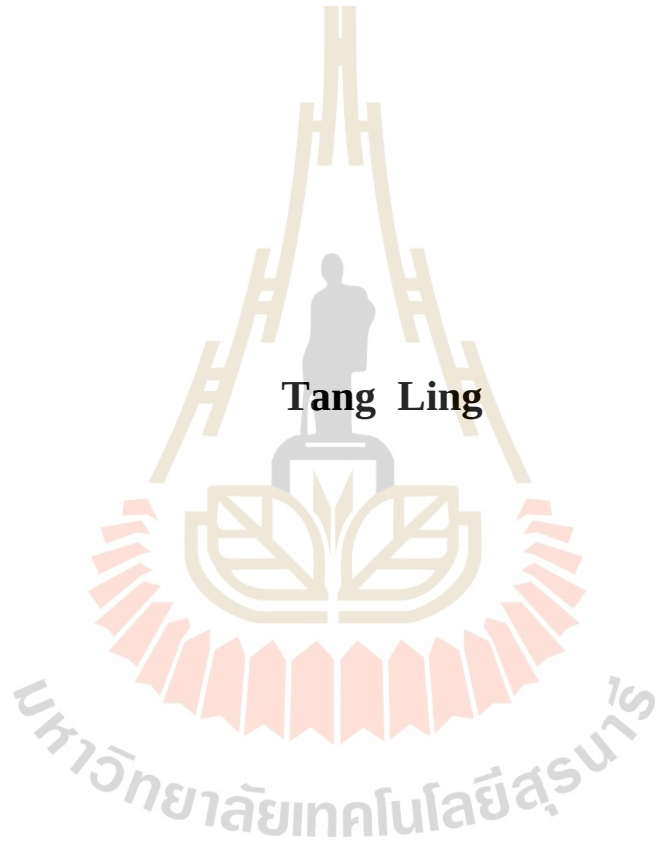
GELATION OF TROPICAL FISH SURIMI UNDER HIGH INTENSITY ULTRASOUND

Tang Ling



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การเกิดเจลของซูริมิปลาเขตร้อนภายใต้อัลตราซาวด์ความเข้มสูง

นางสาวทัง หลิง

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต

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GELATION OF TROPICAL FISH SURIMI UNDER HIGH INTENSITY ULTRASOUND

Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

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ทัง หลิง : การเกิดเจลของซูริมิปลาเขตร้อนภายใต้อัลตร้าซาวด์ความเข้มสูง (GELATION OF TROPICAL FISH SURIMI UNDER HIGH INTENSITY ULTRASOUND)
อาจารย์ที่ปรึกษา : รองศาสตราจารย์ ดร.จิรวัฒน์ ยงสวัสดิกุล, 143 หน้า.

จากการศึกษาการเปลี่ยนแปลงทางเคมีกายภาพของแอคโตไมโอซินจากปลานิล (*Oreochromis niloticus*) ที่ได้รับคลื่นอัลตร้าซาวน์ความเข้มสูง (high intensity ultrasound; HIU) ภายใต้สภาวะที่ความเข้มข้นของเกลือต่ำพบว่า โปรตีนที่สามารถสกัดได้ในสภาวะที่มีความเข้มข้นของโซเดียมคลอไรด์ในช่วง 0.1 – 0.3 โมลลาร์มีค่าเพิ่มขึ้นเมื่อความเข้มของคลื่นอัลต้าซาวน์เพิ่มขึ้น ($p < 0.05$) ปริมาณของหมู่ซัลฟ์ไฮดริล (reactive sulfhydryl; SH) ที่ความเข้มข้นของโซเดียมคลอไรด์ 0.2 โมลลาร์ เพิ่มขึ้นหลังจากได้รับคลื่นอัลตร้าซาวน์ความเข้มสูงติดต่อกันเป็นเวลานาน ซึ่งบ่งชี้ถึงการเปลี่ยนแปลงทางโครงสร้างของโปรตีนภายใต้คลื่นอัลตร้าซาวน์ความเข้มสูง ค่าพื้นผิวไฮโดรโฟบิก (surface hydrophobicity; S_0-ANS) ของแอคโตไมโอซินที่ความเข้มข้นของโซเดียมคลอไรด์ 0.6 โมลลาร์ เพิ่มขึ้นเมื่อความเข้มของคลื่นอัลตร้าซาวน์และเวลาที่ได้สัมผัสเพิ่มขึ้นในระดับที่สูงกว่าในสภาวะที่โซเดียมคลอไรด์เข้มข้น 0.2 โมลลาร์ ความสามารถในการละลายของโปรตีนไมโอซินสายหนัก (myosin heavy chain; MHC) และ แอคติน (actin) ที่โซเดียมคลอไรด์เข้มข้น 0.2 โมลลาร์เพิ่มขึ้นอย่างเด่นชัดภายใต้การใช้คลื่นอัลตร้าซาวน์ความเข้มสูง สารละลายแอคโตไมโอซินที่ความเข้มข้นของโซเดียมคลอไรด์ 0.6 โมลลาร์ถูกทำลายด้วยคลื่นอัลตร้าซาวน์มากกว่าที่ความเข้มข้นของโซเดียมคลอไรด์ 0.2 โมลลาร์ จะเห็นได้ว่าแอคโตไมโอซินสามารถถูกสกัดได้อย่างมีประสิทธิภาพในสภาวะที่มีความเข้มข้นของโซเดียมคลอไรด์ต่ำโดยใช้คลื่นอัลตร้าซาวน์ความเข้มสูง ซึ่งแสดงถึงศักยภาพของเทคโนโลยีคลื่นอัลตร้าซาวน์ความเข้มสูงในการผลิตเจลซูริมิ (surimi gel) ที่มีปริมาณเกลือลดลง

การศึกษาเกิดเจลของซูริมิจากปลาทรายแดง (*Nemipterus* spp.) ที่ความเข้มข้นของโซเดียมคลอไรด์ในระดับ 0.5, 1, และ 2% ภายใต้ความเข้มของคลื่นอัลตร้าซาวน์ที่แตกต่างกัน (10.01, 13.28, และ 16.45 W/cm^2) พบว่า ความสามารถในการสกัดโปรตีนที่ความเข้มข้นของโซเดียมคลอไรด์ 0.5% มีค่าเพิ่มขึ้นเมื่อเพิ่มความเข้มของคลื่นอัลตร้าซาวน์ ($p < 0.05$) ปริมาณของหมู่ซัลฟ์ไฮดริลและค่าพื้นผิวไฮโดรโฟบิกเพิ่มขึ้นหลังจากการใช้คลื่นอัลตร้าซาวน์ความเข้มสูง ในขณะที่กิจกรรมของเอนไซม์แคลเซียมเอทีพีเอส (Ca^{2+} - ATPase) ลดลง ซึ่งบ่งชี้ถึงการเปิดตัวและการเปลี่ยนแปลงโครงร่างของโปรตีนภายใต้คลื่นอัลตร้าซาวน์ความเข้มสูง คุณสมบัติด้านเนื้อสัมผัสของเจลซูริมิที่ความเข้มข้นโซเดียมคลอไรด์ในระดับ 0.5% ดีขึ้นเมื่อความเข้มของคลื่นอัลตร้าซาวน์เพิ่มขึ้น ($p < 0.05$) ในขณะที่คลื่นอัลตร้าซาวน์ความเข้มสูงส่งผลคุณภาพเจลลดลงในซูริมิที่เติมโซเดียมคลอไรด์ในระดับ 1 และ 2% การวิเคราะห์จุลทรรศน์อิเลคตรอนแบบกวาดแสดงให้เห็นถึง

โครงข่ายร่างแหที่เป็นระเบียบในในระดับที่เพิ่มสูงขึ้นในตัวอย่างที่ความเข้มข้น 0.5% โซเดียมคลอไรด์ที่ได้รับคลื่นอัลตร้าซาวน์ความเข้มสูง ผลการศึกษาฟูเรียร์ทรานส์ฟอร์มอินฟราเรดสเปคโตรสโคปี (Fourier transform infrared; FT-IR, spectroscopy) บ่งชี้ว่าโครงสร้างเกลียวแอลฟา (α-helix) ของเจลซูริมิลดลง ในขณะที่โครงสร้างที่ไม่มีระเบียบ (random coil) มีปริมาณเพิ่มขึ้น เมื่อเพิ่มความเข้มของคลื่นอัลตร้าซาวน์ ซึ่งยืนยันถึงการเปลี่ยนแปลงโครงสร้างโปรตีนที่ถูกเหนี่ยวนำด้วยคลื่นอัลตร้าซาวน์ความเข้มสูงที่มากขึ้นตามความเข้มของคลื่น ผลการศึกษานี้บ่งชี้ว่าเทคโนโลยีอัลตร้าซาวน์ความเข้มสูงสามารถประยุกต์เพื่อปรับปรุงเจลซูริมิที่มีโซเดียมคลอไรด์เข้มข้นในระดับ 0.5% เท่านั้น

เมื่อศึกษาผลของระยะเวลาในการสัมผัสคลื่นอัลตร้าซาวน์ (15, 30, และ 45 นาที) ของซูริมิปลาทรายแดงที่ใช้คลื่นอัลตร้าซาวน์ความเข้มสูงในระดับ 13.28 W/cm^2 เพื่อเหนี่ยวนำให้เกิดเจลพบว่า ความสามารถในการสกัดโปรตีน และค่าพื้นผิวไฮโดรโฟบิกเพิ่มขึ้นเมื่อเพิ่มระยะเวลาการสัมผัส โดยที่กิจกรรมของเอนไซม์แคลเซียมเอทีพีเอสลดลง ($p < 0.05$) การใช้คลื่นอัลตร้าซาวน์ความเข้มสูงส่งผลให้ค่ามอดูลัสสะสม (storage modulus; G') ของซูริมิที่เติมโซเดียมคลอไรด์เข้มข้น 0.5% เพิ่มขึ้น ซึ่งบ่งชี้ว่าโครงข่ายร่างแหของเจลดีขึ้นเมื่อใช้คลื่นอัลตร้าซาวน์ความเข้มสูง โดยเฉพาะอย่างยิ่งเมื่อได้รับคลื่นอัลตร้าซาวน์เป็นเวลา 30 นาที นอกจากนี้ สมบัติด้านเนื้อสัมผัสของเจลซูริมิที่มีโซเดียมคลอไรด์เข้มข้นในระดับ 0.5% เหมาะสมที่สุดเมื่อได้รับคลื่นอัลตร้าซาวน์เป็นเวลา 30 นาที จากผลการศึกษาฟูเรียร์ทรานส์ฟอร์มอินฟราเรดสเปคโตรสโคปี การเพิ่มเวลาในการสัมผัสคลื่นอัลตร้าซาวน์ส่งผลให้ปริมาณโครงสร้างเกลียวแอลฟาลดลง ในขณะที่ โครงสร้างที่ไม่มีระเบียบสูงขึ้น ซึ่งแสดงถึงการเปิดตัวของโปรตีนที่มากขึ้น การศึกษาครั้งนี้แสดงให้เห็นว่า เจลซูริมิเกลือต่ำหรือเจลที่มีปริมาณเกลือลดลงสามารถผลิตได้โดยการใช้เทคโนโลยีคลื่นอัลตร้าซาวน์ความเข้มสูงและการให้คลื่นอัลตร้าซาวน์ที่ความเข้ม 13.28 W/cm^2 เป็นเวลา 30 นาที เป็นสภาวะที่เหมาะสมสำหรับการเหนี่ยวนำให้เกิดเจลซูริมิที่ความเข้มข้นโซเดียมคลอไรด์ในระดับ 0.5%

สาขาวิชาเทคโนโลยีอาหาร ลายมือชื่อนักศึกษา Ling Tang

ปีการศึกษา 2562 ลายมือชื่ออาจารย์ที่ปรึกษา _____

TANG LING : GELATION OF TROPICAL FISH SURIMI UNDER HIGH INTENSITY ULTRASOUND. THESIS ADVISOR : ASSOC. PROF. JIRAWAT YONGSAWATDIGUL, Ph.D., 143 PP.

TROPICAL SURIMI/HIGH INTENSITY ULTRASOUND/GELATION/ MYOFIBRILLAR PROTEINS/REDUCED SALT

Physicochemical changes of tilapia (*Oreochromis niloticus*) actomyosin exposed to high intensity ultrasound (HIU) under low salt concentrations were investigated. The extractable protein in 0.1-0.3 M NaCl increased with increasing ultrasonic intensity ($p < 0.05$). Reactive sulfhydryl (SH) content at 0.2 M NaCl increased after a prolonged exposure to HIU, suggesting conformational changes induced by HIU. Surface hydrophobicity (S_0-ANS) of actomyosin in 0.6 M NaCl increased with increasing ultrasonic intensity and exposure time to a higher degree than that in 0.2 M NaCl. A drastic increase in the solubility of myosin heavy chain (MHC) and actin with 0.2 M NaCl were evident under HIU treatments. Actomyosin in 0.6 M NaCl underwent more disruption by HIU than that in 0.2 M NaCl. Actomyosin can be efficiently extracted in low NaCl concentration by HIU, suggesting the potential of HIU technology in production of low/reduced-salt surimi gel.

Gelation of threadfin bream (*Nemipterus* spp.) surimi at 0.5, 1 and 2% NaCl under different ultrasonic intensities (10.01, 13.28 and 16.45 W/cm^2) were elucidated. Protein extractability at 0.5% NaCl was increased with increasing ultrasonic intensity ($p < 0.05$). Reactive SH content and S_0-ANS values increased after HIU treatments and were accompanied by a decrease in Ca^{2+}-ATPase activity, indicating greater protein

unfolding and conformational changes induced by HIU. Textural properties of surimgels at 0.5% NaCl were improved with an increase in ultrasonic intensity ($p < 0.05$), whereas HIU resulted in inferior gels at 1 and 2% NaCl. Scanning electron microscopy (SEM) revealed that HIU resulted in higher levels of regular networks at 0.5% NaCl. Fourier transform infrared (FT-IR) spectroscopy indicated that α-helix content of surimi gels decreased, while the random coil content increased with an increase in ultrasonic intensity, confirming that structural changes induced by HIU were more profound at higher ultrasonic intensity. The results suggested that HIU technology can be applied to improve only the 0.5% NaCl surimi gel.

When the effect of ultrasonic exposure time (15, 30 and 45 min) of HIU-assisted gelation of 0.5% NaCl threadfin bream surimi at ultrasonic intensity of 13.28 W/cm^2 was elucidated, protein extractability and S_0-ANS increased with time, accompanied by a decrease in Ca^{2+}-ATPase activity ($p < 0.05$). HIU treatments increased storage modulus (G') of 0.5% NaCl surimi, indicating gel network formation was improved by HIU, particularly at ultrasonic exposure time of 30 min. Textural properties of 0.5% NaCl surimi gel ($p < 0.05$) was also optimal at ultrasonic exposure time of 30 min. According to FT-IR spectroscopy, an increase in ultrasonic exposure time resulted in lower α-helix but higher random coil content, suggesting that a longer ultrasonic exposure time induced greater protein unfolding. This study demonstrated that low/reduced-salt surimi gel could possibly be achieved by HIU technology, and ultrasonic exposure time of 30 min at ultrasonic intensity of 13.28 W/cm^2 optimally induced surimi gelation at 0.5% NaCl.

School of Food Technology Student's Signature Ling Tang

Academic Year 2019 Advisor's Signature

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LIST OF ABBREVIATIONS

%	Percent
μg	Microgram
μl	Microliter
μmol	Micromole
a^*	redness/greenness
ANOVA	Analysis of Variance
ANS	8-Anilino-1-naphthalenesulfonic acid
ATP	Adenosine 5′-triphosphate disodium salt hydrate
ATR	Attenuated total reflectance
b^*	yellowness/blueness
BSA	Bovine serum albumin
ºC	Degree centigrade
Ca^{2+}	Calcium cation
cm	Centimeter
cm^{-1}	Reciprocal centimeter
DMRT	Duncan's multiple-range test
DTNB	5-5'-Dithiobis-(2-nitrobenzoic acid)
EDTA	Ethylenediaminetetraacetic acid
FT-IR	Fourier Transform Infrared Spectroscopy
×g	Times gravity (Centrifugal force)

LIST OF ABBREVIATIONS (Continued)

g	Gram
G'	Storage modulus
G"	Loss modulus
h	Hour
·H	Hydrogen radical
HIU	High intensity ultrasound
H_2O_2	Hydrogen peroxide
IR	Infrared radiation
kDa	Kilodalton
KH_2PO_4	Potassium phosphate
kHz	Kilohertz
kPa	Kilopascal
L^*	Lightness
m	Meter
M	Molar
mg	Milligram
MHC	Myosin heavy chain
min	Minute
ml	Milliliter
mm	Millimeter
mM	Millimolar
MT	Metric ton

LIST OF ABBREVIATIONS (Continued)

NaCl	Sodium chloride
nm	Nanometer
·OH	Hydroxyl radical
pH	Potential of hydrogen
PMSF	Phenylmethanesulfonyl fluoride
s	Second
S_0-ANS	Surface hydrophobicity
SD	Standard deviation
SDS	Sodium sodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SEM	Scanning electron microscopy
SH	Sulfhydryl group
SPSS	Statistical Product and Service Solutions
S-S	Disulfide
TCA	Trichloroacetic acid
TGase	Transglutaminase
V	Voltage
v/v	volume/volume
W	Watt
W/cm^2	Watts per square centimeter
w/v	Weight per volume
w/w	Weight per weight

CHAPTER I

INTRODUCTION

1.1 Background and significance of the study

Surimi is stabilized fish myofibrillar proteins. During manufacture of surimi, fish flesh is washed with cold water to remove most of blood, lipids, enzymes and sarcoplasmic proteins after mechanical deboning, and then blended with cryoprotectants (Park, Graves, Draves, & Yongsawatdigul, 2013). Over the past decades, surimi production and surimi industry have changed a lot because of the variability of global fish stocks (Guenneugues & Ianelli, 2013). New species of fish have been explored and utilized for surimi production. In particular, the tropical fish surimi has become a major species in competition with the cold water species, such as Alaska pollock (*Theregra chalcogramma*) due to the continually increased production (Guenneugues et al., 2013; Tadpitchayangkoon, Park, & Yongsawatdigul, 2012). Nowadays, Southeast Asia has provided the largest fish resource for surimi production, which occupies 60% of surimi production around the world (Guenneugues et al., 2013). Threadfin bream (*Nemipterus* spp.) is a typical tropical fish, which has become the second largest fish resource for manufacture of surimi, only after Alaska pollock. It plays an important role in surimi markets, especially in Thailand, which contributes to the major production of threadfin bream surimi in the world (Poowakanjana & Park, 2013; Yongsawatdigul, Worratao, & Park, 2002). Threadfin bream is preferred as raw material for surimi production because it has white color, smooth texture, good flavor,

strong gel-forming ability and is resistance to temperature. Thus, the characteristics of threadfin bream surimi has been concerned.

Gelation is a critical parameter determining the quality of surimi because it governs the textural and sensory properties of surimi seafoods. Gelation of surimi is directly related to myofibrillar proteins. Myosin is the major part of total myofibrillar proteins, constituting around 55-60%. The postmortem myosin binds to actin tightly, resulting in the formation of actomyosin, which makes it the predominant protein in surimi. Hence, the concentration and properties of actomyosin will largely contribute to the heat-induced surimi gelation (Lanier, Yongsawatdigul, & Carvajal-Rondanelli, 2013; Yasui, Ishioroshi, & Samejima, 1982).

Generally, surimi gelation involves a partial unfolding of protein and subsequent irreversible aggregation, resulting in formation of a three-dimensional gel network (Lanier et al., 2013). Myofibrillar proteins are typically heat-gelled in manufacture of surimi seafood. During heating, unfolding of myofibrillar proteins occurs, which results in exposure of reactive groups of proteins, forming intermolecular interactions. A three-dimensional gel network will be formed when abundant intermolecular interactions exists (Lanier et al., 2013). Therefore, protein unfolding (denaturation) followed by protein aggregation leads to surimi gel formation through intermolecular covalent and noncovalent interactions. In order to obtain desirable surimi gel, it is necessary to understand the basic knowledge of physicochemical changes of myofibrillar proteins, which could affect the denaturation and aggregation behaviors of myofibrillar proteins, ultimately governing textural properties.

The solubilization of myofibrillar proteins needs a NaCl concentration that is higher than 0.3 M since myofibrillar proteins are salt-soluble proteins. Hence, in order

to form surimi gel, myofibrillar proteins must be extracted first. Sodium chloride at 2-3% is typically required to facilitate solubilization of the myofibrillar proteins in surimi. A decrease in NaCl content results in poor gel-forming ability of surimi because myofibrillar proteins are not adequately solubilized and are not properly unfolded (Lanier et al., 2013). According to Fu et al. (2012), the mechanical properties of silver carp surimi gel became worse when NaCl content was decreased to 1%. Thereby, sodium chloride is an ingredient essential for surimi gelation and gel quality largely depends on sodium chloride concentration.

Sodium chloride provides approximately 90% of the sodium in human diet (Kloss, Meyer, Graeve, & Vetter, 2015). Currently, consumption of excessive sodium has become a public concern since it increases risk of hypertension and cardiovascular diseases. In view of these health problems, the intake of sodium chloride in adults should be less than 5 g/day recommended by the World Health Organization (WHO) in 2012, which equivalents to 2 g/day of sodium (Inguglia, Zhang, Tiwari, Kerry, & Burgess, 2017). Surimi seafood products are typical processed muscle foods. It has been estimated that muscle foods account for about 20-30% of daily sodium consumption (Petracci, Bianchi, Mudalal, & Cavani, 2013). Consequently, the necessity to reduce sodium in surimi-based product is particularly important to fit with the raising consumer awareness and WHO recommendations. In order to meet consumers' demand, efforts have been attempted to reduce salt/sodium content in surimi gels based on various strategies. Initial exploration focused on a partial replacement of sodium chloride with other salts, such as potassium chloride and magnesium chloride. Potassium chloride is typically used to replace partial of NaCl, but it causes negative sensory characteristics due to its bitterness and metallic taste (Tahergorabi & Jaczynski,

2012). Sodium reduction has also been reported by addition of different ingredients, such as whey protein concentrate (Uresti, Téllez-Luis, Ramírez, & Vázquez, 2004), tetra-sodium pyrophosphate (Deysi Cando, Herranz, Borderías, & Moreno, 2016) and lysine (D. Cando, Borderías, & H. M. Moreno, 2016). In recent years, much attention has been given towards the applications of novel physical techniques in food research and industry. Fu et al. (2012) reported that 1% NaCl silver carp surimi gel was improved under microwave heating. High pressure processing technique has been explored to improve Alaska pollock surimi gel at 0.3% NaCl (Deysi Cando, Herranz, Borderías, & Moreno, 2015) and was reported to strengthen the water retention of myosin gel in 0.3 M NaCl (Jianyi Wang, Li, Zheng, Zhang, & Guo, 2019). However, exploration of low/reduced salt surimi still faces challenges because salt plays a critical role in surimi gelation and gel quality. Therefore, low/reduced salt surimi needs to be further investigated, providing more approaches feasible for surimi industries.

In recent years, high intensity ultrasound (HIU) has been applied in food research and industry because it is a safe, nontoxic and green technology. HIU refers to low frequency (16-100 kHz) with high power (10-1000 W/cm^2) (Soria & Villamiel, 2010). Cavitation phenomenon, formation of micro-streaming and reactive free radicals typically occur during sonication, which lead to energy accumulation (high temperature and pressure), high shear energy waves and turbulence. Furthermore, some chemical reactions might also take place (Soria et al., 2010). These would eventually affect the physicochemical properties of myofibrillar proteins. HIU has been reported to cause protein dissociation and improve solubility of myofibrillar proteins at high ionic strength (Saleem & Ahmad, 2016). It has also been reported that HIU could induce the unfolding of myofibrillar proteins, exposing interior hydrophobic and sulfhydryl groups

(Jingyu Wang, Yang, Tang, Ni, & Zhou, 2017; Zou et al., 2018). Moreover, HIU has been proven to accelerate the salt diffusion in meat tissue (McDonnell, Lyng, & Allen, 2014). Besides, the potential of HIU to improve water holding capacity of chicken myofibrillar protein gel (Zhang, Regenstein, Zhou, & Yang, 2017) has also been investigated. These studies focused on a typical high salt concentration, e.g., 0.6 M NaCl. However, little information is available on application of HIU technology in myofibrillar proteins under low salt conditions, where myofibrillar proteins has only limited protein solubility and behave in a different way on denaturation and aggregation. Due to the potential to increase the soluble myofibrillar proteins under low salt, it is assumed that HIU technology should positively contribute to the surimi gel under reduced salt concentration because soluble myofibrillar proteins are thought to be the base element of gel formation. Moreover, the exposure of hydrophobic and sulfhydryl groups induced by HIU is expected to lead to strong protein interactions and gel networks. This would eventually improve gel properties of myofibrillar proteins at low/reduced-salt contents. Therefore, HIU technology could be a potential processing-aid to produce healthier surimi seafood products with reduced sodium content without the use of chemicals and/or enzymes.

Fourier transform infrared (FT-IR) spectroscopy depends on IR absorption which needs a change in the intrinsic dipole moment with molecular vibration (Li-Chan, 1996). It has been applied in the structural analysis of myofibrillar proteins under various conditions, such as aging (Kirschner, Ofstad, Skarpeid, Høst, & Kohler, 2004), heating (Bertram, Kohler, Böcker, Ofstad, & Andersen, 2006; Wu, Bertram, Böcker, Ofstad, & Kohler, 2007), salting (Böcker, Ofstad, Bertram, Egelandsdal, & Kohler, 2006; Wu et al., 2006), and storage (Moosavi-Nasab, Alli, Ismail, & Ngadi, 2005),

where the estimation of protein secondary structure is the major application. Besides, the investigations of FT-IR on secondary structural changes of myofibrillar proteins as affected by high pressure (Larrea-Wachtendorff, Tabilo-Munizaga, Moreno-Osorio, Villalobos-Carvajal, & Pérez-Won, 2015), oxidative stress (Zhou, Zhao, Su, Cui, & Sun, 2014), and addition of polysaccharides (Fan et al., 2017; Y.-z. Zhou et al., 2014) and MTGase (D. Cando et al., 2016) were also reported. Therefore, FT-IR spectroscopy has potential to analyze and characterize the secondary structural changes of fish myofibrillar proteins related to surimi gelation.

1.2 Research objectives

1.2.1 To investigate physicochemical changes of tilapia myofibrillar proteins in low salt concentrations exposed to HIU.

1.2.2 To elucidate the gelation behavior of threadfin bream surimi as a function of NaCl contents and HIU intensities.

1.2.3 To explore the effects of various HIU time on gelation of 0.5% NaCl threadfin bream surimi.

1.3 Research hypotheses

1.3.1 HIU could induce dissociation of protein aggregates and disintegration of myofibril, improving solubility of myofibrillar proteins at low salt contents, which might be beneficial for gelation of low/reduced-salt surimi .

1.3.2 HIU could promote unfolding of myofibrillar proteins, exposing additional hydrophobic and sulfhydryl groups, and thus lead to strong protein interactions by changing aggregation behaviors of muscle proteins during heating .

1.3.3 An increase in the soluble myofibrillar proteins and a greater extent of protein unfolding under HIU would lead to optimal gel network formation under low/reduced salt content.

1.4 Scope of the study

1.4.1 Physicochemical changes of tilapia actomyosin under HIU in low NaCl concentrations were investigated. The extracted actomyosin was quantified by measurement of protein content. Ca^{2+}-ATPase activity, total and reactive SH contents, surface hydrophobicity and *zeta* potential were determined to indicate unfolding and conformational changes of actomyosin. Particle size was measured to evaluate size changes of protein aggregates. SDS-PAGE was performed to show protein patterns under HIU. Changes in microstructure as affected by HIU were also observed based on light microscopy.

1.4.2 Gelation behavior of threadfin bream surimi under HIU at various NaCl contents were elucidated by investigating the effects of different HIU intensities on gel properties of threadfin bream surimi under low/reduced salt and by illustrating physicochemical changes in threadfin bream surimi pastes as a function of NaCl contents and HIU. Gel properties were investigated by texture and color measurements, proteolysis, FT-IR analysis and SEM. Physicochemical changes were studied by protein extractability, remaining Ca^{2+}-ATPase activity, total and reactive SH contents, and surface hydrophobicity.

1.4.3 HIU-assisted gelation of threadfin bream surimi subjected to reduced NaCl content and its related physicochemical properties were studied. Effects of various ultrasonic exposure time on gelation of 0.5% NaCl threadfin bream surimi with 2%

NaCl as a control were explored. The physicochemical properties were examined by determinations of extractable protein content, residual Ca^{2+}-ATPase activity and surface hydrophobicity. The surimi gels were evaluated by rheological properties, texture and whiteness, TCA-soluble oligopeptide and SDS-PAGE. The secondary structural changes of surimi pastes and gels after HIU treatments were also analyzed by FT-IR.

1.5 Expected results

Results from this study would provide an effective way to extract myofibrillar proteins in low NaCl concentrations using HIU technology. Understanding of gelation behavior of surimi under HIU would lay a theoretical foundation for utilization of HIU technology in production of surimi-based products at low/reduced salt levels. Furthermore, the outcome of the study would be helpful in manufacturing healthier surimi seafood with reduced sodium content without the use of chemicals and/or enzymes.

1.6 References

Bertram, H. C., Kohler, A., Böcker, U., Ofstad, R., & Andersen, H. J. (2006). Heat-induced changes in myofibrillar protein structures and myowater of two pork qualities. A combined FT-IR spectroscopy and low-field NMR relaxometry study. **Journal of Agricultural and Food Chemistry.** 54 (5), 1740-1746.

Böcker, U., Ofstad, R., Bertram, H. C., Egelandsdal, B., & Kohler, A. (2006). Salt-induced changes in pork myofibrillar tissue investigated by FT-IR microspectroscopy and light microscopy. **Journal of Agricultural and Food Chemistry.** 54 (18), 6733-6740.

Cando, D., Borderías, A. J., & H.M.Moreno. (2016). Combined effect of aminoacids and microbial transglutaminase on gelation of low salt surimi content under high pressure processing. **Innovative Food Science & Emerging Technologies.** 36, 10-17.

Cando, D., Herranz, B., Borderías, A. J., & Moreno, H. M. (2015). Effect of high pressure on reduced sodium chloride surimi gels. **Food Hydrocolloids.** 51 (Supplement C), 176-187.

Cando, D., Herranz, B., Borderías, A. J., & Moreno, H. M. (2016). Different additives to enhance the gelation of surimi gel with reduced sodium content. **Food Chemistry.** 196, 791-799.

Fan, M., Hu, T., Zhao, S., Xiong, S., Xie, J., & Huang, Q. (2017). Gel characteristics and microstructure of fish myofibrillar protein/cassava starch composites. **Food Chemistry.** 218, 221-230.

Fu, X., Hayat, K., Li, Z., Lin, Q., Xu, S., & Wang, S. (2012). Effect of microwave heating on the low-salt gel from silver carp (*Hypophthalmichthys molitrix*) surimi. **Food Hydrocolloids.** 27 (2), 301-308.

Guenneugues, P., & Ianelli, J. (2013). Surimi Resources and Market. In J. W. Park (Ed.), Surimi and Surimi Seafood (3rd Edition) (pp. 26-53). Boca Raton, FL: CRC Press.

Inguglia, E. S., Zhang, Z., Tiwari, B. K., Kerry, J. P., & Burgess, C. M. (2017). Salt reduction strategies in processed meat products – A review. **Trends in Food Science & Technology.** 59, 70-78.

Kirschner, C., Ofstad, R., Skarpeid, H.-J., Høst, V., & Kohler, A. (2004). Monitoring of denaturation processes in aged beef loin by Fourier transform infrared microspectroscopy. **Journal of Agricultural and Food Chemistry.** 52 (12), 3920-3929.

Kloss, L., Meyer, J. D., Graeve, L., & Vetter, W. (2015). Sodium intake and its reduction by food reformulation in the European Union — A review. **NFS Journal.** 1 (Supplement C), 9-19.

Lanier, T. C., Yongsawatdigul, J., & Carvajal-Rondanelli, P. (2013). Surimi gelation chemistry. In J. W. Park (Ed.), Surimi and Surimi Seafood (3rd 3 Edition) (pp. 101-139). Boca Raton, FL: CRC Press.

Larrea-Wachtendorff, D., Tabilo-Munizaga, G., Moreno-Osorio, L., Villalobos-Carvajal, R., & Pérez-Won, M. (2015). Protein Changes Caused by High Hydrostatic Pressure (HHP): A Study Using Differential Scanning Calorimetry (DSC) and Fourier Transform Infrared (FTIR) Spectroscopy. **Food Engineering Reviews.** 7 (2), 222-230.

Li-Chan, E. C. Y. (1996). The applications of Raman spectroscopy in food science. **Trends in Food Science & Technology.** 7 (11), 361-370.

McDonnell, C. K., Lyng, J. G., & Allen, P. (2014). The use of power ultrasound for accelerating the curing of pork. **Meat Science.** 98 (2), 142-149.

Moosavi-Nasab, M., Alli, I., Ismail, A. A., & Ngadi, M. O. (2005). Protein structural changes during preparation and storage of surimi. **Journal of Food Science.** 70 (7).

Park, J. W., Graves, D., Draves, R., & Yongsawatdigul, J. (2013). Manufacture of Surimi: Harvest to Frozen Block. In J. W. Park (Ed.), Surimi and Surimi Seafood (3rd Edition) (pp. 33-106). Boca Raton, FL: CRC Press.

Petracci, M., Bianchi, M., Mudalal, S., & Cavani, C. (2013). Functional ingredients for poultry meat products. **Trends in Food Science & Technology.** 33 (1), 27-39.

Poowakanjana, S., & Park, J. W. (2013). Biochemical characterisation of Alaska pollock, Pacific whiting, and threadfin bream surimi as affected by comminution conditions. **Food Chemistry.** 138 (1), 200-207.

Saleem, R., & Ahmad, R. (2016). Effect of low frequency ultrasonication on biochemical and structural properties of chicken actomyosin. **Food Chemistry.** 205 (Supplement C), 43-51.

Soria, A. C., & Villamiel, M. (2010). Effect of ultrasound on the technological properties and bioactivity of food: a review. **Trends in Food Science & Technology.** 21 (7), 323-331.

Tadpitchayangkoon, P., Park, J. W., & Yongsawatdigul, J. (2012). Gelation characteristics of tropical surimi under water bath and ohmic heating. **LWT - Food Science and Technology.** 46 (1), 97-103.

Tahergorabi, R., & Jaczynski, J. (2012). Physicochemical changes in surimi with salt substitute. **Food Chemistry.** 132 (3), 1281-1286.

Uresti, R. o. M., Téllez-Luis, S. J., Ramírez, J. A., & Vázquez, M. (2004). Use of dairy proteins and microbial transglutaminase to obtain low-salt fish products from filleting waste from silver carp (*Hypophthalmichthys molitrix*). **Food Chemistry.** 86 (2), 257-262.

Wang, J., Li, Z., Zheng, B., Zhang, Y., & Guo, Z. (2019). Effect of ultra-high pressure on the structure and gelling properties of low salt golden threadfin bream (*Nemipterus virgatus*) myosin. **LWT - Food Science and Technology.** 100, 381-390.

Wang, J., Yang, Y., Tang, X., Ni, W., & Zhou, L. (2017). Effects of pulsed ultrasound on rheological and structural properties of chicken myofibrillar protein. **Ultrasonics Sonochemistry.** 38 (Supplement C), 225-233.

Wu, Z., Bertram, H. C., Böcker, U., Ofstad, R., & Kohler, A. (2007). Myowater dynamics and protein secondary structural changes as affected by heating rate in three pork qualities: a combined FT-IR microspectroscopic and 1H NMR relaxometry study. **Journal of Agricultural and Food Chemistry.** 55 (10), 3990-3997.

Wu, Z., Bertram, H. C., Kohler, A., Böcker, U., Ofstad, R., & Andersen, H. J. (2006). Influence of aging and salting on protein secondary structures and water distribution in uncooked and cooked pork. A combined FT-IR microspectroscopy and 1H NMR relaxometry study. **Journal of Agricultural and Food Chemistry.** 54 (22), 8589-8597.

Yasui, T., Ishioroshi, M., & Samejima, K. (1982). Effect of actomyosin on heat-induced gelation of myosin. **Agricultural and Biological Chemistry.** 46 (4), 1049-1059.

Yongsawatdigul, J., Worratao, A., & Park, J. W. (2002). Effect of endogenous transglutaminase on threadfin bream surimi gelation. **Journal of Food Science.** 67 (9), 3258-3263.

Zhang, Z., Regenstein, J. M., Zhou, P., & Yang, Y. (2017). Effects of high intensity ultrasound modification on physicochemical property and water in myofibrillar protein gel. **Ultrasonics Sonochemistry.** 34, 960-967.

Zhou, F., Zhao, M., Su, G., Cui, C., & Sun, W. (2014). Gelation of salted myofibrillar protein under malondialdehyde-induced oxidative stress. **Food Hydrocolloids.** 40, 153-162.

Zhou, Y.-z., Chen, C.-g., Chen, X., Li, P.-j., Ma, F., & Lu, Q.-h. (2014). Contribution of three ionic types of polysaccharides to the thermal gelling properties of chicken breast myosin. **Journal of Agricultural and Food Chemistry.** 62 (12), 2655-2662.

Zou, Y., Xu, P., Wu, H., Zhang, M., Sun, Z., Sun, C., Wang, D., Cao, J., & Xu, W. (2018). Effects of different ultrasound power on physicochemical property and functional performance of chicken actomyosin. **International Journal of Biological Macromolecules.** 113, 640-647.

CHAPTER II

LITERATURE REVIEW

2.1 Surimi

Surimi is stabilized fish myofibrillar proteins. During manufacture of surimi, after mechanical deboning, fish flesh is washed with cold water, removing most of blood, lipids, enzymes and sarcoplasmic proteins, and then blended with cryoprotectants (Park, Graves, Draves, & Yongsawatdigul, 2013). Surimi production involves fish sorting, heading, gutting, deboning, filleting and mincing, washing, refining and freezing processes. Due to its excellent gel properties, surimi is used for production of many kinds of surimi seafoods, such as crabstick and fish ball. Surimi-based products are popular and widely consumed worldwide, such as in the United States, Southeast Asia, Japan, Korea and China.

2.2 Tropical fish surimi

2.2.1 Fish resources for commercial surimi production

A number of different species of fish have been utilized as raw material in surimi production, including cold water fish (e.g., Alaska pollock), temperate water fish (e.g., Pacific whiting), and warm water fish (e.g., threadfin bream). Alaska pollock (*Theregra chalcogramma*) is the first large-scale fish source used in manufacture of surimi, which is known as a premium grade product with high gel quality (Guenneugues & Ianelli, 2013; Poowakanjana & Park, 2013). Nevertheless, with the decreased harvests and increased fishing costs of Alaska pollock, some new species of fish have

been explored and utilized for commercial surimi production, even though the fish with low gel-forming ability and/or dark color, such as lizardfish and sardine (Guenneugues et al., 2013; Guenneugues & Morrissey, 2005). Nowadays, Southeast Asia has provided the largest fish resource for commercial surimi production, which occupies 60% of surimi production around the world (Guenneugues et al., 2013). In Southeast Asia, the production of tropical fish surimi increased from 320,000 MT in 2005 to 550,000 MT in 2014 (http://www.Groundfishforum.com).

2.2.2 Characteristics of tropical fish surimi

2.2.2.1 Threadfin bream surimi

Threadfin bream (*Nemipterus* spp.) is a typical tropical fish, which has become the second largest fish resource for manufacture of surimi, only after Alaska pollock. It plays an important role in surimi markets, especially in Thailand, which contributes to the major production of threadfin bream surimi in the world (Poowakanjana et al., 2013; Yongsawatdigul, Worratao, & Park, 2002). The production of threadfin bream surimi increased from 65,000 MT in 1978 to 94,000 MT in 2011 (http://www.Groundfishforum.com). Threadfin bream is preferred as raw material for surimi production because it has white color, smooth texture, good flavor, strong gel-forming ability and is resistance to temperature. Thus, the characteristics of threadfin bream surimi has been concerned. The effects of endogenous transglutaminase (TGase), medium (25 °C) and high (40 °C) setting temperatures, comminution conditions, high pressure, and ohmic heating on heat-induced gel properties of threadfin bream surimi have been reported. The gel strength increased when pre-incubation at either 25 and 40 °C, and the optimal pre-incubation time was 4 and 2 h, respectively (Yongsawatdigul et al., 2002). An increase in setting time (0–8 h) at 25 °C led to higher values of breaking

force and deformation of surimi gel, and the highest increase in breaking force was observed in 1 h when setting at 40 °C (Benjakul, Chantarasuwan, & Visessanguan, 2003; Benjakul, Visessanguan, & Chantarasuwan, 2004). Compared with Alaska pollock and Pacific whiting surimi, a better gel texture of threadfin bream surimi was exhibited when a longer chopping at a higher temperature was conducted (Poowakanjana, Mayer, & Park, 2012; Poowakanjana et al., 2013). With a high-pressure pretreatment, the degradation of protein increased, but a more compact microstructure of surimi gel was observed (Zhu, Lanier, & Farkas, 2015). When compared with water bath heating, 1.3 times increase was found at both values of breaking force and deformation of threadfin bream surimi after ohmic heating (Tadpitchayangkoon et al., 2012).

2.2.2.2 Other species

The other tropical fish used in manufacture of surimi in Southeast Asia mainly include lizardfish (*Saurida* spp.), croakers (*Pennahia* and *Johnius* spp.), bigeye snapper (*Priacanthus* spp.) and goat fish/red mullet (*Upeneus* spp. and *Parupeneus* spp.). The characteristics of tropical surimi are varied with fish species because different fish are differed in thermal stability of protein, endogenous enzymes (TGase and proteinase), and color and fat content of muscle.

2.3 Myofibrillar proteins

Fish muscle proteins are composed of 66-77% myofibrillar proteins, 20-30% sarcoplasmic proteins, and 3-5% stroma proteins (Suzuki, 1981). Myofibrillar proteins are the predominant proteins in fish muscle which function as structural proteins. These proteins are salt-soluble proteins, which can be extracted using neutral salt solutions with an ionic strength that is higher than 0.15, and an ionic strength of 0.3-0.7 is usually

required. Myofibrillar proteins are superior gelling proteins, playing a key role in desirable textural properties in muscle foods. In myofibril protein system, myosin and actomyosin is the major protein contributing to the gel-forming capacity in prerigor and postrigor stage, respectively (Strasburg, Xiong, & Chiang, 2008).

2.3.1 Myosin

Myosin constitutes 55-60% of myofibrillar proteins, which is the protein that forms the thick filament (Figure 2.1) (Lanier, Yongsawatdigul, & Carvajal-Rondanelli, 2013). The molecular weight of myosin is around 470 kDa. A myosin molecule is constituted by two heavy chains with 220 kDa and two pairs of different light chains with17-22 kDa, which are assembled in quaternary structure of myosin. The two heavy chains interact to form globular "head" and "rod", and alpha-helix is the main secondary structure in the globular head (Lanier et al., 2013). The actin binding site, ATPase site and DTNB [(5,5-dithiobis)-2-(nitrobenzoic acid)] light chain site are located in the globular head of myosin (Strasburg et al., 2008).

2.3.2 Actin

Actin is the major protein of the thin filament (Figure 2.1), comprising 15-30% of myofibrillar proteins, which is normally associated with troponin and tropomyosin complex in muscle tissue (Lanier et al., 2013). Actin also contains a binding site with myosin where myosin can form a temporary complex with actin during muscle contractile or form a permanent myosin-actin complex in postmorterm (Xiong, 1997).

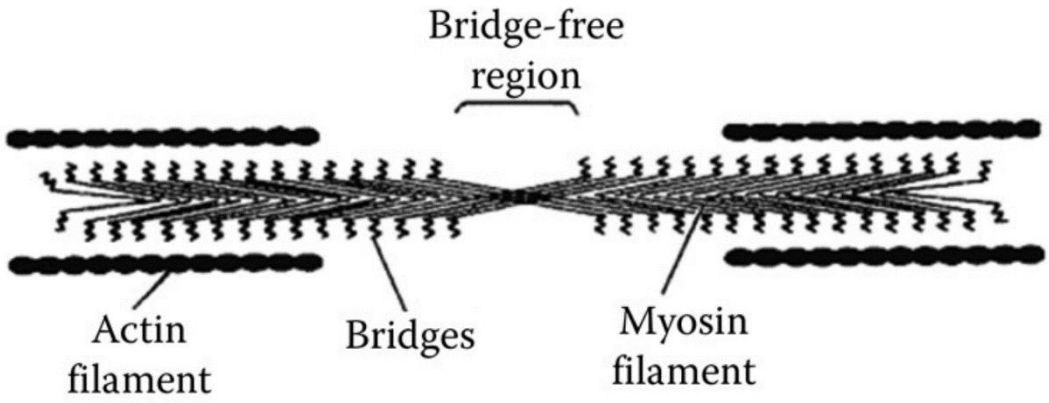


Figure 2.1 Arrangements between actin filament and myosin filament in muscle.

Adapted from: Lanier et al. (2013).

2.3.3 Actomyosin

Myosin constitutes the majority of the total myofibrillar proteins. However, postmortem myosin binds to actin tightly, forming myosin-actin complex, the so-called "actomyosin" (Lanier et al., 2013). Therefore, actomyosin is the predominant protein in surimi, which largely contributes to the heat-induced surimi gelation (Lanier et al., 2013; Yasui, Ishioroshi, & Samejima, 1982).

2.3.4 Other myofibrillar proteins

Other myofibrillar proteins in fish muscle include tropomyosin, troponin complexes, actinins, titin, nebulin, M-proteins, and C-proteins, which play key roles in the integrity of sarcomere structure (Strasburg et al., 2008). In manufacture of surimi-based products, the disassembly of sarcomeres as well as the selective solubilization and/or degradation of these small fractions are also essential to obtain a desirable heat-induced gel structure (Lanier et al., 2013).

2.4 Gelation of surimi

Gelation is a critical parameter determining the quality of surimi because it governs the textural and sensory characteristics of the surimi-based products, such as tenderness and juiciness during mastication. Gelation of surimi is directly related to myofibrillar proteins. The extraction of myofibrillar proteins is the first step to form surimi gel, which is typically conducted by chopping surimi and then adding with salt and phosphates. The former, usually sodium chloride or potassium chloride, assists solubilization of myofibrillar proteins, and the latter facilitates the separation of myosin from actomyosin complex. Usually, the concentration of sodium chloride used is 1.7-3.5% (Lanier et al., 2013).

In manufacturing of surimi seafoods, myofibrillar proteins are typically heat-gelled that is involved with both globular head and tail portions of myosin molecule (Figure 2.2). The excellent heat-induced gel properties of surimi make it useful as a food ingredient in processing of many kinds of surimi seafoods. The mechanism of heat-induced surimi gelation is closely associated with myosin, which is the major gelling protein extracted during comminution stage of surimi. Under a typical surimi processing condition, the gelation begins with the partial unfolding of protein, which is induced by heat treatment. The unfolding of protein results in exposure of reactive groups of proteins, leading to the protein aggregation via intermolecular interactions to form a three-dimensional gel network. Hydrogen bonds, ionic linkages, hydrophobic interactions and covalent bonds (e.g., disulfide bond) are the main kinds of chemical bonds involving in surimi gelation (Lanier et al., 2013). Hydrogen bonds are unstable and break down during temperature rising. Disulfide bonds are the predominant covalent bond at the temperature above 40 °C. As temperature increases, thermal

aggregation is related to the increase in surface hydrophobicity of protein, and hydrophobic interactions become the major force when the temperature rises to around 60 °C (Lanier et al., 2013).

Therefore, protein unfolding (denaturation) followed by protein aggregation leads to formation of surimi gel through intermolecular covalent and noncovalent interactions. In order to obtain desirable surimi gel, it is necessary to understand the basic knowledge of physicochemical changes to the myofibrillar proteins, which could affect the denaturation and aggregation behaviors of myofibrillar proteins, ultimately governing textural properties.

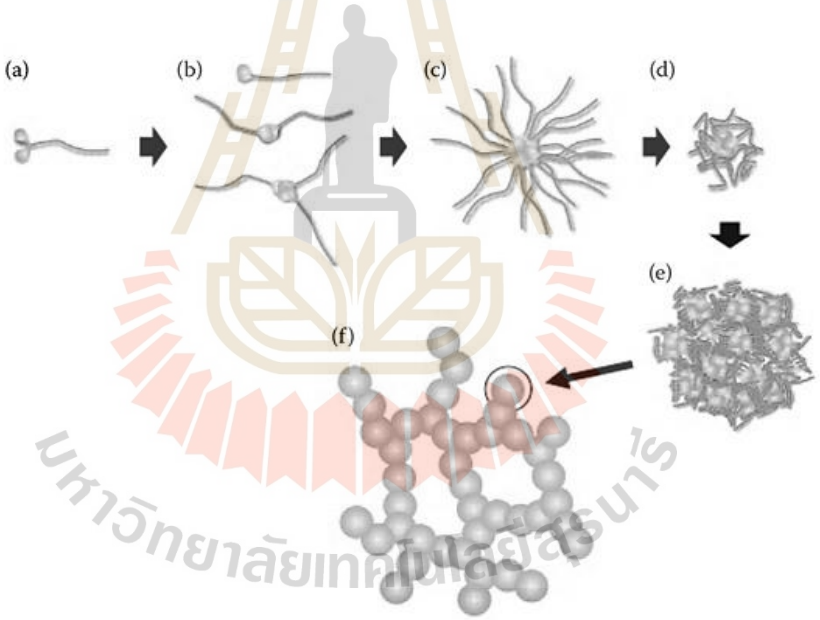


Figure 2.2 Six steps of gel formation of myosin molecule induced by heating. (a) Unheated myosin monomer; (b) formation of cluster in early stage of heating; (c) formation of Daisy wheel by head-to-head of myosin molecules; (d) denaturation of tail portions of myosin; (e) clusters bound via denatured tails; (f) gel network formation.

Adapted from: Lanier et al. (2013).

2.5 Low/reduced salt surimi seafoods

2.5.1 Challenges in production of low/reduced salt surimi

Sodium chloride is the salt used in food industry, consisting of 40% of sodium and 60% of chloride by weight. Sodium chloride provides approximately 90% of sodium in human diet (Kloss, Meyer, Graeve, & Vetter, 2015). Currently, consumption of excessive sodium has become a public concern since it increases risk of hypertension and cardiovascular diseases. In view of these health problems, the intake of salt (sodium chloride) in adults should be less than 5 g/day recommended by the World Health Organization (WHO) in 2012, which equivalents to 2 g/day of sodium (Inguglia, Zhang, Tiwari, Kerry, & Burgess, 2017). Based on the standard of Food & Drug Administration (2017), low sodium on food represents 140 mg of sodium or less per serving, and reduced sodium on food represents at least 25% less sodium than the regular product (Table 2.1).

Table 2.1 Classification of claims on food based on sodium content.

Claims on food	Sodium content
Salt/Sodium-free	Less than 5 mg of sodium per serving
Very low sodium	35 mg of sodium or less per serving
Low sodium	140 mg of sodium or less per serving
Reduced sodium	At least 25% less sodium than the regular product
Light in sodium or lightly salted	At least 50% less sodium than the regular product
No salt added or unsalted	No salt is added during processing, but these products may not be salt/sodium-free unless stated

Adapted from: Food & Drug Administration (2017).

Surimi seafood products are typical processed muscle foods. It has been estimated that muscle foods account for about 20-30% of daily sodium consumption (Petracci, Bianchi, Mudalal, & Cavani, 2013). Consequently, the necessity to reduce sodium in surimi-based product is particularly important to fit with the raising consumer awareness and WHO recommendations.

Sodium chloride at 2-3% is typically required to facilitate solubilization of the myofibrillar proteins in surimi (Lanier et al., 2013). A decrease in NaCl content results in poor gel-forming ability of surimi because myofibrillar proteins are not adequately solubilized and are not properly unfolded (Figure 2.3). Fu et al. (2012) reported that the mechanical properties of silver carp surimi gel became worse when NaCl content was decreased to 1%. Thereby, sodium chloride is an ingredient essential for surimi gelation and gel quality largely depends on sodium chloride concentration. In addition, the preservative properties and the capacity to affect taste and improve product flavor of sodium chloride are also important in surimi seafood products.

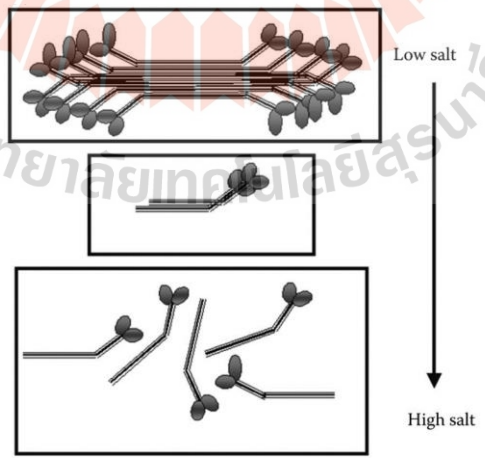


Figure 2.3 Successive disassembly of myosin in solution with increasing of salt concentration.

Adapted from: Lanier et al. (2013).

2.5.2 Salt/sodium reduction strategies in surimi

In order to meet consumers' demand, efforts have been made to reduce salt/sodium content in surimi gels based on various strategies. Initial exploration focused on a partial replacement of sodium chloride with other salts, such as potassium chloride and magnesium chloride. Potassium chloride is typically used to partially replace NaCl, but it causes negative sensory characteristics due to its bitterness and metallic taste (Tahergorabi & Jaczynski, 2012). Sodium reduction has also been reported by addition of different ingredients, such as whey protein concentrate (Uresti, Téllez-Luis, Ramírez, & Vázquez, 2004), tetra-sodium pyrophosphate (Deysi Cando, Herranz, Borderías, & Moreno, 2016) and lysine (D. Cando, Borderías, & H. M. Moreno, 2016). Recently, much attention has been given towards the applications of novel physical techniques. Fu et al. (2012) reported that 1% NaCl silver carp surimi gel was improved after microwave heating because faster heating process of microwave inhibited autolysis and the intense heat provided by microwave expanded protein aggregates which served as substrate for crosslinking reactions. High pressure processing technique has been explored to improve Alaska pollock surimi gel at 0.3% NaCl (Deysi Cando, Herranz, Borderías, & Moreno, 2015) and was reported to strengthen the water retention of myosin gel in 0.3 M NaCl (Jianyi Wang, Li, Zheng, Zhang, & Guo, 2019), which were due to that high pressure induced protein unfolding by decreasing protein volume and then promoted surimi gelation via protein-protein interactions. However, exploration of low/reduced salt surimi still faces challenges because salt plays a critical role in surimi gelation and gel quality. Therefore, low/reduced salt surimi needs to be further investigated, providing more approaches feasible for surimi industries.

2.6 High intensity ultrasound (HIU)

2.6.1 Principle of ultrasound

In recent years, the applications of ultrasound technology in food research and industry have been widely concerned because it is considered as a safe, nontoxic and green technology. The effects of ultrasound energy are mainly due to cavitation phenomenon, micro-streaming and reactive free radicals produced from water molecules. Ultrasound is propagated by a sequence of compression and rarefaction cycles, which are induced on the molecules in the medium (Figure 2.4) (Mason, Riera, Vercet, & Lopez-Buesa, 2005). During sonication, the rarefaction wave may exceed the attractive force between liquid molecules under a adequately high power, resulting in formation of cavitation bubbles (H. Hu, Cheung, Pan, & Li-Chan, 2015; Soria & Villamiel, 2010). These bubbles grow over several cycles to a larger size until becoming unstable and then violently collapse, which leads to energy accumulations in the cavitation zone, generating not only high temperature and pressure, but also very high shear energy wave and turbulence (Liu, Liu, Xiong, Fu, & Chen, 2017; Soria et al., 2010). Furthermore, the variation of bubble size and the diffusion of gases into and out of the bubbles will generate strong micro-currents which are associated with alteration of media characteristics due to high-velocity gradient and shear stress (Soria et al., 2010; Zheng & Sun, 2006). In addition, numerous reactive free radicals ($H_2O \rightarrow \cdot H + \cdot OH$) can also be generated from water molecules during sonication, which may react with and/or modify other molecules and/or reactive groups, such as sulfhydryl groups of proteins (H. Hu, Wu, et al., 2013; Milne, Stewart, & Bremner, 2013; Riesz & Kondo, 1992).

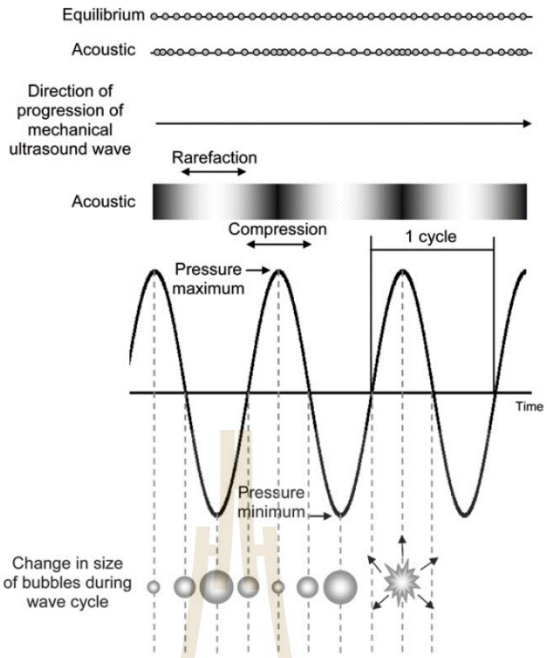


Figure 2.4 Ultrasonic cavitation.

From: Mason et al. (2005).

2.6.2 Applications of HIU in myofibrillar proteins

Ultrasound is based on the mechanical waves at a frequency that exceeds the hearing limit of human (i.e., above 16 kHz), which can be categorized into low intensity ultrasound (LIU) and high intensity ultrasound (HIU) according to different frequency ranges. LIU refers to a high frequency (100 kHz-1 MHz) with a low intensity (usually $<$1 W/cm^2) and is usually applied as a non-destructive analytical technique in food quality assessment. HIU refers to a low frequency (100 kHz-1 MHz) with a high intensity (typically 10-1000 W/cm^2) (Soria et al., 2010), which is most commonly used in food processing (Y. Hu et al., 2014; C. K. McDonnell, Allen, Morin, & Lyng, 2014) and modifications of food proteins (H. Hu et al., 2015; H. Hu, Wu, et al., 2013; Liu et al., 2017; Saleem & Ahmad, 2016; Z. Zhang, Regenstein, Zhou, & Yang, 2017).

HIU has been reported to modify physicochemical and functional properties of food proteins, including soy proteins (H. Hu et al., 2015; H. Hu, Li-Chan, Wan, Tian, & Pan, 2013; Morales, Martínez, Ruiz-Henestrosa, & Pilosof, 2015; P. Zhang et al., 2016), whey proteins (Frydenberg, Hammershøj, Andersen, & Wiking, 2016; Shen, Zhao, & Guo, 2017), black bean isolate (Jiang et al., 2014), plum seed protein isolate (Xue, Wang, Liu, & Li, 2018), quinoa seed protein isolates (Mir, Riar, & Singh, 2019) and myofibrillar proteins (Liu et al., 2017; Saleem et al., 2016; Z. Zhang et al., 2017).

HIU has been recently explored to modify physicochemical properties of myofibrillar proteins, such as solubility (Liu et al., 2017; Z. Zhang et al., 2017). HU has also been reported to induce unfolding of myofibrillar proteins, exposing additional hydrophobic and sulfhydryl groups (Jingyu Wang, Yang, Tang, Ni, & Zhou, 2017; Zou et al., 2018). Moreover, HIU has been proven to accelerate the salt duffusion in meat tissues (Cárcel, Benedito, Bon, & Mulet, 2007; C. K. McDonnell, Lyng, & Allen, 2014; Ciara K. McDonnell, Lyng, Arimi, & Allen, 2014; Ozuna, Puig, García-Pérez, Mulet, & Cárcel, 2013). Besides, the potential of HIU to improve water holding capacity of chicken myofibrillar protein gel (Z. Zhang et al., 2017) has also been investigated.

These studies of myofibrillar proteins focused on a typical high salt concentration, e.g., 0.6 M NaCl. However, little information is available on application of HIU technology in myofibrillar proteins under low salt conditions, where myofibrillar proteins has only limited protein solubility and behave in a different way on denaturation and aggregation. Due to the potential to increase the soluble myofibrillar proteins exposed to low salt, it is assumed that HIU technology should positively contribute to the surimi gel under reduced salt concentration because soluble myofibrillar proteins are thought to be the base element of gel formation. Moreover, the

exposure of more hydrophobic and sulfhydryl groups induced by HIU is expected to lead to strong protein interactions and gel networks. This would eventually improve gel properties at low/reduced-salt contents. Therefore, HIU technology could be a potential processing-aid to produce healthier surimi seafood products with reduced sodium content without the use of chemicals and/or enzymes.

2.7 Fourier transform infrared (FT-IR) spectroscopy

2.7.1 Principle of FT-IR spectroscopy

Infrared (IR) spectroscopy depends on the measurement of IR absorption by solids, liquids or gases, which needs a change in the intrinsic dipole moment with molecular vibration (Li-Chan, 1996; Wehling, 2010). IR is one of the well- established techniques that is used for analysis of protein secondary structures (Kong & Yu, 2007). Nevertheless, H_2O is a polar molecule with strong IR absorption in three obvious bands at around 3400 cm^{-1}, 2125 cm^{-1} and 1645 cm^{-1}, which stand for O-H stretching, water association and H-O-H bending, respectively. Thus, the H-O-H bending vibrational band of H_2O at 1645 cm^{-1} directly overlaps with the amide I region (1600-1700 cm^{-1}) of proteins, and it shows higher intensity than the amide I region. Due to it, water absorption in 1600−1700 cm^{-1} is the biggest interference during IR analysis of protein samples dissolved in H_2O solution. Therefore, IR is commonly used for analysis of samples in dry or non-aqueous forms (Kong et al., 2007).

The Fourier transform (FT) has been developed because of the use of a stable and powerful laser, which is a mathematical treatment used for data acquisition and reliable digital subtraction of IR (Kong et al., 2007; Wehling, 2010). Since FT-IR spectroscopy measures molecular structure on the basis of IR energy absorption of

molecular vibration related to chemical bonds and functional groups at the specific frequencies, it allows the investigation of the structure of peptides and proteins and can provide both qualitative and quantitative analysis (Böcker, Ofstad, Bertram, Egelandsdal, & Kohler, 2006; Wu, Bertram, Böcker, Ofstad, & Kohler, 2007).

2.7.2 Spectra analysis

As shown in Table 2.2, there are nine characteristic IR absorption bands of polypeptides and proteins. Among them, the most useful vibrational bands to determine the secondary structures of proteins are amide I (1600-1690 cm^{-1}) and amide II (1480-1575 cm^{-1}). Since the C=O stretch vibrations of the peptide linkages account for approximately 80%, the amide I region is the most sensitive band to the changes of secondary structures of proteins. Moreover, it has been found that the frequencies of amide I components are closely correlated with each secondary structure of proteins. Whereas, the amide II region is mainly derived from the in-plane NH bending (40−60% of the potential energy) and the CN stretching vibration (18−40% of the potential energy), which show less sensitivity of protein conformational changes than that in amide I (Kong et al., 2007). In addition, FT-IR can provide not only direct correlations between frequencies of amide I band and each secondary structure of proteins, but also the information on structural stability and dynamics of proteins.

Table 2.2 Characteristic infrared bands of peptide linkage.

Designation	Approximate frequency (cm^{-1})	Description
Amide A	3300	NH stretching
Amide B	3100	NH stretching
Amide I	1600-1690	C=O stretching
Amide II	1480-1575	CN stretching, NH bending
Amide III	1229-1301	CN stretching, NH bending
Amide IV	625-767	OCN bending
Amide V	640-800	Out-of-plane NH bending
Amide VI	537-606	Out-of-plane C=O bending
Amide VII	200	Skeletal torsion

From: Kong et al. (2007).

2.7.3 Applications of FT-IR spectroscopy in myofibrillar proteins

FT-IR spectroscopy has been used in structural analysis of myofibrillar proteins related to gelation, where the estimation of protein secondary structures is the main application. Kobayashi, Mayer, and Park (2017) reported that a higher β-sheet content of tilapia surimi gel was exhibited when surimi was chopped at 25 ºC for 18 min compared with that chopped at 5 ºC for 6 min, indicating that a longer chopping at a higher temperature resulted in greater protein unfolding and disintegration of protein before gelation. The characteristic peak of Alaska pollock surimi gels at 1645 cm^{-1} was judged as random coil under high temperature treatments of 100 and 120 ºC, suggesting that random coil was the main secondary structure of surimi gels when treated at these heating conditions (Zhang, Zhang, & Wang, 2016). Alaska pollock surimi gel showed

an increase in β-sheet content with a decrease in α-helix content (Cando, Herranz, Borderías, & Moreno, 2015). Similar results were also reported by Núñez-Flores, Cando, Borderías, and Moreno (2018) that Alaska pollock surimi gel showed obvious decreased α-helix content compared with raw surimi due to heating at 90 ºC for 30 min, which caused an increase in β-sheet content during gelation. Besides, Cando, Herranz, Borderías, and Moreno (2016) reported that a higher β-sheet content was observed at 3% NaCl surimi paste than that at 0.3% NaCl, and the greater protein solubilization and protein unfolding at the higher NaCl content induced an increase in β-sheet content and a decrease in α-helix content during surimi gelation.

Moreover, FT-IR spectroscopy has also been applied to analyze secondary structural changes of myofibrillar proteins under various conditions, such as aging (Kirschner, Ofstad, Skarpeid, Høst, & Kohler, 2004), heating (Bertram, Kohler, Böcker, Ofstad, & Andersen, 2006; Wu et al., 2007), salting (Böcker et al., 2006; Wu et al., 2006), and storage (Moosavi-Nasab, Alli, Ismail, & Ngadi, 2005). In addition, the investigations of FT-IR on secondary structural changes of myofibrillar proteins as affected by high pressure (Larrea-Wachtendorff, Tabilo-Munizaga, Moreno-Osorio, Villalobos-Carvajal, & Pérez-Won, 2015), oxidative stress (Zhou, Zhao, Su, Cui, & Sun, 2014), and addition of polysaccharides (Fan et al., 2017; Y.-z. Zhou et al., 2014) and MTGase (D. Cando et al., 2016) were reported as well. Therefore, FT-IR spectroscopy has potential to analyze and characterize the secondary structural changes of fish myofibrillar proteins related to surimi gelation, which would be a useful tool to monitor the surimi gelation at molecular level.

2.8 References

Benjakul, S., Chantarasuwan, C., & Visessanguan, W. (2003). Effect of medium temperature setting on gelling characteristics of surimi from some tropical fish. **Food Chemistry.** 82 (4), 567-574.

Benjakul, S., Visessanguan, W., & Chantarasuwan, C. (2004). Effect of high-temperature setting on gelling characteristic of surimi from some tropical fish. **International Journal of Food Science & Technology.** 39 (6), 671-680.

Bertram, H. C., Kohler, A., Böcker, U., Ofstad, R., & Andersen, H. J. (2006). Heat-induced changes in myofibrillar protein structures and myowater of two pork qualities. A combined FT-IR spectroscopy and low-field NMR relaxometry study. **Journal of Agricultural and Food Chemistry.** 54 (5), 1740-1746.

Böcker, U., Ofstad, R., Bertram, H. C., Egelandsdal, B., & Kohler, A. (2006). Salt-induced changes in pork myofibrillar tissue investigated by FT-IR microspectroscopy and light microscopy. **Journal of Agricultural and Food Chemistry.** 54 (18), 6733-6740.

Cando, D., Borderías, A. J., & H. M. Moreno. (2016). Combined effect of aminoacids and microbial transglutaminase on gelation of low salt surimi content under high pressure processing. **Innovative Food Science & Emerging Technologies.** 36, 10-17.

Cando, D., Herranz, B., Borderías, A. J., & Moreno, H. M. (2015). Effect of high pressure on reduced sodium chloride surimi gels. **Food Hydrocolloids.** 51 (Supplement C), 176-187.

Cando, D., Herranz, B., Borderías, A. J., & Moreno, H. M. (2016). Different additives to enhance the gelation of surimi gel with reduced sodium content. **Food Chemistry.** 196, 791-799.

Cárcel, J. A., Benedito, J., Bon, J., & Mulet, A. (2007). High intensity ultrasound effects on meat brining. **Meat Science.** 76 (4), 611-619.

Fan, M., Hu, T., Zhao, S., Xiong, S., Xie, J., & Huang, Q. (2017). Gel characteristics and microstructure of fish myofibrillar protein/cassava starch composites. **Food Chemistry.** 218, 221-230.

Food & Drug Administration, U. S. (2017). Sodium in Your Diet: Use the Nutrition Facts Label and Reduce Your Intake. https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/UCM315471.pdf In.

Frydenberg, R. P., Hammershøj, M., Andersen, U., & Wiking, L. (2016). High intensity ultrasound effects on heat-induced whey protein isolate gels depend on α-lactalbumin: β-lactoglobulin ratio. **International Dairy Journal.** 56, 1-3.

Fu, X., Hayat, K., Li, Z., Lin, Q., Xu, S., & Wang, S. (2012). Effect of microwave heating on the low-salt gel from silver carp (*Hypophthalmichthys molitrix*) surimi. **Food Hydrocolloids.** 27 (2), 301-308.

Guenneugues, P., & Ianelli, J. (2013). Surimi Resources and Market. In J. W. Park (Ed.), Surimi and Surimi Seafood (3rd Edition) (pp. 26-53). Boca Raton, FL: CRC Press.

Guenneugues, P., & Morrissey, M. T. (2005). Surimi Resources. In J. W. Park (Ed.), Surimi and Surimi Seafood (2nd Edition) (pp. 4-32). Boca Raton, FL: CRC Press.

Hu, H., Cheung, I. W. Y., Pan, S., & Li-Chan, E. C. Y. (2015). Effect of high intensity ultrasound on physicochemical and functional properties of aggregated soybean β-conglycinin and glycinin. **Food Hydrocolloids.** 45, 102-110.

Hu, H., Fan, X., Zhou, Z., Xu, X., Fan, G., Wang, L., Huang, X., Pan, S., & Zhu, L. (2013). Acid-induced gelation behavior of soybean protein isolate with high intensity ultrasonic pre-treatments. **Ultrasonics Sonochemistry.** 20 (1), 187-195.

Hu, H., Li-Chan, E. C. Y., Wan, L., Tian, M., & Pan, S. (2013). The effect of high intensity ultrasonic pre-treatment on the properties of soybean protein isolate gel induced by calcium sulfate. **Food Hydrocolloids.** 32 (2), 303-311.

Hu, H., Wu, J., Li-Chan, E. C. Y., Zhu, L., Zhang, F., Xu, X., Fan, G., Wang, L., Huang, X., & Pan, S. (2013). Effects of ultrasound on structural and physical properties of soy protein isolate (SPI) dispersions. **Food Hydrocolloids.** 30 (2), 647-655.

Hu, Y., Yu, H., Dong, K., Yang, S., Ye, X., & Chen, S. (2014). Analysis of the tenderisation of jumbo squid (*Dosidicus gigas*) meat by ultrasonic treatment using response surface methodology. **Food Chemistry.** 160 (Supplement C), 219-225.

Inguglia, E. S., Zhang, Z., Tiwari, B. K., Kerry, J. P., & Burgess, C. M. (2017). Salt reduction strategies in processed meat products – A review. **Trends in Food Science & Technology.** 59, 70-78.

Jiang, L., Wang, J., Li, Y., Wang, Z., Liang, J., Wang, R., Chen, Y., Ma, W., Qi, B., & Zhang, M. (2014). Effects of ultrasound on the structure and physical properties of black bean protein isolates. **Food Research International.** 62, 595-601.

Kirschner, C., Ofstad, R., Skarpeid, H.-J., Høst, V., & Kohler, A. (2004). Monitoring of denaturation processes in aged beef loin by Fourier transform infrared microspectroscopy. **Journal of Agricultural and Food Chemistry.** 52 (12), 3920-3929.

Kloss, L., Meyer, J. D., Graeve, L., & Vetter, W. (2015). Sodium intake and its reduction by food reformulation in the European Union — A review. **NFS Journal.** 1 (Supplement C), 9-19.

Kobayashi, Y., Mayer, S. G., & Park, J. W. (2017). FT-IR and Raman spectroscopies determine structural changes of tilapia fish protein isolate and surimi under different comminution conditions. **Food Chemistry.** 226, 156-164.

Kong, J., & Yu, S. (2007). Fourier transform infrared spectroscopic analysis of protein secondary structures. **Acta biochimica et biophysica Sinica.** 39 (8), 549-559.

Lanier, T. C., Yongsawatdigul, J., & Carvajal-Rondanelli, P. (2013). Surimi gelation chemistry. In J. W. Park (Ed.), Surimi and Surimi Seafood (3rd 3 Edition) (pp. 101-139). Boca Raton, FL: CRC Press.

Larrea-Wachtendorff, D., Tabilo-Munizaga, G., Moreno-Osorio, L., Villalobos-Carvajal, R., & Pérez-Won, M. (2015). Protein Changes Caused by High Hydrostatic Pressure (HHP): A Study Using Differential Scanning Calorimetry (DSC) and Fourier Transform Infrared (FTIR) Spectroscopy. **Food Engineering Reviews.** 7 (2), 222-230.

Li-Chan, E. C. Y. (1996). The applications of Raman spectroscopy in food science. **Trends in Food Science & Technology.** 7 (11), 361-370.

Liu, R., Liu, Q., Xiong, S., Fu, Y., & Chen, L. (2017). Effects of high intensity unltrasound on structural and physicochemical properties of myosin from silver carp. **Ultrasonics Sonochemistry.** 37 (Supplement C), 150-157.

Mason, T., Riera, E., Vercet, A., & Lopez-Buesa, P. (2005). Application of ultrasound. In D. W. Sun (Ed.), Emerging Technologies for Food Processing (pp. 323-350). California: Elsevier Academic Press.

McDonnell, C. K., Allen, P., Morin, C., & Lyng, J. G. (2014). The effect of ultrasonic salting on protein and water–protein interactions in meat. **Food Chemistry.** 147 (Supplement C), 245-251.

McDonnell, C. K., Lyng, J. G., & Allen, P. (2014). The use of power ultrasound for accelerating the curing of pork. **Meat Science.** 98 (2), 142-149.

McDonnell, C. K., Lyng, J. G., Arimi, J. M., & Allen, P. (2014). The acceleration of pork curing by power ultrasound: A pilot-scale production. **Innovative Food Science & Emerging Technologies.** 26 (Supplement C), 191-198.

Milne, L., Stewart, I., & Bremner, D. H. (2013). Comparison of hydroxyl radical formation in aqueous solutions at different ultrasound frequencies and powers using the salicylic acid dosimeter. **Ultrasonics Sonochemistry.** 20 (3), 984-989.

Mir, N. A., Riar, C. S., & Singh, S. (2019). Structural modification of quinoa seed protein isolates (QPIs) by variable time sonification for improving its physicochemical and functional characteristics. **Ultrasonics Sonochemistry.** 58, 104700.

Moosavi-Nasab, M., Alli, I., Ismail, A. A., & Ngadi, M. O. (2005). Protein structural changes during preparation and storage of surimi. **Journal of Food Science.** 70 (7).

Morales, R., Martínez, K. D., Ruiz-Henestrosa, V. M. P., & Pilosof, A. M. R. (2015). Modification of foaming properties of soy protein isolate by high ultrasound intensity: particle size effect. **Ultrasonics Sonochemistry.** 26, 48-55.

Núñez-Flores, R., Cando, D., Borderías, A. J., & Moreno, H. M. (2018). Importance of salt and temperature in myosin polymerization during surimi gelation. **Food Chemistry.** 239, 1226-1234.

Ozuna, C., Puig, A., García-Pérez, J. V., Mulet, A., & Cárcel, J. A. (2013). Influence of high intensity ultrasound application on mass transport, microstructure and textural properties of pork meat (*Longissimus dorsi*) brined at different NaCl concentrations. **Journal of Food Engineering.** 119 (1), 84-93.

Park, J. W., Graves, D., Draves, R., & Yongsawatdigul, J. (2013). Manufacture of Surimi: Harvest to Frozen Block. In J. W. Park (Ed.), Surimi and Surimi Seafood (3rd Edition) (pp. 33-106). Boca Raton, FL: CRC Press.

Petracci, M., Bianchi, M., Mudalal, S., & Cavani, C. (2013). Functional ingredients for poultry meat products. **Trends in Food Science & Technology.** 33 (1), 27-39.

Poowakanjana, S., Mayer, S. G., & Park, J. W. (2012). Optimum chopping conditions for Alaska pollock, Pacific whiting, and threadfin bream surimi paste and gel based on rheological and Raman spectroscopic analysis. **Journal of Food Science.** 77 (4), E88-E97.

Poowakanjana, S., & Park, J. W. (2013). Biochemical characterisation of Alaska pollock, Pacific whiting, and threadfin bream surimi as affected by comminution conditions. **Food Chemistry.** 138 (1), 200-207.

Riesz, P., & Kondo, T. (1992). Free radical formation induced by ultrasound and its biological implications. **Free Radical Biology and Medicine.** 13 (3), 247-270.

Saleem, R., & Ahmad, R. (2016). Effect of low frequency ultrasonication on biochemical and structural properties of chicken actomyosin. **Food Chemistry.** 205 (Supplement C), 43-51.

Shen, X., Zhao, C., & Guo, M. (2017). Effects of high intensity ultrasound on acid-induced gelation properties of whey protein gel. **Ultrasonics Sonochemistry.** 39, 810-815.

Soria, A. C., & Villamiel, M. (2010). Effect of ultrasound on the technological properties and bioactivity of food: a review. **Trends in Food Science & Technology.** 21 (7), 323-331.

Strasburg, G., Xiong, Y. L., & Chiang, W. (2008). Physiology and chemistry of edible muscle tissues. In S. Damodaran, K. L. Parkin & O. R. Fennema (Eds.), Fennema's Food Chemistry (4th Edition) (pp. 923-973). Boca Raton, FL: CRC Press.

Suzuki, T. (1981). Fish and krill protein: processing technology. London, UK: Applied science publisher.

Tadpitchayangkoon, P., Park, J. W., & Yongsawatdigul, J. (2012). Gelation characteristics of tropical surimi under water bath and ohmic heating. **LWT - Food Science and Technology.** 46 (1), 97-103.

Tahergorabi, R., & Jaczynski, J. (2012). Physicochemical changes in surimi with salt substitute. **Food Chemistry.** 132 (3), 1281-1286.

Uresti, R. o. M., Téllez-Luis, S. J., Ramírez, J. A., & Vázquez, M. (2004). Use of dairy proteins and microbial transglutaminase to obtain low-salt fish products from filleting waste from silver carp (*Hypophthalmichthys molitrix*). **Food Chemistry.** 86 (2), 257-262.

Wang, J., Li, Z., Zheng, B., Zhang, Y., & Guo, Z. (2019). Effect of ultra-high pressure on the structure and gelling properties of low salt golden threadfin bream (*Nemipterus virgatus*) myosin. **LWT - Food Science and Technology.** 100, 381-390.

Wang, J., Yang, Y., Tang, X., Ni, W., & Zhou, L. (2017). Effects of pulsed ultrasound on rheological and structural properties of chicken myofibrillar protein. **Ultrasonics Sonochemistry.** 38 (Supplement C), 225-233.

Wehling, R. L. (2010). Infrared Spectroscopy. In Food Analysis (4th Edition) (pp. 409-419).

Wu, Z., Bertram, H. C., Böcker, U., Ofstad, R., & Kohler, A. (2007). Myowater dynamics and protein secondary structural changes as affected by heating rate in three pork qualities: a combined FT-IR microspectroscopic and 1H NMR relaxometry study. **Journal of Agricultural and Food Chemistry.** 55 (10), 3990-3997.

Wu, Z., Bertram, H. C., Kohler, A., Böcker, U., Ofstad, R., & Andersen, H. J. (2006). Influence of aging and salting on protein secondary structures and water distribution in uncooked and cooked pork. A combined FT-IR microspectroscopy and 1H NMR relaxometry study. **Journal of Agricultural and Food Chemistry.** 54 (22), 8589-8597.

Xiong, Y. L. (1997). Structure-function relationships of muscle proteins. In S. Damodaran & A. Paraf (Eds.), Food proteins and their applications (pp. 341-392). New York, USA: Marcel Dekker.

Xue, F., Wang, S., Liu, H., & Li, C. (2018). Effects of high-intensity ultrasound treatment on functional properties of plum (*Pruni domesticae semen*) seed protein isolate. **Journal of the Science of Food and Agriculture.** 98(15), 5690-5699.

Yasui, T., Ishioroshi, M., & Samejima, K. (1982). Effect of actomyosin on heat-induced gelation of myosin. **Agricultural and Biological Chemistry.** 46 (4), 1049-1059.

Yongsawatdigul, J., Worratao, A., & Park, J. W. (2002). Effect of endogenous transglutaminase on threadfin bream surimi gelation. **Journal of Food Science.** 67 (9), 3258-3263.

Zhang, L., Zhang, F., & Wang, X. (2016). Changes of protein secondary structures of pollock surimi gels under high-temperature (100 °C and 120 °C) treatment. **Journal of Food Engineering.** 171 (Supplement C), 159-163.

Zhang, P., Hu, T., Feng, S., Xu, Q., Zheng, T., Zhou, M., Chu, X., Huang, X., Lu, X., Pan, S., Li-Chan, E. C. Y., & Hu, H. (2016). Effect of high intensity ultrasound on transglutaminase-catalyzed soy protein isolate cold set gel. **Ultrasonics Sonochemistry.** 29 (Supplement C), 380-387.

Zhang, Z., Regenstein, J. M., Zhou, P., & Yang, Y. (2017). Effects of high intensity ultrasound modification on physicochemical property and water in myofibrillar protein gel. **Ultrasonics Sonochemistry.** 34, 960-967.

Zheng, L., & Sun, D.-W. (2006). Innovative applications of power ultrasound during food freezing processes-a review. **Trends in Food Science & Technology.** 17 (1), 16-23.

Zhou, F., Zhao, M., Su, G., Cui, C., & Sun, W. (2014). Gelation of salted myofibrillar protein under malondialdehyde-induced oxidative stress. **Food Hydrocolloids.** 40, 153-162.

Zhou, Y.-z., Chen, C.-g., Chen, X., Li, P.-j., Ma, F., & Lu, Q.-h. (2014). Contribution of three ionic types of polysaccharides to the thermal gelling properties of chicken breast myosin. **Journal of Agricultural and Food Chemistry.** 62 (12), 2655-2662.

Zhu, Z., Lanier, T. C., & Farkas, B. E. (2015). High pressure effects on heat-induced gelation of threadfin bream (*Nemipterus* spp.) surimi. **Journal of Food Engineering.** 146 (Supplement C), 23-27.

Zou, Y., Xu, P., Wu, H., Zhang, M., Sun, Z., Sun, C., Wang, D., Cao, J., & Xu, W. (2018). Effects of different ultrasound power on physicochemical property and functional performance of chicken actomyosin. **International Journal of Biological Macromolecules.** 113, 640-647.

CHAPTER III

PHYSICOCHEMICAL PROPERTIES OF TILAPIA (*Oreochromis niloticus*) ACTOMYOSIN SUBJECTED TO HIGH INTENSITY ULTRASOUND IN LOW NaCl CONCENTRATIONS

3.1 Abstract

Effects of high intensity ultrasound (HIU) on physicochemical properties of tilapia (*Oreochromis niloticus*) actomyosin in low NaCl concentrations were investigated. The protein content extracted in low NaCl concentrations (0.1-0.3 M NaCl) increased with increasing HIU intensity up to 20.62 W/cm^2 ($p < 0.05$). The effect of HIU on actomyosin extractability in high NaCl concentrations (0.6 and 1.2 M NaCl) was less obvious. Ca^{2+}-ATPase activity and total sulfhydryl (SH) group content decreased in both 0.2 and 0.6 M NaCl. HIU showed more pronounced effect on oxidation of the SH groups in 0.6 M NaCl, while the reactive SH content at 0.2 M NaCl increased after a prolonged exposure to HIU, suggesting conformational changes induced by HIU. Surface hydrophobicity of actomyosin in 0.6 M NaCl increased with increasing ultrasonic intensity and exposure time to a higher degree than that in 0.2 M NaCl. A greater absolute value of the *zeta* potential of actomyosin subjected to HIU were also observed. The HIU treatments decreased the turbidity of actomyosin incubated at 40 and 60 °C. A drastic increase in the solubility of myosin heavy chain

(MHC) and actin with 0.2 M NaCl were evident when HIU treatments were applied, but degradation of MHC occurred in both 0.2 and 0.6 M NaCl. Based on particle size and microstructure, actomyosin in 0.6 M NaCl underwent more disruption by HIU than that in 0.2 M NaCl. HIU induced protein unfolding and protein dissociation, enabling better extraction in a lower NaCl concentration.

Keywords: NaCl, actomyosin, high intensity ultrasound, physicochemical properties

3.2 Introduction

Myofibrillar proteins are salt-soluble proteins requiring > 0.3 M NaCl concentrations for solubilization and gelation. Hence, sodium chloride plays a key role in the gelation of muscle foods. Currently, the reduction of sodium in foods has been driven by more consumers since excessive sodium intake can cause health problems, such as hypertension and cardiovascular diseases (Kloss, Meyer, Graeve, & Vetter, 2015). To meet consumer demand, the production of low-salt or reduced-salt muscle food gels has been developed based on various strategies. The partial substitution of NaCl with other salts, especially KCl, is commonly practiced, but has negative effects on flavor and texture (Tahergorabi & Jaczynski, 2012). The addition of various additives, such as sodium pyrophosphate (Chang, Hultin, & Dagher, 2001), microbial transglutaminase (Uresti, Téllez-Luis, Ramírez, & Vázquez, 2004) and amino acids (e.g., lysine, cysteine and histidine) (Cando, Herranz, Borderías, & Moreno, 2016), has also been studied to improve the gel properties of myofibrillar proteins at low-salt or reduced-salt levels. In addition, some alternative processing techniques such as microwave (Fu et al., 2012) and high pressure (Cando, Herranz, Borderías, & Moreno, 2015) processes have been applied.

Recently, applications of high intensity ultrasound (HIU) have been explored in food research, because HIU is regarded as a safe, nontoxic and environmentally friendly technology. HIU has a low frequency (16-100 kHz) and high power (typically 10-1000 W/cm^2) (Soria & Villamiel, 2010). Cavitation, the formation of microstreaming and highly reactive free radicals ($H_2O \rightarrow \cdot H + \cdot OH$), typically take place during sonication, leading to energy accumulation, high shear energy waves, and possible chemical reactions (Soria et al., 2010). This would eventually affect the physicochemical properties of food components, especially myofibrillar proteins.

Based on HIU principle, it is possible to increase solubility of myofibrillar proteins at relatively low salt concentrations. This would, in turn, improve gel properties at low-salt or reduced-salt levels. It has been reported that HIU not only causes protein dissociation and improves the solubility of myofibrillar proteins at high ionic strength (Saleem & Ahmad, 2016) but also induces the exposure, aggregation and/or oxidation of some functional groups of myofibrillar proteins (e.g., hydrophobic and sulfhydryl groups) (Liu, Liu, Xiong, Fu, & Chen, 2017). Furthermore, HIU has been proven to accelerate the mass transfer of NaCl in meat tissues (McDonnell, Lyng, & Allen, 2014). In addition, the potential of HIU to improve the gel properties of myofibrillar proteins (Zhang, Regenstein, Zhou, & Yang, 2017) has also been reported. The aforementioned studies focused on a typical high salt concentration, e.g., 0.6 M NaCl. To the best of our knowledge, little information is available on the application of HIU to myofibrillar proteins exposed to low salt concentrations, which myofibrillar proteins show limited solubility. It is, thus, necessary to gain more understandings on how myofibrillar proteins behave in low salt concentrations under HIU treatment. This

would ultimately pave a way for development of low-salt and/or reduced-salt actomyosin gel without any sodium substitutes. Generally, the gel formation of food proteins involves the partial denaturation of proteins and subsequent irreversible aggregation (Lanier, Carvajal, & Yongsawatdigul, 2005). Thus, the physicochemical changes to the myofibrillar proteins that are induced by HIU could affect their denaturation and aggregation, which ultimately govern textural properties. Therefore, this study aimed at investigating changes of physicochemical properties of tilapia myofibrillar proteins exposed to HIU under low salt concentrations.

3.3 Materials and methods

3.3.1 Materials

Live tilapia (*Oreochromis niloticus*), with sizes of 1-1.5 kg, were purchased from a local market in Nakhon Ratchasima, Thailand. The fish were transported within 30 min of purchase to the laboratory at Suranaree University of Technology in a polystyrene foam box filled with ice. Upon arrival, live fish were humanely killed by percussion to the brain, then eviscerated, deskinned and washed with tap water. The whole process was conducted on ice. Subsequently, the dorsal muscle was manually collected and vacuum-packed. The actomyosin was prepared immediately.

Phenylmethanesulfonyl fluoride (PMSF, CAS: 329-98-6), adenosine 5′-triphosphate disodium salt hydrate (ATP, CAS: 34369-07-8) and 8-anilino-1-naphthalenesulfonic acid (ANS, CAS: 82-76-8) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Bovine serum albumin (BSA, CAS: 9048-46-8) and 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB, CAS: 69-78-3) were purchased from Fluka

(Buchs, Switzerland). The reagents used for gel electrophoresis were purchased from Bio-Rad (Hercules, CA, USA). All the other chemicals used were of analytical grade.

3.3.2 Actomyosin preparation

Actomyosin was prepared according to the method of Yongsawatdigul and Park (2003), with some modifications. The minced fish (100 g) was homogenized with 500 ml of a low ionic strength buffer (20 mM sodium phosphate containing 50 mM NaCl and 0.05 mM PMSF, pH 7.0) for 2 min using a homogenizer (T25 digital ULTRA-TURRAX, IKA Works, Inc., Wilmington, NC, USA). The homogenate was centrifuged at 5,000×g (CR 22 GIII, Hitachi Ltd., Tokyo, Japan) for 10 min at 4 °C. The supernatant containing sarcoplasmic proteins was discarded. The obtained precipitate was homogenized with the same buffer and centrifuged once more. Then, the resultant residue was homogenized in 1 L of a high ionic strength buffer (20 mM sodium phosphate containing 0.6 M NaCl, pH 7.0) and centrifuged at 10,000×g for 10 min at 4 °C. Subsequently, the supernatant was collected and diluted with 3 volumes of cold distilled water (4 °C) to precipitate the actomyosin. The precipitate was collected by centrifugation at 10,000×g for 10 min at 4 °C. Water was removed from the precipitate by centrifugation at 12,500×g for 15 min at 4 °C. The obtained pellet was referred to as actomyosin and used within 24 h. Three independent extractions using different lots of fish were carried out.

3.3.3 HIU treatments

The HIU treatments were performed using an ultrasonic processor (Q500 sonicator, Qsonica L.L.C, Newtown, CT, USA) equipped with a titanium probe (12 mm-diameter) and at a frequency of 20 kHz. During sonication, samples were immersed in an ice bath to maintain sample temperature around 4-8 ºC. The sonicator

probe was immersed to a depth of 2.5-3 cm in the middle of the container. Actomyosin (5 g) was dissolved in 30 ml of 20 mM sodium phosphate buffer (pH 7.0) containing various NaCl concentrations (0.1, 0.2, 0.3, 0.6 and 1.2 M). The samples in the 50 ml centrifuge tubes were subjected to HIU treatments at amplitudes of 40, 70 and 100% for sonication time of 3 min using a pulse mode of on for 2 s and off for 5 s. Subsequently, the samples were centrifuged at 8,000×g for 20 min at 4 °C. The supernatants were analyzed to determine the extractable protein content (mg/ml), which was measured by the Bradford method using BSA as the standard (Bradford, 1976).

Based on the first trial, the NaCl concentrations of 0.2 and 0.6 M and amplitudes of 40 and 70% were selected for the sonication time evaluation. Actomyosin (5 g) was dissolved in 30 ml of a 20 mM sodium phosphate buffer (pH 7.0) containing either 0.2 M or 0.6 M NaCl. The samples were treated at amplitudes of 40 and 70% for sonication times of 3, 6 and 9 min using the pulse mode, as mentioned above. Subsequently, the samples were centrifuged at 8,000×g for 20 min at 4 °C, and the supernatants were collected and analyzed to determine the extractable protein content, Ca^{2+}-ATPase activity, total and reactive sulfhydryl group content, and surface hydrophobicity. The particle size, *zeta* potential, and turbidity were measured, and SDS-PAGE and light microscopy were also performed for the samples subjected to HIU at amplitudes of 40 and 70% for 6 min. The controls (not subjected to the HIU treatment) were also prepared.

The ultrasonic intensity was determined by calorimetry using a thermocouple (54 Series II thermometer, Fluke Corporation, Everett, WA, USA) and expressed in watts per unit area of the emitting surface (W/cm^2) according to Jambrak, Lelas, Mason, Krešić, and Badanjak (2009). In this study, an HIU amplitude of 40, 70 and 100%

corresponded to an ultrasonic intensity of 7.04, 13.37 and 20.62 W/cm^2, respectively.

3.3.4 Ca^{2+}-ATPase activity

The Ca^{2+}-ATPase activity of actomyosin was determined using the method described by Benjakul, Seymour, Morrissey, and An (1997)_with some modifications. The samples prepared in either 0.2 M or 0.6 M NaCl were diluted to 4 mg/ml with extraction buffer containing NaCl. The diluted sample (500 µl) was added to 250 µl of 0.6 M Tris-maleate (pH 7.0), 250 µl of 0.1 M $CaCl_2$, and 3.75 ml of deionized water. The mixture was incubated at 25 °C for 5 min, and 250 µl of 20 mM ATP was subsequently added to the mixture and incubated at 25 °C for exactly 8 min. The reaction was stopped by the addition of 2.5 ml of chilled 15% (w/v) trichloroacetic acid (TCA). The reaction mixture was then centrifuged at 3,500×g for 5 min, and the inorganic phosphate (Pi) liberated in the supernatant was measured by a UV/Vis spectrophotometer (GENESYS 10S, Thermo Scientific, Waltham, MA, USA) using KH_2PO_4 as a standard. The absorbance was measured at 640 nm. The Ca^{2+}-ATPase activity was expressed as µmol of Pi/mg protein/min at 25 °C. A blank was prepared by adding chilled TCA prior to the addition of ATP.

3.3.5 Total and reactive sulfhydryl group content

The total and reactive sulfhydryl (SH) group contents of actomyosin were determined using the method described by Yongsawatdigul et al. (2003) with slight modifications. To measure the total SH group content, 0.5 ml of the sample was mixed with 4.5 ml of the corresponding extraction buffers (0.2 M or 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 7.0) containing 8 M urea and 10 mM ethylenediaminetetraacetic acid (EDTA). To 2 ml of the mixture, 0.2 ml of Ellman's reagent (0.1% DTNB in 50 mM NaH_2PO_4 buffer, pH 7.0) was added. The reaction

mixture was incubated at 40 °C for 25 min. The reactive SH group content was determined by incubating the reaction mixtures in the buffer without urea at 4 °C for 1 h. The absorbance was measured at 412 nm, and the total and reactive SH group contents were calculated using the molar extinction coefficient of 13,600 M^{-1} cm^{-1} and expressed as mol/10^5 g protein (Ellman, 1959).

3.3.6 Surface hydrophobicity measurement

According to the slightly modified method of Yongsawatdigul et al. (2003), the surface hydrophobicity of actomyosin was determined using ANS, a hydrophobic fluorescent probe. Actomyosin was diluted with a buffer (20 mM sodium phosphate containing either 0.2 M or 0.6 M NaCl, pH 7.0) to obtain a series of protein concentrations (0.1, 0.15, 0.2, 0.25, and 0.3 mg/ml). To 2 ml of the protein solution, 10 µl of 8 mM ANS in a 0.1 M sodium phosphate buffer (pH 7.0) was added and mixed well. The samples were kept in the dark at room temperature for 10 min. The fluorescence intensity of the samples were measured using a spectrofluorometer (FP-8300, JASCO, Easton, MD, USA) at excitation and emission wavelengths of 374 and 485 nm, respectively, and a 5 nm width was used for both the excitation and emission slits. The surface hydrophobicity (S_0-ANS) of the sample was calculated from the slope of the relative fluorescence (R) versus the percentage of the protein concentration by linear regression analysis. R was defined according to Monahan, German, and Kinsella (1995) as follows:

$$R=(F-F_o) / F_o,$$

where F is the fluorescence intensity of the sample containing ANS, and F_o is the fluorescence intensity of the ANS solution prepared without the sample.

3.3.7 Particle size and *zeta* potential

Actomyosin was diluted to 0.2 mg/ml with a buffer (20 mM sodium phosphate containing either 0.2 M or 0.6 M NaCl, pH 7.0). The particle size and *zeta* potential of actomyosin (0.2 mg/ml) were measured using a Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK) instrument, with the corresponding buffer (20 mM sodium phosphate containing either 0.2 M or 0.6 M NaCl, pH 7.0) used as a dispersant. The measurements were performed at 25 °C.

3.3.8 Turbidity determination

The turbidity was measured according to the method of Yongsawatdigul and Park (1999), with some modifications. Actomyosin was diluted to 0.2 mg/ml with a buffer (20 mM sodium phosphate containing either 0.2 M or 0.6 M NaCl, pH 7.0). The diluted actomyosin (0.2 mg/ml) was placed in a quartz cuvette (light path length of 10 mm) and heated at 40 and 60 °C for 30 min. The designated temperatures were obtained within 1 min for all the samples. The turbidity changes were monitored at 320 nm using a UV/Vis spectrophotometer (GENESYS 10S, Thermo Scientific, Waltham, MA, USA) connected to an air cooled single cell Peltier cooler (SPG 1A, Thermo Scientific, USA). The absorbance at 320 nm was automatically recorded at 1-min intervals using the kinetics mode and was used to reflect the turbidity changes.

3.3.9 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The protein patterns of actomyosin with and without receiving the HIU treatments were analyzed by SDS–PAGE (Laemmli, 1970) using a Mini-Protein electrophoresis system (Bio-Rad Laboratories, Inc., USA). Briefly, the sample was

mixed with the treatment buffer (0.125 M Tris–HCl, 4% SDS, and 20% glycerol, pH 6.8) at a ratio of 1:1 (v/v), which was followed by incubating the sample at 95 °C for 5 min. Since the sample dissolved in 0.2 M NaCl without receiving the HIU treatments showed a limited solubility of 0.3 mg/ml, only 2.25 μg of the protein was loaded onto the polyacrylamide gel, which comprised 10% running gel and 4% stacking gel. For the other samples, 20 μg of the protein was loaded. The gels were run at a constant voltage of 100 V. The gels were stained in 0.125% Coomassie Brilliant Blue R-250 and destained in a solution containing 25% methanol and 10% acetic acid. The gels were photographed by a UVITEC imaging system (Fire reader V4, Uvitec Ltd., Cambridge, UK).

3.3.10 Light microscopy

The microstructures of actomyosin with and without receiving the HIU treatments were visualized using a light microscope (Eclipse Ts2, Nikon Instruments Inc., Tokyo, Japan). The sample (50 μl) was spread onto a glass slide and viewed under the light microscope at room temperature. The image was taken at a 400× magnification.

3.3.11 Statistical analysis

Three independent triplicates were performed for all the experiments. The statistical analysis was conducted using SPSS version 23.0 (SPSS Inc., Chicago, IL, USA). The difference between the means was analyzed by analysis of variance (ANOVA) and Duncan's multiple-range test (DMRT) at a significance level of $p < 0.05$.

3.4 Results and discussion

3.4.1 Protein extractability

At lower NaCl concentrations (0.1, 0.2 and 0.3 M NaCl), the extractable

protein content continuously increased compared to the control with increasing ultrasonic intensity (Figure 3.1A). Nevertheless, no obvious effect of HIU was found at higher NaCl concentrations (0.6 and 1.2 M NaCl) (Figure 3.1A). Myofibrillar proteins are likely to be fully extracted at higher NaCl concentrations, and an increase in the ultrasonic intensity, thus, did not further improve extraction. At ultrasonic intensities of 7.04 and 13.37 W/cm^2, the extractable protein content determined using 0.2 M NaCl gradually increased with increasing sonication time up to 9 min, and a more pronounced effect was observed at 13.37 W/cm^2 (Figure 3.1B). During sonication, ultrasonic energy triggers extremely high alterations in pressure, resulting in formation of small rapidly growing bubbles, which expand during the negative pressure excursion and implode violently during the positive excursion, leading not only to high temperatures (5000 K) and pressures (1000 atm), but also high shear energy waves and turbulence in cavitation zone (James; Soria et al., 2010). The shear energy waves and turbulence generated by cavitation may disrupt myofibrils and dissociate myosin aggregates, leading to higher extractability at lower NaCl concentrations. Ultrasonic energy has also been reported to change the spatial conformation and microstructure of chicken actomyosin, resulting in an increased protein solubility (Zou et al., 2018). A higher ultrasonic intensity and longer sonication time resulted in a more pronounced effect due to more accumulated energy. Protein solubility of chicken actomyosin in 0.1 M NaCl also increased with increasing sonication time (Saleem, Hasnain, & Ahmad, 2015).

However, using 0.6 M NaCl, the protein content extracted by applying HIU for 6 min only increased by 22% compared to the control. A longer sonication time of 9 min resulted in a decrease in the extracted protein content (Figure 3.1C). The extractable protein content of actomyosin in 0.2 M NaCl treated with an ultrasonic

intensity of 13.37 W/cm^2 for 9 min was comparable to that in 0.6 M NaCl without receiving the HIU treatments. HIU increased the solubility of actomyosin in 0.2 M NaCl by up to 14.7 times (Figure 3.1B), while an increase of only 1.2 times was observed in 0.6 M NaCl (Figure 3.1C). The extraction of myofibrillar proteins in 0.6 M NaCl may reach the plateau, resulting in a slight increase in protein extractability after HIU treatments. At lower NaCl concentrations, HIU was likely to disrupt myofibrils and dissociate myosin aggregates, leading to more actomyosin extractability. Our study demonstrated the potential of HIU for extraction myofibrillar proteins at lower NaCl concentrations.

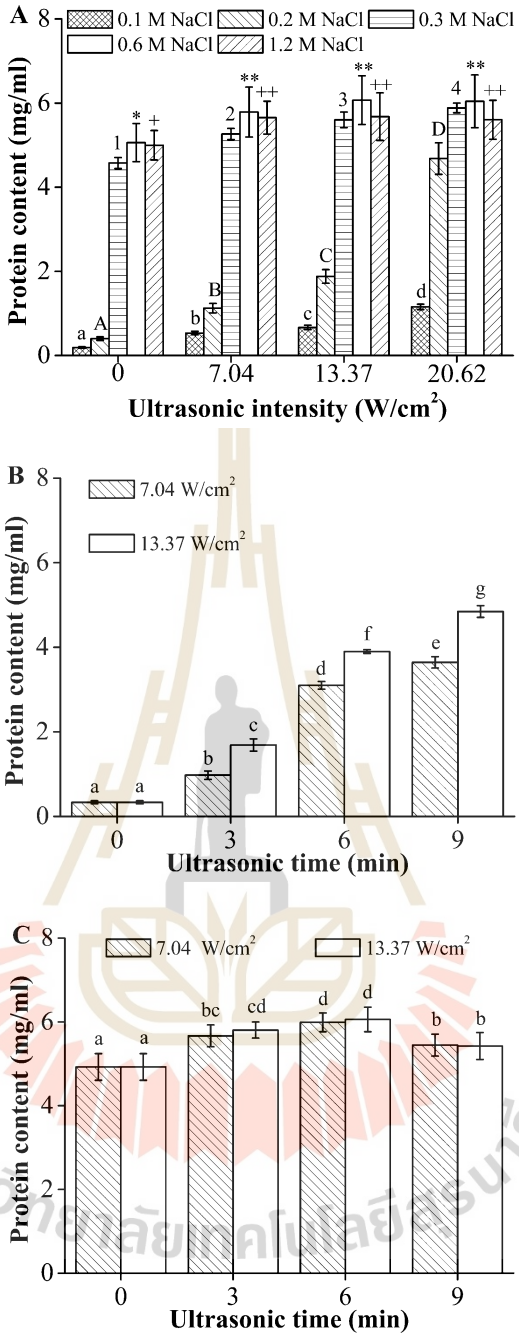


Figure 3.1 Effects of the HIU treatments on the extractable protein content of actomyosin using various NaCl concentrations (A) and various sonication times with ultrasonic intensities of 7.04 and 13.37 W/cm^2 for actomyosin extracted in 0.2 M NaCl (B) and 0.6 M NaCl (C). The different letters and symbols indicate the differences between the treatments ($p < 0.05$).

3.4.2 Ca^{2+}-ATPase activity

The Ca^{2+}-ATPase activity of actomyosin decreased with increasing ultrasonic intensity and time at both studied concentrations of NaCl ($p < 0.05$, Figure 3.2). The Ca^{2+}-ATPase activity reflects the integrity of the globular head of myosin (Chan, Gill, Thompson, & Singer, 1995). Thus, the decrease in the Ca^{2+}-ATPase activity suggested that the conformation of the myosin globular head changed after sonication, which progressed as the ultrasonic intensity and time increased. High pressure, high shear energy waves and turbulence generated by ultrasound vitally contributed to conformational changes of myosin globular head, leading to decreased Ca^{2+}-ATPase activity. The Ca^{2+}-ATPase activity of chicken actomyosin in 0.6 M NaCl decreased after receiving ultrasonic treatments for 5-30 min (Saleem et al., 2016). Liu et al. (2017) also reported that the Ca^{2+}-ATPase activity of silver carp myosin in 0.5 M NaCl significantly decreased with increasing ultrasonic power and time. The remaining Ca^{2+}-ATPase activity of actomyosin in 0.2 M NaCl was higher than that in 0.6 M NaCl for all the HIU conditions (Figure 3.2). These results indicated that fully soluble actomyosin in 0.6 M NaCl was more susceptible to conformational changes induced by cavitation. The larger extent of protein unfolding at 0.6 M NaCl exposed more active sites at myosin globular head, which were likely to be destroyed during sonication due to the cavitation effects, resulting in greater reduction in Ca^{2+}-ATPase activity. A decrease in the Ca^{2+}-ATPase activity of chicken actomyosin in a higher ionic strength buffer was also greater than that in a lower ionic strength buffer (Saleem et al., 2015).

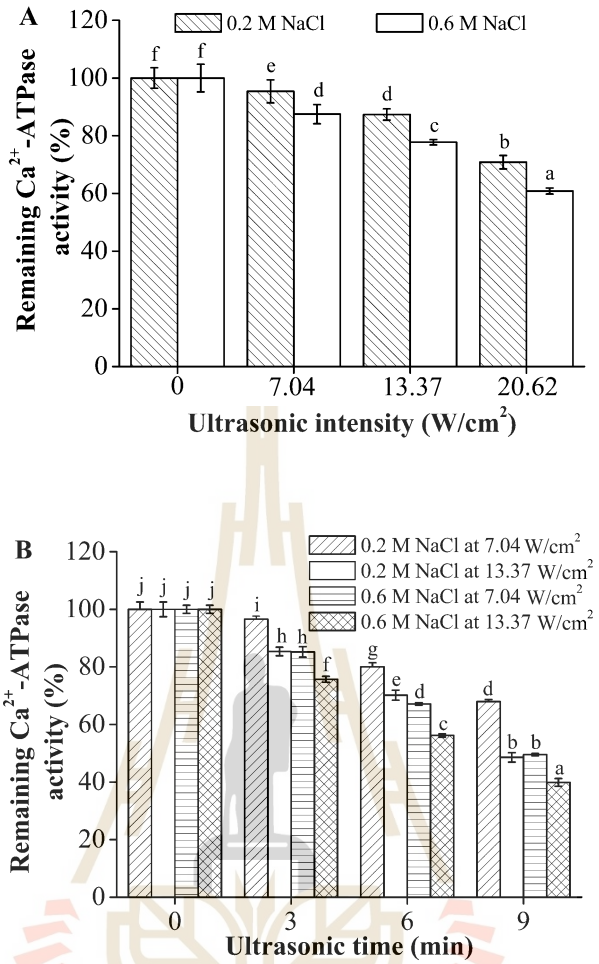


Figure 3.2 The remaining Ca^{2+}-ATPase activity of actomyosin in 0.2 and 0.6 M NaCl and subjected to various ultrasonic intensities (A) and ultrasonic times (B).The different superscripts indicate significant differences between the treatments ($p < 0.05$). The Ca^{2+}-ATPase activity of actomyosin receiving no HIU treatments in 0.2 and 0.6 M NaCl was considered to be 100%.

3.4.3 Total and reactive sulfhydryl groups

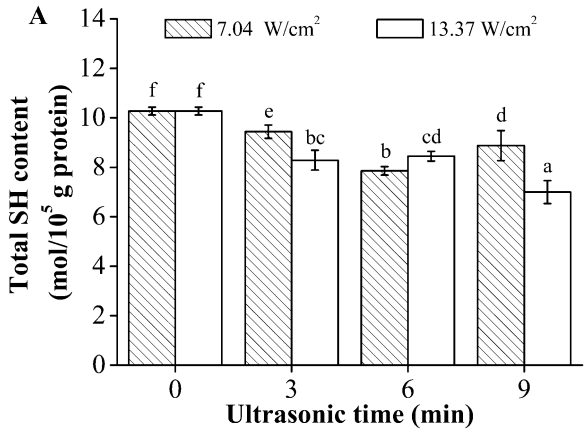
The total sulfhydryl (SH) groups represent all the SH groups in the protein structure, whereas the reactive SH groups refer to the SH groups that are available to react with DTNB. Generally, the total SH group content of the sample after

receiving the HIU treatments in both 0.2 and 0.6 M NaCl decreased compared to the control (Figure 3.3A and B), indicating that HIU induced the oxidation of the SH groups. During sonication, reactive free radicals can be produced from water molecules ($H_2O \rightarrow \cdot H + \cdot OH$), which can further react to form hydrogen peroxide (H_2O_2) (Hu et al., 2013; Milne, Stewart, & Bremner, 2013; Riesz & Kondo, 1992). H_2O_2 is a strong oxidizing agent that can promote the oxidation of SH groups. The oxidation of SH groups (Figure 3.3A and B) also implied that disulfide bonds might be formed during sonication probably through myosin globular heads, causing decreased Ca^{2+}-ATPase activity (Figure 3.2). Zhang et al. (2017) reported that the total SH content of chicken myofibrillar protein in 0.6 M KCl decreased as the ultrasonic intensity increased up to 193 W/cm^2. The total SH content of silver carp myosin in 0.5 M NaCl also decreased when the ultrasonic power and time increased (Liu et al., 2017). Our results also indicated that a higher ultrasonic intensity (13.37 W/cm^2) induced more oxidation of SH groups than a lower intensity for samples in both the studied NaCl solutions with different concentrations.

Similarly, the reactive SH content of actomyosin in 0.6 M NaCl after the HIU treatments was lower than that of the control and decreased with increasing sonication time (Figure 3.3D). Some SH groups were exposed on the surface of the protein due to the partial unfolding of myosin in 0.6 M NaCl and were susceptible to being oxidized during sonication. Longer HIU exposures induced more oxidation of SH groups, leading to a decrease in the reactive SH content. In addition, the reactive SH content of silver carp myosin in 0.5 M NaCl gradually decreased as the ultrasonic power and time increased (Liu et al., 2017). The reduction in the total SH content of samples exposed to HIU (13.37 W/cm^2 for 9 min) and in 0.6 M NaCl was approximately 31%, which

was greater than that of the reactive SH content (15%). A decrease in both the total and reactive SH contents suggested the HIU-induced oxidation of SH groups in 0.6 M NaCl. In contrast, the reactive SH content of the samples in 0.2 M NaCl increased after a prolonged exposure to HIU (Figure 3.3C). This implied that buried SH groups were brought to the surface due to the protein unfolding induced by the HIU treatment. Based on ultrasound principle, the physical forces (e.g., pressure and shear) resulted from cavitation are likely the main cause of this conformational changes. A longer HIU exposure was required to increase the reactive SH content when a lower ultrasonic intensity (7.04 W/cm^2) was used.

Our study revealed that HIU showed more pronounced effect on oxidation of the SH groups than on exposure of buried SH groups in 0.6 M NaCl. It was speculated that oxidation of SH was promoted under high NaCl as actomyosin exposed more buried SH groups, resulting in a greater reduction in total SH content. In addition, free radicals generated from cavitation accelerated oxidation of exposed SH groups. However, actomyosin in solutions with low NaCl concentrations (0.2 M) was in the aggregated form and underwent lesser degree of unfolding, leading to lower degree of SH oxidation, particularly at shorter exposure time of 3 min.



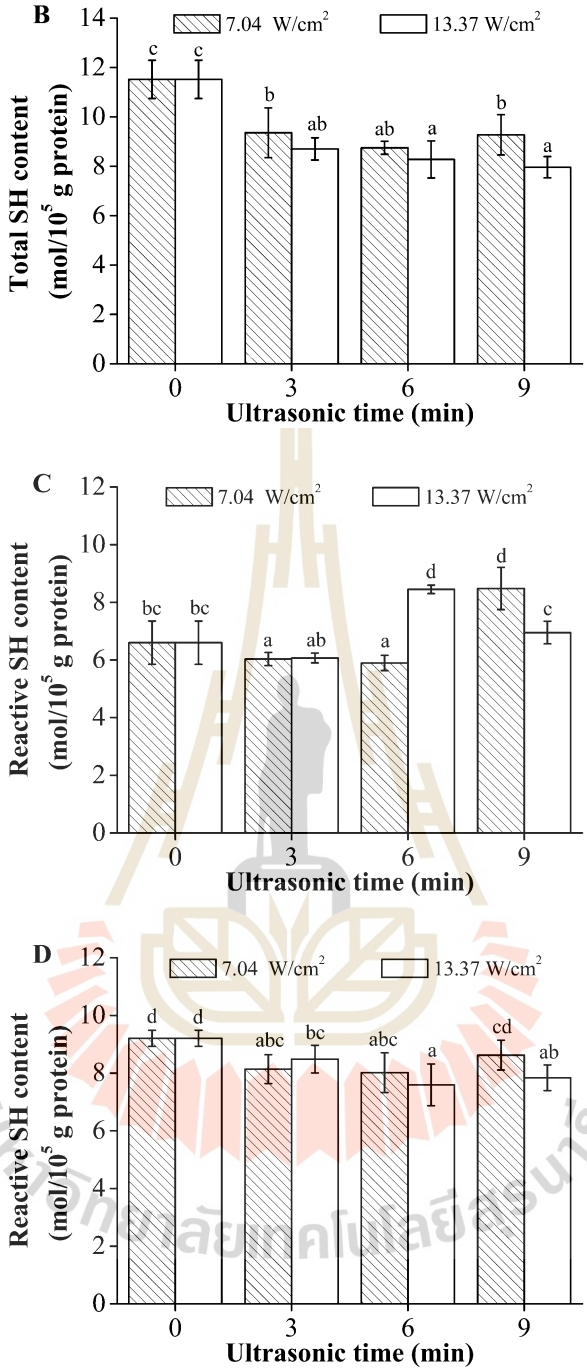


Figure 3.3 Effects of the HIU treatments on the total and reactive sulfhydryl group contents of actomyosin in 0.2 M NaCl (A, C) and 0.6 M NaCl (B, D).The different superscripts indicate significant differences between the treatments ($p < 0.05$).

3.4.4 Surface hydrophobicity

HIU induced the exposure of hydrophobic residues of actomyosin in both 0.2 and 0.6 M NaCl, as seen by the increase in the S_0-ANS (Figure 3.4). The exposure of more hydrophobic groups suggested that the myosin rod unfolded, as the hydrophobic groups of natural myosin are buried in the α-helical regions (Taguchi, Ishizaka, Tanaka, Nagashima, & Amano, 1987). This is mainly due to cavitation generated by HIU. High pressure generated from cavitation could compress protein, causing void disruption and conformational changes. Moreover, incorporation of air bubbles and adsorption of protein molecules to the air–liquid interface occurred when shear force generated during sonication, which also gave rise to conformational changes of proteins. These physical forces might also dissociate myosin aggregates, resulting in more exposure of hydrophobic groups. These events contributed to greater protein unfolding under HIU treatments, which might also lead to more protein extractability (Figure 3.1).

The S_0-ANS values obtained in 0.2 M NaCl were lower than those in 0.6 M NaCl. Myosin was found to assemble into filaments in lower salt concentrations due to hydrophobic interactions (G. Wang et al., 2018), resulting in samples having lower S_0-ANS values in 0.2 M NaCl. In contrast, a high ionic strength leads to more hydrophobic residues of the myosin molecules being exposed. Actomyosin in 0.6 M NaCl had a S_0-ANS that increased by 236% after subjected to a higher ultrasonic intensity (13.37 W/cm^2). Myosin molecules disperse as monomers in 0.6 M NaCl (Ishioroshi, Samejima, & Yasui, 1983), leading to larger extent of protein unfolding, and hydrophobic groups were more easily exposed when HIU energy was applied. In contrast, protein unfolding induced by HIU was still limited in 0.2 M NaCl, in which myosin existed as aggregates.

The results of S_0-ANS suggested that HIU induced higher degree of unfolding for actomyosin in 0.6 M NaCl than that in 0.2 M NaCl. This could be one of reasons explaining a drastic decrease in the Ca^{2+}-ATPase activity (Figure 3.2A). When excessive exposure of hydrophobic groups in 0.6 M NaCl occurred (Figure 3.4B), it may lead to aggregate formation through hydrophobic interactions, causing lower protein solubility after longer HIU exposure (Figure 3.1C).

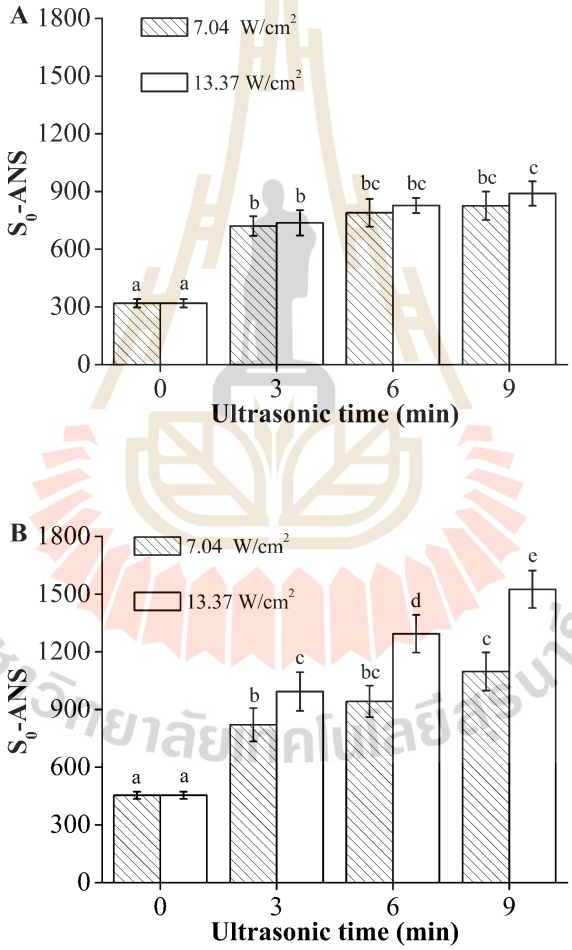


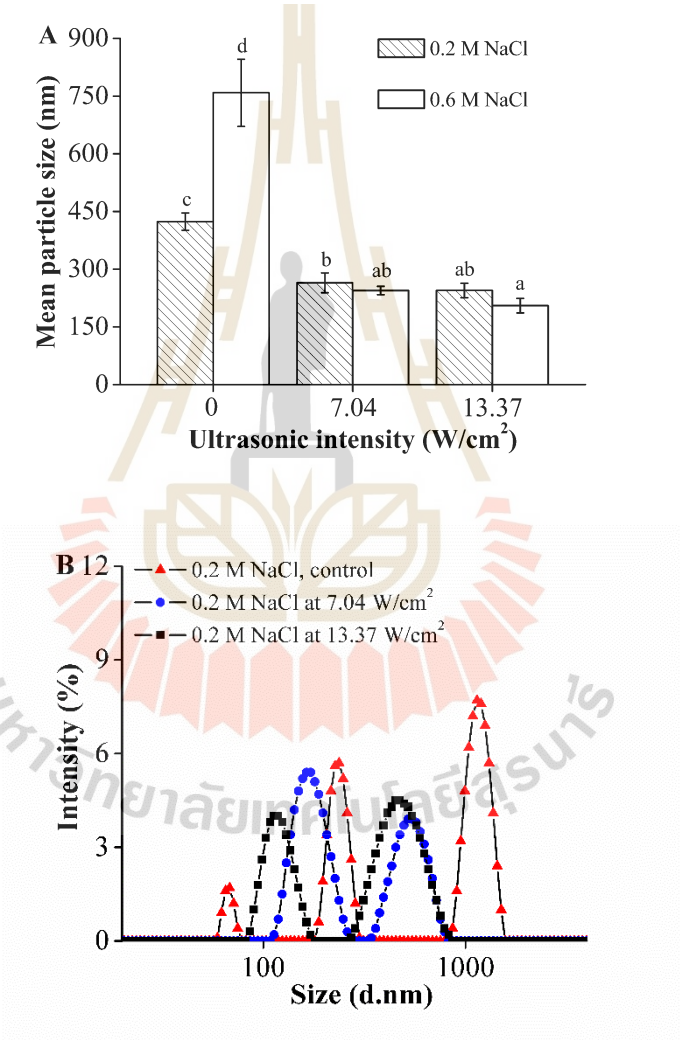
Figure 3.4 Effects of the HIU treatments on the surface hydrophobicity of actomyosin in 0.2 M NaCl (A) and 0.6 M NaCl (B). The different superscripts indicate significant differences between the treatments ($p < 0.05$).

3.4.5 Particle size

The mean particle size of actomyosin in both 0.2 and 0.6 M NaCl decreased after the HIU treatments ($p < 0.05$), but an increase in the ultrasonic intensity from 7.04 to 13.37 W/cm^2 had no effect on the particle size ($p > 0.05$, Figure **3**.5A). Actomyosin subjected to HIU was violently agitated by a high shear energy and turbulence, resulting in disruption of myofibrils and dissociation of myosin aggregates. The average particle size of chicken myofibrillar protein in 0.6 M KCl decreased from 2084.6 to 271.3 nm after an HIU treatment with an ultrasonic intensity of 193 W/cm^2 (Zhang et al., 2017). Liu et al. (2017) reported that the mean particle size of silver carp myosin in 0.5 M NaCl decreased by approximately 32% when treated with an ultrasound power of 250 W for 12 min. Without applying HIU, the particle size of actomyosin in 0.2 M NaCl was smaller than that in 0.6 M NaCl. In addition, the size reduction induced by HIU for samples in 0.6 M NaCl was more remarkable compared with that of samples in 0.2 M NaCl. In 0.6 M NaCl, filament aggregates were looser due to a higher extent of protein unfolding and, thus, were more susceptible to be broken down by HIU, leading to a greater decrease in the mean particle size. A decrease in the particle size could also contribute to the increased extractable protein content (Figure **3**.1), as a larger surface area of protein might improve the protein-water interactions.

HIU not only reduced the particle size of actomyosin but also improved the uniformity of the particle size distribution (Figure **3**.5B and C). In 0.2 M NaCl, actomyosin receiving no HIU treatment had a size distribution with a dominant peak at approximately 1,100 nm and two other smaller peaks, while only double peaks at 120-540 nm were observed for the HIU-treated sample size distribution (Figure **3**.5B). Actomyosin in 0.6 M NaCl also had a size distribution with double peaks at 250 and

1,690 nm, but only one dominant peak at 340-380 nm was evident after the HIU treatment (Figure **3**.5C). The more uniform size distribution might also be due to a shearing effect resulting from cavitation. The particle size distribution of silver carp myosin in 0.5 M NaCl also became narrower with increasing ultrasonic power and time (Liu et al., 2017).



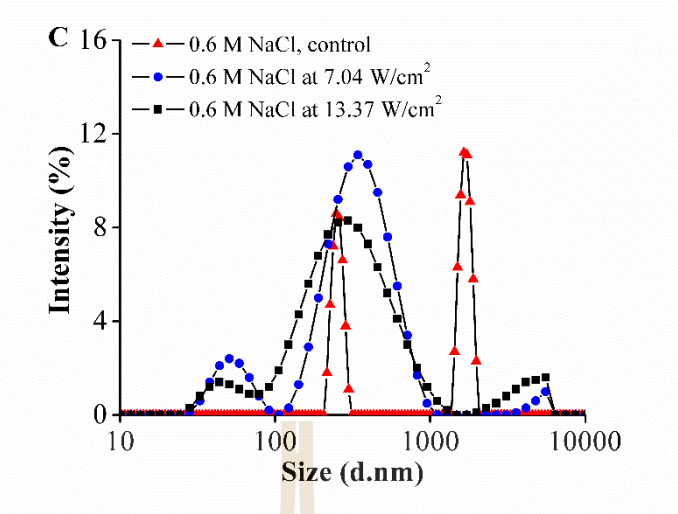


Figure 3.5 Effects of the HIU treatments on the mean particle size (A) and size distribution of actomyosin in 0.2 M NaCl (B) and 0.6 M NaCl (C). The different superscripts indicate significant differences between the treatments ($p < 0.05$). The HIU exposure time was 6 min.

3.4.6 *Zeta* potential

Actomyosin in 0.2 M NaCl had larger absolute values of the *zeta* potential than actomyosin in 0.6 M NaCl regardless of the HIU treatment (Figure 3.6). The negative *zeta* potential values indicated that more amino acids with negative charges than positive charges were present on the protein surface of actomyosin. A higher salt concentration (0.6 M NaCl) resulted in lower absolute values of the *zeta* potential. This was likely due to the decreased thickness of the electrical double layer, which resulted from increasing the ionic strength of the medium (Salgin, Salgin, & Bahadir, 2012). Wu et al. (2016) also reported that at a pH of 7.5, the *zeta* potential of pork myofibrillar proteins decreased when the NaCl concentration increased from 0 to 0.8 M. HIU increased the absolute values of the *zeta* potential of actomyosin in 0.2 M

NaCl ($p \leq 0.05$, Figure 3.6). This could be attributed to the disruption of protein aggregates and exposure of more charged groups, which was induced by the cavitaion. The increased absolute values of the *zeta* potential were in agreement with previous study on chicken myofibrillar proteins in 0.6 M KCl (Zhang et al., 2017). The absolute value of the *zeta* potential of actomyosin in 0.6 M NaCl increased when the applied ultrasonic intensity was 7.04 W/cm^2 ($p < 0.05$, Figure 3.6) and further increasing the intensity did not further increase the *zeta* potential ($p \geq 0.05$, Figure 3.6). It seemed that HIU had less of an effect on the *zeta* potential of the samples in 0.6 M NaCl, which was probably because the extent of the unfolding induced by HIU was greater for actomyosin in 0.2 M NaCl than in 0.6 M NaCl (Figure 3.3C).

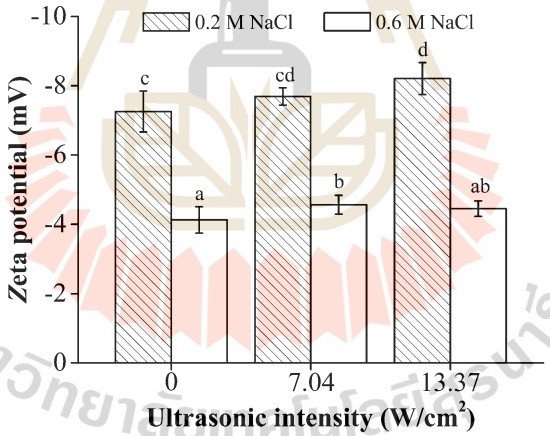


Figure 3.6 Effects of the HIU treatments on the *zeta* potential of actomyosin in 0.2 and 0.6 M NaCl. The different superscripts indicate significant differences between the treatments ($p < 0.05$). The HIU exposure time was 6 min.

3.4.7 Turbidity

Generally, the HIU treatments decreased the turbidity of actomyosin at

40 and 60 °C for samples in both NaCl concentrations ($p < 0.05$, Figure 3.7). As HIU reduced the particle size and improved the size uniformity of actomyosin in both 0.2 and 0.6 M NaCl (Figure 3.5), smaller protein aggregates resulted in decreased turbidity. HIU treatments also decreased the turbidity of chicken myofibrillar protein at an intensity of 193 W/cm^2 (Zhang et al., 2017). The turbidity of sample receiving no HIU treatment in 0.2 M NaCl was higher than that of sample receiving no HIU treatment in 0.6 M NaCl when the samples were incubated at 40 °C (Figure 3.7A), but was lower when the samples were incubated at 60 °C (Figure 3.7B). A high NaCl concentration (0.6 M) decreased the electrostatic interactions due to screening effects (Haug, Draget, & Smidsrød, 2004), which might promote the dissolution of myosin and result in a lower turbidity at 40 °C. A lower turbidity of chicken actomyosin (Saleem et al., 2015) and silver carp myosin (G. Wang et al., 2018) were also reported in solutions with a higher ionic strength. The greater extent of protein unfolding that occurred in 0.6 M NaCl led to more hydrophobic residues being exposed, which promoted hydrophobic interactions at a higher temperature (60 °C). This would, in turn, result in a higher turbidity than that observed for the samples in 0.2 M NaCl. Nevertheless, the HIU-treated samples did not show significant higher turbidities when the temperature increased from 40 to 60 °C. Comparing the extent of aggregation of the HIU-treated actomyosin with that of the control (without HIU) would not be plausible. HIU reduced the particle size of actomyosin (Figure 3.5A), which resulted in a lower extent of light scattering and lower absorbance at 320 nm. HIU-treated actomyosin was expected to unfold to a greater extent (Figure 3.4), in which case aggregates could readily form.

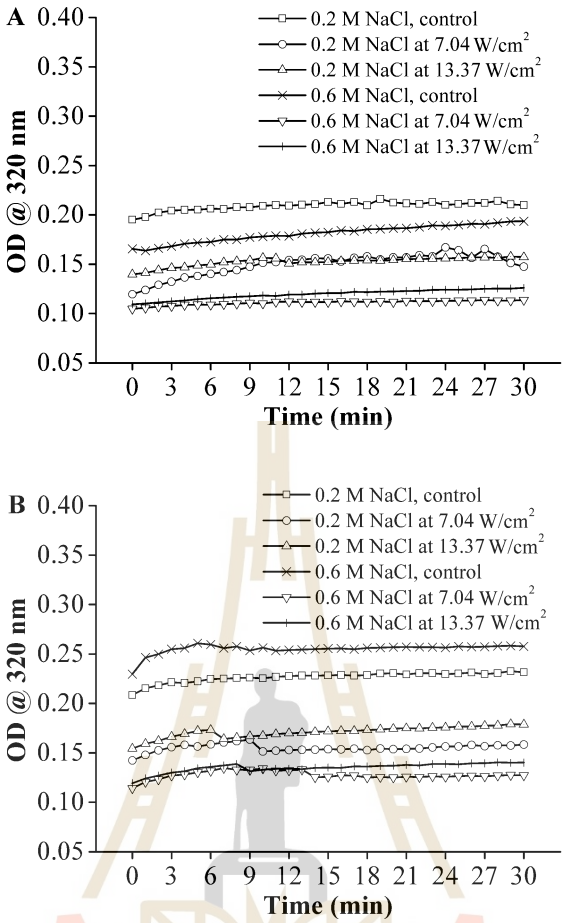


Figure 3.7 Effects of the HIU treatments on the turbidity changes of actomyosin incubated at 40 (A) and 60 °C (B) for 30 min in 0.2 and 0.6 M NaCl. The HIU exposure time was 6 min.

3.4.8 SDS-PAGE

Without HIU, myosin and actin were scarcely extracted in 0.2 M NaCl, but more protein was solubilized after the 6 min HIU treatments with intensities of 7.04 and 13.37 W/cm^2 (Figure 3.8). This was consistent with the extractable protein content (Figure 3.1). These findings confirmed that HIU could facilitate actomyosin extraction in 0.2 M NaCl. The myosin heavy chain (MHC) bands of HIU-treated actomyosin in both 0.2 and 0.6 M NaCl became thinner when the ultrasonic intensity was increased

from 7.04 to 13.37 W/cm^2. In addition, bands with smaller molecular weights (66-200 kDa) were evident and appeared to increase with increasing ultrasonic intensity. This phenomenon indicated that MHC underwent degradation to some extent under HIU. This is probably due to high shearing effect and/or cleavage of peptide bonds by ultrasonic-generated free radicals (Zhang, Zhang, Chen, & Zhou, 2018). Liu et al. (2017) reported that HIU induced the degradation of the MHCs of silver carp myosin. Based on SDS-PAGE, HIU not only improved the solubility of myosin and actin in 0.2 M NaCl but also induced the degradation of MHCs in both NaCl concentrations.

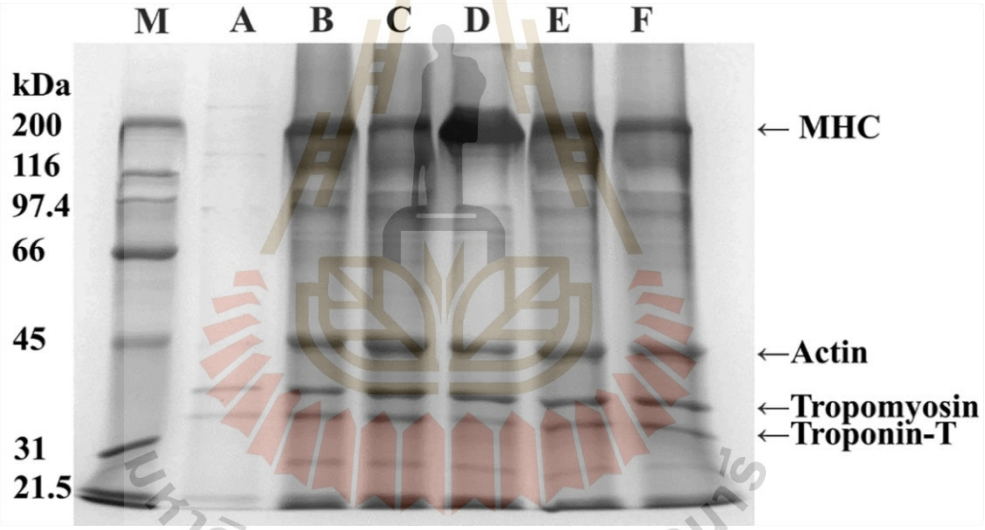


Figure 3.8 SDS-PAGE patterns of actomyosin subjected to the HIU treatments. M = protein markers; A = 0.2 M NaCl/control; B = 0.2 M NaCl treated with an ultrasonic intensity of 7.04 W/cm^2; C = 0.2 M NaCl treated with an ultrasonic intensity of 13.37 W/cm^2; D = 0.6 M NaCl/control; E = 0.6 M NaCl treated with an ultrasonic intensity of 7.04 W/cm^2; F = 0.6 M NaCl treated with an ultrasonic intensity of 13.37 W/cm^2. The HIU exposure time was 6 min.

3.4.9 Microstructure

Without HIU, actomyosin in 0.2 M NaCl contained denser filament aggregates compared to that in 0.6 M NaCl (Figure 3.9A and D). It was evident that HIU significantly changed the shape and filament length of the actomyosin aggregates in both NaCl concentrations. When an ultrasonic intensity of 7.04 W/cm^2 was applied, actomyosin in 0.2 M NaCl formed looser aggregates with shorter and smaller filaments compared to the control (Figure 3.9B). As the ultrasonic intensity increased to 13.37 W/cm^2, shorter filaments with less packed actomyosin were evident (Figure 3.9C). Strong shear energy waves and turbulence generated by ultrasound disrupted myofibril filaments, loosening the filamentous structure and shortening the length. Moreover, high pressure induced by ultrasonic waves probably promoted separation of filament aggregates. Ultrasound has been reported to induce the dispersion of chicken muscle proteins in 0.6 M KCl (Saleem et al., 2016; J. Wang, Yang, Tang, Ni, & Zhou, 2017; Zou et al., 2018). The HIU-treated actomyosin in 0.6 M NaCl appeared to be more filamentous, and the filaments had shorter lengths and smaller sizes compared with those in 0.2 M NaCl, indicating that the actomyosin complex dissociated or disrupted more easily in 0.6 M NaCl than in 0.2 M NaCl. This could probably due to the greater extent of conformational changes in 0.6 M NaCl. These findings further proved that HIU induced, to a greater extent, protein dissociation and the breakdown of aggregates in both 0.2 and 0.6 M NaCl. The cavitation effect of HIU would be more pronounced in 0.6 M NaCl, in which the actomyosin filament structures were looser, and thus more likely to be disrupted by HIU. The results were also in consistent with the changes in the particle size (Figure 3.5), which showed that the reduction of the mean particle size, which was affected by HIU, was more obvious for actomyosin in 0.6 M NaCl.

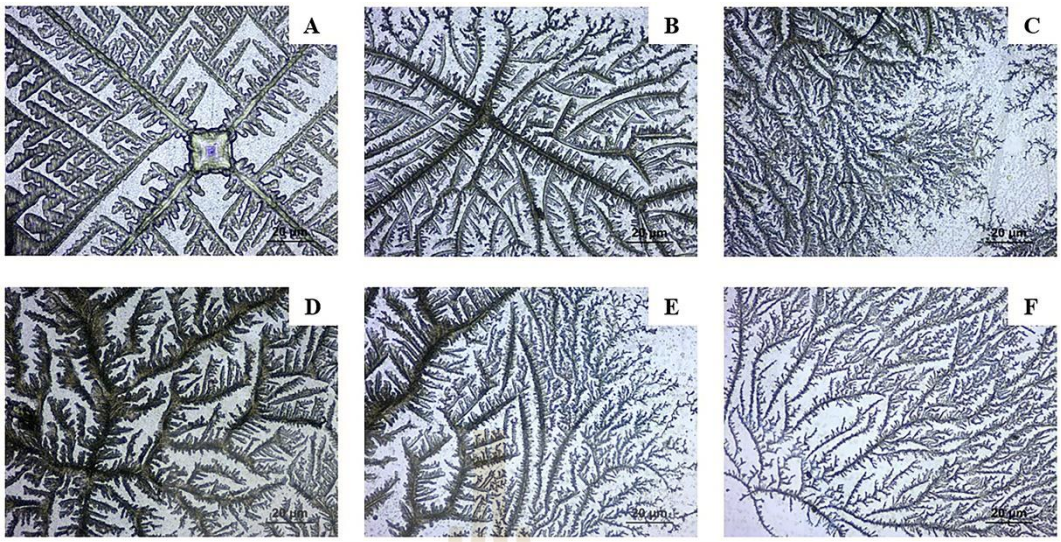


Figure 3.9 Microstructures of actomyosin treated by HIU treatments (magnification: 400×). A = 0.2 M NaCl/control; B = 0.2 M NaCl treated with an ultrasonic intensity of 7.04 W/cm^2; C = 0.2 M NaCl treated with an ultrasonic intensity of 13.37 W/cm^2; D = 0.6 M NaCl/control; E = 0.6 M NaCl treated with an ultrasonic intensity of 7.04 W/cm^2; F = 0.6 M NaCl treated with an ultrasonic intensity of 13.37 W/cm^2. The HIU exposure time was 6 min. Scale bar = **20** μm.

3.5 Conclusions

The protein solubility of actomyosin in low NaCl concentration (0.2 M) was improved after HIU treatments. HIU showed more pronounced effect on oxidation of SH groups than exposure of buried SH groups in 0.6 M NaCl. HIU also induced larger degree of protein unfolding and conformational changes in 0.6 M NaCl than that in 0.2 M NaCl. Smaller aggregates of HIU-treated actomyosin were observed at 40 and 60 °C, which was probably due to HIU reducing the particle size and improving the size uniformity of actomyosin. HIU caused the MHCs to degrade in both NaCl

concentrations, but disruption of actomyosin induced by HIU appeared to be greater in 0.6 M NaCl. The results demonstrated that actomyosin can be efficiently extracted in 0.2 M NaCl by HIU, suggesting the potential of HIU technology in production of low/reduced-salt actomyosin gel.

3.6 References

Benjakul, S., Seymour, T. A., Morrissey, M. T., & An, H. (1997). Physicochemical changes in Pacific whiting muscle proteins during iced storage. **Journal of Food Science.** 62 (4), 729-733.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry.** 72 (1), 248-254.

Cando, D., Herranz, B., Borderías, A. J., & Moreno, H. M. (2015). Effect of high pressure on reduced sodium chloride surimi gels. **Food Hydrocolloids.** 51 (Supplement C), 176-187.

Cando, D., Herranz, B., Borderías, A. J., & Moreno, H. M. (2016). Different additives to enhance the gelation of surimi gel with reduced sodium content. **Food Chemistry.** 196, 791-799.

Chan, J. K., Gill, T. A., Thompson, J. W., & Singer, D. S. (1995). Herring surimi during low temperature setting, physicochemical and textural properties. **Journal of Food Science.** 60 (6), 1248-1253.

Chang, H. s., Hultin, H. O., & Dagher, S. M. (2001). Effect of MgCl2/sodium pyrophosphate on chicken breast muscle myosin solubilization and gelation. **Journal of Food Biochemistry.** 25 (5), 459-474.

Ellman, G. L. (1959). Tissue sulfhydryl groups. **Archives of Biochemistry and Biophysics.** 82 (1), 70-77.

Fu, X., Hayat, K., Li, Z., Lin, Q., Xu, S., & Wang, S. (2012). Effect of microwave heating on the low-salt gel from silver carp (*Hypophthalmichthys molitrix*) surimi. **Food Hydrocolloids.** 27 (2), 301-308.

Haug, I. J., Draget, K. I., & Smidsrød, O. (2004). Physical and rheological properties of fish gelatin compared to mammalian gelatin. **Food Hydrocolloids.** 18 (2), 203-213.

Hu, H., Wu, J., Li-Chan, E. C. Y., Zhu, L., Zhang, F., Xu, X., Fan, G., Wang, L., Huang, X., & Pan, S. (2013). Effects of ultrasound on structural and physical properties of soy protein isolate (SPI) dispersions. **Food Hydrocolloids.** 30 (2), 647-655.

Ishioroshi, M., Samejima, K., & Yasui, T. (1983). Heat-induced gelation of myosin filaments at a low salt concentration. **Agricultural and Biological Chemistry.** 47 (12), 2809-2816.

Jambrak, A. R., Lelas, V., Mason, T. J., Krešić, G., & Badanjak, M. (2009). Physical properties of ultrasound treated soy proteins. **Journal of Food Engineering.** 93 (4), 386-393.

James, M. T. (1990). A General Introduction to Sonochemistry. In Sonochemistry: The Uses of Ultrasound in Chemistry (1st Edition) (pp. 1-8). Cambridge, UK: Royal Society of Chemistry.

Kloss, L., Meyer, J. D., Graeve, L., & Vetter, W. (2015). Sodium intake and its reduction by food reformulation in the European Union — A review. **NFS Journal.** 1 (Supplement C), 9-19.

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

Lanier, T. C., Carvajal, P., & Yongsawatdigul, J. (2005). Surimi Gelation Chemistry. In J. W. Park (Ed.), Surimi and Surimi Seafood (2nd Edition) (pp. 435-489). Boca Raton, FL: CRC Press.

Liu, R., Liu, Q., Xiong, S., Fu, Y., & Chen, L. (2017). Effects of high intensity unltrasound on structural and physicochemical properties of myosin from silver carp. **Ultrasonics Sonochemistry.** 37 (Supplement C), 150-157.

McDonnell, C. K., Lyng, J. G., & Allen, P. (2014). The use of power ultrasound for accelerating the curing of pork. **Meat Science.** 98 (2), 142-149.

Milne, L., Stewart, I., & Bremner, D. H. (2013). Comparison of hydroxyl radical formation in aqueous solutions at different ultrasound frequencies and powers using the salicylic acid dosimeter. **Ultrasonics Sonochemistry.** 20 (3), 984-989.

Monahan, F. J., German, J. B., & Kinsella, J. E. (1995). Effect of pH and temperature on protein unfolding and thiol/disulfide interchange reactions during heat-induced gelation of whey proteins. **Journal of Agricultural and Food Chemistry.** 43 (1), 46-52.

Riesz, P., & Kondo, T. (1992). Free radical formation induced by ultrasound and its biological implications. **Free Radical Biology and Medicine.** 13 (3), 247-270.

Saleem, R., & Ahmad, R. (2016). Effect of low frequency ultrasonication on biochemical and structural properties of chicken actomyosin. **Food Chemistry.** 205 (Supplement C), 43-51.

Saleem, R., Hasnain, A., & Ahmad, R. (2015). Solubilisation of muscle proteins from chicken breast muscle by ultrasonic radiations in physiological ionic medium. **Cogent Food & Agriculture.** 1 (1), 1046716.

Salgin, S., Salgin, U., & Bahadir, S. (2012). Zeta potentials and isoelectric points of biomolecules: the effects of ion types and ionic strengths. **Int. J. Electrochem. Sci.** 7 (12), 12404-12414.

Soria, A. C., & Villamiel, M. (2010). Effect of ultrasound on the technological properties and bioactivity of food: a review. **Trends in Food Science & Technology.** 21 (7), 323-331.

Taguchi, T., Ishizaka, H., Tanaka, M., Nagashima, Y., & Amano, K. (1987). Protein-Protein Interaction of Fish Myosin Fragments. **Journal of Food Science.** 52 (4), 1103-1104.

Tahergorabi, R., & Jaczynski, J. (2012). Physicochemical changes in surimi with salt substitute. **Food Chemistry.** 132 (3), 1281-1286.

Uresti, R. o. M., Téllez-Luis, S. J., Ramírez, J. A., & Vázquez, M. (2004). Use of dairy proteins and microbial transglutaminase to obtain low-salt fish products from filleting waste from silver carp (*Hypophthalmichthys molitrix*). **Food Chemistry.** 86 (2), 257-262.

Wang, G., Liu, M., Cao, L., Yongsawatdigul, J., Xiong, S., & Liu, R. (2018). Effects of different NaCl concentrations on self-assembly of silver carp myosin. **Food Bioscience.** 24, 1-8.

Wang, J., Yang, Y., Tang, X., Ni, W., & Zhou, L. (2017). Effects of pulsed ultrasound on rheological and structural properties of chicken myofibrillar protein. **Ultrasonics Sonochemistry.** 38 (Supplement C), 225-233.

Wu, L., Wu, T., Wu, J., Chang, R., Lan, X., Wei, K., & Jia, X. (2016). Effects of cations on the “salt in” of myofibrillar proteins. **Food Hydrocolloids.** 58, 179-183.

Yongsawatdigul, J., & Park, J. W. (1999). Thermal aggregation and dynamic rheological properties of Pacific whiting and cod myosins as affected by heating rate. **Journal of Food Science.** 64 (4), 679-683.

Yongsawatdigul, J., & Park, J. W. (2003). Thermal denaturation and aggregation of threadfin bream actomyosin. **Food Chemistry.** 83 (3), 409-416.

Zhang, Z., Regenstein, J. M., Zhou, P., & Yang, Y. (2017). Effects of high intensity ultrasound modification on physicochemical property and water in myofibrillar protein gel. **Ultrasonics Sonochemistry.** 34, 960-967.

Zhang, Z., Zhang, X., Chen, W., & Zhou, P. (2018). Conformation stability, in vitro digestibility and allergenicity of tropomyosin from shrimp (*Exopalaemon modestus*) as affected by high intensity ultrasound. **Food Chemistry.** 245, 997-1009.

Zou, Y., Xu, P., Wu, H., Zhang, M., Sun, Z., Sun, C., Wang, D., Cao, J., & Xu, W. (2018). Effects of different ultrasound power on physicochemical property and functional performance of chicken actomyosin. **International Journal of Biological Macromolecules.** 113, 640-647.

CHAPTER IV

GELATION BEHAVIOR OF THREADFIN BREAM SURIMI UNDER HIGH INTENSITY ULTRASOUND AT VARIOUS NaCl CONTENTS

4.1 Abstract

Effects of high intensity ultrasound (HIU) treatments at ultrasonic intensities of 10.01, 13.28 and 16.45 W/cm^2 for 30 min on gelation behavior of threadfin bream (*Nemipterus* spp.) surimi at various NaCl contents (0.5, 1 and 2% NaCl) were elucidated. Protein extractability at 0.5% NaCl was increased with an increase in ultrasonic intensity ($p < 0.05$); a decrease in the extractable protein content at 1 and 2% NaCl was observed at a higher intensity of 16.45 W/cm^2. At all tested NaCl contents, reactive SH content and S_0-ANS of the surimi pastes were increased after HIU treatments and were accompanied by a decrease in the Ca^{2+}-ATPase activity and total SH content, indicating a greater extent of unfolding and conformational changes induced by HIU, particularly at 1 and 2% NaCl. Textural properties and color of surimi gels at 0.5% NaCl were improved concomitant to an increase in ultrasonic intensity ($p < 0.05$), whereas HIU treatments resulted in inferior gels at 1 and 2% NaCl, which was mainly attributed to an excessive protein unfolding. Scanning electron microscopy (SEM) revealed that HIU resulted in higher levels of regular networks at 0.5% NaCl, while the coarser gel networks were observed at 1 and 2% NaCl. HIU treatments showed minimal effect on proteolysis of the surimi gels. Fourier transform infrared (FT-

IR) spectroscopy indicated that the α-helix content of the surimi gels was decreased, while the random coil levels were increased with an increase in ultrasonic intensity and NaCl content, confirming that structural changes induced by HIU treatments were more profound at higher NaCl contents (1 and 2% NaCl). The results suggested that HIU technology could be applied to improve 0.5% NaCl surimi gel, whereas HIU had a detrimental effect on the gels containing 1 and 2% NaCl.

Keywords: surimi, high intensity ultrasound, protein conformation, reduced salt

4.2 Introduction

Gelation is a critical parameter determining the quality of surimi because it governs the textural and sensory characteristics of the surimi-based products. Gelation of surimi is directly related to the myofibrillar proteins. Sodium chloride at 2-3% is typically required to facilitate solubilization of the myofibrillar proteins (Lanier, Carvajal, & Yongsawatdigul, 2005). A decrease in NaCl content results in poor gel-forming ability of surimi becaue the protein is not adequately solubilized and is not properly unfolded (Lanier et al., 2005). Thus, sodium chloride is an ingredient essential for surimi gelation and gel quality largely depends on sodium chloride concentration.

High intensity ultrasound (HIU) is a green processing technology with low frequency (16-100 kHz) and high power (10-1000 W/cm^2) (Soria & Villamiel, 2010). During sonication, the formation and collapse of cavitation bubbles results in energy accumulation and strong microstreaming (Soria & Villamiel, 2010). Simultaneously, a quantity of highly reactive free radicals ($H_2O \rightarrow \cdot H + \cdot OH$) could be produced from water molecules; these radicals may react with and modify other molecules/reactive groups (e.g., sulfhydryl groups of proteins) (Soria & Villamiel, 2010). HIU has been

recently applied to modify physicochemical properties of the myofibrillar proteins, such as solubility (Liu, Liu, Xiong, Fu, & Chen, 2017; Zhang, Regenstein, Zhou, & Yang, 2017). It has been reported that HU induces unfolding of myofibrillar proteins, exposing additional hydrophobic and sulfhydryl groups (Wang, Yang, Tang, Ni, & Zhou, 2017; Zou et al., 2018). Hence, an increase in the soluble myofibrillar proteins and the extent of protein unfolding are expected to lead to strong protein interactions and gel networks. In addition, the previous chapter (Chapter III) suggested a potential use of HIU technology in extraction of actomyosin in low NaCl concentrations. Thus, it is assumed that HIU should positively contribute to the surimi gel under reduced salt concentration due to increased solubility of the myofibrillar proteins, which are thought to be the base element of gel formation. Nevertheless, myosin globular head might undergo severe conformational changes in high NaCl concentration (0.6 M NaCl) after subjected to HIU treatments (Figure 3.2 in Chapter III), implying that HIU may not be good for typical surimi gel produced with 2-3% NaCl.

At present, only a few studies have focused on the application of HIU technology in surimi gelation; HIU can be a promising technology that can improve the surimi gel without the use of chemicals and/or enzymes. HIU-induced physicochemical changes of fish actomyosin in different NaCl concentrations was illustrated in Chapter III. However, how HIU treatment would affect gel properties of fish proteins exposed to various NaCl contents has not yet been elucidated thus far. Therefore, the objectives of this study were: (1) to investigate the effects of HIU treatments on gel properties of threadfin bream surimi under low/reduced salt using 2% NaCl as a control; (2) to illustrate physicochemical changes in threadfin bream surimi as a function of NaCl contents and HIU. Understanding of gelation behavior of surimi under HIU is expected

to provide the fundamental basis for utilization of the HIU technology in production of the surimi-based products at low/reduced salt levels.

4.3 Materials and methods

4.3.1 Materials and reagents

Frozen threadfin bream surimi (*Nemipterus* spp.) was purchased from a surimi plant at Samutsakorn, Thailand. Surimi samples did not contain egg white protein. Samples were packed in a polystyrene foam box filled with ice and immediately transported to a laboratory at Suranaree University of Technology. Upon arrival, surimi was cut into 500-g blocks, vacuum-packed, and then kept at -40 °C until use.

Bovine serum albumin (BSA) and 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB) were obtained from Fluka (Buchs, Switzerland). 8-anilino-1-naphthalenesulfonic acid (ANS) and Adenosine 5′-triphosphate disodium salt hydrate (ATP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Reagents used for gel electrophoresis were bought from Bio-Rad (Hercules, CA, USA). All other reagents were of analytical grade.

4.3.2 Preparation of surimi paste at various NaCl contents

Frozen surimi was thawed at 4 °C overnight and cut into approximately 3-cm cubes. Surimi cubes were chopped in a Stephan vacuum cutter (UM 5 universal, Stephan Machinery Co., Columbus, OH, USA) at low speed for 1 min. Subsequently, sodium chloride was added at 0.5, 1, and 2% (w/w) of the total weight of the surimi paste and chopping was continued at low speed for 1 min. Then, the moisture content of the 0.5, 1 and 2% NaCl samples was adjusted to 81.5, 81 and 80% (w/w), respectively, using ice, and the samples were chopped at low speed for another 1 min. All treatments

contained the same amount of surimi. After addition of ice, chopping was conducted at a high speed for 3 min under vacuum condition. The total chopping time was 6 min. Chopping temperature was maintained below 10 °C using a chiller (RTE-101, NesLab, Newington, NH, USA). Surimi paste was then vacuum-packed to remove air bubbles.

4.3.3 HIU treatments of surimi paste

HIU treatments of surimi paste were carried out using an ultrasonic processor (Q500 sonicator, Qsonica L.L.C, Newtown, CT, USA) at a frequency of 20 kHz. Vacuum-packed surimi paste (540 g) was put into a container containing 1.2 L water and then immersed in an ice bath to maintain sample temperature at approximately 4-8 ºC. A titanium probe (25 mm in diameter) connected with a booster were immersed at a depth of 2.5-3cm in the middle of the container during sonication. Samples were sonicated at an amplitude of 70, 85 and 100% for 30 min. A pulse mode of on for 5 s and off for 5 s was used. Afterwards, surimi paste was used for measurement of protein extractability (as detailed in section 2.4) and subsequent gel preparation (section 2.8). Controls (without HIU treatment) were also prepared.

In this study, amplitude of 70, 85 and 100% with booster corresponded to ultrasonic intensity of 10.01, 13.28 and 16.45 W/cm^2, respectively using the method detailed by Jambrak, Lelas, Mason, Krešić, and Badanjak (2009), which were measured using calorimetry by a thermocouple (54 Series II thermometer, Fluke Corporation, Everett, WA, USA).

4.3.4 Protein extractability

Surimi paste with different salt contents of 0.5, 1, and 2% NaCl was homogenized with 20 mM Tris-maleate buffer (pH 7.0) at a ratio of 1:9 (w/v) using a homogenizer (T25 digital ULTRA-TURRAX, IKA Works, Inc., Wilmington, NC,

USA). Buffer containing 0.1, 0.21 and 0.43 M NaCl was used for the samples containing 0.5, 1 and 2% NaCl, respectively. Then, the homogenate was centrifuged at 8,000×g (Sorvall ST 16R Centrifuge, Thermo Scientific, Waltham, MA, USA) for 20 min at 4 °C. Supernatants were referred to as extractable protein and the protein content (mg/ml) was determined by the Bradford method (Bradford, 1976). BSA was used as a standard. Ca^{2+}-ATPase activity, total and reactive sulfhydryl group contents, and surface hydrophobicity of extractable protein were also analyzed.

4.3.5 Remaining Ca^{2+}-ATPase activity

Protein samples were diluted to 4 mg/ml with the corresponding extraction buffer (20 mM sodium phosphate buffer containing 0.1, 0.21 or 0.43 M NaCl, pH 7.0). Buffer containing 0.1, 0.21 and 0.43 M NaCl was used for the samples containing 0.5, 1 and 2% NaCl, respectively. The diluted sample (500 µl) was added with 250 µl of 0.6 M Tris-maleate (pH 7.0) and 250 µl of 0.1 M $CaCl_2$. The NaCl concentration in the reaction mixtures of 0.5, 1 and 2% NaCl samples was 0.1, 0.21 and 0.43 M NaCl, respectively. Deionized water was then added, making a total volume of 4.75 ml. Subsequently, measurement of Ca^{2+}-ATPase activity was conducted using the method of Endoo and Yongsawatdigul (2014) and the results were expressed as µmol of Pi/mg protein/min at 25 °C. Remaining Ca^{2+}-ATPase activity (%) of threadfin bream surimi paste was calculated as follows:

$$\text{Remaining } Ca^{2+}\text{-ATPase activity (\%)} = (A \times 100)/A_0,$$

where A_0 and A is Ca^{2+}-ATPase activity of sample without HIU treatment and after HIU treatments at each NaCl content, respectively.

4.3.6 Measurement of total and reactive sulfhydryl group

For measurement of total sulfhydryl group (SH) content, 0.5 ml of protein sample was added to 4.5 ml of the corresponding extraction buffer (0.1, 0.21 or 0.43 M NaCl, 50 mM sodium phosphate buffer, pH 7.0) with 8 M urea and 10 mM ethylenediaminetetraacetic acid (EDTA). Buffer containing 0.1, 0.21 and 0.43 M NaCl was used for the samples containing 0.5, 1 and 2% NaCl, respectively. Reactive SH content was assayed by preparing samples using buffers without urea. Then, total and reactive SH contents were measured using Ellman's reagent (0.1% DTNB, 5, 5'-dinitrobis [2-nitrobenzoic acid] in 50 mM NaH_2PO_4 buffer, pH 7.0) based on the method detailed by J. Yongsawatdigul and Park (2003). The results were expressed as mol/10^5 g protein.

4.3.7 Surface hydrophobicity determination

To determine surface hydrophobicity, protein samples were diluted with buffers (20 mM sodium phosphate containing 0.1, 0.21 or 0.43 M NaCl, pH 7.0) to obtain different concentrations of protein (0.1, 0.15, 0.2, 0.25, and 0.3 mg/ml). Buffer containing 0.1, 0.21 and 0.43 M NaCl was used for the 0.5, 1 and 2% NaCl samples, respectively. Surface hydrophobicity was then measured using ANS according to the method described by J. Yongsawatdigul and Park (2003). The initial slope of the plot of relative fluorescence (Monahan, German, & Kinsella, 1995) versus percentage of protein concentration (%, w/v) was referred to as surface hydrophobicity (S_0-ANS), which was calculated by linear regression analysis.

4.3.8 Surimi gel preparation and gel measurements

Surimi paste was stuffed into a polyvinylidine chloride casing (3 cm in diameter) and then heated at 90 °C for 30 min in a water bath. After heating, the samples

were immediately submerged in ice water for 20 min and then stored in a refrigerator (4 °C) overnight. Surimi gels were equilibrated at room temperature for 2 h before texture evaluation and color measurement. A texture analyzer (TA. XT *plus* Stable Micro Systems, Surrey, UK) was used to evaluate the textural properties of the surimi gels. Samples were cut into 3 cm long pieces. Breaking force (g) and distance (mm) were determined using a 5-mm diameter spherical plunger probe at a test speed of 1 mm/s (J. Yongsawatdigul & Piyadhammaviboon, 2004). Five measurements were carried out for each sample. Color values (L^*, a^* and b^*) of the samples were determined by a colorimeter (ColorQuest XE, HunterLab, Reston, VA, USA). Whiteness was then calculated using the equation L^*-3b^* (Park, 1994), where L^* is the lightness, and b^* is the yellowness/blueness.

4.3.9 Trichloroacetic acid (TCA)-soluble oligopeptide

TCA-soluble oligopeptide contents of surimi gels were determined according to the method of Jirawat Yongsawatdigul and Piyadhammaviboon (2005) with slight modifications. Three grams of sample was added to 27 ml of 5% cold TCA solution. The mixture was homogenized using a homogenizer (T25 digital ULTRA-TURRAX) and then centrifuged at 8, 000×g (Sorvall ST 16R) for 15 min at 4 °C. The supernatant was collected for measurement of TCA-soluble oligopeptide content by the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951) with tyrosine as a standard. The results were expressed as μmol tyrosine/g sample.

4.3.10 SDS-PAGE

SDS-PAGE was conducted to analyze the protein patterns of surimi gels according to the protocol of Laemmli (1970). Sample solubilization was carried out according to the method detailed by J. Yongsawatdigul and Piyadhammaviboon (2004)

with slight modifications. One gram of surimi gel was added to 10 ml of hot 10% SDS solution and then homogenized using a homogenizer (T25 digital ULTRA-TURRAX). The homogenates were heated at 90 °C for 1 h and subsequently centrifuged at 10,000×g (Sorvall ST 16R) for 10 min at room temperature. The supernatants were collected and diluted 1:1 (v/v) in treatment buffer (0.125 M Tris–HCl, 4% SDS, and 20% glycerol, pH 6.8). Then, the mixtures were heated at 95 °C for 5 min and cooled down prior to gel running. A polyacrylamide gel comprising of 10% running gel and 4% stacking gel was used. Protein (20 µg) was loaded onto the gel. Gel electrophoresis was conducted at a constant voltage of 100 V. Gels were stained with 0.125% Coomassie Brilliant Blue R-250 for 30 min and destained with a solution containing 25% methanol and 10% acetic acid. Gels were imaged by a UVITEC imaging system (Fire reader V4, Uvitec Ltd., Cambridge, UK).

4.3.11 Fourier transform infrared (FT-IR) spectroscopy

Secondary structures of surimi proteins were analyzed using a FT-IR spectrometer (Bruker Tensor 27, Karlsruhe, Germany) equipped with an attenuated total reflectance (ATR) platinum accessory. After lyophilization, the surimi gels were scanned for 64 times in the range of 500 to 4000 cm^{-1} at a resolution of 4 cm^{-1}. The spectra were collected at room temperature. Background interference was removed before recording spectra, which were then analyzed using the OPUS software version 7.5 (Bruker Optics GmbH, Ettlingen, Germany). Thirty measurements from triplicate samples were collected to obtain an average spectrum. The average spectrum was normalized, adjusted by baseline correction, and then curve-fitted in the 1600-1700 cm^{-1} region by appropriate Gaussian and Lorentzian functions using the OPUS software version 7.5 (Bruker Optics GmbH). The relative percentage of each secondary structure was calculated.

4.3.12 Scanning electron microscopy (SEM)

The microstructures of the surimi gels with and without HIU treatments were observed using a field emission scanning electron microscope (ZEISS Gemini, Carl Zeiss, Germany). Surimi gels were cut into 2-3 mm thick pieces and lyophilized. Dried samples were mounted on a bronze stub using carbon tape and then sputter-coated with gold. The specimens were observed at an accelerating voltage of 3 kV and a magnification of 200.

4.3.13 Statistical analysis

All the experiments were carried out in three replicates and the values were expressed as the mean ± SD. The SPSS version 23.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Analysis of variance (ANOVA) was used to analyze the data and the mean comparisons were conducted using Duncan's multiple-range test (DMRT). Significant level was $p < 0.05$. All the calculations and figures were performed with OriginPro 2018 (OriginLab Corporation, Northampton, MA, USA).

4.4 Results and discussion

4.4.1 Protein extractability

The extractable protein content of the 0.5% NaCl surimi paste was continuously increased concomitant to an increase in ultrasonic intensity (10.01-16.45 W/cm^2) compared to the control without ultrasound ($p < 0.05$, Figure 4.1). Myofibrillar proteins are salt-soluble proteins which require a NaCl concentration higher than 0.3 M to achieve a good solubilization. Thus, a limited protein solubility at low NaCl content (0.5% NaCl) was shown without HIU treatment (Figure 4.1). Myofibrils can be

disrupted by the physical forces (high shear energy and turbulence) produced during sonication, improving protein extraction. Moreover, hydrogen bonds and hydrophobic interactions may also be disrupted by ultrasound (Maity, Rasale, & Das, 2012), which are responsible for intermolecular association of natural myosin aggregates, and thus, more protein could be solubilized. In addition, the spatial conformational changes of the myofibrillar proteins, which resulted from the physical forces induced by cavitation, were also reported to lead to an increase in protein extraction (Zou et al., 2018). Higher ultrasonic intensity was associated with higher protein extractability (Figure 4.1) probably due to greater ultrasonic energy produced. Protein solubility of silver carp myosin (Liu et al., 2017) and chicken myofibrillar protein (Zhang et al., 2017) were also increased with applied HIU intensity.

At 1 and 2% NaCl, the extractable protein content was gradually increased concomitant to an increase in ultrasonic intensity from 10.01 to 13.28 W/cm^2 ($p \leq 0.05$, Figure 4.1), while a decrease in the protein extraction was observed at higher ultrasonic intensity (16.45 W/cm^2). Myofibrillar proteins were expected to unfold to a greater extent at higher NaCl contents (1 and 2% NaCl), exposing additional hydrophobic groups. This exposure may induce the formation of macromolecular aggregates through hydrophobic interactions, resulting in lower protein solubility. Wang et al. (2017) reported that protein solubility of the chicken myofibrillar proteins was increased after ultrasound treatment for 3 and 6 min ($p < 0.05$), while a prolonged treatment time (9-15 min) decreased the solubility. It should be noted that extractable protein content of 0.5% NaCl sample was increased by 126% after HIU treatments, while an increase of only 35 and 10% was observed at 1 and 2% NaCl, respectively (Figure 4.1). Our results indicated that HIU can extract more soluble myofibrillar proteins at lower NaCl (0.5%

NaCl); however, a higher ultrasonic intensity of 16.45 W/cm^2 decreased protein extractability at higher NaCl contents (1 and 2%).

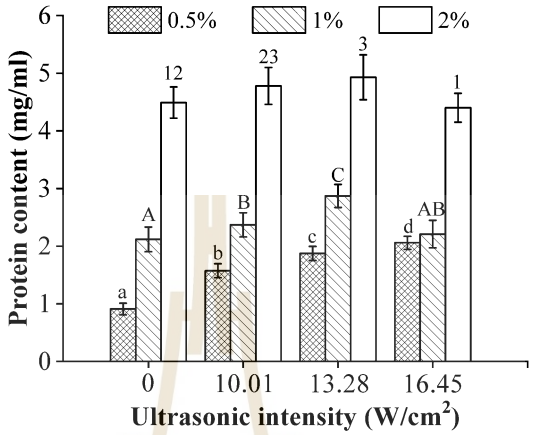


Figure 4.1 Effects of HIU treatments on protein extractability of threadfin bream surimi at various NaCl contents. Different superscripts in the case of the same NaCl content indicate significant differences ($p < 0.05$).

4.4.2 Remaining Ca^{2+}-ATPase activity

The remaining Ca^{2+}-ATPase activity of the surimi pastes with 0.5, 1 and 2% NaCl was decreased concomitant to an increase in ultrasonic intensity ($p < 0.05$, Figure 4.2). The reduction in remaining Ca^{2+}-ATPase activity indicated that HIU induced conformational changes of myosin globular head where the active site for Ca^{2+}-ATPase is located. Higher ultrasonic intensity had more pronounced effect on disturbance of the myosin conformation. The main cause of Ca^{2+}-ATPase activity reduction could be the physical effects (e.g., high pressure and turbulence) resulted from cavitation phenomenon. The conformational changes of myosin might also induce rearrangement of myosin molecules through protein–protein interactions (Benjakul &

Bauer, 2000), contributing to the decline of Ca^{2+}-ATPase activity. Ca^{2+}-ATPase activity of silver carp myosin was notably decreased concomitant to an increase in ultrasonic intensity ranging from 22.81-55.50 W/cm^2 (Liu et al., 2017). The remaining Ca^{2+}-ATPase activity at 0.5% NaCl was the highest, while that of a sample containing 2% NaCl was the lowest at all the ultrasonic intensities (Figure 4.2). After subjected to ultrasonic intensity of 16.45 W/cm^2, the remaining Ca^{2+}-ATPase activity at 0.5, 1 and 2%NaCl was 89, 81and 72%, respectively (Figure 4.2), suggesting that the protein at higher NaCl was more susceptible to ultrasonic energy. Higher NaCl levels resulted in a higher extent of protein unfolding to expose additional active sites; thus, the proteins can be easily influenced by cavitation. This phenomenon also revealed that HIU might induce severe destruction of myosin globular head in surimi samples containing higher NaCl contents, which may cause negative effect on surimi gelation. Changes in remaining Ca^{2+}-ATPase activity in surimi pastes are consistent with our previous study in tilapia actomyosin, which also showed that the residual Ca^{2+}-ATPase activity in 0.2 M NaCl was higher than that in 0.6 M NaCl after HIU treatments (Tang & Yongsawatdigul, 2020).

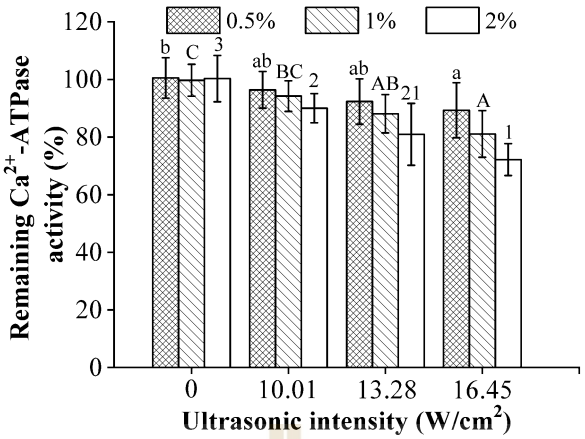


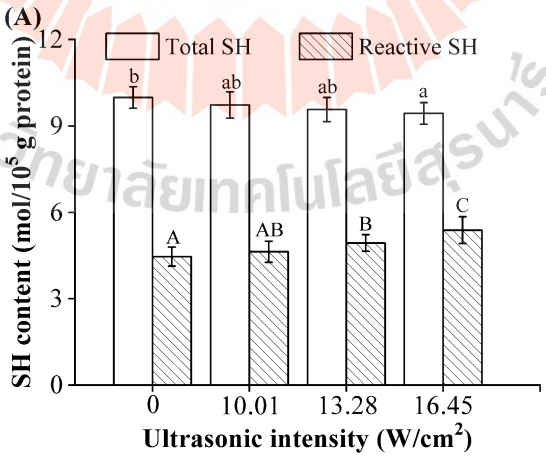
Figure 4.2 Remaining Ca^{2+}-ATPase activity of threadfin bream surimi at various NaCl contents after sonication with various intensities. Different superscripts in the case of the same NaCl content indicate significant differences ($p < 0.05$). Ca^{2+}-ATPase activity without HIU treatments at each NaCl content was considered to be 100%.

4.4.3 Total and reactive sulfhydryl group contents

Total sulfhydryl group (SH) content at all studied NaCl levels was slightly decreased concomitant to an increase in ultrasonic intensity from 0 to 16.45 W/cm^2 ($p \leq 0.05$, Figure 4.3), suggesting that HIU may induce oxidation of SH groups and that higher ultrasonic intensity had a more pronounced effect. A number of reactive radicals (·H and ·OH) could be produced from water molecules and subsequently recombine to form hydrogen peroxide (H_2O_2) (Hu et al., 2013; Milne, Stewart, & Bremner, 2013; Riesz & Kondo, 1992). H_2O_2 can rapidly degrade to oxygen and water, providing possibility to oxidize some SH groups to disulfide bonds. Liu et al. (2017) reported a reduction of total SH content in silver carp myosin when ultrasonic intensity increased from 22.81-55.50 W/cm^2. Moreover, the total SH content of chicken

myofibrillar proteins also decreased concomitant to an increase in ultrasonic intensity (Zhang et al., 2017; Zou et al., 2018).

In contrast, reactive SH content at 3 studied NaCl levels was continuously increased concomitant to an increase in ultrasonic intensity (Figure 4.3), suggesting that additional buried SH groups were exposed on the protein surface after HIU treatments. High pressure and shear force generated by cavitation induce a more pronounced protein unfolding, exposing additional SH groups. Zhang et al. (2017) reported that reactive SH content of the chicken myofibrillar protein was increased from 3.67 to 7.33 mol/10^4 g protein when ultrasonic intensity of 193 W/cm^2 was applied. In addition, an increase in the reactive SH content induced by HIU was 21, 26 and 27% in the samples containing 0.5, 1 and 2% NaCl, respectively. The results suggested that HIU induced protein unfolding at higher NaCl (1 and 2% NaCl) to a greater extent than that observed at lower NaCl (0.5% NaCl).



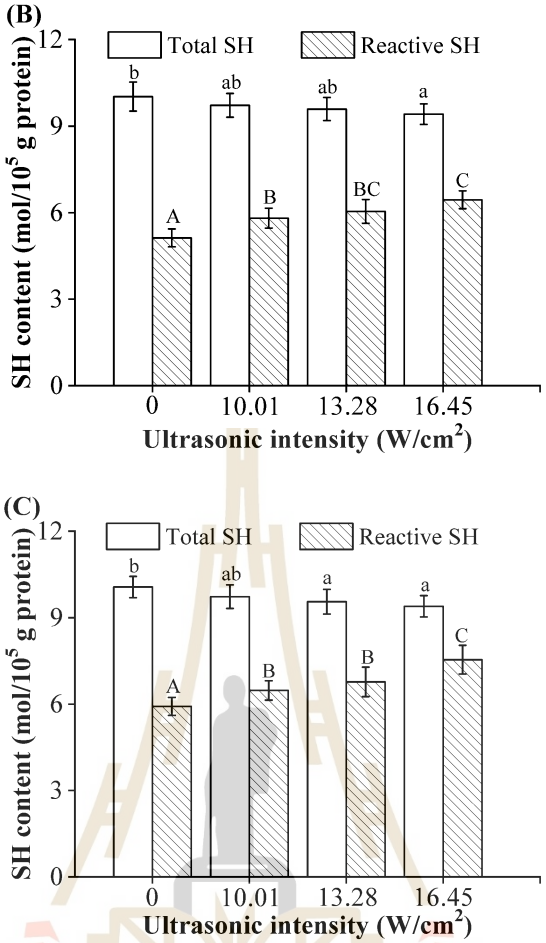


Figure 4.3 Effects of HIU treatments on the total and reactive SH contents of threadfin bream surimi containing 0.5 (A), 1 (B) and 2% (C) NaCl. Different lowercase and uppercase letters indicate significant differences in the total and reactive SH content ($p < 0.05$), respectively.

4.4.4 Surface hydrophobicity

An increase in the S_0-ANS values of the samples with 3 tested NaCl levels indicated that hydrophobic groups of the surimi paste were exposed after HIU treatments ($p < 0.05$, Figure 4.4). Moreover, at the 3 studied NaCl levels, the S_0-ANS values continuously increased concomitant to an increase in ultrasonic intensity

compared with the control ($p < 0.05$, Figure 4.4), illustrating that higher ultrasonic intensity induced more exposure of buried hydrophobic groups. As mentioned previously, high pressure and shear force generated during sonication might be the main probable cause of the conformational changes of the protein and dissociation of myosin aggregates, which induced exposure of the hydrophobic groups, resulting in an increased S_0-ANS value. Liu et al. (2017) reported that S_0-ANS of silver carp myosin significantly increased with inreasing ultrasonic intensity up to 55.50 W/cm^2. An increase in S_0-ANS induced by HIU treatments has also been found in previous studies on chicken myofibrillar proteins (Wang et al., 2017; Zhang et al., 2017).

Surimi paste with higher NaCl content showed higher S_0-ANS values regardless of HIU treatments, indicating a higher extent of unfolding was induced at high NaCl content (Figure 4.4). Moreover, the effect of HIU on protein unfolding was less pronounced at 0.5% NaCl; at this NaCl level, myosin mainly exists as the aggregate forms thus limiting the exposure of the hydrophobic groups. Thus, higher HIU energy is required for protein unfolding. An increase in S_0-ANS induced by HIU was up to 56, 61 and 63% at 0.5, 1 and 2% NaCl, respectively, after HIU treatment at 16.45 W/cm^2 (Figure 4.4). This was in accordance with the results of remaining Ca^{2+}-ATPase activity (Figure 4.2) and reactive SH content (Figure 4.3), which further verified that a greater extent of protein unfolding at higher NaCl (1 and 2% NaCl) was induced by HIU. In addition, unfolded proteins can participate in hydrophobic interactions to form large aggregates, which are precipitated after centrifugation. This may explain why at higher NaCl content (1 and 2% NaCl), a decrease in protein extractability (Figure 4.1) was observed in samples exposed to a higher ultrasonic intensity of 16.45 W/cm^2.

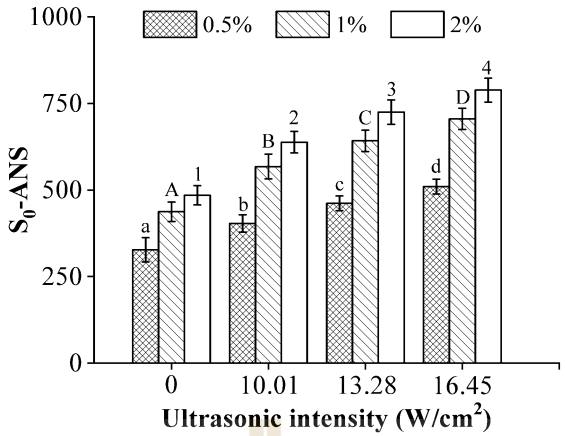


Figure 4.4 Effects of HIU treatments on surface hydrophobicity of threadfin bream surimi with various NaCl contents. Different superscripts in the case of the same NaCl content indicate significant differences ($p < 0.05$).

4.4.5 Textural properties

Without HIU treatments, surimi gel at 0.5% NaCl showed the lowest values of breaking force and distance, whereas the highest values were observed in surimi gel containing 2% NaCl (Figure 4.5). Gel formation of the food proteins involves partial denaturation followed by irreversible aggregation (Lanier et al., 2005). Solubilization of the myofibrillar proteins was lower at 0.5% NaCl (Figure 4.1), resulting in limited protein solubilization and aggregation. Higher NaCl contents (1 and 2% NaCl) yield higher levels of extractable proteins, particularly myofibrillar proteins (Figure 4.1), and a higher degree of protein unfolding (Figure 4.4). These factors enable aggregation and formation of a proper gel network. It was reported that both breaking force and distance of the Alaska pollock surimi gel at 0.3% NaCl were lower than those at 3% NaCl (Cando, Herranz, Borderías, & Moreno, 2015, 2016). Both values of breaking force and distance of the surimi gels at 0.5% NaCl were continuously

increased concomitant to an increase in ultrasonic intensity ($p < 0.05$, Figure 4.5). Our study indicated that HIU treatments improved the gelation of surimi at reduced NaCl content (0.5% NaCl) probably due to an increase in protein extractability (Figure 4.1) and in protein unfolding (Figure 4.4). An increase in the concentration of the soluble myofibrillar proteins should be beneficial to the heat-induced surimi gelation. In addition, higher extent of protein unfolding should expose additional interior groups (e.g., hydrophobic groups), which are expected to facilitate intermolecular covalent/noncovalent interactions thus promoting the development of a better gel network.

In contrast, HIU treatment resulted in inferior textural properties of the surimi gels at 1 and 2% NaCl (Figure 4.5) despite higher protein extractability (Figure 4.1). Gelation of surimi under HIU treatments does not depend only on the salt-soluble proteins. Excessive unfolding of the myofibrillar proteins at 1 and 2% NaCl, compared with unfolding observed at 0.5% NaCl, was likely to occur after HIU treatments as indicated by a dramatic decrease in the Ca^{2+}-ATPase activity (Figure 4.2) and a higher increase in the S_o-ANS values (Figure 4.4). Conformation of myosin at 1 and 2% NaCl was expected to be severely disturbed by HIU, which should eventually yield an inferior gel network. Zhang et al. (2017) reported that chicken myofibrillar protein at 0.6 M KCl was severely denatured after higher intensity HIU treatments (173 and 193 W/cm^2 for 15 min) resulting in a loose microstructure and lower water-holding capacity of protein gel. Therefore, it can be speculated that gelation of HIU-treated surimi is related to the quantity of the salt-extractable proteins and to the conformation of the soluble myofibrillar proteins. Severe conformational changes of the proteins observed in surimi containing 1 and 2% NaCl subjected to HIU may hamper protein interactions thus

causing inappropriate protein aggregation and a poor gel network.

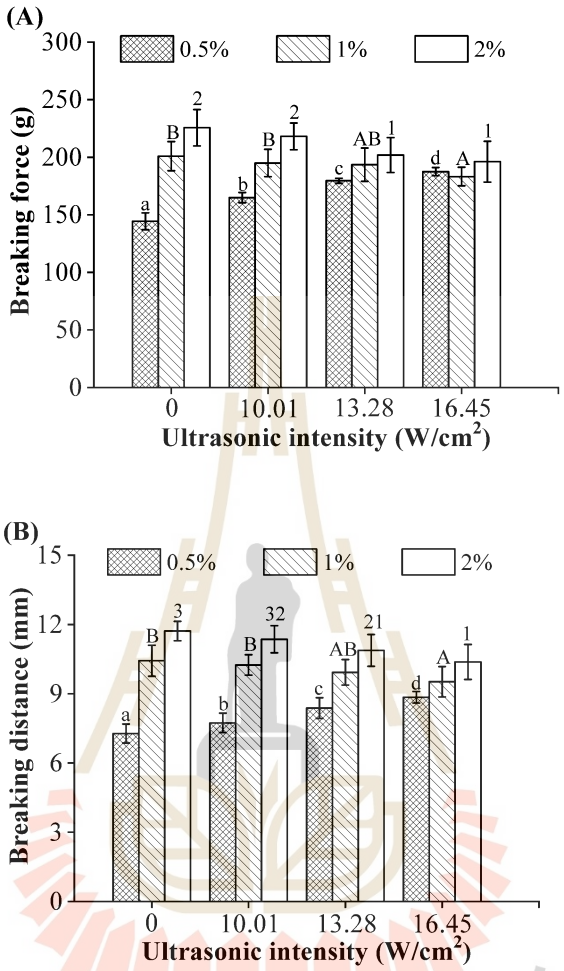


Figure 4.5 Effects of HIU treatments on textural properties of the threadfin bream surimi gels at various NaCl contents. Different superscripts in the case of the same NaCl content indicate significant differences ($p < 0.05$).

4.4.6 Color

Whiteness is likely to be related to protein aggregation and to the gel network (Cando et al., 2015; Tadpitchayangkoon, Park, & Yongsawatdigul, 2012). Without HIU treatments, surimi gel at 0.5% NaCl was characterized by the lowest

whiteness and lightness (Figure 4.6A and B). Higher NaCl content resulted in higher levels of the solubilized protein leading to a higher order gel network which reflected more light thus resulting in higher whiteness. Cando et al. (2015) reported that lightness value (L^*) of the Alaska pollock surimi gel at 0.3% NaCl was lower than that of the gel at 3% NaCl. Whiteness and lightness of the surimi gels at 0.5% NaCl were continuously increased concomitant to an increase in ultrasonic intensity compared with that in the control ($p < 0.05$, Figure 4.6A and B). This increase implied that HIU-treated surimi gels at 0.5% NaCl may have a higher level of protein aggregation and a uniform gel network probably due to an increase in the extractable proteins (Figure 4.1) and a higher degree of protein unfolding induced by HIU (Figure 4.4). However, higher intensity of HIU treatments reduced whiteness of the surimi gels at 1 and 2% NaCl ($p \leq 0.05$, Figure 4.6A). Since an excessive unfolding of surimi at higher NaCl contents (1 and 2% NaCl) occurred under higher intensity (Figure 4.4), the rate of protein aggregation may be substantially faster than the rate of unfolding resulting in a coarser and irregular gel network. These events can lead to lower luminosity of the surimi gels ($p \leq 0.05$, Figure 4.6B). It should be noted that changes in whiteness and lightness were consistent with the changes in the textural properties (Figure 4.5).

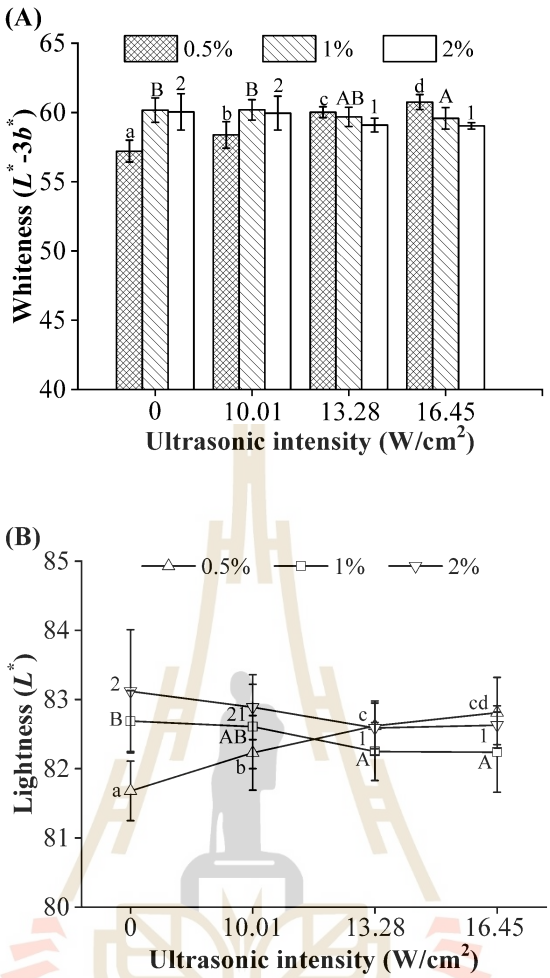


Figure 4.6 Effects of HIU treatments on whiteness (A) and lightness (B) of the threadfin bream surimi gels at various NaCl contents. Different superscripts in the case of the same NaCl content indicate significant differences ($p < 0.05$).

4.4.7 Proteolysis

The TCA-soluble oligopeptide content indicates the extent of proteolytic degradation resulted from endogenous proteinases (Tadpitchayangkoon et al., 2012). Generally, the TCA-soluble oligopeptide content of the surimi gels was slightly increased concomitant to an increase in ultrasonic intensity at all the studied NaCl

contents ($p \leq 0.05$), while the effect of NaCl content was subtle ($p > 0.05$, Figure 4.7). The results suggested that proteolysis of the surimi gels may be promoted by HIU to an extent. It was found that serine proteinases were responsible for proteolysis of threadfin bream (*Nemipterus* spp.) (Kinoshita, Toyohara, & Shimizu, 1990). Cavitation effects induced protein unfolding (Figure 4.4) leading to higher exposure of the catalytic sites to proteolysis. Moreover, proteolysis can alter enzyme conformation in such a way that the catalytic activity is promoted. Uluko et al. (2013) reported that ultrasonic pretreatment (800 W, 1-8 min) led to a higher degree of hydrolysis of a milk protein concentrate probably due to an increase in the catalytic efficiency of trypsin and Alcalase as a result of dissociation of the protein aggregates and conformational changes of the enzymes induced by ultrasound. Ultrasound (20 kHz, 20-40% amplitude) treatments increased the susceptibility of β-lactoglobulin to pepsin and trypsin proteolysis (Ma, Wang, & Guo, 2018). It should be mentioned that the TCA-soluble oligopeptide contents were relatively low. Endogenous proteolytic activity varies with the fish species. Threadfin bream surimi has substantially lower proteolytic activity compared with that of lizardfish surimi, which is considered as a high proteolytic surimi. Tadpitchayangkoon et al. (2012) reported that the levels of the TCA-soluble oligopeptides of a threadfin beam gel were 1.08 nmol/mg protein, which was approximately 33% of that of a lizardfish gel (3.35 nmol/mg protein).

HIU-treated surimi gels did not show any noticeable changes in the intensity of MHC at 3 studied NaCl contents (Figure 4.8). The results were in agreement with the changes in the TCA-soluble oligopeptide content (Figure 4.7). Saleem and Ahmad (2016) reported that chicken actomyosin remained intact after ultrasound treatment at 120 W for up to 30 min. In addition, no significant changes were observed in the

chicken myofibrillar proteins treated with ultrasound at 240 W for up to 15 min (Wang et al., 2017). Based on the TCA-soluble oligopeptide content (Figure 4.7) and SDS-PAGE analysis (Figure 4.8), HIU showed only a minimal effect on proteolysis of the surimi gels, and the effect of HIU-induced proteolysis on a decrease in the gel texture at 1 and 2% NaCl can be ruled out.

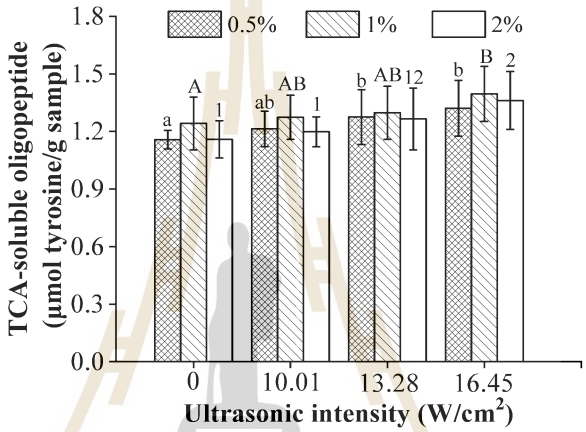


Figure 4.7 Effects of HIU treatments on the TCA-soluble oligopeptide content of the threadfin bream surimi gels at various NaCl contents. Different superscripts in the case of the same NaCl content indicate significant differences ($p < 0.05$).

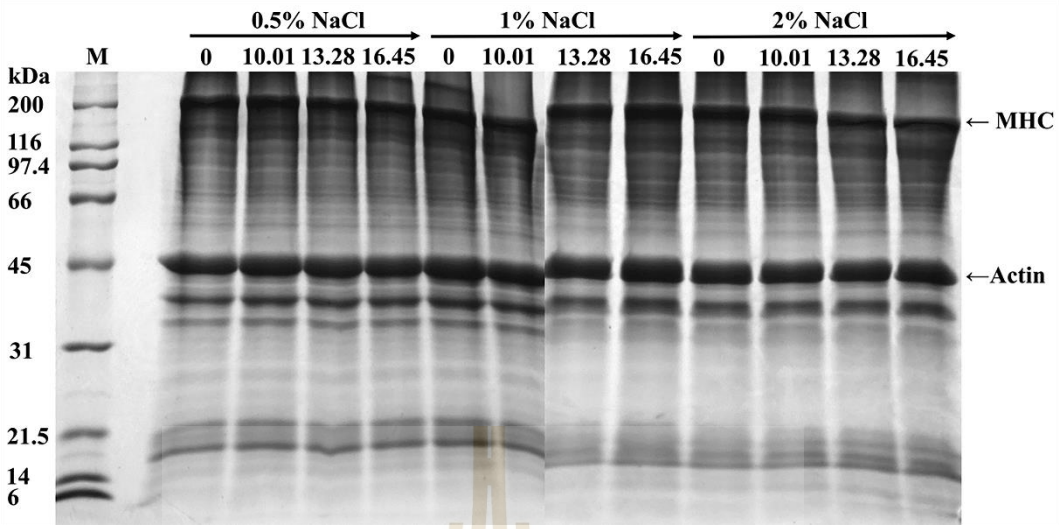


Figure 4.8 SDS-PAGE patterns of the threadfin bream surimi gels subjected to HIU treatments. M indicates protein markers. Numbers (0, 10.01, 13.28 and 16.45) refer to ultrasonic intensity (W/cm^2).

4.4.8 Changes of protein secondary structures

Changes in protein secondary structures were examined by analyzing the amide I region (1600-1700 cm^{-1}). Surimi gels with higher NaCl contents exhibited lower α-helix levels but higher levels of β-sheet, β-turn and random coil regardless of HIU treatments ($p < 0.05$, Figure 4.9), confirming that higher NaCl content can lead to a greater protein unfolding. The β-sheet content of the Alaska pollock surimi gel at 3% NaCl was higher than that at 0.3% NaCl (Cando et al., 2015, 2016). HIU treatments resulted in a decline in the α-helix content in the surimi gels with all studied NaCl contents concomitant with an increase in β-sheet, β-turn and random coil contents compared with those determined in the control ($p < 0.05$, Figure 4.9). Moreover, higher ultrasonic intensity showed a more pronounced effect. These findings suggested that the protein underwent enhanced unfolding upon HIU treatments. A decrease by

approximately 19% in the α-helix levels was observed at 2% NaCl, while only approximately 13% reduction was observed in the samples containing 0.5 NaCl, suggesting a higher protein unfolding was induced by HIU at higher NaCl content. Jiang et al. (2014) reported that a reduction in α-helix content and an increase in β-sheet content of black bean protein isolate were observed after ultrasonic treatments (150-450 W, 12 and 24 min). Similarly, the α-helix content of the beef protein was decreased after sonication with intensity ranging from 2.39 to 20.96 W/cm^2 for 30-120 min (Kang et al., 2016). However, an increase in the β-sheet content was similar in all studied NaCl contents. In addition, an 18% increase in the random coil levels was observed in 0.5% NaCl sample after HIU treatment, while higher NaCl contents resulted in a greater increase in the random coil structure by 21-25%. This result indicated that HIU caused an excessive protein unfolding at higher NaCl contents, which can be partly accountable for the inferior gel textures observed at 1 and 2% NaCl (Figure 4.5). Changes in secondary structures were consistent with the changes in the reactive SH contents (Figure 4.3) and S_0-ANS values (Figure 4.4). These results confirmed that HIU treatments induced unfolding of the myofibrillar proteins to a higher extent and a more obvious effect was observed at higher NaCl content.

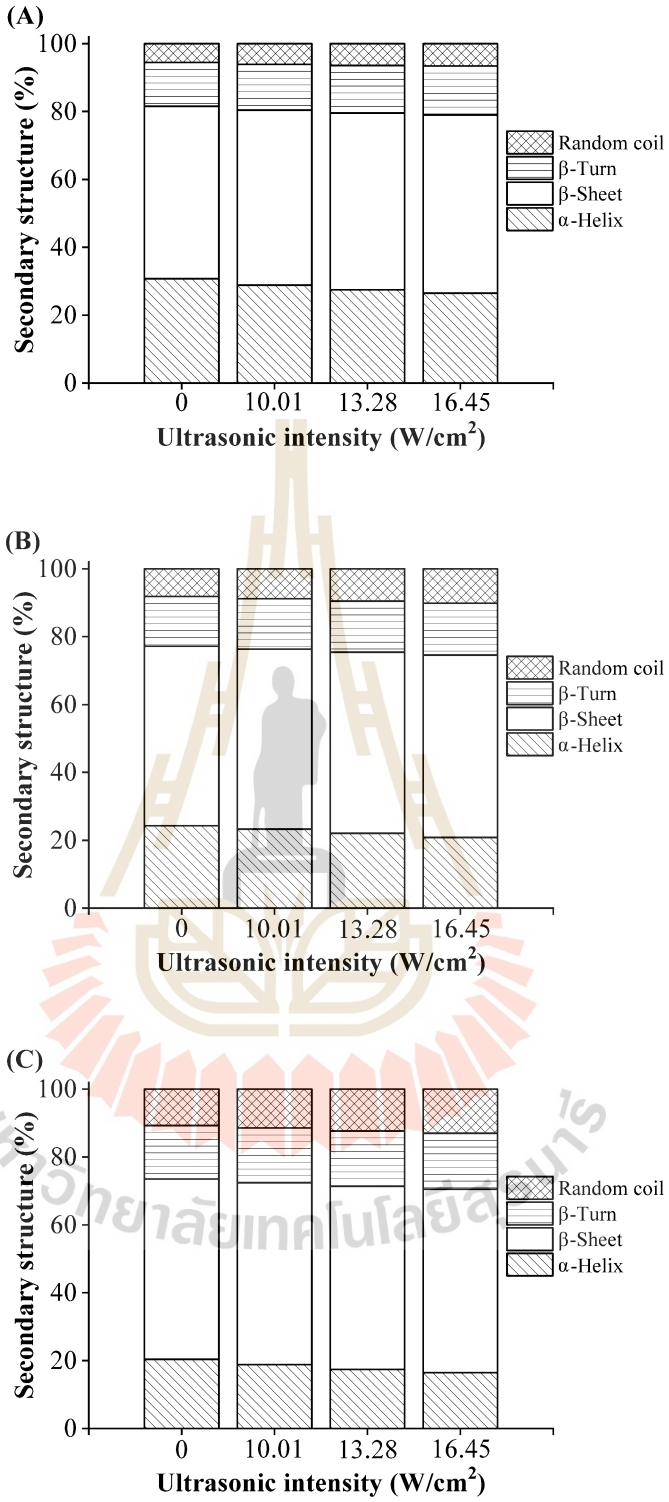


Figure 4.9 Effects of HIU treatments on the protein secondary structures of the threadfin bream surimi gels with various contents of 0.5 (A), 1 (B) and 2% (C) NaCl.

4.4.9 Microstructure of surimi gels

Without HIU, surimi gel at 0.5% NaCl had a coarse microstructure with larger and irregular pores (Figure 4.10). The gel network became denser and the pores were more uniform concomitant to an increase in the NaCl content to 1 and 2%. Finer gel structures with smaller pores were observed in the 0.5% NaCl samples subjected to the HIU treatment (Figure 4.10). The change was in agreement with the textural properties (Figure 4.5) and color (Figure 4.6). The change can be attributed to the exposure of the hydrophobic groups (Figure 4.4) induced by HIU as mentioned previously, which facilitates the hydrophobic interactions between the molecules thus promoting the gel network development. However, HIU induced a gel network of higher heterogeneity with larger pores at 1 and 2% NaCl corresponding to the inferior surimi gels induced by HIU (Figure 4.5). A higher extent of protein unfolding after HIU (Figures 4.2, 4.4 and 4.9) may partly contribute to the inferior gel networks.

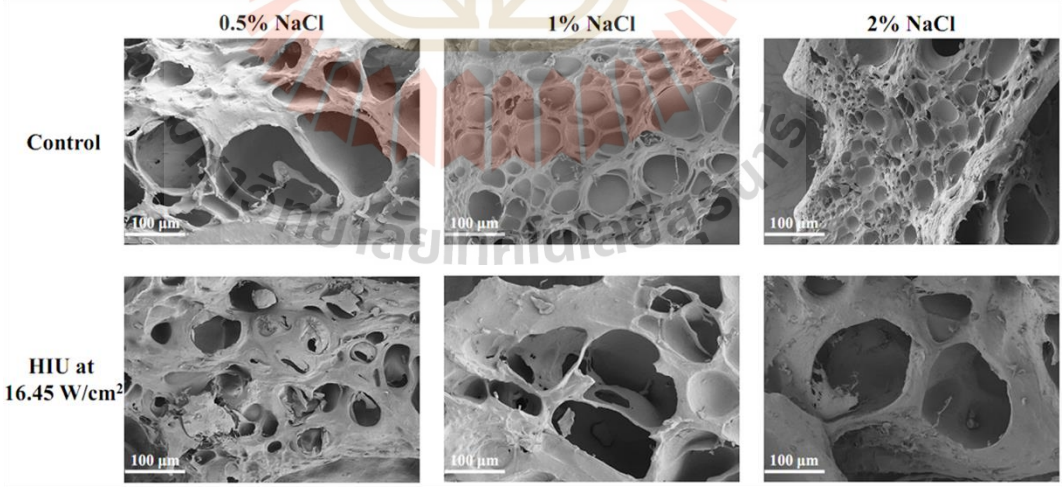


Figure 4.10 Effects of HIU treatment (ultrasonic intensity of 16.45 W/cm^2, 30 min) on the microstructure of the threadfin bream surimi gels at various NaCl contents (magnification: 200×). Scale bar = 100 µm.

4.5 Conclusions

Gel properties of surimi at 0.5% NaCl were improved after HIU treatments due to enhanced protein extractability and a higher extent of protein unfolding induced by HIU. An excessive unfolding occurred in HIU-treated surimi at 1 and 2% NaCl resulting in inferior surimi gels. HIU-induced protein unfolding and conformational changes were more pronounced at higher NaCl contents. HIU showed only a minimal effect on proteolysis of the threadfin bream surimi gels at the 3 studied NaCl contents. HIU technology could improve threadfin bream surimi gel at low NaCl content (0.5% NaCl); however, HIU is detrimental to the gels containing 1 and 2% NaCl, and 2% NaCl is the typical NaCl content in surimi-based seafood.

4.6 References

Benjakul, S., & Bauer, F. (2000). Physicochemical and enzymatic changes of cod muscle proteins subjected to different freeze-thaw cycles. **Journal of the Science of Food and Agriculture.** 80 (8), 1143-1150.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry.** 72 (1), 248-254.

Cando, D., Herranz, B., Borderías, A. J., & Moreno, H. M. (2015). Effect of high pressure on reduced sodium chloride surimi gels. **Food Hydrocolloids.** 51 (Supplement C), 176-187.

Cando, D., Herranz, B., Borderías, A. J., & Moreno, H. M. (2016). Different additives to enhance the gelation of surimi gel with reduced sodium content. **Food Chemistry.** 196, 791-799.

Endoo, N., & Yongsawatdigul, J. (2014). Comparative study on chemical and gel-forming properties of surimi from freshwater and marine fish during frozen storage. **Food and Applied Bioscience Journal.** 2 (3), 192-202.

Hu, H., Wu, J., Li-Chan, E. C. Y., Zhu, L., Zhang, F., Xu, X., Fan, G., Wang, L., Huang, X., & Pan, S. (2013). Effects of ultrasound on structural and physical properties of soy protein isolate (SPI) dispersions. **Food Hydrocolloids.** 30 (2), 647-655.

Jambrak, A. R., Lelas, V., Mason, T. J., Krešić, G., & Badanjak, M. (2009). Physical properties of ultrasound treated soy proteins. **Journal of Food Engineering.** 93 (4), 386-393.

Jiang, L., Wang, J., Li, Y., Wang, Z., Liang, J., Wang, R., Chen, Y., Ma, W., Qi, B., & Zhang, M. (2014). Effects of ultrasound on the structure and physical properties of black bean protein isolates. **Food Research International.** 62, 595-601.

Kang, D.-c., Zou, Y.-h., Cheng, Y.-p., Xing, L.-j., Zhou, G.-h., & Zhang, W.-g. (2016). Effects of power ultrasound on oxidation and structure of beef proteins during curing processing. **Ultrasonics Sonochemistry.** 33, 47-53.

Kinoshita, M., Toyohara, H., & Shimizu, Y. (1990). Purification and properties of a novel latent proteinase showing myosin heavy chain-degrading activity from threadfin-bream muscle. **The Journal of Biochemistry.** 107 (4), 587-591.

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

Lanier, T. C., Carvajal, P., & Yongsawatdigul, J. (2005). Surimi Gelation Chemistry. In J. W. Park (Ed.), Surimi and Surimi Seafood (2nd Edition) (pp. 435-489). Boca Raton, FL: CRC Press.

Liu, R., Liu, Q., Xiong, S., Fu, Y., & Chen, L. (2017). Effects of high intensity unltrasound on structural and physicochemical properties of myosin from silver carp. **Ultrasonics Sonochemistry.** 37 (Supplement C), 150-157.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. **Journal of Biological Chemistry.** 193 (1), 265-275.

Ma, S., Wang, C., & Guo, M. (2018). Changes in structure and antioxidant activity of β-lactoglobulin by ultrasound and enzymatic treatment. **Ultrasonics Sonochemistry.** 43, 227-236.

Maity, I., Rasale, D. B., & Das, A. K. (2012). Sonication induced peptide-appended bolaamphiphile hydrogels for in situ generation and catalytic activity of Pt nanoparticles. **Soft Matter.** 8 (19), 5301-5308.

Milne, L., Stewart, I., & Bremner, D. H. (2013). Comparison of hydroxyl radical formation in aqueous solutions at different ultrasound frequencies and powers using the salicylic acid dosimeter. **Ultrasonics Sonochemistry.** 20 (3), 984-989.

Monahan, F. J., German, J. B., & Kinsella, J. E. (1995). Effect of pH and temperature on protein unfolding and thiol/disulfide interchange reactions during heat-induced gelation of whey proteins. **Journal of Agricultural and Food Chemistry.** 43 (1), 46-52.

Park, J. W. (1994). Functional protein additives in surimi gels. **Journal of Food Science.** 59 (3), 525-527.

Riesz, P., & Kondo, T. (1992). Free radical formation induced by ultrasound and its biological implications. **Free Radical Biology and Medicine.** 13 (3), 247-270.

Saleem, R., & Ahmad, R. (2016). Effect of low frequency ultrasonication on biochemical and structural properties of chicken actomyosin. **Food Chemistry.** 205 (Supplement C), 43-51.

Soria, A. C., & Villamiel, M. (2010). Effect of ultrasound on the technological properties and bioactivity of food: a review. **Trends in Food Science & Technology.** 21 (7), 323-331.

Tadpitchayangkoon, P., Park, J. W., & Yongsawatdigul, J. (2012). Gelation characteristics of tropical surimi under water bath and ohmic heating. **LWT - Food Science and Technology.** 46 (1), 97-103.

Tang, L., & Yongsawatdigul, J. (2020). Physicochemical properties of tilapia (*Oreochromis niloticus*) actomyosin subjected to high intensity ultrasound in low NaCl concentrations. **Ultrasonics Sonochemistry.** 63, 104922.

Uluko, H., Zhang, S., Liu, L., Chen, J., Sun, Y., Su, Y., Li, H., Cui, W., & Lv, J. (2013). Effects of microwave and ultrasound pretreatments on enzymolysis of milk protein concentrate with different enzymes. **International Journal of Food Science & Technology.** 48 (11), 2250-2257.

Wang, J., Yang, Y., Tang, X., Ni, W., & Zhou, L. (2017). Effects of pulsed ultrasound on rheological and structural properties of chicken myofibrillar protein. **Ultrasonics Sonochemistry.** 38 (Supplement C), 225-233.

Yongsawatdigul, J., & Park, J. W. (2003). Thermal denaturation and aggregation of threadfin bream actomyosin. **Food Chemistry.** 83 (3), 409-416.

Yongsawatdigul, J., & Piyadhammaviboon, P. (2004). Inhibition of autolytic activity of lizardfish surimi by proteinase inhibitors. **Food Chemistry.** 87 (3), 447-455.

Yongsawatdigul, J., & Piyadhammaviboon, P. (2005). Effect of microbial transglutaminase on autolysis and gelation of lizardfish surimi. **Journal of the Science of Food and Agriculture.** 85 (9), 1453-1460.

Zhang, Z., Regenstein, J. M., Zhou, P., & Yang, Y. (2017). Effects of high intensity ultrasound modification on physicochemical property and water in myofibrillar protein gel. **Ultrasonics Sonochemistry.** 34, 960-967.

Zou, Y., Xu, P., Wu, H., Zhang, M., Sun, Z., Sun, C., Wang, D., Cao, J., & Xu, W. (2018). Effects of different ultrasound power on physicochemical property and functional performance of chicken actomyosin. **International Journal of Biological Macromolecules.** 113, 640-647.

CHAPTER V

HIU-ASSISTED GELATION OF THREADFIN BREAM SURIMI SUBJECTED TO REDUCED NaCl CONTENT UNDER VARIOUS ULTRASONIC EXPOSURE TIME

5.1 Abstract

Effect of ultrasonic exposure time (15, 30 and 45 min) of HIU-assisted gelation of 0.5% NaCl threadfin bream (*Nemipterus* spp.) surimi at ultrasonic intensity of 13.28 W/cm^2 was investigated. Protein extractability and surface hydrophobicity increased with increasing time up to 30 min, accompanied by a decrease in Ca^{2+}-ATPase activity ($p < 0.05$). HIU treatments increased storage modulus (G') of 0.5% NaCl surimi, indicating gel network formation was improved by HIU, particularly at ultrasonic exposure time of 30 min. Textural properties and whiteness of 0.5% NaCl surimi gel ($p < 0.05$) was also optimal at ultrasonic exposure time of 30 min. HIU treatments showed negative effect on 2% NaCl surimi gel probably due to the excessive protein unfolding caused by ultrasound. HIU treatments did not obviously affect proteolysis of both surimi pastes and gels based on trichloroacetic acid (TCA)-soluble oligopeptide content and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). According to Fourier transform infrared (FT-IR) spectroscopy, an increase in ultrasonic exposure time resulted in lower α-helix content but higher random coil content in both surimi pastes and gels, suggesting that a longer ultrasonic exposure time induced greater protein unfolding. The results demonstrated that ultrasonic exposure time of 30 min at

ultrasonic intensity of 13.28 W/cm^2 optimally induced surimi gelation at 0.5% NaCl.

Keywords: ultrasound, reduced NaCl, threadfin bream surimi, gel, myofibrillar proteins

5.2 Introduction

The amount of NaCl typically used for surimi gelation is 2-3%, which is required to solubilize myofibrillar proteins (Lanier, Carvajal, & Yongsawatdigul, 2005). The mechanical and functional properties of silver carp surimi gel reduced when lowering NaCl content to 1% (Fu et al., 2012). Thereby, reduced or low sodium surimi-based gel is typically suffered from inferior textural characteristics.

Nowadays, consumption of excessive sodium has become a public concern since it increases risks of hypertension and cardiovascular diseases (Kloss, Meyer, Graeve, & Vetter, 2015). The intake of sodium chloride in adults should be less than 5 g/day according to the World Health Organization (WHO), which equivalents to 2 g/day of sodium. Efforts have been attempted to reduce sodium content in surimi gels to fit with the raising consumer awareness. Potassium chloride can be used to partially replace of NaCl, but it causes negative sensory characteristics due to its bitterness and metallic taste (Tahergorabi & Jaczynski, 2012). Sodium reduction has also been reported by addition of different ingredients, such as whey protein concentrate (Uresti, Téllez-Luis, Ramírez, & Vázquez, 2004), tetra-sodium pyrophosphate (Deysi Cando, Herranz, Borderías, & Moreno, 2016) and lysine (D. Cando, Borderías, & H. M. Moreno, 2016). Recently, much attention has been given towards the applications of novel physical techniques. Fu et al. (2012) reported that 1% NaCl silver carp surimi gel was improved after microwave heating because faster heating process of microwave

inhibited autolysis and the intense heat provided by microwave expanded protein aggregates which served as substrate for crosslinking reactions. High pressure processing technique has been explored to improve Alaska pollock surimi gel at 0.3% NaCl (Deysi Cando, Herranz, Borderías, & Moreno, 2015) and was reported to strengthen the water retention of myosin gel in 0.3 M NaCl (Jianyi Wang, Li, Zheng, Zhang, & Guo, 2019), which were due to that high pressure induced protein unfolding by decreasing protein volume and then promoted surimi gelation via protein-protein interactions.

Ultrasound is a promising eco-friendly processing technique. Ultrasound technology is based on the mechanical waves at a frequency that exceeds the hearing limit of human (i.e., above 16 kHz), which can be divided into low intensity ultrasound (100 kHz-1 MHz with intensity of 0.1-1 W/cm^2) and high intensity ultrasound (HIU, 16-100 kHz with intensity of 10-1000 W/cm^2) (Soria & Villamiel, 2010). HIU has been proven to modify physicochemical and functional properties of myofibrillar proteins, such as solubility (Liu, Liu, Xiong, Fu, & Chen, 2017; Zou et al., 2018), turbidity (Zhang, Regenstein, Zhou, & Yang, 2017) and rheological properties (Amiri, Sharifian, & Soltanizadeh, 2018; Jingyu Wang, Yang, Tang, Ni, & Zhou, 2017). This is due to cavitation effect that generates strong micro-streaming and highly reactive ·OH during sonication (Soria et al., 2010). Moreover, it has also been reported to induce the unfolding of myofibrillar proteins, leading to exposure of interior hydrophobic and sulfhydryl groups (Liu et al., 2017; Zhang et al., 2017). In addition, the previous work in Chapter III found that actomyosin could be efficiently extracted in low NaCl concentrations by HIU. Hence, HIU technology provides the possibility for developing low/reduced-salt muscle foods with desirable gel properties.

Based on the foregoing information, it could be inferred that HIU technology would be worth exploring to develop low/reduced-salt surimi gel, which might be the onset of breakthrough of sodium reduction in not only surimi-based products, but also the other muscle foods. Based on the work of chapter IV, HIU treatments at different ultrasonic intensities (10.01, 13.28 and 16.45 W/cm^2, 30 min) improved surimi gel at 0.5% NaCl. Nevertheless, myofibrillar proteins underwent severe conformational changes under the higher intensity of 16.45 W/cm^2 (Figure 4.2). Therefore, this study aimed to look for a milder but effective condition by exploring the effect of various ultrasonic time on gelation of 0.5% NaCl threadfin bream surimi under the medium intensity of 13.28 W/cm^2. In-depth understanding of an optimal ultrasonic condition applied to low/reduced-salt surimi would be essential in development of reduced sodium surimi seafoods.

5.3 Materials and methods

5.3.1 Materials

Threadfin bream surimi (grade AA) was supplied by a surimi plant at Samutsakorn, Thailand. The surimi sample contained no egg white protein. Frozen surimi blocks of 20 kg were packed in a polystyrene foam box, filled with ice bags, and transported to a laboratory at Suranaree University of Technology immediately. Frozen surimi was cut into 500-g blocks and vacuum-packed upon arrival, and then stored in a freezer (-40 °C) during this study. All chemicals used in this study were of analytical grade.

5.3.2 Preparation of surimi pastes

Frozen surimi was partially thawed in a refrigerator (4 °C) overnight before being chopped for 6 min in a Stephan vacuum silent cutter (UM 5 universal, Stephan Machinery Co., Columbus, OH, USA). Sodium chloride was added at 0.5 and 2% (w/w) of the total weight of surimi paste, respectively. Ice was added to adjust the moisture content of 0.5 and 2% NaCl samples to 81.5 and 80% (w/w), respectively. All samples contained the same amount of surimi. A chiller (RTE-101, NesLab, Newington, NH, USA) was used to maintain sample temperature below 10 °C during chopping. The surimi pastes prepared were vacuum-packed into a polyethylene bag to eliminate air bubbles.

5.3.3 HIU treatments of surimi pastes with various ultrasonic exposure time

An ultrasonic processor (Q500 sonicator, Qsonica L.L.C, Newtown, CT, USA) was used for HIU treatments of surimi pastes at a frequency of 20 kHz. The vacuum-packed surimi paste (540 g) was put into a container containing 1.2 L water, which was immersed in an ice bath to keep the sample temperature around 4-8 ºC during sonication. A titanium probe (25-mm diameter) connected to a booster was placed at the middle of the container with 2.5-3cm depth. Samples were subjected to an output power of 130 W (amplitude of 85%) for various ultrasonic exposure time of 15, 30 and 45 min. A pulse mode of on-time for 5 s and off-time for 5 s was applied. Subsequently, the surimi pastes were used for measurement of extractable protein content (as detailed in 5.3.4), rheological measurements (as described in 5.3.7) and surimi gel preparation (as described in 5.3.8). Samples without HIU treatments were employed as the control.

In this study, amplitude of 85% with booster corresponded to ultrasonic intensity of 13.28 W/cm^2, which was measured using the method detailed by Jambrak, Lelas, Mason, Krešić, and Badanjak (2009).

5.3.4 Determination of extractable protein content

Preparation of extractable protein of threadfin bream surimi paste with 0.5 and 2% NaCl was carried out according to the method of Endoo and Yongsawatdigul (2014) with slight modifications. A 20 mM Tris-maleate buffer (pH 7.0) containing 0.1 and 0.43 M NaCl was used for the 0.5 and 2% NaCl sample, respectively. The extractable protein content (mg/ml) was determined using the Bradford method with bovine serum albumin (BSA) as a standard (Bradford, 1976).

5.3.5 Residual Ca^{2+}-ATPase activity

Ca^{2+}-ATPase activity of the extractable protein was measured using the method described by Endoo et al. (2014) with some modifications. Protein samples were diluted to 4 mg/ml with the extraction buffer of 20 mM sodium phosphate buffer containing 0.1 M or 0.43 M NaCl, pH 7.0 for mesurement of Ca^{2+}-ATPase activity of 0.5 and 2% NaCl sample, respectively. The results of Ca^{2+}-ATPase activity were expressed as μmol of Pi/mg protein/min at 25 °C. Residual Ca^{2+}-ATPase activity (%) of the extractable protein was calculated as follows:

$$\text{Residual } Ca^{2+}\text{-ATPase activity (\%)} = (A \times 100) / A_o,$$

where A_o and A is Ca^{2+}-ATPase activity of sample without ultrasound treatment and after ultrasound treatments, respectively.

5.3.6 Surface hydrophobicity

Surface hydrophobicity of the extractable protein was measured using ANS using the method of J. Yongsawatdigul and Park (2003) with slight modifications. Protein sample was diluted with 20 mM sodium phosphate containing 0.1 M or 0.43 M NaCl, pH 7.0 to obtain 5 different protein concentrations (0.1, 0.15, 0.2, 0.25, and 0.3 mg/ml). Buffer containing 0.1 and 0.43 M NaCl was used for the 0.5 and 2% NaCl sample, respectively. Surface hydrophobicity (S_0-ANS) was calculated from the slope of relative fluorescence (Monahan, German, & Kinsella, 1995) versus percentage of protein concentration (%, w/v) by linear regression analysis.

5.3.7 Dynamic rheological measurements

Threadfin bream surimi pastes (with and without ultrasound treatments) were subjected to a dynamic rheological measurement using a HAAKE MARS 40 rheometer (Thermo Scientific, Waltham, MA, USA). A parallel plate (40-mm diameter) geometry was applied. Samples were put on the lower plate and the higher plate was set with a gap of 1 mm. Then, sample table was covered with a thin film of cooking oil to avoid moisture evaporation during measurement. Samples were heated from 10 to 90 °C at a heating rate of 1 °C/min at a fixed frequency of 1 Hz and shear stress of 10 Pa, which were found in the linear viscoelastic range. Storage modulus (G'), loss modulus (G"), and tan (δ) were measured. Three replicates were applied for each sample.

5.3.8 Surimi gel preparation and gel measurements

Threadfin bream surimi pastes were stuffed into polyvinylidine chloride casing (3 cm-diameter) and both ends were sealed tightly prior to heating at 90 °C for 30 min. Subsequently, surimi gels were immediately chilled in ice water for 20 min and stored overnight in a refrigerator (4 °C). Surimi gels were equilibrated at room

temperature for 2 h prior to gel measurements. Surimi gels were cut into 30-mm long specimens for evaluation of textural properties using a Texture analyzer (TA. XT *plus* Stable Micro Systems, Surrey, UK) equipped with a 5-mm diameter spherical plunger probe. Breaking force (g) and distance (mm) were recorded using a test speed of 1 mm/s (J. Yongsawatdigul & Piyadhammaviboon, 2004). A colorimeter (ColorQuest XE, HunterLab, Reston, VA, USA) was used to measure color values (L^*, a^* and b^*) of gel samples. Whiteness of surimi gel was calculated using the equation L^*-3b^* (Park, 1994). Three replicates were applied, and five samples were measured for each replicate.

5.3.9 Trichloroacetic acid (TCA)-soluble oligopeptide

TCA-soluble oligopeptide contents of threadfin bream surimi pastes and gels were measured according to the method of Jirawat Yongsawatdigul and Piyadhammaviboon (2005). TCA-soluble oligopeptide content was determined using Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951) with tyrosine as a standard and expressed as μmol tyrosine/g sample.

5.3.10 SDS-PAGE

SDS-PAGE was conducted according to J. Yongsawatdigul et al. (2004), with slight modifications. The surimi pastes and gels were chopped into small pieces and 1 g of each sample was solubilized in 10 ml of hot 10% SDS solution. The samples were homogenized using a homogenizer (T25 digital ULTRA-TURRAX, IKA Works, Inc., Wilmington, NC, USA) prior to heating at 90 °C for 1 h. Afterwards, the homogenates were centrifuged at 10,000×g (Sorvall ST 16R Centrifuge, Thermo Scientific, Waltham, MA, USA) for 10 min at room temperature to collect the supernatants. The protein content of the supernatants was measured by the Bradford method (Bradford, 1976). Then, the protein samples were mixed with treatment buffer

(0.125 M Tris–HCl, 4% SDS, and 20% glycerol, pH 6.8) in a ratio of 1:1 (v/v) prior to heating at 95 °C for 5 min, and then stored at -18 °C until use. Gel electrophoresis was performed by a polyacrylamide gel comprised of 4% (w/v) stacking gel and 10% (w/v) separating gel. The amount of loaded protein was 20 µg. A constant voltage of 100 V was applied. Gels were stained in 0.125% (w/v) Coomassie Brilliant Blue R-250 and destained in a solution containing 25% (v/v) methanol and 10% (v/v) acetic acid. The gels were viewed and photographed using a UVITEC imaging system (Fire reader V4, Uvitec Ltd., Cambridge, UK).

5.3.11 FT-IR spectroscopy

Threadfin bream surimi pastes and gels were lyophilized. An attenuated total reflectance (ATR) FT-IR spectrometer (Bruker Tensor 27, Karlsruhe, Germany) was used. Samples were scanned for 64 times from 500 to 4000 cm^{-1} with a resolution of 4 cm^{-1} to obtain spectra. Thirty measurements from triplicate samples were collected. The spectra of each sample were averaged before normalization, and then adjusted by baseline correction prior to curve-fitting in region of 1600 to 1700 cm^{-1} by appropriate Gaussian and Lorentzian functions. OPUS software version 7.5 (Bruker Optics GmbH, Ettlingen, Germany) was used to analyze the spectra. The relative percentage of each secondary structure was calculated.

5.3.12 Statistical analysis

All measurements were conducted in three independent triplicates. The data were expressed as mean ± standard deviation (SD). SPSS software version 23.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. A significant level of $p < 0.05$ was used to compare differences among treatments by Duncan's multiple-range test (DMRT).

5.4 Results and discussion

5.4.1 Extractable protein content

The extractable protein content at 0.5% NaCl gradually increased with increasing ultrasonic exposure time up to 30 min ($p < 0.05$, Figure 5.1), while a longer time of 45 min did not result in a further increase of protein content ($p > 0.05$). Without ultrasound treatments, myofibrillar proteins showed much lower solubility at 0.5% NaCl compared with that at 2% NaCl. Cavitation generates high shear force and turbulence, which are expected to disrupt intermolecular forces between proteins, such as hydrogen bonds (Maity, Rasale, & Das, 2012). The physical forces also induced conformational changes of myofibrillar proteins (Zou et al., 2018). Both events could lead to greater protein solubilization, improving the extractable protein content. Furthermore, it was reported that ultrasound energy resulted in exposure of more charged groups of proteins (Jambrak, Mason, Lelas, Herceg, & Herceg, 2008), which improved protein-water interactions, leading to higher protein solubility. Besides, C.-H. Tang, Wang, Yang, and Li (2009) found that ultrasonic treatment induced formation of soluble aggregates, leading to an increase of soluble protein content. Liu et al. (2017) reported that protein solubility of silver carp myosin increased when ultrasonic exposure time increased from 3 to 12 min. Similar results were also found in beef myofibrillar proteins after subjected to ultrasonic time of 10, 20 and 30 min (Amiri et al., 2018).

The protein extractability at 2% NaCl increased when ultrasound treatment was applied for 30 min ($p \leq 0.05$, Figure 5.1), whereas a longer time (45 min) resulted in a slight decrease in extractable protein content. A higher NaCl content (2% NaCl) exposed more hydrophobic groups due to greater protein unfolding, which was likely

to induce formation of aggregates with larger molecular weight, resulting in a decrease in protein extractability. Moreover, Jingyu Wang et al. (2017) reported that a longer ultrasonic exposure time of 9-15 min at power of 240 W also resulted in increased protein solubility of chicken myofibrillar proteins. In addition, protein content of 0.5% NaCl sample increased up to 108% after ultrasonic time for 30 min, while only 12% increase was observed in the 2% NaCl sample (Figure 5.1). A higher NaCl content (2% NaCl) led to more extraction of myofibrillar proteins, and the protein extraction at 2% NaCl may reach the maximum after ultrasound treatments, resulting in a less increase in protein content. Our results illustrated that more myofibrillar proteins at 0.5% NaCl could be extracted after subjected to ultrasound treatments, and ultrasonic exposure time of 30 min showed the highest extraction efficiency. It was also demonstrated that 108% increase of protein extractability at 0.5% NaCl was improved by ultrasound, which was much greater than the 12% increase at 2% NaCl.

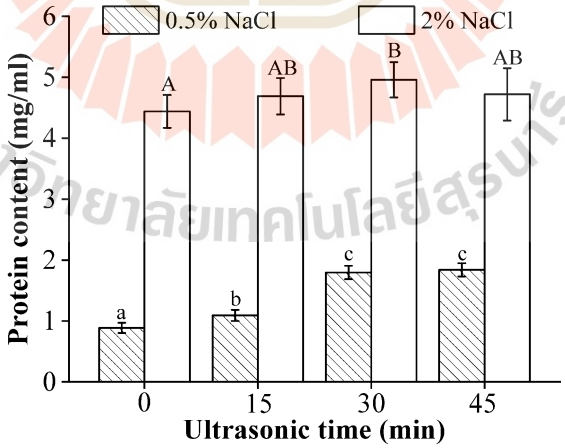


Figure 5.1 Effects of various ultrasonic time on extractable protein content of threadfin bream surimi pastes at 0.5 and 2% NaCl. Different superscripts in the same NaCl content indicate significant differences ($p < 0.05$).

5.4.2 Residual Ca^{2+}-ATPase activity

Residual Ca^{2+}-ATPase activity of threadfin bream surimi pastes at both NaCl contents decreased with increasing ultrasonic exposure time up to 30 min ($p < 0.05$), but no further decrease was observed at 45 min ($p > 0.05$, Figure 5.2). A decrease in residual Ca^{2+}-ATPase activity indicated that myosin globular head underwent conformational changes when ultrasound energy was applied. This was mainly due to the physical forces that were produced during sonication, such as high pressure and high shear force. Saleem and Ahmad (2016) reported that Ca^{2+}-ATPase activity of chicken actomyosin continuously decreased with increasing ultrasonic time ranging from 5 to 30 min. In addition, the Ca^{2+}-ATPase activity of silver carp myosin also obviously declined when ultrasonic time increased up to 12 min at the power of 100-250 W (Liu et al., 2017). It should be noted that the reduction of residual Ca^{2+}-ATPase activity at 2% NaCl was 18% after 45-min exposure, while 11% reduction was observed at 0.5% NaCl (Figure 5.2), implying that myofibrillar proteins at 2% NaCl underwent to conformational changes to a greater extent. The greater decrement of Ca^{2+}-ATPase activity was also observed after ultrasonic time for 30 min when chicken actomyosin was suspended in Tris-maleate buffer containing higher ionic strength (Saleem, Hasnain, & Ahmad, 2015).

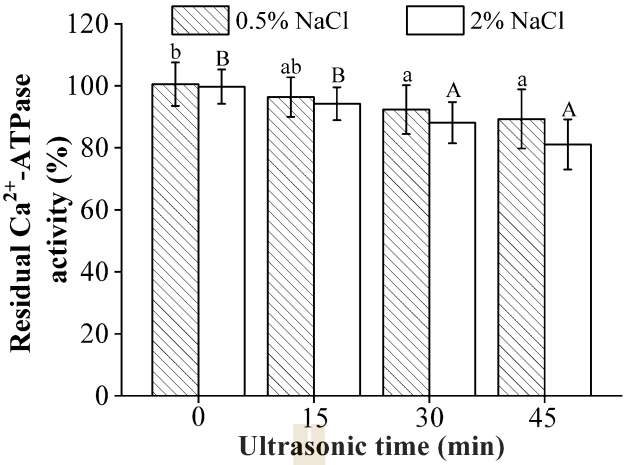


Figure 5.2 Effects of various ultrasonic time on residual Ca^{2+}-ATPase activity of threadfin bream surimi pastes at 0.5 and 2% NaCl. Different superscripts in the same NaCl content indicate significant differences ($p < 0.05$). Ca^{2+}-ATPase activity without ultrasound treatments at each NaCl content was regarded as 100%.

5.4.3 Surface hydrophobicity

Hydrophobic residues of threadfin bream surimi pastes at 0.5 and 2% NaCl were both exposed after subjected to ultrasound treatments, showing an increase in S_0-ANS values ($p<0.05$, Figure 5.3). S_0-ANS value increased when ultrasonic exposure time of 15 and 30 min were applied ($p < 0.05$), while, no obvious increase was observed as ultrasonic exposure time further increased ($p > 0.05$). The exposure of hydrophobic groups suggested that ultrasound treatments induced protein unfolding to a greater extent, in which ultrasonic exposure time of 30 min showed a prominent effect. An increase in S_0-ANS induced by ultrasound energy has also been reported in chicken myofibrillar proteins (Jingyu Wang et al., 2017; Zhang et al., 2017) and silver carp myosin (Liu et al., 2017). In addition, our results were consistent with previous studies

which reported that ultrasound treatments also induced exposure of hydrophobic groups in soy protein isolate (Hu et al., 2013), egg white protein (Arzeni et al., 2012) and black bean protein isolates (Jiang et al., 2014).

With and without ultrasound treatments, a higher S_0-ANS value was observed at 2% NaCl (Figure 5.3), indicating that reduced NaCl induced less protein unfolding. At low ionic strength, myosin filaments exist in bundle, exposing less hydrophobic groups (41%). Moreover, S_0-ANS of 2% NaCl sample showed 53% increase after subjected to ultrasonic time for 45 min, whereas only 41% increase was observed in 0.5% NaCl sample (Figure 5.3), indicating that ultrasound treatments induced less extent of protein unfolding at reduced NaCl (0.5% NaCl). Based on the changes in residual Ca^{2+}-ATPase activity (Figure 5.2) and S_0-ANS values (Figure 5.3), ultrasound resulted in greater protein unfolding and conformational changes of myofibrillar proteins.

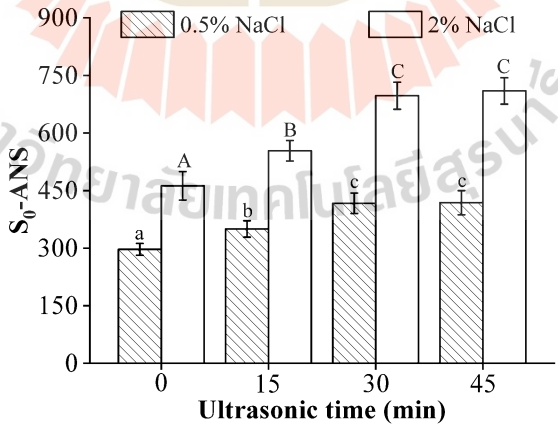


Figure 5.3 Effects of various ultrasonic time on surface hydrophobicity of threadfin bream surimi pastes at 0.5 and 2% NaCl. Different superscripts in the same NaCl content indicate significant differences ($p < 0.05$).

5.4.4 Dynamic rheological properties of surimi pastes

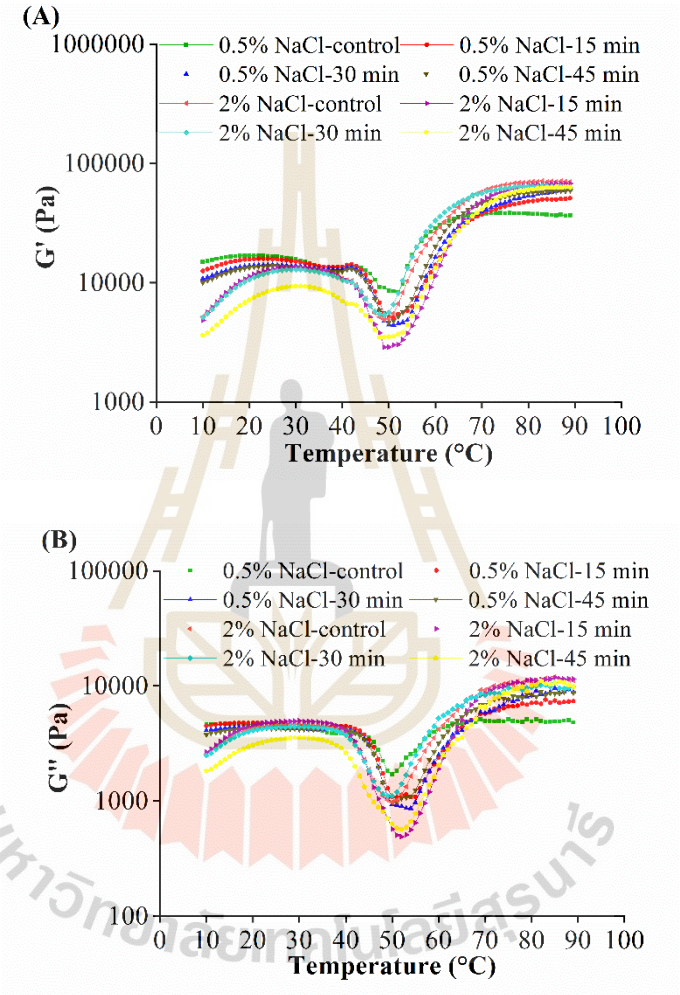
Without ultrasound treatments, 0.5% NaCl surimi paste showed both higher storage modulus (G') and loss modulus (G") values (Figure 5.4A and B). This was due to limited myofibrillar protein solubilization at low ionic strength. Deysi Cando et al. (2016) reported that G' and G" of Alaska pollock surimi at 0.3% NaCl were also higher than those at 3% NaCl. In addition, G' value of myosin gel in 0.3 M NaCl was significantly higher than that in 0.6 M NaCl (Jianyi Wang et al., 2019). More myofibrillar proteins were solubilized after subjected to ultrasound treatments (Figure 5.1), resulting in decreased values of G' and G" at both 0.5 and 2% NaCl. The first increase in G' started at 36-37 °C, which was due to the aggregation of partially unfolded protein (Jirawat Yongsawatdigul et al., 2005). Ultrasound treatments resulted in greater protein unfolding (Figure 5.3), leading to lower onset temperature and higher G'_p (the G' value that begins to decrease to the minimum) were noted in ultrasound-treated 0.5% NaCl samples compared with control. This semi gel-like structure was easily disrupted as the temperature increased to around 42 °C. The dissociation of actomyosin complex and denaturation of myosin also occurred (Jirawat Yongsawatdigul et al., 2005). Both led to increased fluidity, resulting in decreased G' around 42 °C. A higher temperature of onset of G'_{min} was shown in 0.5% NaCl samples after ultrasound treatments (Table 5.1), indicating stronger aggregation formed resulted from greater protein unfolding induced by ultrasound. The increase of G'_{max} (the G' value at 90 °C) was attributed to formation of permanent myosin gel networks, and its onset point indicated the temperature where surimi paste transformed from sol to gel (Poowakanjana et al., 2012; Jirawat Yongsawatdigul et al., 2005). A decrease in onset of G'_{max} (°C) was noted with increasing ultrasonic time in 0.5% NaCl samples (Table

5.1), suggesting that lower thermal energy was required for unfolding prior to aggregation due to greater protein unfolding after ultrasound treatments. Normally higher G', at the point where gelation is completed, determines a better gel network formation with higher elasticity (Poowakanjana et al., 2012). At 90 °C, G'_{max} value of ultrasound-treated 0.5% NaCl samples was comparable to that of the control, and the highest G'_{max} was observed when applied to ultrasonic time of 30 min (Figure 5.4A and Table 5.1), implying that gel network formation was improved by ultrasound, particularly at 30 min.

However, different patterns of G' were observed in 2% NaCl samples. The 2% NaCl samples reached G'_p at around 30 °C and declined to the minimum at about 49 °C before continuously reaching the maximum (Figure 5.4A). Protein at 2% NaCl was not able to form a semi gel-like structure as protein was fully unfolded and partially aggregated at high NaCl during paste preparation. Besides, the maximum G' decreased when 2% NaCl samples were subjected to ultrasound treatments, especially those treated at ultrasonic time of 30 and 45 min (Figure 5.4A and Table 5.1). Ultrasound might result in an excessive protein unfolding at 2% NaCl, particularly at ultrasonic time of 30 and 45 min (Figure 5.3), causing weaker gel network. Zhang et al. (2017) also reported that ultrasound treatments with power of 200-1,000 W resulted in greater denaturation of chicken myofibrillar proteins, leading to decreased values of both G' and G''. Based on G' and G'', it suggested that ultrasound treatments improved gel network formation at 0.5% NaCl, but it induced worse gel network at 2% NaCl.

In addition, the progress patterns of G'' were similar to G' (Figure 5.4B). Tan (δ) of all the samples increased in the range of 30-40 °C and then declined until the end of heating (Figure 5.4C). The profiles of tan (δ) in ultrasound-treated samples were higher

than the control regardless of NaCl content. Higher values of tan (δ) in ultrasound-treated 2% NaCl surimi gels suggested that protein networks were not well stabilized and organized, resulting in less elastic gels.



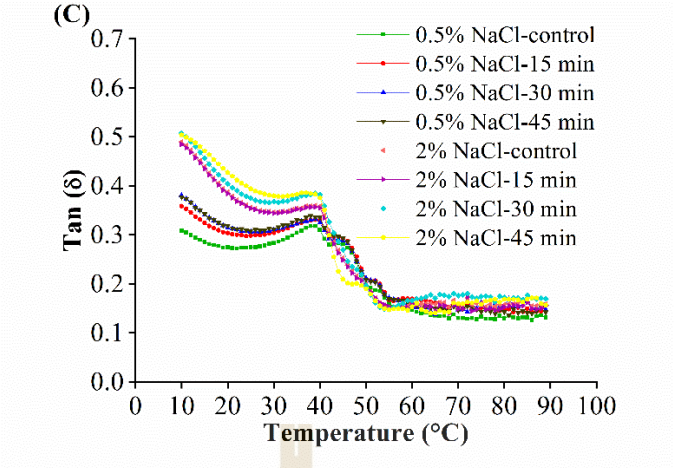


Figure 5.4 Effects of various ultrasonic time on rheological properties of threadfin bream surimi pastes at 0.5 and 2% NaCl.

Table 5.1 Typical parameters in G' profiles.

Salt content (%)	Ultrasonic time (min)	Onset of G'_{min} (°C)	Onset of G'_{max} (°C)	G'_{max} (kPa)
0.5	0	41.97 ± 0.006^{d}	52.85 ± 0.010^{g}	36.66 ± 2.74^{a}
	15	42.80 ± 0.004^{e}	51.85 ± 0.021^{f}	50.98 ± 6.34^{b}
	30	42.94 ± 0.012^{f}	51.04 ± 0.015^{e}	59.97 ± 6.36^{c}
	45	42.89 ± 0.007^{f}	50.92 ± 0.006^{e}	59.15 ± 6.11^{c}
2	0	30.79 ± 0.005^{a}	49.87 ± 0.010^{d}	70.14 ± 7.88^{ef}
	15	30.89 ± 0.006^{b}	49.47 ± 0.005^{c}	67.74 ± 6.76^{de}
	30	30.90 ± 0.011^{b}	48.85 ± 0.006^{b}	63.28 ± 6.12^{cd}
	45	31.89 ± 0.006^{c}	48.48 ± 0.005^{a}	63.05 ± 6.15^{cd}

Onset of G'_{min} represents the temperature at which G' value begins to decrease to the minimum; Onset of G'_{max} represents the temperature at which G' value begins to increase to the maximum; G'_{max} represents the G' value at 90 °C. All the values were expressed as mean ± SD of three replicates. Different superscripts in the same column indicate significant differences ($p < 0.05$).

5.4.5 Textural properties of surimi gels

The 0.5%-NaCl surimi gels showed lower breaking force and distance compared with 2%-NaCl samples regardless of ultrasound treatments (Figure 5.5). Without ultrasound treatments, a limited protein extractability was observed at 0.5% NaCl sample (Figure 5.1). Solubility of myofibrillar proteins is thought to be a premise element for formation of surimi gel. In addition, less extent of protein unfolding at 0.5% NaCl was shown (Figure 5.3). For both reasons, poor gelation was observed in the 0.5%-NaCl sample. At 2% NaCl, higher myofibrillar proteins were extracted (Figure 5.1) and greater extent of exposure of hydrophobic residues, requiring for gel networks (Figure 5.3). Fu et al. (2012) reported that the gel strength of silver carp surimi increased when the salt level was increased. Lower values of breaking force and distance were also observed in Alaska pollock surimi gel containing 0.3% NaCl than 3% NaCl (Deysi Cando et al., 2015, 2016).

Breaking force and distance of 0.5%-NaCl sample gradually increased at ultrasonic time of 15 to 30 min ($p < 0.05$, Figure 5.5), but no further increase was observed when a longer exposure time of 45 min was applied ($p > 0.05$). An increase in extractable protein (Figure 5.1) and the extent of protein unfolding (Figure 5.3) induced by ultrasound would lead to more soluble myofibrillar proteins and stronger protein-protein interactions, improving gel texture at 0.5% NaCl. Our results suggested that ultrasound would positively contribute to surimi gel at reduced NaCl content (0.5% NaCl), and ultrasonic time of 30 min was the optimal time. However, both breaking force and distance of 2%-NaCl sample decreased at all the studied ultrasonic time compared to the control, particularly at ultrasonic time of 30 and 45 min ($p \leq 0.05$, Figure 5.5), indicating that ultrasound treatments caused inferior surimi gels at 2%

NaCl especially the sample with a longer ultrasonic exposure time. The results of textural properties were also consistent with the G'_{max} (Figure 5.4A and Table 5.1), which further confirmed that reduced NaCl (0.5% NaCl) surimi gel was improved under ultrasound, but surimi gel at high NaCl (2% NaCl) showed inferior gel texture after ultrasound treatments.

In addition, our results (Figures 5.1 and 5.5) revealed that textural properties of surimi gels were not positively correlated with extractable protein content. As mentioned previously, severe conformational changes (Figure 5.2) and excessive protein unfolding (Figure 5.3) at 2% NaCl probably occurred after subjected to ultrasound treatments. This suggested that myofibrillar proteins at 2% NaCl might undergo severe denaturation under ultrasound, resulting in inferior gel texture. Chicken myofibrillar proteins in high ionic strength were also severely denatured after ultrasound treatment at 800 and 1,000 W for 15 min, causing inferior gel properties (Zhang et al., 2017). Hence, it is inferred that surimi gelation under ultrasound might be not solely depend on the extractable protein content, but also relate to conformational structure of soluble myofibrillar proteins, in which the latter seemed to have a more pronounced effect in 2%-NaCl sample.

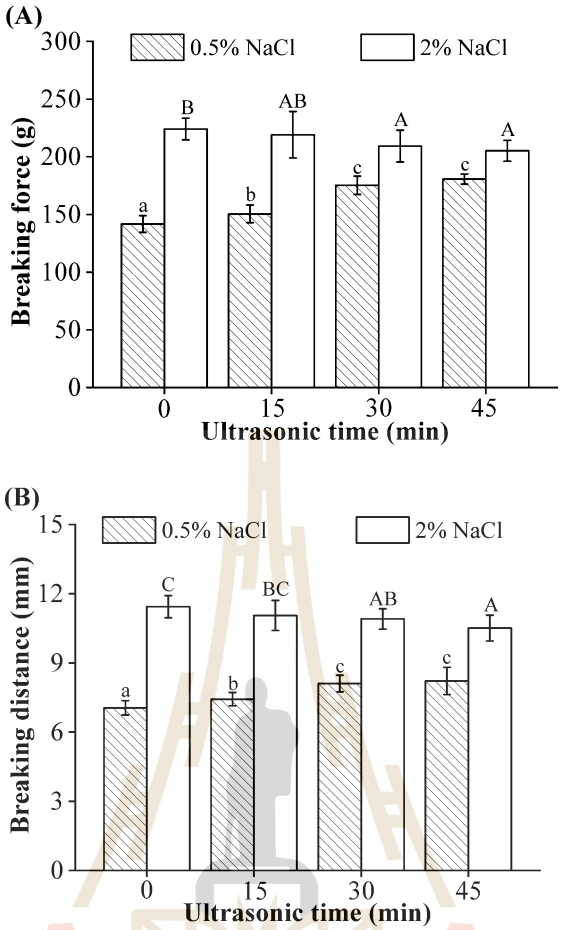


Figure 5.5 Effects of various ultrasonic time on textural properties of threadfin bream surimi gels at 0.5 and 2% NaCl. Different superscripts in the same NaCl content indicate significant differences ($p < 0.05$).

5.4.6 Whiteness of surimi gels

Without ultrasound treatments, 0.5%-NaCl surimi gel showed lower whiteness values than 2%-NaCl (Figure 5.6). Low NaCl resulted in disorderly gel networks due to extensive aggregation of less solubilized proteins, and thus, less light would pass through, lowering whiteness. Alaska pollock surimi gel at 0.3% NaCl also showed lower lightness value than 3% NaCl (Deysi Cando et al., 2015). Whiteness values of 0.5% NaCl surimi gel increased after subjected to ultrasound treatments, and

the highest increase appeared at ultrasonic time of 30 and 45 min ($p < 0.05$, Figure 5.6). An increase in salt soluble proteins (Figure 5.1) and greater extent of protein unfolding (Figure 5.3) promoted by ultrasound at 0.5% NaCl improved more uniform gel networks, making more light pass through. Nevertheless, an excessive unfolding of myofibrillar proteins was likely to occur in ultrasound-treated 2% NaCl surimi, particularly the sample with a longer exposure time (Figure 5.3), which might induce disorder gel network by a much faster rate of protein aggregation, causing lower whiteness of surimi gels ($p < 0.05$, Figure 5.6).

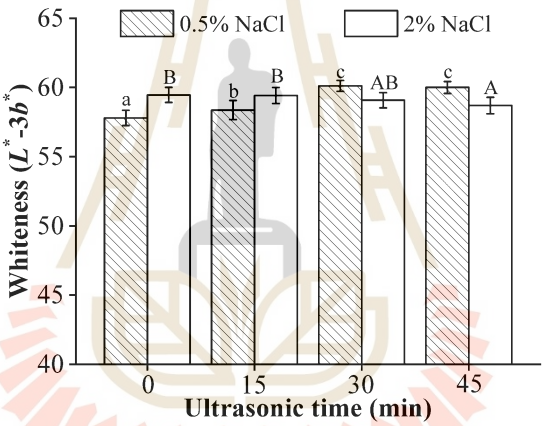


Figure 5.6 Effects of various ultrasonic time on whiteness of threadfin bream surimi gels at 0.5 and 2% NaCl. Different superscripts in the same NaCl content indicate significant differences ($p < 0.05$).

5.4.7 TCA-soluble oligopeptide content

At either 0.5 or 2% NaCl, there were no significant differences in TCA-soluble oligopeptide content among surimi paste samples with and without ultrasound treatments ($p > 0.05$, Figure 5.7), suggesting that proteolysis did not severely occur during sonication. Proteolysis was induced during heating, as, surimi gels at both NaCl

contents showed higher TCA-soluble oligopeptide contents compared with their respective paste samples ($p \leq 0.05$, Figure 5.7), implying that ultrasound induced proteolysis during thermally-induced gelation. This would be likely due to exposure of more catalytic sites for proteolysis, induced by ultrasound energy (Figure 5.3). Serine proteinases, which could induce proteolytic degradation of threadfin bream (*Nemipterus* spp.) (Kinoshita, Toyohara, & Shimizu, 1990), might also undergo conformational changes, promoting catalytic activity. Ma, Wang, and Guo (2018) reported that ultrasound treatments at amplitude of 20-40% increased the proteolysis of pepsin and trypsin on β-lactoglobulin. In addition, a larger extent of hydrolysis of milk protein concentrate was also observed after ultrasound pretreatment at 800 W for 1-8 min (Uluko et al., 2013).

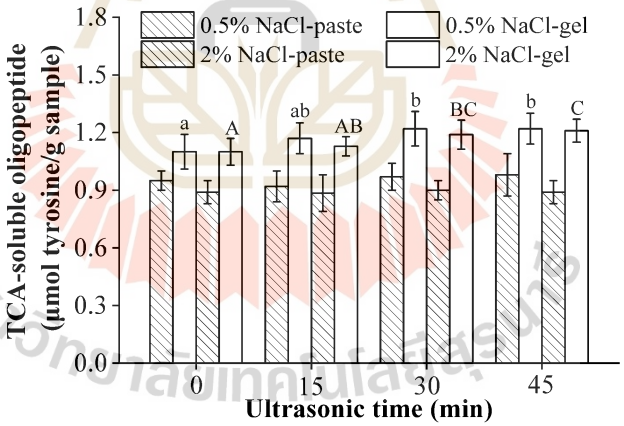


Figure 5.7 Effects of various ultrasonic time on TCA-soluble oligopeptide content of threadfin bream surimi pastes and gels containing 0.5 and 2% NaCl. Different superscripts in each group indicate significant differences ($p < 0.05$).

5.4.8 SDS-PAGE patterns

Compared to surimi paste, degradation of myosin heavy chain (MHC) and actin was slightly observed in 0.5 and 2% NaCl surimi gel (Figure 5.8). No obvious changes on MHC were observed in surimi pastes treated by ultrasound at both salt contents (Figure 5.8), which were consistent with the results of TCA-soluble oligopeptide content (Figure 5.7). It was also reported that chicken myofibrillar proteins showed no protein degradation after ultrasound treatments at 120 W for 30 min (Saleem et al., 2016) and 240 W for 15 min (Jingyu Wang et al., 2017), respectively. Similarly, no evident changes in protein patterns were shown in 0.5 and 2% NaCl surimi gels after ultrasound treatments for various ultrasonic time (Figure 5.8). In addition, only minor changes of TCA-soluble oligopeptide contents were shown when compared to control (Figure 5.7) since threadfin bream surimi shows much lower proteolytic activity compared with other high proteolytic surimi (e.g., lizardfish) (Tadpitchayangkoon, Park, & Yongsawatdigul, 2012). This could be the reason explaining no noticeable changes in SDS-PAGE patterns (Figure 5.8). Therefore, results of TCA-soluble oligopeptide content (Figure 5.7) and SDS-PAGE (Figure 5.8) suggested that ultrasound did not increase proteolysis of surimi paste, and only a slight increase in proteolysis of surimi gels was induced.

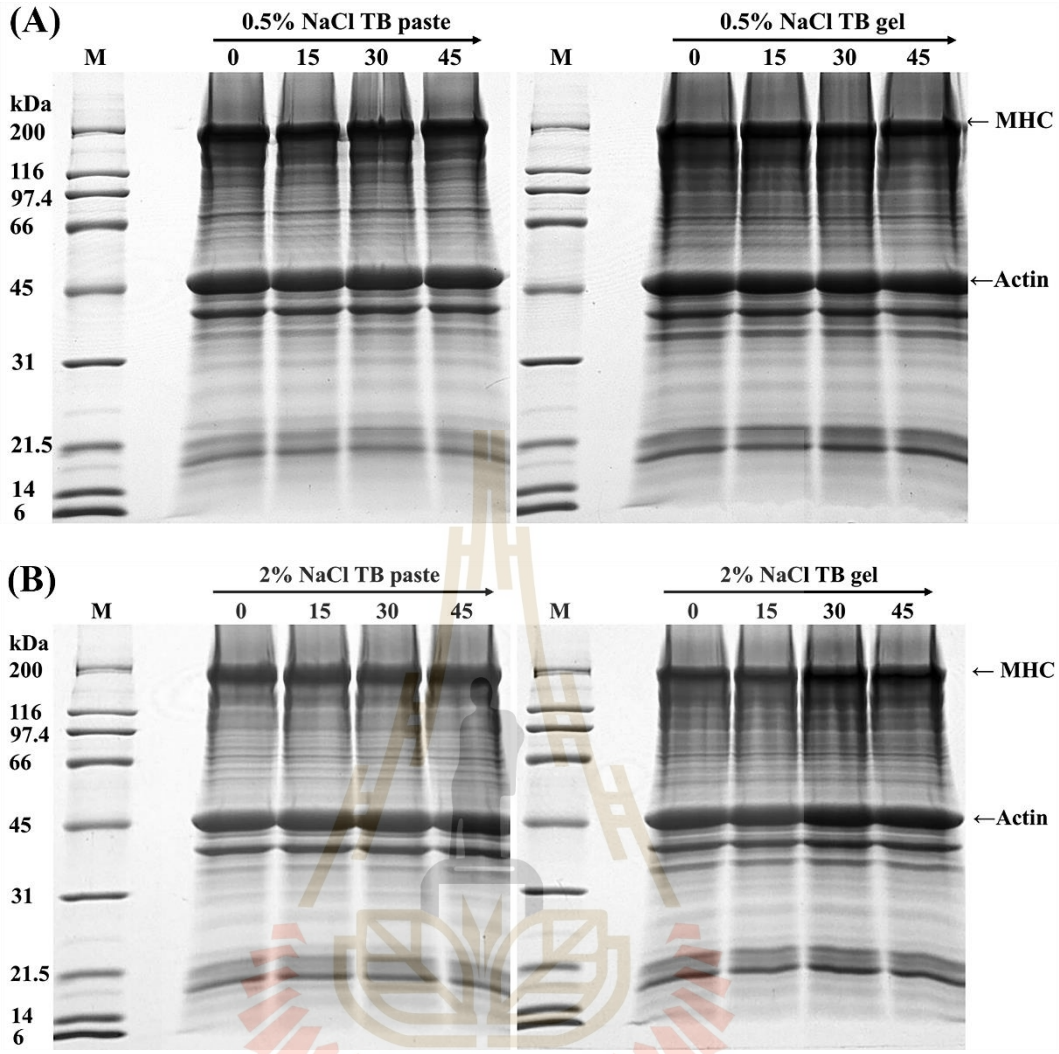


Figure 5.8 SDS-PAGE patterns of threadfin bream surimi pastes and gels containing 0.5 (A) and 2% (B) NaCl subjected to various ultrasonic time. M indicates protein markers. Numbers (0, 15, 30 and 45) refer to ultrasonic time (min).

5.4.9 Changes of secondary structures

With or without ultrasound treatments, surimi gels at both 0.5 and 2% NaCl showed lower α-helix content but higher contents of β-sheet, β-turn and random coil compared to their paste counterparts (Figure 5.9), indicating that heating led to greater extent of protein unfolding during gelation. Both surimi pastes and gels at 2%

NaCl showed lower α-helix but higher β-sheet, β-turn and random coil contents than those at 0.5% NaCl (Figure 5.9), which further verified that 2% NaCl resulted in a greater protein unfolding. A lower β-sheet content of Alaska pollock surimi gel was also reported at 0.3% NaCl than 3% NaCl (Deysi Cando et al., 2015). After subjected to ultrasound treatments for up to 30 min, a continuous decline of α-helix content was shown in surimi pastes and gels at both studied NaCl contents, accompanied by an increase in β-sheet, β-turn and random coil contents ($p < 0.05$), whereas, a longer exposure time of 45 min did not result in a further change ($p > 0.05$, Figure 5.9). The results suggested that myofibrillar proteins underwent unfolding to a greater extent after ultrasound treatments, and ultrasonic time of 30 min showed the most pronounced effect.

Moreover, the greater changes of protein secondary structures were evident in 2% NaCl samples than 0.5% NaCl samples. About 14% reduction in α-helix was shown in 2% NaCl surimi paste after treated at ultrasonic time for 45 min, whereas, only 11% decrease was observed in 0.5% NaCl surimi paste, which further illustrated that a greater protein unfolding occurred in high NaCl (2% NaCl) sample, corresponding to the changes of S_0-ANS value (Figure 5.3). Similarly, a greater reduction in α-helix content (16%) was also observed in 2% NaCl surimi gel than 0.5% NaCl surimi gel (11%) after ultrasound treatments. Kang et al. (2016) reported that α-helix content of beef protein declined with increasing ultrasonic time from 30 to 120 min. In addition, α-helix content of black bean protein isolate also decreased after subjected to ultrasonic time for 12 and 24 min (Jiang et al., 2014). However, a much higher increment in random coil content were also noted in ultrasound-treated 2% NaCl samples, displaying 15 and 18% increase in paste and gel, respectively. In contrast, the 0.5% NaCl surimi

paste and gel after ultrasound treatments showed approximately 2 and 3% increase in random coil content, respectively. The findings could be part of the reasons explaining the inferior textural properties of 2% NaCl surimi gels upon ultrasound treatments (Figure 5.5).

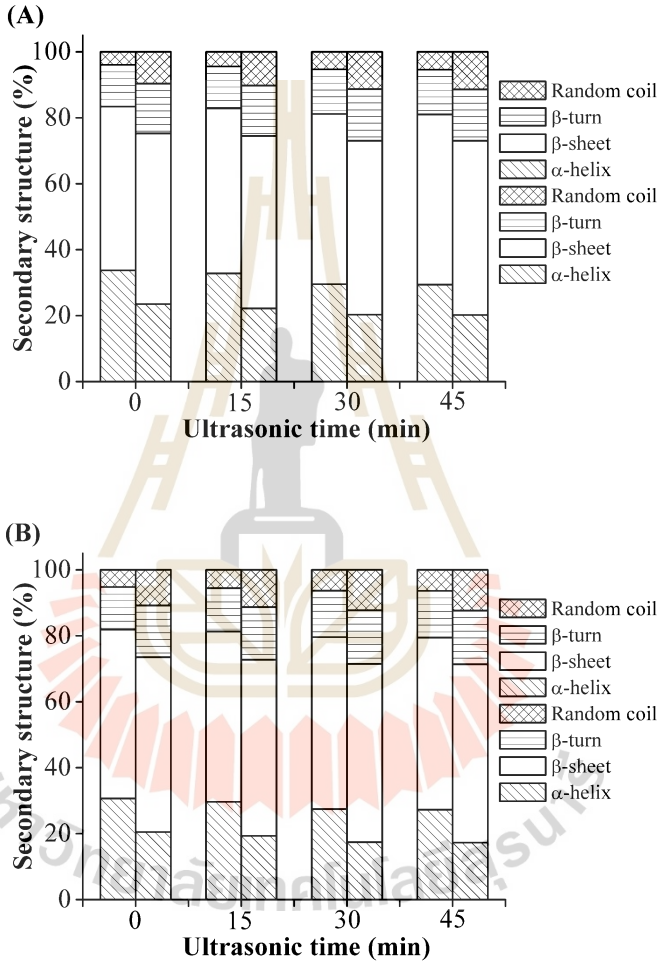


Figure 5.9 Effects of various ultrasonic time on protein secondary structures of threadfin bream surimi pastes (A) and gels (B) containing 0.5 and 2% NaCl. White and gray bars refer to samples at 0.5 and 2% NaCl, respectively.

5.5 Conclusions

HIU showed different effects on threadfin bream surimi gels containing reduced and high NaCl contents. HIU treatments with various ultrasonic time improved reduced NaCl (0.5% NaCl) threadfin bream surimi gel with higher elasticity, and ultrasonic time of 30 min was the optimal. This was due to HIU increased extractable protein content and induced a greater protein unfolding at 0.5% NaCl. However, HIU showed detrimental effect on threadfin bream surimi gel at 2% NaCl. HIU induced greater protein unfolding and conformational changes at 2% NaCl than 0.5% NaCl. HIU did not increase proteolysis of surimi paste, and only slight increase in proteolysis of surimi gels was induced. This study suggested that low/reduced-salt threadfin bream surimi gel could possibly be produced using HIU technology, and ultrasonic exposure time of 30 min optimally induced surimi gelation at 0.5% NaCl.

5.6 References

Amiri, A., Sharifian, P., & Soltanizadeh, N. (2018). Application of ultrasound treatment for improving the physicochemical, functional and rheological properties of myofibrillar proteins. **International Journal of Biological Macromolecules.** 111, 139-147.

Arzeni, C., Martínez, K., Zema, P., Arias, A., Pérez, O. E., & Pilosof, A. M. R. (2012). Comparative study of high intensity ultrasound effects on food proteins functionality. **Journal of Food Engineering.** 108 (3), 463-472.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry.** 72 (1), 248-254.

Cando, D., Borderías, A. J., & H.M.Moreno. (2016). Combined effect of aminoacids and microbial transglutaminase on gelation of low salt surimi content under high pressure processing. **Innovative Food Science & Emerging Technologies.** 36, 10-17.

Cando, D., Herranz, B., Borderías, A. J., & Moreno, H. M. (2015). Effect of high pressure on reduced sodium chloride surimi gels. **Food Hydrocolloids.** 51 (Supplement C), 176-187.

Cando, D., Herranz, B., Borderías, A. J., & Moreno, H. M. (2016). Different additives to enhance the gelation of surimi gel with reduced sodium content. **Food Chemistry.** 196, 791-799.

Endoo, N., & Yongsawatdigul, J. (2014). Comparative study on chemical and gel-forming properties of surimi from freshwater and marine fish during frozen storage. **Food and Applied Bioscience Journal.** 2 (3), 192-202.

Fu, X., Hayat, K., Li, Z., Lin, Q., Xu, S., & Wang, S. (2012). Effect of microwave heating on the low-salt gel from silver carp (*Hypophthalmichthys molitrix*) surimi. **Food Hydrocolloids.** 27 (2), 301-308.

Hu, H., Wu, J., Li-Chan, E. C. Y., Zhu, L., Zhang, F., Xu, X., Fan, G., Wang, L., Huang, X., & Pan, S. (2013). Effects of ultrasound on structural and physical properties of soy protein isolate (SPI) dispersions. **Food Hydrocolloids.** 30 (2), 647-655.

Jambrak, A. R., Lelas, V., Mason, T. J., Krešić, G., & Badanjak, M. (2009). Physical properties of ultrasound treated soy proteins. **Journal of Food Engineering.** 93 (4), 386-393.

Jambrak, A. R., Mason, T. J., Lelas, V., Herceg, Z., & Herceg, I. L. (2008). Effect of ultrasound treatment on solubility and foaming properties of whey protein suspensions. **Journal of Food Engineering.** 86 (2), 281-287.

Jiang, L., Wang, J., Li, Y., Wang, Z., Liang, J., Wang, R., Chen, Y., Ma, W., Qi, B., & Zhang, M. (2014). Effects of ultrasound on the structure and physical properties of black bean protein isolates. **Food Research International.** 62, 595-601.

Kang, D.-c., Zou, Y.-h., Cheng, Y.-p., Xing, L.-j., Zhou, G.-h., & Zhang, W.-g. (2016). Effects of power ultrasound on oxidation and structure of beef proteins during curing processing. **Ultrasonics Sonochemistry.** 33, 47-53.

Kinoshita, M., Toyohara, H., & Shimizu, Y. (1990). Purification and properties of a novel latent proteinase showing myosin heavy chain-degrading activity from threadfin-bream muscle. **The Journal of Biochemistry.** 107 (4), 587-591.

Kloss, L., Meyer, J. D., Graeve, L., & Vetter, W. (2015). Sodium intake and its reduction by food reformulation in the European Union — A review. **NFS Journal.** 1 (Supplement C), 9-19.

Lanier, T. C., Carvajal, P., & Yongsawatdigul, J. (2005). Surimi Gelation Chemistry. In J. W. Park (Ed.), Surimi and Surimi Seafood (2nd Edition) (pp. 435-489). Boca Raton, FL: CRC Press.

Liu, R., Liu, Q., Xiong, S., Fu, Y., & Chen, L. (2017). Effects of high intensity unltrasound on structural and physicochemical properties of myosin from silver carp. **Ultrasonics Sonochemistry.** 37 (Supplement C), 150-157.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. **Journal of Biological Chemistry.** 193 (1), 265-275.

Ma, S., Wang, C., & Guo, M. (2018). Changes in structure and antioxidant activity of β-lactoglobulin by ultrasound and enzymatic treatment. **Ultrasonics Sonochemistry.** 43, 227-236.

Maity, I., Rasale, D. B., & Das, A. K. (2012). Sonication induced peptide-appended bolaamphiphile hydrogels for in situ generation and catalytic activity of Pt nanoparticles. **Soft Matter.** 8 (19), 5301-5308.

Monahan, F. J., German, J. B., & Kinsella, J. E. (1995). Effect of pH and temperature on protein unfolding and thiol/disulfide interchange reactions during heat-induced gelation of whey proteins. **Journal of Agricultural and Food Chemistry.** 43 (1), 46-52.

Park, J. W. (1994). Functional protein additives in surimi gels. **Journal of Food Science.** 59 (3), 525-527.

Poowakanjana, S., Mayer, S. G., & Park, J. W. (2012). Optimum chopping conditions for Alaska pollock, Pacific whiting, and threadfin bream surimi paste and gel based on rheological and Raman spectroscopic analysis. **Journal of Food Science.** 77 (4), E88-E97.

Saleem, R., & Ahmad, R. (2016). Effect of low frequency ultrasonication on biochemical and structural properties of chicken actomyosin. **Food Chemistry.** 205 (Supplement C), 43-51.

Saleem, R., Hasnain, A., & Ahmad, R. (2015). Solubilisation of muscle proteins from chicken breast muscle by ultrasonic radiations in physiological ionic medium. **Cogent Food & Agriculture.** 1 (1), 1046716.

Soria, A. C., & Villamiel, M. (2010). Effect of ultrasound on the technological properties and bioactivity of food: a review. **Trends in Food Science & Technology.** 21 (7), 323-331.

Tadpitchayangkoon, P., Park, J. W., & Yongsawatdigul, J. (2012). Gelation characteristics of tropical surimi under water bath and ohmic heating. **LWT - Food Science and Technology.** 46 (1), 97-103.

Tahergorabi, R., & Jaczynski, J. (2012). Physicochemical changes in surimi with salt substitute. **Food Chemistry.** 132 (3), 1281-1286.

Tang, C.-H., Wang, X.-Y., Yang, X.-Q., & Li, L. (2009). Formation of soluble aggregates from insoluble commercial soy protein isolate by means of ultrasonic treatment and their gelling properties. **Journal of Food Engineering.** 92 (4), 432-437.

Tang, L., & Yongsawatdigul, J. (2020). Physicochemical properties of tilapia (*Oreochromis niloticus*) actomyosin subjected to high intensity ultrasound in low NaCl concentrations. **Ultrasonics Sonochemistry.** 63, 104922.

Uluko, H., Zhang, S., Liu, L., Chen, J., Sun, Y., Su, Y., Li, H., Cui, W., & Lv, J. (2013). Effects of microwave and ultrasound pretreatments on enzymolysis of milk protein concentrate with different enzymes. **International Journal of Food Science & Technology.** 48 (11), 2250-2257.

Uresti, R. o. M., Téllez-Luis, S. J., Ramírez, J. A., & Vázquez, M. (2004). Use of dairy proteins and microbial transglutaminase to obtain low-salt fish products from filleting waste from silver carp (*Hypophthalmichthys molitrix*). **Food Chemistry.** 86 (2), 257-262.

Wang, G., Liu, M., Cao, L., Yongsawatdigul, J., Xiong, S., & Liu, R. (2018). Effects of different NaCl concentrations on self-assembly of silver carp myosin. **Food Bioscience.** 24, 1-8.

Wang, J., Li, Z., Zheng, B., Zhang, Y., & Guo, Z. (2019). Effect of ultra-high pressure on the structure and gelling properties of low salt golden threadfin bream (*Nemipterus virgatus*) myosin. **LWT - Food Science and Technology.** 100, 381-390.

Wang, J., Yang, Y., Tang, X., Ni, W., & Zhou, L. (2017). Effects of pulsed ultrasound on rheological and structural properties of chicken myofibrillar protein. **Ultrasonics Sonochemistry.** 38 (Supplement C), 225-233.

Yongsawatdigul, J., & Park, J. W. (2003). Thermal denaturation and aggregation of threadfin bream actomyosin. **Food Chemistry.** 83 (3), 409-416.

Yongsawatdigul, J., & Piyadhammaviboon, P. (2004). Inhibition of autolytic activity of lizardfish surimi by proteinase inhibitors. **Food Chemistry.** 87 (3), 447-455.

Yongsawatdigul, J., & Piyadhammaviboon, P. (2005). Effect of microbial transglutaminase on autolysis and gelation of lizardfish surimi. **Journal of the Science of Food and Agriculture.** 85 (9), 1453-1460.

Zhang, Z., Regenstein, J. M., Zhou, P., & Yang, Y. (2017). Effects of high intensity ultrasound modification on physicochemical property and water in myofibrillar protein gel. **Ultrasonics Sonochemistry.** 34, 960-967.

Zou, Y., Xu, P., Wu, H., Zhang, M., Sun, Z., Sun, C., Wang, D., Cao, J., & Xu, W. (2018). Effects of different ultrasound power on physicochemical property and functional performance of chicken actomyosin. **International Journal of Biological Macromolecules.** 113, 640-647.

CHAPTER VI

SUMMARY

Physicochemical changes of tilapia actomyosin exposed to HIU under low salt concentrations were investigated. The protein solubility of actomyosin in low NaCl concentration (0.2 M) was improved after HIU treatments. HIU showed more pronounced effect on oxidation of SH groups than exposure of buried SH groups in 0.6 M NaCl. HIU also induced larger degree of protein unfolding and conformational changes in 0.6 M NaCl than that in 0.2 M NaCl. Smaller aggregates of HIU-treated actomyosin were observed at 40 and 60 °C, which was probably due to HIU reducing the particle size and improving the size uniformity of actomyosin. HIU caused the MHCs to degrade in both NaCl concentrations, but disruption of actomyosin induced by HIU appeared to be greater in 0.6 M NaCl. The results demonstrated that actomyosin can be efficiently extracted in 0.2 M NaCl by HIU, suggesting the potential of HIU technology in production of low/reduced-salt actomyosin gel.

Gelation behavior of threadfin bream surimi as a function of NaCl contents and HIU intensities were elucidated. Gel properties of surimi at 0.5% NaCl were improved after HIU treatments due to enhanced protein extractability and a higher extent of protein unfolding induced by HIU. An excessive unfolding occurred in HIU-treated surimi at 1 and 2% NaCl resulting in inferior surimi gels. HIU-induced protein unfolding and conformational changes were more pronounced at higher NaCl contents. HIU showed only a minimal effect on proteolysis of the threadfin bream surimi gels at the 3 studied NaCl contents. HIU technology could improve threadfin bream surimi gel

at reduced NaCl content (0.5% NaCl); however, HIU is detrimental to the surimi gels containing 1 and 2% NaCl, and 2% NaCl is the typical NaCl content in surimi-based seafood.

HIU-assisted gelation of threadfin bream surimi subjected to reduced NaCl content under various ultrasonic exposure time were studied. HIU showed different effects on threadfin bream surimi gels containing reduced and high NaCl contents. HIU treatments with various ultrasonic time improved reduced NaCl (0.5% NaCl) threadfin bream surimi gel with higher elasticity, and ultrasonic time of 30 min was the optimal. This was due to HIU increased extractable protein content and induced a greater protein unfolding at 0.5% NaCl. However, HIU showed detrimental effect on threadfin bream surimi gel at 2% NaCl. HIU induced greater protein unfolding and conformational changes at 2% NaCl than 0.5% NaCl. HIU did not increase proteolysis of surimi paste, and only slight increase in proteolysis of surimi gels was induced. This study suggested that low/reduced-salt threadfin bream surimi gel could possibly be produced using HIU technology, and ultrasonic exposure time of 30 min optimally induced surimi gelation at 0.5% NaCl.

Therefore, the outcome of this research would provide an effective way to extract myofibrillar proteins in low NaCl concentrations using HIU technology. Furthermore, understanding of gelation behavior of surimi under HIU would lay a theoretical foundation for utilization of HIU technology in production of surimi-based products at low/reduced salt levels. In addition, the outcome of the study would be helpful in manufacturing healthier surimi seafood with reduced sodium content without the use of chemicals and/or enzymes.

BIOGRAPHY

Ling Tang was born in January 20, 1987 in Xingyi, Guizhou Province, China. She studied for her high school diploma at Xingyi No. 1 School (1999-2005) and Panjiang School (2005-2006) in Xingyi, Guizhou Province, China. She received the degree of Bachelor of Science (Food Quality and Safety) in 2010 and received the degree of Master of Agriculture (Food Science) in 2013 from Guizhou University, China. After graduation, she has taken a lecturer position in School of Food Quality and Safety, College of Public Health, Zunyi Medical University, China.

In 2015, she pursued for the degree of Doctor of Philosophy in Food Technology supervised by Assoc. Prof. Dr. Jirawat Yongsawatdigul at Suranaree University of Technology and received One Research One Grant (OROG) scholarship from Suranaree University of Technology to financially support her research. During her Ph.D. study, she obtained opportunities to present her research work including at The 6th SUT International Colloquium on Agricultural Technology 2018 in Thailand, The 21st Food Innovation Asia Conference 2019 in Thailand with awarded “First Place in Oral Presentation”, and The 22nd Food Innovation Asia Conference 2020 in Thailand. She has also published her research work entitled “Physicochemical properties of tilapia (*Oreochromis niloticus*) actomyosin subjected to high intensity ultrasound in low NaCl concentrations” in Ultrasonics Sonochemistry in 2020.