reducing condition according to the method of Laemmli (1970). To the finely chopped gel samples (3 g), 27 mL of heated SDS solution (85C) were added. The mixture was then homogenized at a speed of 11,000 rpm for 2 min. The homogenate was incubated at 85C for 1 h to dissolve total proteins. The mixtures were centrifuged at 3,500 ×g for 20 min to remove undissolved matters. Protein concentration of the supernatant was determined by the Biuret method (Robinson and Hogden, 1940) using bovine serum albumin as standard. SDS-PAGE gel consisted of 10% running gel and 4% stacking gel. After separation, the proteins were stained with 0.02% (w/v) Coomassie Brilliant Blue R-250 in 50% (v/v) methanol and 7.5% (v/v) acetic acid and destained with 50% methanol and 7.5% (v/v) acetic acid, followed by 5% methanol (v/v) and 7.5% (v/v) acetic acid.

4.3.5.7 Microstructures

The selected gel samples including the positive control gel (without EGTA and ECHE), the control gel (with EGTA), gel added with EGTA in combination with ECHE at levels of 0.15 and 0.25% were examined for microstructure using a scanning electron microscope (SEM). The samples were cut into cubic shape with a thickness of 2-3 mm. The gels were fixed with 2.5% (v/v) glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 3 h at room temperature. The samples were rinsed with distilled water. Fixed specimens were dehydrated in ethanol with serial concentrations of 25, 50, 70, 80, 90 and 100%. Samples were critical point dried using CO_2 as transition fluid. The prepared samples were mounted on a bronze stub, sputter-coated with gold and visualized using SEM (Quanta 400, FEI, Eindhoven, the Netherlands).

4.3.6 Dynamic rheological study of low setting surimi paste as affected by ECHE incorporation during setting

Surimi pastes containing EGTA (0.57%) and ECHE at different levels (0-0.25%) were prepared as previously described and were subjected to dynamic rheological measurement following the method of Rawdkuen *et al*. (2008) with a slight modification. A rheometer (HAAKE RheoStress1, ThermoFisher Scientific, Karlsruhe, Germany) with 35 mm, 4^{o} slope cone and plate geometry was used for monitoring the changes in storage or elastic modulus (G'). An oscillation of 1 Hz with 1% deformation was used for testing. This condition yielded a linear response in the viscoelastic region. The temperature sweep was recorded during heating up from 20 to 40 °C with heating rate 1 °C /min and the temperature was held at 40 °C for 30 min. G' was recorded during setting at 40 °C for 30 min. To minimize water evaporation of the samples during measurement, silicon oil was applied to cover the pastes.

4.3.7 Statistical analysis

Experiments were carried out using three different lots of samples. Data were subjected to analysis of variance (ANOVA). Comparison of means was performed by the Duncan's multiple range test (Steel and Torrie, 1980). Statistical analysis was conducted using SPSS for Windows (SPSS Inc., Chicago, IL, USA). Data with $P < 0.05$ were considered to be statistically significant.

4.4 Results and discussion

4.4.1 Effect of ECHE on gel properties of low setting surimi

4.4.1.1 Breaking Force and deformation

Breaking force of surimi gel from sardine in the presence of EGTA (0.57%) was markedly decreased, compared with that of the positive control (without EGTA and ECHE). On the other hand, deformation of gel containing EGTA was higher than that of the positive control ($P<0.05$). The result indicated that EGTA at 0.57% effectively inhibited endogenous TGase via Ca^{2+} chelation. Endogenous TGase has been known to require Ca^{2+} for activation. It plays a role in introducing non-disulfide covalent bond (ε-(γ-glutamyl)lysine linkage) in surimi gel with high setting phenomenon (Benjakul *et al.,* 2004). Those bonds could strengthen the gel matrix as

indicated by the high breaking force as found in the positive control gel. It was noted that EGTA addition resulted in the higher deformation of gel (P<0.05). Weak bonds such as H-bond, hydrophobic interaction and other bonds more likely contributed to the less rigid gel network as evidenced by higher deformation or elasticity. In the present study, EGTA was employed to inactivate endogenous TGase, in which low setting surimi could be prepared. When ECHE at various levels was added in surimi gel containing EGTA, breaking force increased when ECHE was added up to 0.15%. (P<0.05) as well. However, the sharp decrease in breaking force was observed when ECHE at levels above 0.15% was incorporated (P<0.05). It was postulated that the excessive cross-linking caused by the high amount of ECHE might induce the protein coagulation, in which the ordered and fine network of surimi could not be formed. Moreover, the self-aggregation of the component in ECHE, particularly phenolic compounds, might occur when the higher level was introduced. As a result, lower cross-linking activity was obtained. In this study, phenolics in the reduced form were used as protein cross-linkers. Interaction between hydroxyl group of polyphenol and hydrogen acceptor in protein molecules by hydrogen bonds, and also protein-polyphenol hydrophobic interactions likely contributed to the increased gel strength (Rattaya *et al*., 2009). However, the addition of ECHE caused the decrease in deformation of surimi gel in dose-dependent manner (P<0.05). When proteins underwent cross-linking at higher extent, the gel became more rigid as indicated by lower deformation. The results suggested that the use of ECHE at the optimum level (0.15%) was able to enhance gel strength of low setting surimi, whose endogenous TGase was not involved in protein cross-linking.

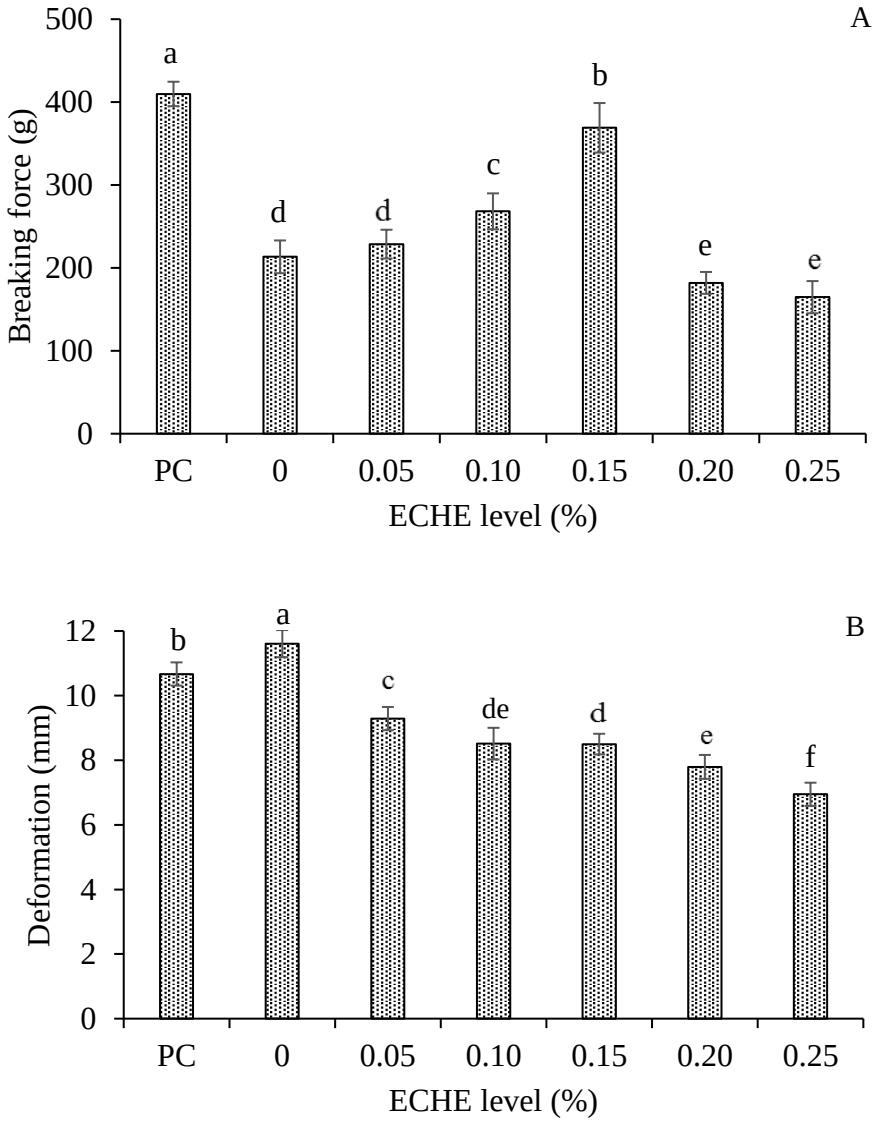


Figure 19. Breaking force (A) and deformation (B) of low setting sardine surimi gel as affected by ECHE at different levels. PC: positive control (without EGTA and ECHE). Bars represent the standard deviation (n=3). Different letters on the bars indicate significant differences (P<0.05).

4.4.1.2 Texture profile analysis

Hardness, springiness, cohesiveness, gumminess and chewiness of low setting surimi gels were lower than those of positive control gel (without EGTA and ECHE) as shown in Table 8. When ECHE was added into gel containing EGTA, hardness, representing the force required to compress sample to attain a given

deformation, of resulting gels was increased when level of ECHE increased up to 0.15% (P<0.05). With further increasing level of ECHE (0.20-0.25%), all aforementioned parameters had the decreases in values. This result was in agreement with cohesiveness, capability to breakdown the internal structure, gumminess, the energy used for a semi-solid food for swallowing, and chewiness, the energy used for chewing the sample to the point that able to swallow it (P<0.05). Nevertheless, the decrease in springiness, elastic recovery that occurs when the compressive force is removed, was noticeable when ECHE at all levels was added (P<0.05). The result suggested that ECHE addition resulted in the lower springiness. This was coincidental with the decreased deformation of gel added with ECHE (Fig. 19). The addition of 0.15% ECHE in surimi gel containing EGTA rendered the highest hardness. This was in accordance with the highest breaking force (Fig. 19). Therefore, the addition of ECHE could improve the texture property of low setting surimi gel by inducing the interaction between protein molecules.

Table 8. Textural profiles of low setting sardine surimi gel as affected by ECHE at different levels.

ECHE Level (%)	Hardness (N)	Springiness (cm)	Cohesiveness (ratio)	Gumminess (N)	Chewiness (N cm)
PC	160.47±11.80^{a}	0.94±0.02^{b}	0.84 ± 0.05^{a}	139.59±8.08^{a}	127.27±7.00^{a}
0	98.39±2.31^{d}	0.98±0.01^{a}	0.71±0.02^{c}	67.20±2.01^{d}	60.14±1.46^{d}
0.05	106.70±1.91^{c}	0.86±0.02^{d}	0.72±0.01^{c}	73.35±7.09^{cd}	68.60±0.44^{c}
0.10	108.35±2.70^{c}	0.86±0.01^{d}	0.72±0.01^{c}	77.57±2.62^{c}	69.03±2.49^{c}
0.15	138.20±2.94^{b}	0.88±0.03^{d}	0.79±0.04^{b}	106.62±2.66^{b}	96.56±2.01^{b}
0.20	95.75±4.36^{d}	0.90±0.01^{c}	0.73±0.02^{c}	69.91±1.91^{d}	61.96±1.67^{d}
0.25	85.70±1.20^{e}	0.89±0.01^{c}	0.69±0.01^{c}	59.62±1.91^{e}	52.24±1.43^{e}

Values are mean ± SD (n=3). Different superscripts in the same column denote the significant differences (P<0.05). PC: Positive control gel (without EGTA and ECHE).

4.4.1.3 Expressible moisture content

Expressible moisture content of low setting gel from sardine surimi was higher than that of positive control gel as shown in Table 9. Without the action of endogenous TGase, the gel network showed the lower ability to imbibe water as evidenced by the higher expressible moisture content. Surimi gels containing EGTA (low setting surimi gel) showed the continuous decreases in expressible moisture content when the levels of ECHE increased up to 0.15% ($P<0.05$). However, the increase in expressible moisture content was observed when ECHE at 0.20 and 0.25% was incorporated ($P<0.05$). It was noted that expressible moisture content of low setting gel added with 0.15% ECHE was not different from that of positive control gel ($P>0.05$). As the gel was added with the appropriate level of ECHE, the cross-linking of proteins could be promoted by the phenolics in ECHE via hydrogen bond and hydrophobic interaction, leading to the formation of ordered and stronger network with increasing water holding capacity (Balange and Benjakul, 2009). The excessive coagulation or precipitation of proteins could lead to lower water holding capacity when high levels of ECHE or phenolic was added into surimi (Balange and Benjakul, 2009; Arfat and Benjakul, 2013). Thus, ECHE at a proper level also improved water holding capacity of low setting surimi gel.

4.4.1.4 Whiteness

There was no difference in whiteness between the positive control gel and gel containing EGTA ($P>0.05$) (Table. 9). Low setting surimi gel (with EGTA) showed a decrease in whiteness when ECHE levels increased ($P<0.05$). The decreases in whiteness of surimi gel were due to the presence of phenolic compounds and tannin in ECHE. Addition of oxidized phenolic compounds resulted in the decrease in whiteness of gel of bigeye snapper surimi (Balange and Benjakul, 2009) and fish emulsion sausage (Maqsood *et al*., 2012). Plant phenolic compounds generally have dark color. As a consequence, the uses of those phenolics might cause the darkening of the final products. Although sardine surimi had a high content of dark muscle and myoglobin (Kudre *et al.,* 2013), the addition of ECHE higher than 0.05% led to the lower whiteness of low setting surimi gel ($P<0.05$). Therefore, the addition of ECHE slightly resulted in whiteness lowering of surimi gel from sardine.

Table 9. Expressible moisture content, whiteness and TCA-soluble peptide content of low setting sardine surimi as affected by ECHE at different levels.

ECHE Level (%)	Expressible moisture content (%)	Whiteness	TCA-soluble peptide content (µmole tyrosine equivalent/g)
PC	4.40±0.28^{e}	68.87±0.56^{a}	4.12±0.03^{d}
0	5.82±0.22^{a}	68.28±0.60^{ab}	5.19±0.02^{a}
0.05	5.34±0.35^{b}	67.77±0.56^{bc}	4.55±0.04^{b}
0.10	4.80±0.29^{d}	67.18±0.56^{cd}	4.39±0.02^{c}
0.15	4.38±0.26^{e}	66.86±0.75^{d}	4.15±0.05^{d}
0.20	4.89±0.18^{d}	64.95±0.42^{e}	3.96±0.04^{e}
0.25	5.26±0.17^{c}	62.79±0.43^{f}	3.81±0.04^{f}

Values are mean ± SD (n=3). Different superscripts in the same column denote the significant differences (P<0.05). PC: Positive control gel (without EGTA and ECHE).

4.4.1.5 TCA-Soluble peptide content

TCA-soluble peptide content of low setting surimi gel added with different levels of ECHE is presented in Table. 9. TCA-soluble peptide content indicates proteolytic degradation occurring during setting and gelation. Amongst all low setting surimi gels, that without ECHE showed the highest TCA-soluble peptide content (P<0.05). The result suggested that the degradation mediated by endogenous proteases in surimi gels was impeded in the presence of ECHE. Kudre *et al*. (2013) reported that proteolysis in surimi from sardine could be enhanced at 40 °C and reached the maximum at 65 °C. When myofibrillar proteins were cross-linked, the cleavage sites for proteases were less available, leading to more resistance to hydrolysis. Furthermore, the phenolics might interact directly with proteases, resulting in the loss in their activity. Surimi gel from bigeye snapper incorporated with phenolic compounds extracted from kiam wood had the decreased protein solubility associated with the enhanced protein cross-linking (Balange and Benjakul, 2011). When comparing with the positive control gel, the gel containing EGTA (without ECHE) had the higher TCA-soluble peptide content (P<0.05). Cross-linking mediated by endogenous TGase in the former might provide the polymerized proteins, which were more resistant to

proteolysis induced by proteases in surimi. As a result, the lower degradation took place in the positive control gel. Therefore, protein degradation in low setting surimi gels from sardine could be lowered by the addition of ECHE, especially at high levels.

4.4.1.6 Protein patterns

Protein patterns of positive control gel and low setting surimi gel without and with the addition of ECHE at various levels in comparison with surimi paste are depicted in Fig. 20. Myosin heavy chain (MHC) and actin are the major proteins in surimi paste. In the positive control gel, MHC completely disappeared, but actin was still retained. The disappearance of MHC was mostly mediated by endogenous transglutaminase, suggesting the formation of polymerized proteins (Kaewudom *et al.*, 2013). When EGTA at 0.57% was present, MHC of gels was more remained. The result indicated that EGTA more likely chelated Ca^{2+} in the surimi, causing the inactivation of endogenous TGase. As a consequence, the cross-linking of protein via non-disulfide covalent bonds was prevented. Seki *et al.* (1998) reported that the addition of EGTA, a Ca^{2+}chelator, resulted in the suppression of endogenous TGase in walleye pollack surimi. When considering the protein pattern of surimi gel containing EGTA in the presence of ECHE at various levels, similar patterns were noticeable. Although the addition of ECHE affected the textured properties, no differences in protein patterns were found. The result suggested that most of bondings stabilizing the network by phenolics in ECHE were weak bonds such as H-bond, hydrophobic-hydrophobic interaction, etc. Under electrophoretic condition, those bondings were destroyed as evidenced by the similar proteins band. Additionally, as endogenous TGase was mostly inhibited, non-disulfide covalent bonds could not be formed. In general, no marked changes in actin were observed, suggesting that actin was not polymerized by endogenous TGase or phenolics from ECHE. Actin was also reported to be resistant to proteolysis (Balange and Benjakul, 2009). Thus, protein cross-linking mediated by phenolic in ECHE, mainly via weak bonds, primarily contributed to the increased breaking force of surimi gels in the absence of endogenous TGase.

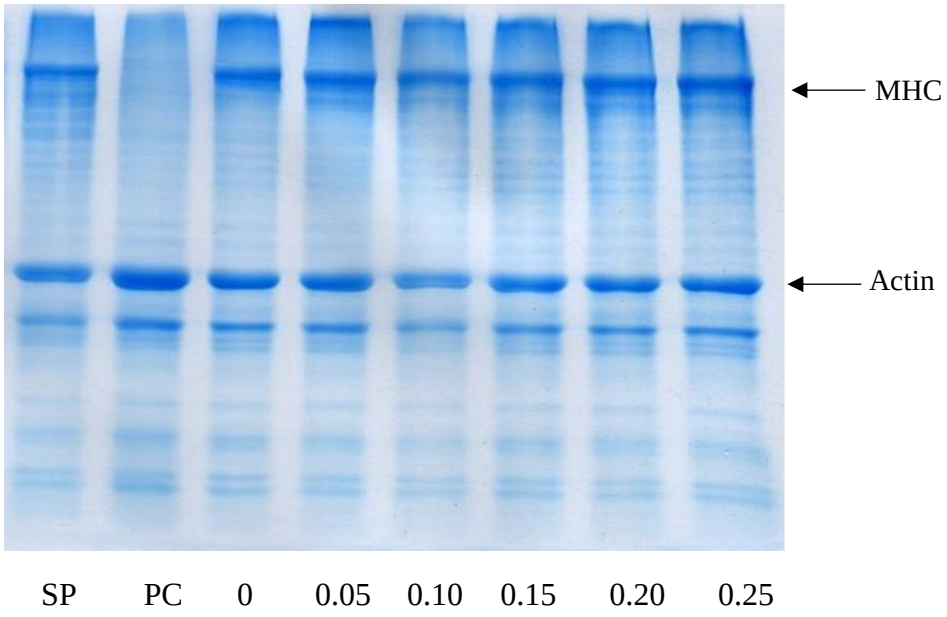


Figure 20. Protein patterns of low setting sardine surimi gel as affected by ECHE at different levels. MHC, myosin heavy chain; SP, surimi paste; PC, positive control (without EGTA and ECHE). Numbers denote the levels of ECHE (%).

4.4.1.7 Microstructures

Microstructures of the positive control (without EGTA and ECHE), gel containing EGTA without and with ECHE at 0.15 and 0.25% are illustrated in Fig. 21. The positive control gel had a fine network with the smaller void or cavities. Surimi gel network became coarser and larger voids with the addition of EGTA, as compared with the positive control gel. Nevertheless, the network of gel containing EGTA became finer and denser when 0.15% ECHE was added. The results suggested that phenolic compounds might induce the cross-linking of myofibrillar proteins effectively (Shitole *et al.*, 2014; Buamard and Benjakul, 2015). Fine and ordered surimi gel network more likely imbibed water as evidenced by the lowest expressible moisture content (Table 9). In the presence of ECHE at a level of 0.25%, the coagulated or precipitated proteins were formed within the gel network. This disconnected network with larger cavity showed the lower gel strength with poor water holding capacity. Therefore, the addition of ECHE at an appropriate level could yield the gel with low setting phenomenon,

however the excessive level resulted in the formation of coagulation associated with poor gel property.

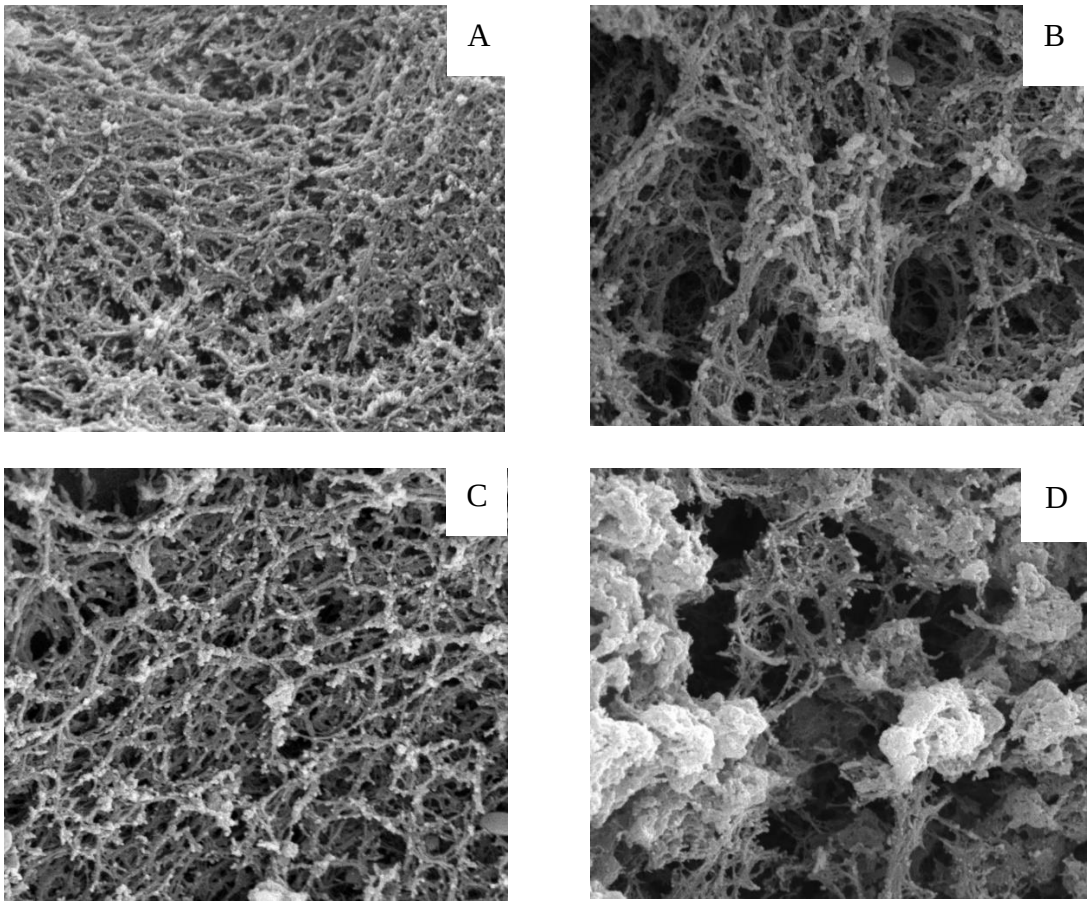


Figure 21. Electron microscopic images of low setting surimi gel added with ECHE at different levels (magnification: 10,000×): (A) positive control gel (without EGTA and ECHE); (B) surimi gel added with EGTA; (C) surimi gel added with EGTA and 0.15% ECHE and (D) surimi gel added with EGTA and 0.25% ECHE.

4.4.2 Impact of ECHE on dynamic rheological properties of low setting surimi paste during setting

Elastic modulus (G') of sardine surimi paste containing EGTA in the absence and presence of ECHE at various levels during setting at 40 °C is shown in Fig. 22. The positive control paste (without EGTA and ECHE) showed the higher G'

than the paste added with EGTA. G', the stored energy of a viscoelastic material during the formation process, increased continuously during the setting in all samples. This indicated the formation of protein network via several bonds between protein molecules (Zhang *et al.*, 2013). When the samples were held at 40 °C, the highest G' was observed in the positive control paste. G' of paste added with EGTA increased when ECHE was incorporated in a dose-dependent manner. The results indicated that the addition of ECHE in surimi paste without TGase activity could enhance G' during setting period. At 40 °C, proteins were partially unfolded and the formation of protein network occurred. Unfolded proteins might favor the interaction or aggregation via reactive groups or domains. Hydrophobic domains plausibly underwent interaction via hydrophobic-hydrophobic interaction (Benjakul *et al.*, 2005). For surimi containing endogenous TGase, gel network formation is more pronounced in the presence or addition of Ca^{2+}, resulting in the stronger gel (Yin and Park, 2014). It was noted that the higher G' was noticeable when ECHE was added, indicating the enhanced protein cross-linking by phenolics in the ECHE. During setting at 40 °C, the denatured or unfolded muscle proteins were more polymerized by those phenolics in ECHE, especially at high levels. As a result, gel network was more strengthened. However, the paste added with 0.25% ECHE having the highest G' did not show the highest breaking force (Fig. 19). The enhanced entanglement or cross-linking of protein as indicated by high G' was not related well with the ordered alignment of proteins in gel network. The result reconfirmed that excessive cross-linking or interaction of proteins induced by ECHE led to the weaker gel with poorer water holding capacity.

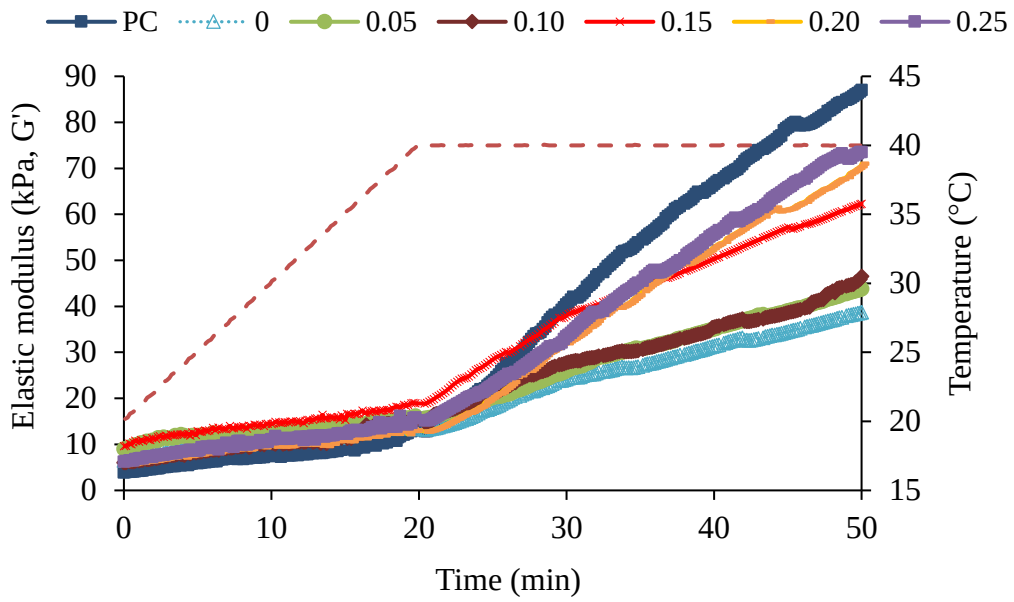


Figure 22. Elastic modulus (G´) of low setting sardine surimi paste as affected by ECHE at different levels. Numbers denote the levels of ECHE (%).

4.5 Conclusion

Addition of ECHE at an appropriate level could strengthen the surimi gel from sardine with low setting phenomenon by increasing breaking force, hardness, gumminess, chewiness and water holding capacity. However, the addition of ECHE resulted in the decreases in deformation and whiteness, particularly when increasing levels were used. Therefore, the extract from coconut husk could be used as a natural additive to improve the gel properties of surimi with low setting phenomenon.

4.6 References

Arfat, Y. A. and Benjakul, S. 2013. Gel strengthening effect of zinc salts in surimi from yellow stripe trevally. Food Biosci. 3: 1-9.

Balange, A. and Benjakul, S. 2009. Enhancement of gel strength of bigeye snapper (*Priacanthus tayenus*) surimi using oxidized phenolic compounds. Food Chem. 113: 61-70.

Balange, A. and Benjakul, S. 2011. Effect of kiam wood extract as influenced by pH during oxygenation on mackerel surimi gel. J. Food Biochem. 35: 574-595.

Banlue, K., Morioka, K. and Itoh, Y. 2010. Effect of inorganic oxidizing reagents on gel-forming properties of walleye pollack surimi through low temperature setting. J. Biol. Sci. 10: 18-24.

Benjakul, S. and Visessanguan, W. 2003. Transglutaminase-mediated setting in bigeye snapper surimi. Food Res. Int. 36: 253–266.

Benjakul, S., Visessanguan, W. and Pecharat, S. 2004. Suwari gel properties as affected by transglutaminase activator and inhibitors. Food Chem. 85: 91-99.

Benjakul, S., Visessanguan, V. and Srivilai, C. 2007. Gel properties of bigeye snapper (*Priacanthus tayenus*) surimi as affected by setting and plasma proteins. J. Food Qual. 24: 453-471.

Benjakul, S., Visessanguan, V., Thongkaew, C. and Tanaka, M. 2005. Effect of frozen storage on chemical and gel-forming properties of fish commonly used for surimi production in Thailand. Food Hydrocolloid. 19: 197-207.

Benjakul, S., Yarnpakdee, S., Vissessanguan, W. and Phatcharat, S. 2010. Combination effects of whey protein concentrate and calcium chloride on properties of goatfish surimi gel. J. Texture Stud. 41: 341-357.

Buamard, N. and Benjakul, S. 2015. Improvement of gel properties of sardine (*Sardinella albella*) surimi using coconut husk extracts. Food Hydrocolloid. 51: 146-155.

Carvalho, O. V., Botelho, C. V., Ferreira, C. G. T., Ferreira, H. C. C., Santos, M. R., Diaz, M. A. N., Oliveira, T. T., Soares-Martins, J. A. P., Almeida, M. R. and Júnior, A. S. 2013. *In vitro* inhibition of canine distemper virus by flavonoids and phenolic acids: Implications of structural differences for antiviral design. Res. Vet. Sci. 95: 717-724.

Gawlik-Dziki, U., Jeżyna, M., Świeca, M., Dziki, D., Baraniak, B. and Czyż, J. 2012. Effect of bioaccessibility of phenolic compounds on *in vitro* anticancer activity of broccoli sprouts. Food Res. Int. 49: 469-476.

Hossain, M. A., Kalbani, M. S. A. A., Farsi, S. A. J. A., Weli, A. M. and Al-Riyami, Q. 2014. Comparative study of total phenolics, flavonoids contents and evaluation of antioxidant and antimicrobial activities of different polarities fruits crude extracts of *Datura metel* L. Asian Pac. J. Trop. Dis. 4: 378-383.

Hu, Y., Liu, W., Yuan, C., Morioka, K., Chen, S., Liu, D. and Ye, X. 2015. Enhancement of the gelation properties of hairtail (*Trichiurus haumela*) muscle protein with curdlan and transglutaminase. Food Chem. 176: 115-122.

Hu, Y., Morioka, K. and Itoh, Y. 2010. Participation of cysteine protease cathepsin L in the gel disintegration of red bulleye (*Priacanthus macracanthus*) surimi gel paste. J. Sci. Food Agric. 90: 370-375.

Kaewudom, P., Benjakul, S. and Kijroongrojana, K. 2013. Properties of surimi gel as influenced by fish gelatin and microbial transglutaminase. Food Biosci. 1: 39-47.

Kudre, T., Benjakul, S. and Kishimura, H. 2013. Effect of protein isolates from black bean and mungbean on proteolysis and gel properties of surimi from sardine (*Sardinella albella*). LWT-Food Sci. Technol. 50: 511-518.

Kuhn, C. R., Prentice-Hernández, C., Vendruscolo, J. L. and SOARES, G. J. D. 2004. Surimi of king weakfish (*Macrodon ancylodon*) wastes: Texture gel evaluation with protease inhibitors and transglutaminase. Braz. Arch. Biol. Technol. 47: 895-901.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of head of bacteriophage T4. Nature. 227: 680-685.

Lobbes, J. M., Fitznar, H. P. and Kattner, G. 1999. High-performance liquid chromatography of lignin-derived phenols in environmental samples with diode array detection. Anal. Chem. 71: 3008-3012.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. 193: 256-275.

Mansfield, B. 2003. Fish, factory trawlers, and imitation crab: the nature of quality in the seafood industry. J. Rural Stud. 19: 9-21.

Maqsood, S., Benjakul, S. and Balange, A. K. 2012. Effect of tannic acid and kiam wood extract on lipid oxidation and textural properties of fish emulsion sausages during refrigerated storage. Food Chem. 130: 408-416.

Rattaya, S., Benjakul, S. and Prodpran, T. 2009. Properties of fish skin gelatin film incorporated with seaweed extract. J. Food Eng. 95: 151-157.

Rawdkuen, S., Benjakul, S., Vissessanguan, W. and Lanier, T. C. 2008. Rheological and textural properties of Pacific whiting surimi gels as influenced by chicken plasma. Int. J. Food Prop. 11: 820-832.

Rodrigues, S. and Pinto, G. A. S. 2007. Ultrasound extraction of phenolic compounds from coconut (*Cocos nucifera*) shell powder. J. Food Eng. 80: 869-872.

Sakamoto, H., Kumazawa, Y., Toiguchi, S., Seguro, K., Soeda, T. and Motoki, M. 1988. Gel strength enhancement by addition of microbial transglutaminase. J. Food Sci. 53: 924-928.

Seki, N., Nozawa, H. and Ni, S. 1998. Effect of transglutaminase on the gelation of heat-denatured surimi. Fish. Sci. 64: 959-963.

Seymour, T. A., Morissey, M. T. Peters, M. Y. and An, H. 1994. Purification and characterization of Pacific whiting proteases. J. Agric. Food Chem. 42: 2421-2427.

Shitole, S. S., Balange, A. K. and Gangan, S. S. 2014. Use of seaweed (*Sargassum tenerrimum*) extract as a gel enhancer for lesser sardine (*Sardinella brachiosoma*) surimi. Int. Aquat. Res. doi: s40071-014-0055-9.

Slinkard, K. and Singleton, V. L. 1997. Total phenol analyses: Automation and comparison with manual methods. Am. J. Enol. Vitic. 28: 49-55.

Steel, R. G. D. and Torrie, J. H. 1980. Principles and procedures of statistics. McGraw-Hill Book, New York.

Temdee, W. and Benjakul, S. 2014. Effect of oxidized kiam wood and cashew bark extracts on gel properties of gelatin from cuttlefish skins. Food Biosci. 7: 95-104.

Vázquez-Torres, H., Canché-Escamilla, G. and Cruz-Ramos, C. A. 1992. Coconut husk lignin. II. Characterization by infrared and nuclear magnetic resonance spectroscopy. J. Appl. Polym. Sci. 45: 645-653.

Visessanguan, W., Benjakul, S. and An, H. 2003. Purification and characterization of cathepsin L in arrowtooth flounder (*Atheresthes stomias*) muscle. Comp. Biochem. Physiol. B. Biochem. Mol. Biol. 134: 477-487.

Yin, T. and PARK, J. W. 2014. Effects of nano-scaled fish bone on the gelation properties of Alaska pollock surimi. Food Chem. 150: 463-468.

Yongsawatdigul, J., Wooratao, A. and Park, J. W. 2002. Effect of endogenous transglutaminase on threadfin bream surimi gelation. J. Food Sci. 67: 3258-3263.

Zhang, F., Fang, L., Wang, C., Shi, L., Chang, T., Yang, H. and Cui, M. 2013. Effects of starches on the textural, rheological, and color properties of surimi-beef gels with microbial transglutaminase. Meat Sci. 93: 533-537.

CHAPTER 5

COMBINATION EFFECT OF HIGH PRESSURE TREATMENT AND ETHANOLIC EXTRACT FROM COCONUT HUSK ON GEL PROPERTIES OF SARDINE SURIMI

5.1 Abstract

Effects of ethanolic coconut husk extract (ECHE) at various levels (0–0.10%, based on protein) on gel properties of sardine (*Sardinella albella*) surimi under different gelling conditions including pressurization at 300 MPa, 30 min (HP); and pressurization, followed by heating (90 °C, 20 min) (HP/H) were investigated. At the same level of ECHE, HP/H gel had the higher breaking force ($P<0.05$) than HP counterpart. The increases in breaking force and water holding capacity were observed as the levels of ECHE were increased up to 0.075% for all gels, regardless of gelling processes used ($P<0.05$). With the addition of 0.075% ECHE, breaking force of HP and HP/H were 435 and 577 g, respectively. Lower autolysis of surimi gel was also found in HP/H gel in the presence of ECHE. With addition of ECHE at a concentration of 0.075%, HP/H gel had a network with higher connectivity than HP gel and traditional two-step heated gel. Pressurization prior to heating could thus improve quality of sardine surimi gel when ECHE (0.075%) was incorporated, in which the breaking force was increased by 69%, compared with that of traditional control gel (without ECHE).

5.2 Introduction

Coconut husk is a fibrous external portion of the fruit of coconut and is considered as underutilized natural resource (Panyakaew and Fotios, 2011). Various phenolic compounds have been found in coconut husk, namely 4-hydroxybenzoic acid, ferulic acid, tannic acid, catechin. Lignin precursor such as vanillic acid, *ρ*-coumaric acid and syringic acid were also identified (Viju *et al.*, 2013). Phenolic compounds have been known to possess antioxidant (Maqsood *et al.*, 2014), antimicrobial and anticancer activities (Lima *et al.* 2015). Furthermore, phenolic compounds have been demonstrated as protein cross-linking agents, which were able to strengthen the protein gels, especially surimi (Temdee and Benjakul, 2014).

Recently, ethanolic coconut husk extract (ECHE) has been reported to induce cross-linking of myofibrils from sardine. It was able to strengthen the gel from sardine surimi prepared by two-step heating (Buamard and Benjakul 2017a, Buamard *et al*., 2017). The addition of ECHE up to 0.125% protein had no impact on taste liking score of sardine surimi gel (Buamard and Benjakul, 2015).

Gel-forming properties of myofibrillar proteins are essential for the development muscle-based product from surimi (Sun and Holley, 2011). Gelling property of surimi depends on several factors including species and freshness of raw materials, additives used, etc. Dark fleshed fish such as sardine and mackerel, which contain high fat and endogenous proteases, yield surimi with poor gel properties (Sriket, 2014). Technologies used for enhancement of gelation, especially for surimi with poor gelling property, have been developed. High pressure processing has been known as a non-thermal processing technology to preserve food products. It has also been employed to modify or improve the functional properties of proteins, particularly gelation (Sun and Holley, 2011).

High pressure has been used to induce gelation of surimi, in which gel with high elasticity was obtained (Angsupanich *et al.*, 1999). At the initially pressurizing stage, the hydrophobic interactions of native protein structure are disrupted because of the volume decrease (Balny and Masson, 1993). During pressurization, disulfide bonds are formed via decreasing the distances between sulfhydryl groups (Cheftel and Culioli, 1997). When the pressure is released, proteins unfold and hydrogen bonds and hydrophobic interactions are subsequently formed (Grigera and McCarthy, 2010). Therefore, pressure-induced fish protein gelation is mainly constructed by disulfide bonds, hydrogen bonds and hydrophobic interactions (Sun and Holley, 2011). Formation of hydrophobic interactions induced by high pressure treatment plays an important role in surimi gel with reduced sodium chloride (Cando *et al.*, 2015). Blue whiting surimi gel had the highest breaking force when its paste was subjected to pressurization at 375 MPa for 20 min (Pérez-Mateos *et al.*, 1997). Recently, Liang, Guo *et al.* (2016) reported that bighead carp gels treated at pressures above 300 MPa for 30 min exhibited higher gel strength, compared to traditional two-step heated gels. High pressurization was able to unfold the muscle proteins in surimi paste. The

exposed reactive groups on domains of proteins could undergo cross-linking by phenolics incorporated. As a consequence, the gel strength of surimi could be enhanced. However, no information regarding the use of phenolic extract in conjunction with pressurization for preparation of surimi gel exists. Therefore, this study aimed to investigate the effect of ECHE at different levels on the properties of surimi gel from sardine induced by pressurization without and with subsequent heating in comparison with typical (two-step) heating.

5.3 Materials and methods

5.3.1 Chemicals

All chemicals were of analytical grade. Sodium dodecyl sulfate (SDS) and β-mercaptoethanol (β-ME) were obtained from Sigma Aldrich, Inc (St. Louis, MO, USA). *N,N,N′,N′*-tetramethyl ethylene diamine (TEMED), acrylamide and bisacrylamide were purchased from Fluka (Buchs, Switzerland). Ethanol, trichloroacetic acid and acetic acid were procured from Merck (Darmstadt, Germany). Sodium chloride and urea were obtained from RCI Labscan (Bangkok, Thailand).

5.3.2 Preparation of coconut husk

Husk of coconut (*Cocos nucifera* Linn.) fruit with the age of about 11 months was collected from a local market in Hat Yai, Songkhla Province, Thailand and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai, Thailand. Husk sample was prepared as per the method of Buamard and Benjakul (2015). Husk was manually defibered and ground using a mill (IKA Labortechnik colloid mill, Selangor, Malaysia). The prepared sample was sieved using a stainless-steel screen tray with a sieve size of 0.18 mm. The obtained powder was placed in a polyethylene bag, sealed and kept at room temperature until use.

5.3.3 Preparation of ethanolic coconut husk extract

Coconut husk powder (10 g) was subjected to extraction using 350 mL of 60% ethanol according to the method of Buamard and Benjakul (2015). The extraction was performed at room temperature (28–30 °C) for 3 h by continuous stirring, followed by filtration through a Whatman filter paper No.1 (Whatman

International Ltd., Maidstone, UK). The filtrates were evaporated at 40 °C using an Eyela rotary evaporator (Tokyo Rikakikai, Co. Ltd., Tokyo, Japan) and purged with nitrogen gas to remove the residual ethanol. The extract was then dried using a Scanvac Model Coolsafe 55 freeze dryer (Coolsafe, Lynge, Denmark) to obtain the dry extract. Dried extract was powdered using a mortar and pestle. The powder named 'ECHE' was transferred into an amber bottle and stored in a desiccator until use.

5.3.4 Preparation of surimi gel added with ECHE using different gelation processes

Frozen surimi, AA grade, from sardine (*S. albella*) was purchased from Man A Frozen Food Co., Ltd. (Songkhla, Thailand) and kept at −20 °C until use, but not longer than one month. Surimi was tempered in running water (25–28 °C) until the core temperature reached 0-2 °C. The surimi was chopped into small pieces. Subsequently, 2.5% of salt was added into the prepared surimi. The mixture was blended using a mixer (National Model MK-5080M, Selangor, Malaysia) at a speed of 2,200 rpm for 1 min. During the blending, the temperature was maintained below 10 °C.

ECHE was firstly dissolved in cold distilled water and pHs of solution was adjusted to 7. ECHE solution was added into surimi paste to obtain different levels (0.025, 0.05, 0.075 and 0.10%, based on protein content). The moisture content of surimi paste was adjusted to 80% with cold distilled water. Subsequently, the mixture was chopped for 30 s, followed by 10 s of a rest interval for a total time of 3 min to avoid heat generated. The paste was stuffed into a polyvinylidene chloride casing with a diameter of 2.5 cm. Both ends were sealed tightly and subjected to different gelation processes.

To prepare high pressure treated gel, casings containing surimi paste were firstly heat sealed in a high-pressured polyethylene bag. The packed samples were transferred into the working chamber of a high-pressure rig (S-FL-850-9-W model, Stansted Fluid Power Ltd, Essex, UK) containing low-compressibility fluid (1 mL castor oil/4 mL ethanol). The surimi pastes containing ECHE at various levels were pressurized at 300 MPa for 30 min. From the preliminary study, pressurization

at 300 MPa for 30 min yielded sardine surimi gel with the highest breaking force (data not shown). The temperature in the pressurization chamber was below 30 °C. The core temperature of surimi gels was approximately 20 °C at the end of pressurization. The resulting gels were termed ‘HP’. After being pressurized, another portion of resulting gels was subsequently heated at 90 °C for 20 min in a temperature-controlled water bath (Memmert, D-91126, Schwabach, Germany). Gels were then cooled in iced water for 30 min. The gels obtained were referred to as ‘HP/H’.

For gel prepared by conventional method, paste stuffed in casing was set at 40 °C for 30 min, followed by heating at 90 °C for 20 min. Gels were cooled in iced water for 30 min. Gel was termed ‘S/H’. S/H gel added with ECHE at a level of 0.075% was also prepared. This level of ECHE yielded S/H gel with the highest breaking force (Buamard and Benjakul, 2015).

All gels including HP, HP/H and S/H gels were stored at 4 °C for 24 h prior to analyses.

5.3.5 Analyses

5.3.5.1 Breaking force and deformation

Breaking force and deformation of surimi gels were determined using a texture analyzer (Model TA-XT2, Stable MicroSystems, Godalming, Surrey, UK) equipped with a spherical plunger (diameter 5 mm) as described by Benjakul *et al.* (2007). The force to puncture into the gel (breaking force) and the distance, at which the plunger punctured into the gel (deformation), were both recorded.

5.3.5.2 Texture profile analysis

Gel samples were subjected to texture profile analysis (TPA) following the method of Kaewudom *et al.* (2013) using a texture analyzer (Model TA-XT2, Stable MicroSystems, Godalming, Surrey, UK) with a slight modification. A cylinder probe (diameter 35 mm) was used for determination of hardness, springiness, cohesiveness, gumminess and chewiness.

5.3.5.3 Expressible moisture contents

Expressible moisture content of surimi gel was measured according to the method of Benjakul *et al.* (2007). Cylindrical gel samples (a thickness of 5 mm and weight of X g) were placed between three pieces of Whatman filter paper No.1 at the bottom and two pieces on the top. After the standard weight (5 kg) was placed on the top of the sample for 2 min, the weight of sample (Y) was measured. Expressible moisture content was calculated as follows:

$$\text{Expressible moisture (\%)} = [(X\text{-}Y)/X] \times 100$$

5.3.5.4 Whiteness

Whiteness of gel samples was determined using a colourimeter (HunterLab, Colorflex, Hunter Associates Laboratory, Reston, VA, USA). CIE L^*, a^* and b^* values were measured and whiteness was then calculated using the following equation:

$$\text{Whiteness} = 100\text{-}[(100\text{-}L^*)^2 + a^{*2} + b^{*2}]^{1/2}$$

where L^* is the lightness; a^* is the redness/greenness; and b^* is the yellowness/blueness.

5.3.5.5 Trichloroacetic acid (TCA)-soluble peptide contents

TCA-soluble peptide content was determined according to the method of Benjakul *et al.* (2007). Gel sample (3 g) was homogenized with 27 mL of 5% cold TCA at a speed of 11,000 rpm for 2 min using a homogenizer (IKA Labortechnik, Selangor, Malaysia). The homogenate was allowed to store in ice for 1 h and centrifuged at 8,000 ×g for 10 min. TCA-soluble peptide content in the supernatant was measured according to the Lowry method (Lowry, Rosebrough, Farr, and Randall, 1951) and expressed as μmol tyrosine equivalent/g sample.

5.3.6 Characterization of surimi gel added with ECHE at the selected concentration prepared by different gelation processes

Gels including HP, HP/H and S/H added without and with ECHE at a concentration of 0.075% were prepared and subjected to analyses.

5.3.6.1 Solubility

Solubility of protein in surimi gels was determined as described by Pérez-Mateos *et al.* (1997). Various solvents including S1 (0.6 M KCl), S2 (20 mM Tris–HCl, pH 8.0), S3 (20 mM Tris– HCl, pH 8.0 containing 1% SDS), S4 (20 mM Tris–HCl, pH 8.0 containing 1% SDS and 8 M urea) and S5 (20 mM Tris–HCl, pH 8.0 containing 1% SDS, 2% β-mercaptoethanol and 8 M urea) were used for analysis. Solubility of protein in surimi samples was expressed as the percentage of total protein in surimi gels solubilized directly in 0.5 M NaOH.

5.3.6.2 Protein patterns

Protein patterns of surimi gels were analyzed by SDS-Polyacrylamide gel electrophoresis under the reducing condition according to the method of Laemmli (1970) as modified by Buamard and Benajkul (2015). Protein (15 µg) was loaded on gel consisting of 4% stacking gel and 10% running gel. After separating, proteins were stained and destained.

5.3.6.3 Microstructure

The gel samples were examined for microstructure using a scanning electron microscope (Quanta 400, FEI, Eindhoven, the Netherlands) according to the method of Buamard and Benjakul (2015). The samples with a thickness of 2–3 mm were fixed with 2.5% glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 3 h at room temperature. The samples were rinsed with distilled water. Fixed specimens were dehydrated in ethanol with serial concentrations of 25, 50, 70, 80, 90 and 100%. Samples were critical point dried using CO_2 as transition fluid. The prepared samples were mounted on a bronze stub and sputter-coated with gold. The specimens were visualized using SEM (Quanta 400, FEI, Eindhoven, the Netherlands).

5.3.7 Statistical analysis

Analyses were conducted in triplicate. Data were subjected to analysis of variance (ANOVA). Comparison of means was performed by the Duncan's multiple range test (Steel and Torrie, 1980). Significance of differences was defined at

$P \leq 0.05$. Statistical analysis was carried out using SPSS package version 11.0 for Windows (SPSS Inc., Chicago, IL, USA).

5.4. Results and discussion

5.4.1 Effect of ECHE at different levels on properties of high pressure treated surimi gels

5.4.1.1 Breaking force and deformation

Breaking force and deformation of surimi gels with and without the addition of ECHE at various levels obtained from different gelation processes are shown in Table 10. Generally, HP gels had the lowest breaking force, compared to S/H and HP/H samples ($P<0.05$). When pressure was progressively introduced to surimi paste, electrostatic and hydrophobic interactions were readily broken. Hydrogen bonds might be ruptured completely when pressurization was still continued (Pérez-Mateos *et al.,* 1997). Nevertheless, hydrogen bonds and other reversible interactions such as van de Waals attractions and hydrophobic interactions were dominantly formed when pressure was released (Angsupanich *et al.,* 1999, Cao *et al.,* 2012). Thus, this contributed to the less rigid gel network as evidenced by lower breaking force but higher deformation or elasticity, compared to gels prepared by two-step heating or pressurization in combination with heating. For gel prepared by conventional method using two-step heating, endogenous transglutaminase might play a role in formation of isopeptides, ε-γ-glutamyl lysine (Zhu *et al.,* 2014). The oxidation of SH groups of proteins during heating also led to the formation of disulfide bonds (Balange and Benjakul, 2009). Among all gels, HP/H sample showed the highest breaking force ($P<0.05$). Proteins were more likely unfolded due to the applied pressure. This might expose the reactive groups for TGase mediated cross-linking. Furthermore, the interaction between those reactive domains could take place during subsequent heating. As a result, the highest breaking force was obtained with coincidental decrease in deformation.

When ECHE was added, breaking force of all gels increased when ECHE levels increased up to 0.075% ($P<0.05$), irrespective of gelation processes. Condensed tannin (492.2 mg catechin equivalent/g dry extract) were the main

phenolics in ECHE. Tannic acid and catechin were detected as the major free phenolics in ECHE (0.21 and 0.10 mg/g dry extract, respectively) (Buamard and Benjakul, 2017b). The interaction between hydroxyl group of those polyphenols and hydrogen acceptor in protein molecules via hydrogen bond, and protein-polyphenol hydrophobic interactions more likely occurred (Buamard and Benjakul, 2015). It was noted that HP/H gels containing ECHE, especially at 0.075%, had the highest breaking force ($P<0.05$). Unfolding of proteins induced by high pressure might favor the exposure of lysine and glutamine, which contributed to the formation of ε-(γ-glutamyl)-lysine linkage. As a result, the gel strength could be increased (Zhu *et al*., 2014). During heating, phenolic compounds in ECHE might convert to quinone or its derivatives due to the autoxidation to some extent. These substances could react with a nucleophilic domain in proteins to form a covalent bond (Rohn *et al.,* 2004). Also, those unfolded proteins could be cross-linked by phenolics more effectively. HP gels had higher deformation, compared to HP/H gels at all ECHE concentrations used ($P<0.05$). It was noted that ECHE concentrations had no effect on deformation, when the same gelation process was used. Since weak bonds induced by addition of phenolics were dominants, the deformation was not much affected by the addition of ECHE (Buamard and Benjakul, 2015). It was noted that breaking force of HP and HP/H gels decreased as ECHE at high level (0.1%) was added. This might be due to the excessive aggregation of proteins, leading to the formation of coagulum type gel with lower gel strength. Furthermore, the phenolics might interact with endogenous TGase, thus resulting in the loss in activity of TGase. Buamard and Benjakul (2015) also reported that ECHE at level above 0.125% yielded the two-step heated surimi gel with the decreased breaking force. The result suggested that pressurization, followed by heating, could strengthen surimi gel from sardine, especially when added with 0.075% ECHE.

Table 10. Breaking force, deformation, expressible moisture content, whiteness and TCA-soluble peptide contents of pressurized surimi gel without and with subsequent heating in the absence or presence of ECHE at different levels.

Treatment	ECHE levels (%)	Breaking force (g)	Deformation (mm)	Expressible moisture content (%)	Whiteness	TCA-soluble peptide contents (μmole tyrosine equivalent/g sample)
HP	0	304.23±11.34^{g}	10.64±0.71^{ab}	11.61±0.84^{a}	60.37±0.61^{d}	7.69±0.51^{a}
	0.025	376.15±10.96^{e}	11.38±0.67^{a}	11.05±0.79^{a}	60.01±0.69^{d}	7.34±0.44^{a}
	0.050	391.08±18.77^{d}	11.96±0.76^{a}	10.92±0.91^{ab}	57.57±0.88^{e}	6.98±0.39^{b}
	0.075	435.41±11.54^{c}	11.84±0.88^{a}	10.34±0.84^{b}	54.59±0.74^{f}	6.35±0.42^{b}
	0.100	306.44±17.12^{g}	12.51±0.94^{a}	10.65±0.56^{ab}	51.59±0.94^{g}	6.21±0.31^{bc}
HP/H	0	402.47±11.34^{cd}	7.33±0.69^{c}	10.61±0.46^{ab}	69.37±0.80^{a}	6.08±0.34^{c}
	0.025	431.44±20.21^{c}	8.46±0.71^{bc}	9.54±0.39^{b}	66.79±0.71^{b}	5.94±0.42^{c}
	0.050	472.85±19.31^{b}	9.31±0.78^{b}	8.12±0.51^{c}	66.75±0.94^{b}	5.36±0.39^{d}
	0.075	577.34±17.66^{a}	8.61±0.68^{bc}	7.12±0.44^{d}	63.68±0.93^{c}	5.01±0.44^{d}
	0.100	434.17±16.48^{c}	9.01±0.81^{b}	8.64±0.50^{c}	59.90±0.85^{d}	4.87±0.38^{de}
S/H	0	341.78±19.78^{f}	11.62±0.97^{a}	9.33±0.78^{b}	69.94±0.79^{a}	5.58±0.32^{cd}
	0.075	451.39±14.44^{b}	7.36±0.65^{c}	8.19±0.35^{a}	65.98±0.97^{b}	5.17±0.48^{a}

Value are mean ± SD (n=3). Different superscripts in the same column indicate the significant differences (P<0.05). S/H: two-steps heating gel (40°C 30 min, followed by 90 °C 20 min), HP: High pressure treated gel (300 MPa for 30 min), HP/H: High pressure treated gel (300 MPa for 30 min), followed by heating (90 °C for 20 min).

5.4.1.2 Texture profiles

In the absence of ECHE, HP/H and S/H gels showed the higher hardness than HP gel ($P<0.05$) (Table 11). However, HP gel had the highest springiness ($P<0.05$). For cohesiveness, HP/H gel showed the highest value ($P<0.05$). No differences in gumminess and chewiness were found between HP/H gels and S/H gels ($P>0.05$). When pressurization was employed, weak bonds were dominant. This contributed to high elasticity as indicated by high springiness, elastic recovery that occurs when the compressive force is removed. These results were coincidental with the lower breaking force of HP gel, compared to S/H and HP/H gels (Table 10). For S/H gel, setting phenomenon, mediated by endogenous TGase, play a role in strengthening the gel via the formation of ε-(γ-glutamyl)-lysine linkage (Zhu *et al.*, 2014). For HP/H gel, high pressure applied more likely unfolded proteins and favored the interaction between protein chains via several bondings.

Regardless of gelation processes, the values of all aforementioned parameters, except springiness, were increased as ECHE was added up to 0.075% ($P<0.05$). Cohesiveness is capability to breakdown the internal structure. Gumminess represents the energy used for a semi-solid food for swallowing. Chewiness represents the energy used for chewing the sample to the point that is able to swallow it ($P<0.05$). The result reconfirmed that heating after pressurization could strengthen the gel matrix, particularly in the presence of ECHE at 0.075%. HP/H containing ECHE at a concentration of 0.075% had the higher hardness, cohesiveness, gumminess and chewiness than S/H added with ECHE at the same level ($P<0.05$). Therefore, the addition of ECHE affected textural property of pressurized surimi gel, in which the interaction between protein molecules and phenolics of unfolded proteins could be enhanced.

Table 11. Textural properties of pressurized surimi gel without and with subsequent heating in the absence and presence of ECHE at different levels.

Treatment	ECHE levels (%)	Hardness (N)	Springiness (cm)	Cohesiveness	Gumminess (N)	Chewiness (N.cm)
HP	0	66.49±4.99^{f}	0.98±0.01^{a}	0.74±0.04^{b}	52.64±1.87^{f}	60.23±3.66^{e}
	0.025	67.53±3.34^{f}	0.98±0.01^{a}	0.72±0.02^{c}	53.45±2.21^{f}	63.83±2.94^{e}
	0.050	70.00±4.77^{e}	0.98±0.01^{a}	0.73±0.04^{b}	67.88±1.68^{de}	65.10±3.01^{de}
	0.075	79.84±5.34^{d}	0.97±0.02^{a}	0.76±0.05^{b}	69.55±2.38^{d}	69.35±3.14^{d}
	0.100	73.66±5.70e	0.97±0.01^{a}	0.77±0.04^{b}	68.67±3.14^{de}	65.00±2.99^{de}
HP/H	0	99.13±6.94^{c}	0.93±0.02^{b}	0.89±0.06^{a}	70.72±2.26^{d}	80.96±3.09^{c}
	0.025	100.31±5.38^{c}	0.92±0.01^{b}	0.88±0.03^{a}	80.64±2.39^{c}	85.04±1.79^{b}
	0.050	106.44±5.31^{b}	0.94±0.02^{b}	0.87±0.03^{a}	83.34±1.97^{b}	83.24±2.07^{b}
	0.075	140.59±6.21^{a}	0.94±0.01^{b}	0.87±0.02^{a}	91.70±3.62^{a}	99.36±3.64^{a}
	0.100	108.11±5.46^{b}	0.93±0.01^{b}	0.86±0.04^{a}	83.96±1.14^{b}	84.76±3.82^{b}
S/H	0	98.34±5.88^{c}	0.94±0.03^{b}	0.78±0.03^{b}	73.39±1.63^{d}	81.42±2.58^{c}
	0.075	106.86±4.03^{b}	0.95±0.01^{b}	0.79±0.02^{b}	77.65±2.98^{b}	86.10±2.88^{b}

Value are mean ± SD (n=3). Different superscripts in the same column indicate the significant differences (P<0.05). S/H: two-steps heating gel (40°C 30 min, followed by 90 °C 20 min), HP: High pressure treated gel (300 MPa for 30 min), HP/H: High pressure treated gel (300 MPa for 30 min), followed by heating (90 °C for 20 min).

5.4.1.3 Expressible moisture content

Expressible moisture contents of HP gels from sardine surimi were higher than that of S/H gels as shown in Table 10. However, there was no difference in expressible moisture content between HP and HP/H gels (P>0.05). Without heat treatment, the gel network showed the lower ability to imbibe water as indicated by the higher expressible moisture content. Gelation induced by high pressure treatment is attributed to a decrease in the volume of the protein molecules. This leads to the rearrangement of water molecules around amino acid residues in pressure-induced gels, which are glossy, compared to opaque gels obtained by heat treatment (Ngarize,

et al., 2005). When ECHE was incorporated up to 0.075%, HP/H gels showed the continuous decreases in expressible moisture content. The result indicated higher water binding capacity of gel. However, the increase in expressible moisture content was noticed when ECHE at 0.1% was added ($P<0.05$). The decrease in expressible moisture content was also found in HP gel added with 0.075% ECHE ($P<0.05$). The presence of phenolics in ECHE could promote the cross-linking of proteins via hydrogen bond and hydrophobic interaction. This led to the formation of stronger network, which was able to imbibe more water (Buamard *et al.,* 2017). Moreover, subsequent heat treatment after pressurization could induce the formation of ordered gel with high ability in holding water. For HP gel without heating, the gel network could not form in the fashion, which entrapped water effectively. Increased water holding capacity was also observed in S/H gel added with 0.075% ECHE ($P<0.05$) as indicated by the lower expressible moisture content. Thus, ECHE at a proper level was able to improve water holding capacity of high pressure treated surimi gel, particularly HP/H gel.

5.4.1.4 Whiteness

Among all gels, HP/H and S/H gels showed the higher whiteness than HP gel ($P<0.05$). The result indicated that heat treatment resulted in the increased whiteness. This was more likely due to heat coagulation of proteins, leading to light scattering. Hwang *et al.* (2007) reported that whiteness of fish gels was higher when treated with two-step heating, as compared to gels induced only by high pressure. Tabilo-Munizaga and Barbosa-Cánovas (2004) suggested that the increase in whiteness was attributable to heating. This was mainly associated with protein denaturation of surimi gels. The decreases in whiteness of surimi gels were observed as the concentrations of ECHE were increased, regardless of pressure or heat applied (Table 10). ECHE added in surimi had a reddish-brown color. As a consequence, the gels thus became darker when ECHE was incorporated as indicated by decreased whiteness. With addition of 0.075% ECHE, the higher whiteness was found in S/H gel, compared to others ($P<0.05$). Thus, ECHE addition had the impact on whiteness of surimi gels to some degree.

5.4.1.5 TCA-soluble peptide content

HP gels showed the higher TCA-soluble peptide content, compared to HP/H and S/H gels ($P<0.05$) (Table 10). TCA-soluble peptide content indicates proteolytic degradation occurring in gels. This was probably caused by greater accessibility of the unfolded proteins acting as substrate as induced by high pressure. Pressurization brought about structural change of protein and disruption of lysosomal membranes. As a result, proteases could be enhanced and increased proteolysis (Hernández-Andrés *et al.,* 2008). However, the myofibrillar proteins were cross-linked in S/H and HP/H gels due to the formation of disulfide bond and some covalent bonds during heating. As a consequence, the cleavage sites for proteases were less available. This resulted in more resistance to hydrolysis (Zhu *et al.*, 2014).

TCA-soluble peptide content of HP and HP/H gels decreased when the concentration of ECHE increased ($P<0.05$). These results suggested that ECHE could retard muscle protein degradation in a dose-dependent manner. Cross-linking induced by phenolics in ECHE might lead to the formation of large aggregate, which was resistant to proteolysis. Cleavage sites were hindered and hydrolysis became less. The phenolics might also interact directly with proteases. As a result, the enzymes lost their activity. Resistance to proteolysis became more pronounced when further heat was applied. Balange and Benjakul (2009) also reported that surimi gel from bigeye snapper incorporated with phenolic compounds extracted from kiam wood had the decreased protein solubility associated with the enhanced protein cross-linking. The lower proteolysis was also observed in S/H gel containing 0.075% ECHE, compared to the control (without ECHE) ($P<0.05$). Therefore, protein degradation in high pressure treated gels from sardine surimi could be impeded by the addition of ECHE, especially at high concentrations.

5.4.2 Characteristics of high pressure treated surimi gel added with ECHE at the selected level

5.4.2.1 Protein patterns

Protein patterns of HP and HP/H gels in comparison with S/H gel in the absence and presence of 0.075% ECHE are illustrated in Fig. 23. Myosin heavy chain (MHC) and actin are the major proteins in surimi paste. The lower band intensity of MHC was observed when surimi paste was subjected to two-step heating (S/H), pressurization (HP) or pressurization, followed by heating (HP/H). Among all gels, HP/H gel had higher cross-linked proteins, compared to other gels. With addition of 0.075% ECHE, the formation of cross-linked protein was obvious in HP gel. Nevertheless, no differences in protein patterns were observed in HP/H gel without and with ECHE. The oxidized phenolics could induce the formation of non-disulfide covalent bonds between proteins (Balange and Benjakul, 2009). Liang *et al*. (2016) reported that MHC of bighead carp decreased with increasing pressure above 300 MPa. During sardine paste was pressurized, the protein molecules were unfolded and ECHE could cross-link those proteins via hydrogen bond and hydrophobic interaction. However, some phenolics might undergo oxidation and the resulting quinones could induce protein cross-linking in high pressure treated gel (HP) via non-disulfide covalent bond. This was indicated by the formation of cross-linked proteins under reducing condition. Nevertheless, no marked changes in actin were observed in all gels, regardless of gelation processes or addition of ECHE.

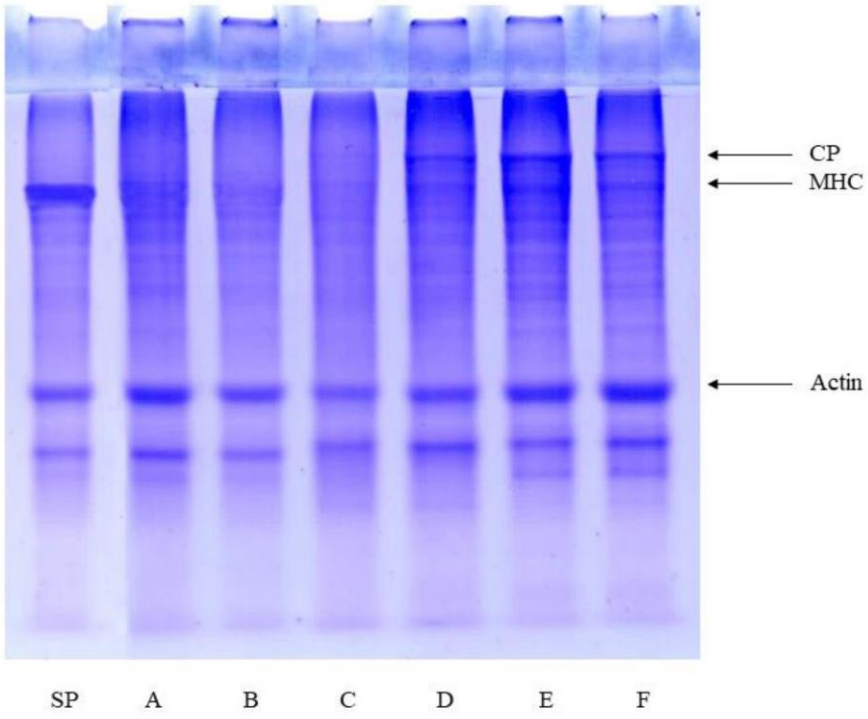


Figure 23. SDS-PAGE patterns of gels from sardine surimi prepared by different gelation processes without and with 0.075% ECHE. SP: surimi paste; (A): two-step heated gel (40 °C 30 min, followed by 90 °C 20 min); (B): two-step heated gel added with 0.075% ECHE; (C): high pressure treated gel (300 MPa for 30 min); (D): high pressure treated gel added with 0.075% ECHE; (E): high pressure treated gel (300 MPa for 30 min), followed by heating (90 °C for 20 min) and (F): high pressure treated gel (300 MPa for 30 min), followed by heating (90 °C for 20 min) added with 0.075% ECHE; MHC, myosin heavy chain and CP: cross-linked protein.

5.4.2.2 Solubility

Solubility of high pressure treated surimi gels without (HP) and with subsequent heating (HP/H), in the absence and presence of 0.075% ECHE in different solubilizing agents is shown in Table 12. Solubility was found to be lower than 20% in all gels with and without ECHE prepared by all gelation processes when solubilized with 0.6 M KCl (S1) and 20 MM Tris-HCl (pH 8.0) (S2). The decrease in solubility was caused by the formation of protein networks during pressurizing and/or

heating. When the gels were solubilized in 20 mM Tris-HCl (pH 8.0) containing 1% SDS (S3), solubility was increased to 49% for S/H gel and 47% for HP gel. For HP/H gel, the lowest solubility was found (39%). Hydrogen bond and some hydrophobic interaction are ruptured by SDS (Liang *et al.*, 2016). The solubility was further increased in S4 containing SDS and urea, indicating the presence of hydrogen bonds and hydrophobic interaction in surimi gels. HP/H gel showed the lowest solubility in S4, compared to gels with other gelation processes. HP/H gel added with 0.075% ECHE showed the lowest solubility among all samples ($P<0.05$). This suggested that hydrophobic interaction was more involved when phenolics in ECHE were incorporated during pressurization and heating. When the samples were solubilized in S5, containing SDS, urea and β-ME, further increases in solubility were observed. The result indicated the presence of disulfide bonds in the gel. During heating, proteins further underwent aggregation via disulfide bonds. It was noted that the largest increase in solubility of HP/H gel was found when β-ME was added in solubilizing agent, compared to other samples. This indicated that high pressurization in combination with heating could induce the formation of disulfide bond to the highest extent. Unfolded proteins induced by high pressure could undergo oxidation of sulfhydryl groups more effectively, especially during subsequent heating. The decrease in solubility of HP/H gel, especially in the presence of ECHE was related with the highest breaking force (Table 10) and hardness (Table 11). EHCE might also be oxidized to quinone and then attached to protein molecules. Altered protein molecules might be oxidized and disulfide bond formation was enhanced. Among all gels, HP/H gels incorporated with 0.075% ECHE had the lowest solubility in S5 ($P<0.05$). Pressurization prior to heating might change protein conformation, in which the interaction between protein molecules via non-disulfide covalent bonds, especially mediated by endogenous TGase. This phenomenon occurred along with the cross-linking by quinones generated from the oxidation of phenolics in ECHE.

Table 12. Solubility of gels from sardine surimi prepared by different gelation processes without and with ECHE at a level of 0.075%.

Treatment	ECHE levels (%)	Solvents*				
		S1	S2	S3	S4	S5
HP	0	15.36±1.04^{a}	11.43±0.99^{a}	47.16±0.99^{a}	76.17±1.26^{b}	84.00±1.58^{a}
	0.075	14.47±1.07^{a}	10.20±1.00^{a}	44.58±1.96^{b}	75.38±1.87^{b}	82.69±1.26^{ab}
HP/H	0	8.44±1.06^{c}	8.01±0.96^{b}	38.94±1.74^{d}	64.59±1.90^{d}	79.35±0.98^{b}
	0.075	7.36±1.00^{c}	7.19±0.80^{b}	33.32±0.99^{c}	60.32±1.58^{e}	76.12±1.10^{c}
S/H	0	11.14±1.12^{b}	10.98±0.79^{a}	48.74±1.26^{a}	79.68±1.18^{a}	83.11±1.73^{a}
	0.075	10.62±0.98^{b}	9.48±0.91^{b}	43.01±1.38^{b}	73.34±0.94^{bc}	80.16±0.99^{b}

Value are mean ± SD (n=3). Different superscripts in the same column indicate the significant differences (P<0.05). S/H: two-steps heating gel (40°C 30 min, followed by 90 °C 20 min), HP: High pressure treated gel (300 MPa for 30 min), HP/H: High pressure treated gel (300 MPa for 30 min), followed by heating (90 °C for 20 min).

* S1 (0.6 M KCl); S2 (20 mM Tris– HCl pH 8.0); S3 (20 mM Tris–HCl, pH 8.0, containing 1% SDS); S4 (20 mM Tris–HCl, pH 8.0, containing 1% SDS and 8 M urea) and S5 (20 mM Tris–HCl, pH 8.0, containing 1% SDS, 2% β-mercaptoethanol and 8 M urea).

5.4.2.3 Microstructures

Microstructures of HP and HP/H gels without and with ECHE at 0.075% are depicted in Fig. 24. HP gel had a looser network and larger voids, while S/H and HP/H gels had a finer network with the smaller voids or cavities. These fine and ordered gel networks more likely had higher strength and imbibed more water. This was evidenced by the higher breaking force, hardness and the lower expressible moisture content. When 0.075% ECHE was incorporated, slightly denser network was developed, regardless of gelation processes used. The results suggested that phenolic compounds induced the cross-linking of myofibrillar proteins in sardine surimi especially that subjected to pressurization with subsequent heating. Phenolics have been reported to strengthen surimi networks (Shitole *et al.*, 2014). Thus, the addition of ECHE in combination with pressurization, followed by heating increased the connectivity and rigidity of proteins in surimi gel networks. As a consequence, gel strength of surimi gels could be increased.

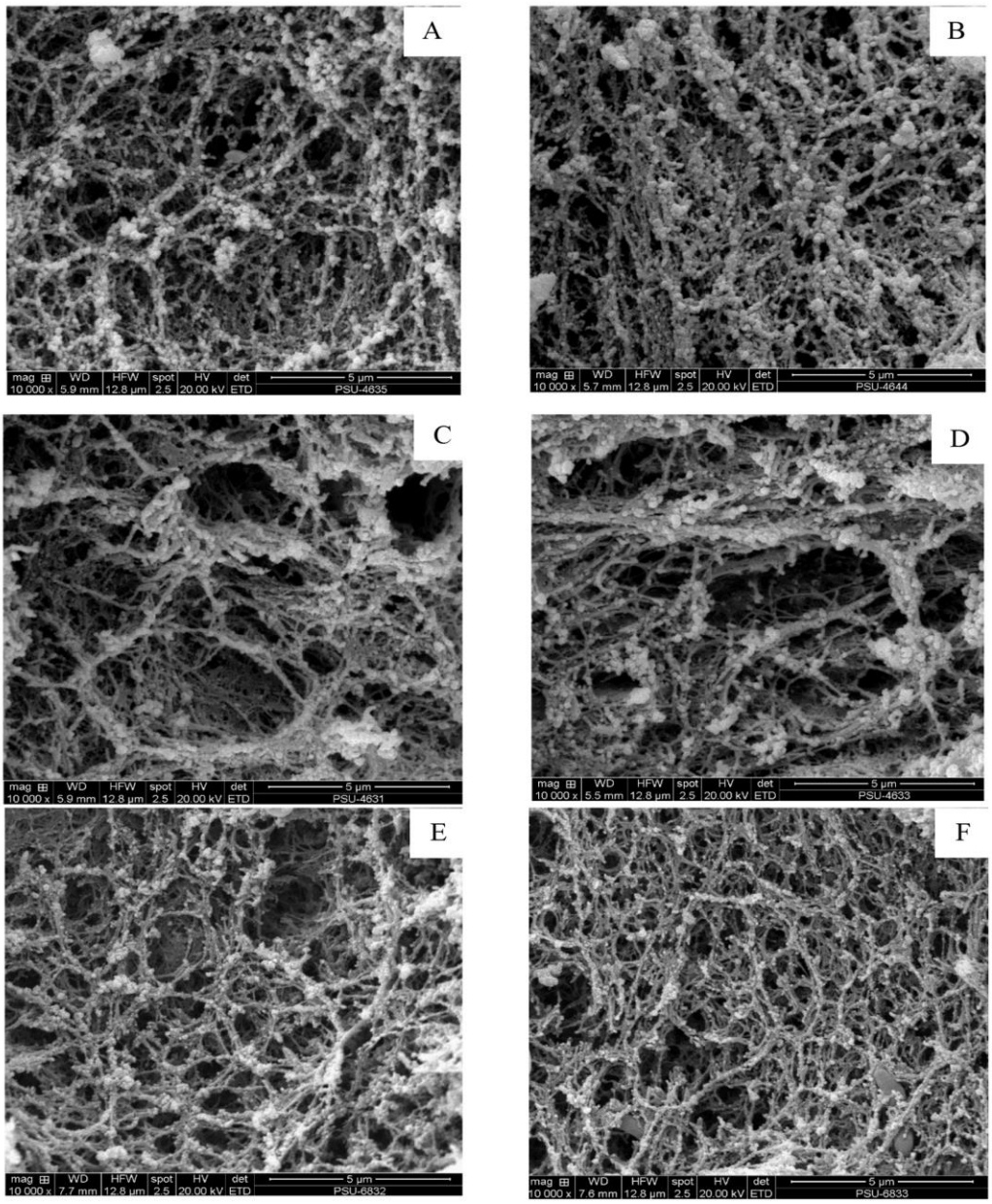


Figure 24. Electron microscopic images of gels from sardine surimi prepared by different gelation processes without and with 0.075% ECHE (magnification: 10,000×): (A): two-step heated gel (40 °C 30 min, followed by 90 °C 20 min); (B): two-step heated gel added with 0.075% ECHE; (C): high pressure treated gel (300 MPa for 30 min); (D): high pressure treated gel added with 0.075% ECHE; (E): high pressure treated gel (300 MPa for 30 min), followed by heating (90 °C for 20 min) and (F): high pressure treated gel (300 MPa for 30 min), followed by heating (90 °C for 20 min) added with 0.075% ECHE.

5.5 Conclusion

ECHE at the appropriate level could be used as the new natural gel strengthener for surimi, especially when used in conjunction with high pressurization with subsequent heating. Nevertheless, ECHE, particularly at an excessive amount, had the negative impact on elasticity and whiteness of surimi gel. The extract from coconut husk could be used along with commercial cross-linking enzymes in surimi or related industry. Also, coconut husk, agricultural waste, could be better utilized and exploited.

5.6 References

Angsupanich, K., Edde, M. and Ledward, D. A. 1999. Effects of high pressure on the myofibrillar proteins of cod and turkey muscle. J. Agric. Food Chem. 47: 92-99.

Balange, A. and Benjakul, S. 2009. Enhancement of gel strength of bigeye snapper (*Priacanthus tayenus*) surimi using oxidized phenolic compounds. Food Chem. 113: 61-70.

Balny, C. and Masson, P. 1993. Effects of high pressure on proteins. Food Rev. Int. 9: 611-628.

Benjakul, S., Visessanguan, V. and Srivilai, C. 2007. Gel properties of bigeye snapper (*Priacanthus tayenus*) surimi as affected by setting and plasma proteins. J. Food Quality. 24: 453-471.

Buamard, N. and Benjakul, S. 2015. Improvement of gel properties of sardine (*Sardinella albella*) surimi using coconut husk extracts. Food Hydrocolloid. 51: 146-155.

Buamard, N. and Benjakul, S. 2017a. Cross-linking activity of ethanolic coconut husk extract toward sardine (*Sardinella albella*) muscle proteins. J. Food Biochem. 41: e12283.

Buamard, N. and Benjakul, S. 2017b. Ethanolic coconut husk extract: *In vitro* antioxidative activity and effect on oxidative stability of shrimp oil emulsion. Eur. J. Lipid Sci. Technol. 119: 1700131.

Buamard, N., Benjakul, S. and Konno, K. 2017. Improvement of gel quality of sardine surimi with low setting phenomenon by ethanolic coconut husk extract. J. Texture Stud. 48: 47-56.

Cando, D., Herranz, B., Borderías, A. J. and Moreno, H. M. 2015. Effect of high pressure on reduced sodium chloride surimi gels. Food Hydrocolloids. 51: 176-187.

Cao, Y., Xia, T., Zhou, G. and Xu, X. 2012. The mechanism of high pressure-induced gels of rabbit myosin. Innovative Food Sci. Emerg. Technol. 16: 41-46.

Cheftel, J. C., and Culioli, J. 1997. Effects of high pressure on meat: A review. Meat Sci. 46: 211-236.

Grigera, J. R., and McCarthy, A. N. 2010. The behavior of the hydrophobic effect under pressure and protein denaturation. Biophysic. J. 98: 1626-1631.

Hernández-Andrés, A., Pérez-Mateos, M., Montero, P., and Gómez-Guillén, M. D. C. 2008. A comparative study of the effects of high pressure on proteolytic degradation of sardine and blue whiting muscle. Fisheries Sci. 74: 899-910.

Herranz, B., Tovar, C. A., Borderias, J., and Moreno, H. M. 2013. Effect of high-pressure and/or microbial transglutaminase on physicochemical, rheological and microstructural properties of flying fish surimi. Innov. Food Sci. Emerging Technol. 20: 24-33.

Hwang, J. S., Lai, K. M., and Hsu, K. C. 2007. Changes in textural and rheological properties of gels from tilapia muscle proteins induced by high pressure and setting. Food Chem. 104: 746-753.

Kaewudom, P., Benjakul, S., and Kijroongrojana, K. 2013. Properties of surimi gel as influenced by fish gelatin and microbial transglutaminase. Food Biosci. 1: 39-47.

Laemmli, U. K. 1970. Cleavage of structural proteins during assembly of head of bacteriophage T4. Nature. 227: 680–685.

Liang, Y., Guo, B., Zhou, A., Xiao, S. and Liu, X. 2016. Effect of high pressure treatment on gel characteristics and gel formation mechanism of bighead carp (*Aristichthys nobilis*) surimi gels. J. Food Process. Preserv. doi:10.1111/jfpp .13155.

Lima, E. B. C., Sousa, C. N. S., Meneses, L. N., Ximenes, N. C., Santos Júnior, M. A., Vasconcelos, G. S. and Vasconcelos, S. M. M. 2015. *Cocos nucifera* (L.) (Arecaceae): A phytochemical and pharmacological review. Brazilian J. Med. Biol. Res. 4: 953-964.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. 193: 256-275.

Maqsood, S., Benjakul, S., Abushelaibi, A. and Alam, A. 2014. Phenolics compounds and plant phenolic extracts as natural antioxidants in prevention of lipid oxidation in seafood: A detailed review. Comp. Rev. Food Sci. Food Safety. 13: 1125-1140.

Ngarize, S., Adam, A. and Howell, N. 2005. A comparative study of heat and high pressure induced gels of whey and egg albumen proteins and their binary mixtures. Food Hydrocolloids. 19: 984-996.

Panyakaew, S. and Fotios, S. 2011. New thermal insulation boards made from coconut husk and bagasse. Energ. Buildings. 43: 1732-1739.

Pérez-Mateos, M., Lourenço, H., Montero, P. and Borderias, J. 1997. Rheological and biochemical characteristic of high pressure and heat-induced gels from blue whiting (*Micromesistius poutassou*) muscle protein. J. Agric. Food Chem. 45: 44–49.

Rohn, S., Rawel, H. M. and Kroll, J. 2004. Antioxidant activity of protein-bound quercetin. J. Agric. Food Chem. 52: 4725-4729.

Shitole, S. S., Balange, A. K. and Gangan, S. S. 2014. Use of seaweed (*Sargassum tenerrimum*) extract as gel enhancer for lesser sardine (*Sardinella brachiosoma*) surimi. Int. Aquat. Res. 6: 55-59.

Sriket, C. 2014. Proteases in fish and shellfish: Role on muscle softening and prevention. Int. Food Res. J. 21: 433-445.

Steel, R. G. D. and Torrie, J. H. 1980. Principles and procedures of statistics. McGraw-Hill Book, New York.

Sun, X. D. and Holley, R. A. 2011. Factor influencing gel formation by myofibrillar proteins in muscle foods. Comp. Rev. Food Sci. Food Safe. 10: 33-51.

Tabilo-Munizaga, G. and Barbosa-Cánovas, G. V. 2004. Color and textural parameters of pressurized and heat-treated surimi gels as affected by potato starch and egg white. Food Res. Int. 37: 767-775.

Temdee, W. and Benjakul, S. 2014. Effect of oxidized kiam wood and cashew bark extracts on gel properties of gelatin from cuttlefish skins. Food Biosci. 7: 95-104.

Viju, N., Satheesh, S. and Vincent, S. G. P. 2013. Antibiofilm activity of coconut (*Cocos nucifera* Linn.) husk fibre extract. Saudi. J. Biol. Sci. 20: 85-91.

Zhu, Z., Lanier, T. C., Farkas, B. E. and Li, B. 2014. Transglutaminase and high-pressure effects on heat-induced gelation of Alaska pollock (*Theragra chalcogramma*) surimi. J. Food Eng. 131: 154-160.

CHAPTER 6

ETHANOLIC COCONUT HUSK EXTRACT: *IN VITRO* ANTIOXIDATIVE ACTIVITY AND EFFECT ON OXIDATIVE STABILITY OF SHRIMP OIL EMULSION

6.1 Abstract

Ethanolic coconut husk extract (ECHE) was characterized and its effect on lipid oxidation of shrimp oil-in-water emulsion was studied. Condensed tannin was abundant in ECHE (492.2 and 441.9 mg catechin equivalent/g extract as determined by vanillin and BuOH-HCl assays, respectively). The major free phenolics in ECHE were tannic acid and catechins (205.98 and 103.56 mg/kg dry extract, respectively). Antioxidative activities of ECHE at different levels (50-200 mg/L) tested by all *in vitro* assays increased as its concentration increased ($P<0.05$). 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activity and metal chelating activity were decreased up to 50% when heated at temperature higher than 90 °C for longer than 60 min ($P<0.05$). Impact of ECHE (200 and 400 mg/L) on lipid oxidation of shrimp oil-in-water emulsion was monitored throughout 12 days of storage at 30 °C. Lipid oxidation of emulsion added with ECHE was retarded as evidenced by the lower conjugated diene (CD), thiobarbituric acid-reactive substances (TBARS) value and *ρ*-anisidine value (AnV). Eicosapentaenoic acid (EPA; C20:5 *n*-3) and docosahexaenoic acid (DHA; C22:6 *n*-3) were more retained in the sample added with ECHE (200 mg/L) at the end of storage ($P<0.05$). ECHE could therefore be used as a natural antioxidant, particularly in emulsion.

6.2 Introduction

Lipid oxidation is a severe problem in foods, especially those containing unsaturated or polyunsaturated fatty acids, which are susceptible to oxidation (Takeungwongtrakul *et al.,* 2012). Lipid oxidation causes negative effects on organoleptic properties, depletes nutritional properties and generates toxic compounds in foods. Moreover, oxidation of lipid is associated with aging, membrane damage, cancer and heart disease (Ramarathnam *et al.,* 1995). This phenomenon is much more

accentuated in oil-in-water emulsion because a large interfacial area is produced during the emulsification process. As a consequence, lipid oxidation is enhanced in emulsion (McClements and Decker, 2000). Therefore, incorporation of antioxidant is needed to retard such a deteriorative reaction and to prolong shelf-life of food products.

Nowadays, antioxidants from natural sources such as α-tocopherol, ascorbic acid, carotenoids, peptides, protein hydrolysates and other phenolic compounds have been paid more interest since they have no negative effects on consumer's health. These antioxidants also show physiological and dietary effects (Maqsood and Benjakul, 2010; Kittiphatanabawon *et al.*, 2012). Natural antioxidants, especially phenolic compound, has been known to possess a wide range of biological effects including antioxidative, antimicrobial, antiviral (Ha *et al.*, 2016) antimutagenicity and anticancer activities (Wahle *et al*., 2010).

Coconut husk, a fibrous external portion of the coconut fruit, is considered as the underutilized resource from fruit separation. Generally, coconut husk has been employed for the textile production such as brushes, floor mats, upholstery padding and rope (Wang and Huang, 2009). Apart from those utilizations, coconut husk has been reported to be a potential source of phenolic compounds such as tannins, flavonoids and phlobatannins (Rodrigues and Pinto, 2007). Recently, ethanolic coconut husk extract (ECHE) has been reported to induce the aggregation of muscle protein from sardine (Buamard and Benjakul, 2017). Additionally, the extract acted as a potential protein cross-linker to improve the quality of gelatin gel from yellowfin tuna swim bladder as well as sardine surimi gel (Buamard and Benjakul, 2015; Kaewdang and Benjakul, 2015). It also improved the mechanical property of nanocomposite film from tilapia skin gelatin (Nagarajan *et al*., 2015). However, no information regarding the antioxidative activity of ethanolic coconut husk extract exists. Thus, this study aimed to examine *in vitro* antioxidative activities of ECHE and to investigate its preventive effect toward lipid oxidation in shrimp oil-in-water emulsion during the extended storage.

.

6.3 Materials and methods

6.3.1 Chemicals

All chemicals were of analytical grade. 2,2′-azinobis(3-ethylbenzo thiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tripyridyltriazine (TPTZ), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4″-disulfonic acid sodium salt (ferrozine), *N*,*N*-dimethyl *p*-nitrosoaniline (DPN), Trolox, fluorescein, 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH) and sodium caseinate were purchased from Sigma–Aldrich Canada Ltd. (Oakville, ON, Canada). Ethylenediaminetetraacetic acid (EDTA) was obtained from Merck (Darmstadt, Germany). Hydrogen peroxide (H_2O_2) and sodium hypochlorite (NaOCl) were procured from Lab-Scan (Bangkok, Thailand).

6.3.2 Preparation of coconut husk

Husk of coconut (*Cocos nucifera* Linn.) fruit with the age about 11 months was collected from a local market in Hat Yai, Songkhla Province, Thailand and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai, Thailand. Coconut husk powder was prepared as per the method of Buamard and Benjakul (2015). The powder was placed in a polyethylene bag, sealed and kept at room temperature until use.

6.3.3 Preparation of ethanolic coconut husk extract

Coconut husk powder was subjected to extraction according to the method of Buamard and Benjakul (2015). To husk powder (10 g), 350 mL of 60% ethanol (v/v) were added. The mixture was stirred at room temperature (28-30 °C) for 3 h at low speed using a magnetic stirrer (IKA-Werke, Staufen, Germany). The mixture was centrifuged at 5,000 ×g for 30 min at room temperature using an Allegra. 25R centrifuge (Beckman Coulter, Palo Alto, CA, USA). Subsequently, the supernatant was then filtered through a Whatman filter paper No.1 (Whatman International Ltd., Maidstone, UK). The filtrate was evaporated at 40 °C using an Eyela rotary evaporator (Tokyo Rikakikai, Co. Ltd., Tokyo, Japan) and purged with nitrogen gas to remove the residual ethanol. The extract was then dried using a

Scanvac Model Coolsafe 55 freeze dryer (Coolsafe, Lynge, Denmark). Dried extract named ‘ECHE’ was powdered using a mortar and pestle.

6.3.4 Characterization of ECHE

6.3.4.1 Determination of condensed tannin

Condensed tannin or proanthocyanidin content was determined by the vanillin assay as described by Saad *et al*. (2012) with a slight modification. ECHE solution (0.5 mL, 50 mg/mL) was added with 3 mL of 4% vanillin-methanol solution and 1.5 mL of 12 M HCl. The mixture was allowed to stand for 15 min at room temperature (25-28 °C) in the dark. A standard curve was prepared using catechin in the range of 0-100 mg/L. The absorbance of the mixture was read at 500 nm using a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). The content was expressed as mg catechin equivalent/g extract.

Condensed tannin content was also measured using the BuOH-HCl assay according to the method of Bucić-Koji *et al.* (2011) with a slight modification. ECHE solution (0.5 mL, 50 mg/mL) was added to 5 mL of an acid solution of ferrous sulfate (77 mg of $FeSO_4 \cdot 7H_2O$ in 500 mL of 2:3 (12.04 M HCl:*n*-Butanol). The tubes containing reaction mixture were loosely covered and placed in a water bath at 95 °C for 15 min. The absorbance was read at 530 nm. The result was reported as cyanidin equivalent/g extract. The condensed tannin content was calculated using the equation as follows:

Condensed tannin content (mg cyanidin equivalent/g extract) = $(A \times MW \times Df)/(\varepsilon \times l)$

where A denotes absorbance of the extract; MW is molecular weight of cyanidin (287 g/mol); Df is dilution factor; ε is molar extinction coefficient of cyanidin (34,700/M/cm); and l is pathlength (cm).

6.3.4.2 Identification and quantification of phenolic compounds

Phenolic compounds in ECHE was analyzed using a LC/DAD/MSD equipped with a diode array detector (DAD) and a scan mode 100-700 *m/z* MS detector. Sample was firstly separated on LiChroCART® Purospher® STAR RP-18e column (Merck, USA) (150 x 4.6 mm, diameter 5 µm) with an Agilent 1100 series

(Agilent Technologies, Waldbronn, Germany). Mobile phase A and B were acetonitrile and 10 mM ammonium formate buffer pH 4 with formic acid, respectively (flow rate: 1.0 mL/min; temperature: 40˚C). The gradient program was as follows: 100% B constant (0-5 min), 0-20% A (5-10 min), 20% A constant (10-20 min), and 20-40% A (20-60 min). The detection was carried out at 270, 330, 350 and 370 nm. MS detection was done in the positive ionization mode, where an electrospray ionizing source with nitrogen as drying gas was used. The conditions used were as follows: the capillary voltage: 4000 V; gas temperature: 320˚C; drying gas flow: 13 L/min and nebulizer pressure 60 psi. Quantitative analysis by MSD was operated in the SIM (Selected Ion Monitoring) mode. Standards were as follows: 188, 209 *m/z* for gallic acid; 185, 329, 503, 649 *m/z* for catechin, isoquercetin, tannic acid and rutin; 289, 327, 341 *m/z* for hydroquinin, eriodictyol and quercetin. The peaks detected were quantified by comparing the retention time and peak area with those of phenolic standards.

6.3.5 Study on antioxidative activities of ECHE

Prior to assays, the ECHE was dissolved in distilled water to obtain the designated concentrations (0-200 mg/L). Thereafter, the obtained solutions were neutralized to pH 7 using either 1 N HCl or 1 N NaOH. Antioxidative activities were determined.

6.3.5.1 ABTS radical scavenging activity

ABTS radical scavenging activity was determined as described by Kittiphatanabawon *et al.* (2012). A standard curve was prepared using Trolox in the range of 0-600 μM. The absorbance of the resulting solution was measured at 734 nm. The activity was expressed as μmol Trolox equivalents (TE)/mL.

6.3.5.2 DPPH radical scavenging activity

DPPH radical scavenging activity was analyzed according to the method of Wettasinghe and Shahidi (2000). Trolox with the range of 0–60 μM was used for standard curve preparation. The resulting solution was read at 517 nm and the activity was expressed as μmol trolox equivalents (TE)/mL.

6.3.5.3 Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity was assayed as per the method of Wettasinghe and Shahidi (2000). Trolox (0–10 mM) was used as the standard. Absorbance was read at 230 nm. The activity was expressed as μmol Trolox equivalents (TE)/mL.

6.3.5.4 Singlet oxygen scavenging activity

The capacity of samples to scavenge singlet oxygen was measured as described by Kittiphatanabawon *et al.* (2012). The absorbance was read at 440 nm. A standard curve of trolox (0–10 mM) was prepared. The activity was expressed as μmol Trolox equivalents (TE)/mL.

6.3.5.5 Ferric reducing antioxidant power (FRAP)

FRAP was determined according to the method of Benzie and Strain (1999). The standard curve was prepared using Trolox ranging from 0 to 500 μM. The absorbance of ferrous tripyridyltriazine complex (color product) was read at 593 nm. The activity was expressed as μmol Trolox equivalents (TE)/mL.

6.3.5.6 Metal chelating activity

Ferrous ion chelating activity was determined as described by Boyer and McCleary (1987). The standard curve was prepared using EDTA ranging from 0 to 30 μM. The absorbance was read at 562 nm. The activity was expressed as μmol EDTA equivalents (EE)/mL.

6.3.5.7 Oxygen radical absorbance capacity

Oxygen radical absorbance capacity (ORAC) was measured as detailed by Kittiphattanabawon *et al.* (2012) with a slight modification. Twenty microliters of ECHE (200 mg/L) solution were loaded onto black polystyrene, nontreated 96-wells microplate (Cellstar, Grenier, Bio-One, Monroe, NC, USA). The loaded microplate was inserted to a FLUOstar Omega microplate reader (BMG Labtechnologies GmbH, Offenberg, Germany) equipped with FLUOstar Omega evaluation software version 5.10. The samples were equilibrated at 37 °C for 10 min. Thereafter, 200 μL of 0.11

μM fluorescein dissolved in 75 mM phosphate buffer (pH 7.0) were added to the sample. The reaction was started by the addition of 75 μL of 60 mM AAPH. The reaction was performed at 37 °C. The fluorescence intensity was measured every 120 s for 75 cycles with excitation and emission filters of 485 and 520 nm, respectively. The control was prepared in the same manner, except that 75 mM phosphate buffer (pH 7.0) was used instead of the sample. The kinetic curve (AUC) of all samples was plotted between fluorescence intensity and the number of cycles. Control was also prepared in the same manner except that the sample was omitted and distilled water was used instead. Trolox at a concentration of 80 mg/L was used as the reference.

6.3.6 Study on thermal stability of ECHE

ECHE (50 mg/L) was incubated at different temperatures (80, 90 and 100 °C) for various times (20, 40 and 60 min) in a temperature-controlled water bath (Memmert, D-91126, Schwabach, Germany). The solution was cooled suddenly in iced water. The residual activity was determined for DPPH radical scavenging activity, FRAP and metal chelating activity.

6.3.7 Effect of ECHE addition on oxidative stability of shrimp oil emulsion

6.3.7.1 Preparation of shrimp oil-in-water emulsion incorporated with ECHE

Shrimp oil-in-water emulsion was prepared according to the method of Takeungwongtrakul and Benjakul (2013) with a slight modification. Sodium caseinate (6 % w/w) and sodium azide (0.02% w/w) were dissolved in 10 mM sodium phosphate buffer (pH 7.0). To the solution (90 mL), shrimp oil (10 mL) was gradually added within 1 min. The mixture was homogenized continuously using an IKA Labortechnik homogenizer at a speed of 13,500 rpm for totally 5 min. ECHE was then added into emulsion to obtain different final concentrations (100, 200 and 400 mg/L). Tocopherol or ascorbic acid at a final concentration of 200 mg/L were used as the positive controls. Control was also prepared in the same manner except that no additives were added and distilled water was used instead. All samples were stored at 30 °C and were taken randomly for analyses at day 0, 3, 6, 9 and 12.

6.3.7.2 Analyses

6.3.7.2.1 Peroxide value

Peroxide value (PV) was determined according to the method of Chaijan *et al*. (2006) with a slight modification. To 1 mL of emulsion sample, 2 mL of chloroform/methanol (2:1, v/v) were added. The mixture was mixed using a vortex mixer for 3 min to separate the sample into two phases. The organic solvent phase (20 μL) was mixed with 2.35 mL of chloroform/methanol (2:1, v/v), followed by 50 μL of 30% ammonium thiocyanate (w/v) and 50 μL of 20 mM ferrous chloride solution in 3.5% HCl (w/v). After 20 min, the absorbance of the colored solution was read at 500 nm. Blank was prepared in the same manner, except that distilled water was used instead of ferrous chloride. A standard curve was prepared using cumene hydroperoxide with the concentration range of 0.5–2 mg/L. PV was calculated after blank subtraction and expressed as mg cumene hydroperoxide/L emulsion.

6.3.7.2.2 Conjugated diene

Conjugated dienes (CD) were measured according to the method of Takeungwongtrakul and Benjakul (2013) with a slight modification. The sample (0.1 mL) was dissolved in methanol (5 mL) and the absorbance at 234 nm was read. The content of conjugated dienes was expressed as the increase in absorbance at 234 nm.

6.3.7.2.3 Thiobarbituric acid reactive substances

Thiobarbituric acid reactive substances (TBARS) were determined as described by Chaijan *et al*. (2006). Emulsion sample (0.5 mL) was mixed with 2.5 mL of a solution containing 0.375% thiobarbituric acid (w/v), 15% trichloroacetic acid (w/v) and 0.25 M HCl. The mixture was heated in a boiling water (95–100°C) for 10 min to develop a pink color, cooled with running tap water and centrifuged at 3,600 ×g at 25 °C for 20 min using a Microcentrifuge 22 (Krefeld, North Rhine-Westphalia, Germany). The absorbance of the supernatant was measured at 532 nm. A standard curve was prepared using 1,1,3,3-tetramethoxypropane at the

concentrations ranging from 0 to 6 mg/L. TBARS were calculated and expressed as mg malonaldehyde/L emulsion.

6.3.7.2.4 *ρ*-Anisidine value

ρ-Anisidine value (AnV) of sample was analyzed according to the method of Takeungwongtrakul and Benjakul (2013). The sample (0.5 g) was mixed with 25 mL of isooctane. The solution (2.5 mL) was mixed with 0.5 mL of 0.5% *ρ*-anisidine in acetic acid for 10 min. The absorbance was read at 350 nm. The *ρ*-anisidine value was calculated using the following formula:

$$\rho\text{-anisidine value} = 25\times[(1.2\times A_2)-A_1]/W$$

where A_1 and A_2 are A_{350} before and after adding *ρ*-anisidine, respectively; W is the weight of sample (g).

6.3.7.2.5 Fatty acid profile

Lipid extracted from emulsion sample at day 0 and those without additive (control) and with ECHE (200 mg/L) addition after 12 days of storage were used for analysis. Fatty acid profile was determined as fatty acid methyl esters (FAMEs). FAMEs were prepared according to the method of AOAC. The prepared methyl ester was injected to the GC (Shimadzu, Kyoto, Japan) equipped with the flame ionization detector (FID) at a split ratio of 1:20. A fused silica capillary column (30 m × 0.25 mm), coated with bonded polyglycol liquid phase, was used. The analytical conditions were: injection port temperature of 250 °C and detector temperature of 270 °C. The oven was programmed from 170 to 225 °C at a rate of 1 °C/min (no initial or final hold). Retention times of FAME standards were used to identify chromatographic peaks of the samples. Fatty acid content was calculated, based on the peak area ratio and expressed as g fatty acid/100 g oil.

6.3.8 Statistical analysis

All experiments were run in triplicate. The experimental data were subjected to Analysis of Variance (ANOVA) and the differences between means were evaluated by the Duncan's Multiple Range Test (Steel and Torrie, 1960). Data

analysis was performed using a SPSS package version 11.0 for Windows (SPSS Inc., Chicago, IL, USA).

6.4 Results and discussion

6.4.1 Condensed tannin content in ECHE

Condensed tannin content was 492.2 ± 9.3 and 441.9 ± 7.8 mg catechin equivalent/g extract, when determined by vanillin and BuOH-HCl assays, respectively. Based on the result, the content obtained from the former assay was higher than the latter. Vanillin assay has been used to analyze condensed tannin, however simple flavonoids such as quercetin, kaempferol and tangeritin, are also detected. BuOH-HCl assay determines only condensed tannins, in which more complex polyphenols form the group of tannins (Bucić-Kojić *et al.,* 2011; Wettasinghe and Shahidi, 2000). Thus, the extraction of coconut husk powder by 60% ethanol yielded the resulting ECHE, which contained condensed tannins as the major component and simple flavonoids and free phenolic acids were found at a low content. Maritime pine bark (Nurulhuda *et al*., 1990) and fig fruit (Bucić-Kojić *et al*., 2011) also had condensed tannin as the dominant constituent. Tunisian pomegranate peels were reported to contain high amount of condensed tannin or proanthocyanidin (Bucić-Kojić *et al.,* 2011). Tannin contents varies with cultivars, ages, maturity and extraction solvents (Buamard and Benjakul, 2015, Nurulhuda *et al*., 1990). Thus, ECHE was rich in condensed tannin.

6.4.2 Phenolic compounds in ECHE

Phenolic compounds of ECHE identified by LC/DAD/MSD are shown in Table 13. Seven phenolic compounds were detected in ECHE. Tannic acid and catechin were found as major phenolics (205.98 and 103.56 mg/kg, respectively). Gallic acid, eriodictyol, isoquercetin, quercetin and hydroquinn were also detected at low content. This result suggested that ECHE was rich in condensed tannin and had low content of free phenolic compounds. Wood bark and fruit shell are the potential sources of phenolic compounds (Bucić-Kojić *et al*., 2011, Nurulhuda *et al.,* 1990). Tannic acid has been found as a major phenolic acid in *Punica granatum* L. fruit peel, *Quercus ilex* L. root bark as well as kiam wood (Gharzouli *et al*., 1999; Balange and

Benjakul, 2009). Nevertheless, several plant species and parts might have different phenolic compounds. Phenolics from argan fruit shell contained epicatechin and isoquercitrin (Monfalouti *et al.*, 2012). Liimatainen *et al.* (2012) reported that bark from silver birch had a wide range of phenolics including flavonols, lignans, procyanidins and arylbutanoids. Cat's claw bark had protocatechuic acid, catechin, epicatechin, procyanidin as major phenolics (Hoyos *et al.*, 2015). Thus, ECHE could serve as an alternative source of phenolics, especially tannic acid and catechin.

Table 13. Phenolic compounds in ECHE

Phenolic compounds	Time of retention (min)	Phenolic content* (mg/kg dry extract)
Gallic acid	6.7-7.2	41.30 ± 3.89
Catechin	12.4-12.6	103.56 ± 7.76
Tannic acid	12.7-13.0	205.98 ± 10.14
Quercetin	16.4-16.5	21.17 ± 2.02
Hydroquinin	24.0-24.3	25.39 ± 2.18
Eriodictiol	31.2-31.3	28.66 ± 2.39
Quercetin	33.9-34.0	36.73 ± 2.88

* Values are given as means ± SD (n=3).

6.4.3 *In vitro* antioxidative activities of ECHE

Antioxidative activities of ECHE at different concentrations are shown in Fig. 1. The activities of ECHE tested by different assays were more pronounced with increasing concentrations (P<0.05). However, hydrogen peroxide radical scavenging was not increased when ECHE concentration was higher than 100 mg/L (P>0.05). ECHE showed ABTS, DPPH radical and singlet oxygen scavenging activities in a dose dependent manner (Fig. 25A, B, F). ABTS radical has been used to determine both hydrophilic and hydrophobic antioxidant capacities, while DPPH radical has been employed for investigating antioxidant properties in organic solvent (Hoyos *et al*., 2015). The extracts from *Pinus radiata* bark (Chupin *et al*., 2013), Alaska cedar bark (Rosales-Castro *et al*., 2014) and açai fruit (Pacheco-Palencia *et al*., 2008) showed higher antioxidative activity at higher concentrations. Singlet oxygen,

which is a highly reactive, electrophilic and non-radical molecule, can be formed by the reaction between photosensitizers and triple oxygen in the presence of light (Kittiphatanabawon *et al*., 2012). The singlet oxygen scavenging activity of ECHE was also increased when ECHE concentration increased (Fig. 25F) ($P<0.05$). Phenolics in ECHE, especially at a high concentration, might be able to trap or bind with the singlet oxygen. ECHE also exhibited hydrogen peroxide scavenging activity. Hydrogen peroxide, a potential oxidizing agent, is the precursor for the generation of hydroxyl radical, which is a strong initiator of lipid oxidation (Choe and Min, 2005). It was suggested that ECHE was capable of retarding lipid oxidation via scavenging hydrogen peroxide.

Apart from radical scavenging activities, ECHE also showed FRAP and chelating activity toward ferrous ions in a dose-dependent manner ($P<0.05$) (Fig.25C, D). The result indicated that ECHE could easily donate the electron to Fe^{3+}, thus reducing it to Fe^{2+}. Maqsood and Benjakul (2010) reported that tannic acid showed the highest reducing capacity, followed by caffeic acid, ferulic acid and catechin, respectively. However, catechin showed the highest metal chelating activity because of its polyhydroxylated structure and number of hydroxyl group in *ortho*-position of phenolics (Maqsood and Benjakul, 2010) Thus, ECHE containing tannic acid, catechin and other phenolic compounds could act as the antioxidant with radical scavenging activity, reducing power as well as metal chelating property.

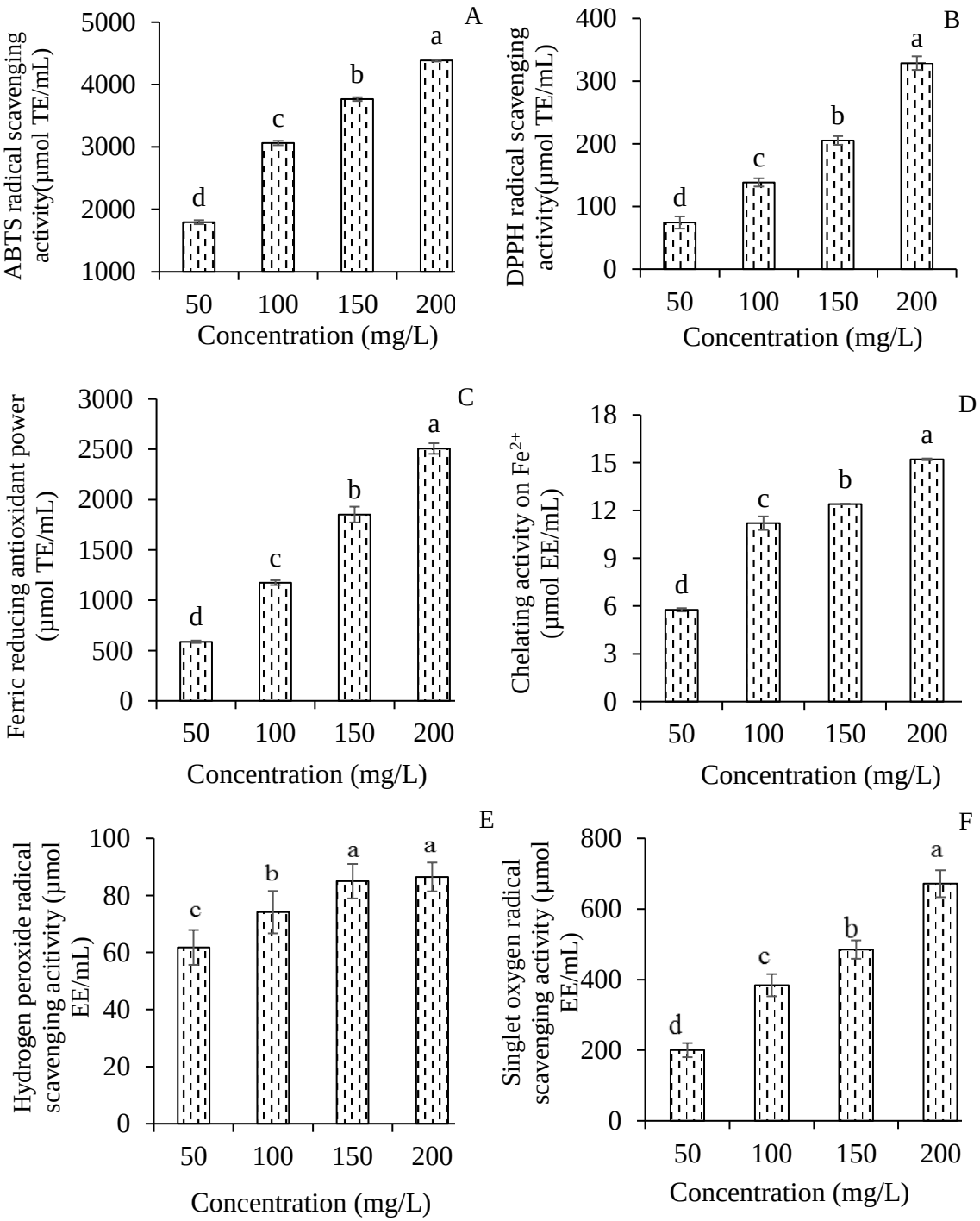


Figure 25. ABTS radical scavenging activity (A), DPPH radical scavenging activity (B), ferric reducing antioxidant power (C), chelating activity on ferrous ion (D), hydrogen peroxide scavenging activity (E) and singlet oxygen scavenging activity (F) of ECHE at different levels. Bars represent the standard deviation (n=3). Different letters on the bars indicate significant differences (P<0.05).

6.4.4 Oxygen radical absorbance activity of ECHE

ECHE also possessed peroxyl radical scavenging activity as evaluated by ORAC assay (Fig. 26). ORAC assay has been used to determine the antioxidant activity of compounds by scavenging peroxyl radical, which is thermally generated through the decomposition of the azo compound, AAPH. Fluorescein, which has been used as a fluorescence probe, reacts with peroxyl radical, hence causing fluorescence decay. The fluorescence decay was inhibited in the presence of antioxidants (Kittiphatanabawon *et al*., 2012). The fluorescence decay was highest for the control. The presence of ECHE at a concentration of 200 mg/L showed the inhibition effect toward such a decay, equivalent to Trolox at a concentration of 80 mg/L. It has been reported that natural material rich in phenolic compounds such as coffee (regular and decaffeinated coffees) and berry fruits showed ORAC (Vicente *et al.*, 2014; Colak *et al.*, 2016). Therefore, ECHE could play a role in scavenging peroxyl radicals. As a result, the lipid oxidation could be impeded using ECHE.

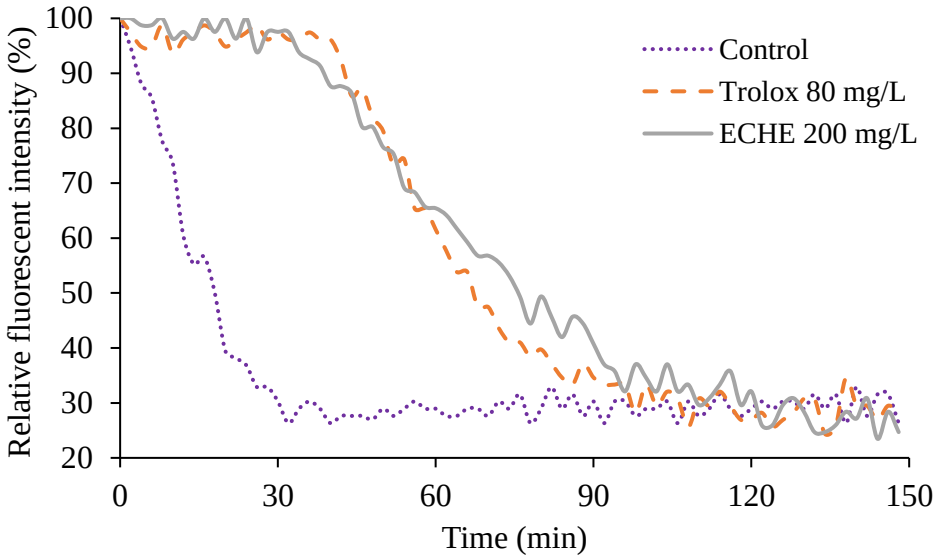


Figure 26. Fluorescence decay curve of fluorescein in the presence of ECHE and Trolox. ECHE and Trolox at the concentrations of 200 and 80 mg/L, respectively, was tested for oxygen radical absorbance capacity (ORAC) assay. Control: without addition of antioxidant.

6.4.5 Thermal stability of ECHE

Stability of ECHE as affected by heating temperature and time is shown in Fig. 27. In general, the decreases in DPPH radical scavenging activity, FRAP and chelating activity were observed when ECHE was subjected to heating at higher temperature for longer time ($P<0.05$). At temperature of 80 °C, there were no differences in DPPH radical scavenging and ferrous chelating activity when incubation duration increased from 20 to 40 min (Fig. 3A, C) ($P>0.05$). When heated at 90 and 100 °C, all activities were sharply decreased as the time increased ($P<0.05$). Pacheco-Plalencia *et al.* (2008) reported that the extract from açai fruit was stable when heated at 150 and 170 °C for up to 20 min, in which only 10% losses in phenolics and antioxidant capacity were found. Polyphenols extracted from highbush blueberry fruit were decreased by 23.1% when heated at 115 °C for 60 s (Kucner *et al*., 2014). These results suggested that antioxidative activity of ECHE was still retained to some degree in foods after thermal processing.

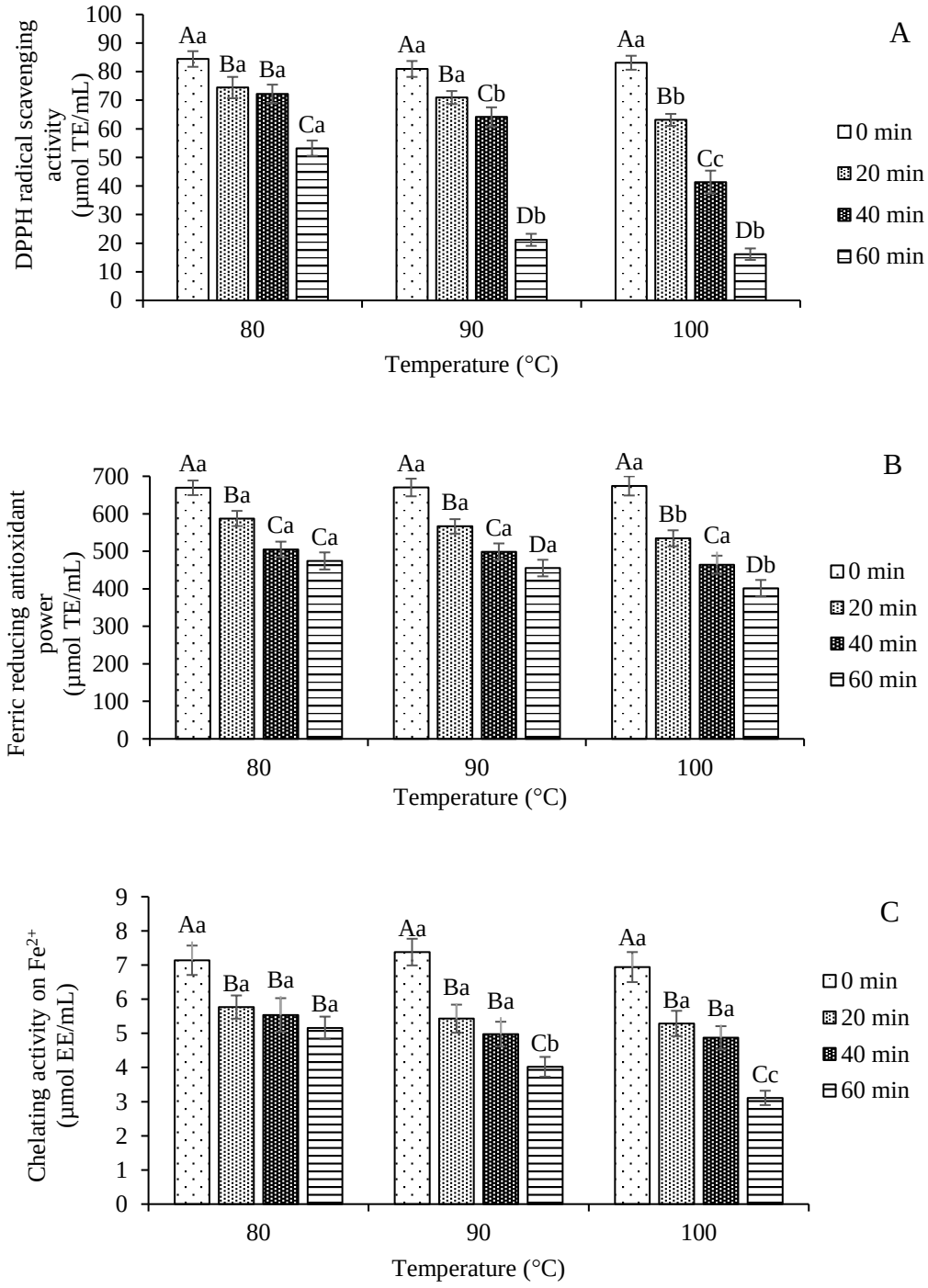


Figure 27. DPPH radical scavenging activity (A), ferric reducing antioxidant power (B) and chelating activity (C) of ECHE after heating at different temperatures for various times. Bars represent the standard deviation (n=3). Different capital letters within the same heating temperature denote the significant differences (P<0.05). Different small letters within the same heating time denote significant differences (P<0.05).

6.4.6 Oxidative stability of shrimp oil-in-water emulsion containing ECHE

6.4.6.1 Peroxide value (PV)

PV of shrimp oil-in-water emulsion with and without ECHE incorporated during 12 days of storage at 30 °C is shown in Fig. 28. PV of the control and the samples incorporated with ECHE at different concentrations increased within the first 8 days of storage (Fig. 28A) ($P<0.05$). PV remained constant or subsequently decreased during 10-12 days. Nevertheless, the emulsion incorporated with α-tocopherol had a sharp increase in PV up to day 10 and PV was slightly decreased at day 12 ($P<0.05$). The increase in PV of the samples indicated the increasing formation of hydroperoxide, a primary lipid oxidation product (Shahidi and Zhong, 2005). ECHE containing catechin and tannic acid, which had a large number of hydrophobic domains, could align themselves at the interface of oil droplet and functioned as a radical scavenger. At the end of storage time, the sample added with α-tocopherol yielded the highest PV than the others ($P<0.05$). Generally, tocopherols can donate hydrogen atom to lipid and/or peroxide radicals (Chaijan *et al*., 2006). In the present study, the accumulation of hydroperoxide was higher in the sample added with α-tocopherol at the end of storage. This might be due to the lower decomposition of hydroperoxide formed in the sample. It was noted that α-tocopherol showed the excellent preventive effect on oxidation within the first 4 days. Thus, it was postulated that α-tocopherol might loss the activity with the extended storage time in the emulsion tested. Among all samples, that added with 400 mg/L ECHE had the lowest PV at the end of storage ($P<0.05$).

6.4.6.2 Conjugated diene (CD)

CD is another indicator of oxidation in the early stage of oxidation. Almost immediately after hydroperoxides are formed, the non-conjugated double bonds present in natural unsaturated lipids are converted to conjugated double bonds (Chaijan *et al.*, 2006). In this study, there was no difference of CD among all samples during the first 4 days of storage (Fig. 28B). After day 6, CD values of sample added with ECHE and the selected antioxidants, especially ascorbic acid, were lower than

those of the control (P<0.05). However, sample added with α-tocopherol had similar CD value to the control from day 8 to the end of storage. This might be associated with the formation of tocopheroxyl radicals, reactive radical from tocopherol, during the storage of this emulsion system. Based on the result, ECHE at 400 mg/L showed the highest activity in retardation of CD formation at day 12. However, its efficacy was equivalent to ascorbic acid (200 mg/L)

6.4.6.3 Thiobarbituric acid reactive substances (TBARS)

The control had the continuous increases in TBARS (P<0.05) throughout the storage of 12 days (Fig. 28C). TBARS values have been used to indicate the formation of the secondary lipid oxidation products, especially aldehydes (Kucner *et al*., 2014). The lower increased in TBARS was found in the sample added with α-tocopherol within the first 4 days. Nevertheless, the higher TBARS values were noticeable in this sample, compared with those added with other additives after 8 days of storage (P<0.05). The increases in TBARS were coincidental with the slight decrease in PV when storage time increased. This was owing to the decomposition of hydroperoxides into the aldehydes or other secondary oxidation products (Chaijan *et al.,* 2006). In general, the generation of secondary oxidation products was more suppressed as the concentration of ECHE increased. At day 12, the lowest TBARS value was found in the sample added with ECHE at 400 mg/L. The result suggested that ECHE was effective in preventing the oxidation of shrimp oil-in-water emulsion.

6.4.6.4 *ρ*-Anisidine value (AnV)

The AnV of shrimp oil-in-water emulsion with and without added ECHE or the selected antioxidants increased when the storage time increased (P<0.05) (Fig. 28D). In general, a similar trend was observed in comparison with the aforementioned lipid oxidation products tested by other assays. The addition of ECHE and the antioxidants lowered the generation of secondary oxidation products including nonvolatile compounds. AnV is a tool to indicate non-volatile lipid oxidation product (Shahidi and Zhong, 2005). AnV of the emulsion without ECHE or the antioxidants was noticeably higher than that of sample added with ECHE or the antioxidants throughout the storage (P<0.05). Phenolics such as tannic acid or catechin were

reported to be efficient in prevention of unsaturated lipids against oxidation. It plays a role as a scavenger of radicals associated with lipid oxidation (Maqsood and Benjakul, 2010). ECHE (400 mg/L) exhibited the highest efficacy in retarding AnV in emulsion. Nevertheless, its activity was similar to ascorbic acid (200 mg/L) throughout the storage of 12 days.

Overall, the efficiency in inhibition of lipid oxidation was in accordance with *in vitro* antioxidative activity of ECHE (Fig. 25 and 26). The incorporation of ECHE potentially prevented the oxidation of lipids in shrimp oil-in-water emulsion, which was rich in PUFAs.

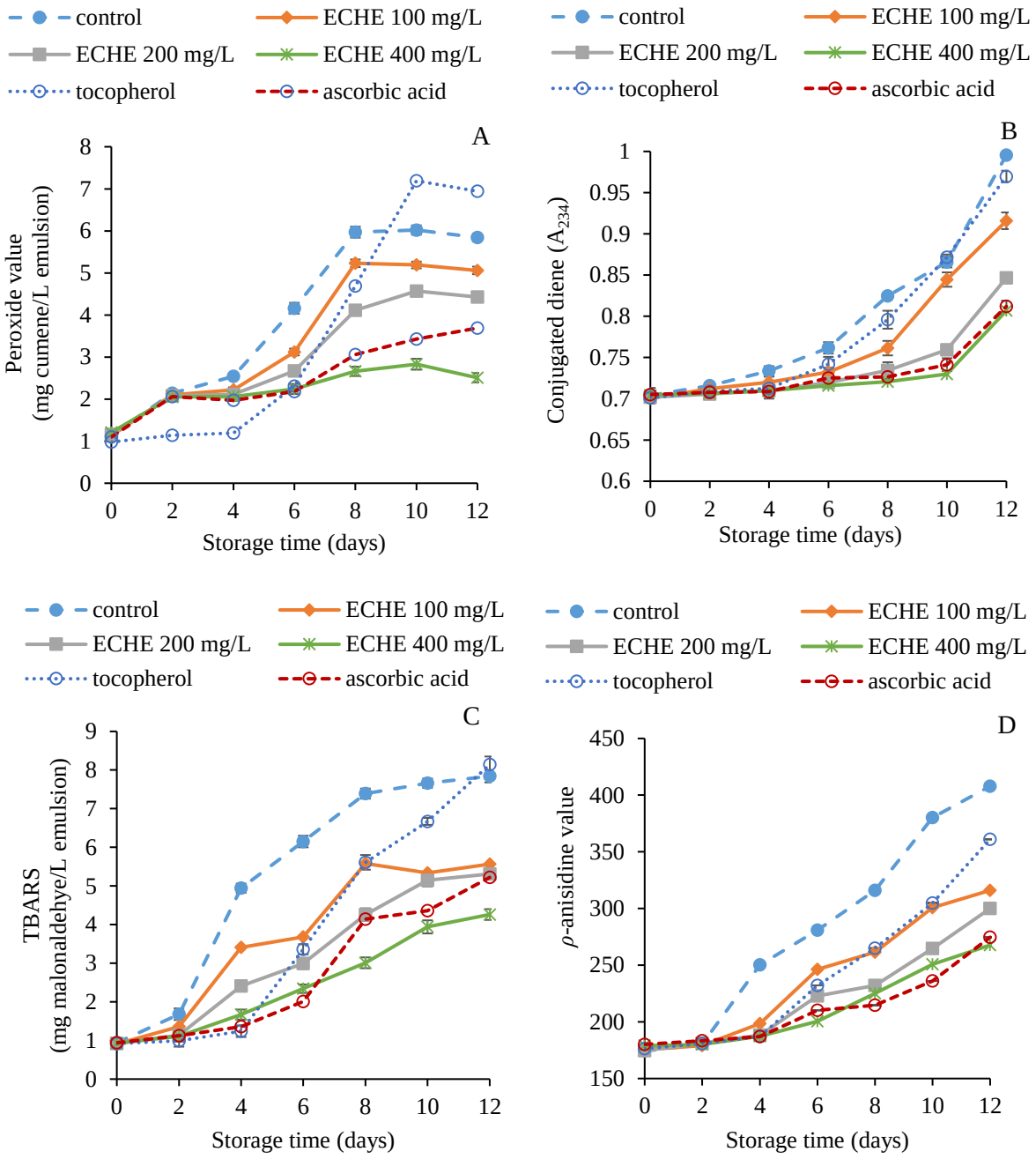


Figure 28. Effect of α-tocopherol, ascorbic acid and ECHE at different levels on the formation of lipid oxidation products in shrimp oil-in-water emulsion stored at 30 °C for a period of 12 days. Peroxide value (A), conjugated diene (B), thiobarbituric acid-reactive substances (TBARS) value (C) and *ρ*-anisidine value (D). Bars represent the standard deviation (*n*=3).

6.4.6.5 Fatty acid profiles

Fatty acid profiles of lipids extracted from shrimp oil-in-water emulsion added with and without ECHE at 200 mg/L stored for 0 and 12 days are shown in Table 14. At day 0, lipid from the emulsion contained 25.09% SAT, 28.99% MUFA and 33.38% PUFA. Lipids from shrimp oil-in-water emulsion contained linoleic acid (C18:2 *n*-6) as the most abundant fatty acid, followed by palmitic acid (C16:0) and oleic acid (C18:1 *n*-9), respectively. Shrimp oil from Pacific white shrimp hepatopancreas contained linoleic acid and oleic acid as the dominant fatty acids. It had EPA and DHA of 2.15 and 6.20 g/100 g lipids, respectively (Takeungwongtrakul *et al*., 2012). When the emulsion was stored for 12 days, the decreases in unsaturated fatty acids (MUFA and PUFA) of lipids from emulsion without ECHE addition were observed ($P<0.05$). DHA and EPA in the control sample decreased by 5.55 and 34.00%, respectively. Lower decreases in DHA and EPA were found in the sample added with ECHE, compared to the control (0.92 and 31.50%, respectively). Thiansilakul *et al.* (2010) reported that the decreases in DHA and EPA contents were related with their susceptibility to the oxidation during the extended storage. These results were in accordance with the lower primary and secondary oxidation products present in the emulsion added with ECHE, compare to the control (Fig. 28). The addition of oregano or rosemary extract into menhaden oil could lower the oxidation of PUFAs, in which EPA and DHA were much more maintained (Bhale *et al*., 2007). The result confirmed that ECHE was effective in retarding the lipid oxidation of shrimp oil-in-water emulsion, thereby preventing the formation of lipid oxidation products and loss in PUFAs. As a result, the oxidative deterioration of shrimp oil-in-water emulsion was retarded by the addition of ECHE.

Table 14. Fatty acid profiles of lipid extracted from shrimp oil in-water emulsion added with and without ECHE at day 0 and 12 of storage at 30 °C.

Fatty acids (g/100 g oil)	Day 0	Day 12	
		Without ECHE (200 mg/L)	With ECHE (200 mg/L)
C10:0	0.15 ± 0.01 *	ND	ND
C12:0	0.04 ± 0.01 c**	0.10 ± 0.01 a	0.07 ± 0.00 b
C14:0	0.81 ± 0.02 b	0.88 ± 0.01 a	0.87 ± 0.02 a
C14:1	0.19 ± 0.01 a	0.18 ± 0.00 a	0.19 ± 0.00 a
C15:0	0.37 ± 0.01 c	0.74 ± 0.02 a	0.48 ± 0.00 b
C16:0	19.86 ± 0.01b	19.99 ± 0.01 a	19.86 ± 0.01 b
C16:1 *n-7*	2.11 ± 0.02 a	1.99 ± 0.02 b	2.09 ± 0.01 a
C17:0	0.45 ± 0.01 c	0.65 ± 0.01 b	0.69 ± 0.01 a
C18:0	2.33 ± 0.01 c	3.43 ± 0.00 a	3.11 ± 0.01 b
C18:1 *n*-9	20.13 ± 0.01 b	19.42 ± 0.02 c	20.39 ± 0.00 a
C18:1 *n*-7	2.83 ± 0.01 a	2.30 ± 0.01 c	2.62 ± 0.01 b
C18:2 *n*-6	20.19 ± 0.00 a	19.12 ± 0.01 c	20.13 ± 0.00 b
C18:3 *n*-3 (ALA)	1.88 ± 0.02 a	1.28 ± 0.02 c	1.34 ± 0.02 b
C18:3 *n*-6	0.18 ± 0.01 c	0.27 ± 0.01 b	0.29 ± 0.00 a
C18:4 *n*-3	0.07 ± 0.00 b	0.10 ± 0.01 a	0.11 ± 0.01 a
C20:0	0.20 ± 0.01 b	0.33 ± 0.02 a	0.21 ± 0.00 b
C20:1 *n*-7	0.29 ± 0.00 a	0.27 ± 0.01 b	0.29 ± 0.01 a
C20:1 *n*-9	1.50 ± 0.01 a	1.32 ± 0.01 c	1.41 ± 0.00 b
C20:1 *n*-11	0.73 ± 0.01 a	0.63 ± 0.00 c	0.68 ± 0.00 b
C20:2 *n*-6	1.84 ± 0.01 a	1.68 ± 0.01 c	1.80 ± 0.02 b
C20:3 *n*-6	0.08 ± 0.00 b	0.12 ± 0.01 a	0.12 ± 0.00 a
C20:1 *n*-7	0.19 ± 0.00 c	0.27 ± 0.01 b	0.29 ± 0.01 a
C20:3 *n*-3	0.92 ± 0.02 a	0.62 ± 0.01 c	0.65 ± 0.01 b
C20:4 *n*-6 (ARA)	0.29 ± 0.02 a	0.27 ± 0.02 a	0.29 ± 0.03 a
C20:4 *n*-3	0.13 ± 0.01 b	0.19 ±0.01 a	0.20 ± 0.02 a
C20:5 *n*-3 (EPA)	2.00 ± 0.00 a	1.32 ± 0.01 b	1.37 ± 0.01 a
C21:0	0.12 ± 0.01 b	0.19 ± 0.00 a	0.11 ± 0.00 b
C22:0	0.20 ± 0.01 b	0.31 ± 0.00 a	0.21 ± 0.02 b
C22:1 *n*-9	0.19 ± 0.00 a	0.16 ± 0.01 b	0.16 ± 0.00 b
C22:1 *n*-11, *n*-13	0.79 ± 0.02 a	0.57 ± 0.01 c	0.61 ± 0.00 b
C22:2	0.02 ± 0.00 b	0.03 ± 0.00 a	0.03 ± 0.00 a
C22:4 *n*-6	0.16 ± 0.01 b	0.23 ± 0.01 a	0.25 ± 0.01 a
C22:5 *n*-6	0.32 ± 0.02 c	0.48 ± 0.01 b	0.51 ± 0.01 a
C22:6 *n*-3 (DHA)	5.41 ± 0.02 a	5.11 ± 0.01 c	5.36 ± 0.01 b
C23:0	0.12 ± 0.00 b	0.20 ± 0.01 a	0.19 ± 0.00 a
C24:0	0.43 ± 0.01 c	0.63 ± 0.01 b	0.67 ± 0.02 a
C24:1	0.22 ± 0.01 a	0.19 ± 0.00 b	0.19 ± 0.00 b
Saturated fatty acid (SFA)	25.09 ± 0.02 c	27.44 ± 0.03 a	26.47 ± 0.01 b
Monounsaturated fatty acid (MUFA)	28.99 ± 0.03 a	27.02 ± 0.04 c	28.64 ± 0.01 b
Polyunsaturated fatty acid (PUFA)	33.38 ± 0.01 a	30.80 ± 0.01 c	32.43 ± 0.02 b

* Values are given as means ± SD for triplicate determinations. ** Different lowercase letters in the same row indicate significant differences ($P<0.05$). ND: Non-detectable.

6.5 Conclusion

ECHE contained the condensed tannin as well as free phenolic compounds including tannic acid and catechin, etc. ECHE showed antioxidant activities with a dose-dependent manner as tested by various *in vitro* assays. Moreover, the use of ECHE could retard lipid oxidation and maintain polyunsaturated fatty acids in shrimp oil-in-water emulsion throughout 12 days of storage at 30 °C due to its antioxidant property. Therefore, ECHE could be used as a natural antioxidant, which prevented lipid oxidation in food emulsion system.

6.6 References

Balange, A. K. and Benjakul, S. 2009. Use of kiam wood extract as gel enhancer for mackerel (*Rastrelliger kanagurta*) surimi. Int. J. Food Sci. Technol. 44: 1661-1669.

Benzie, I. F. F. and Strain, J. J. 1999. Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Method. Enzymol. 299: 15–27.

Bhale, S. D., Xu, Z., Prinyawiwatkul, W., King, J. M. and Godber, J. S. 2007. Oregano and rosemary extracts inhibit oxidation of long-chain *n*-3 fatty acids in menhaden oil. J. Food Sci. 72: C504-508.

Boyer, R. F. and McClearly, C. J. 1987. Superoxide ion as a primary reductant in ascorbate-mediated ferritin iron release. Free Radic. Biol. Med. 3: 389-395.

Buamard, N. and Benjakul, S. 2015. Improvement of gel properties of sardine (*Sardinella albella*) surimi using coconut husk extracts. Food Hydrocolloids. 51: 146–155.

Buamard, N. and Benjakul, S. 2017. Cross-linking activity of ethanolic coconut husk extract toward sardine (*Sardinella albella*) muscle proteins. J. Food Biochem. 41: e12283.

Bucić-Kojić, A., Planinić1, M., Tomas, S., Jokić, S., Mujić, I., Bilić, M. and Velić, D. 2011. Effect of extraction conditions on the extractability of phenolic compounds from lyophilised fig fruits (*Ficus Carica* L.). Pol. J. Food Nutr. Sci. 61: 195-199.

Chaijan, M., Benjakul, S., Visessanguan, W. and Faustman, C. 2006. Changes of lipids in sardine (*Sardinella gibbosa*) muscle during iced storage. Food Chem. 99: 89-91.

Choe, E. and Min, D. B. 2005. Chemistry and reactions of reactive oxygen species in foods. J. Food Sci. 70: R142-159.

Chupin, L., Motillon, C., Bouhtoury, C., Pizzi, A. and Charrier, B. 2013. Characterization of maritime pine (*Pinus pinaster*) bark tannins extracted under different conditions by spectroscopic methods, FTIR and HPLC. Ind. Crop. Prod. 49: 897-903.

Colak, N., Torun, H., Gruz, J., Strnad, M., Subrtova, M., Inceer, H. and Ayaz, F. A. 2016. Comparison of phenolics and phenolic acid profiles in conjunction with oxygen radical absorbing capacity (ORAC) in berries of *Vaccinium arctostaphylos* L. and *V. myrtillus* L. Pol. J. Food Nutr. Sci. 66: 85-92.

Gharzouli, K., Khennouf, S., Amira, S., Gharzouli, A. 1999. Effects of aqueous extracts from *Quercus ilex* L. root bark, *Punica granatum* L. fruit peel and *Artemisia herba-alba* Asso leaves on ethanol-induced gastric damage in rats. Phytother. Res. 13: 42-45.

Ha, T. K. Q., Dao, T. T., Nguyen, N. H., Kim, J., Kim, E., Cho, T. O. and Oh, W. K. 2016. Antiviral phenolics from the leaves of *Cleistocalyx operculatus*. Fitoterapia. 110: 135-141.

Hoyos, M. N., Sánchez-Patán, F., Masis, R. M., Martín-Álvarez, P. J., Ramirez, W. Z., Monagas, M. J. and Bartolomé, B. 2015. Phenolic assessment of *Uncaria tomentosa* L. (Cat's Claw): Leaves, stem, bark and wood extracts. Molecules. 20: 22703-22717.

Kaewdang, O. and Benjakul, S. 2015. Effect of ethanolic extract of coconut husk on gel properties of gelatin from swim bladder of yellowfin tuna. LWT-Food Sci. Technol. 62: 955-961.

Kittiphatanabawon, P., Benjakul, S., Visessanguan, W. and Shahidi, F. 2012. Gelatin hydrolysate from blacktip shark skin prepared using papaya latex enzyme: Antioxidant activity and its potential in model systems. Food Chem. 135: 1118-1126.

Kucner, A., Papiewska, A., Klewicki, R., Sójka, M. and Klewicka, E. 2014. Influence of thermal treatment on the stability of phenolic compounds and the microbiological quality of sucrose solution following osmotic dehydration of highbush blueberry fruits. Acta. Sci. Pol. Technol. Aliment. 13: 79-88.

Liimatainen, J., Karonen, M., Sinkkonen, J., Helander, M., Salminen, J.-P. 2012. Phenolic compounds of the inner bark of *Betula pendula*: Seasonal and genetic variation and induction by wounding. J. Chem. Ecol. 38: 1410-1418.

Maqsood, S. and Benjakul, S. 2010. Comparative studies of four different phenolic compounds on *in vitro* antioxidative activity and the preventive effect on lipid oxidation of fish oil emulsion and fish mince. Food Chem. 119: 123-132.

Martysiak-Żurowska, D. and Wenta, W. 2012. A comparison of ABTS and DPPH methods for assessing the total antioxidant capacity of human milk. Acta Sci. Pol. Technol. Aliment. 11: 83-89.

McClements, D. J. and Decker, E. A. 2000. Lipid oxidation in oil-in-water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food systems. J. Food Sci. 65: 1270-1282.

Monfalouti, H. E., Charrouf, Z., Belviso, S., Ghirardello, D., Scursatone, B., Guillaume, D., Denhez, C. and Zeppa, G. 2012. Analysis and antioxidant capacity of the phenolic compounds from argan fruit (*Argania spinose* (L.) Skeels). Eur. J. Lipid Sci. Technol. 114: 446-452.

Nagarajan, M., Benjakul, S., Prodpran, T. and Songtipya, P. 2015. Properties and characteristics of nanocomposite films from tilapia skin gelatin incorporated with ethanolic extract from coconut husk. J. Food Sci. Technol. 52: 7669-7682.

Nurulhuda, M. N., Chew, L. T., Mohd, N. M. Y. and Abdul, R. M. A. 1990. Tannin properties of *Rhizophora mucronata* barks of different ages. Eur. J. Wood Prod. 10: 381-383.

Pacheco-Palencia, L. A., Mertens-Talcott, S. and Talcott, S. T. 2008. Chemical composition, antioxidant properties, and thermal stability of a phytochemical enriched oil from açai (*Euterpe oleracea* Mart.). J. Agric. Food Chem. 56: 4631-4636.

Pena-Ramos, E. A. and Xiong, Y. L. L. 2003. Whey and soy protein, hydrolysates inhibit lipid oxidation in cooked pork patties. Meat Sci. 64: 259-263.

Ramarathnam, N., Osawa, T., Ochi, H. and Kawakishi, S. 1995. The contribution of plant food antioxidants to human health. Trends Food Sci. Technol. 6: 75-82.

Rodrigues, S. and Pinto, G. A. S. 2007. Ultrasound extraction of phenolic compounds from coconut (*Cocos nucifera*) shell powder. J. Food Eng. 80: 869-872.

Rosales-Castro, M., González-Laredo, R. F., Bae, Y.-S., Kim, J. K., Morre, J. and Karchesy, J. J. 2014. Characterization and antioxidant properties of the condensed tannins from Alaska cedar inner bark. Rec. Nat. Prod. 8: 217-227.

Saad, H., Charrier-El Bouhtoury, F., Pizzi, A., Rode, K., Charrier, B. and Ayed, N. 2012. Characterization of pomegranate peels tannin extractives. Ind. Crop. Prod. 40: 239-246.

Shahidi, F. and Zhong, Y. 2005. Lipid oxidation: Measurement methods in Bailey's industrial oil and fat products. John Wiley and Sons, Inc. New Jersey.

Steel, R. G. D. and Torrie, J. H. 1980. Principles and procedures of statistics. McGraw-Hill Book, New York.

Takuengwongtrakul, S., Benjakul, S. and H-Kitiikun, A. 2012. Lipids from cephalothorax and hepatopancreas of Pacific white shrimp (*Litopenaeus vannamei*): Compositions and deterioration as affected by iced storage. Food Chem. 134: 2066-2074.

Takeungwongtrakul, S. and Benjakul, S. 2013. Oxidative stability of shrimp oil-in-water emulsions as affected by antioxidant incorporation. Int. Aquat. Res. 5: 14.

Thiansilakul, Y., Benjakul, S., Richards, M. P. 2010. Changes in heme proteins and lipids associated with off-odour of seabass (*Lates calcarifer*) and red tilapia (*Oreochromis mossambicus* × *O. niloticus*) during iced storage. Food Chem. 124: 1109-1119.

Vicente, S. J. V., Queiroz, Y. S., Gotlieb, S. L. D. and Silva Torres, E. A. F. 2014. Stability of phenolic compounds and antioxidant capacity of regular and decaffeinated coffees. Braz. Arch. Biol. Technol. 57:110-118.

Wahle, K. W., Brown, I., Rotondo, D. and Heys, S. D. 2010. Plant phenolics in the prevention and treatment of cancer. Adv. Exp. Med. Biol. 698: 36-51.

Wang, W. and Huang, G. 2009. Characterization and utilization of natural coconut fibres composites. Mater. Design. 30: 2741-2744.

Wettasinghe, M. and Shahidi, F. 2000. Scavenging of reactive-oxygen species and DPPH free radicals by extracts of borage and evening primrose meals. Food Chem. 70: 17–26.

CHAPTER 7

EFFECT OF ETHANOLIC COCONUT HUSK EXTRACT AND PRE-EMULSIFICATION ON PROPERTIES AND STABILITY OF SURIMI GEL FORTIFIED WITH SEABASS OIL DURING REFRIGERATED STORAGE

7.1 Abstract

The effects of ethanolic coconut husk extract (ECHE) at various levels (0-0.25%) and pre-emulsification on gel properties and stability of sardine (*Sardinella albella*) surimi gel fortified with seabass oil during refrigerated storage of 10 days were investigated. Addition of seabass oil pre-emulsified with soy protein isolate (SPI) in the presence of ECHE at levels of 0.20-0.25%, which had the average major mean diameter (d_{43}) of 17.18-33.01 µm, yielded surimi gel with the highest breaking force and had the increases in hardness, cohesiveness, gumminess and chewiness ($P<0.05$). Decrease in whiteness was found in surimi gel added with ECHE, especially with increasing ECHE levels ($P<0.05$). When surimi gel added with ECHE at different levels were stored at 4 °C for 10 days, lipid oxidation as determined by peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) of surimi gel was lowered as the levels of ECHE increased ($P<0.05$). Nevertheless, addition of ECHE did not affect total viable count and psychrophilic bacterial count in surimi gels. Thus, the incorporation of seabass oil pre-emulsified using SPI in combination with ECHE (0.2-0.25%) could improve textural properties and oxidative stability of surimi gels from sardine.

7.2 Introduction

Surimi is an important proteinaceous source, commonly used for preparing various products with nutritive values. The increasing popularity of surimi-based products is due to variety of products, especially form and texture. Although proteins become more concentrated and some lipids are removed during washing, the remaining lipids rich in n-3 polyunsaturated fatty acids (PUFA) are prone to oxidation, thus causing off-odor and reducing consumer acceptability. Recently, an increasing interest in the fortification of food products with n-3 PUFAs has been paid for health benefits

(Wang *et al*., 2015). n-3 PUFAs in fish oils play an important role in reducing the risk of a number of diseases, including atherosclerosis, coronary heart disease, hypertension, inflammation, diabetes, etc. Currently, Sae-leaw and Benjakul (2017) reported that oil extracted from seabass visceral depot fat, a byproduct from seabass evisceration, contained high amount of n-3 PUFAs, particularly eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3). It can be used as food supplement to increase the nutritional value of foods, especially surimi-based products. Chicken surimi prepared from spent hen breast protein was fortified with smelt oil at 10%. However, the surimi added with fish oil underwent higher lipid oxidation than those incorporated with flaxseed or soybean oil during storage at -15 to -10 °C (Wang *et al*., 2016). Thus, the addition of antioxidant is a common practice to prevent lipid oxidation. Another strategy to improve the oxidative stability of *n*-3 PUFAs-enriched foods is to protect the oil in a delivery system. Pre-emulsification technique, the prior preparation of fat mixture in emulsified form before introducing it into a meat product, is an approach to stabilize the lipid core in food matrix (Kang *et al*., 2017) Among the emulsifying agents, soy protein isolate at level of 0.5-1.0% was found to render the corn oil-in-water emulsion with higher stability, compared with milk casein (Hu et al., 2003). Cáceres *et al.* (2008) prepared bologna sausage fortified with pre-emulsified fish oil to give the oil levels of 1-6% in the final products, in which TBARS values was within the acceptable levels (0.1-0.3 mg MDA/kg). Pre-emulsified fish oil with sodium caseinate could therefore improve the oxidative stability of sausage (Cáceres *et al.*, 2008).

Coconut husk, a fibrous external portion of the fruit of coconut, is considered as underutilized natural resource (Panyakaew and Fotios, 2011). Various phenolic compounds have been found in coconut husk, namely tannic acid, gallic acid and catechin. Lignin precursors such as vanillic acid, *ρ*-coumaric acid and syringic acid were also identified (Buamard and Benjakul, 2017; Viju *et al*., 2013). Phenolic compounds have been known to possess antioxidant (Maqsood *et al*., 2013), antimicrobial and anticancer activities (Jose *et al*., 2014). Recently, ethanolic coconut husk extract (ECHE), containing phenolic compounds, have been demonstrated as protein cross-linking agents that were able to strengthen surimi gels (Buamard and

Benjakul, 2015). Moreover, ECHE at 200 mg/L played a role in retardation of lipid oxidation in shrimp oil-in-water emulsion and retained the *n*-3 fatty acids including EPA and DHA during 12 days of storage at 37 °C (Buamard and Benjakul, 2017). Pre-emulsification of fish oil in the presence of ECHE could introduce phenolics to be localized at oil droplet surface, hence acting as antioxidant more effectively at the interface. The incorporation of pre-emulsified fish oil using soy protein isolate containing ECHE could increase oxidative stability of surimi gel fortified with fish oil. Therefore, the objective of this study was to examine the effect of ECHE and pre-emulsification on properties and oxidative stability of surimi gel fortified with seabass oil extracted from visceral depot fat during refrigerated storage of 10 days.

7.3 Materials and methods

7.3.1 Chemicals and surimi

Cumene hydroperoxide was procured from Fluka (Buchs, Switzerland). Methanol and chloroform were purchased from RCI Lab-Scan (Bangkok, Thailand). 2-Thiobarbituric acid was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Trichloroacetic acid, anhydrous sodium sulfate and ferrous chloride were purchased from Merck (Darmstadt, Germany).

Frozen sardine surimi (B grade) was procured from Chaichareon Marine Co., Ltd. (Pattani, Thailand), placed in polyethylene bag and kept at -20 °C for not longer than 2 months.

7.3.2 Preparation of oil from seabass visceral depot fat

Oil extraction was performed according to the method of Sae-leaw and Benjakul (2017). Depot fat from seabass was placed in a round bottom flask equipped with a rotary evaporator (N-1000, EYELA, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) and subjected to heating under vacuum at 70 °C for 20 min with a continuous swirling. Subsequently, the resulting mixture was centrifuged at 10,000 ×g for 20 min at 4 °C using a refrigerated centrifuge (CR22N, Hitachi, Hitachi Koki Co., Ltd., Tokyo, Japan) in order to separate oil from residual debris. The oil was then collected and used for fortification into surimi gels.

7.3.3 Preparation of coconut husk powder

Husk of coconut (*Cocos nucifera* Linn.) fruit with the age of about 11 months was obtained from a local market in Hat Yai, Songkhla Province, Thailand and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai, Thailand. Husk sample was prepared as per the method of Buamard and Benjakul (2015). The prepared sample was sieved using a stainless-steel screen tray with a sieve size of 0.18 mm. The obtained powder was placed in a polyethylene bag, sealed and kept at room temperature (28–30 °C) until use.

7.3.4 Preparation of ethanolic coconut husk extract

Coconut husk powder (10 g) was subjected to extraction using 350 mL of 60% (v/v) ethanol as per the method of Buamard and Benjakul (2015). The extraction was conducted at room temperature for 3 h by continuous stirring, followed by filtration through a Whatman filter paper No.1 (Whatman International Ltd., Maidstone, UK). The filtrate was evaporated at 40 °C using an Eyela rotary evaporator (Tokyo Rikakikai, Co. Ltd., Tokyo, Japan) and purged with nitrogen gas to remove the residual ethanol. The extract was thereafter dried using a Scanvac Model Coolsafe 55 freeze dryer (Coolsafe, Lynge, Denmark) to obtain the dry extract. Dried extract was powdered using a mortar and pestle. The powder named ‘ECHE’ was transferred into an amber bottle, capped tightly and stored in a desiccator until use.

7.3.5 Effect of ECHE on gel properties and oxidative stability of surimi gel fortified with pre-emulsified seabass oil during refrigerated storage

7.3.5.1 Preparation of gels containing pre-emulsified seabass oil

7.3.5.1.1 Pre-emulsification

To prepare the pre-emulsified seabass oil, emulsifier solution was firstly prepared by dissolving soy protein isolate (SPI) in 10 mM phosphate buffer (pH 7) to obtain a concentration of 4% (w/v). The emulsifier solution (30 mL) was then mixed with seabass oil (10 mL) in the absence or presence of ECHE at various levels (0.25-1.25%,) using an IKA homogenizer (T 25 digital ULTRA-TURRAX®, IKA-Werke GmbH & Co. KG, Staufen, Germany) at a speed of 19,000 rpm for 5 min.

7.3.5.1.2 Particle size distribution

Particle size distribution of emulsions was determined using a laser particle size analyzer (LPSA) (Model LS 230, Beckman Coulter, Fullerton, CA, USA) as described by Castellani *et al.* (2006). A volume-weighted mean particle diameter (d_{43}) representing the mean diameter of an emulsion droplet with the same volume was recorded.

7.3.5.1.3 Confocal laser scanning microscopy (CLSM)

The microstructures of emulsion samples were examined with a CLSM (Model LSM 800; ZEISS, Jena, Germany). The samples were dissolved in Nile blue A solution (1:10) and manually stirred until uniformity was achieved. Fifty microliters of sample were smeared on the microscopy slide. The CLSM was operated in the fluorescence mode at the excitation wavelength of 533 nm and the emission wavelength of 630 nm using a Helium Neon Red laser (HeNe-R) for lipid analysis. A magnification of 200× was used.

7.3.5.1.4 Preparation of surimi gel containing pre-emulsified seabass oil and ECHE

Frozen sardine surimi was thawed by running water until the core temperature reached 0−2 °C. The surimi was cut into small piece and mixed with 2.5% salt in a mixer (National Model MK-5080M, Selangor, Malaysia) for 2 min. During chopping, the temperature was maintained below 10 °C. All prepared emulsions (40 mL) were added into surimi paste (200 g) to obtain the final seabass oil level of 5% (based on surimi gel) and ECHE at the final levels of 0.05-0.25% (based on surimi gel) during chopping and mixing for 3 min. The moisture content of surimi paste was finally adjusted to 80% with cold distilled water. Subsequently, the mixture was chopped for 30 s, followed by 10 s of a rest interval for a total time of 3 min to avoid heat generated. The paste was stuffed into a polyvinylidene chloride casing with a diameter of 2.5 cm. Both ends were sealed tightly. The prepared samples were incubated at 40 °C for 30 min, followed by heating at 90 °C for 20 min. All gels were then cooled in iced water for 30 min and stored at 4 °C for 18−24 h. Surimi gels containing pre-emulsified seabass oil in the absence or presence of ECHE at various levels were packaged separately in

zip lock bags and stored at 4 °C. Gel samples were randomly taken every 2 days up to 10 days for analyses, except textural profile analysis which was performed at day 0, 6 and 10 of storage.

7.3.5.2 Analyses

7.3.5.2.1 Breaking force and deformation

Breaking force and deformation of surimi gels were determined using a texture analyzer (Model TA-XT2, Stable Micro Systems, Surrey, UK), following the method of Benjakul *et al.* (2004). Surimi gels were equilibrated at room temperature before analysis for 1 h. Cylindrical samples (2.5 cm in diameter and 2.5 cm in length) were prepared. A spherical probe with a diameter of 5 mm was pressed into the cut surface of a gel specimen perpendicularly at a constant depression speed of 60 mm min^{-1} until the puncture took place. The breaking force was defined as the force required in grams to break the gel, and the breaking deformation represented the distance in millimeters travelled by probe from surface of the gel at the point of breakage.

7.3.5.2.2 Texture profile analysis

Texture profile analysis (TPA) of the gels was carried out according to Singh and Benjakul (2017). Cylindrical gels (2.5 cm in diameter and 2.5 cm in length) were used for the TPA measurement. Gel samples were subjected to two-cycle compression at 50% compression using the texture analyzer with a 70 mm TPA compression plate attachment moving at a speed of 127 mm/min. From the force–time curves, hardness, springiness, cohesiveness, chewiness and resilience were calculated.

7.3.5.2.3 Whiteness

Whiteness of gel samples was determined using a colorimeter (HunterLab, Colorflex, Hunter Associates Laboratory, Reston, VA, USA). CIE L^*, a^* and b^* values were measured and whiteness was then calculated using the following equation:

$$\text{Whiteness} = 100-[(100-L^*)^2 + a^{*2} + b^{*2}]^{1/2}$$

where L^* is the lightness; a^* is the redness/greenness; and b^* is the yellowness/blueness.

7.3.5.2.4 Peroxide value (PV)

PV was determined according to the method of Richards and Hultin (2002) as adopted by Vate *et al.* (2015). A standard curve was prepared using cumene hydroperoxide at the concentration range of 0.5-2 mg/L. PV was expressed as milligrams of hydroperoxide equivalents per kilogram of surimi gel.

7.3.5.2.5 Thiobarbituric acid reactive substances (TBARS)

TBARS of gel samples were determined using the distillation method following the method of Vate *et al.* (2015). Distillate was reacted with TBA reagent and blank was also prepared. After heating, the reaction mixture was cooled in water for 10 min and the absorbance at 532 nm was measured. A standard curve was prepared using 1,1,3,3-tetramethoxypropane at the concentration range of 0-6 mg/L. TBARS were calculated and expressed as mg malonaldehyde/kg surimi.

7.3.5.2.6 Microbiological analyses

Microbiological analyses were performed by the spread plate method (Sallam, 2007). Surimi gels (25 g) were chopped into a small piece and mixed with 225 mL of 0.85% sterile saline solution. The samples were then homogenized in a Stomacher blender (Model 400, Seward Ltd., Worthington, UK) for 1 min at 220 rpm. For all homogenates, ten-fold serial dilutions were made using 0.85% saline solution and appropriate dilutions (0.1 mL) were used for the microbiological analyses. Total viable count (TVC) was determined using plate count agar (PCA) with the incubation at 35 °C for 2 days (Maturin and Peeler, 1998). Psychrophilic bacteria count (PBC) was enumerated using PCA and the plates were incubated at 4 °C for 10 days.

7.3.6 Statistical analysis

All experiments were performed in triplicate using three different lots of samples. The data were subjected to one-way analysis of variance (ANOVA). Comparison of means was carried out using the Duncan's multiple range test. Statistical analysis was done using the Statistical Package for Social Science (SPSS 11.0 for

windows, SPSS Inc., Chicago, IL, USA). Differences between means at the 5% ($P<0.05$) level were considered significant.

7.4 Results and discussion

7.4.1 Characteristics of pre-emulsified seabass oil

The particle size distribution of seabass oil pre-emulsified with soy protein isolate (SPI) in the absence and presence of ECHE at various levels is depicted in Fig. 29A. Particle size (d_{43}) of all the emulsions, regardless of ECHE incorporation, showed a bimodal distribution (Fig. 29A), suggesting that emulsion droplets were varied in sizes with two distinct oil droplet sizes. The mean diameters of the sample without ECHE were 1.52-2.43 and 18.86-22.73 μm as the minor and major mean diameter, respectively. D_{43} of samples added with 0.20% were in the range of 1.67-3.02 and 17.18-24.95 for minor and major mean diameters, respectively (data not shown). When 0.25% ECHE was present, the emulsion had a minor mean diameter range of 1.83-3.06 μm with a major mean diameter of 18.86-33.01 μm, respectively. It was found that oil droplet sizes were similar, irrespective of ECHE levels used, probably due to the same emulsification condition used for all samples. Nevertheless, droplet aligned more closely or become crowded as the level of ECHE increased (Fig. 29B). When ECHE was incorporated in the resulting emulsion at a higher level, the excessive phenolics in the ECHE more likely acted as protein cross-linker, which could connect proteins localized at the interface. As a consequence, the droplets were more linked each other. Friesen *et al.* (2015) reported that phenolic compounds such as rutin or epicatechin could induce cross-linking of film prepared from soy protein. Thus, free phenolics could affect the agglomeration of oil droplets in pre-emulsified oil as evidence by the increased particulate cluster formation of oil droplet.

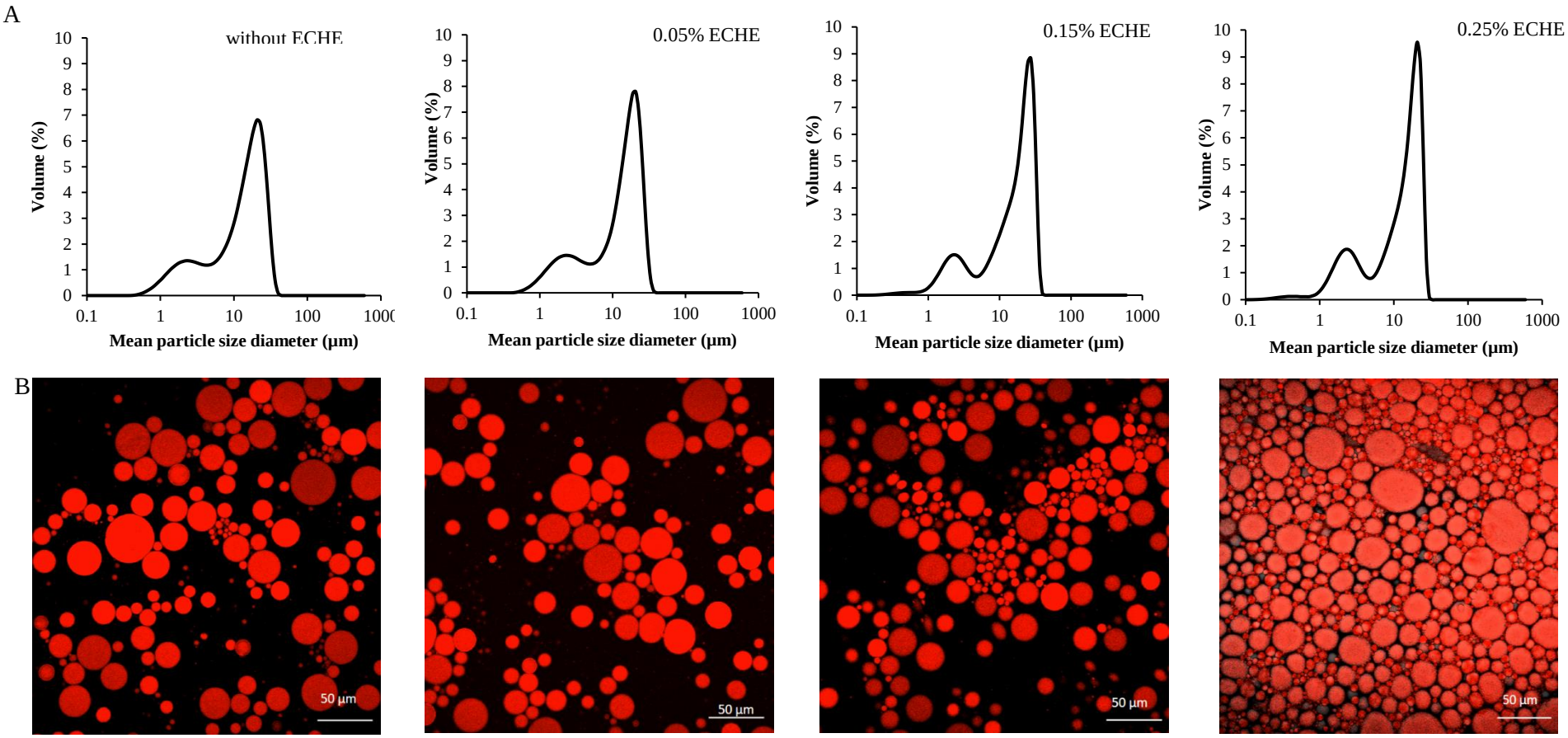


Figure 29. Particle size distribution (A) and microstructures (B) of seabass oil pre-emulsified using soy protein isolate in the absence and presence of ECHE at different levels.

7.4.2 Effect of ECHE on gel properties of surimi gel fortified with pre-emulsified seabass oil during refrigerated storage

7.4.2.1 Breaking force and deformation

Breaking force and deformation of surimi gel fortified with seabass oil pre-emulsified using soy protein isolate (SPI) in the absence and presence of ECHE at various levels are shown in Fig. 30 At day 0, the highest breaking force was found in sample added with ECHE at a concentration of 0.25% ($P<0.05$). Nevertheless, there was no difference between the sample added with ECHE at 0.20 and 0.25% ($P>0.05$). Emulsion surimi gels added with ECHE generally had higher breaking force than those without ECHE. The result suggested that ECHE could enhance gel strength of emulsion surimi gel. ECHE containing phenolic compounds, which were able to interact with the protein in surimi via some bondings such as hydrogen bonding, hydrophobic interaction and covalent bonding (Buamard and Benjakul, 2015). These phenolic compounds possess hydrogen donors that potentially form hydrogen bonds with carboxyl groups of proteins (Buitimea-Cantúa *et al*., 2017). In the present study, ECHE was incorporated into pre-emulsified oil using soy protein isolate as emulsifier. Thus, ECHE was mostly localized at oil interface. However, some ECHE might liberate to surimi during chopping of pre-emulsified oil with surimi paste. Liu *et al*. (2017) reported that tannins could form a covalent interaction with SPI via pyrocatechol oxidation on tannins B ring. Furthermore, SPI has been known to exhibit the protease inhibitory activity in surimi. This might also contribute to the increased gel strength. Luo *et al.* (2008) reported that the use of SPI could deplete the development of modori or gel weakening in silver carp surimi. In addition, setting at 40 °C was implemented for all the gels, in which non-disulfide covalent bond formation mediated by indigenous transglutaminase occurred, thus strengthening the gel (Benjakul *et al.*, 2003). For deformation, the addition of ECHE up to 0.10% had no impact on the values ($P>0.05$). Nevertheless, the deformation decreased when ECHE at 0.15-0.25% was incorporated *(*$P<0.05$). This was in line with the increased breaking force. Gels with high rigidity governed by strong bonds had the less deformability or elasticity as indicated by the decreased deformation.

During the extended storage of surimi gel added with pre-emulsified seabass oil, the gel without ECHE had the slight increase in breaking force up to day 6 ($P<$ 0.05), followed by slight decrease until the end of storage. However, breaking force of emulsion gels containing ECHE remained constant until the end of storage (day 10). For emulsion gels, free oils, which were prone to oxidation, might contain lipid oxidation products, especially aldehydes or ketones. Those compounds were able to induce the protein cross-linking as evidenced by increased breaking force. When the oil was pre-emulsified by proteins, the oil was less exposed to the air, thereby having the lower oxidation, particularly in the presence of ECHE possessing antioxidant activity.

For deformation, there was no difference between gels added without or with ECHE until day 4 of storage ($P>0.05$). Subsequently, slight changes in deformation were found at varying extents, depending on samples (Fig. 30B). As the storage time increased, higher proteolysis mediated by microbial proteases might contribute to less deformation. Therefore, pre-emulsification of seabass oil as well as ECHE had the impact on textural properties of sardine surimi gel during extended storage to some degrees.

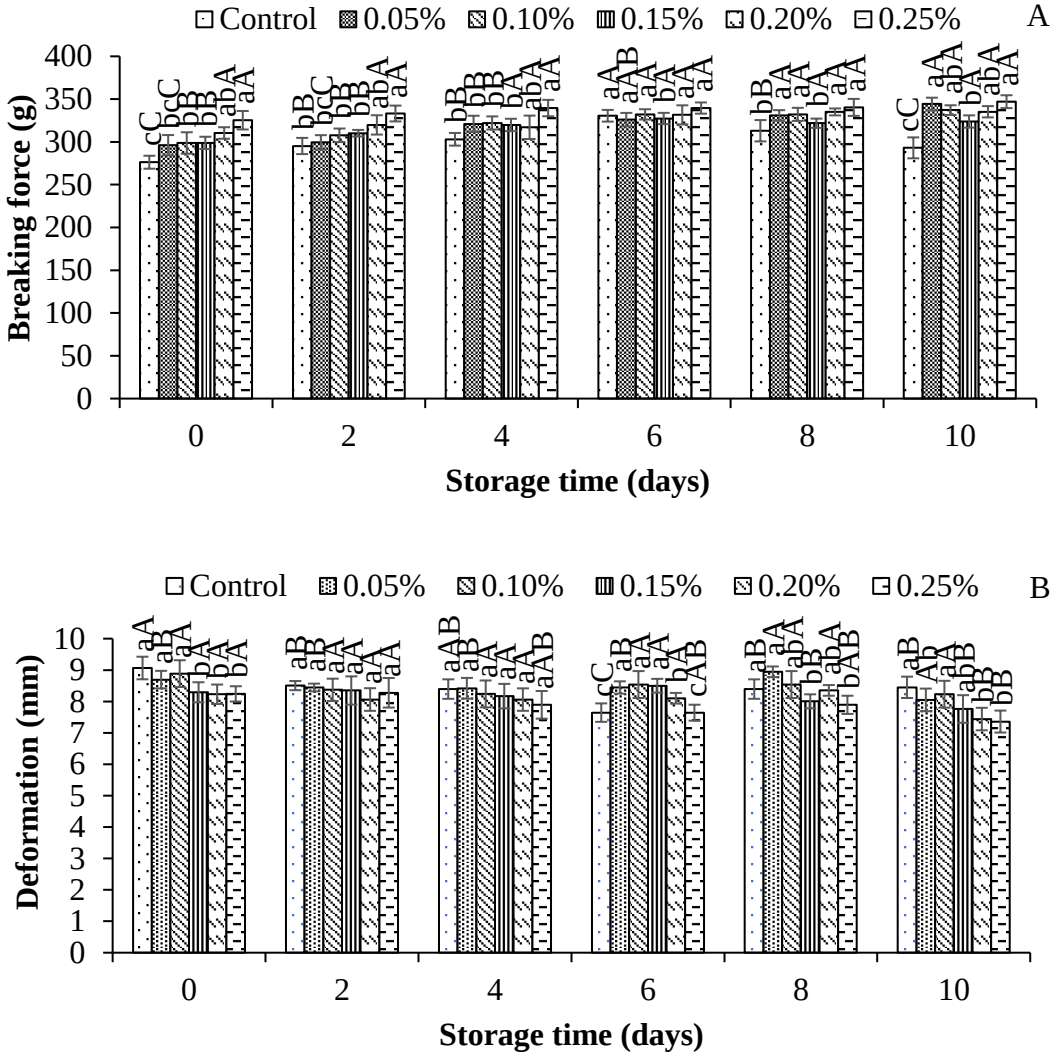


Figure 30. Changes in breaking force (A) and deformation (B) of surimi gels fortified with pre-emulsified seabass oil in the absence and presence of ECHE at different levels during the refrigerated storage at 4 °C for 10 days. Bars represent the standard deviation (n=3). Different lowercase letters on the bars within the same storage time indicate significant differences (P<0.05). Different uppercase letters on the bars within the same sample indicate significant differences (P<0.05).

7.4.2.2 Textural properties

Hardness, gumminess and chewiness of surimi gels fortified with pre-emulsified seabass oil were increased as ECHE levels were increased in all samples (P<0.05) as shown in Table 15. This was in agreement with the increased

breaking force of gel samples added with ECHE at higher levels (Fig. 30A). No marked changes in cohesiveness, capability to breakdown the internal structure, were noticeable as a function of storage time. Hardness, representing the force required to compress sample to attain a given deformation, of the emulsion gel was increased as a level of ECHE used increased ($P<0.05$). With increasing ECHE levels in pre-emulsified oil, cross-linking of protein in surimi was more pronounced. Furthermore, SPI has been reported to act as protease inhibitor in surimi (Luo *et al*., 2008). Hence, the degradation of protein in the gel was lowered. Gels added with pre-emulsified oil containing 0.15% ECHE or higher level had lower springiness, elastic recovery that occurs when the compressive force is removed ($P<0.05$). This was in line with the lower deformation when ECHE levels increased. For gumminess, the energy used for a semi-solid food for swallowing, and chewiness, the energy used for chewing the sample to the point that is able to swallow it, gels added with pre-emulsified oil containing ECHE showed higher values, especially when the levels of ECHE were increased ($P<0.05$). Therefore, the addition of ECHE in pre-emulsified oil had the impact on textural property of resulting sardine surimi gel.

During the storage, hardness, gumminess and chewiness of the control gel (without ECHE) increased at day 6 ($P<0.05$). However, at day 10, the decreases in those aforementioned parameters were observed ($P<0.05$). Protease might play an important role in proteolysis, which induced the degradation of protein network. For the emulsion gels, particularly those containing ECHE, no marked changes were noticeable after 6 days of storage ($P>0.05$). Phenolic compounds in ECHE more likely cross-linked the proteins. Those protein aggregates were less prone to hydrolysis. As a result, textural properties were maintained during the extended storage.

Table 15. Textural profiles of surimi gels fortified with pre-emulsified seabass oil in the presence or absence of ECHE at different levels during the refrigerated storage at 4 °C for 10 days.

ECHE levels (g/100 g)	Storage time (days)	Attributes				
		Hardness (N)	Springiness (cm)	Cohesiveness (Ratio)	Gumminess (N)	Chewiness (N cm)
0	0	23.34 ± 1.28^{dB}	0.79 ± 0.06^{abA}	0.87 ± 0.03^{cB}	21.62 ± 1.53^{dB}	18.98 ± 2.14^{cB}
	6	40.38 ± 2.01^{bA}	0.70 ± 0.03^{cB}	0.92 ± 0.01^{aA}	37.14 ± 1.22^{bA}	26.99 ± 1.98^{bA}
	10	22.19 ± 1.46^{dB}	0.69 ± 0.02^{bB}	0.86 ± 0.05^{bB}	20.08 ± 0.99^{cB}	14.96 ± 1.16^{dC}
0.05	0	24.81 ± 0.95^{dB}	0.81 ± 0.03^{aA}	0.90 ± 0.03^{bA}	22.37 ± 2.13^{dB}	18.76 ± 1.19^{cB}
	6	39.71 ± 1.77^{bA}	0.82 ± 0.04^{aA}	0.92 ± 0.04^{aA}	36.53 ± 3.02^{bA}	29.95 ± 2.27^{abA}
	10	41.22 ± 0.89^{bA}	0.77 ± 0.02^{aB}	0.95 ± 0.01^{aA}	39.16 ± 3.71^{bA}	30.15 ± 2.74^{bA}
0.10	0	34.36 ± 1.37^{cB}	0.84 ± 0.04^{aA}	0.91 ± 0.03^{bA}	33.91 ± 2.42^{cB}	29.27 ± 2.15^{bA}
	6	40.96 ± 0.81^{bA}	0.83 ± 0.02^{aA}	0.94 ± 0.02^{aA}	38.50 ± 2.96^{bA}	31.96 ± 3.08^{aA}
	10	39.97 ± 1.12^{bA}	0.80 ± 0.03^{aA}	0.96 ± 0.02^{aA}	38.37 ± 2.24^{bA}	30.70 ± 2.54^{bA}
0.15	0	36.18 ± 1.82^{cA}	0.77 ± 0.01^{bA}	0.95 ± 0.02^{aA}	35.81 ± 3.22^{bcA}	26.31 ± 1.43^{bA}
	6	38.36 ± 2.18^{bA}	0.75 ± 0.06^{abA}	0.90 ± 0.03^{aA}	34.52 ± 3.16^{bA}	24.81 ± 1.50^{bA}
	10	37.44 ± 0.97^{cA}	0.73 ± 0.05^{abA}	0.94 ± 0.01^{aA}	35.19 ± 2.19^{bA}	23.58 ± 2.06^{cA}
0.20	0	39.95 ± 1.79^{bA}	0.72 ± 0.03^{cA}	0.95 ± 0.01^{aA}	38.53 ± 3.69^{bA}	30.61 ± 3.99^{abA}
	6	41.79 ± 1.00^{aA}	0.73 ± 0.02^{bA}	0.95 ± 0.04^{aA}	39.70 ± 3.33^{bA}	31.63 ± 2.47^{aA}
	10	42.00 ± 0.85^{bA}	0.72 ± 0.05^{abA}	0.94 ± 0.03^{aA}	39.48 ± 2.08^{bA}	30.40 ± 2.63^{bA}
0.25	0	49.92 ± 1.49^{aA}	0.70 ± 0.02^{cA}	0.94 ± 0.03^{aA}	46.07 ± 3.15^{aA}	35.47 ± 3.26^{aA}
	6	49.58 ± 2.13^{aA}	0.69 ± 0.04^{cA}	0.96 ± 0.02^{aA}	47.59 ± 3.52^{aA}	35.69 ± 3.06^{aA}
	10	50.04 ± 1.91^{aA}	0.67 ± 0.05^{bA}	0.95 ± 0.04^{aA}	47.53 ± 3.36^{aA}	34.81 ± 2.97^{aA}

*Values are expressed as mean ± SD (n = 3). Different lowercase superscripts in the same column within the same storage time indicate significant differences (P<0.05). Different uppercase superscripts in the same column within the same ECHE level indicate significant differences (P<0.05).

7.4.2.3 Whiteness

Whiteness of gel fortified with pre-emulsified seabass oil in the absence or presence of ECHE at different levels during the storage of 10 days is shown in Table 16. Among all samples, gel without ECHE showed the highest whiteness ($P<0.05$). Decrease in whiteness was noticeable when gel was incorporated with pre-emulsified oil containing higher level of ECHE. ECHE was brownish in color and it resulted in browner pre-emulsified oil. This resulted in the less whiteness in resulting gels. The whiteness of all gels decreased as the storage time increased ($P<0.05$). Occurrence of lipid oxidation product also caused the discoloration of surimi gel (Li *et al*., 2016). Yu *et al*. (2005) reported that discoloration could be due to non-enzymatic browning reactions between lipid oxidation products and the amine in the phospholipid head groups or the amine in protein. This resulted in the lower whiteness during refrigerated storage of emulsion surimi gel. Naturally, sardine muscle had high content of myoglobin (Chaijan *et al*., 2004). When myoglobin undergoes autooxidation to the ferric form or metmyoglobin, the dark brown color is also generated. It was noted that emulsion gel samples containing ECHE showed a slow decrease in whiteness, especially when ECHE at a higher level was incorporated ($P<0.05$). Thiansilakul *et al*. (2012) reported that tannic acid at 200 mg/L effectively prevented the discoloration of bighead carp mince containing myoglobin. This might be related with the lower oxidation of lipids and myoglobin in samples added with ECHE. As a consequence, the discoloration associated with lipid and myoglobin oxidation might be decreased. Therefore, ECHE could prevent the decrease in whiteness of emulsion surimi gel during the extended storage.

Table 16. Whiteness of surimi gels fortified with pre-emulsified seabass oil in the presence or absence of ECHE at different levels during refrigerated storage at 4 °C for 10 days.

ECHE levels (%)	Storage time (days)					
	0	2	4	6	8	10
0	73.63 ± 0.22^{aA}	73.01 ± 0.16^{aB}	70.98 ± 0.09^{cC}	69.66 ± 0.17^{cD}	68.45 ± 0.13^{cE}	65.08 ± 0.13^{bF}
0.05	72.24 ± 0.24^{bA}	72.30 ± 0.09^{bA}	72.22 ± 0.09^{aA}	71.03 ± 0.11^{aB}	70.34 ± 0.09^{aC}	70.18 ± 0.16^{aC}
0.10	70.70 ± 0.19^{cA}	70.68 ± 0.18^{cA}	70.52 ± 0.11^{bA}	70.35 ± 0.15^{bAB}	69.59 ± 0.21^{bB}	69.38 ± 0.08^{aB}
0.15	68.12 ± 0.21^{dA}	68.09 ± 0.13^{dA}	67.88 ± 0.08^{dA}	67.97 ± 0.11^{dA}	67.76 ± 0.08^{dB}	67.57 ± 0.13^{cB}
0.20	65.22 ± 0.31^{eA}	65.38 ± 0.17^{eA}	65.26 ± 0.14^{cA}	64.79 ± 0.29^{cB}	64.75 ± 0.15^{eB}	64.63 ± 0.21^{dB}
0.25	64.78 ± 0.20^{eA}	64.65 ± 0.16^{fA}	64.44 ± 0.20^{fA}	64.15 ± 0.18^{fAB}	64.03 ± 0.17^{fB}	63.99 ± 0.11^{eB}

*Values are expressed as mean ± SD (n = 3). Different lowercase superscripts in the same column indicate significant differences (P<0.05). Different uppercase superscripts in the same row indicate significant differences (P<0.05).

7.4.3 Oxidative stability

Peroxide values (PV) of surimi gels fortified with pre-emulsified seabass oil without and with ECHE at different levels are shown in Fig. 31A. At day 0, there was no differences in PV between the samples added with ECHE (P>0.05), in which slightly higher PV was found in the sample without ECHE (P<0.05). PV of emulsion surimi gel was lower as the level of ECHE increased at all storage times tested (P<0.05), except at day 10, in which the sample added with 0.05% ECHE had higher PV than that without ECHE (P<0.05). Seabass oil from visceral depot fat was rich in long chain polyunsaturated fatty acids (PUFAs), particularly EPA and DHA. Those fatty acids are prone to lipid oxidation. As a consequence, the development of undesirable off-flavors occurred (Sae-leaw and Benjakul, 2017). Apart from causing negative effect on the quality and shelf-life of food products, lipid oxidation also lowers its nutritional value (Hęś and Gramza-Michałowska, 2017). For gel containing pre-emulsified seabass oil in the presence of ECHE at higher levels, the lower PV was observed throughout 10 days of refrigerated storage (P<0.05). Since ECHE was added

into pre-emulsified oil, phenolic compounds could locate close to oil droplets and functioned as antioxidant effectively. The result suggested that ECHE could retard the formation of primary lipid oxidation products in emulsion surimi gel during the extended storage. Nevertheless, at day 8, PV of the control gel and that added with ECHE at a level of 0.25% decreased ($P<0.05$). This was plausibly due to the decomposition of hydroperoxides to the low molecular weight secondary lipid oxidation products, including aldehydes, ketones, alcohols, etc. (Bosselli *et al*., 2005).

Changes in TBARS of surimi gels fortified with pre-emulsified seabass oil in the absence and presence of ECHE at different levels are depicted in Fig. 31B. At day 0, TBARS were lower as the level of ECHE was higher than 0.10% ($P<0.05$). ECHE at higher level more likely prevented oxidation of seabass oil during gel preparation. During the first 4 days, the decrease in TBARS of samples added with ECHE at 0.20 and 0.25% decreased continuously. This might be associated with the loss in volatile lipid oxidation products. The sample without ECHE showed the noticeable increase in TBARS up to 6 day of storage time. This was plausibly due to the decomposition of hydroperoxides into secondary lipid oxidation products. Thereafter, TBARS were decreased until the end of storage. TBARS of samples added ECHE was lower than that without ECHE at all storage time. At higher level of ECHE, the lower TBARS values were obtained ($P<0.05$).

The oxidative stability of emulsion surimi gel was increased when ECHE was added, due to the antioxidant activity of ECHE. ECHE was reported to possess antioxidant activities, including 2,2′-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activities, ferric reducing antioxidant power and chelating activity (Buamard and Benjakul, 2017). Moreover, pre-emulsification might facilitate the localization of ECHE at the interface. As a consequence, ECHE could act as antioxidant more potentially. Thus, the preparation of pre-emulsified seabass oil in the presence of ECHE prior to the addition into surimi gel could be an effective means to improve oxidative stability of emulsion surimi gel rich in PUFA during the extended storage.

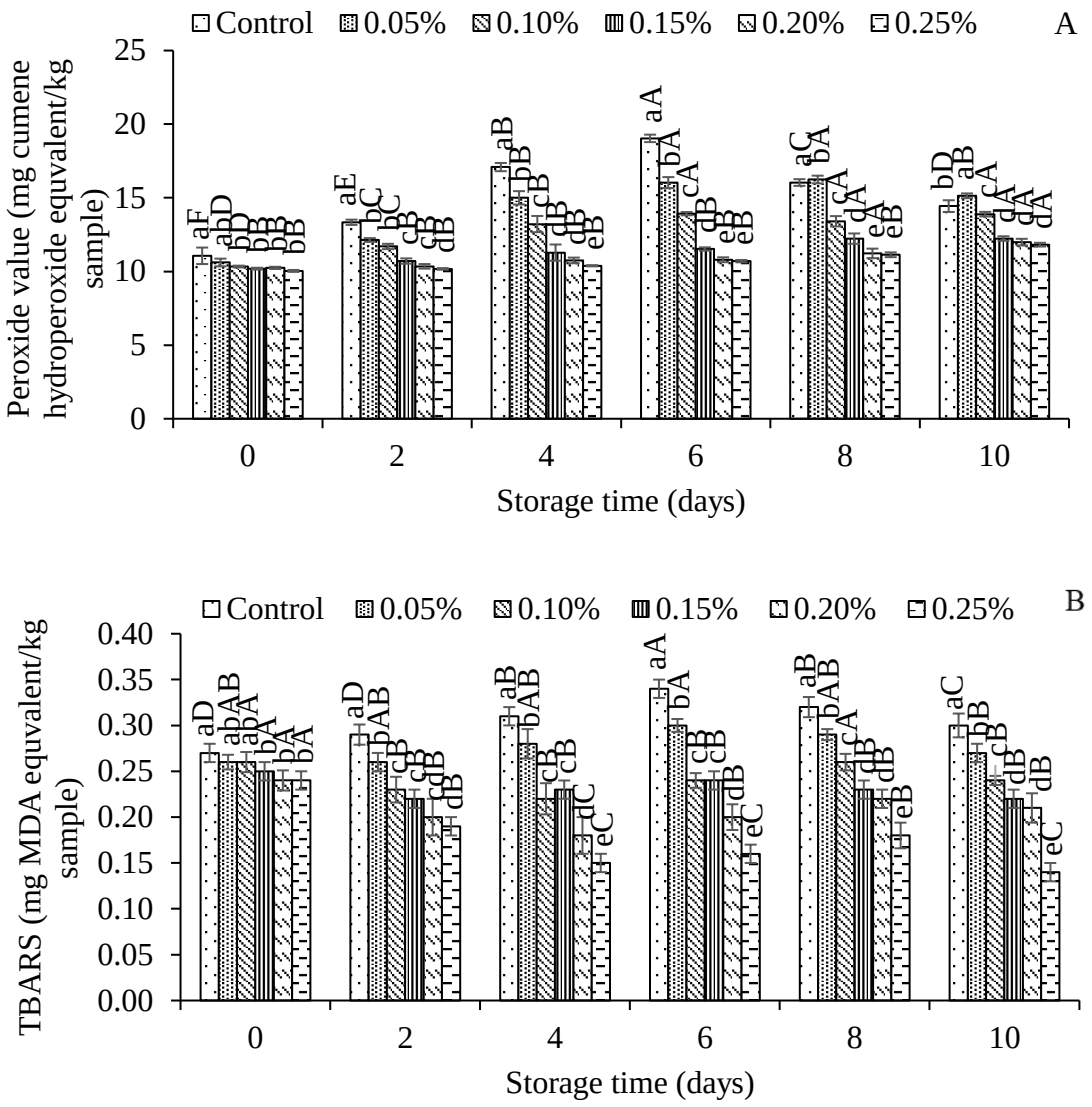


Figure 31. Changes in PV (A) and TBARS (B) of surimi gels fortified with pre-emulsified seabass oil in the absence and presence of ECHE at different levels during the refrigerated storage at 4 °C for 10 days. Bars represent the standard deviation (n=3). Different lowercase letters on the bars within the same storage time indicate significant differences (P<0.05). Different uppercase letters on the bars within the same sample indicate significant differences (P<0.05).

7.4.4 Microbiological quality

The initial TVC for all samples were 2.29-3.37 log CFU/g. After 10 days of storage, TVC were in the range of 4.57-4.69 log CFU/g, which were lower than the

limit. The maximal limit of 6 log CFU/g is recommended for TVC in fish and fish products (Sanjee and Karim, 2016). PBC of all samples at day 0 was found ranging from 2.09 to 2.19 log CFU/g. At the end of storage (day 10), PBC with the range of 4.71-4.93 log CFU/g were attained. At the same storage time, there were no differences in both TVC and PBC among all the samples ($P>0.05$). The psychrophilic bacterial count is one of major microorganisms that are responsible for spoilage of aerobically stored fish at chilled temperatures (Odeyemi *et al*., 2018). Bacterial growth is related with the spoilage of fish and fish products. The addition of emulsified seabass oil and ECHE did not affect TVC and PBC in emulsion surimi gels. Polyphenols might become less available when pre-emulsified or embedded in surimi gel matrix. As a result, the antimicrobial activity of ECHE was negligible, thus having the low impact on microbial inhibition in emulsion gels.

Table 17. Total viable count (TVC) and psychrophilic bacterial count (PBC) of surimi gels fortified with pre-emulsified seabass oil in the presence or absence of ECHE at different levels before and after storage at 4 °C for 10 days.

Microbial	Storage time (days)	ECHE levels (%)					
		0	0.05	0.10	0.15	0.20	0.25
TVC	0	2.29±0.09*,aB	2.31±0.09aB	2.37±0.08aB	2.34±0.04aB	2.30±0.08aB	2.35±0.05aB
	10	5.66±0.14aA	5.69±0.09aA	5.70±0.04aA	5.51±0.10aA	5.64±0.07aA	5.67±0.03aA
PBC	0	2.19±0.03aB	2.14±0.18aB	2.10±0.05aB	2.15±0.11aB	2.09±0.11aB	2.13±0.12aB
	10	4.89±0.09aA	4.87±0.05aA	4.91±0.09aA	4.86±0.11aA	4.93±0.11aA	4.71±0.18aA

*Values are expressed as CFU/g (n = 3).

Different lowercase superscripts in the same row indicate significant differences (P<0.05). Different uppercase superscripts in the same column within the same count tested indicate significant differences (P<0.05).

7.5 Conclusion

Pre-emulsification of seabass oil in the presence of ECHE before adding into surimi gel was an effective method to lower deterioration caused by lipid oxidation, especially when ECHE was used at a higher level. Addition of pre-emulsified seabass oil along with ECHE yielded surimi gel with the increased breaking force with lowered changes in whiteness during the storage. Nonetheless, pre-emulsification without and with ECHE had no effect on microbial load of emulsion gels. Therefore, surimi gel fortified with seabass oil possessing improved gel property and oxidative stability could be prepared using pre-emulsification in conjunction with ECHE.

7.6 References

Benjakul, S., Chantarasuwan, C. and Visessanguan, W. 2003. Effect of medium temperature setting on gelling characteristics of surimi from some tropical fish. Food Chem. 82: 567-574.

Benjakul, S., Visessanguan, W., Tueksuban, J. and Tanaka, M. 2004. Effect of some protein additives on proteolysis and gel-forming ability of lizardfish (*Saurida tumbil*). Food Hydrocolloid. 18: 395-401.

Boselli, E., Caboni, M. F., Rodriguez-Estrada, M. T., Toschi, T. G., Daniel, M. and Lercker, G. 2005. Photoxidation of cholesterol and lipids of turkey meat during storage under commercial retail conditions. Food Chem. 91: 705-713.

Buamard, N. and Benjakul, S. 2015. Improvement of gel properties of sardine (*Sardinella albella*) surimi using coconut husk extracts. Food Hydrocolloids. 51: 146-155.

Buamard, N. and Benjakul, S. 2017. Ethanolic coconut husk extract: *In vitro* antioxidative activity and effect on oxidative stability of shrimp oil emulsion. Eur. J. Lipid. Sci. Technol. 119: 1700131.

Buitimea-Cantúa, N. E., Gutiérrez-Uribe J. A. and Serna-Saldívar, S. O. 2017. Phenolic-protein interactions: Effect on food properties and health benefits. J. Med. Food. 21: 1-11.

Cáceres, E., García, M. L. and Selgas, M. D. 2008. Effect of pre-emulsified fish oil – as source of PUFA *n*−3 – on microstructure and sensory properties of *mortadella*, a Spanish bologna-type sausage. Meat Sci. 80: 183-193.

Castellani, O., Belhomme, C., David-Briand, E., Guérin-Dubiard, C. and Anton, M. 2006. Oil-in-water emulsion properties and interfacial characteristics of hen egg yolk phosvitin. Food Hydrocolloid. 20: 35-43.

Chaijan, M., Benjakul, S., Visessanguan, W. and Faustman, C. 2004. Characteristics and gel properties of muscles from sardine (*Sardinella gibbosa*) and mackerel (*Rastrelliger kanagurta*) caught in Thailand. Food Res. Int. 37: 1021–1030.

Friesen, K., Chang, C. and Nickerson, M. 2015. Incorporation of phenolic compounds, rutin and epicatechin, into soy protein isolate films: Mechanical, barrier and cross-linking properties. Food Chem. 172: 18-23.

Hęś, M. and Gramza-Michałowska, A. 2017. Effect of plant extracts on lipid oxidation and changes in nutritive value of protein in frozen-stored meat products. J. Food Proc. Preserve. 41: e12989.

Hu, M., McClements, D. J. and Decker, E. A. 2003. Lipid oxidation in corn oil-in-water emulsions stabilized by casein, whey protein isolate, and soy protein isolate. J. Agric. Food Chem. 51: 1696-1700.

Jose, M., Cyriac, M. B., Pai, V. and Shantaram, M. 2014. Antimicrobial properties of *Cocos nucifera* (coconut) husk: An extrapolation to oral health. J. Nat. Sci. Biol. Med. 5: 359-364.

Kang, Z. L., Zhu, D. Y., Li, B., Ma, H. J. and Song, Z. J. 2017. Effect of pre-emulsified sesame oil on physical-chemical and rheological properties of pork batters. Food Sci. Technol. Campinas. 37: 620-626.

Li, P., Yang, H., Zhu, Y., Wang, Y., Bai, D., Dai, R., Ren, X., Yang, H. and Ma, L. 2016. Influence of washing and cold storage on lipid and protein oxidation in catfish (*Clarias lazera*) surimi. J. Aquat. Food Prod. Technol. 25: 790-801.

Liu, C., Zhang, Y., Li, X., Luo, J., Gao, Q. and Li, J. 2017. A high-performance bio-adhesive derived from soy protein isolate and condensed tannins. RSC Adv. 7: 21226-21233.

Luo, Y., Shen, H., Pan, D. and Bu, G. 2008. Gel properties of surimi from silver carp (*Hypophthalmichthys molitrix*) as affected by heat treatment and soy protein isolate. Food Hydrocolloid. 22: 1513-1519.

Maqsood, S., Benjakul, S. and Shahidi, F. 2013. Emerging role of phenolic compounds as natural food additives in fish and fish product. Crit. Rev. Food Sci. Nutr. 53: 162-179.

Maturin, L. J. and Peeler, J. T. 1998. Aerobic plate count. *In* R. I. Mecker, G. J. Jackson and R, Bandler (Eds.), Food and drug administration bacteriological analytical manual. Gaithersburg, MD: US Food and Drug Administration. 3.01-3.10.

Odeyemi, O. A., Burke, C. M., Bolch, C. C. J. and Stanley, R. 2018. Seafood spoilage microbiota and associated volatile organic compounds at different storage temperatures and packaging conditions. Int. J. Food Microbiol. https://doi.org/10.1016/j.ijfoodmicro. 2017.12.029

Panyakaew, S. and Fotios, S. 2011. New thermal insulation boards made from coconut husk and bagasse. Energ. Buildings. 43: 1732-1739.

Richards, M. P. and Hultin, H. O. 2002. Contribution of blood and blood components to lipid oxidation in fish muscle. J. Agric. Food Chem. 50: 555-564.

Sae-leaw, T. and Benjakul, S. 2017. Lipids from visceral depot fat of Asian seabass (*Lates calcarifer*): Compositions and storage stability as affected by extraction methods. Eur. J. Lipid Sci. Technol. 119: 1700198.

Sallam, K. I. 2007. Antimicrobial and antioxidant effects of sodium acetate, sodium lactate, and sodium citrate in refrigerated sliced salmon. Food Control. 18: 566-575.

Sanjee, S. A. and Karim, E. 2016. Microbiological quality assessement of frozen fish and fish processing materials from Bangladesh. Int. J. Food Sci: http://dx.doi.org/10.1155/2016 /8605689.

Singh, A. and Benjakul, S. 2017. Effect of serine protease inhibitor from squid ovary on gel properties of surimi from Indian mackerel. J. Texture Stud. 48: 541-549.

Suvanich, V., Jahncke, M. L. and Marshall, D. L. 2000. Changes in selected chemical quality characteristics of channel catfish frame mince during chill and frozen storage. J. Food Sci. 65: 24-29.

Thiansilakul, Y., Benjakul, S., Grunwald, E. W. and Richards, M. P. 2012. Retardation of myoglobin and haemoglobin-mediated lipid oxidation in washed bighead carp by phenolic compounds. Food Chem. 134: 789-796.

Vate, N. K., Benjakul, S. and Agustini, T. W. 2015. Application of melanin-free ink as a new antioxidative gel enhancer in sardine surimi gel. J. Sci. Food Agric. 95: 2201-2207.

Viju, N., Satheesh, S. and Vincent, S. G. P. 2013. Antibiofilm activity of coconut (*Cocos nucifera* Linn.) husk fibre extract. Saudi J. Biol. Sci. 20: 85-91.

Wang, H.-L., Chou, C.-S., Yu, Y.-S., Hsu, C.-L., Wang, S.-Y., Ko, Y.-F. and Chen, Y.-C. 2016. Chicken surimi fortified by omega-3 fatty acid addition: Manufacturing and quality properties. J. Sci. Food Agric. 96: 1609-1617.

Yu, L. H., Lee, E. S., Jeong, J. Y., Paik, H. D., Choi, J. H. and Kim, C. J. 2005. Effects of thawing temperature on the physicochemical properties of pre-rigor frozen chicken breast and leg muscles. Meat Sci. 71: 375-382.

CHAPTER 8

CONCLUSION AND SUGGESTION

8.1 Conclusion

1. Surimi gel strengthening agent could be extracted from coconut husk using 60% ethanol (ECHE). Addition of ECHE yielded the surimi gel from sardine with the increased breaking force, textural and sensory characteristics. However, the addition of ECHE caused a slight decrease in whiteness, particularly with increasing level.

2. ECHE effectively induced the aggregation of NAM during heating as evidenced by the increased turbidity and larger particle size of aggregated proteins. Protein aggregation was enhanced via hydrophobic interactions and disulfide bond when ECHE was incorporated.

3. Addition of ECHE at an appropriate level could strengthen the surimi gel from sardine with low setting phenomenon by increasing breaking force, hardness, gumminess, chewiness and water holding capacity. However, the addition of ECHE resulted in the decreases in deformation and whiteness, particularly with increasing levels.

4. ECHE at the appropriate concentration could be used as the gel strengthener for surimi subjected to high pressurization with subsequent heating. Breaking force and water holding capacity of resulting gel were much improved.

5. ECHE contained the condensed tannin and free phenolic compounds. ECHE showed antioxidant activities with a dose-dependent manner as tested by various *in vitro* assays. Moreover, the use of ECHE could retard lipid oxidation and maintain polyunsaturated fatty acids in shrimp oil-in-water emulsion throughout 12 days of storage at 30 °C.

6. Pre-emulsification of seabass oil in the presence of ECHE before adding into surimi gel was an effective method to lower the deterioration caused by lipid oxidation, especially when ECHE was used at a higher level. Addition of pre-emulsified

seabass oil along with ECHE yielded surimi gel with the increased breaking force with lowered changes in whiteness during the storage.

8.2 Suggestion

1. The antimicrobial activity of ECHE should be studied, in which the controlled release of active compound should be investigated in the gel or emulsion gel.

2. Improvement of color of ECHE via fractionation or bleaching to lower the negative impact of ECHE on discoloration should be further investigated.

3. Prevention of protein degradation (modori) in surimi gel by ECHE should be studied.

VITAE

Name Mr. Natchaphol Buamard

Student ID 5611030001

Education Attainment

Degree	Name of Institution	Year of Graduation
Bachelor of Science (Agro-Industry, First Class Honor)	Prince of Songkla University	2011

Scholarship Awards during Enrollment

- Prince of Songkla University Ph.D. Scholarship, Graduate School, Prince of Songkla University.
- Oversea Thesis Research Study Scholarship, Graduate School, Prince of Songkla University.
- Scholarship of Academic Distinction, Prince of Songkla University.

List of Publication and Proceedings

Publication

1. Buamard, N. and Benjakul, S. 2015. Improvement of gel properties of sardine (*Sardinella albella*) surimi using coconut husk extracts. Food Hydrocolloids. 51: 146-155.
2. Buamard, N. and Benjakul, S. 2017. Cross-linking activity of ethanolic coconut husk extract toward sardine (*Sardinella albella*) muscle proteins. Journal of Food Biochemistry. 41: e12283.
3. Buamard, N., Benjakul, S. and Konno, K. 2017. Improvement of gel quality of sardine surimi with low setting phenomenon by ethanolic coconut husk extract. Journal of Texture Studies: 48: 47-56.

4. Buamard, N. and Benjakul, S. 2017. Ethanolic coconut husk extract: *In vitro* antioxidative activity and effect on oxidative stability of shrimp oil emulsion. European Journal of Lipid Science and Technology. 119: 1700131.

5. Buamard, N. and Benjakul, S. 2018. Combination effect of high pressure treatment and ethanolic extract from coconut husk on gel properties of sardine surimi. LWT-Food Science and Technology. 91: 361-367.

6. Buamard, N. and Benjakul, S. 2018. Effect of ethanolic coconut husk extract and pre-emulsification on properties and stability of surimi gel fortified with seabass oil during refrigerated storage. LWT-Food Science and Technology. Submitted.

Proceeding

1. Buamard, N. and Benjakul, S. 2017. Ethanolic coconut husk extract: Antioxidative activity and enhancement of oxidative stability of shrimp oil-in-water emulsion. The 19th Food Innovation Asia Conference 2017. BITEC Bangna, Bangkok, Thailand. 15-17th June 2017. Poster presentation.