EFFECT OF ASCORBIC ACID AND HYDROGEN PEROXIDE ON GEL-FORMING ABILITY OF TROPICAL SURIMI

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ผลของกรดแอสคอร์บิกและไฮโดรเจนเปอร์ออกไซด์ต่อความสามารถในการเกิดเจลของซูริมิจากปลาเขตร้อน

นายดานู ปาว

มหาวิทยาลัยเทคโนโลยีสุรนารี

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

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Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for a Master's Degree.

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ดานู ปาว : ผลของกรดแอสคอร์บิกและไฮโดรเจนเพอร์ออกไซด์ต่อความสามารถในการเกิดเจลซูริมิจากปลาเขตร้อน (EFFECT OF ASCORBIC ACID AND HYDROGEN PEROXIDE ON GEL-FORMING ABILITY OF TROPICAL SURIMI) อาจารย์ที่ปรึกษา : รองศาสตราจารย์ ดร. จิรวัฒน์ ยงสวัสดิกุล, 146 หน้า.

งานวิจัยนี้มีจุดประสงค์เพื่อศึกษาผลของกรดแอสคอร์บิกและไฮโดรเจนเพอร์ออกไซด์ต่อความสามารถในการเกิดเจลซูริมิจากปลาทรายแดง (*Nemipterus* spp.) ปลาปากคม (*Saurida* spp.) และปลาซาร์ดีน (*Sardinella* spp.) โดยตรวจสอบคุณภาพเจลซูริมิทางเนื้อสัมผัสด้วยวิธีวัดแรงกดทะลุ (penetration test) และวัดค่าสี (L^*, a^* and b^*) นอกจากนี้วิเคราะห์ปริมาณหมู่ซัลฟ์ไฮดริลทั้งหมด (Total sulfhydryl group) และวิเคราะห์รูปแบบโปรตีนโดยวิธีเจลอิเลคโตรโฟรีซีส (SDS-polyacrylamide gel electrophoresis) และติดตามการเปลี่ยนแปลงโครงสร้างโปรตีนด้วยเทคนิคฟูเรียร์ทรานสฟอร์มอินฟราเรด สเปกโทรสโกปี (Fourier Transform Infrared spectroscopy) และรามานสเปกโทรสโกปี (Raman spectroscopy) ค่าความเป็นกรดด่าง (pH) ของซูริมิจากปลาสามชนิดลดลง เมื่อปริมาณกรดแอสคอร์บิกเพิ่มขึ้นโดยตัวอย่างที่กรดแอสคอร์บิกในระดับ 0.2% มีค่า pH 6.7-6.5 การเติมกรดแอสคอร์บิกในระดับ 0.15% ร่วมกับไฮโดรเจนเพอร์ออกไซด์ในระดับ 0.1% มีผลให้ค่าแรงที่ใช้ในการกด และระยะทางการเปลี่ยนรูปร่างของซูริมิปลาปากคม เพิ่มสูงถึง 300% และ 55% ตามลำดับ เมื่อให้ความร้อนที่อุณหภูมิ 90 °ซ การเติมกรดแอสคอร์บิก 0.2% และไฮโดรเจนเพอร์ออกไซด์ 0.15% ส่งผลให้ค่าความสามารถในการเกิดเจลของซูริมิจากปลาทรายแดงเพิ่มขึ้น 150% และ 90% ตามลำดับ อย่างไรก็ตาม การเติมกรดแอสคอร์บิกและไฮโดรเจนเพอร์ออกไซด์ไม่มีผลปรับปรุงการเกิดเจลของซูริมิปลาซาร์ดีนเมื่อได้รับความร้อนที่ 90 °ซ การเซ็ทตัว (setting) ที่อุณหภูมิ 40 °ซ ไม่มีผลในการปรับปรุงการเกิดเจลของซูริมิปลาปากคมและปลาทรายแดง แต่มีผลเพิ่มคุณภาพเจลของซูริมิปลาซาร์ดีน ความขาวของเจลซูริมิจากปลาทั้งสามชนิดเพิ่มขึ้นเล็กน้อยเมื่อเติมไฮโดรเจนเพอร์ออกไซด์เพียงอย่างเดียว ความขาวของเจลซูริมิปลาปากคมและปลาทรายแดงลดลงเมื่อเติมแอสคอร์บิกร่วมกับไฮโดรเจนเพอร์ออกไซด์ เนื่องจากเกิดการออกซิเดชั่นของกรดแอสคอร์บิก การปรับปรุงคุณภาพเจลสัมพันธ์กับปริมาณพันธะไดซัลไซด์ที่เพิ่มขึ้น ไม่พบไมโอซินสายหนัก (Myosin heavy chain, MHC) ในเจลจากปลาทั้งสามชนิดที่ให้ความร้อนโดยตรงเมื่อเพิ่มปริมาณกรดแอสคอร์บิกและไฮโดรเจนเพอร์ออกไซด์ปริมาณโอลิโกเพปไทด์ที่ละลายได้ในสารละลายกรดไตรคลอโรอะซิติก (TCA-soluble oligopeptide) ของซูริมิจากปลาทั้งสามชนิดเพิ่มขึ้นเมื่อเติมกรดแอสคอร์บิกเพิ่มขึ้น หมู่ซัลฟ์ไฮดริลทั้งหมด ของเจลซูริมิจากปลาทั้งสามชนิดลดลงอย่างมากเมื่อเติมกรดแอสคอร์บิก

ร่วมกับไฮโดรเจนเพอร์ออกไซด์ ผลวิเคราะห์จากเทคนิคฟูเรียร์ทรานส์ฟอร์มอินฟราเรดสเปกโตรสโกปี พบว่าปริมาณแอลฟา-ฮีลิกซ์ (α-helix) ลดลงและเบต้าชีท (β-sheet) ในเจลซูริมิสามชนิดเพิ่มขึ้นเมื่อเติมกรดแอสคอร์บิกในระดับ 0.2% และไฮโดรเจนเพอร์ออกไซด์ในระดับ 0.15% ผลรามานสเปกโตรสโกปี บ่งชี้ว่าแรงกระทำไฮโดรโฟบิก (hydrophobic interactions) ของซูริมิสามชนิดเพิ่มขึ้น ขณะที่กลุ่มไฮโดรคาร์บอนสายตรง (aliphatic residues) ลดลงเมื่อเติมกรดแอสคอร์บิก 0.2% และ/หรือไฮโดรเจนเพอร์ออกไซด์ 0.15% ซึ่งพิจารณาจากเลขคลื่น 2930 และ 1450 ซม$^{-1}$ ปริมาณกลุ่มไทโรซีน และฟีนิลอะลานิน ในซูริมิจากปลาทั้งสามชนิดลดลงเมื่อเติมกรดแอสคอร์บิกและ/หรือไฮโดรเจนเพอร์ออกไซด์ กรดแอสคอร์บิกและ/หรือไฮโดรเจนเพอร์ออกไซด์ส่งผลต่อการเพิ่มพันธะไดซัลไฟด์ของซูริมิปลาปากคมและปลาทรายแดงซึ่งบ่งชี้ได้จากการเปลี่ยนแปลงของการสั่นแบบยืดของพันธะไดซัลไฟด์ ในขณะที่ไม่ผลต่อการเปลี่ยนแปลงในเจลซูริมิปลาซาร์ดีน ผลการวิเคราะห์องค์ประกอบหลัก (Principle component analysis, PCA) แสดงให้เห็นว่าคุณภาพเนื้อสัมผัสของเจลซูริมิปลาปากคมและปลาทรายแดงมีความสัมพันธ์เชิงบวกกับปริมาณเบต้าชีทและสัมพันธ์เชิงลบกับปริมาณแอลฟา-ฮีลิกซ์และปริมาณหมู่ซัลไฮดริลทั้งหมด ผลการวิจัยบ่งชี้ว่ากรดแอสคอร์บิกและ/หรือไฮโดรเจนเพอร์ออกไซด์ส่งเสริมคุณสมบัติการเกิดเจลของซูริมิปลาปากคมและปลาทรายแดง ซึ่งความเหมาะสมของปริมาณที่ใช้แปรเปลี่ยนตามชนิดของปลา การเซ็ตตัวมีผลต่อการปรับปรุงคุณภาพเจลของปลาซาร์ดีนมากกว่าการเติมการเติมสารทั้งสองชนิดที่ศึกษา

มหาวิทยาลัยเทคโนโลยีสุรนารี

สาขาวิชาเทคโนโลยีอาหาร ลายมือชื่อนักศึกษา_____

ปีการศึกษา 2562 ลายมือชื่ออาจารย์ที่ปรึกษา_____

DANOU PAO : EFFECT OF ASCORBIC ACID AND HYDROGEN PEROXIDE ON GEL-FORMING ABILITY OF TROPICAL SURIMI. THESIS ADVISOR : ASSOC. PROF. JIRAWAT YONGSAWATDIGUL, Ph.D., 146 PP.

ASCORBIC ACID/HYDROGEN PEROXIDE/GEL

The objective of this study was to investigate the effect of ascorbic acid (AsA) and hydrogen peroxide (H_2O_2) on the gel-forming ability of lizardfish (*Saurida* spp.; LZ), threadfin bream (*Nemipterus* spp.; TB), and sardine (*Sardinella* spp., SD) surimi. Texture of surimi gels was monitored using the punch test and the color was measured using a colorimeter (L^*, a^* and b^*). Total sulfhydryl group (TSH) content was determined along with SDS-polyacrylamide gel electrophoresis. Changes of protein structures were analyzed using Fourier Transform Infrared (FT-IR) and Raman spectroscopies. pH of surimi pastes from all species decreased with increasing AsA with pH 6.7-6.5 at 0.2% AsA. Combination of 0.15% AsA and 0.1% H_2O_2 greatly increased the breaking force and distance of LZ surimi by 300% and 55%, respectively, when direct heating at 90 °C was applied. Addition of 0.2% AsA and 0.15% H_2O_2 resulted in the maximum gel improvement with 150% and 90% increase, respectively, in TB directly cooked gel. However, these additives did not improve gel formation of SD. Setting at 40 °C had no effect on the gel improvement of both LZ and TB species as compared to directly cooked gels, but greatly increased textural properties of SD surimi. Whiteness of all surimi gels slightly increased with the addition of H_2O_2 alone. Whiteness of LZ and TB gels decreased upon the addition of AsA combined with H_2O_2 due to ascorbic acid oxidation. Gel improvement was associated with an increase in

disulfide linkages. Myosin heavy chain (MHC) of all species was not observed with increasing AsA and H_2O_2 content when direct heating was applied, suggesting that these additives promoted polymerization of MHC. TCA-soluble oligopeptide of all surimi species increased with addition of AsA. Total sulfhydryl (TSH) groups of all surimi gels drastically reduced with the combination of AsA and H_2O_2. FT-IR spectroscopy revealed that α-helix content decreased, but β-sheet of all species increased with the addition of 0.2% AsA and 0.15% H_2O_2. Raman spectroscopy showed that hydrophobic interactions of all surimi species enhanced in concomitant with decreasing aliphatic residues after the addition of 0.2% AsA and/or 0.15% H_2O_2, as observed near wavenumbers 2930 and 1450 cm^{-1}. Tyr and Phe of aromatic bands in these surimi species also decreased by adding AsA and/or H_2O_2. AsA and/or H_2O_2 increased the total disulfide bond of LZ and TB surimi gel as indicated by changes in disulfide bond stretching, while did not change in SD gel. Principal component analysis revealed that textural properties of LZ and TB surimi gels were positively correlated to the β-sheet content and negatively correlated to α-helix content, aliphatic and aromatic residues, and TSH groups. The results suggested that AsA and/or H_2O_2 enhanced gel formation properties of LZ and TB surimi, which the optimum use of each additive and cooking process depended mainly on fish species. Setting has a greater effect on gel improvement of SD than additives.

School of Food Technology Student's Signature_____

Academic Year 2019 Advisor's Signature_____

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LIST OF ABBREVIATIONS

%	Percent
μmol	Micromole
×g	Times gravity (Centrifugal force)
a*	Green to Red value
AM	Actomyosin
ANOVA	Analysis of Variance
μm	Micrometer
AsA	Ascorbic Acid
ATR	Attenuated Total Reflectance
b*	Blue to Yellow
βME	β-mercaptoethanol
Ca^{2+}	Calcium cation
cm	Centimeter
cm^{-1}	Reciprocal centimeter
Cu^{2+}	Copper ion
DHA	Dehydro-L-ascorbic Acid
DI	Distilled water
DKA	2,3-diketo-L-gulonic acid
DTNB	5-5'-dithiobis-(2-nitrobenzoic acid)
EDTA	Ethylene Diamine Tetraacetic Acid

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LIST OF ABBREVIATIONS (Continued)

Fe (II)	Ferrous
Fig	Figure
g	Gram
h	Hour
FT-IR	Fourier Transform Infrared Spectroscopy
H·	Hydrogen radical
H_2O_2	Hydrogen peroxide
HMM-S1	Heavy-Meromyosin Subfragment-1
kDa	kilodalton
L*	Black to light, Lightness
LMM	Light-Meromyosin
LZ	Lizardfish
m	Meter
M	Molar
MANOVA	Multivariate Analysis of Variance
MDA	Monodehydro-L-ascorbic Acid
MHC	Myosin Heavy Chain
min	Minute
ml	Milliliter
NaCl	Sodium chloride
NaOH	Sodium Hydroxide

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LIST OF ABBREVIATIONS (Continued)

nm	Nanometer
$O_2^{-}\cdot$	Superoxide anion radical
ºC	Degree centigrade
pH	Potential of Hydrogen
rpm	Revolutions Per Minute
s	Second
SD	Sardine
SDS	Sodium Dodecyl Sulphate
SDS-PAGE	Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis
SH	Sulfhydryl
S-S	Disulfide
TB	Threadfin bream
TCA	Trichloroacetic acid
TGase	Transglutaminase
v/v	volume/volume
w/v	Weight per Volume
w/w	Weight per Weight

มหาวิทยาลัยเทคโนโลยีสุรนารี

CHAPTER I

INTRODUCTION

1.1 Introduction

Thailand is one of the largest surimi producers in Southeast Asia and white-fleshed fish species are commonly used as a raw material (Guenneugues and Morrissey, 2005). Threadfin bream (*Nemipterus* spp., TB) and lizardfish (*Saurida* spp., LZ) are important species for tropical surimi production in various countries, including Thailand, Vietnam, India, Indonesia, the Philippines, and Malaysia (Morrissey and Tan, 2000; Guenneugues and Morrissey, 2005). Other species of tropical fish as sardine (*Sardinella* spp., SD) is also used as a resource to produce surimi products. TB surimi gel usually gives white color and good gel-forming ability. LZ surimi typically undergoes textural degradation by high endogenous proteolytic activity, which leads to degradation and denaturation of myofibrillar proteins and poor gel-forming ability (Yongsawatdigul and Piyadhammaviboon, 2004). SD is classified as pelagic dark-fleshed fish associated with high lipid and myoglobin, which contribute to difficulties in making high-quality surimi (Chaijan et al., 2004). Color of surimi gel is another important quality attribute. The dark color is not preferred by consumers, resulting in lower of the commercial value of SD (Chen et al., 1996; Chen et al., 1998). To increase the surimi gel-forming ability, various food grade additives have been used. But addition of food additives poses adverse effects on the gel such as off flavor or off-color development (Rawdkuen and Benjakul, 2008). Moreover, surimi gel strength can be

increased by subjecting surimi paste to setting prior to cooking (An et al., 1996; Kimura et al., 1991).

Ascorbic acid (AsA) is a reducing agent that can affect surimi gel properties (Nishimura et al., 1996). Addition of AsA to surimi paste results in an increase in gel strength of various species, such as carp, walleye pollack, Alaska pollock, and milkfish, (Kishi et al., 1995; Lee et al., 1997; Ikeuci et al., 2007). AsA is auto-oxidized in the presence of transition metals to monodehydro-L-ascorbic acid (MDA), and spontaneously disproportionate to AsA and dehydro-L-ascorbic acid (DHA). In this reaction, superoxide anion radical ($O_2^{-}\cdot$) is generated, which oxidizes sulfhydryl (SH) groups on myosin heavy chain (MHC), generating disulfide (S-S) bond between MHC (Nishimura et al., 1996). However, the role of AsA on surimi gel improvement is still contradictory and appears to vary with species.

Hydrogen peroxide (H_2O_2) is known as an oxidizing agent with rapid degradation to oxygen and water (Jaruga and Dizdaroglu, 1996; Chen et al., 1999). This reagent can promote oxidation of SH group of muscle proteins to intermolecular S-S bonds during surimi gel formation (Phatcharat et al., 2006). Chen et al. (1999) reported that reducing agent or oxidizing agent did not only improve surimi gel-forming ability but also enhance gel color. However, the improvement mechanism of surimi gel-forming ability using reducing and oxidizing agent has not been systematically studied.

In surimi gelation, setting (suwari) has been typically practiced to enhance gel-forming ability. Surimi pasts are incubated at relatively low temperatures (5-40 °C) prior to heating at higher temperatures (80-90 °C). The setting phenomenon is closely related to fish habitat temperature (Morales et al., 2001). Polymerization of MHC occurs through cross-linking by endogenous transglutaminase (TGase) which imparts

the improvement of gel strength after heating at higher temperatures (Benjakul and Visessanguan, 2003). MHC is covalently cross-linked by the catalysis of TGase via non-disulfide covalent bonds. In addition, S-S bonds and hydrophobic interactions are involved in gelation (Hossain et al., 1998). However, TGase-mediated cross-linking reaction of MHC varies, depending upon species (Araki and Seki, 1993; Morales et al., 2001). According to Benjakul et al. (2004), setting at 40 °C for an appropriate time is a promising means to improve the gelling property of surimi produced from tropical fish. On the other hand, proteolytic disintegration of myofibrillar proteins can also occur during setting, which adversely affects gel quality. This is known in Japanese as "modori" (Alvarez et al., 1999). Proteolysis is caused by heat-stable proteases that hydrolyze myofibrillar proteins to small peptides, preventing the development of three-dimensional gel network (An et al., 1996). However, the effect of a reducing reagent (AsA) and an oxidizing reagent (H_2O_2) during setting at high temperature and direct heating on surimi gel formation has not been widely studied.

1.2 Research objectives

This study is aimed at investigating the effect of AsA and H_2O_2 on gelation and structural changes of tropical surimi, namely lizardfish, threadfin bream, and sardine.

1.3 Research hypotheses

1. AsA promotes polymerization of MHC via S-S linkages.
2. H_2O_2 accelerates the oxidation of MHC protein, affecting the textural and color properties of surimi gel.

3. AsA and H_2O_2 induce conformational changes of muscle proteins, resulting in changes of textural properties.

1.4 Scope of the study

Tropical surimi gels of LZ, TB and SD as affected by various concentrations of AsA (0.1, 0.15 and 0.2%) and H_2O_2 (0.05, 0.1 and 0.15%) were studied. Gels were prepared by direct heating at 90 °C for 30 min or setting at 40 °C for 30 min before heating at 90 °C for 30 min. Tropical surimi gels were characterized based on textural and color properties, protein solubility, protein pattern by SDS-PAGE, total SH group, and TCA-soluble oligopeptide. Structural changes induced by AsA and H_2O_2 were monitored by FT-IR and Raman spectroscopy.

1.5 References

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CHAPTER II

LITERATURE REVIEWS

2.1 Tropical fish surimi

Tropical fish used in surimi production in Southeast Asia are threadfin bream (*Nemipterus* spp., TB), bigeye snapper (*Priacanthus* spp.), croakers (*Sciaenidae* spp.), and lizardfish (*Saurida* spp., LZ). Other species are also used, depending on availability (seasonality) and price, including conger eel (*Congresoxs* spp), barracuda (*Sphyraena* spp), leather jacket (*Stephanoleptis cirrhifer, Navodon modestus*), various types of mackerel and sardines (Guenneugues and Ianelli, 2014). Surimi made from dark flesh fish results in low gel strength and a strong "fishy taste" (Guenneugues and Morrissey, 2005). Dark muscle fish species made up 40-50% of the total fish catch in the world (Bone, 1978, Hultin and Kelleher, 2000).

TB lives in warm water (20–30 °C) and forms an important part of the trawl catch in Southeast Asia (Guenneugues and Ianelli, 2014). This species can be made to a high-quality surimi product. TB surimi has replaced approximately one-third of the pollock surimi supply. However, surimi gel produced from this species might contain heat-activated proteases (Kinoshita et al., 1990). Setting at 25 °C or 40 °C for an appropriate time should be compromised to improve its gelling properties (Benjakul et al., 2003).

LZ is one of the most important species for surimi production in Southeast Asia. It has a high meat yield, white color, and good flavor when only fresh raw materials are used (Benjakul et al., 2003). LZ surimi is low value with poor gel-forming ability

depending on storage time and condition (Morrissey and Tan, 2000). Formaldehyde is rapidly accumulated during the storage of whole fish, resulting in an inferior quality for surimi production (Kurokawa, 1979). Yasui et al. (1987) reported that the gel-forming ability of LZ fell immediately during ice storage, due to the formation of formaldehyde and dimethyamine. Yongsawatdigul and Piyadhammaviboon (2004) reported that LZ surimi is known for its high endogenous proteolytic activity, leading to inferior gel properties.

SD is a small coastal pelagic species with dark muscle. SD can be used for surimi production with relatively low gel quality (Kudre et al., 2013). In addition, this pelagic dark-fleshed fish has high lipid, myoglobin, and sarcoplasmic proteins, which contribute to the difficulties in making high-quality surimi (Chaijan et al., 2010). It also contains high levels of proteases, particularly heat-activated proteases, which show an adverse effect on gel formation (Hu et al., 2010). Dark muscle also has higher proteolytic activity than white muscle (Shimizu et al., 1992). These proteases induce degradation of myofibrillar proteins, resulting in gel weakening (Buamard and Benjakul, 2015). The high amount of lipid content leads to lipid oxidation, which may be the primary factor limiting its shelf-life, causing the formation of undesirable flavors and decreased gelling ability (Lanier, 2000). The texture and sensory evaluation of surimi gel prepared from very fresh SD were markedly enhanced by setting at 37 °C for 30 min or 4 °C for 24 h. But setting effect decreased with freshness quality of raw material (Ishikawa et al., 1977; Roussel and Cheftel, 1988).

2.2 Fish protein

Fish muscle proteins are known as myofibrillar proteins containing myosin, actin, sarcoplasmic, and connective tissue (Kamata and Shibasaki, 1978). Myosin is about 55-60% of the total myofibrillar proteins which is responsible for gelation and water-holding capacity. Actomyosin (AM) is a complex protein between actin and myosin, which forms after death when high energy (adenosine triphosphate "ATP") compounds are depleted. Myosin and actin are linked by noncovalent bonds, that are easily split by ATP. AM retains most of the myosin ATPase activity (Galluzzo and Regenstein, 1978). AM rather than myosin is the protein dictating the thermal gelation of surimi products.

Myosin is a double-headed enzyme, which possesses two pear-shaped globular heads (S1) and a characteristic coiled-coil tail (rod, α-helical) (Xiong, 1997). It has three biological functions which are ATPase activity, actin-binding ability and thick filament formability under the physiological ionic environment. Myosin is also divided into two components which are heavy-meromyosin subfragment-1 (HMM-S1) and light-meromyosin (LMM). HMM-S1 has ATPase-activity (releases the energy for muscle contraction) and binds to actin in the absence of ATP. ATPase activity has been used as an indicator of the extent of myosin unfolding and aggregation during storage. There are two small light chains noncovalently attached to a globular head. These light chains are not important in the gelation of muscle proteins (Lanier et al., 2014). Myosin ATPase is activated exponentially at 20-40 °C and the activity was lost almost completely at 40 °C.

Surimi gels are composed of a three-dimensional protein network of AM formed by thermal treatment (Sano et al., 1988; Ogawa et al., 1993, 1995). Thermal gelation of myosin exists in two steps: 1. denaturation and 2. aggregation of the globular head

segments of myosin molecules through oxidation of SH groups (Samejima et al., 1981). myofibrillar proteins have highly reactive surfaces. Unfolding in the S1 region occurs easily when heating at 20-40 °C (Figure 2.1.b). The aggregation of myosin molecules is initiated by disulfide (S-S) cross-linking of denatured HMM-S1 (head region of myosin) (Figure 2.1.c) (Yasui and Samejima, 1990; Yamamoto, 2008); then intermolecular aggregation occurred in the S1 region and caused a subsequent unfolding of LMM above 40 °C (tail to tail region of myosin interaction) during heating (Figure 2.1.d) (Taguchi et al., 1987). The exposed regions of LMM can aggregate and contribute to the formation of the gel network (Figure 2.1.e, f).

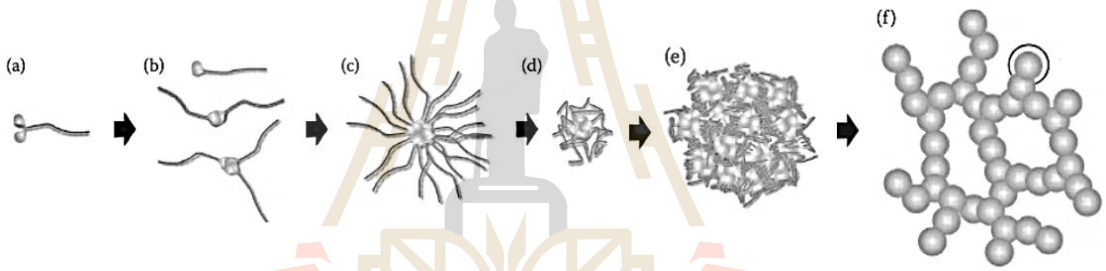

Figure 2.1 Heat-induced gel formation of myosin monomer. (a) Unheated; (b) cluster formation in early heating; (c) Daisy wheel formation of myosin head-to-head; (d) denaturation of tail portions; (e) clusters bound through denatured tails; (f) gel network formation (Yamamoto, 2008).

Intermolecular S-S bonding can be induced by reducing agents, such as bromate or ascorbate. The addition of potassium bromate resulted in the formation of elastic gels from fish proteins (Okada and Nakayama, 1961). Potassium bromate induced oxidation of SH compounds to form intermolecular S-S bonds. Yoshinaka et al. (1972) and Lee et al. (1992) suggested that the improving action of ascorbic acid (AsA) in the gel formation of Alaska pollock surimi may be due to the oxidation of SH groups in fish

proteins by dehydroascorbic acid (DHA), and that DHA oxidizes sulfhydryl (SH) groups in the AM molecule.

2.2.1. Protein oxidation

Oxidation processes generally affect the quality of meat products via inducing several changes in proteins such as modification of amino acid side chains, protein fragmentation, polymerization, and structural alteration. The main oxidative modifications of protein take place at the side chains of amino acids, including thiol oxidation, aromatic hydroxylation, and formation of carbonyl groups (Stadtman, 1990). Cysteine and methionine contain reactive sulfur atoms which are the most susceptible to oxidation (Shacter, 2000). Sulfur anion is the most powerful nucleophile and rich in electrons, which can be easily removed by reactive oxygen species (ROS) include free radicals (•OH, $O_2^{\bullet -}$, RS•, and ROO•), nonradical species (H_2O_2 and ROOH), and reactive aldehydes and ketones (Butterfield and Stadtman, 1997). These oxidants directly attack the backbone of a protein to cause fragmentation and conformational changes in the secondary and tertiary structure of the protein; inducing S-S, dityrosine, and other intermolecular bridges, resulting in protein aggregation and polymerization (Martinaud et al., 1997; Morzel et al., 2006).

Myosin is susceptible to oxidation, causing cross-linking between individual protein molecules (Liu and Xiong, 2000a). The increase in carbonyl contents was associated with reductions in the thermal stability of myosin (Liu and Xiong, 2000b). Oxidized proteins have varying functional properties, particularly gelation. Decker et al. (1993) reported that the oxidation of turkey white muscle myofibrillar proteins by iron or copper and ascorbate caused a decreased gel strength. Chanarat et al. (2015) suggested that carbonyl content of myosin from tilapia via Fenton's reaction using H_2O_2

(0, 0.05, 0.1, 1 and 5 mM) resulted in decreased sulfhydryl group content and Ca^{2+}-ATPase activity.

2.3 Surimi gelation

Gelation is an important function at a property of protein, which is required for surimi products. Gel formation involves denaturation and aggregation, giving rise to matrices capable of holding water through physicochemical forces (Mulvihill and Kinsella, 1987). Denaturation and degradation of proteins contribute aggregation through intermolecular covalent and noncovalent interactions including S-S, hydrophobic interactions, and hydrogen bonds (Cheftel, 1992; Lee and Lanier, 1995). These proteins are known as myofibrillar proteins, including myosin and actin, found as an AM complex of fish mince and surimi (Niwa, 1992). Thermal gelation of fish muscle proteins has occurred in three steps, including (1) dissociation of myofibril structures by protein solubilization in the presence of salt; (2) partial unfolding of protein structure induced by heat treatment; and (3) aggregation of unfolded protein via both S-S bonds and non-disulfide covalent bonds to form a three-dimensional network (Stone and Stanley, 1992). This gelation mechanism undergoes S-S bond formation and hydrophobic interaction. Total number of free SH groups in the myosin heads decreases with increasing temperatures and crosslinks of S-S bonds (Samejima et al., 1981). Itoh et al. (1979) demonstrated that oxidation of SH groups took place above 40 °C during the gelation of carp AM. The development of the gel network with further heating was supported by Yasui and Samejima (1990) who reported that S-S bonds are indeed involved in the initiation of gelation.

When surimi pastes are heated, proteins unfold and expose reactive surfaces, which then interact to form intermolecular bonds (Lanier, 2000). During sufficient bonding occurs, a three-dimensional gel network is formed as a gel. Four main types of chemical bonds involved in gelation: hydrogen bonds, ionic linkages, hydrophobic interactions, and covalent bonds (Lanier et al., 2014). Hydrogen bonds become less stable and break down during temperature rises. Hydrogen bonds can be important in the stabilization of bound water within the hydrogel and add gel strength during cooling and aging of surimi seafood (Howe, 1994). Rising temperature to near 60 °C, intermolecular hydrophobic interactions among proteins occur, which is a primary force for the surimi gel formation. At > 40 °C, S-S bond is the predominant covalent bond. An intermolecular S-S bond is formed by the oxidation of two cysteine residues on neighboring protein chains (Lanier et al., 2005).

Thermal aggregation of fish myosin was coincidental with an increase in surface hydrophobicity as temperature increased (Chan et al., 1992). The extent of myosin aggregation depends on the amount of hydrophobic surface exposed on the headed molecules (Wicker *et al.*, 1986). Incubation of surimi paste below 40 °C increased firmness, cohesiveness, and water-holding capacity (Kimura et al., 1991). The setting can be achieved at either high-temperature setting, within a short period of time (2-4 h) near 40 °C; or low-temperature setting, below 40 °C within an extended time (12-24 h). This is related to the TGase activity and thermal stability of fish proteins (Wu et al., 1985). Atlantic croaker and sand trout surimi gels greatly increased gel strength and elasticity at 40 °C (Lanier, 1986). Furthermore, the gel strength was highly correlated with the decrease in α-helicity. Ogawa et al. (1995) proposed that the setting of surimi is initiated by the unfolding of α-helix.

Analysis of the set gel on polyacrylamide or agarose gel revealed that myosin heavy chain (MHC) was cross-linked to higher molecular weight polymers. TGase is involved in the reaction (Seki et al., 1990). This enzyme was found in Alaska pollock surimi and other species (Imai et al., 1996).

2.4 Gel characteristics during setting

Setting is known as an important process in the production of surimi-based products which is responsible for the formation of the final products with greater gel strength and elasticity. Fish species, freshness, seasonal variation, handling of the materials are factors that can affect the gel-forming properties of surimi (Shimizu, 1984). Setting of surimi is partly contributed from transglutaminases (TGase, protein-glutamine-γ-glutamyltransferase, EC 2.3.2.13) (Figure 2.2). TGase are transferase enzymes which promote acyl transfer reactions between free amino groups, and the γ-carboxyamide groups of glutamines (Motoki and Seguro, 1998). The γ-carboxyamide group acts as an acyl donor and several primary amines are acyl acceptors.

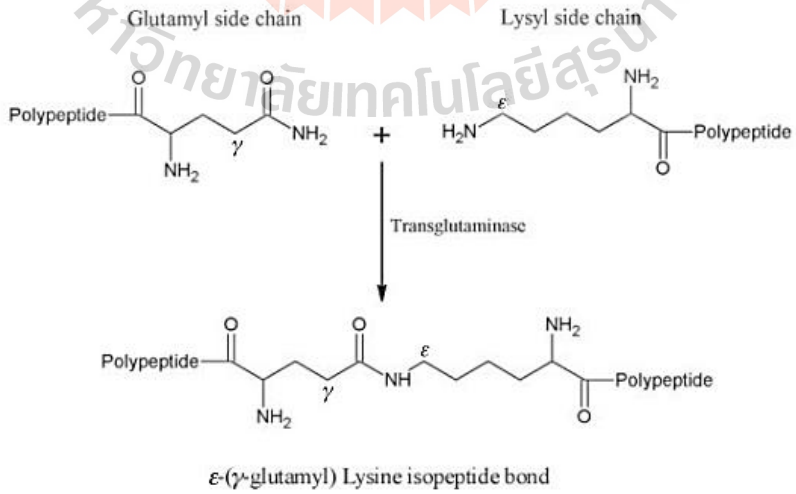

Figure 2.2 Cross-linking of proteins by transglutaminase.

Relationship between thermal stability of myosin and habitat temperature at where the fish lives, has been reported (Hashimoto et al., 1982). Fish as Alaska pollock live in the cold water of 0-5 °C while Pacific whiting lives in temperature of 5-15 °C. Tropical fish such as threadfin bream have a habitat temperature of 20-30 °C (Esturk et al., 2004). Fish myosin from cold water species is consequently more vulnerable to thermal denaturation than warm water species (Tsuchimoto et al., 1988). Lee (1984) suggested that warm water species could tolerate higher temperatures than cold water species. Generally, setting is performed at low temperature (0-4 °C), medium (25 °C) or high temperature (40 °C) depending on the temperature habitat of fish (Lanier, 1992). Setting at different temperatures may lead to different gel characteristics, especially with different fish species. Different species have a different optimum setting temperature, as determined by the heat stability of myosin. Low temperature setting is a property unique to fish proteins (Lanier, 1986). Differences in surface hydrophobicity and SH group exposure have also been implicated with various setting temperatures (Wicker et al. 1986, 1989; Kim, 1987; Niwa 1992). More recently, the changes in gel properties induced by setting have been associated with changes in MHC content (Numakura et al., 1985, 1987; Katoh et al. 1986; Nishimoto et al., 1988). A decrease in MHC content during setting was converted by non-disulfide cross-linking of MHC mediated by a TGase (Kimura et al. 1991; Kishi et al. 1991). Pollock (cold water fish) and croaker (warm water fish) showed optimum setting temperatures of 25 and 40 °C, respectively (Kamath et al., 1992), whereas croaker surimi showed no setting response at 4 °C (Kim et al., 1986). Alaska pollock had the highest shear stress values at 5 °C or lower; while LZ and TB had a higher shear stress value at 25 °C or higher (Esturk et al., 2004). Setting at 40 °C for 1 h increased breaking force of TB (Benjakul, 2004).

Setting SD surimi at 40 °C for 30 min could increase the cross-linking of proteins (Buamard et al., 2017). High-temperature settings for a shorter time is more commonly practiced in tropical surimi, but protein degradation induced by protease can occur (Jiang et al., 2000).

TGase is a calcium-dependent enzyme (Folk, 1980). Ca^{2+} ions must be sufficient for TGase optimum activity, known as calcium-activated TGase (Lee and Park, 1998). Ca^{2+}-dependent enzyme catalyzes acyl-transfer reaction of the crosslinking of MHC during setting (Kishi et al., 1991; Kamath et al., 1992). Optimal Ca^{2+} ion content varies with fish species (Nozawa et al., 1997; Benjakul and Visessanguan, 2003).

It is not only protein-protein interactions by covalent dipeptide linkages which stabilize during setting, but intermolecular hydrophobic interactions also occur (Niwa, 1992). These hydrophobic interactions by setting were observed by Raman spectroscopy with a decreased intensity of a band near 2930 cm^{-1} (Bouraoui et al., 1997).

2.5 Surimi gel softening

Proteolytic disintegration of myofibrillar proteins has an adverse effect on the gel-forming properties of surimi. Breakdown of myofibrillar proteins inhibits the development of a three-dimensional gel network (An et al., 1996). Proteolytic degradation of surimi gels is increased at temperatures above 50 °C with severe degradation of myofibrillar proteins, particularly myosin (Jiang et al., 2000). When gel heated at 50-70 °C, proteolytic degradation of myofibrillar proteins occurs, resulting in the disintegration of gel structure known as ‘modori’ (Alvarez et al., 1999). Modori gel is mainly due to the proteolysis caused by heat-stable proteases, such as cathepsins,

alkaline proteases, and calpains (Benjakul et al., 1997; Jiang et al., 2000). Proteolysis of myosin was shown to lower surimi gel strength (Morrissey et al., 1993). Native conformation of myosin is primarily important for proper gelation and maximum gel strength (Niwa, 1992). Cysteine proteases were reported to hydrolyze muscle proteins of Pacific whiting (Seymour et al., 1994). Serine proteases were found to be responsible for the textural breakdown of TB (Kinoshita et al., 1990). Benjakul et al. (2003) endogenous sarcoplasmic and myofibril-associated proteases play an important role in the degradation of myofibrillar proteins of LZ during heat-induced gelation, which results in a gel weakening. Heat-stable protease is typically inactivated when the temperature exceeds 80 °C, thus it is possible to minimize this proteolytic activity and textural degradation when rapid heating is applied (Choi et al., 2005; Yongsawatdigul et al., 2014). Heat-activated proteases can be classified as sarcoplasmic and myofibril associated proteases based on the extractability (Kinoshita et al., 1990). The water-soluble constituents include sarcoplasmic protein that is removed during washing process, while myofibril associated proteases are not easily removed by washing (Toyoda et al., 1992; Benjakul et al., 1996). Myofibril associated serine protease in muscle showed the highest activity at 55 °C (Osatomi et al., 1997) and it was found in LZ with a molecular weight of 60 kDa. It was able to hydrolyze MHC at 55-60 °C (Cao et al., 1999). Heat-activated proteases are also present in TB with optimal activity at 50-60 °C (Kinoshita et al., 1990).

In addition, heat-stable alkaline proteases are also involved in textural degradation at pH 8.5–9.0 of 60–65 °C (Folco et al., 1984; Kinoshita et al., 1990). Heat-stable alkaline proteases including serine and cysteine proteases were also found in LZ (Suwansakornkul et al., 1993). Benjakul et al. (2004) suggested that surimi gel prepared

by setting at 40 °C decreased proteolytic degradation. Klomklao et al. (2008) reported that in the presence of proteases in SD which was susceptible to rapid autolytic degradation of tissue. Several types of proteolytic enzymes in SD has been reported, such as acid, neutral and alkaline proteinases have been found muscle (Lugo-Sanchez et al., 1997).

2.6 Oxidizing

2.6.1. Ascorbic acid (AsA)

AsA in the L-form promotes the oxidation of bread dough and improves bread quality (Koehler, 2003). AsA is also used as an improver in surimi gel (Nishimura et al., 1992), which improves the quality of heat-induced fish gel (Yoshinaka et al., 1972; Nishimura et al., 1990). Superoxide anion radical (O_2^-) generated during the oxidation of AsA promoted the formation of S-S bonds among the MHC (Figure 2.3) (Nishimura et al., 1994). When AsA is added to surimi, the oxidation of AsA occurs in the presence of a transition metal such as Fe (II). This transition metal catalyzes the auto-oxidation of AsA to MDA. The univalent oxidized product of AsA, is spontaneously disproportionate into AsA and DHA. AsA reduces molecular oxygen to form O_2^-. The O_2^- oxidizes SH groups of the myosin heavy chain (MHC), by removing hydrogen radical (H·) from the SH groups and generating thiyl radicals (S·). The reaction between pairs of S· can form S-S bonds and producing a network structure that increases gel strength of surimi gel (Nishimura et al., 1996).

Yoshinaka et al. (1972), DHA also oxidized SH groups in the AM; when Alaska pollack AM was added 2-10 µM DHA/g of meat and heat at 80 °C. Chen et al. (1999) proposed that L* values of milkfish gel added AsA (0.02-0.08%) steadily increased

with the increment of concentration. However, Benjakul et al. (2005) reported that the addition of AsA slightly increased LZ surimi gel formation with a decrease of SH group content and increased S-S bonds, but it did not improve the gel-forming ability of TB surimi. Ikeuci et al. (2007), carp crude myosin incubated with 0.2% AsA at 40 °C improved S-S bridges and gelation of myosin.

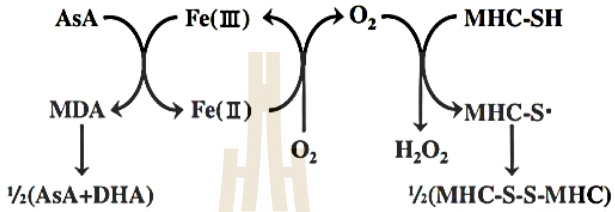

Figure 2.3 Scheme for superoxide anion radical-dependent polymerization of myosin heavy chain. Source: Ikeuci et al. (2007).

2.6.2. Hydrogen peroxide (H_2O_2)

The oxidation of SH groups leads to S-S bond formation (Figure 2.4) (Kishi et al., 1995). H_2O_2 is a strong oxidizing agent that degrades rapidly to oxygen and water (Chen et al., 1999). This oxygen is responsible for SH group oxidation on MHC and enhances the gel formation. According to Chen et al. (1999), adding H_2O_2 (0.01-0.04%) accelerated the oxidation of SH groups by increasing the maximum stress values and water holding capacity of milkfish meat paste and improved the lightness of surimi gel.

$$\text{Protein-SH} + \text{HS-Protein} \xrightarrow{O_2} \text{Protein-S-S-Protein} + H_2O\,(\text{water})$$

Figure 2.4 Formation of intramolecular and intermolecular S-S bonds. Source: Lanier et al. (2005).

2.7 Ascorbic acid browning

Browning of AsA can be defined as thermal decomposition of AsA under both aerobic and anaerobic conditions by oxidative or non-oxidative mechanisms, either in the presence or absence of amino compounds (Wedzicha, 1984). Non-enzymatic browning is occurred by degradation of AsA followed by browning. Factors influencing the nature of AsA degradation including temperature, salt, and sugar concentration, pH, oxygen, enzymes, metal catalysis, amino acids, oxidants or reductants, initial concentration of AsA, and the ratio of AsA to DHA (Corzo-Martinez et al., 2012). Figure 2.5 shows that AsA degraded to DHA in the presence of oxygen. DHA is not stable and spontaneously converted to 2,3-diketo-L-gulonic acid (DKA) (Lee and Nagy, 1996). Under anaerobic condition, AsA is also converted to DKA by keto tautomer, β-elimination at C-4 and decarboxylation to give rise to 3-deoxypentosone, that degraded to furfural. In aerobic conditions, decarboxylation of DKA produced xylosone and converted to reductones. In the presence of amino acids, AsA, DHA, furfural, 3-deoxypentosone and xylosone may contribute to non-enzymatic browning (Belitz and Grosch, 1997). The presence of metal ion including Cu^{2+} and Fe^{2+} catalyzes oxidation of AsA faster than the spontaneous reaction.

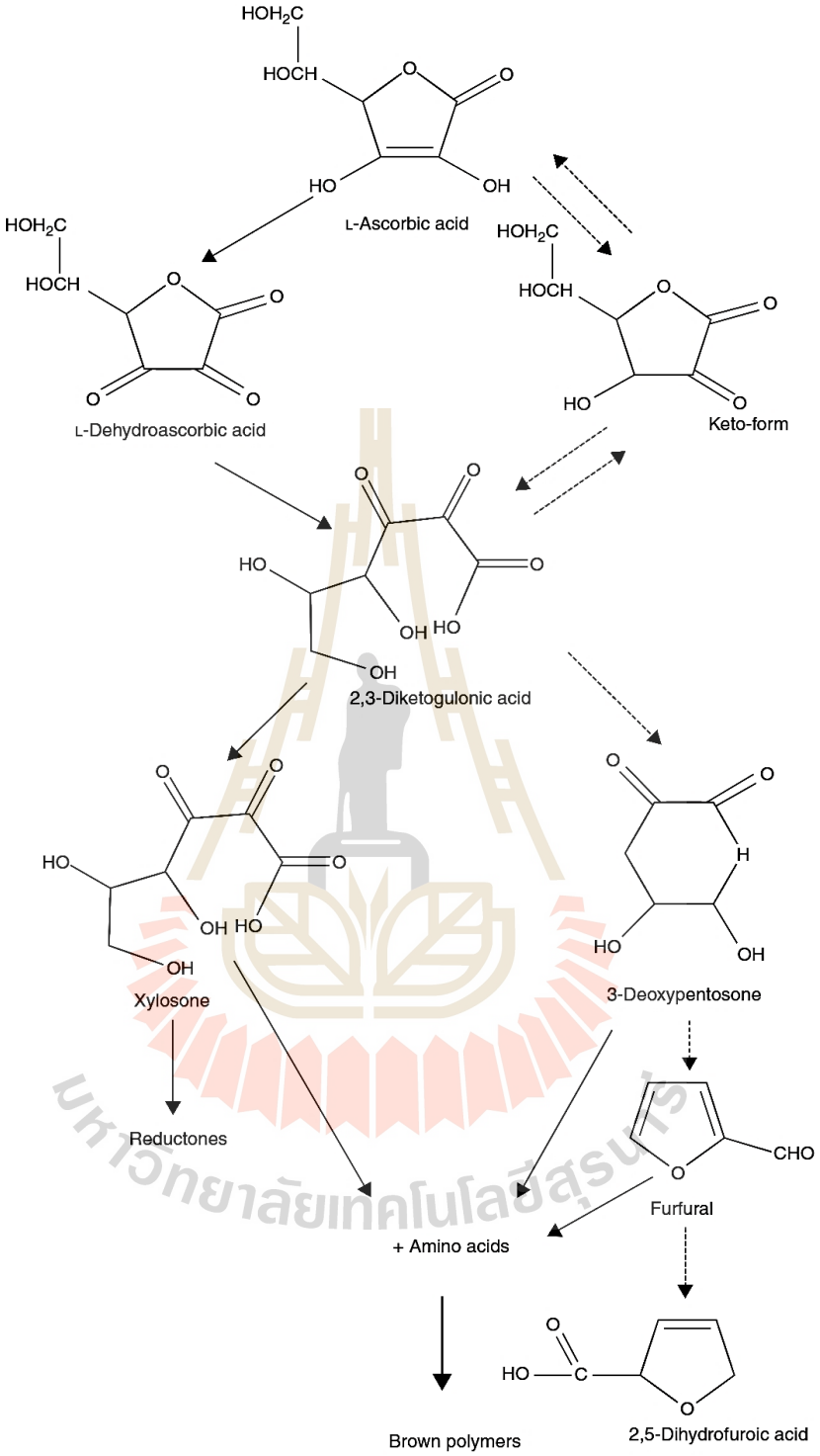

Figure 2.5 Ascorbic acid oxidation to brown color (solid line, anaerobic route; dashed line, aerobic route).

Source: Corzo-Martinez et al., 2012.

2.8 FT-IR and Raman spectroscopy instrument

Vibrational spectroscopy is used to investigate changes in chemical composition and molecular structure of proteins, carbohydrates, nucleic acids, cell membranes, and tissues (Kobayashi et al., 2017). Fourier transform infrared spectroscopy (FT-IR) and Raman spectroscopy is complementary techniques that expose differences between structural macromolecular changes (Yuen et al., 2009, Flores-Morales et al., 2012). Molecular vibrations are infrared-active when the dipole moment of the molecule changes as the molecule vibrates. Vibrations are Raman-active when the polarizability of the molecule changes as the molecule vibrates. Both techniques can be used to nondestructively analyze the sample, with higher sensitivity (Li-Chan, 1996).

FT-IR has been shown to be sensitive to the secondary structure of proteins, making it a powerful technique for studying protein aggregation (Miller et al., 2013). FT-IR spectrum has two prominent features, the Amide I (~1650 cm^{-1}) and Amide II (~1540 cm^{-1}) bands, where the former arise primarily from the C=O stretching, vibration and the latter is attributed to the N-H bending and C-N stretching vibrations of the peptide backbone (Haris and Severcan, 1999). The frequency of the Amide I band is particularly sensitive to the secondary structure based on different hydrogen-bonding environments for α-helix, β-sheet, turn, and unordered conformations.

Raman spectroscopy measures the scattering of monochromatic laser light that ranges from visible (10^{-6} m) to near-ultraviolet (10^{-8} m). The Raman effect is produced through the exchange of energy between incident photons and the vibrational energy levels of the molecule. Raman scattering is the inelastic scattering of laser light impinged onto the sample and is scattered with a transfer of energy between the

excitation light and the sample. Raman scattering occurs when the molecular motion produces a change in the polarizability of the molecule (Li-Chan et al., 1994).

Zhou et al. (2015) suggested that changes in the secondary and tertiary structure of myofibrillar proteins occurred during protein oxidation in meat, leading to changes in the physicochemical state and functional properties. Changes in the frequency and intensity of the Raman bands indicate the change of secondary protein structure and variations in local environments of myofibrillar proteins (Xu et al., 2011). Raman spectroscopy has been used to study the substructure of myosin (Carew et al., 1975) and was successfully employed to monitor structural changes upon gelation (Li-Chan and Nakai, 1991). Raman spectroscopy offers the ability to analyze a very small amount of sample non-destructively with high sensibility.

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มหาวิทยาลัยเทคโนโลยีสุรนารี

CHAPTER III

EFFECT OF ASCORBIC ACID AND HYDROGEN PEROXIDE ON GEL-FORMING ABILITY OF LIZARDFISH AND THREADFIN BREAM SURIMI

3.1 Abstract

The objective of this study was to investigate the effect of ascorbic acid (AsA) and hydrogen peroxide (H_2O_2) on gel-forming ability of lizardfish (LZ) and threadfin bream (TB) surimi. A combination of 0.15% AsA and 0.1% H_2O_2 greatly increased breaking force and distance of LZ surimi by 300% and 55%, respectively. A combination of 0.2% AsA and 0.15% H_2O_2 resulted in the maximum TB surimi gel improvement with 150% and 90% increase, respectively. Setting at 40 °C had no effect on the gel improvement of both species. Whiteness of surimi gels increased with addition of H_2O_2 alone. However, the browning reaction obviously occurred when combined AsA and H_2O_2 was added. Myosin heavy chain (MHC) was not observed and high molecular weight proteins increased in gel added 0.2% AsA and/or 0.15% H_2O_2, when extraction buffer was only SDS. MHC was recovered when buffer containing βME was applied. These results suggested the involvement of disulfide bonds induced by both AsA and H_2O_2. TCA-soluble oligopeptide of surimi gel increased with increasing AsA and was higher in setting. Total sulfhydryl groups drastically reduced in the combined 0.2% AsA and 0.15% H_2O_2. Fourier-transform infrared spectroscopy

revealed a decrease in α-helix and an increase in β-sheet content as AsA and H_2O_2 increased in both species. Hydrophobic interactions and conformation of disulfide bonds in surimi increased with addition of these additives, as revealed by Raman spectroscopy. Based on principal component analysis, textural characteristics were positively correlated with β-sheet structure. AsA and H_2O_2 and direct heating at 90 °C enhanced gel formation of TB and LZ surimi, but the optimum use of each additive depended mainly on fish species.

3.2 Introduction

Threadfin bream (*Nemipterus* spp.; TB) and lizardfish (*Saurida* spp.; LZ) are important species of tropical surimi production in Southeast Asia (Benjakul et al., 2005). Gel quality of tropical surimi is generally lower than cold water surimi. Thus, a means to improve gel quality of tropical surimi should be sought. The improvement of surimi gel properties has been achieved by the addition of protein additives (Park and Morrissey, 2000), applying a proper setting condition and the use of microbial transglutaminase (Visessanguan et al., 2003). An increase in disulfide (S-S) bonds would be one of promising approach to improve surimi gelation. Oxidizing agents have been used to induce the oxidation of proteins, particularly via disulfide (S-S) formation, and degraded proteins could be cross-linked and formed larger aggregates with improved gel-forming ability (Liu and Xiong, 2000). Ascorbic acid (AsA) can be auto-oxidized in the presence of transition metals (iron) to monodehydro-L-ascorbic acid, and spontaneously disproportionate dehydro-L-ascorbic acid (DHA). Superoxide anion radical is generated and removes hydrogen radical from sulfhydryl (SH) groups on myosin heavy chain (MHC), generating thiyl radicals (S·) and form a S-S bond between

MHC (Nishimura et al., 1996). Hydrogen peroxide (H_2O_2) is known as an oxidizing agent that can also induce the oxidation of thiol group to form S-S bond (Chen et al., 1999).

The objective of this study was to investigate the effect of AsA and H_2O_2 on textural, color improvement and protein structural changes of tropical surimi, namely lizardfish, and threadfin bream.

3.3 Materials and Methods

3.3.1 Materials

LZ and TB frozen surimi obtained from Andaman Surimi Industries Co., Ltd. (Samutsakhon, Thailand) and Anuson Mahachai Co., Ltd. (Samutsakhon, Thailand), respectively, were cut to approximately 1 kg blocks, vacuum packed, and stored at -20 °C until used.

3.3.2 Surimi gel preparation

Frozen surimi was thawed in a refrigerator overnight and cut into small pieces (about 1 cm cubes). Surimi samples were chopped for 1 min by a Stephan chopper (UM 5 universal, Stephan U. Söhne GmbH & Co., Hameln, Germany) at a temperature below 10 °C during chopping. The surimi was added 2% (w/w) NaCl and chopping were combined for 1 min. Then ice was added to adjust the final moisture content of the paste to 78% (w/w) and mixture was chopped for 1 min. Different amount of AsA at 0, 0.1, 0.15, 0.2% and H_2O_2 at 0, 0.05, 0.1 and 0.15% were added at the same time of mixing ice. Vacuum was applied for 3 min and chopping was carried out at high speed for 3 min. Surimi pastes were vacuum-packed and stuffed into polyvinylidene chloride casing (3 cm diameter) by vertical sausage stuffer. Two thermal treatments

were applied, including direct heating at 90 °C for 30 min and setting at 40 °C for 30 min before heating at 90 °C for 30 min. Subsequently, surimi gels were cooled immediately in iced water for 20 min and stored at 4 °C overnight prior to further analysis.

3.3.3 pH measurement

pH of surimi pastes was measured using a pH meter (Mettler Toledo™, SevenCompact™ S220, pH/Ion Benchtop Meter, Switzerland) calibrated against standard pH buffers of 4.0 and 7.0 at 25 °C. Sample (2 g) was added 18 ml distilled water and homogenized before pH measurement (Smyth and O'Neill, 1997).

3.3.4 Texture analysis

Texture analysis was measured using an empirical punch test with a 5 mm spherical probe (stable micro systems texture analyzer, TA-XT Plus, Surrey GU7 1YL, UK). Penetration speed of 1 mm/s was used. Gels equilibrate at room temperature (25±2 °C) for 2 h and cut into a cylinder shape with 3 cm diameter and 3 cm long (Park, 2005).

3.3.5 Whiteness

Color of surimi gels was measured using CIE L^*, a^*, and b^* by a HunterLab spectrophotometer (HunterLab, ColorQuest XE, Hunter Associates Laboratory, USA). L^* represents lightness and b^* = yellow (+) to blue (-) axis. Whiteness was calculated using the equation of L* - 3b* (Park, 1994).

3.3.6 Protein patterns by SDS-PAGE

Protein patterns of surimi gels were analyzed using SDS–PAGE according to the method of Laemmli (1970) and modified by Bonham et al. (2015). Hot extraction buffer (10 ml of 10% SDS solution) was added to sample (1 g) and homogenized at a

speed of 11,000 rpm for 2 min and heated at 90 °C for 1-2 h. Extraction buffer was prepared with and without 10% β-mercaptoethanol (βME). Samples were shaken at room temperature overnight (Numakura et al., 1985) and centrifuged at 8,000 ×g for 20 min to remove undissolved debris. Protein concentration of supernatant was determined by the Lowry method (Lowry et al., 1951). Solubilized samples were mixed with treatment buffer (0.125 M Tris-Cl, pH 6.8, containing 4% SDS, 20% glycerol) that either contained or without 10% βME at a ratio 1:1 (v/v). Subsequently, samples were heated at 95 °C for 3 min. Samples (20 μg protein) were separated on 7.5% polyacrylamide gel with 4% stacking gel (Varelas et al., 1991). Electrophoresis was carried out under 100V. After separation, gels were stained with staining solution (0.125% (w/v) Coomassie Brilliant blue R-250 in 40% methanol and 10% acetic acid), for 1 h and destained in 25% methanol and 10% acetic acid until protein bands were visibly clear. Images of gels recorded using UVITEC (Bio-Active Con., Ltd, Cambridge CB4 0WS, UK).

3.3.7 TCA-soluble peptide content

Trichloroacetic acid (TCA) soluble oligopeptide was determined according to the method described by Morrissey et al. (1993). Surimi gels (3 g) were homogenized with 27 ml of cold 5% TCA (w/v) at a speed of 11,000 rpm for 2 min. The homogenate was incubated in ice water for 1 h and centrifuged at 8,000 ×g for 10 min. TCA-soluble oligopeptide content in the supernatant was measured by Lowry method (Lowry et al., 1951) using L-tyrosine as a standard and expressed as μmol tyrosine/g sample.

3.3.8 Total sulfhydryl group content (TSH)

Total sulfhydryl group content of surimi gel was determined based on Ellman method (Ellman, 1959) using 5-5'-dithiobis-(2-nitrobenzoic acid) (DTNB). Protein solubilization was carried out by homogenizing surimi gel (0.5 g) at 11,000 rpm for 2 min with 25 ml of 0.1 M phosphate buffer (pH 7.0) containing 10 mM EDTA (Ethylene diamine tetraacetic acid), 2% SDS and 8 M urea. Bradford assay was used to determine protein content (Bradford, 1976). Supernatant (4 ml) was mixed with 0.4 ml of 0.1% DTNB in 0.1 M phosphate buffer (pH 7.0) containing 8 M urea, 2% SDS and 10 mM EDTA. The mixture was incubated at 40 °C for 15 min before measuring the absorbance at 412 nm. TSH content was calculated using a molar extinction of 13612.5 M^{-1} cm^{-1} for 2-nitro-5-triobenzoic acid (TNB).

3.3.9 FT-IR spectroscopy

Infrared spectra between 4000 and 400 cm^{-1} were recorded an FT-IR spectrometer equipped with an attenuated total reflectance (ATR) accessory (FT-IR, Platinum ATR, T27/Hyp2000, Bruker Limited, Leipzig, Germany). Approximately 1 mg of freeze-dried paste and gel samples were placed on the surface of the ATR crystal and clamped into the mount of an FT-IR spectrometer. Spectra were collected in 64 scans with a resolution of 4 cm^{-1} at room temperature (25 °C). Background interference was removed, and the recorded spectral data were analyzed using OPUS software version 7.0. Data collection for each sample took less than 2 min. Prior to data analysis, the spectra were baseline corrected, normalized according to the amide I peak at 1600-1700 cm^{-1} and the curve-fitting procedure was performed to determine the percentage of protein secondary structures were determined according to the method reported by Alix et al. (1988).

3.3.10 Raman spectroscopy

Raman spectra of surimi gels/paste were collected using a laser Raman spectrometer (FT-Raman spectrometer, Vertex 70-Ram II, Bruker Limited, Leipzig, Germany) at room temperature. Surimi paste and surimi gel samples were applied to a sample holder and placed on the instrument. A spectral resolution was set at 4.0 cm^{-1} from 400-4000 cm^{-1}, the laser energy was 500 mW and the final spectra was an average of 64 scans. The recorded spectra were analyzed using OPUS software version 7.0. The intensity area values of Raman bands were determined after spectral normalization (4000-400 cm^{-1}). In order to increase spectral resolution, a second-derivative spectrum was determined.

3.3.11 Principal component analysis (PCA)

Principal component analysis (PCA) was performed to reduce the dimensionality of the FT-IR spectral data and estimate the relationship between the additives and gel textural quality characteristic and changes of protein structural during direct heating or setting, retaining as much as possible of the variation present in the data set (Jolliffe, 2002). PCA was carried out using the Unscrambler X 10.5 software (Synchrotron Thailand Central Lab, Nakhon Ratchasima 30000). Spectra were justified by Savitzky-Golay smoothing filters before baseline correction in the range of 400-4000 cm^{-1} and Multiplicative Scatter Correction or MSC/EMSC. Then, PCA was analyzed. Sample scores in the first two principal components (PCs) were selected and plotted. Correlation plots and score plots were presented as the final PCA.

3.3.12 Statistical analysis

The effect of concentration of AsA and H_2O_2 on surimi gel-forming properties were evaluated using SPSS 14.0. A one-way analysis of variance (ANOVA)

was employed to determine the statistical difference. Duncan's multiple range test was used to identify the significant differences between means ($p < 0.05$, Cochran and Cox, 1992).

3.4 Results and discussion

3.4.1 pH

AsA and H_2O_2 affected pH of surimi pastes as shown in Figure 3.1. Addition of H_2O_2 alone up to 0.15% did not change pH of LZ and TB surimi pastes. pH of both surimi species decreased as concentration of AsA increased ($P < 0.05$). This was in agreement with Chen et al. (1999). Low pH induced by AsA could affect the texture.

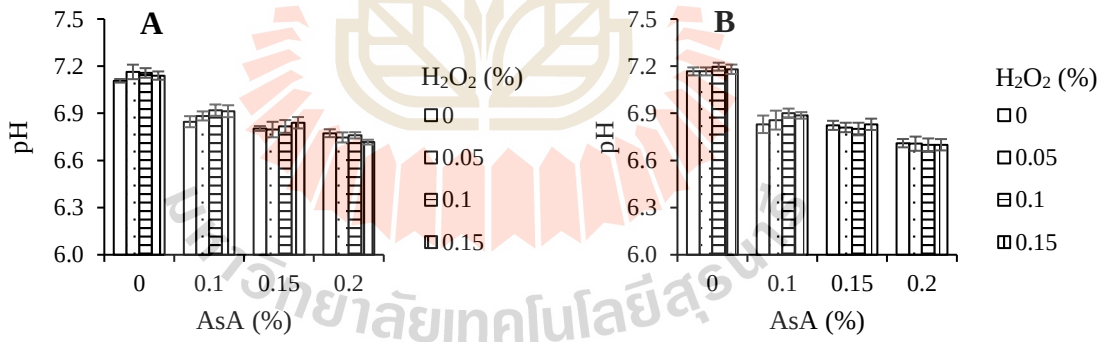

Figure 3.1 Effect of AsA and H_2O_2 on pH of surimi pastes from LZ (A) and TB (B).

3.4.2 Texture

Gel-forming ability of surimi including breaking force and breaking distance at different concentrations of AsA and H_2O_2 are shown in Figure 3.2. Adding H_2O_2 alone increased breaking force and breaking distance of LZ surimi gel cooked by

direct heating, which 0.15% H_2O_2 increased 180% and 30% of breaking force and breaking distance, respectively, compared to the control (without H_2O_2 and AsA), while AsA alone showed less effect (Figure 3.2A and B). A combination of 0.15% AsA and 0.1% H_2O_2 greatly increased breaking force and breaking distance of LZ surimi gel by 300% and 55%, respectively. TB surimi gel cooked at 90 °C for 30 min did not affect textural improvement when H_2O_2 alone was added, whereas addition of 0.2% of AsA increased the breaking force and breaking distance by 65% and 40%, respectively (Figure 3.2C and D). Addition of H_2O_2 alone did not significantly enhance breaking force and breaking distance of TB surimi gel compared to the control. This was in agreement with Nishimura et al. (1990). Adding 0.2% AsA and 0.15% H_2O_2 resulted in the maximum TB surimi gel improvement with 150% and 90% increase of breaking force and breaking distance, respectively. Results indicated that H_2O_2 and AsA improved gel-forming ability of LZ and TB surimi. Chen et al. (1999) reported that addition of 0.01 to 0.04% H_2O_2 in milkfish surimi slightly increased the gel strength and water holding capacity. Bhoite-Solomon et al. (1992) reported that H_2O_2 alone could lead the formation of S-S cross-linked aggregates of myosin. Benjakul et al. (2005) reported that addition of 0.05-0.1% AsA slightly increased gel strength of LZ and TB, due to recover of reactive of SH groups. Those additives produced the covalent bonds formed during SH groups oxidation, enhancing gel strength (Itoh, 1979). In addition, a combination of AsA and H_2O_2 exhibited greatly increase surimi gel-forming ability. AsA was likely to be oxidized in the presence of H_2O_2, generating DHA. DHA proposed to oxidize SH groups, resulting in S-S linkages of MHC and formed intermolecular bonds. Setting at 40 °C for 30 min did not promote the gel formation of control samples of LZ and TB surimi. However, the textural properties of set gels were

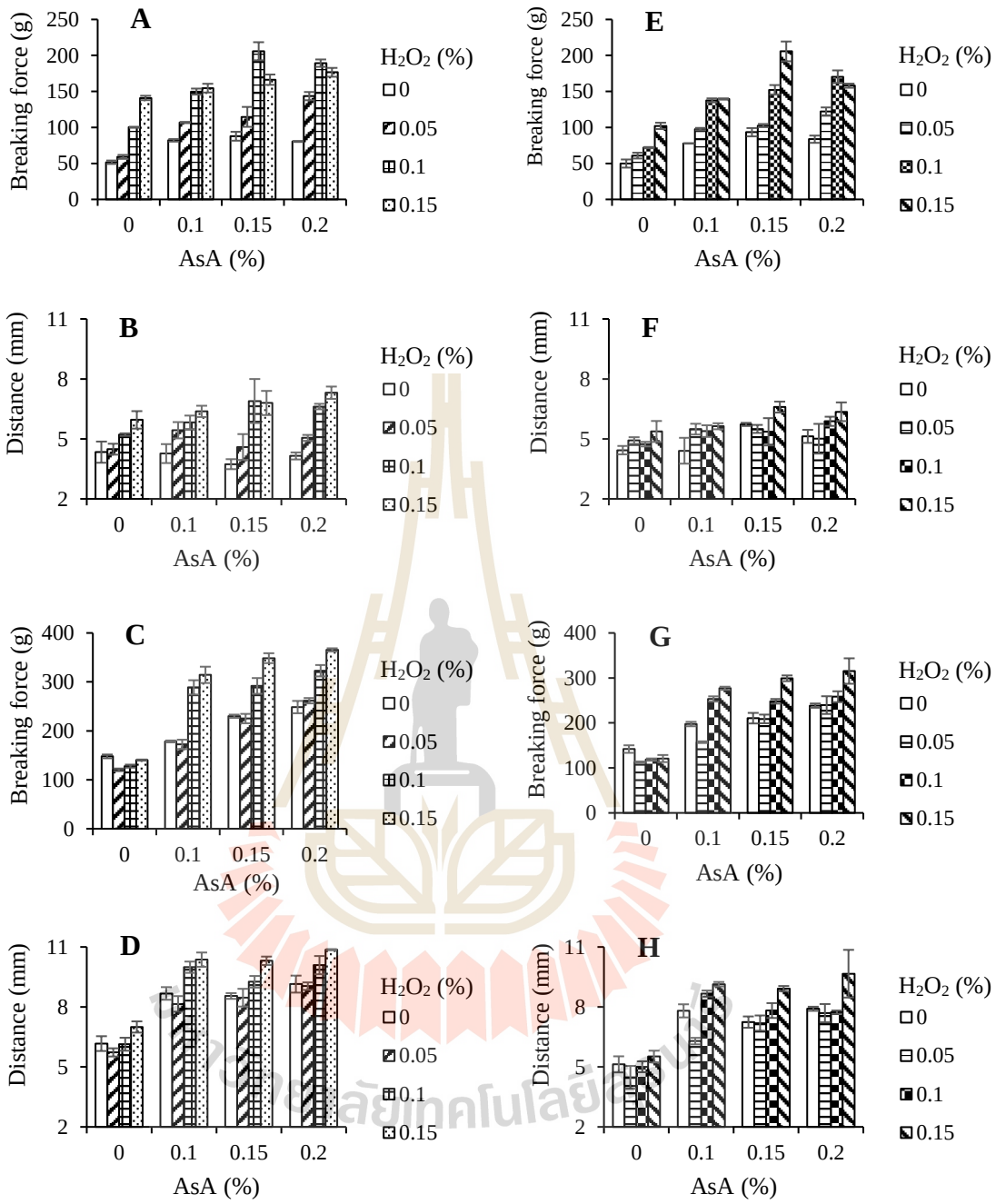

Figure 3.2 Breaking force (A, C, E, G) and distance (B, D, F, H) of surimi gels from LZ (A, B, E, F), and TB (C, D, G, H) added AsA and H_2O_2. A, B, C, D = direct heating at 90 °C for 30 min; E, F, G, H = setting at 40 °C for 30 min before heating at 90 °C for 30 min.

lower than those of directly cooked gel from both species (Figure 3.2E-H). Nishimura et al. (1990) were observed that gel strength of setting and direct heating of Alaska pollack was improved by adding 0.05-0.5% of AsA compared to the control gel. Benjakul et al. (2004) reported that a decrease in surimi gel strength from TB during setting caused by the degradation of protein induced by proteases. Rawdkuen and Benjakul (2008), LZ surimi also contains proteinases which be caused proteolytic degradation. Protein degradation caused by proteases during setting would lead to lesser gel enhancing effect of AsA and H_2O_2 when setting was applied.

3.4.3 Whiteness

Whiteness of all surimi gels is shown in Figures 3.3 and 3.4. Whiteness of LZ and TB surimi gels slightly increased with addition of H_2O_2 alone, when direct heating was applied (Figure 3.3A and B). AsA alone did not affect whiteness improvement of both surimi species. Combination of 0.2% AsA and 0.15% H_2O_2 decreased whiteness of LZ gel from 55.5 of control to 16.7, while whiteness of TB gel decreased from 54.9 to 38.9 ($P < 0.05$). Whiteness improvement by H_2O_2 was likely due to bleaching effect of H_2O_2 myoglobin.

Browning reaction occurred in surimi gels added the combination of 0.15-0.2% AsA and 0.15% H_2O_2 (Figure 3.4). Non-enzymatic browning occurred in the combination of AsA and H_2O_2 as known as ascorbic acid oxidation (Wedzicha 1984). Since H_2O_2 rapidly degrades to oxygen, promoting ascorbic acid oxidation. In the presence of oxygen, AsA degraded to DHA and is converted to xylosone. Interaction between amino acids and xylosone contributed to brown polymers (Corzo-Mart´ınez et al., 2012). Setting LZ and TB gels at 40 °C showed higher whiteness than directly heated gels (Figure 3.3C and D). According to Hwang et al. (2007), whiteness is also

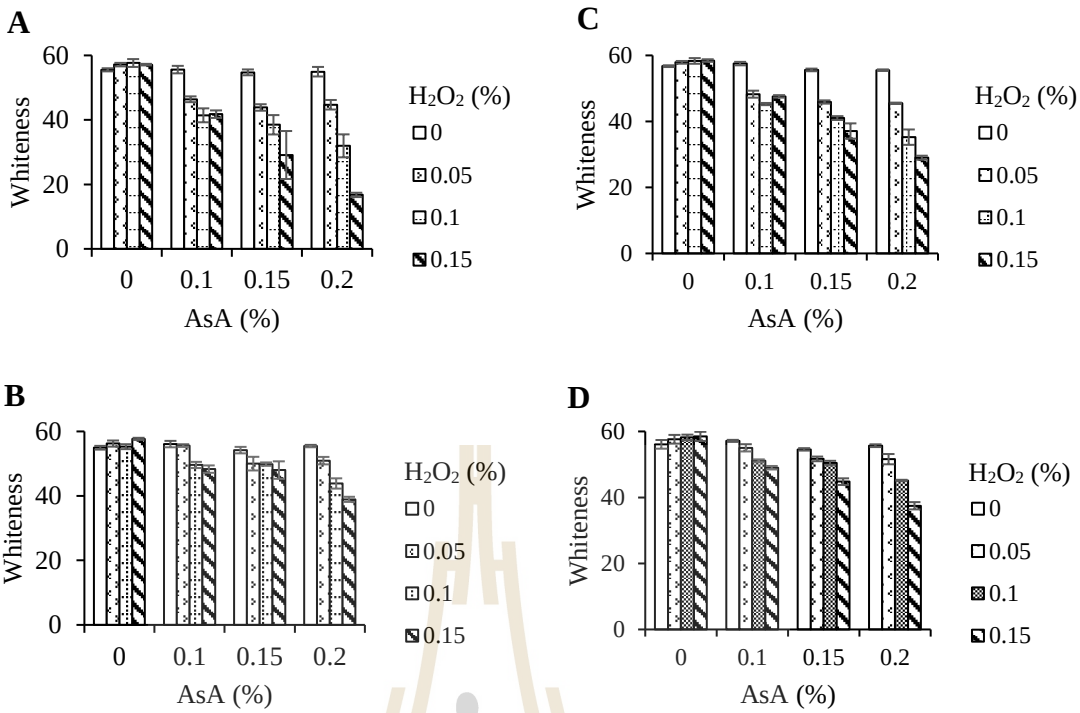

Figure 3.3 Effect of AsA and H_2O_2 on whiteness of surimi gels from LZ (A, C) and TB (B, D) which were cooked at 90 °C for 30 min (A, B) and set at 40 °C for 30 min (C, D).

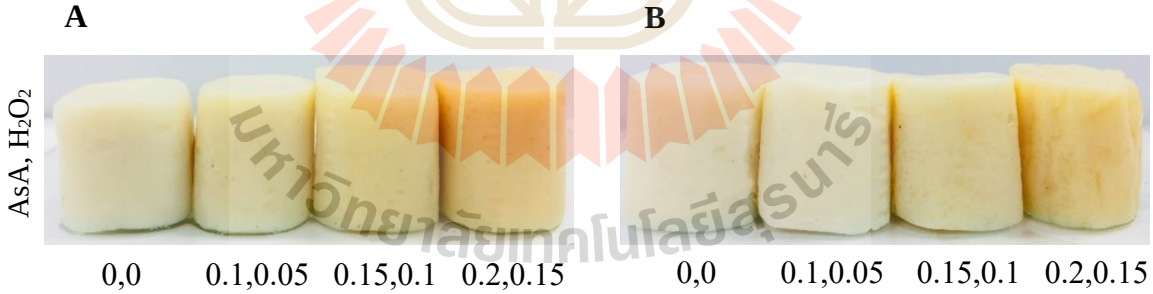

Figure 3.4 Effect of AsA and H_2O_2 on whiteness of surimi gels from LZ (A) and TB (B) which cooked at 90 °C for 30 min.

related to the degree of protein denaturation. Chaijan et al. (2010) reported that oxidation of myoglobin was susceptible by heat treatment, and high whiteness of gel from short-bodied mackerel surimi was found in kamaboko gel (setting at 40 °C for 30

min) as compared to directly cooked gel at 90 °C. H_2O_2 alone could be used to improve whiteness for LZ surimi gel without a negative effect on gel texture. The optimal concentration of AsA and H_2O_2 for both gel strength and whiteness are combined 0.15% AsA and 0.1% H_2O_2.

3.4.4 Disulfide linkage and protein pattern by SDS-PAGE

Protein of cooked gels with the addition of AsA and H_2O_2 were extracted using 10% SDS extraction buffer either in the presence or absence of β-mercaptoethanol (βME). In the buffer without βME, protein solubility of directly heated LZ gels added 0.15% H_2O_2 alone significantly decreased to 9.2 mg/ml as compared to the control gel (12.3 mg/ml), while AsA alone did not affect (Figure 3.5A). Protein solubility content of TB surimi gels extracted with SDS alone did not change by adding H_2O_2, while a slight decrease was noticed when added 0.2% AsA (Figure 3.5G). In the absence of βME, combined 0.2% AsA and 0.15% H_2O_2 provided the minimum amount of protein solubility of LZ and TB surimi directly cooked gels. Protein solubility has been used to detect myosin aggregation (Samejima et al., 1985). A decrease in protein solubility of gels by adding AsA and/or H_2O_2 suggested that protein unfolded and exposed hydrophobic residues to an aqueous solution, then caused protein aggregation through intermolecular disulfides and hydrophobic interactions. Adding βME in the extraction buffer led to an increase in protein solubility. This could be caused βME is a reducing agent which destroys S-S bonds (Hamada, 1992). In the presence of βME, protein solubility of LZ and TB surimi gels added 0.15% H_2O_2 increased when direct heating was applied. Addition of 0.2% AsA increased protein solubility of LZ and TB gels (Figure 3.5E and G). Moreover, protein solubility of LZ and TB directly cooked gels also increased with addition of combined 0.2% AsA and 0.15% H_2O_2. An increase

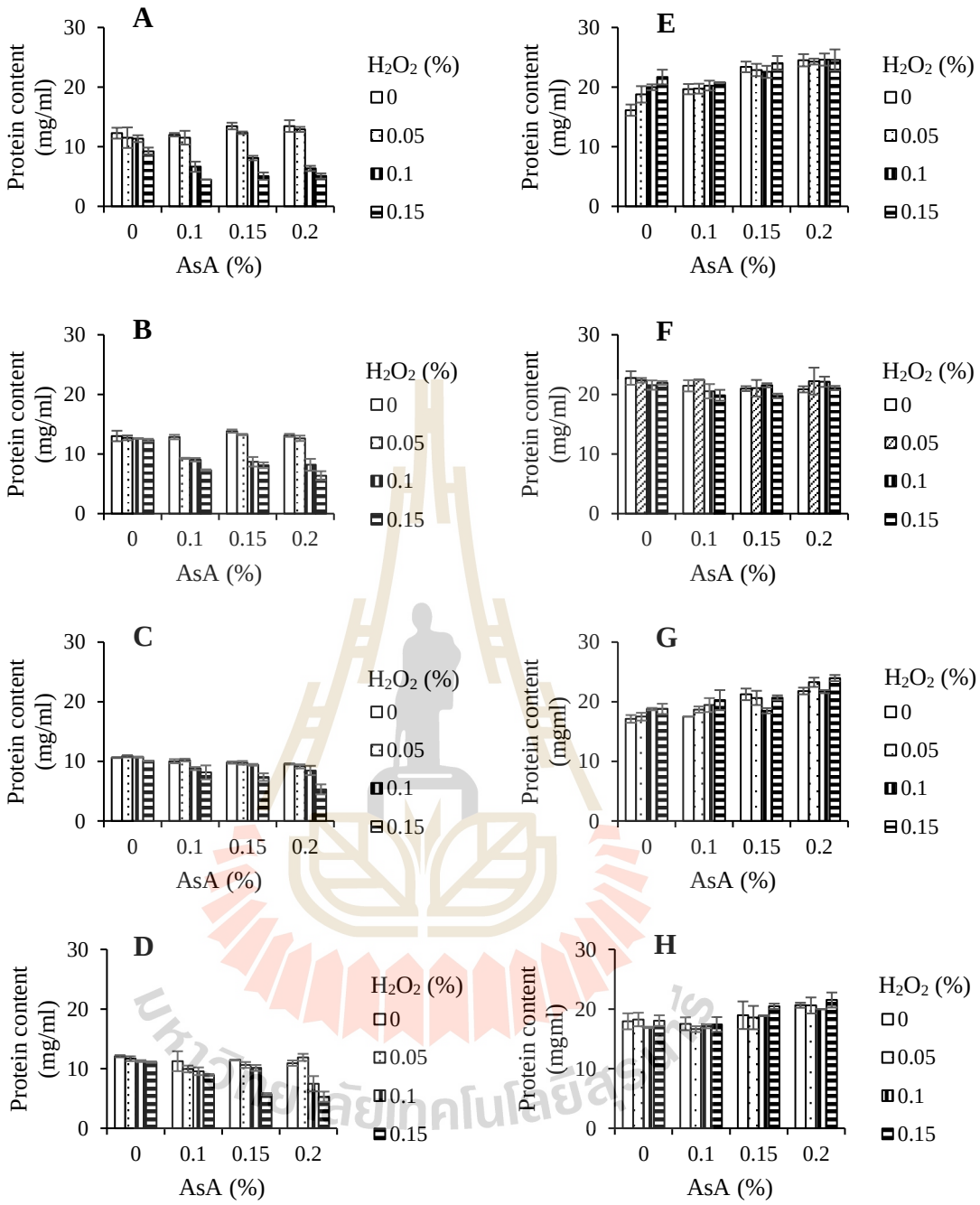

Figure 3.5 Protein solubility of surimi gels from LZ (A, B, F, G) and TB (C, D, G, H) cooked at 90 °C for 30 min (A, C, E, G) and set at 40 °C for 30 min (B, D, F, H), in the absence (A, B, C, D) and presence (E, F, G, H) of β-mercaptoehanol.

in protein solubility of gels by adding combined 0.2% AsA and 0.15% H_2O_2 in the extraction buffer containing βME, indicated that S-S linkages were enhanced by AsA and H_2O_2.

The addition of AsA and H_2O_2 in setting gels from both LZ and TB showed a similar effect on protein content of direct heating gels. However, protein solubility of set gels added AsA and/or H_2O_2 with extraction buffer either an absence or presence βME, showed less effect than direct heating (Figure 3.5B, D, F, and H). A lower effect in protein content was observed in setting gels from both LZ and TB surimi, this result was in agreement with the low gel improvement when setting was applied.

MHC band of LZ surimi gels were not observed in the cooked gel added AsA and H_2O_2 when extraction was made without βME, either cooked by direct heating or setting (Figure 3.6A). MHC band of TB gels decreased with increasing concentration of AsA and H_2O_2, especially, addition of 0.2% AsA and 0.15% H_2O_2 when extraction buffer without βME (Figure 3.6B). However, MHC band of both surimi species was recovered when buffer containing 10% βME was applied, indicating the involvement of S-S bonds on polymerization of MHC by AsA and H_2O_2 during direct heating or setting. These results were similar to Nishimura et al. (1996) who reported that MHC decreased by adding AsA when setting or direct heating was applied. Decreasing of MHC was due to polymerization by disulfide-bridging and the polymerization of MHC in surimi. These results indicated that H_2O_2 could cause the oxidation of MHC via S-S bonds formation, and AsA also accelerated the formation of S-S bonds. LZ is known as naturally undergoes textural degradation by endogenous protease (Suwansakornkul et al., 1993). Therefore, the disappearance of MHC in LZ gels during setting also could be due to proteolysis of MHC by endogenous protease. Figure 3.6B showed that there

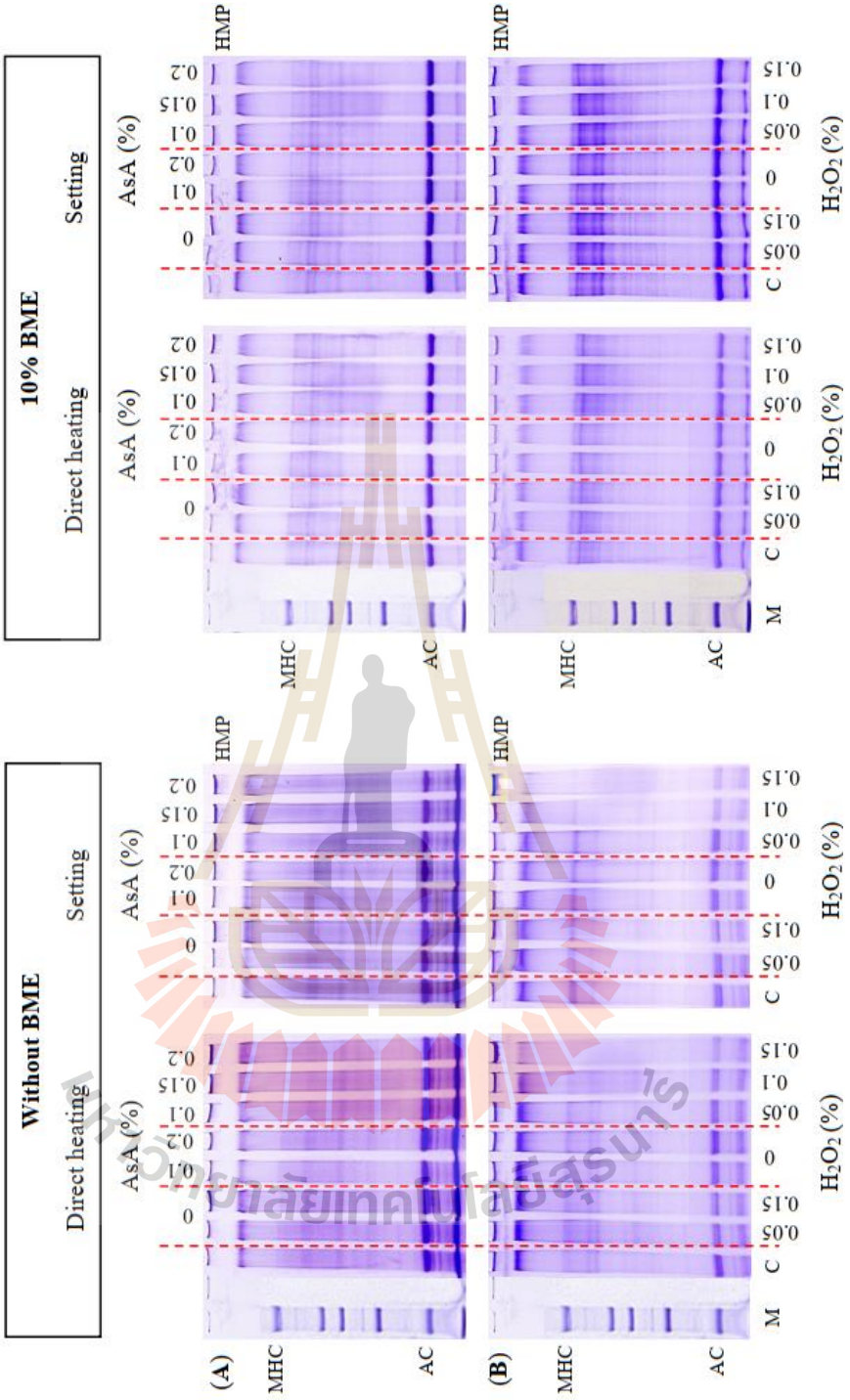

Figure 3.6 Effect of AsA and H_2O_2 on protein patterns of surimi gels from LZ (A) and TB (B) extracted in an absence and a presence of 10% βME, cooked by direct heating and setting. M: standard maker, C: control sample, HMP: high molecular weight proteins, MHC: Myosin heavy chain and AC: Actin.

were several bands under MHC in TB gel during setting when extraction buffer added βME was used. This might be degradation MHC by proteases. Lee et al. (1990) reported that proteolysis was detected during setting of TB surimi. These results suggested that proteolytic degradation occurred in both species when setting was applied. Furthermore, extraction buffer with SDS alone, high molecular weight proteins (HMP>205 kDa) was observed on the top of 7.5% acrylamide gel and increased in the samples of LZ and TB gels with addition 0.2% AsA and/or 0.15% H_2O_2. In the presence of 10% βME, HMP intensity of all samples reduced. These results suggested that MHC was likely to be polymerized via S-S bonds which were catalyzed by AsA and/or H_2O_2. This study suggested that cross-linked MHC was induced by S-S covalent bonds, and combination of 0.2% AsA and 0.15% H_2O_2 was the most effective.

3.4.5 TCA-soluble oligopeptide content

TCA-soluble oligopeptide content of LZ and TB surimi gels increased with increasing AsA concentrations ($P < 0.05$, Figure 3.7A and B) and slightly decreased by adding H_2O_2. These results suggested that AsA promoted proteolysis which could be induced by acid proteases, which are quite active at pH 5.5 or between 6.5-7.2 (An et al., 1994). Nevertheless, proteolytic degradation in surimi gels could be prevented by addition of H_2O_2. Taggart et al. (2000) reported that H_2O_2 can be used as a protease inhibitor by oxidation of protease. Setting at 40 °C for 30 min resulted in higher TCA-soluble oligopeptide content of both species as compared to the direct heating samples ($P < 0.05$, Figure 3.7C, D), suggesting that endogenous proteases were active during setting. Benjakul et al. (2004) reported that proteolytic activity of tropical fish including TB occurred during setting at 40 °C. TCA-soluble oligopeptide contents were in accordance with lower breaking force and distance of the LZ and TB surimi

gels during setting. Proteolysis during setting led to the degradation of myofibrillar proteins. Surimi gels set at 40 °C showed degradation products on the SDS-PAGE (Figure 3.6). It should be noted that although TCA-soluble oligopeptide content of LZ and TB surimi gels increased by addition of AsA, but their gel strength was increased. Result was in accordance with Benjakul et al. (2003), this caused the protein cross-linking occurs a greater extent than proteolysis. Result also showed that TCA-soluble oligopeptide of LZ was higher than TB species. LZ contains high endogenous proteolytic activity, leading it weaker gel textural as compared to TB gel (Yongsawatdigul and Piyadhammaviboon, 2004).

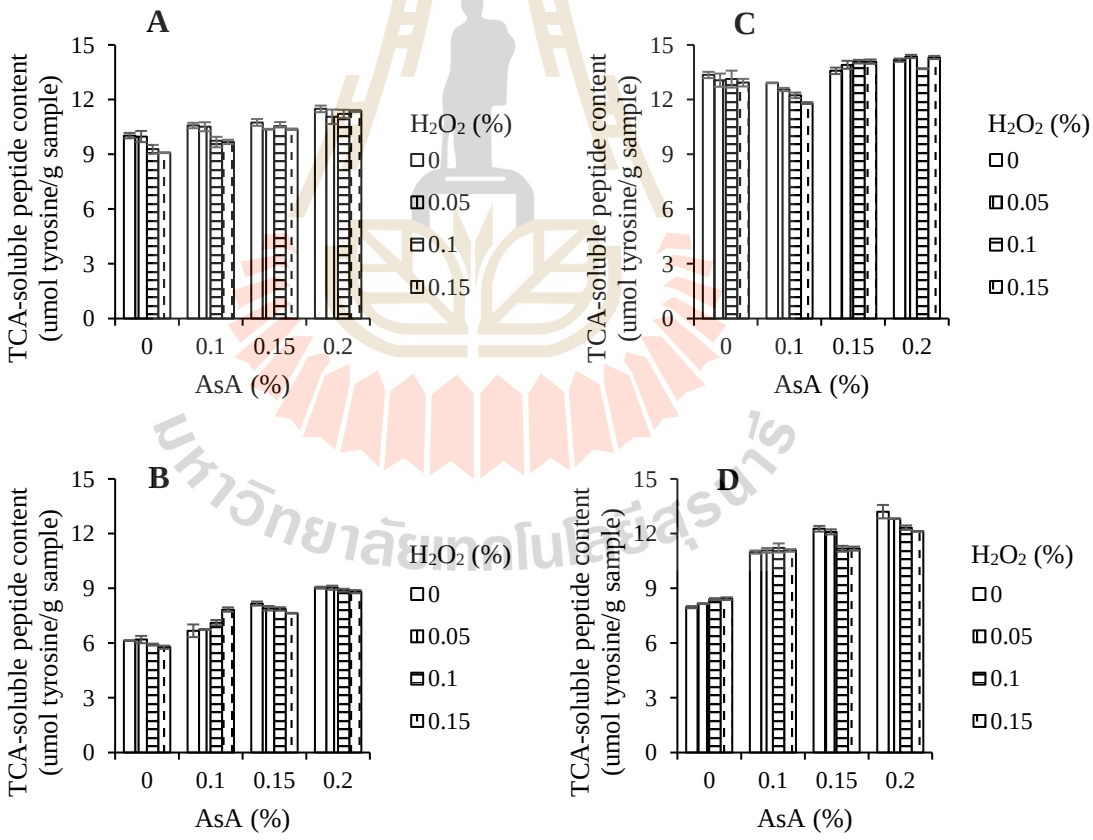

Figure 3.7 TCA-soluble oligopeptide content of surimi gels from LZ (A, C) and TB (B, D) cooked at 90 °C for 30 min (A, B) and set at 40 °C for 30 min (C, D).

3.4.6 Total SH group

To confirm the formation of S-S bonds in surimi gels, the content of TSH group was determined. Opstvedt et al. (1984) reported that the decrease in protein SH content is a result of the formation of S-S bonds through the oxidation of SH group. TSH group contents of LZ surimi gels decreased by adding 0.15% H_2O_2, when direct heating was applied, while it did not change by addition of AsA ($P < 0.05$, Figure 3.8A). H_2O_2 seems to affect TSH group contents, indicated that H_2O_2 is a stronger oxidizing agent that oxidized SH groups. A decrease of TSH group contents of TB surimi gels added AsA alone and directly cooked, occurred to a more extent than H_2O_2 alone (Figure 3.8B). This result was in accordance with that Nishimura and Ohtsuru (1990) who found that AsA promoted S-S bond formation. Combination of 0.2% AsA and 0.15% H_2O_2 minimum decreased TSH contents in both species. TSH group changes in setting at 40 °C exhibited a similar trend. Runglerdkriangkrai et al. (1999) reported that proteins unfolded, and the intra-SH group exposed to the surface of protein molecules and coincided with the formation of polymer by S-S bonds during heating. These results were coincidental with gel textural improvement added AsA and/or H_2O_2 (Figure 3.2). Combination of both additives was associated with the minimum TSH group contents.

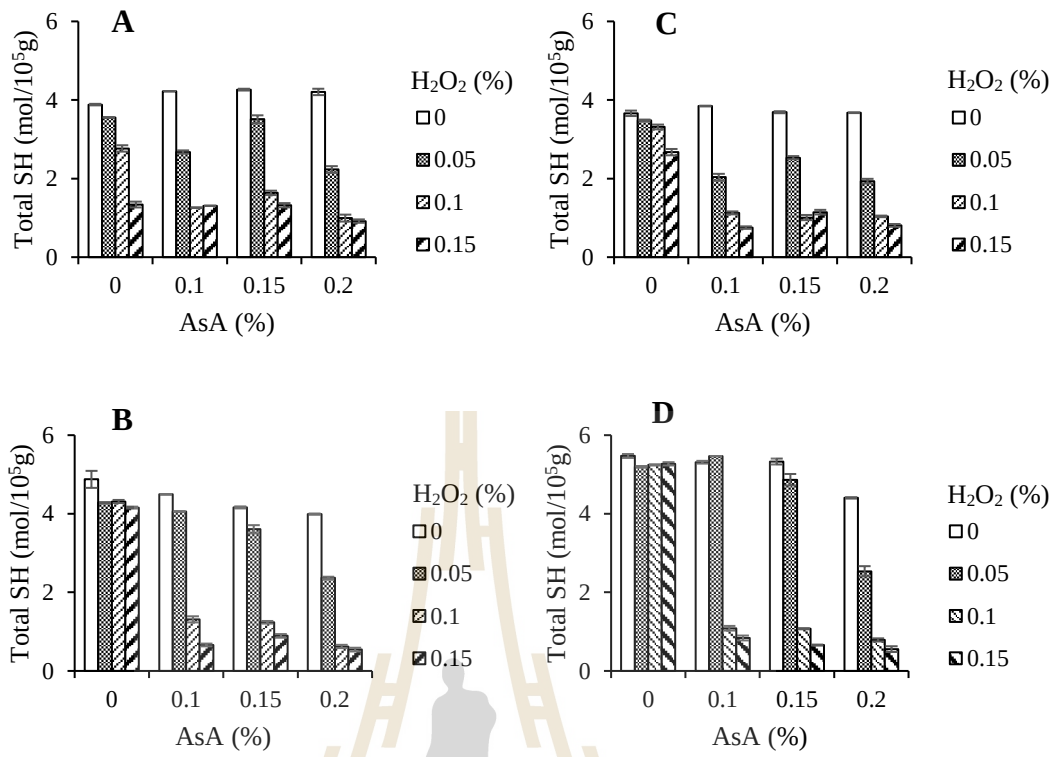

Figure 3.8 Total sulfhydryl group (TSH) content of surimi gels from LZ (A, C) and TB (B, D) cooked at 90 °C for 30 min (A, B) and set at 40 °C for 30 min (C, D).

3.4.7 Changes in secondary structure

Secondary structural changes were estimated by the amide I band (1600-1700 cm^{-1}) of FT-IR spectra. It involves mainly C=O stretching, C-N stretching, C-C-N bending, and N-H in-plane bending of peptide groups (Herrero, 2008). The amide I band generally contains information of α-helix (1650-1660 cm^{-1}), β-sheet (1665-1680 cm^{-1} and 1614-1637 cm^{-1}), β-turn (1680-1690 cm^{-1}) and random coil (1640-1642 cm^{-1}) structures (Cando et al., 2016). Addition of 0.15% H_2O_2 and/or 0.2% AsA decreased α-helix and increased β-sheet content of LZ pastes as compared to the control ($P < 0.05$, Figure 3.11A), whereas these additives did not affect β-turn. For TB pastes, α-helix also slightly a decreased and β-turn increased with the addition of AsA and H_2O_2, whereas

β-sheet was not affected ($P < 0.05$, Figure 3.11D). Both LZ and TB surimi gels cooked by direct heating or setting showed a decrease in α-helix and an increase in β-sheet with addition of 0.2% AsA and 0.15% H_2O_2 ($P < 0.05$, Figure 3.11B-F). The β-turn also slightly increased with increasing additives. The increase of β-sheet content in surimi gel suggested that surimi gels might be more involved with the formation of H-bondings (Kobayashi et al., 2017). These results demonstrated that H_2O_2 has a greater effect on secondary structural changes than AsA. Unfolding and changes in secondary structure occurred to a greater extent when H_2O_2 was added. AsA showed a minimal effect on structure, suggesting that protein unfolding is less affected by AsA. An increase in β-sheet content indicates that denatured protein might be aggregated. In addition, the greater extent of changes of α-helix and β-sheet of LZ and TB surimi gels were evident in 90 °C-cooked gel (Figure 3.11B and E). Moreover, changes of α-helix and β-sheet content of both surimi species after cooked by direct heating or setting were greater than paste. Secondary structure changes upon heating was similar to Bouraoui et al. (1997) and Sanchez-Gonzalez et al. (2008). Gelation occurs via the unfolding of α-helix structure to β-sheet upon setting or direct heating at 90 °C.

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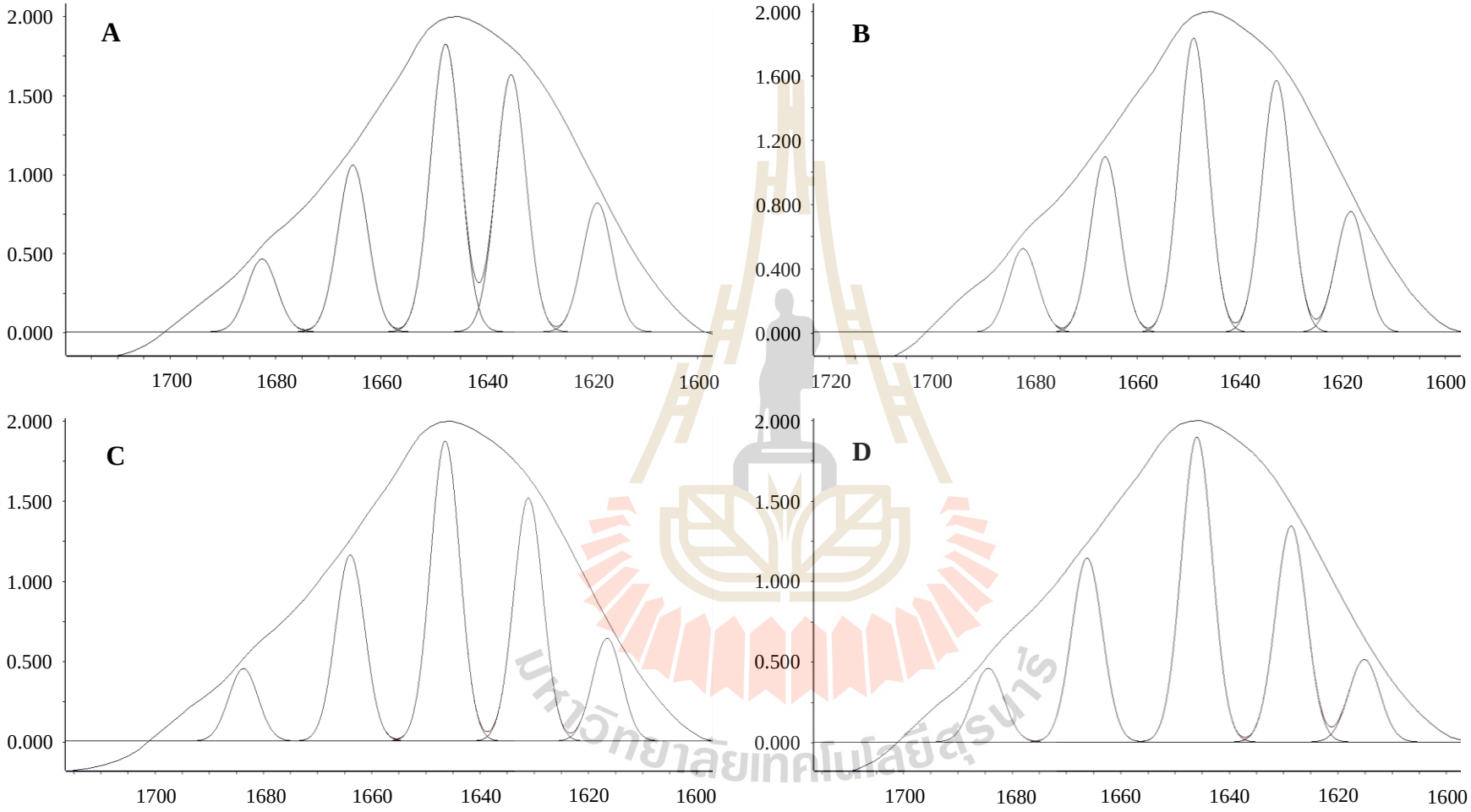

Figure 3.9 Curve-fitted amide I FT-IR bands of lyophilized LZ surimi paste added various additives: control (A), 0.15% H_2O_2 (B), 0.2% AsA (C), and combined 0.2% AsA and 0.15% H_2O_2.

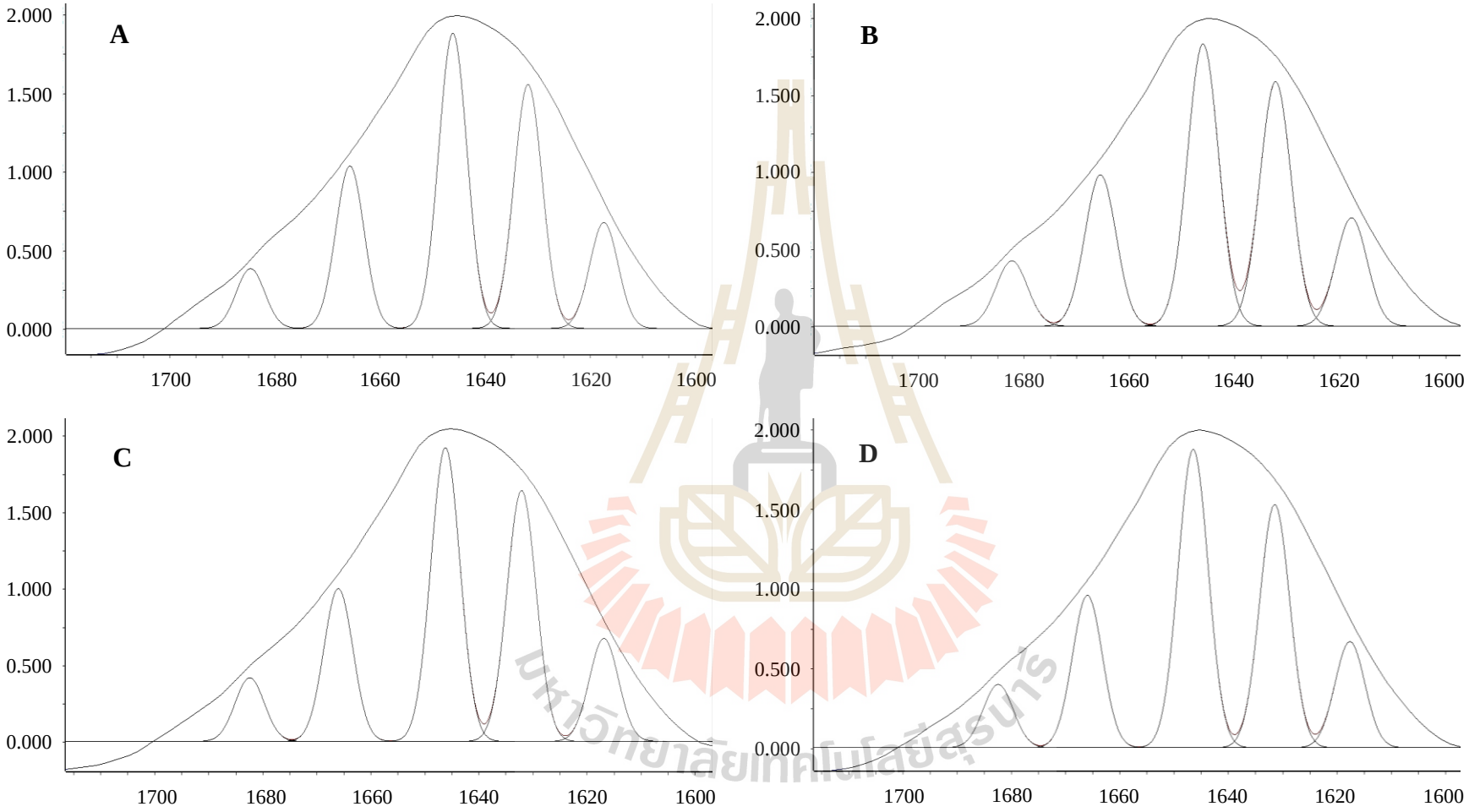

Figure 3.10 Curve-fitted amide I FT-IR bands of lyophilized TB surimi paste added various additives: control (A), 0.15% H_2O_2 (B), 0.2% AsA (C), and combined 0.2% AsA and 0.15% H_2O_2.

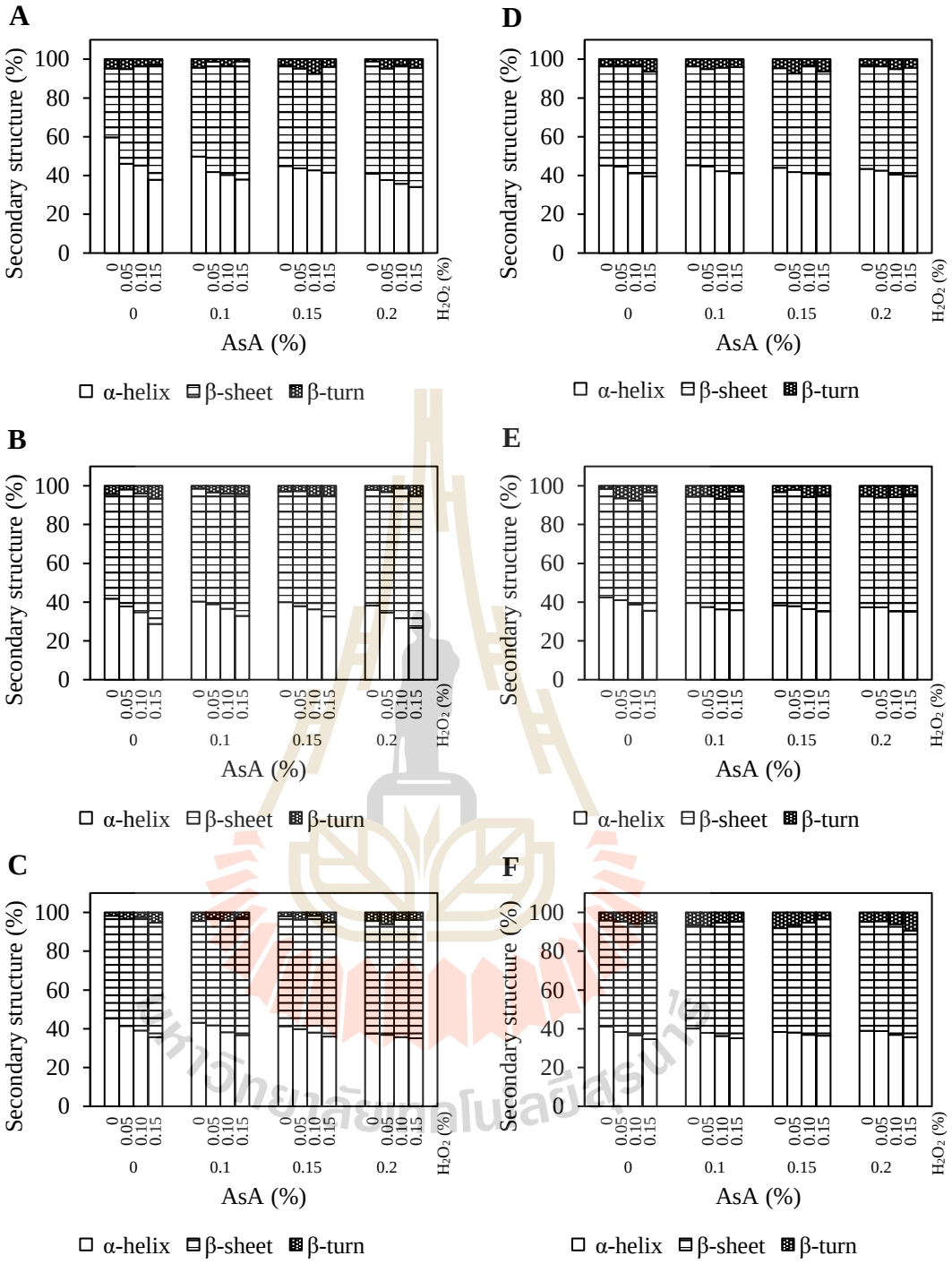

Figure 3.11 Changes of secondary structure determination from FT-IR spectra of surimi pastes (A, D), gels cooked at 90 °C for 30 min (B, E), and gels set at 40 °C for 30 min before cooking at 90 °C for 30 min (C, F). LZ (A, B, C) and TB (D, E, F) surimi.

3.4.8 Tertiary structures

The wavenumber between 2800-3000 cm^{-1} region of the Raman spectrum is assigned to C-H stretching including CH_2 asymmetric and CH_3 symmetric stretching vibrations of aliphatic residues. This provides information about the hydrophobic interactions of aliphatic residues (Li-Chan et al., 1994). Addition of 0.15% H_2O_2 and/or 0.2% AsA decreased C-H band at 2936 cm^{-1} in both LZ and TB surimi ($P < 0.05$, Table appendix 1-6). This was likely C-H group due to hydrophobic interaction by addition of AsA and H_2O_2. Moreover, C-H stretching bands at 2874 and 2980 cm^{-1} of both surimi species also decreased with addition of H_2O_2 and/or AsA, as compared with the control sample ($P < 0.05$). Wang and Damodaran (1991) reported that cooked gel might result in the destruction of the proteins tertiary. These results designated that a decrease in bands near 2878, 2936 and 2980 cm^{-1} demonstrated involvement of hydrophobic interaction enhanced by the addition of H_2O_2 and AsA in direct heating or setting.

Raman band at 1450 cm^{-1} is assigned to C-H bending vibrations. Addition of 0.15% H_2O_2 and/or 0.2% AsA decreased band at 1450 cm^{-1} of LZ and TB pastes, and of directly cooked or set gels as compared to the control (Table appendix 1-6). Therefore, it is postulated that addition of these additives could enhance the protein unfolding of both surimi paste and gel either from direct heating or setting. Kobayashi (2017) reported that a decrease in band intensity of the band at 1450 cm^{-1} could improve the unfolding protein structure. A decrease in band area at 1450 cm^{-1} was observed in gel more than in paste, indicating hydrophobic interactions via aliphatic residues were promoted during gelation. A decrease in band area with the addition of AsA and H_2O_2 indicated that hydrophobic interactions can be enhanced with addition of AsA and H_2O_2.

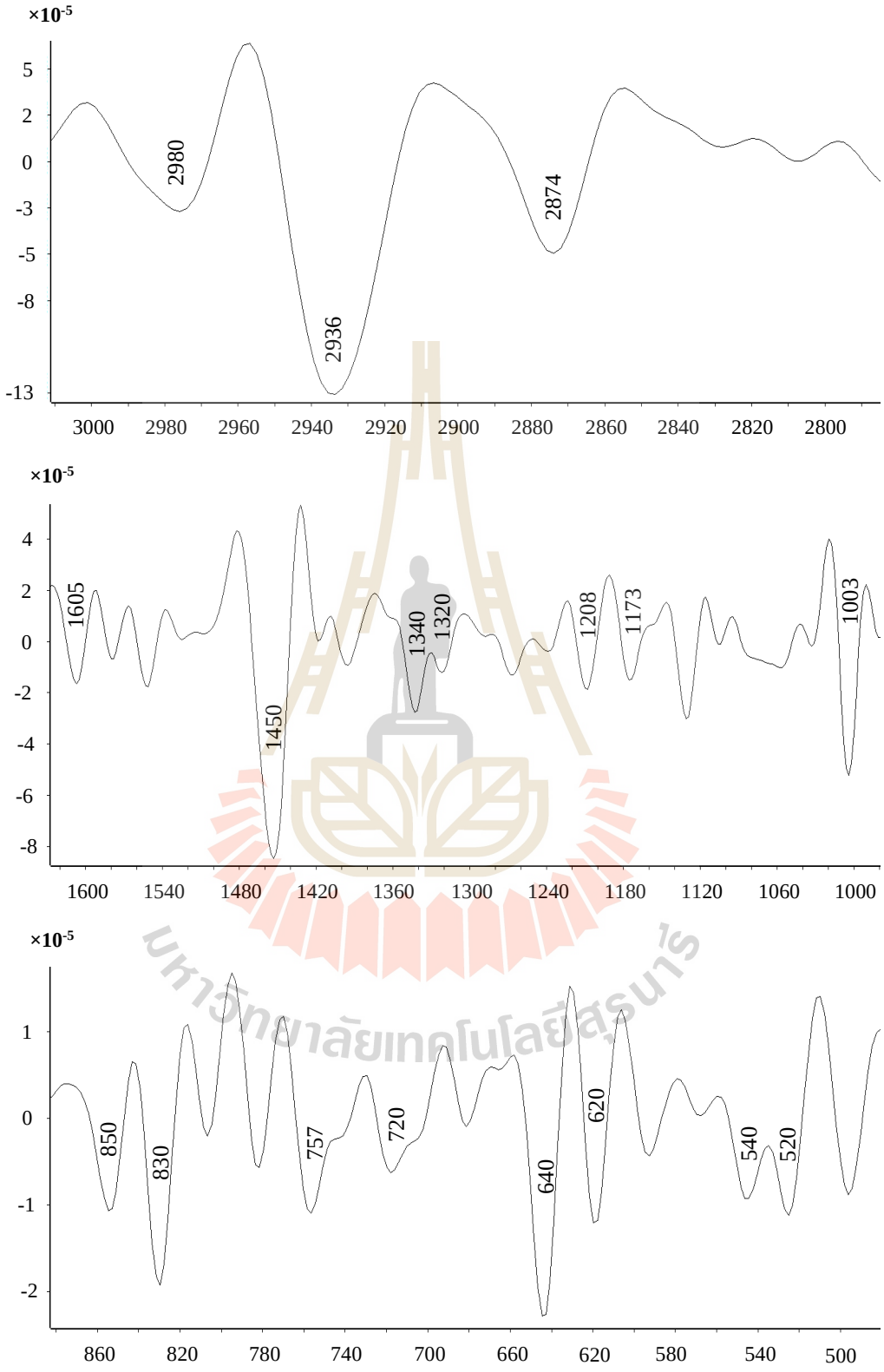

Figure 3.12 Second derivative of control LZ surimi paste of the selected regions.

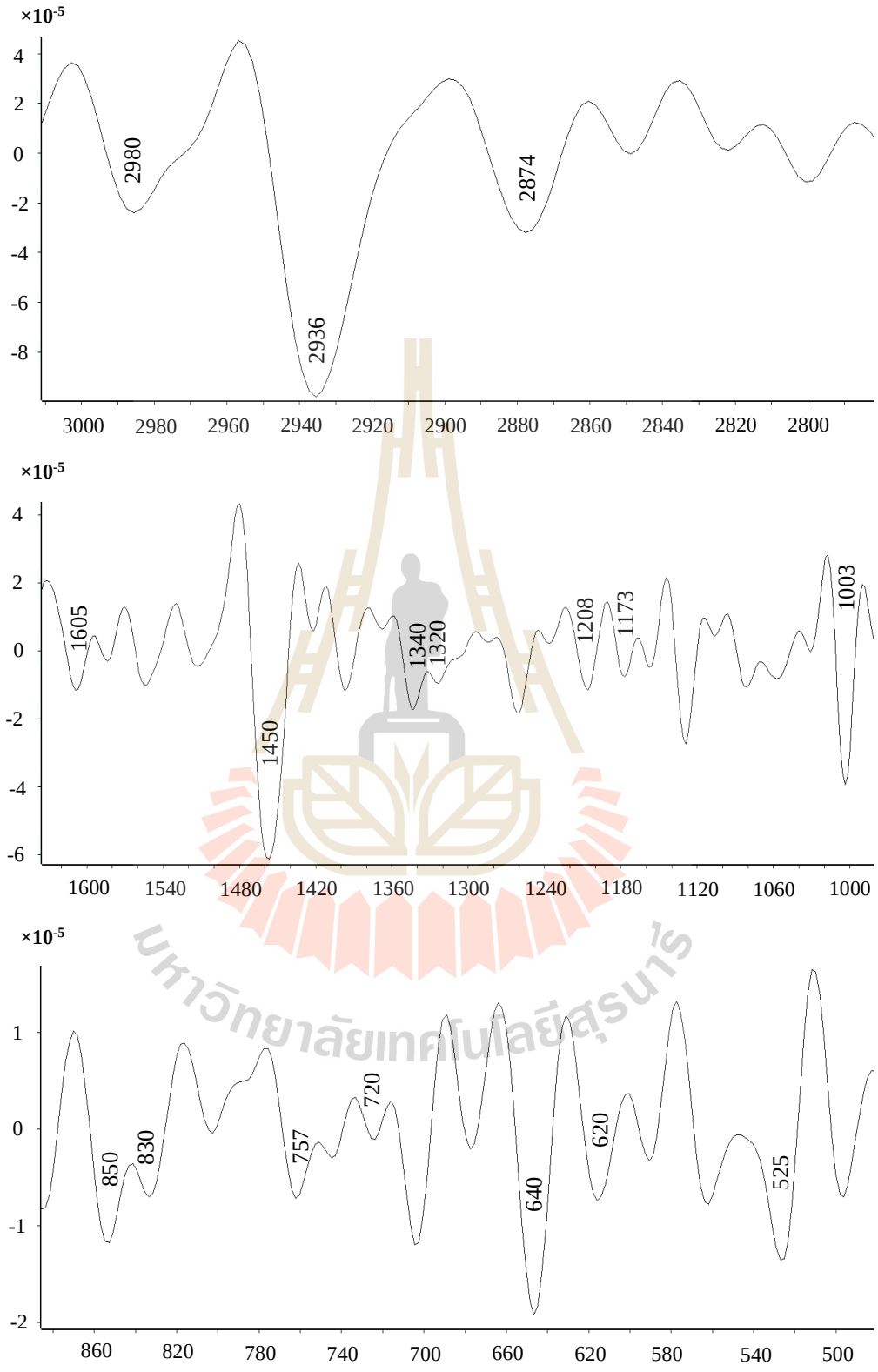

Figure 3.13 Second derivative of control TB surimi paste of the selected regions.

Furthermore, addition of 0.15% H_2O_2 and/or 0.2% AsA decreased the band tryptophan (Trp) at 1340 cm^{-1} of LZ or TB surimi pastes and gel cooked by direct heating or setting ($P < 0.05$). Li-Chan (1996) reported that when Trp residues from a buried hydrophobic microenvironment exposed to the polar aqueous solvent, the band near 760 cm^{-1} regions decreased. The band at 760 cm^{-1} of both pastes and set gels decreased by addition of 0.2% AsA and 0.15% H_2O_2 ($P < 0.05$), as compared to the control sample. However, this band in LZ and TB directly cooked gels did not significantly decrease after added these additives. These changes indicated that Trp residues buried in the tertiary structure exposed and formed hydrophobic interaction during gelation.

The ratio 850/830 cm^{-1} of tyrosine (Tyr) band has been used to monitor the involvement of the phenolic hydroxyl group in hydrogen bonding and structural change of protein (Herrero, 2008). When the ratio is ≥ 1.0, it indicated that the Tyr residues can be considered as “exposed”; conversely, Tyr are “buried” when this ratio is lower than 1.0 (Thawornchinsombut et al., 2006). The Tyr ratio of LZ and TB including pastes and cooked gels were found less than 1.0 (Table 3.1 and 3.2), which indicated that addition of AsA and H_2O_2 caused Tyr residues of proteins buried to the hydrophobic environment. Addition of 0.15% H_2O_2 and/or 0.2% AsA decreased Tyr doublet ratio in surimi paste of both species compared to the control sample ($P < 0.05$). Moreover, the ratio of 850/830 cm^{-1} of gel cooked by direct heating or setting was lower than surimi paste, but it did not significantly change by addition of AsA and/or H_2O_2. Bouraoui et al. (1997) reported that Tyr doublet ratio decreased upon gelation. This might be proposed that Tyr residues decrease during gelation due to hydrophobic environment. Directly cooked gel of LZ surimi showed a higher 850/830 cm^{-1} ratio as compared to

set gels, indicated that set gels appeared to have more interaction of Tyr. However, Tyr ratio of set gel from TB surimi was higher than directly cooked gel, suggesting that directly cooked gel produced more Tyr interaction in TB surimi. Our study showed that Tyr residues were mainly buried in the hydrophobic environment and involved Tyr residues as strong hydrogen bond donors with the addition of AsA and H_2O_2.

Table 3.1 and 3.2 exhibited that changes in the hydrophobic environment around aliphatic and aromatic were indicated by a decrease in total aliphatic and aromatic residues of LZ and TB surimi with increasing AsA and H_2O_2 followed gelation ($P < 0.05$). Additionally, a combination of H_2O_2 and AsA resulted in protein denaturation and hydrophobic interaction in both LZ and TB surimi.

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Table 3.1 Integral area of Raman spectra of LZ surimi as affected by various concentrations of AsA and H_2O_2.

Additives (%)		Paste			Direct heating			Setting		
AsA	**H_2O_2**	**Aliphatic ($\times10^{-3}$)**	**Aromatic ($\times10^{-3}$)**	**850/830**	**Aliphatic ($\times10^{-3}$)**	**Aromatic ($\times10^{-3}$)**	**850/830**	**Aliphatic ($\times10^{-3}$)**	**Aromatic ($\times10^{-3}$)**	**850/830**
0	0	7.29 ± 0.09 ^{a}	2.36 ± 0.27	0.98 ± 0.21 ^{a}	8.68 ± 0.25 ^{a}	2.50 ± 0.15 ^{bc}	0.74 ± 0.09 ^{ab}	8.64 ± 0.13 ^{a}	2.81 ± 0.09 ^{a}	0.54 ± 0.16
	0.05	6.50 ± 0.15 ^{b}	1.91 ± 0.18	0.63 ± 0.17 ^{bcd}	7.97 ± 0.12 ^{b}	2.22 ± 0.23 ^{de}	0.32 ± 0.07 ^{d}	8.21 ± 0.43 ^{ab}	2.63 ± 0.10 ^{abc}	0.53 ± 0.09
	0.1	6.31 ± 0.24 ^{bc}	1.86 ± 0.12	0.94 ± 0.34 ^{ab}	7.69 ± 0.14 ^{cde}	2.18 ± 0.15 ^{de}	0.30 ± 0.09 ^{d}	8.03 ± 0.22 ^{bc}	2.45 ± 0.21 ^{bcd}	0.67 ± 0.16
	0.15	5.67 ± 0.13 ^{def}	1.82 ± 0.05	0.41 ± 0.08 ^{cde}	7.98 ± 0.21 ^{b}	2.12 ± 0.11 ^{e}	0.37 ± 0.05 ^{d}	8.29 ± 0.27 ^{ab}	2.67 ± 0.08 ^{abc}	0.63 ± 0.18
0.1	0	6.13 ± 0.12 ^{bcd}	220 ± 0.19	0.33 ± 0.05 ^{cde}	7.84 ± 0.34 ^{bc}	2.34 ± 0.13 ^{cde}	0.74 ± 0.22 ^{ab}	7.55 ± 0.16 ^{cd}	2.60 ± 0.30 ^{abc}	0.55 ± 0.16
	0.05	5.77 ± 0.09 ^{def}	2.00 ± 0.09	0.48 ± 0.05 ^{cde}	7.72 ± 0.05 ^{bcd}	2.87 ± 0.25 ^{a}	0.80 ± 0.11 ^{a}	7.63 ± 0.15 ^{cd}	2.80 ± 0.03 ^{def}	0.48 ± 0.08
	0.1	5.95 ± 0.25 ^{def}	2.19 ± 0.20	0.53 ± 0.02 ^{cde}	7.78 ± 0.22 ^{bcd}	2.41 ± 0.08 ^{bcd}	0.35 ± 0.08 ^{d}	7.63 ± 0.13 ^{cd}	2.67 ± 0.14 ^{ab}	0.37 ± 0.07
	0.15	5.89 ± 0.19 ^{def}	1.81 ± 0.47	0.42 ± 0.17 ^{cde}	7.51 ± 0.06 ^{def}	2.88 ± 0.09 ^{a}	0.39 ± 0.01 ^{d}	7.45 ± 0.87 ^{cd}	2.49 ± 0.29 ^{bcd}	0.47 ± 0.19
0.15	0	5.89 ± 0.63 ^{def}	1.67 ± 0.25	0.56 ± 0.14 ^{cde}	7.72 ± 0.30 ^{bcd}	2.63 ± 0.31 ^{ab}	0.61 ± 0.08 ^{bc}	7.58 ± 0.13 ^{cd}	2.51 ± 0.11 ^{bcd}	0.37 ± 0.10
	0.05	6.06 ± 0.37 ^{cde}	2.03 ± 0.31	0.27 ± 0.12 ^{e}	7.75 ± 0.21 ^{bcd}	2.35 ± 0.14 ^{cde}	0.43 ± 0.13 ^{cd}	7.34 ± 0.05 ^{de}	2.06 ± 0.05 ^{f}	0.41 ± 0.11
	0.1	5.60 ± 0.42 ^{def}	220 ± 0.39	0.43 ± 0.15 ^{cde}	7.95 ± 0.31 ^{b}	2.46 ± 0.07 ^{bcd}	0.64 ± 0.05 ^{ab}	7.31 ± 0.09 ^{de}	2.10 ± 0.14 ^{f}	0.46 ± 0.07
	0.15	5.66 ± 0.44 ^{def}	1.76 ± 0.17	0.66 ± 0.16 ^{bc}	7.18 ± 0.23 ^{f}	2.14 ± 0.04 ^{e}	0.80 ± 0.11 ^{a}	6.84 ± 0.36 ^{e}	2.06 ± 0.03 ^{f}	0.45 ± 0.29
0.2	0	5.49 ± 0.18 ^{f}	1.83 ± 0.13	0.53 ± 0.18 ^{cde}	7.44 ± 0.14 ^{def}	2.36 ± 0.12 ^{cde}	0.44 ± 0.07 ^{cd}	7.29 ± 0.33 ^{de}	2.28 ± 0.09 ^{def}	0.59 ± 0.14
	0.05	563 ± 0.09 ^{def}	1.81 ± 0.31	0.54 ± 0.40 ^{cde}	7.60 ± 0.11 ^{cde}	2.23 ± 0.02 ^{cde}	0.66 ± 0.11 ^{ab}	7.47 ± 0.25 ^{cd}	2.37 ± 0.08 ^{cde}	0.29 ± 0.13
	0.1	5.57 ± 0.11 ^{ef}	2.09 ± 0.26	0.30 ± 0.10 ^{ade}	7.63 ± 0.13 ^{cde}	2.42 ± 0.15 ^{bcd}	0.43 ± 0.07 ^{cd}	7.45 ± 0.04 ^{cd}	2.40 ± 0.10 ^{bcd}	0.63 ± 0.08
	0.15	5.51 ± 0.31 ^{ef}	1.86 ± 0.07	0.54 ± 0.02 ^{cde}	7.30 ± 0.11 ^{ef}	2.20 ± 0.15 ^{de}	0.43 ± 0.12 ^{cd}	7.10 ± 0.06 ^{de}	2.22 ± 0.07 ^{ef}	0.47 ± 0.14

Data were given as mean ± SD. Different letters within the same column indicate significant differences ($P < 0.05$) between mean values.

Table 3.2 Integral area of Raman spectra of TB surimi as affected by various concentrations of AsA and H_2O_2.

Additives (%)		Paste			Direct heating			Setting		
AsA	**H_2O_2**	**Aliphatic ($\times10^{-3}$)**	**Aromatic ($\times10^{-3}$)**	**850/830**	**Aliphatic ($\times10^{-3}$)**	**Aromatic ($\times10^{-3}$)**	**850/830**	**Aliphatic ($\times10^{-3}$)**	**Aromatic ($\times10^{-3}$)**	**850/830**
0	0	6.29 ± 0.07 ^{a}	1.97 ± 0.14	0.80 ± 0.04 ^{a}	7.63 ± 0.04 ^{a}	2.81 ± 0.04 ^{a}	0.37 ± 0.00	8.42 ± 0.08 ^{a}	3.26 ± 0.03 ^{a}	0.55 ± 0.27
	0.05	5.71 ± 0.24 ^{cde}	1.64 ± 0.00	0.38 ± 0.20 ^{cd}	7.55 ± 0.18 ^{ab}	2.60 ± 0.15 ^{abc}	0.71 ± 0.09	7.16 ± 0.04 ^{cde}	2.59 ± 0.08 ^{bc}	0.32 ± 0.16
	0.1	5.58 ± 0.27 ^{def}	1.73 ± 0.19	0.51 ± 0.12 ^{bcd}	7.05 ± 0.30 ^{cde}	2.71 ± 0.14 ^{a}	0.29 ± 0.08	7.28 ± 0.17 ^{cde}	2.46 ± 0.18 ^{cd}	0.64 ± 0.08
	0.15	5.49 ± 0.19 ^{ef}	1.73 ± 0.37	0.49 ± 0.17 ^{bcd}	6.52 ± 0.23 ^{h}	2.35 ± 0.06 ^{cd}	0.31 ± 0.09	6.79 ± 0.25 ^{f}	2.10 ± 0.04 ^{f}	0.46 ± 0.03
0.1	0	5.99 ± 0.03 ^{abc}	1.99 ± 0.02	0.41 ± 0.17 ^{bcd}	7.42 ± 0.13 ^{abc}	2.64 ± 0.29 ^{ab}	0.46 ± 0.01	7.70 ± 0.04 ^{b}	2.74 ± 0.13 ^{cd}	0.48 ± 0.12
	0.05	5.92 ± 0.23 ^{abc}	2.30 ± 0.09	0.48 ± 0.04 ^{bcd}	7.21 ± 0.30 ^{bcd}	2.43 ± 0.11 ^{bcd}	0.49 ± 0.04	7.36 ± 0.34 ^{bc}	2.29 ± 0.12 ^{def}	0.39 ± 0.12
	0.1	6.18 ± 0.18 ^{ab}	2.23 ± 0.30	0.66 ± 0.24 ^{ab}	7.57 ± 0.07 ^{ab}	2.25 ± 0.04 ^{d}	0.31 ± 0.13	7.42 ± 0.17 ^{bc}	2.30 ± 0.15 ^{def}	0.29 ± 0.07
	0.15	5.73 ± 0.25 ^{cde}	1.85 ± 0.11	0.46 ± 0.09 ^{bcd}	7.31 ± 0.15 ^{abc}	2.32 ± 0.24 ^{d}	0.32 ± 0.19	7.32 ± 0.37 ^{cd}	2.36 ± 0.17 ^{cde}	0.42 ± 0.07
0.15	0	6.13 ± 0.02 ^{abc}	1.90 ± 0.04	0.40 ± 0.12 ^{bcd}	6.83 ± 0.21 ^{fgh}	2.28 ± 0.08 ^{d}	0.36 ± 0.12	7.20 ± 0.12 ^{cde}	2.38 ± 0.20 ^{cde}	0.49 ± 0.11
	0.05	5.80 ± 0.13 ^{bcd}	1.53 ± 0.06	0.60 ± 0.19 ^{abc}	6.84 ± 0.36 ^{efg}	2.23 ± 0.05 ^{d}	0.38 ± 0.20	7.14 ± 0.15 ^{cde}	2.32 ± 0.13 ^{def}	0.41 ± 0.14
	0.1	5.78 ± 0.04 ^{bcd}	1.75 ± 0.16	0.32 ± 0.08 ^{d}	6.80 ± 0.13 ^{fg}	2.32 ± 0.05 ^{d}	0.42 ± 0.11	7.15 ± 0.31 ^{cde}	2.22 ± 0.16 ^{def}	0.37 ± 0.10
	0.15	5.77 ± 0.31 ^{bcd}	1.77 ± 0.12	0.27 ± 0.02 ^{d}	6.90 ± 0.07 ^{efg}	2.37 ± 0.15 ^{cd}	0.37 ± 0.05	6.95 ± 0.22 ^{def}	2.15 ± 0.10 ^{ef}	0.32 ± 0.16
0.2	0	5.90 ± 0.14 ^{abc}	1.79 ± 0.05	0.30 ± 0.12 ^{d}	6.64 ± 0.21 ^{gh}	2.64 ± 0.13 ^{ab}	0.57 ± 0.13	6.91 ± 0.12 ^{ef}	2.17 ± 0.15 ^{def}	0.42 ± 0.11
	0.05	5.89 ± 0.24 ^{abc}	1.95 ± 0.04	0.36 ± 0.11 ^{cd}	7.07 ± 0.07 ^{cde}	2.28 ± 0.14 ^{d}	0.39 ± 0.21	7.10 ± 0.14 ^{cde}	2.47 ± 0.14 ^{cde}	0.44 ± 0.03
	0.1	5.58 ± 0.30 ^{def}	2.16 ± 0.11	0.62 ± 0.13 ^{abc}	7.12 ± 0.15 ^{cde}	2.26 ± 0.19 ^{d}	0.41 ± 0.12	7.20 ± 0.08 ^{cde}	2.32 ± 0.15 ^{def}	0.43 ± 0.06
	0.15	5.26 ± 0.40 ^{f}	2.00 ± 0.08	0.50 ± 0.18 ^{bcd}	6.99 ± 0.19 ^{def}	2.22 ± 0.08 ^{d}	0.24 ± 0.27	6.95 ± 0.18 ^{def}	2.24 ± 0.10 ^{def}	0.43 ± 0.04

Data were given as mean ± SD. Different letters within the same column indicate significant differences ($P < 0.05$) between mean values.

3.4.9 Disulfide bond regions

S-S bond plays an important role in the gelation process. The characteristic frequency of S-S bond in Raman spectra present at bands 500-550 cm^{-1}, containing three types stretching vibration of S-S bridge, including *g-g-g*, *g-g-t*, and *t-g-t*, where "*g*" and "*t*" is gauche and trans, respectively (Li-Chan et al., 1994). In this study, the bands at 525 and 540 cm^{-1} were revealed to S-S formation in the *g-g-t* and *t-g-t* conformation, respectively. The S-S stretching vibration of LZ surimi paste around 525 cm^{-1} increased ($P < 0.05$) and 540 cm^{-1} decreased ($P > 0.05$) with increasing AsA and H_2O_2 (Table 3.3). Addition of AsA and H_2O_2 did not significantly change S-S stretching vibration of LZ directly cooked gel. Moreover, this S-S stretching vibration of LZ set gel around 525 cm^{-1} increased ($P < 0.05$) and 540 cm^{-1} decreased ($P > 0.05$) after added 0.2% AsA and 0.15% H_2O_2. Addition of AsA and/or H_2O_2 increased band area around 525 ($P < 0.05$) and 540 cm^{-1} ($P > 0.05$) of TB surimi pastes (Table 3.4). Addition of 0.2% AsA and 0.15% H_2O_2 of TB surimi gels cooked by direct heating or setting resulted in decrease band at 540 cm^{-1}, while increased 525 cm^{-1} ($P < 0.05$). These results suggested that S-S bonds formation of these surimi were promoted by addition of AsA and/or H_2O_2. Results showed that all pastes and gels did not observe the band at 510 cm^{-1}. This is in agreement with Li-Chan and Nakai (1991) who reported a decrease in the band near 508 cm^{-1} upon gelation. The S-S stretching band at 510 cm^{-1} was observed from natural protein (Tu et al., 1976), thus a decrease in band area at 510 cm^{-1} indicated that protein denaturation promoted by addition of AsA and H_2O_2, and gelation. The band around 525 cm^{-1} was present in surimi pastes and gels from both species, indicating that the S-S bonds were formed into *g-g-t* conformation and it was a regular conformation of the S-S bond in fish muscle. Li-Chan and Nakai (1991) and Xu et al.

(2011) reported that the band near 530 cm^{-1} increased and 545 cm^{-1} decreased when fish proteins underwent thermal denaturation. The S-S formation was formed in *gauche-gauche-trans* S-S bridge by AsA and H_2O_2 in both LZ and TB surimi. Table 3.3 showed that total S-S bond region of LZ surimi paste increased by addition of AsA and H_2O_2 ($P < 0.05$), while LZ gels cooked by direct heating or setting did not significantly increase. Addition of AsA and H_2O_2 increased the total S-S bond of TB surimi paste and directly cooked gel ($P < 0.05$, Table 3.4). However, setting TB surimi at 40 °C did not change the total S-S bond. These results indicated that AsA and H_2O_2 promoted S-S bond conformation in both LZ and TB surimi gels, especially when direct heating at 90 °C was applied.

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Table 3.3 Integral area ($\times 10^{-3}$) of disulfide bond regions of surimi paste and gels (direct heating and setting) from LZ as affected by various contents of AsA and H_2O_2.

Additives (%)		Paste			Direct heating			Setting		
AsA	**H_2O_2**	**540±5**	**525±5**	**Total**	**540±5**	**525±5**	**Total**	**540±5**	**525±5**	**Total**
0	0	0.17 ± 0.01	0.19 ± 0.05 ^{bcd}	**0.35** ^{abc}	0.13 ± 0.04	0.16 ± 0.03	**0.29**	0.20 ± 0.01	0.12 ± 0.02 ^{f}	**0.32**
	0.05	0.14 ± 0.03	0.25 ± 0.04 ^{ab}	**0.39** ^{abc}	0.06 ± 0.00	0.17 ± 0.04	**0.23**	0.12 ± 0.03	0.14 ± 0.01 ^{def}	**0.27**
	0.1	0.15 ± 0.05	0.26 ± 0.04 ^{ab}	**0.41** ^{a}	0.08 ± 0.02	0.24 ± 0.03	**0.32**	0.14 ± 0.01	0.16 ± 0.03 ^{cde}	**0.31**
	0.15	0.14 ± 0.04	0.29 ± 0.04 ^{a}	**0.43** ^{a}	0.08 ± 0.00	0.24 ± 0.04	**0.32**	0.15 ± 0.03	0.24 ± 0.05 ^{a}	**0.38**
0.1	0	0.14 ± 0.03	0.13 ± 0.02 ^{d}	**0.27** ^{de}	0.10 ± 0.08	0.15 ± 0.04	**0.25**	0.16 ± 0.05	0.17 ± 0.01 ^{bcd}	**0.32**
	0.05	0.14 ± 0.02	0.19 ± 0.06 ^{bcd}	**0.33** ^{abc}	0.13 ± 0.00	0.21 ± 0.09	**0.34**	0.14 ± 0.06	0.14 ± 0.01 ^{def}	**0.27**
	0.1	0.15 ± 0.03	0.26 ± 0.06 ^{ab}	**0.41** ^{abc}	0.12 ± 0.02	0.19 ± 0.04	**0.31**	0.10 ± 0.04	0.16 ± 0.04 ^{cde}	**0.26**
	0.15	0.14 ± 0.03	0.24 ± 0.05 ^{abc}	**0.38** ^{abc}	0.10 ± 0.03	0.22 ± 0.04	**0.32**	0.08 ± 0.01	0.16 ± 0.02 ^{cde}	**0.25**
0.15	0	0.13 ± 0.04	0.27 ± 0.05 ^{ab}	**0.40** ^{abc}	0.10 ± 0.01	0.18 ± 0.05	**0.28**	0.12 ± 0.02	0.17 ± 0.05 ^{abc}	**0.30**
	0.05	0.11 ± 0.05	0.24 ± 0.05 ^{abc}	**0.36** ^{abc}	0.09 ± 0.06	0.22 ± 0.03	**0.31**	0.13 ± 0.05	0.22 ± 0.00 ^{abc}	**0.35**
	0.1	0.12 ± 0.02	0.24 ± 0.04 ^{abc}	**0.37** ^{abc}	0.09 ± 0.03	0.21 ± 0.02	**0.30**	0.13 ± 0.04	0.21 ± 0.07 ^{abc}	**0.34**
	0.15	0.14 ± 0.02	0.15 ± 0.03 ^{d}	**0.30** ^{bcd}	0.11 ± 0.04	0.22 ± 0.04	**0.33**	0.12 ± 0.05	0.19 ± 0.04 ^{abc}	**0.31**
0.2	0	0.06 ± 0.03	0.14 ± 0.05 ^{d}	**0.20** ^{e}	0.09 ± 0.01	0.23 ± 0.06	**0.31**	0.06 ± 0.02	0.23 ± 0.03 ^{abc}	**0.29**
	0.05	0.11 ± 0.07	0.17 ± 0.04 ^{cd}	**0.28** ^{cde}	0.09 ± 0.02	0.17 ± 0.01	**0.26**	0.11 ± 0.06	0.19 ± 0.04 ^{abc}	**0.30**
	0.1	0.12 ± 0.06	0.29 ± 0.02 ^{a}	**0.41** ^{ab}	0.10 ± 0.02	0.17 ± 0.03	**0.27**	0.12 ± 0.02	0.20 ± 0.04 ^{abc}	**0.32**
	0.15	0.08 ± 0.05	0.29 ± 0.05 ^{a}	**0.38** ^{abc}	0.09 ± 0.01	0.24 ± 0.06	**0.33**	0.11 ± 0.07	0.23 ± 0.02 ^{ab}	**0.34**

Mean in a column with different superscripts are significantly different ($P < 0.05$).

Table 3.4 Integral area ($\times 10^{-3}$) of disulfide bond regions of surimi paste and gels (direct heating and setting) from TB as affected by various contents of AsA and H_2O_2.

Additives (%)		Paste			Direct heating			Setting		
AsA	**H_2O_2**	**540±5**	**525±5**	**Total**	**540±5**	**525±5**	**Total**	**540±5**	**525±5**	**Total**
0	0	0.10 ± 0.02	0.16 ± 0.00 ^{fg}	**0.27** ^{cde}	0.20 ± 0.02 ^{a}	0.14 ± 0.03 ^{g}	**0.34** ^{cde}	0.17 ± 0.08	0.16 ± 0.05 ^{def}	**0.33**
	0.05	0.08 ± 0.03	0.14 ± 0.03 ^{g}	**0.22** ^{f}	0.12 ± 0.04 ^{bc}	0.21 ± 0.01 ^{efg}	**0.33** ^{def}	0.17 ± 0.02	0.13 ± 0.04 ^{f}	**0.30**
	0.1	0.11 ± 0.04	0.26 ± 0.04 ^{abc}	**0.37** ^{abc}	0.17 ± 0.02 ^{ab}	0.24 ± 0.05 ^{cde}	**0.41** ^{abc}	0.16 ± 0.03	0.19 ± 0.08 ^{bcd}	**0.35**
	0.15	0.18 ± 0.02	0.29 ± 0.01 ^{ab}	**0.47** ^{a}	0.17 ± 0.03 ^{ab}	0.25 ± 0.05 ^{abc}	**0.42** ^{abc}	0.13 ± 0.06	0.20 ± 0.07 ^{bcd}	**0.33**
0.1	0	0.14 ± 0.02	0.23 ± 0.01 ^{bcd}	**0.36** ^{abc}	0.11 ± 0.04 ^{bc}	0.18 ± 0.02 ^{efg}	**0.29** ^{ef}	0.14 ± 0.03	0.24 ± 0.04 ^{abc}	**0.38**
	0.05	0.10 ± 0.02	0.22 ± 0.01 ^{cde}	**0.32** ^{bcd}	0.13 ± 0.05 ^{bc}	0.15 ± 0.03 ^{g}	**0.28** ^{f}	0.12 ± 0.03	0.28 ± 0.01 ^{ab}	**0.40**
	0.1	0.14 ± 0.08	0.25 ± 0.04 ^{abc}	**0.39** ^{abc}	0.09 ± 0.03 ^{cd}	0.25 ± 0.03 ^{abc}	**0.34** ^{cde}	0.16 ± 0.04	0.19 ± 0.07 ^{bcd}	**0.35**
	0.15	0.11 ± 0.04	0.26 ± 0.03 ^{abc}	**0.37** ^{abc}	0.04 ± 0.01 ^{d}	0.16 ± 0.02 ^{fg}	**0.20** ^{g}	0.15 ± 0.04	0.31 ± 0.05 ^{a}	**0.45**
0.15	0	0.13 ± 0.02	0.27 ± 0.04 ^{abc}	**0.40** ^{ab}	0.12 ± 0.00 ^{bc}	0.23 ± 0.01 ^{de}	**0.35** ^{bcd}	0.12 ± 0.01	0.15 ± 0.03 ^{ef}	**0.27**
	0.05	0.08 ± 0.07	0.20 ± 0.05 ^{efg}	**0.27** ^{cde}	0.14 ± 0.02 ^{abc}	0.25 ± 0.04 ^{abc}	**0.39** ^{abc}	0.11 ± 0.07	0.17 ± 0.05 ^{cde}	**0.28**
	0.1	0.09 ± 0.09	0.16 ± 0.03 ^{fg}	**0.25** ^{ef}	0.12 ± 0.05 ^{bc}	0.25 ± 0.01 ^{abc}	**0.38** ^{abc}	0.08 ± 0.03	0.23 ± 0.04 ^{abc}	**0.31**
	0.15	0.12 ± 0.03	0.21 ± 0.05 ^{def}	**0.33** ^{bcd}	0.13 ± 0.02 ^{bc}	0.25 ± 0.04 ^{abc}	**0.38** ^{abc}	0.11 ± 0.03	0.27 ± 0.04 ^{ab}	**0.38**
0.2	0	0.11 ± 0.01	0.31 ± 0.01 ^{a}	**0.42** ^{ab}	0.11 ± 0.01 ^{bc}	0.30 ± 0.04 ^{abc}	**0.41** ^{abc}	0.13 ± 0.01	0.25 ± 0.03 ^{abc}	**0.38**
	0.05	0.14 ± 0.02	0.22 ± 0.05 ^{def}	**0.36** ^{abc}	0.13 ± 0.04 ^{bc}	0.31 ± 0.05 ^{ab}	**0.44** ^{ab}	0.16 ± 0.02	0.17 ± 0.03 ^{cde}	**0.32**
	0.1	0.13 ± 0.02	0.27 ± 0.03 ^{abc}	**0.40** ^{ab}	0.13 ± 0.04 ^{bc}	0.16 ± 0.03 ^{afg}	**0.29** ^{ef}	0.10 ± 0.04	0.24 ± 0.07 ^{abc}	**0.33**
	0.15	0.13 ± 0.02	0.26 ± 0.05 ^{abc}	**0.39** ^{abc}	0.14 ± 0.01 ^{abc}	0.32 ± 0.04 ^{a}	**0.46** ^{a}	0.14 ± 0.05	0.27 ± 0.05 ^{ab}	**0.40**

Mean in a column with different superscripts are significantly different ($P < 0.05$).

3.4.10 Principal component analysis (PCA)

Based on PCA of all samples, PC-1 and PC-2 explained 39% and 19% of variation in data set, respectively (Figure 3.14). The correlation loading plot (Figure 3.14A) showed that the most important variables were explained by breaking force and distance, TSH group, β-sheet, C-H bending at 1425 cm^{-1}, β-sheet at 1235, 1645, 1625 and 1539 cm^{-1}, C-H stretching at 2922 and 2852 cm^{-1}, and O-H stretching at 3228 cm^{-1}. Furthermore, LZ and TB surimi clearly differentially along PC-1 (Figure 3.14B). According to PC-1, TB surimi added combined AsA and H_2O_2 appeared to cluster in a high value of breaking force and distance, which was positively correlated with O-H stretching at 3340 and 3228 cm^{-1}, S-S and β-turn. However, addition of H_2O_2 alone resulted in LZ gels with increased β-sheet 1645, 1625 and 1539 cm^{-1} from FT-IR, and aliphatic regions from Raman, which were correlated with a low value of textural properties. PC-2 showed that LZ gels added combined AsA and H_2O_2 appeared to have a high β-sheet, C-H bending at 1425 cm^{-1}, β-sheet band at 1235 cm^{-1} of amide III, protein solubility in the presence of βME (PS+βME) and TCA-soluble oligopeptide, whereas TB gels added H_2O_2 alone contented showed high correlation with TSH group, whiteness, α-helix, protein solubility in the absence of βME (PS-βME), aromatic from Raman spectroscopy.

Directly cooked gels and set gels of both LZ and TB surimi did not clearly separate (Figure 3.15B and 3.16B), indicating that the heating regime did not affect surimi gel improvement. When only LZ was considered, the textural of LZ gels increased with addition of high concentration of AsA and H_2O_2, which was positively correlated with β-sheet. Moreover, TB surimi gel texture was improved with addition of combined AsA and H_2O_2, positively correlated with β-sheet and PS+βME (Figure

3.16). Texture of both LZ and TB gels was negatively correlated with whiteness, TSH, SP-βME, and α-helix, aliphatic and aromatic monitored by Raman, when AsA or H_2O_2 alone was applied. These results indicated that enhancement of textural characteristics of these surimi species by AsA and H_2O_2 was positively correlated with increasing β-sheet structure, and negatively correlated with TSH, PS-βME, and α-helix, aliphatic and aromatic obtained from Raman spectroscopy. These results suggested that textural improvement of LZ and TB surimi enhanced by the addition of combined AsA and H_2O_2 with involvement of S-S bondings through SH groups oxidation and hydrophobic interactions via aliphatic and aromatic residues.

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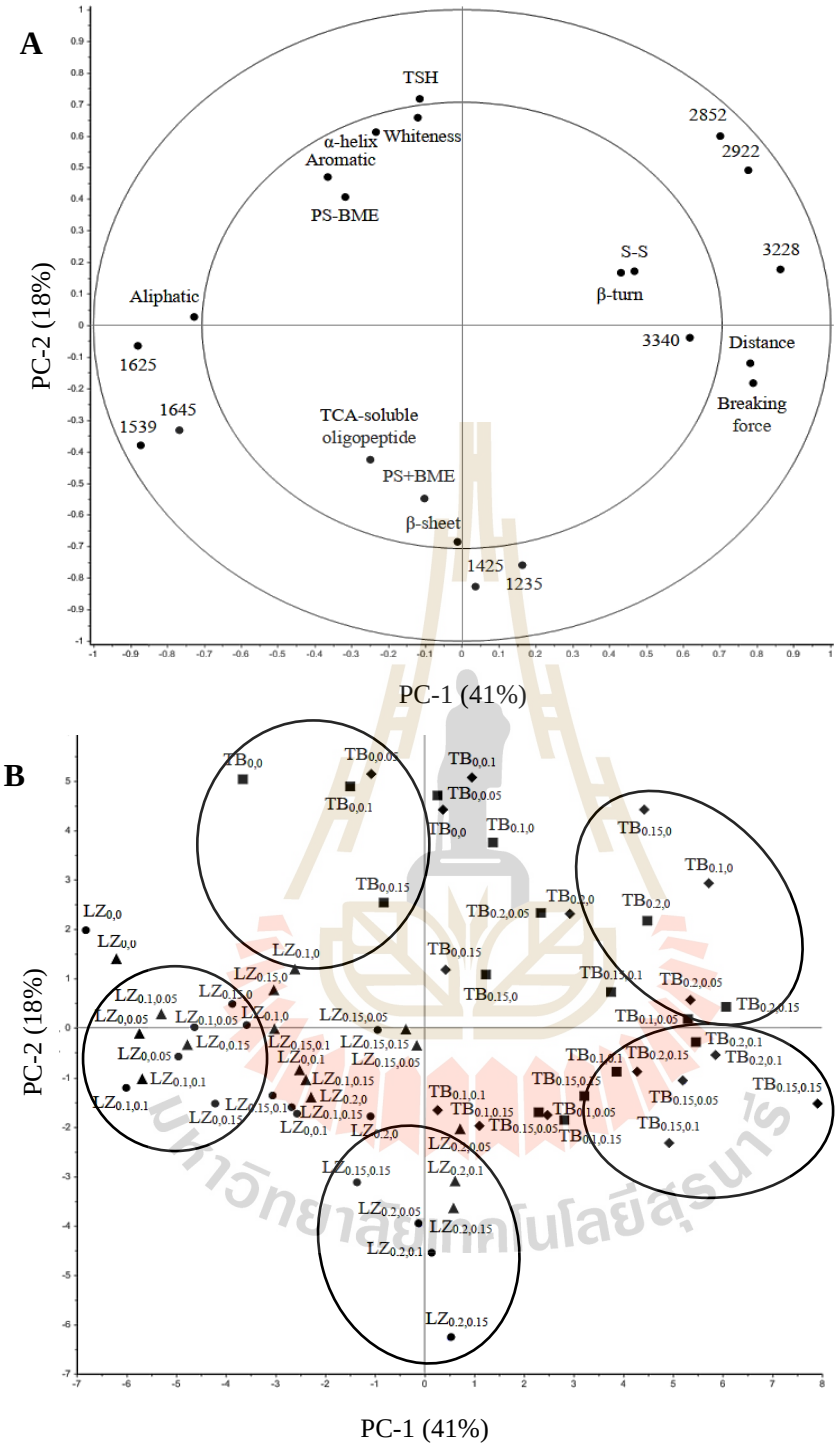

Figure 3.14 PCA correlation loading plots (A) and score plots (B) of LZ and TB surimi gels added AsA and H_2O_2. B: LZ directly cooked gel (●), LZ set gel (▲), TB directly cooked gel (◆), and TB set gel (■). $TB_{(x,y)}$ and $LZ_{(x,y)}$ which x = AsA, y = H_2O_2.

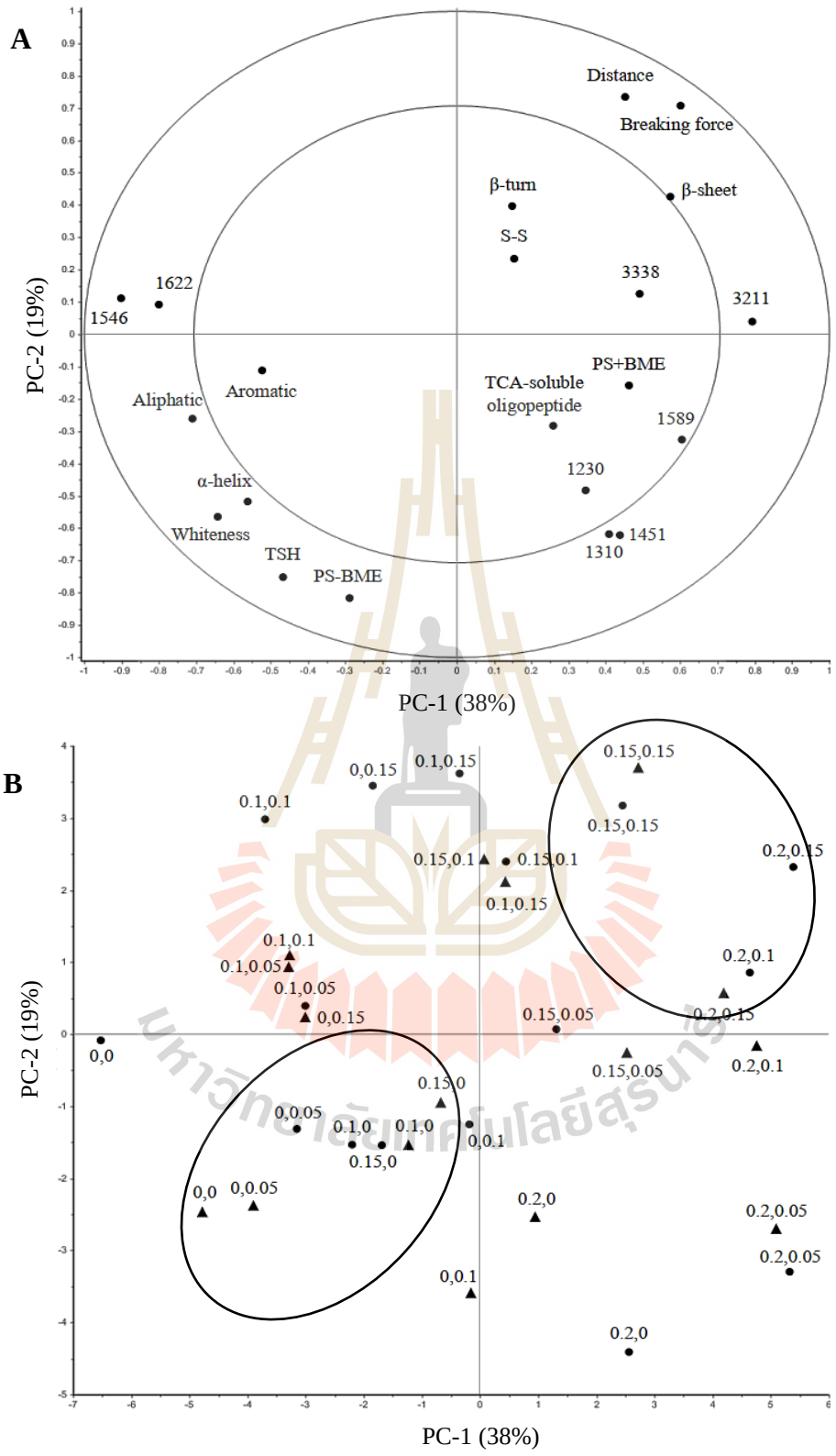

Figure 3.15 PCA correlation loading plots (A) and score plots (B) of LZ surimi gel. B: direct heating (●) and setting (▲). $LZ_{(x,y)}$ which x = AsA, y = H_2O_2.

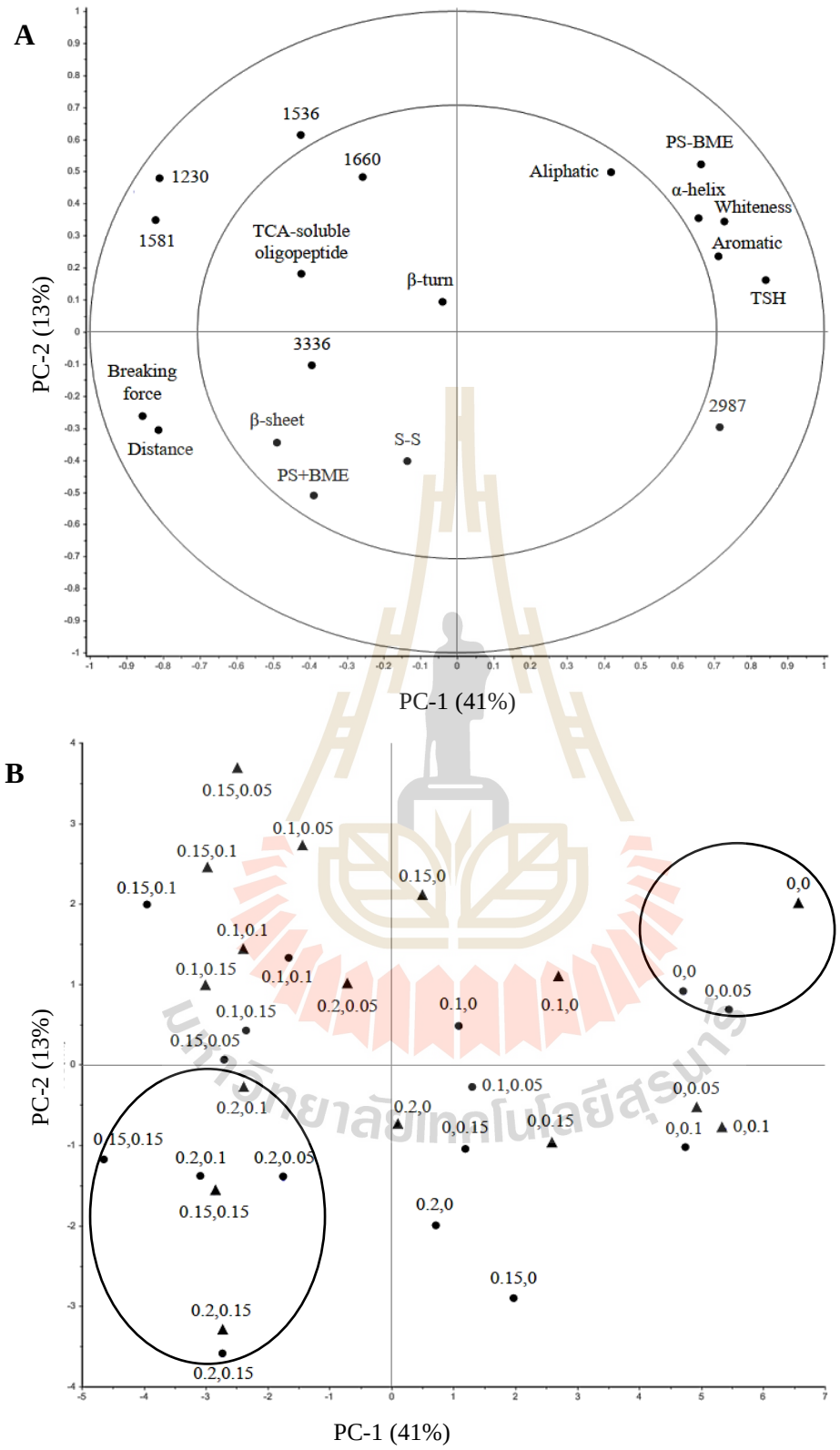

Figure 3.16 PCA correlation loading plots (A) and score plots (B) of TB surimi gel. B: direct heating (●) and setting (▲). $TB_{(x,y)}$ which x = AsA, y = H_2O_2.

3.5 Conclusions

Both the oxidizing and reducing agents improved the LZ and TB surimi gelation. Adding 0.15% H_2O_2 alone increased both breaking force and distance of LZ surimi gel, while adding 0.2% AsA alone increased gel strength of TB surimi cooked either by direct heating at 90 °C or setting at 40 °C. Increasing levels of the combined AsA and H_2O_2 led to maximum gel strength from both surimi species. Addition of H_2O_2 alone increased whiteness of these surimi gels. But combination of AsA and H_2O_2 significantly decreased whiteness of both species caused by ascorbic acid browning. AsA and/or H_2O_2 enhanced the gel-forming ability due to increased MHC cross-linking through disulfide bonding, especially when gel cooked by direct heating. AsA promoted proteolytic activity. FT-IR spectroscopy showed that α-helix decreased and β-sheet of LZ and TB surimi increased with increasing concentration of AsA and H_2O_2. Raman spectroscopy showed that aliphatic and aromatic residues decreased in both surimi species added AsA and H_2O_2. Disulfide bridge of LZ and TB surimi also promoted and formed g-g-t conformation at 525 cm^{-1} by AsA and H_2O_2. Principal component analysis exhibited that the textural of surimi gels from these species were positively correlated to the β-sheet content and negatively correlated to the whiteness, TSH group, α-helix content, aromatic, and aliphatic. Addition of AsA and/or H_2O_2 enhanced gel formation of LZ and TB surimi, and to get the best properties for LZ or TB surimi gel, a combination of AsA and H_2O_2 is recommended.

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CHAPTER IV

EFFECT OF ASCORBIC ACID AND HYDROGEN PEROXIDE ON GEL-FORMING ABILITY OF SARDINE SURIMI

4.1 Abstract

Effects of ascorbic acid (AsA) and hydrogen peroxide (H_2O_2) on gel-forming ability of sardine (SD) surimi were investigated. Addition of AsA and H_2O_2 did not affect breaking force and distance of SD surimi gel directly cooked at 90 °C for 30 min. However, adding 0.15% H_2O_2 alone increased 20% breaking distance of SD surimi gel compared to control, when setting at 40 °C for 30 min was applied. Setting resulted in the maximum breaking force and distance of SD with 100% and 35%, respectively, compared to the direct heating process. Whiteness of surimi gels increased with the addition of a combined 0.2% AsA and 0.15% H_2O_2 by 63.5-66.6%. Gel improvement was likely associated with a slight increase in disulfide linkages as observed by an increase in solubility of protein with addition of 0.15% H_2O_2, when the extraction buffer containing β-mercaptoethanol. Myosin heavy chain (MHC) was not observed when the level of AsA and H_2O_2 increased in combination with setting, suggesting MHC covalent cross-linking induced by these 2 additives. TCA-soluble oligopeptide of surimi gel increased with AsA concentration. FT-IR spectroscopy analysis revealed that α-helix content decreased, and β-sheet increased with addition of 0.2% AsA and 0.15% H_2O_2

in set gel. Raman spectroscopy also indicated that hydrophobic interactions were enhanced by the addition of AsA and H_2O_2. AsA and/or H_2O_2 appeared to induce structural changes of SD muscle protein, but the extent of gel improvement was more pronounced with setting effect.

4.2 Introduction

A tropical fish namely sardine (*Sardinella* spp., SD) is a pelagic dark-fleshed fish and has been used as one of the raw materials for surimi production in Southeast Asia. SD surimi exhibit weaker gel with the darker color as compared to other tropical white fish surimi such as threadfin bream (Chaijan et al., 2004). The weak gel is not acceptable by consumers. Strategies to improve gel quality and color should be sought.

Setting gel has been formed by incubating surimi paste at a relatively low temperature before heating. Setting (suwari) has been used to improve gel strength due to the action of endogenous transglutaminases (TGase) which catalyze non-disulfide covalent cross-linking (Kitakami, et al., 2004). Nishimura et al. (1992) explained that the gel strength of the setting gels depends on the degree and uniformity of cross-linking between protein molecules formed during incubation. Wicker et al. (1989) have proposed that the formation of a setting gel was mainly due to hydrophobic interaction. Furthermore, the addition of oxidants such as ascorbic acid, hydrogen peroxide, sodium hypochlorite, potassium bromate has been shown to improve surimi gel strength by promoting oxidation of sulfhydryl (SH) groups to intermolecular disulfide (S-S) bonds.

Ascorbic acid (AsA) or vitamin C is a reducing agent, which can be oxidized to dehydro-L-ascorbic acid (DHA). The oxygen radicals produced by DHA and promoted the oxidize SH groups on myosin heavy chain (MHC), resulting in S-S bond formation.

Benjakul et al. (2005) reported that addition of AsA slightly increased gel formation of lizardfish surimi gel through the formation of S-S bond. On the other hand, hydrogen peroxide (H_2O_2) is a strong oxidizing agent with degrades rapidly to oxygen and water. H_2O_2 can also promote the oxidation of SH groups to S-S bonds. Chen et al. (1999) showed that H_2O_2 accelerated the oxidation of SH groups, increasing the maximum stress values and water holding capacity of milkfish meat paste. Moreover, H_2O_2 has been used in food processing at a concentration of 0.04-1.25% (21 CFR 184.1366). An application of 1% H_2O_2 has no apparent acute toxicity or subchronic toxicity, and United States Food and Drug Administration (US FDA) (2004) listed H_2O_2 as Generally Recognized as Safe.

This study was aimed at investigating the effect of AsA and H_2O_2 on gelation of sardine surimi.

4.3 Materials and Methods

4.3.1 Materials

Frozen surimi produced form SD, was purchased from Man A Frozen Foods Co., Ltd. (Songkhla, Thailand), cut into small blocks (1 kg) and stored at -20 °C before used.

4.3.2 Surimi gel preparation

Frozen surimi was thawed in a refrigerator overnight and cut into small pieces (about 1 cm cubes). Surimi samples were chopped by a Stephan chopper (UM 5 universal, Stephan U. Söhne GmbH & Co., Hameln, Germany). Ice was added to adjust the final moisture content to 78% (w/w). Different amount of AsA at 0, 0.1, 0.15, 0.2% and H_2O_2 at 0, 0.05, 0.1 and 0.15% were also added. Surimi pastes were stuffed into

polyvinylidene chloride casing (3 cm diameter). Two thermal treatments were applied, including direct heating at 90 °C for 30 min and setting at 40 °C for 30 min before heating at 90 °C for 30 min. Surimi gels were cooled immediately in iced water for 20 min and stored at 4 °C overnight prior to further analysis.

4.3.3 pH measurement

pH of surimi pastes was measured using a pH meter (Mettler Toledo™, SevenCompact™ S220, pH/Ion Benchtop Meter, Switzerland) calibrated against standard pH buffers of 4.0 and 7.0 at 25 °C before use. Sample (2 g) was added 18 ml distilled water and homogenized before pH measurement (Park, 2014).

4.3.4 Texture analysis

Texture analysis was measured using an empirical punch test. Stable micro systems texture analyzer (TA-XT Plus, Surry GU7 1YL, UK) equipped with a 5 mm spherical probe was used to measure breaking force and breaking distance. Penetration speed of 1 mm/s was used. Gels equilibrate at room temperature (25±2 °C) for 2 h and cut into a cylinder shape, with 3 cm diameter and 3 cm long (Park, 2005).

4.3.5 Whiteness

Whiteness of surimi gels was determined using a HunterLab spectrophotometer (HunterLab, ColorQuest XE, Hunter Associates Laboratory, USA). *L**, *a**, and *b** were measured and whiteness was calculated using the equation of $100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$ (Lanier et al., 1991; Park, 1994).

4.3.6 Protein cross-linking by SDS-PAGE

Protein patterns of surimi gels were analyzed using SDS-Polyacrylamide gel electrophoresis (SDS–PAGE) according to the method of Laemmli (1970). Samples (1 g) were solubilized with 10 ml of 10% of hot SDS (extraction buffer) and

homogenized at a speed of 11,000 rpm for 2 min. Extraction buffer was prepared with and without 10% β-mercaptoethanol (βME), and shaken at room temperature overnight (Numakura et al., 1985) and centrifuged at 8,000 ×g for 20 min to remove undissolved debris. Protein concentration of the supernatant was determined by the Lowry method (Lowry et al., 1951). Samples (20 μg protein) were loaded into 10% running gel with 4% stacking gel. Electrophoresis was carried out under 100V. After separation, gels were stained with staining solution (0.125% (w/v) Coomassie Brilliant blue R-250 in 40% methanol and 10% acetic acid).

4.3.7 TCA-soluble peptide content

Surimi gel (3 g) was mixed with 27 ml of cold 5% TCA (w/v) and homogenized for 2 min at a speed of 11,000 rpm. Homogenate was incubated in ice-water for 1 h and centrifuged at 8,000 ×g for 10 min. TCA-soluble oligopeptide content in the supernatant was measured as previously described and expressed as μmol tyrosine/g sample (Morrissey et al., 1993).

4.3.8 Total sulfhydryl group content (TSH)

Total sulfhydryl group content of surimi gel was determined based on Ellman method (Ellman, 1959) using 5-5'-dithiobis-(2-nitrobenzoic acid) (DTNB). 0.5 g surimi gel was solubilized with 25 ml of 0.1 M phosphate buffer (pH 7.0) containing 10mM EDTA (Ethylene diamine tetraacetic acid), 2% SDS and 8 M urea, and centrifuged at 10,000 ×g for 10 min. The supernatant was collected, and the protein content was determined (Bradford, 1976). Supernatant (4 ml) was prepared with 0.4 ml of 0.1% DTNB in 0.1 M phosphate buffer (pH 7.0) and incubated at 40 °C for 15 min. Absorbance at 412 nm was measured. TSH content was calculated using a molar extinction of 13612.5 M^{-1} cm^{-1} for 2-nitro-5-triobenzoic acid (TNB).

4.3.9 FT-IR spectroscopy

Protein secondary structures were determined using an ATR-FTIR (FT-IR, Platinum ATR, T27/Hyp2000, Bruker Hong Kong Limited, Leipzig, Germany) with a collection in 64 scans with a resolution of 4 cm^{-1} at room temperature. Freeze-dried of paste and gel samples were placed on the surface of the ATR crystal and clamped into the mount of FT-IR spectrometer. Infrared spectra between 4000 and 400 cm^{-1} were recorded. Spectral data from the scanning were baseline corrected and normalized according to the amide I peak at 1600-1700 cm^{-1}. Protein secondary structures were calculated by curve fitting was carried out by means of OPUS software version 7.0. Percentages of protein secondary structures were determined according to the method reported by Alix et al. (1988).

4.3.10 Raman spectroscopy

Protein tertiary structure was measured using Raman spectrometer (FT-Raman spectrometer, Vertex 70-Ram II, Bruker Hong Kong Limited, Leipzig, Germany) which described by Poowakanjana et al. (2012). Raman spectra of surimi sample were collected using laser energy (500 mW) at room temperature. Surimi paste and gel samples were applied to a sample holder and placed on the instrument. The spectra were recorded in the range of 400-4000 cm^{-1}. Each spectrum was obtained under the following conditions: 64 scans, 4.0 cm^{-1} resolution. All scanned spectral data were smoothing and normalized at 4000-400 cm^{-1}. Protein tertiary structures were calculated by derivation using a second-derivative spectrum, integration, and area calculation was analyzed using OPUS software version 7.0.

4.3.11 Statistical analysis

Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple range test (Steel and Torrie, 1980). Analysis was performed using SPSS 14.0 software.

4.4 Results and Discussion

4.4.1 pH

Addition of H_2O_2 alone did not affect pH of SD surimi pastes. pH of SD pasts slightly decreased with increasing AsA concentration (Table 4.1, $P < 0.05$). This result was similar to Chen et al. (1999), who reported that AsA slightly lowered pH value of meat paste and its product. Lower pH indicated that AsA might be changed gel formation of SD surimi.

Table 4.1 pH of surimi pastes from sardine (SD).

Additives (%)		pH
AsA	H_2O_2	
0	0	6.84 ± 0.01 ^{ab}
	0.05	6.85 ± 0.01 ^{a}
	0.1	6.82 ± 0.02 ^{b}
	0.15	6.84 ± 0.01 ^{ab}
0.1	0	6.66 ± 0.02 ^{c}
	0.05	6.66 ± 0.03 ^{c}
	0.1	6.64 ± 0.02 ^{cd}
	0.15	6.65 ± 0.02 ^{c}
0.15	0	6.63 ± 0.03 ^{ed}
	0.05	6.62 ± 0.01 ^{ed}
	0.1	6.63 ± 0.01 ^{ed}
	0.15	6.61 ± 0.01 ^{e}
0.2	0	6.52 ± 0.02 ^{f}
	0.05	6.52 ± 0.01 ^{f}
	0.1	6.52 ± 0.01 ^{f}
	0.15	6.51 ± 0.01 ^{f}

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4.4.2 Texture

Addition of H_2O_2 and/or AsA did not affect breaking force and breaking distance of SD surimi gels cooked by direct heating at 90 °C (Figure 4.1A and C). Adding 0.15% H_2O_2 increased breaking distance of surimi gel by 20% when setting was applied, as compared to the control (without H_2O_2 and AsA, $P < 0.05$, Figure 4.1D). This result resembled the previous studies of Chen et al. (1999) who reported that 0.01-0.04% H_2O_2 slightly increased the gel strength from milkfish. Setting at 40 °C increased both breaking force and distance of surimi gels by 100% and 35%, respectively, in the absence or presence H_2O_2 and AsA, compared to gels directly heated ($P < 0.05$). Setting significantly improved breaking force and distance of SD surimi gels. The result exhibited that H_2O_2 increased breaking distance at only 40 °C incubation. Setting increased the gel strength of SD surimi gels; thus, SD might contain a high TGase, proteins was more unfolding and rearrangement during setting (Chaijan et al., 2004). This could be oxidation induced by H_2O_2 promoted proper gel network development at 40 °C. However, addition of combined AsA and H_2O_2 decreased SD surimi gel cooked by setting at 40 °C ($P < 0.05$). According to Kumazawa et al. (1995), Alaska pollock surimi gel strength was suppressed about 50% by the presence of NH_4Cl which was a TGase inhibitor. Therefore, the combination of AsA and H_2O_2 did not affect SD surimi gel improvement, suggested that AsA and H_2O_2 effectively inhibited TGase activity during setting.

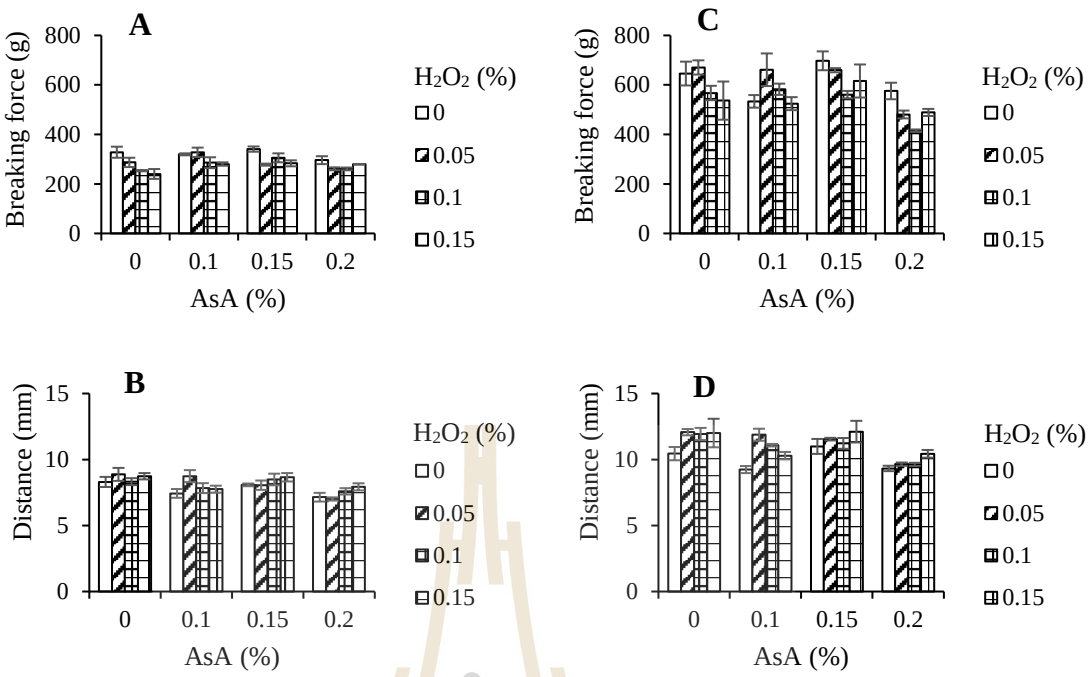

Figure 4.1 Breaking force (A, C) and distance (B, D) of SD surimi gels added AsA and H_2O_2. A and B = direct heating at 90 °C for 30 min; C and D = setting at 40 °C for 30 min before heating at 90 °C for 30 min.

4.4.3 Whiteness

Whiteness of SD surimi gels slightly increased with addition of H_2O_2 alone, while AsA alone showed less effect on color. Combination of 0.2% AsA and 0.15% H_2O_2 increased whiteness of SD surimi gel cooked when direct heating and setting by 2.8% and 5.1%, respectively, compared with control gel ($P < 0.05$, Figure 4.2). Myoglobin was bleached by H_2O_2. Myoglobin also denatured at acidic pH by adding AsA. The effect on gel subjected to setting was similar. Our study demonstrated that H_2O_2 and AsA had more effect on whiteness than gel improvement.

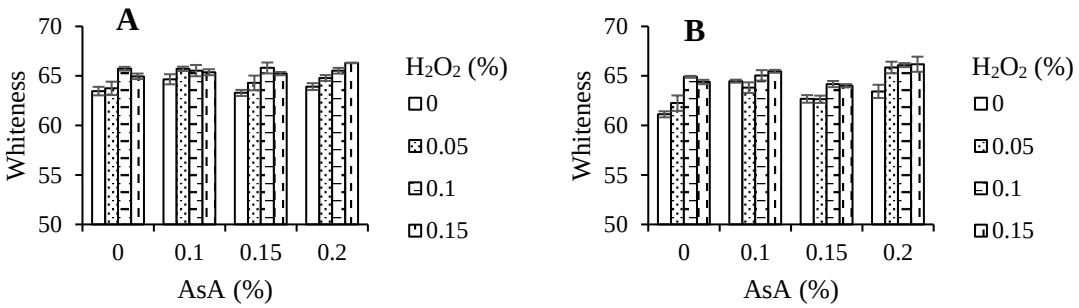

Figure 4.2 Effect of AsA and H_2O_2 on whiteness of SD surimi gels which cooked at 90 °C for 30 min (A) and set at 40 °C for 30 min (B).

4.4.4 Disulfide linkage and protein pattern

Soluble protein content of cooked gels with added AsA and H_2O_2 were extracted by using extraction buffer present and absence β-mercaptoethanol (βME), are shown in Figure 4.3. Extraction buffer containing βME was used to solubilize protein by destroying S-S bonds except for non-disulfide covalent bonds (Benjakul et al., 2001). In the presence of βME, protein solubility of SD surimi gels added H_2O_2 slightly increased when gels directly cooked at 90 °C or set at 40 °C by 20.1-21.8 mg/ml and 15.0-17.6 mg/ml, respectively ($P < 0.05$, Figure 4.3A and B), indicating that disulfide linkages might be enhanced by addition of H_2O_2 alone. Addition of AsA alone or combined with H_2O_2 did not affect protein solubility of SD surimi gels. In the buffer without βME, protein solubility of SD surimi gels from both direct heating and setting were subtle at various H_2O_2 concentrations ($P > 0.05$, Figure 4.3C and D). These results implied that H_2O_2 might have promoted disulfide bond formation via oxidation of cysteine residues. SD surimi gels showed lower protein solubility when setting was applied, as compared to directly heated gels. This could be because cross-linkings catalyzed by TGase through non-disulfide bonds during setting. Non-disulfide covalent

bond through incubating SD surimi at 40 °C was supposed to be a major contributor to the strengthening of SD surimi gels. Coincidentally, setting exhibited greater breaking force and distance SD surimi gels.

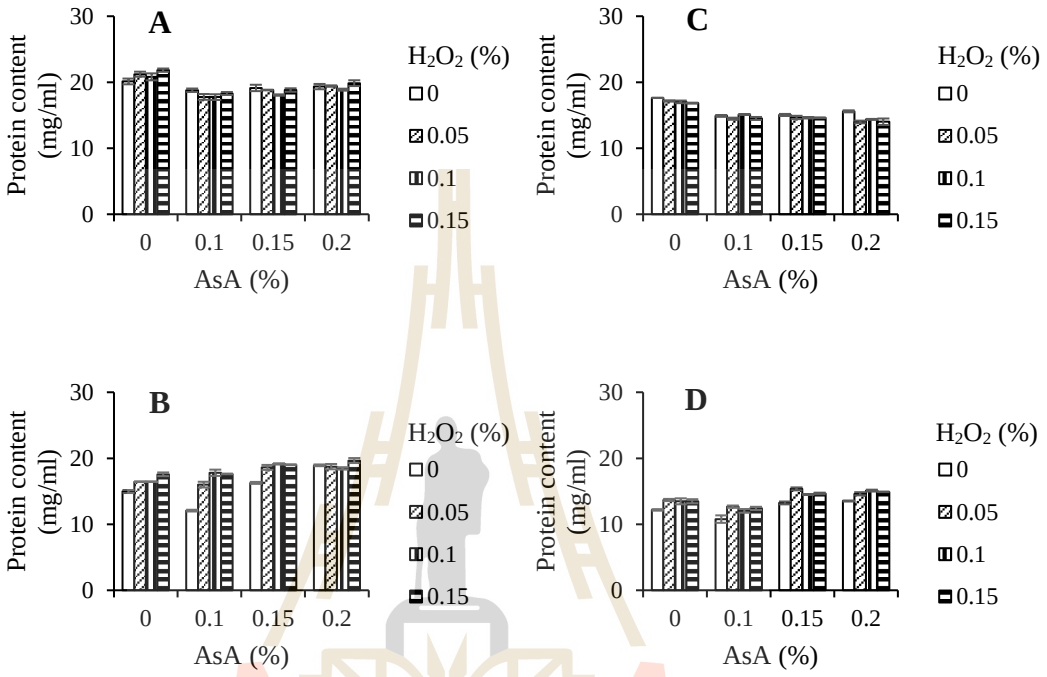

Figure 4.3 Protein solubility of SD surimi gels cooked at 90 °C for 30 min (A, C) and set at 40 °C for 30 min (B, D), in the presence (A, B) and absence (C, D) of β-mercaptoethanol.

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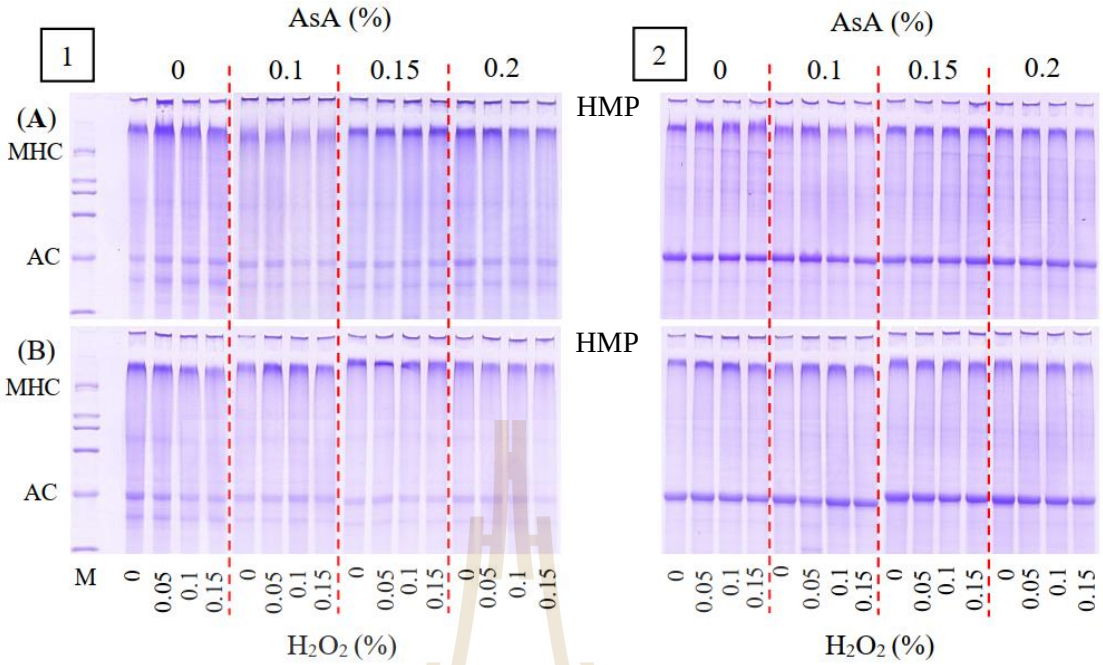

Figure 4.4 Effect of AsA and H_2O_2 on SDS-PAGE patterns of SD surimi gels extracted in an absence (1) and a presence (2) βME, cooked at 90 °C for 30 min (A) and set at 40 °C for 30 min (B). M: standard maker, HMP: high molecular weight proteins, MHC: Myosin heavy chain and AC: Actin.

MHC band was the main protein that changed during gel-forming, while AC was harder affected. MHC of SD surimi gel was not observed with increasing AsA and H_2O_2 when extracted by a buffer without βME, either setting or direct heating (Figure 4.4.1A and B). This result was similar to Nishimura et al. (1996) who reported that MHC decreased by adding AsA when set and direct heating were replied. However, MHC bands from directly cooked gels were recovered when βME was applied (Figure 4.4.2A), indicating that the disappearance of MHC was due to polymerization of MHC. AsA and/or H_2O_2 acted as to accelerate the formation of S-S bridging for promoting the gel-forming ability. Moreover, MHC of samples extracted by a buffer containing 10% βME were completely disappeared when setting applied and HMP did not disrupt by

βME (Figure 4.4.2B). Non-disulfide covalent bonds were not dissociated by SDS, βME and heat, which endogenous TGase has been reported to be responsible for these MHC cross-linked polymers, suggesting MHC polymerization by endogenous TGase through non-disulfide covalent bonding. These results suggested that most MHC was polymerized to high molecule substances by S-S and non-disulfide covalent cross-linking upon setting process.

4.4.5 TCA-soluble oligopeptide content

TCA-soluble oligopeptide content of SD surimi gels increased with an increase of AsA ($P < 0.05$), whereas H_2O_2 did not affect (Figure 4.5). Enzymes are inactivated by H_2O_2 (Taggart et al., 2000). This result suggested that AsA promoted proteolysis which could be induced by acid proteases. Incubation at 40 °C for 30 min led to comparable TCA-soluble oligopeptide to direct heating (Figure 4.5B). Lower gel properties with the addition of AsA could partly be attributed to proteolysis. TCA-soluble oligopeptide content of SD surimi gels cooked at 90 °C was similar to gels set at 40 °C, indicating that proteins cross-linking polymerized by endogenous TGase were more resistant to proteolysis induced by proteases.

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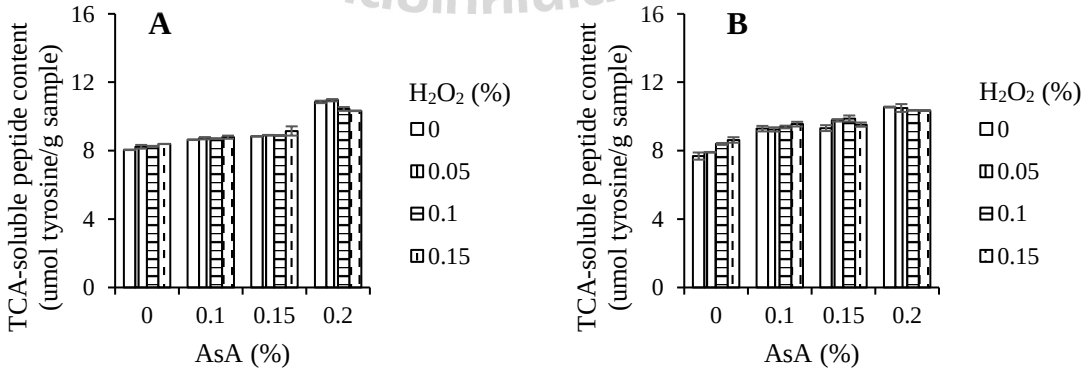

Figure 4.5 TCA-soluble peptide content of SD surimi gels cooked at 90 °C for 30 min (A) and set at 40 °C for 30 min (B).

4.4.6 Total sulfhydryl group (TSH)

Addition of AsA or H_2O_2 alone did not affect TSH group content of SD surimi gels (Figure 4.6). However, TSH group content of SD directly cooked gels significantly decreased with combination of 0.2% AsA and 0.15% H_2O_2 ($P < 0.05$, Figure 4.6A). This result indicated that addition of AsA and H_2O_2 promoted oxidation of SH groups to the formation of S-S bonds when direct heating was applied. However, set surimi gel at 40 °C did not affect TSH group content after added AsA and H_2O_2 (Figure 4.6B). Since, gel strength of SD surimi significantly improved by setting, as compared with direct heating, suggested that gel formation of SD surimi enhanced by TGase enzymes through non-disulfide covalent bonds rather than S-S covalent bonds by SH groups oxidation during setting with or without AsA and H_2O_2. This result was in agreement with Chaijan et al. (2004), SD gel had higher gel strength during setting at 40 °C for 30 min, caused protein cross-linking by endogenous TGase.

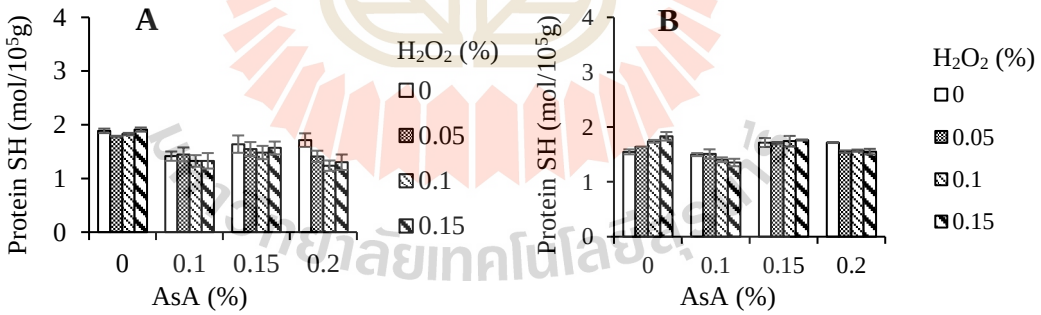

Figure 4.6 Total of protein sulfhydryl groups (TSH) content of SD surimi gels; which A = direct heating; and B = setting.

4.4.7 Changes in secondary structure

In surimi paste, addition of H_2O_2 and/or AsA decreased α-helix content and increased β-sheet and β-turn content as compared to control ($P < 0.05$, Figure

4.7A). Combination of 0.2% AsA and 0.15% H_2O_2 decreased α-helix and increased β-sheet content of SD directly cooked gels, while β-turn did not affect (Figure 4.7B). Compared to control, combination of 0.2% AsA and 0.15% H_2O_2 decreased α-helix of SD surimi gel when setting was applied, but β-sheet and β-turn content did not affect, (Figure 4.7C). Moreover, α-helix and β-sheet content of set gel were lower and higher than directly cooked gel, respectively. A decrease of α-helix and an increase of β-sheet content in surimi gels suggested that protein was unfolding after AsA and/or H_2O_2 addition and gelation. In addition, the greater extent of changes of α- helix and β-sheet were evident in set gel at 40 °C.

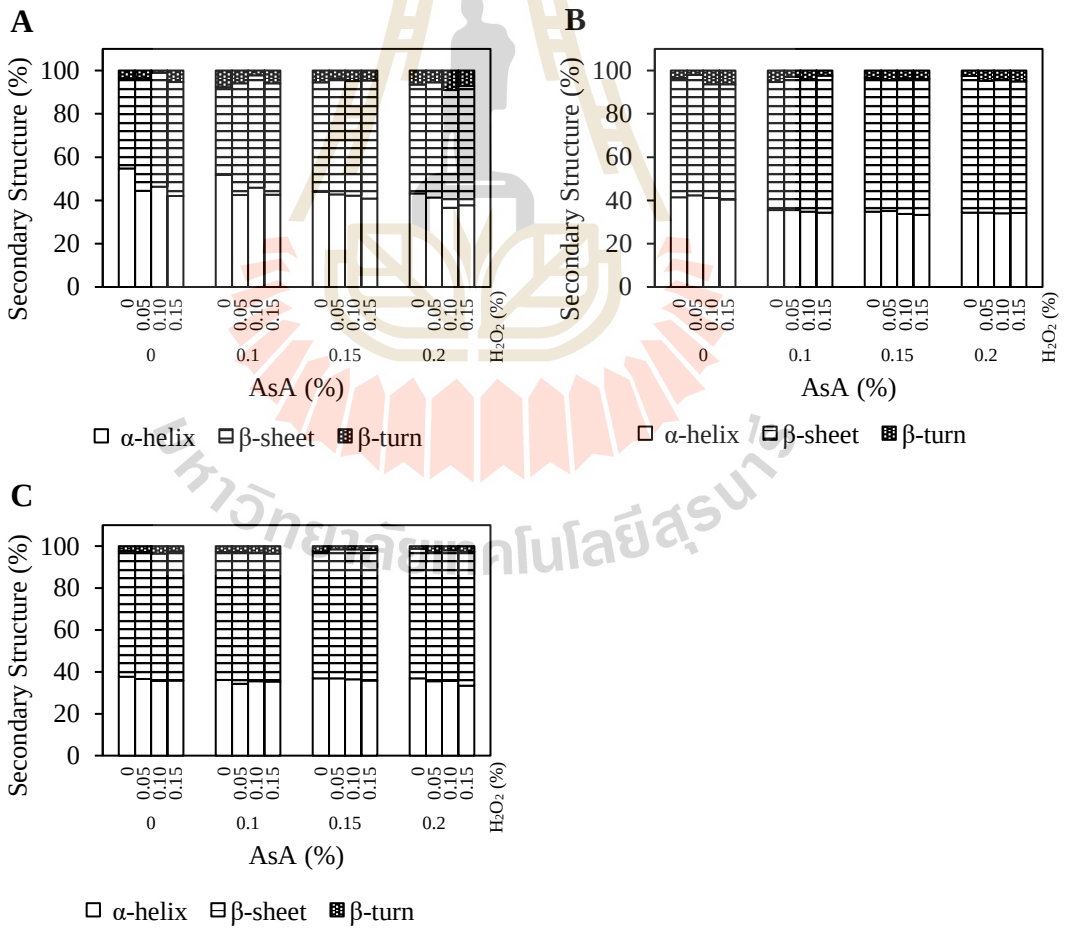

Figure 4.7 Secondary structure fractions from FT-IR spectra which A = surimi paste, B = gels cooked at 90 °C for 30 min, and C = gels set at 40 °C for 30 min.

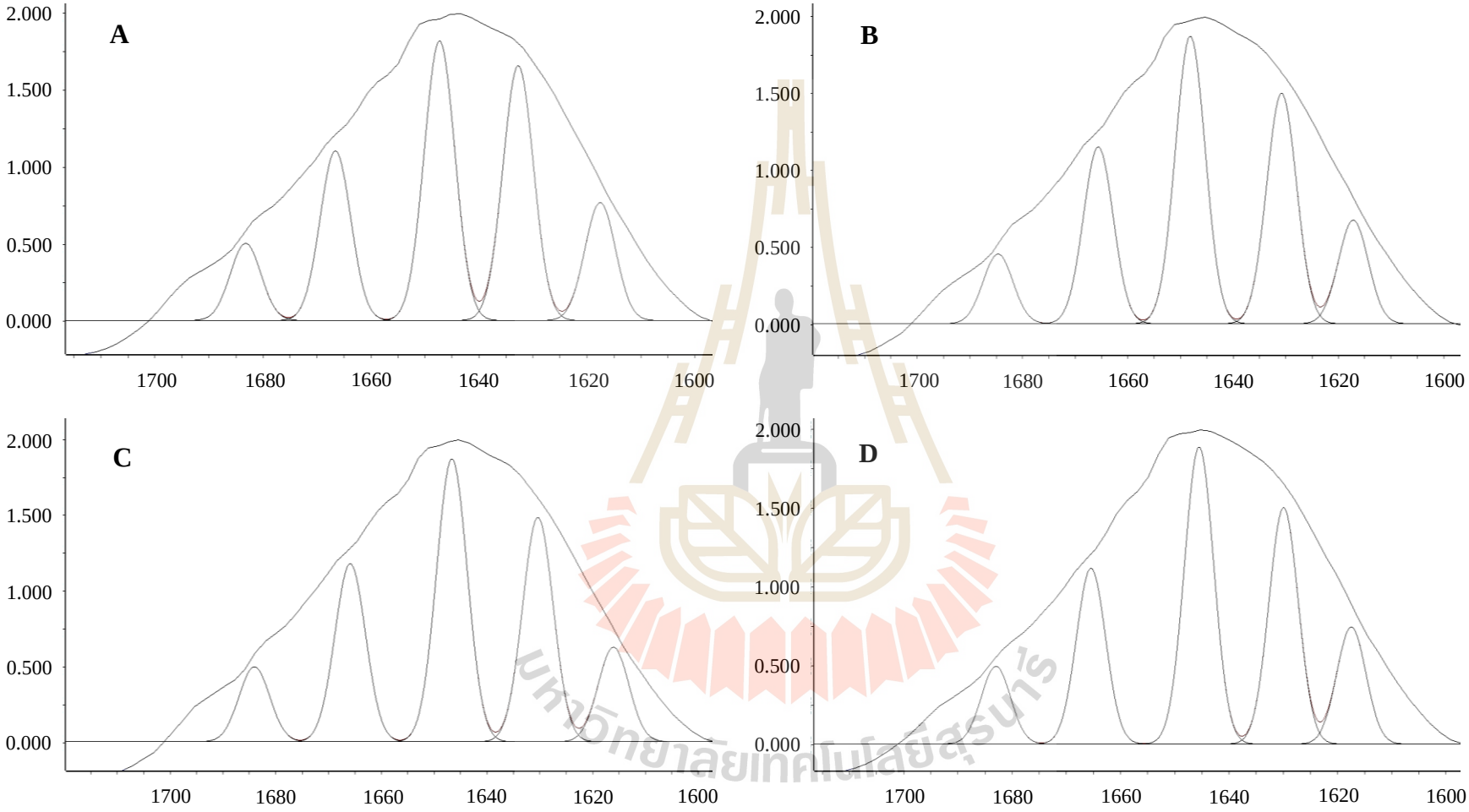

Figure 4.8 Curve-fitted amide I FT-IR bands of lyophilized SD surimi paste added various additives: control (A), 0.15% H_2O_2 (B), 0.2% AsA (C), and combined 0.2% AsA and 0.15% H_2O_2.

4.4.8 Tertiary structures

Aliphatic proteins reveal C–H stretching and bending vibrations at 2800-3000 cm^{-1} and 1450 cm^{-1} region of the Raman spectrum (Howell et al., 1999). The strong C-H band placed at 2936 cm^{-1} of SD surimi paste and gels cooked by direct heating or setting, decreased by addition of 0.15% H_2O_2 and/or 0.2% AsA, as compared to the control sample ($P < 0.05$, Table appendix 7-9). C–H stretching bands at 2980 cm^{-1} of SD surimi paste did not affect, while directly cooked or set gels decreased with increasing H_2O_2 and/or AsA ($P < 0.05$). Additionally, other C–H stretching bands near 2878 cm^{-1} of both SD surimi paste and gels decreased when additives were added ($P < 0.05$). The band area allotted to C–H stretching vibration at 2936 cm^{-1} greatly decreased in set gel as compared to paste or directly cooked gel. Wang and Damodaran (1991) reported that heating proteins above their thermal denaturation temperature resulted in the total destruction of both the tertiary and the H-bonded secondary structures in proteins. Thus, setting SD surimi at 40 °C could be exposed more the hydrophobic amino acids and enhanced involvement of hydrophobic interactions with addition of AsA and H_2O_2. C–H bending at 1450 cm^{-1} also decreased with addition of combined 0.2% AsA and 0.15% H_2O_2 in SD surimi paste or gels as compared to the control sample ($P < 0.05$). These aliphatic residues decrease with addition of H_2O_2 and/or AsA, indicating that these additives enhanced protein denaturation, and hydrophobic interaction between hydrophobic residues of aliphatic groups might be promoted.

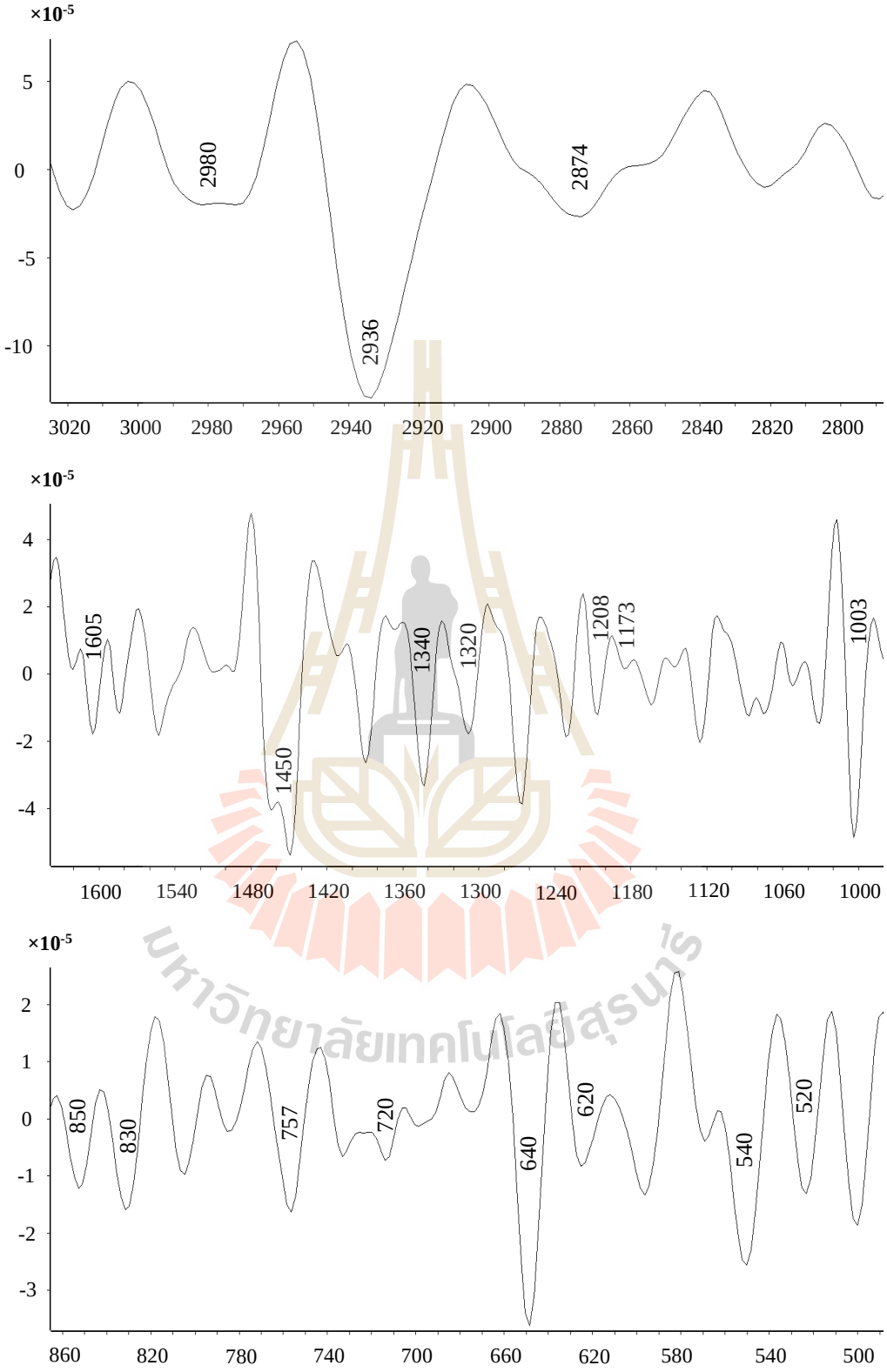

Figure 4.9 Second derivative of control SD surimi paste of the selected regions.

Tryptophan (Trp) residues in proteins located at 1340 of SD surimi paste and gels decreased with increasing combined H_2O_2 and AsA, as compared to the control. A decreased in Trp band at 1340 cm^{-1} perhaps indicated the exposure of Trp residues and involved in the H-bonding of a hydrophilic environment by adding H_2O_2 and/or AsA and gelation processing. Trp band near 760 cm^{-1} of SD paste and set gel at 40 °C decreased by the addition of H_2O_2 and AsA, while directly cooked gel did not significantly change. The decreased of the Trp band near 760 cm^{-1} by addition of H_2O_2 and AsA, indicates Trp residues is buried in the tertiary structure and the hydrophobicity might be involved by adding H_2O_2 and AsA, and gelation. This result is similar to Li-Chan (1996) reported that when Trp residues were moved from a hydrophobic micro-environment to a polar aqueous, the intensity of the band near 760 cm^{-1} regions decreased.

The doublet band of tyrosine (Tyr) is assigned to vibrations of the benzene ring of Tyr residues, which is one of the aromatic amino acid side chains. Tyr doublet ratio 850/830 cm^{-1} of SD surimi paste and directly cooked gel did not affect by adding H_2O_2 and/or AsA, as compared to control sample (Table 4.2). However, this ratio had not greatly decreased with addition of these additives when setting was applied ($P < 0.05$). Furthermore, Tyr ratio of SD paste or gels was lower than 1. These results indicated that the Tyr exposed Tyr residues were buried in the hydrophobic environment and involved as strong hydrogen bond donors upon the addition of AsA and H_2O_2 when SD surimi incubated at 40 °C.

Addition of AsA and H_2O_2 decreased total aliphatic and aromatic of SD surimi paste and gels (Table 4.2), indicated that adding AsA and H_2O_2 could improve protein unfolding and change aliphatic and aromatic groups due to hydrophobic interaction.

Table 4.2 Integral area of Raman spectra of SD as affected by various concentrations of AsA and H_2O_2.

Additives (%)		Paste			Direct heating			Setting		
AsA	**H_2O_2**	**Aliphatic ($\times10^{-3}$)**	**Aromatic ($\times10^{-3}$)**	**850/830**	**Aliphatic ($\times10^{-3}$)**	**Aromatic ($\times10^{-3}$)**	**850/830**	**Aliphatic ($\times10^{-3}$)**	**Aromatic ($\times10^{-3}$)**	**850/830**
0	0	6.59 ± 0.11 ^{a}	2.57 ± 0.18 ^{a}	0.96 ± 0.16	7.01 ± 0.03 ^{a}	2.53 ± 0.04 ^{a}	0.65 ± 0.09	6.94 ± 0.06 ^{b}	2.87 ± 0.07 ^{a}	0.75 ± 0.24 ^{abc}
	0.05	5.72 ± 0.08 ^{b}	2.15 ± 0.05 ^{bc}	0.71 ± 0.22	6.65 ± 0.17 ^{b}	2.37 ± 0.15 ^{abc}	0.66 ± 0.25	6.58 ± 0.04 ^{cd}	2.59 ± 0.07 ^{b}	0.65 ± 0.07 ^{abc}
	0.1	5.36 ± 0.07 ^{c}	1.88 ± 0.14 ^{fg}	0.59 ± 0.23	6.02 ± 0.14 ^{fg}	2.17 ± 0.01 ^{cde}	0.49 ± 0.17	6.15 ± 0.15 ^{e}	2.36 ± 0.07 ^{cd}	0.79 ± 0.09 ^{abc}
	0.15	5.19 ± 0.05 ^{cde}	1.96 ± 0.11 ^{bcd}	0.61 ± 0.23	6.25 ± 0.06 ^{def}	2.16 ± 0.10 ^{cde}	0.48 ± 0.22	5.73 ± 0.05 ^{hi}	2.10 ± 0.19 ^{g}	0.71 ± 0.16 ^{abc}
0.1	0	5.36 ± 0.10 ^{c}	2.31 ± 0.05 ^{b}	0.64 ± 0.14	6.67 ± 0.15 ^{b}	2.25 ± 0.22 ^{bcd}	0.61 ± 0.35	7.34 ± 0.03 ^{a}	2.63 ± 0.06 ^{b}	0.92 ± 0.04 ^{a}
	0.05	5.11 ± 0.07 ^{cde}	2.09 ± 0.07 ^{cde}	0.68 ± 0.12	6.54 ± 0.12 ^{bc}	2.42 ± 0.12 ^{ab}	0.60 ± 0.16	6.74 ± 0.20 ^{c}	2.48 ± 0.16 ^{bc}	0.76 ± 0.02 ^{abc}
	0.1	4.75 ± 0.13 ^{fg}	1.86 ± 0.04 ^{fg}	0.52 ± 0.10	6.07 ± 0.10 ^{efg}	2.00 ± 0.00 ^{ef}	0.66 ± 0.11	5.82 ± 0.04 ^{fgh}	2.17 ± 0.14 ^{efg}	0.65 ± 0.21 ^{abc}
	0.15	4.45 ± 0.06 ^{h}	1.76 ± 0.10 ^{g}	0.46 ± 0.14	6.20 ± 0.15 ^{def}	2.00 ± 0.13 ^{ef}	0.53 ± 0.16	5.37 ± 0.10 ^{j}	2.07 ± 0.06 ^{g}	0.86 ± 0.32 ^{ab}
0.15	0	5.28 ± 0.14 ^{cd}	2.11 ± 0.08 ^{cd}	0.69 ± 0.26	6.33 ± 0.05 ^{cde}	2.20 ± 0.11 ^{cde}	0.58 ± 0.10	5.91 ± 0.20 ^{fgh}	2.46 ± 0.09 ^{bc}	0.43 ± 0.19 ^{efg}
	0.05	5.04 ± 0.18 ^{de}	2.01 ± 0.11 ^{cde}	0.60 ± 0.16	6.14 ± 0.19 ^{def}	2.00 ± 0.01 ^{ef}	0.67 ± 0.15	5.99 ± 0.02 ^{efg}	2.28 ± 0.04 ^{def}	0.42 ± 0.13 ^{fg}
	0.1	5.04 ± 0.08 ^{de}	1.89 ± 0.06 ^{fg}	0.54 ± 0.13	6.17 ± 0.10 ^{def}	2.00 ± 0.10 ^{ef}	0.68 ± 0.22	5.98 ± 0.04 ^{efg}	2.10 ± 0.04 ^{g}	0.46 ± 0.18 ^{def}
	0.15	5.00 ± 0.18 ^{ef}	1.94 ± 0.01 ^{def}	0.51 ± 0.09	6.09 ± 0.33 ^{def}	1.93 ± 0.10 ^{f}	0.58 ± 0.12	5.62 ± 0.11 ^{i}	1.87 ± 0.07 ^{h}	0.26 ± 0.05 ^{g}
0.2	0	5.17 ± 0.19 ^{cde}	1.77 ± 0.05 ^{g}	0.57 ± 0.05	6.35 ± 0.11 ^{cd}	2.06 ± 0.02 ^{def}	0.41 ± 0.15	6.51 ± 0.06 ^{d}	2.34 ± 0.08 ^{cde}	0.53 ± 0.09 ^{cde}
	0.05	5.22 ± 0.27 ^{cde}	1.91 ± 0.26 ^{efg}	0.50 ± 0.16	6.04 ± 0.11 ^{fg}	2.17 ± 0.15 ^{cde}	0.43 ± 0.12	6.01 ± 0.08 ^{ef}	2.33 ± 0.05 ^{cde}	0.54 ± 0.20 ^{cde}
	0.1	4.99 ± 0.13 ^{ef}	1.82 ± 0.04 ^{fg}	0.55 ± 0.26	6.04 ± 0.13 ^{fg}	2.25 ± 0.06 ^{bcd}	0.43 ± 0.08	5.99 ± 0.09 ^{efg}	2.12 ± 0.07 ^{fg}	0.51 ± 0.17 ^{cde}
	0.15	4.64 ± 0.20 ^{gh}	1.80 ± 0.10 ^{g}	0.56 ± 0.10	5.80 ± 0.07 ^{g}	2.05 ± 0.12 ^{def}	0.45 ± 0.04	5.80 ± 0.10 ^{ghi}	2.14 ± 0.10 ^{fg}	0.56 ± 0.20 ^{bcd}

Data were given as mean ± SD. Different letters within the same column indicate significant differences ($P < 0.05$) between mean values.

4.4.9 Disulfide bond regions

The characteristic of S-S bond in Raman spectra existing at bands 500 and 550 cm^{-1}, containing three types stretching vibration of S-S bridge, including *g-g-g*, *g-g-t*, and *t-g-t*, where "*g*" and "*t*" is gauche and trans, respectively (Li-Chan et al., 1994). The S-S band at 510 cm^{-1} assigned for native protein, did not observe in addition of AsA and H_2O_2, or after cooked by direct heating or setting, indicated that protein denaturation was enhanced by addition of AsA and H_2O_2, or gelation. S-S bands of SD surimi paste decreased near 525 cm^{-1} and slightly increased at 540 cm^{-1} with increasing of 0.2% AsA and 0.15% H_2O_2 ($P < 0.05$, Table 4.3). S-S band near 540 cm^{-1} of SD directly cooked gel decreased by addition of AsA and H_2O_2 ($P < 0.05$), while band near 525 cm^{-1} did not change. Moreover, addition of AsA and H_2O_2 did not affect the S-S bond region near 540 and 525 cm^{-1} of SD set gel. Total S-S bond region of SD surimi paste and gels decreased by the addition of AsA and H_2O_2. Results indicating the formation of S-S bonds inferred more *g-g-t* around 525 cm^{-1} of S-S bridges in SD surimi after added AsA and H_2O_2, but S-S bonds conformation did not improve by these additives.

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Table 4.3 Integral area ($\times 10^{-3}$) of disulfide bond regions of Raman spectra of surimi paste and gels (direct heating and setting) from SD as affected by various concentrations of AsA and H_2O_2.

Additives (%)		Surimi paste			Gel (direct heating)			Gel (setting)		
AsA	**H_2O_2**	**540±5**	**525±5**	**Total**	**540±5**	**525±5**	**Total**	**540±5**	**525±5**	**Total**
0	0	0.21 ± 0.08 ^{a}	0.15 ± 0.04 ^{bcd}	**0.36** ^{a}	0.19 ± 0.06 ^{a}	0.13 ± 0.02	**0.32**	0.15 ± 0.02	0.19 ± 0.05 ^{a}	**0.33** ^{a}
	0.05	0.14 ± 0.02 ^{ab}	0.12 ± 0.04 ^{cde}	**0.26** ^{bcd}	0.17 ± 0.03 ^{abc}	0.11 ± 0.02	**0.28**	0.18 ± 0.02	0.19 ± 0.01 ^{a}	**0.37** ^{a}
	0.1	0.12 ± 0.03 ^{b}	0.12 ± 0.04 ^{cde}	**0.24** ^{cde}	0.13 ± 0.02 ^{abc}	0.12 ± 0.03	**0.26**	0.10 ± 0.01	0.13 ± 0.06 ^{bc}	**0.23** ^{c}
	0.15	0.11 ± 0.06 ^{b}	0.19 ± 0.07 ^{abc}	**0.30** ^{abc}	0.19 ± 0.05 ^{ab}	0.10 ± 0.01	**0.29**	0.12 ± 0.02	0.13 ± 0.03 ^{c}	**0.25** ^{bc}
0.1	0	0.08 ± 0.02 ^{b}	0.12 ± 0.02 ^{cde}	**0.20** ^{de}	0.12 ± 0.04 ^{bcd}	0.14 ± 0.01	**0.26**	0.11 ± 0.01	0.10 ± 0.02 ^{c}	**0.21** ^{c}
	0.05	0.09 ± 0.02 ^{b}	0.18 ± 0.01 ^{abc}	**0.28** ^{abc}	0.11 ± 0.03 ^{cd}	0.12 ± 0.01	**0.23**	0.14 ± 0.02	0.11 ± 0.01 ^{c}	**0.25** ^{bc}
	0.1	0.14 ± 0.03 ^{ab}	0.11 ± 0.01 ^{de}	**0.25** ^{cde}	0.09 ± 0.02 ^{cd}	0.14 ± 0.03	**0.23**	0.12 ± 0.04	0.10 ± 0.01 ^{c}	**0.22** ^{c}
	0.15	0.10 ± 0.01 ^{b}	0.12 ± 0.04 ^{cde}	**0.22** ^{de}	0.11 ± 0.04 ^{cd}	0.20 ± 0.01	**0.31**	0.11 ± 0.01	0.11 ± 0.03 ^{c}	**0.22** ^{c}
0.15	0	0.15 ± 0.03 ^{ab}	0.20 ± 0.02 ^{ab}	**0.35** ^{ab}	0.14 ± 0.06 ^{abc}	0.13 ± 0.05	**0.27**	0.11 ± 0.03	0.11 ± 0.03 ^{c}	**0.22** ^{c}
	0.05	0.08 ± 0.04 ^{b}	0.17 ± 0.03 ^{abc}	**0.25** ^{cde}	0.13 ± 0.05 ^{abc}	0.12 ± 0.05	**0.26**	0.12 ± 0.01	0.12 ± 0.01 ^{c}	**0.24** ^{c}
	0.1	0.09 ± 0.03 ^{b}	0.11 ± 0.03 ^{de}	**0.20** ^{de}	0.11 ± 0.06 ^{cd}	0.16 ± 0.04	**0.27**	0.10 ± 0.03	0.12 ± 0.01 ^{c}	**0.22** ^{c}
	0.15	0.15 ± 0.05 ^{ab}	0.14 ± 0.03 ^{bcd}	**0.29** ^{abc}	0.08 ± 0.02 ^{d}	0.16 ± 0.05	**0.25**	0.10 ± 0.03	0.11 ± 0.02 ^{c}	**0.21** ^{c}
0.2	0	0.11 ± 0.02 ^{b}	0.13 ± 0.02 ^{cde}	**0.24** ^{cde}	0.10 ± 0.01 ^{cd}	0.14 ± 0.04	**0.24**	0.12 ± 0.01	0.11 ± 0.01 ^{c}	**0.23** ^{c}
	0.05	0.13 ± 0.04 ^{b}	0.14 ± 0.05 ^{bcd}	**0.26** ^{bcd}	0.11 ± 0.01 ^{cd}	0.12 ± 0.03	**0.24**	0.09 ± 0.01	0.18 ± 0.05 ^{ab}	**0.27** ^{bc}
	0.1	0.12 ± 0.02 ^{b}	0.09 ± 0.01 ^{e}	**0.22** ^{de}	0.11 ± 0.04 ^{cd}	0.12 ± 0.05	**0.23**	0.10 ± 0.01	0.11 ± 0.03 ^{c}	**0.21** ^{c}
	0.15	0.10 ± 0.03 ^{b}	0.22 ± 0.03 ^{a}	**0.33** ^{abc}	0.11 ± 0.01 ^{cd}	0.14 ± 0.04	**0.25**	0.13 ± 0.05	0.19 ± 0.04 ^{a}	**0.31** ^{ab}

Data were given as mean ± SD. Different letters within the same column indicate significant differences ($P < 0.05$) between mean values.

4.5 Conclusions

Addition of AsA and/or H_2O_2 did not improve both breaking force and distance of SD directly cooked gel at 90 °C. Addition of 0.15% H_2O_2 alone slightly increased breaking distance of SD surimi gel when setting at 40 °C was applied. Combination of 0.2% AsA and/or 0.15% H_2O_2 enhanced whiteness of surimi gels. Proteolytic activity in SD surimi was high with addition of 0.2% AsA. Based on FT-IR spectroscopy, gels added AsA and H_2O_2 and set surimi gel at 40 °C showed a reduction of α-helical structure in concomitant with an increase β-sheet structure. Moreover, aliphatic residues monitored by Raman decreased, suggesting involvement of hydrophobic interaction of SD surimi induced by addition of AsA and H_2O_2. Disulfide bond conformation did not enhance in this surimi gel. Therefore, addition of H_2O_2 alone and setting improved gel-forming ability and structural changes of SD surimi.

4.6 References

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CHAPTER V

SUMMARY

Ascorbic acid (AsA) and hydrogen peroxide (H_2O_2) have been shown to have a different effect on tropical surimi gel-forming ability. Addition of H_2O_2 alone increased both breaking force and distance of lizardfish (LZ) surimi gel, while AsA alone increased gel strength of threadfin bream (TB) surimi, when gel either cooked by direct heating at 90 °C or setting at 40 °C. Combination of AsA and H_2O_2 resulted in the greatly enhanced the gel strength of both LZ and TB surimi. However, addition of AsA and/or H_2O_2 did not affect gel improvement of sardine (SD) surimi. In contrast, setting greatly enhanced gel texture of SD surimi. Whiteness of SD gel improved by combined AsA and H_2O_2, but this combination decreased whiteness in LZ and TB gels through ascorbic acid browning. AsA and/or H_2O_2 enhanced polymerization of MHC in all surimi species by promoting oxidation of sulfhydryl groups during direct heating rather than setting. Proteolytic activity occurred in all surimi with addition of AsA subjected to setting process. A decrease in α-helix and an increase in β-sheet content of all surimi species from FT-IR spectroscopy were observed with addition of AsA and H_2O_2. Raman spectra showed that the involvement of hydrophobic interactions increased by these additives in all species. Principle component analysis showed that textural characteristics were positively correlated with β-sheet content of LZ and TB gels. This study indicated that AsA and H_2O_2 improved gel formation, and hydrophobic interactions and S-S bonds involvement of LZ and TB surimi. Gel improvement of SD surimi was more enhanced with setting at 40 °C rather than addition of additives.

APPENDIX

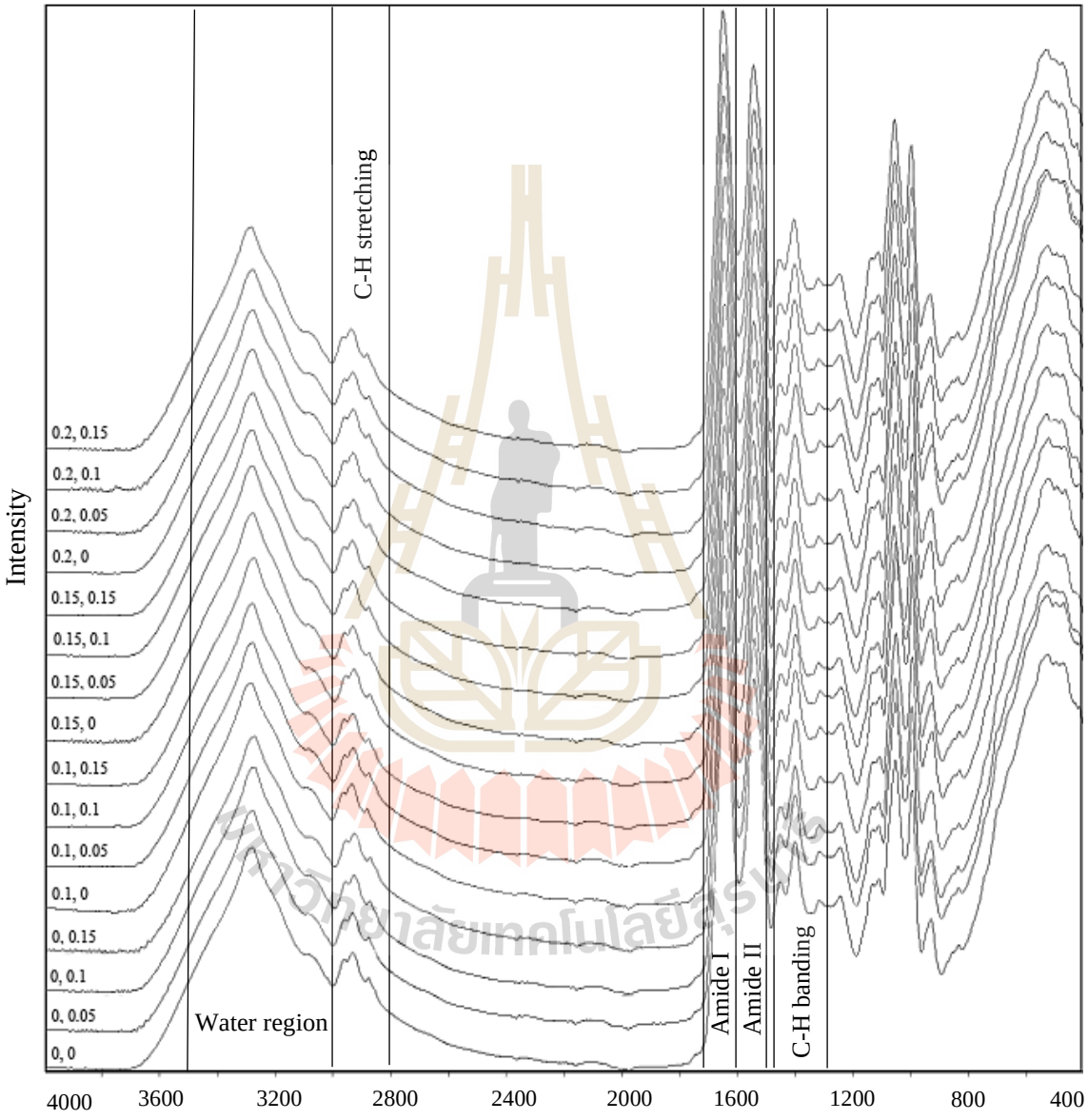

Figure 1 FT-IR spectra of lyophilized LZ surimi pastes added various concentration of AsA, H_2O_2.

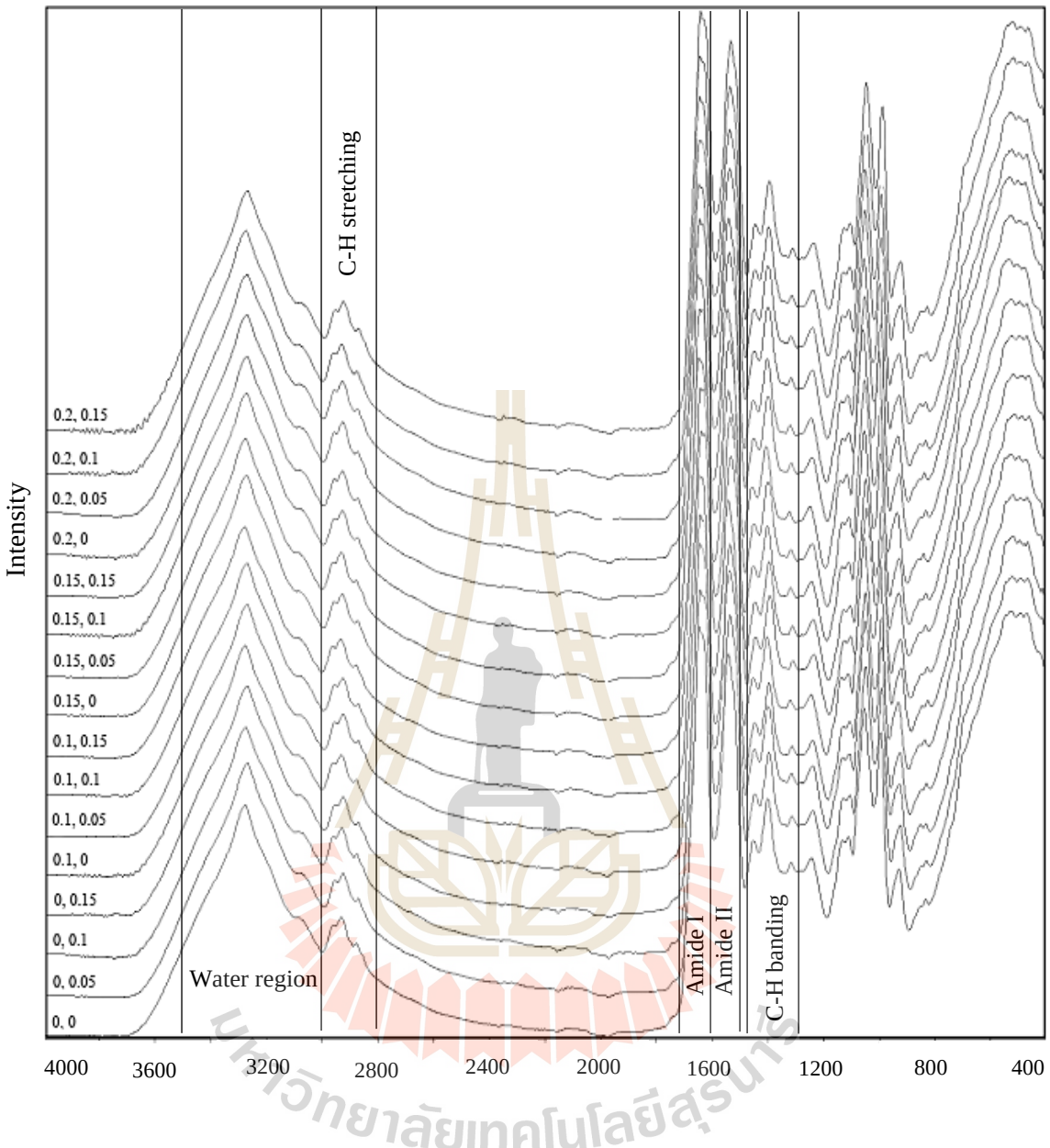

Figure 2 FT-IR spectra of lyophilized LZ directly cooked gels added various concentration of AsA, H_2O_2.

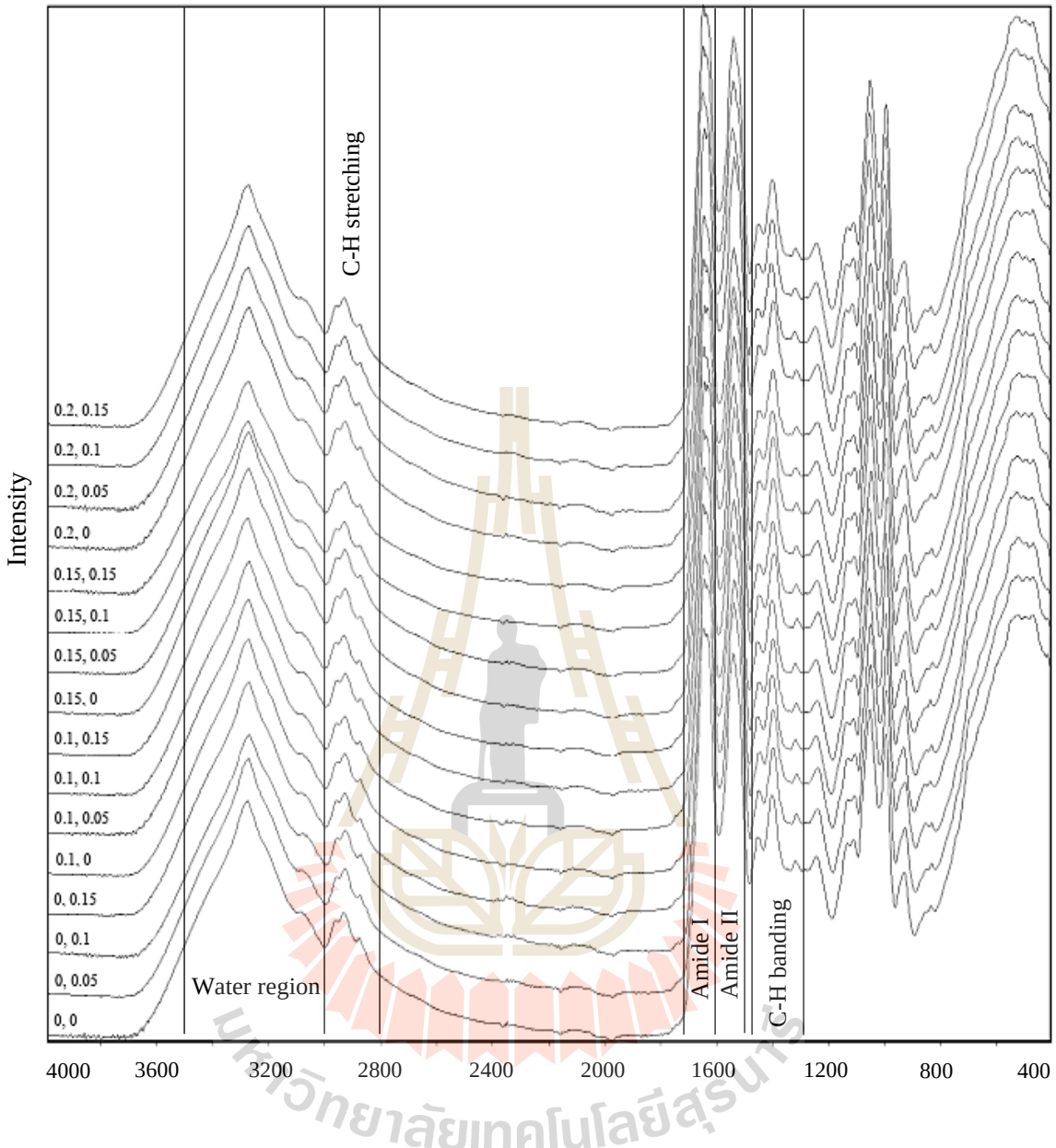

Figure 3 FT-IR spectra of lyophilized LZ set gels added various concentration of AsA, H_2O_2.

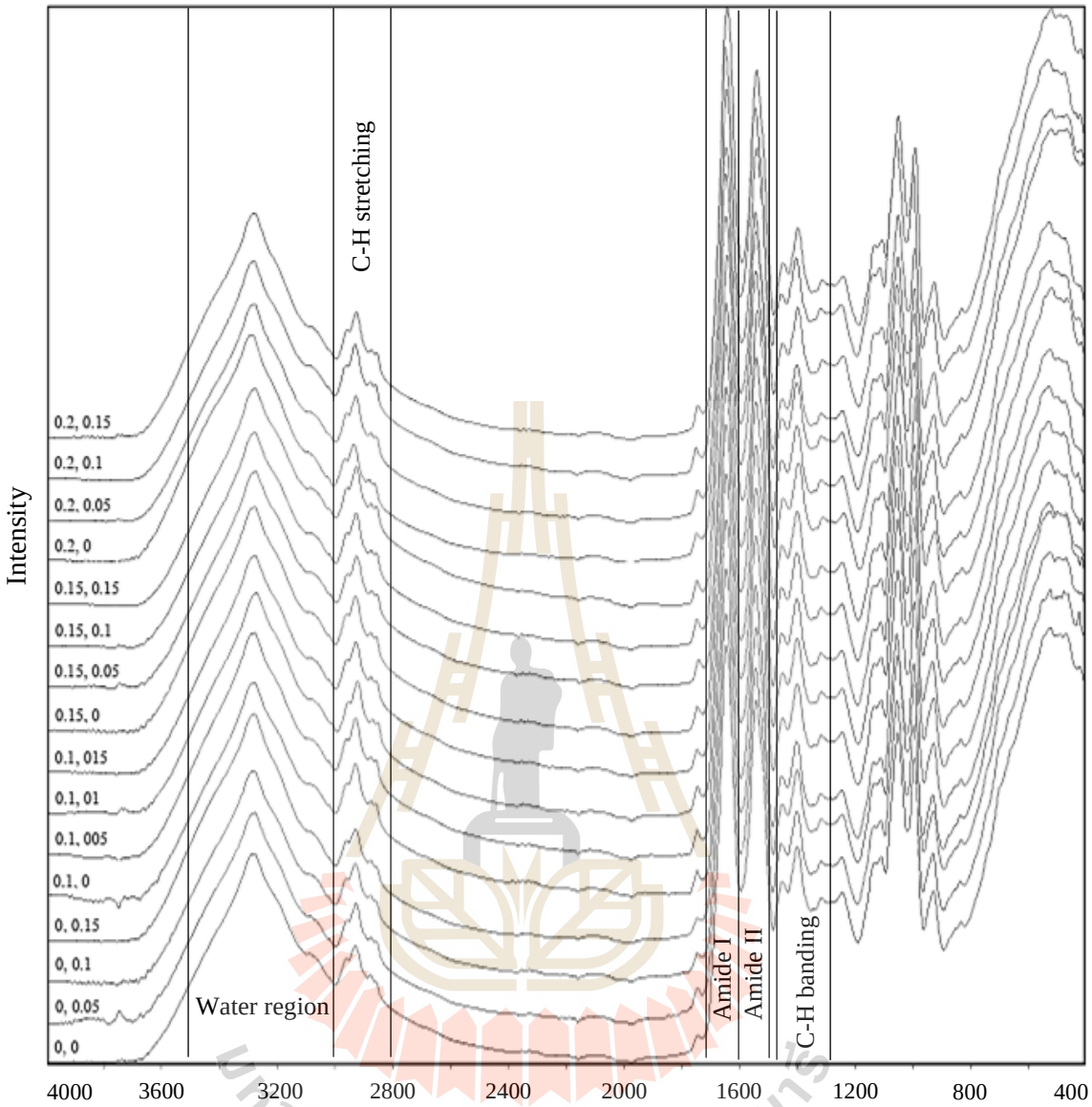

Figure 4 FT-IR spectra of lyophilized TB surimi pastes added various concentration of AsA, H_2O_2.

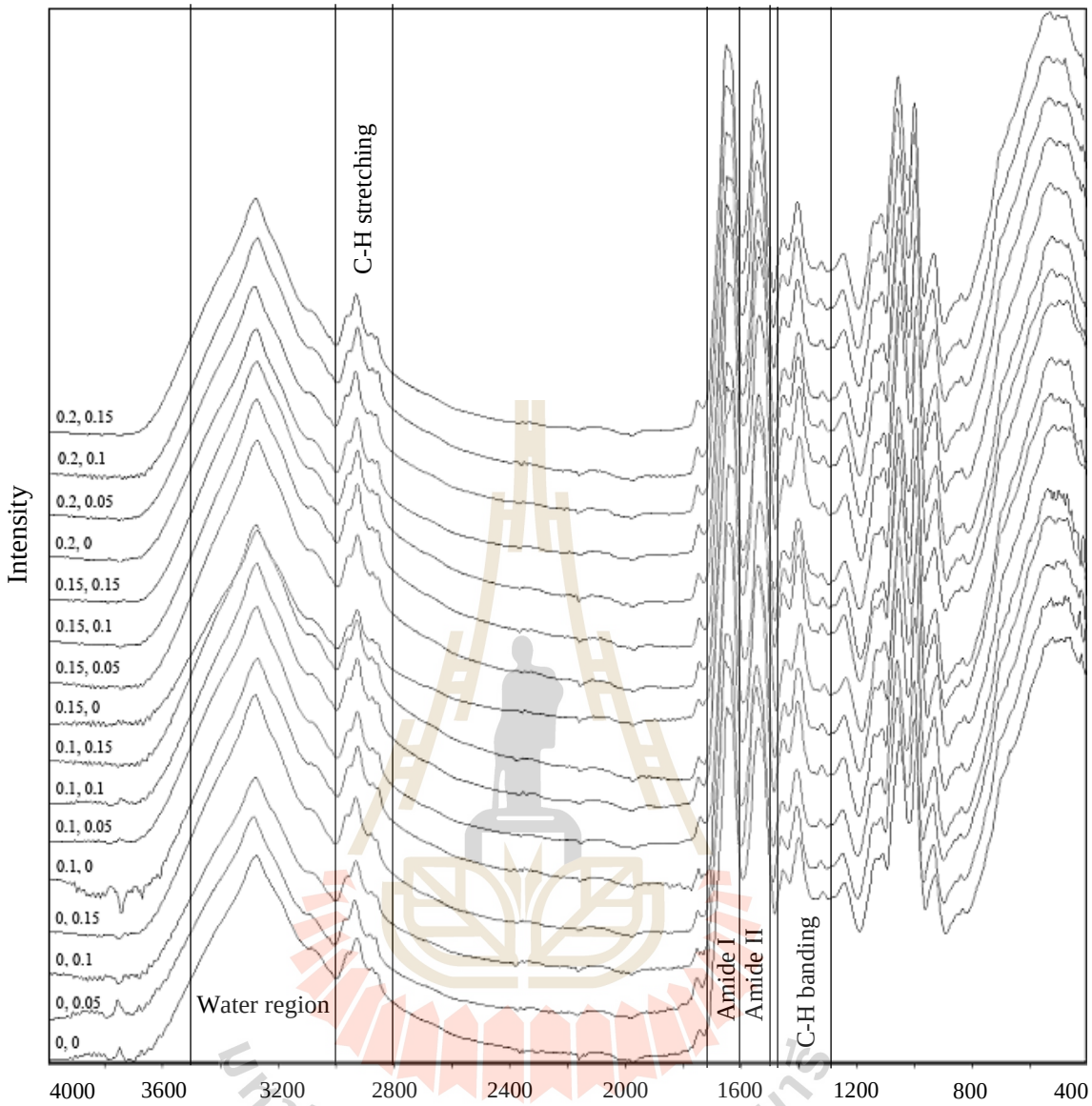

Figure 5 FT-IR spectra of lyophilized TB directly cooked gels added various concentration of AsA, H_2O_2.

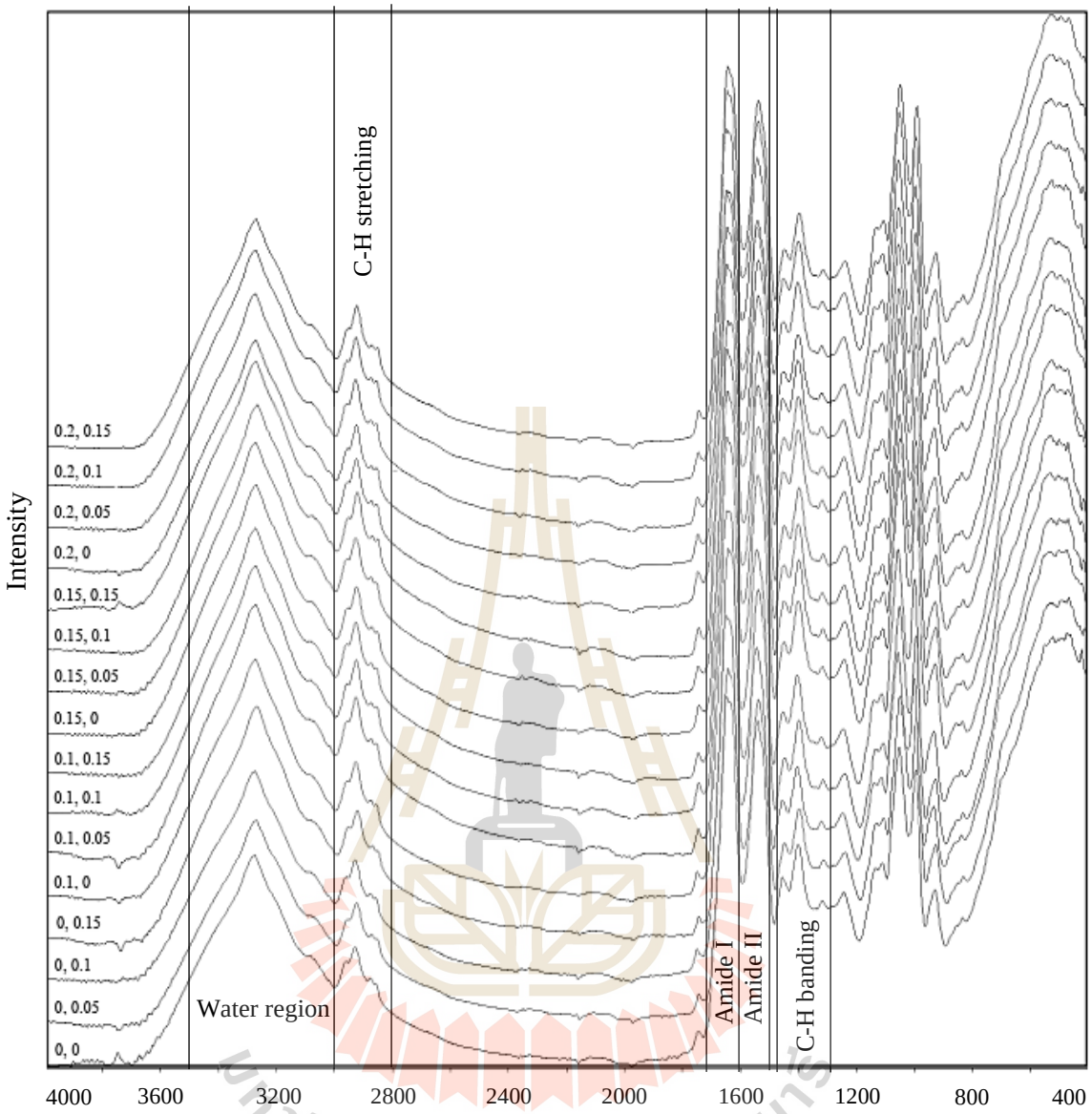

Figure 6 FT-IR spectra of lyophilized TB set gels added various concentration of AsA, H_2O_2.

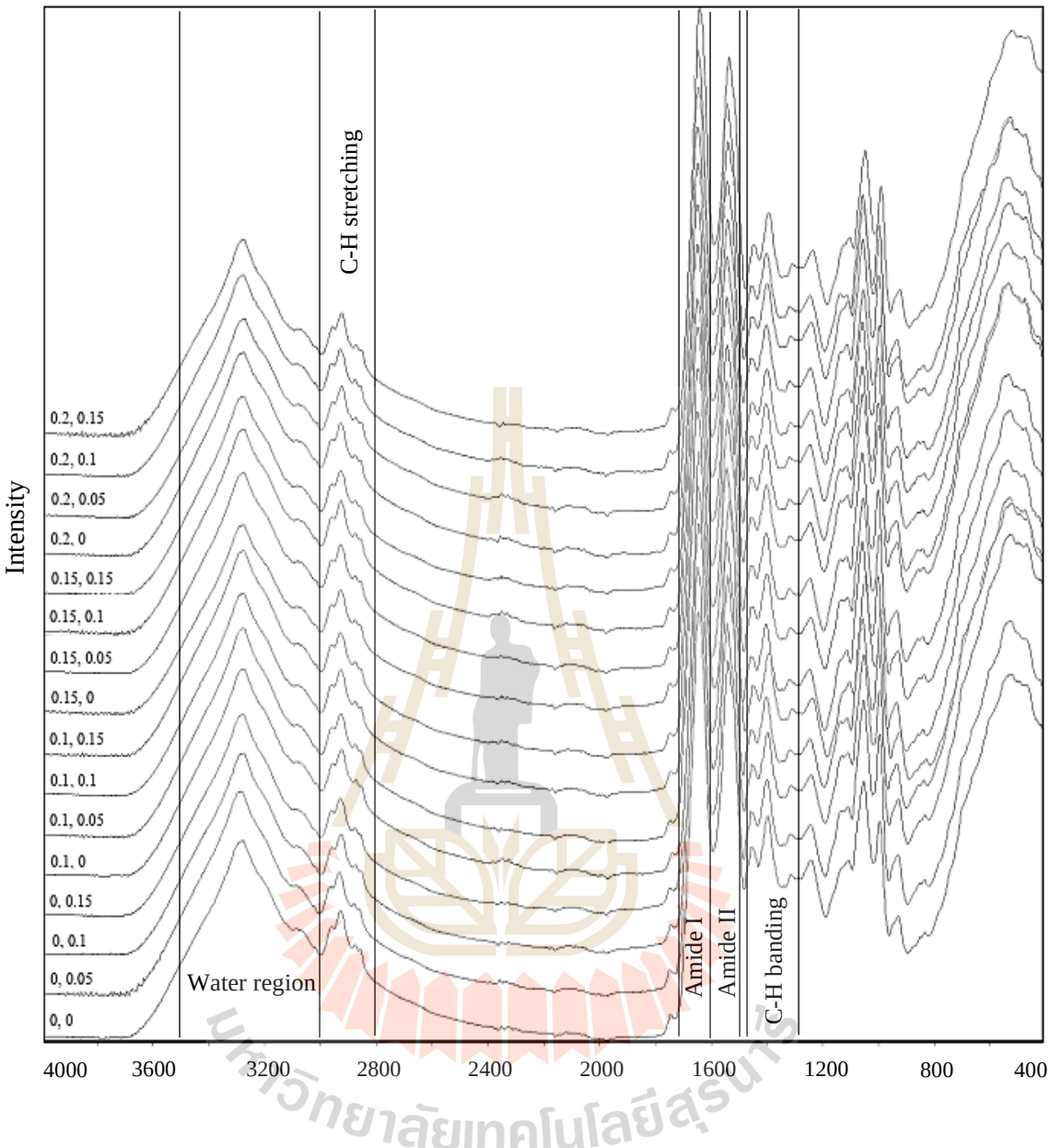

Figure 7 FT-IR spectra of lyophilized SD surimi pastes added various concentration of AsA, H_2O_2.

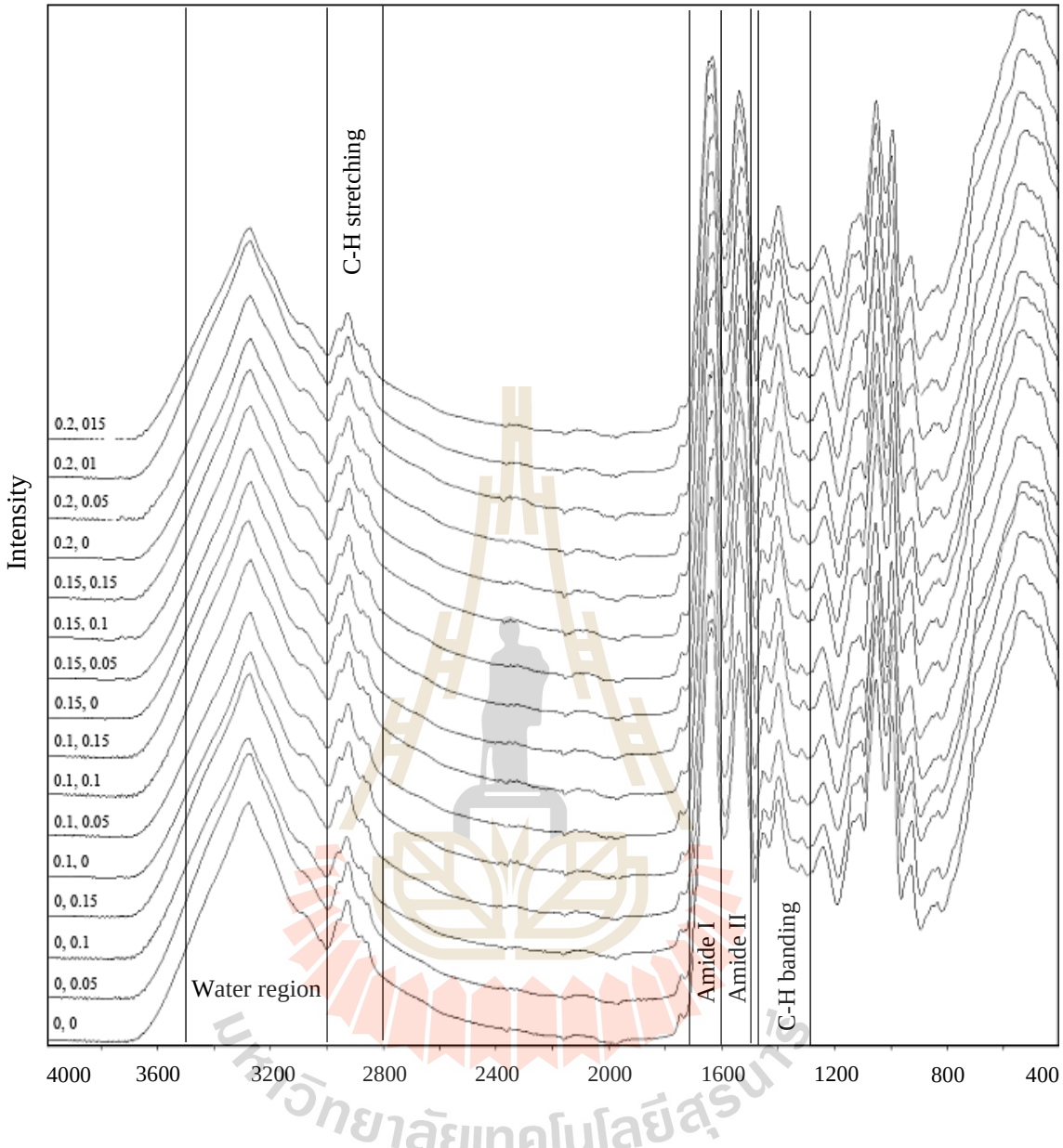

Figure 8 FT-IR spectra of lyophilized SD directly cooked gels added various concentration of AsA, H_2O_2.

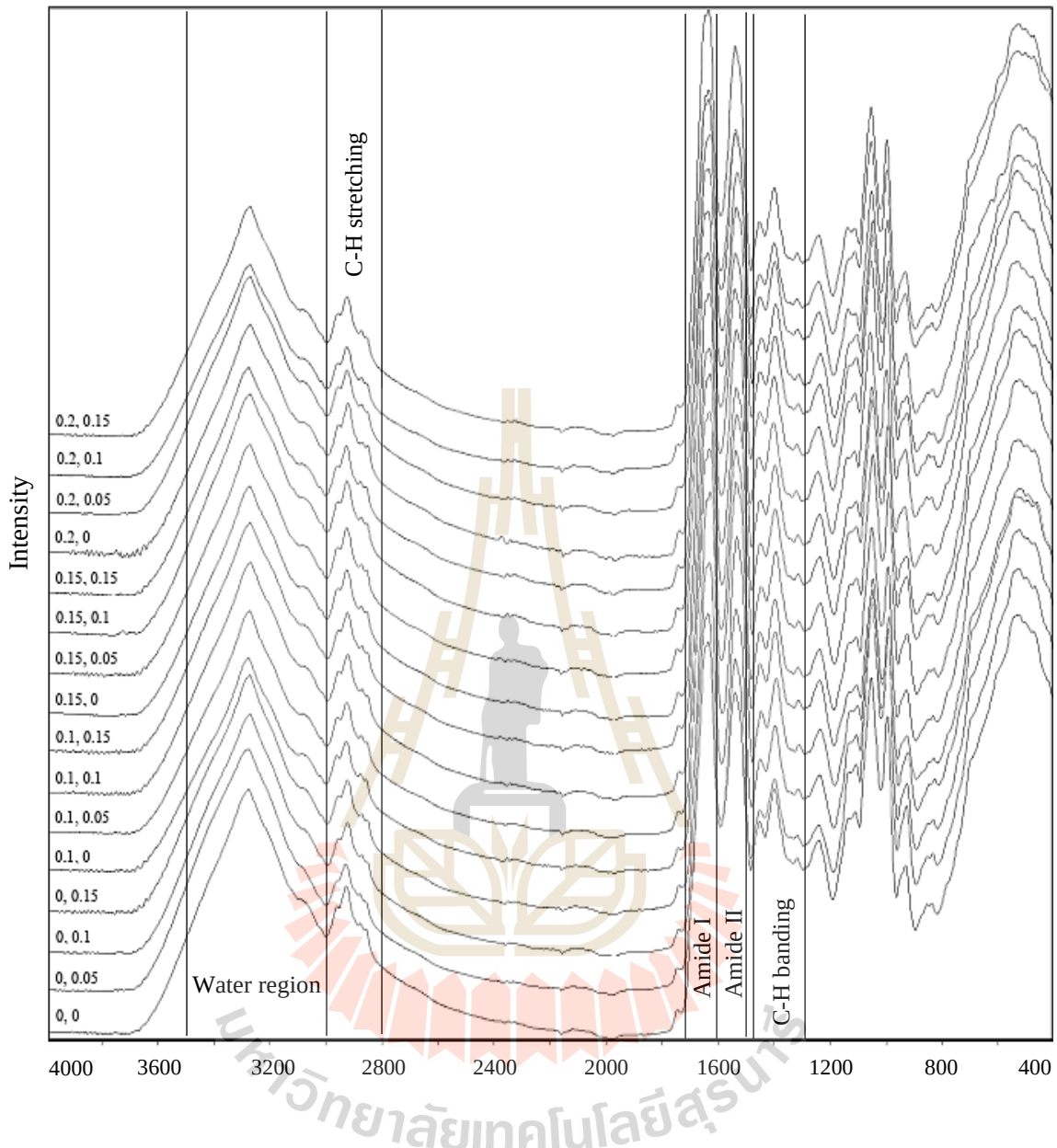

Figure 9 FT-IR spectra of lyophilized SD set gels added various concentration of AsA, H_2O_2.

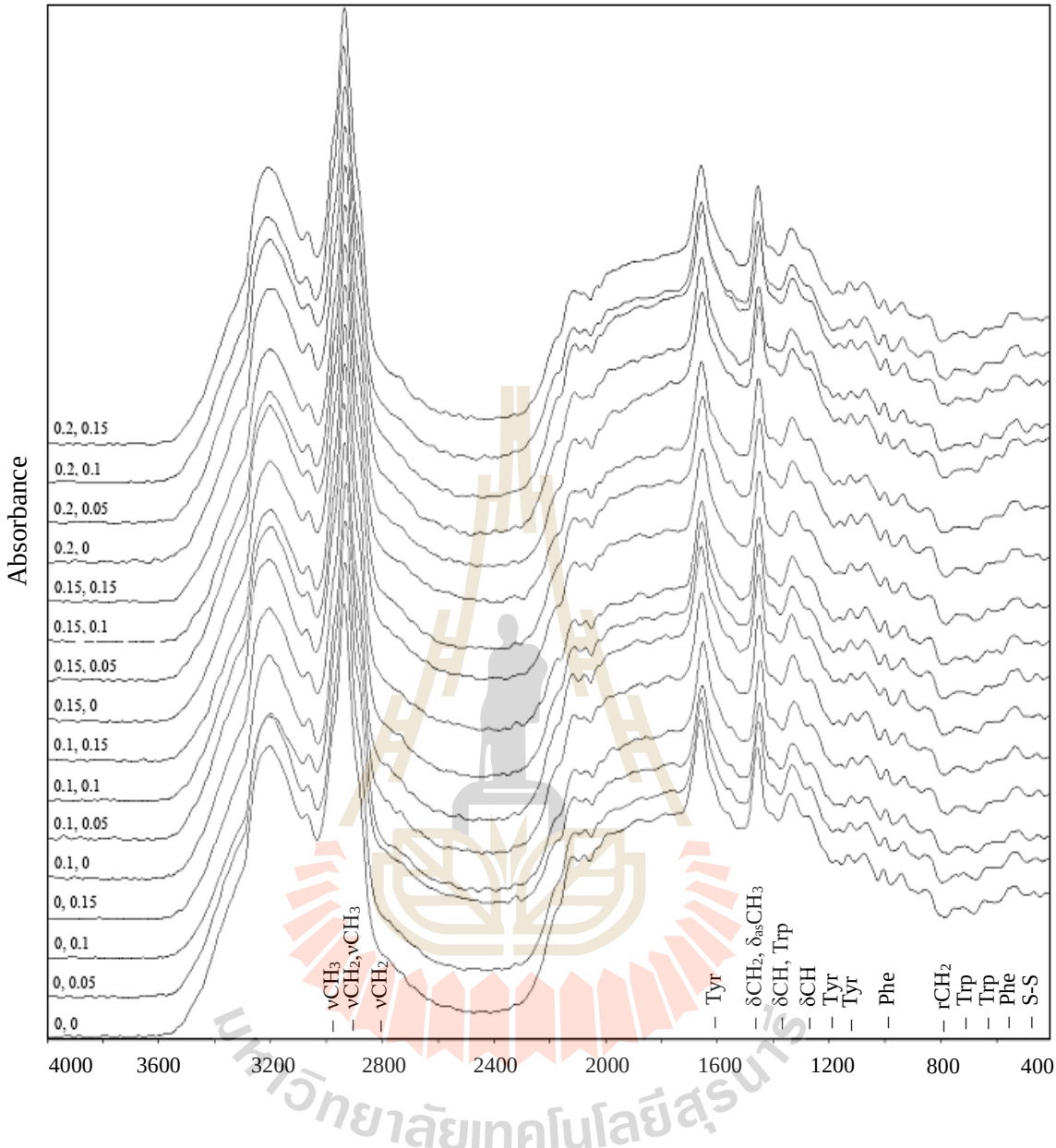

Figure 10 Raman spectra of LZ surimi pastes added various concentrations of AsA, H_2O_2.

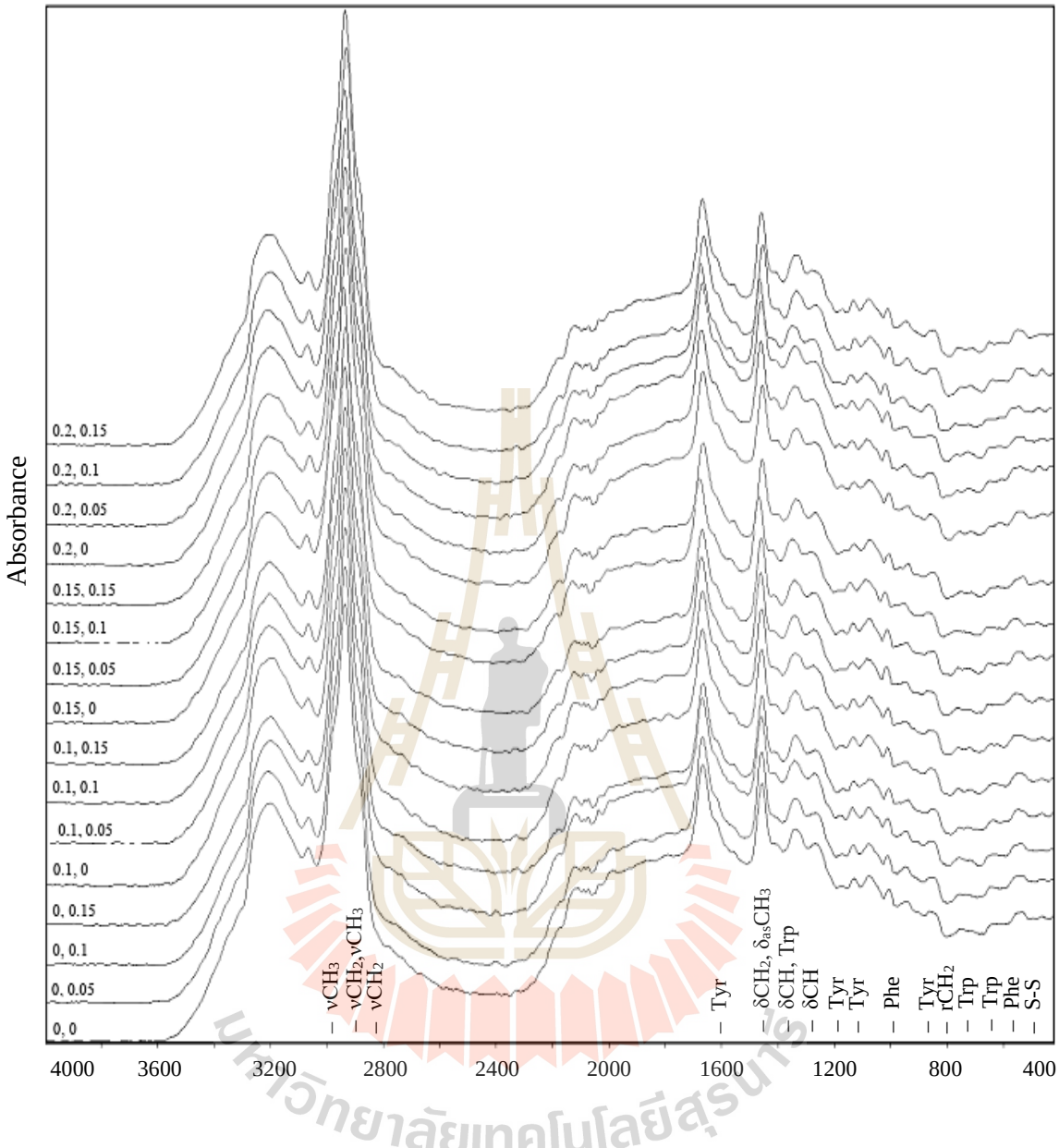

Figure 11 Raman spectra of LZ directly cooked gels cooked added various concentrations of AsA, H_2O_2.

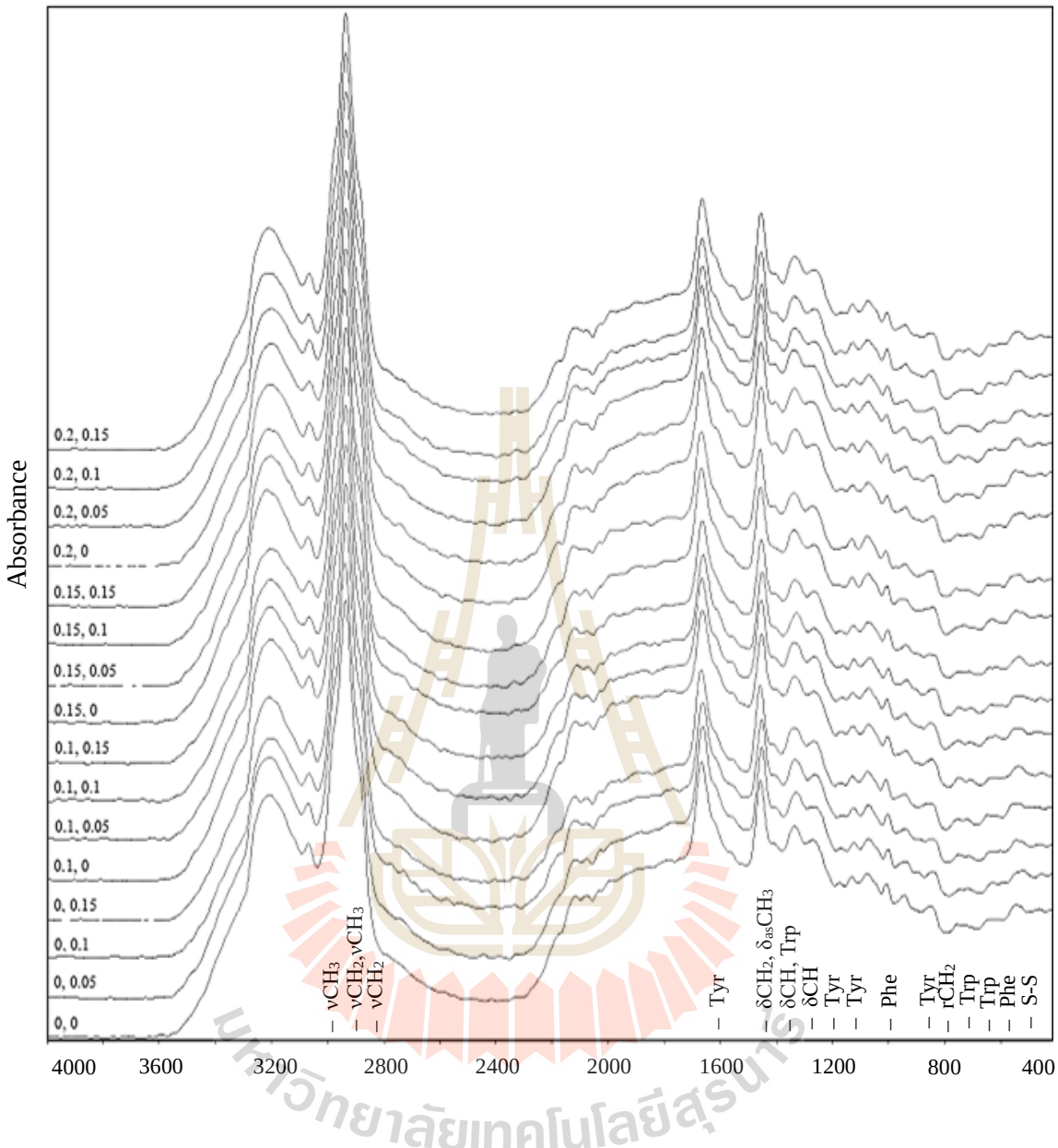

Figure 12 Raman spectra of LZ set gels added various concentrations of AsA, H_2O_2.

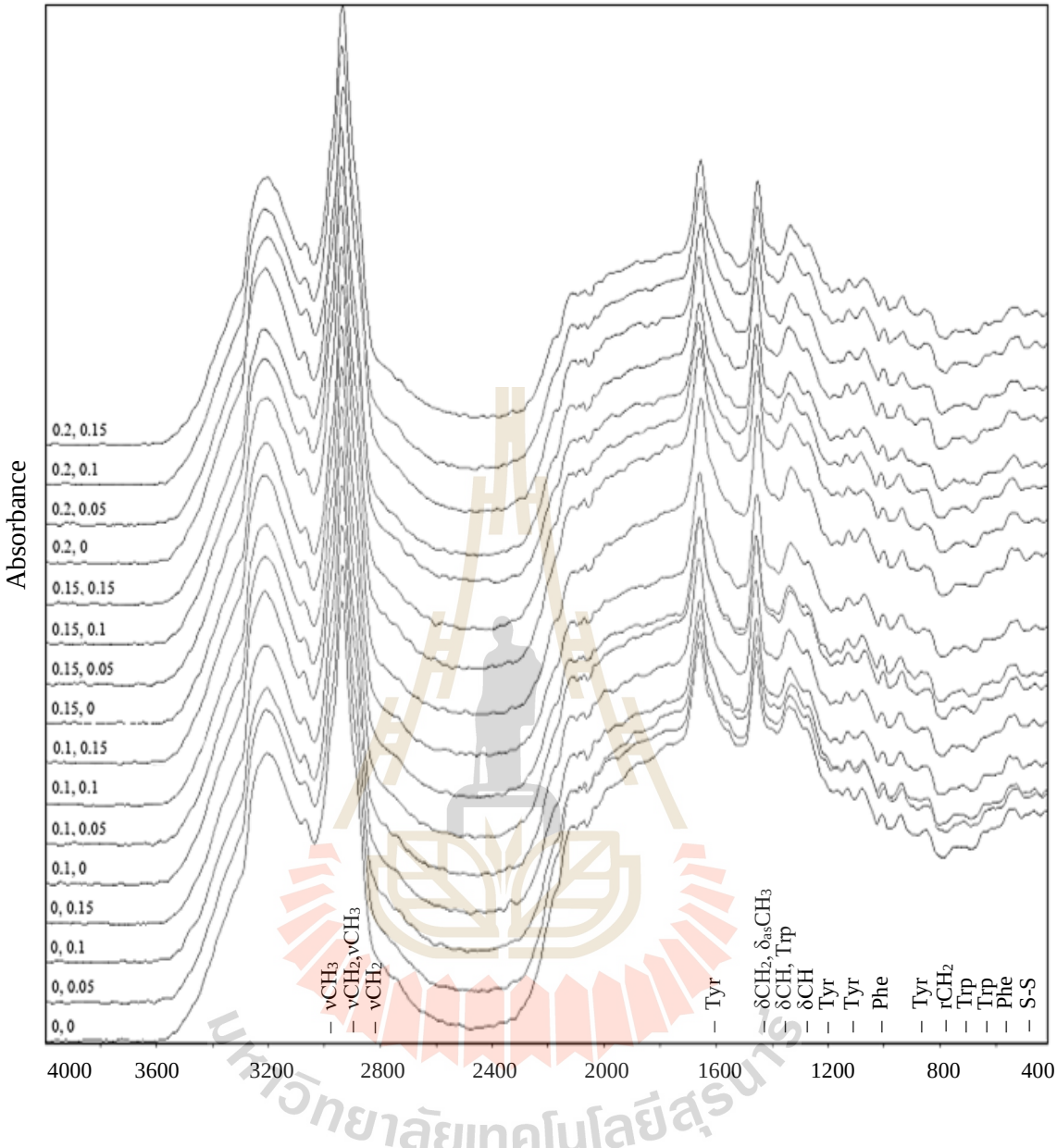

Figure 13 Raman spectra of TB surimi pastes added various concentrations of AsA, H_2O_2.

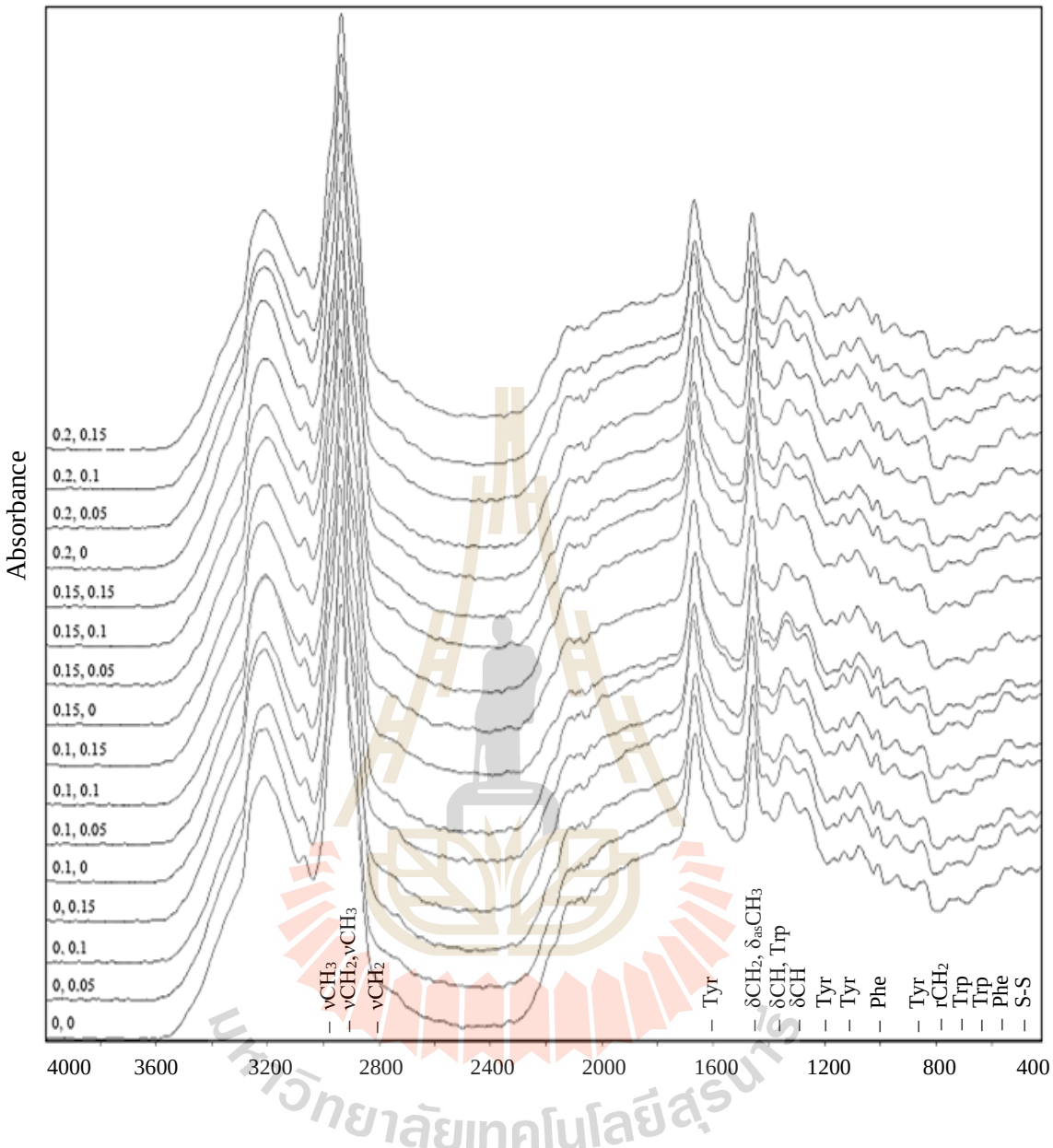

Figure 14 Raman spectra of TB directly cooked gels added various concentrations of AsA, H_2O_2.

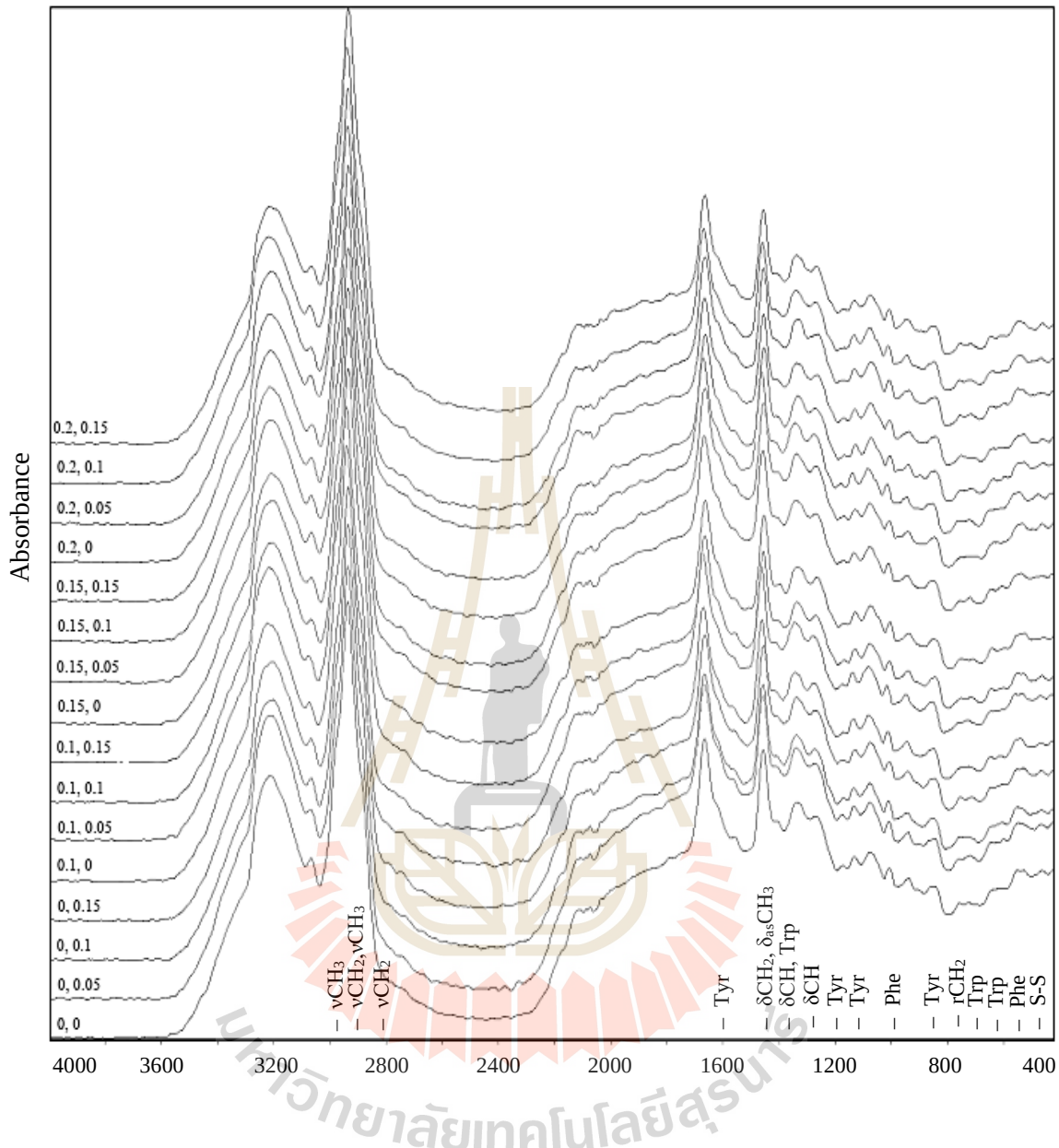

Figure 15 Raman spectra of TB set gels added various concentrations of AsA, H_2O_2.

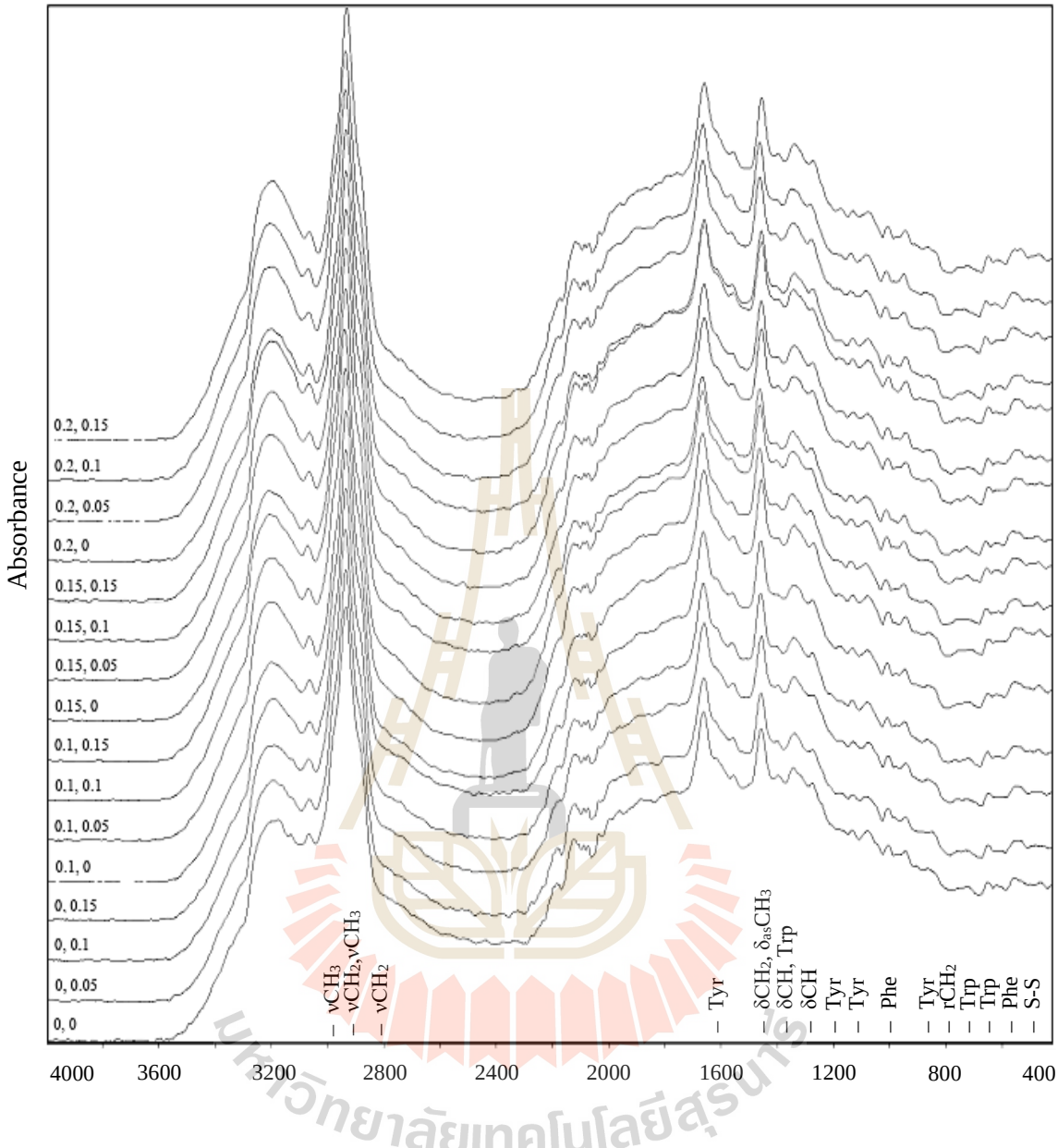

Figure 16 Raman spectra of SD surimi pastes added various concentrations of AsA, H_2O_2.

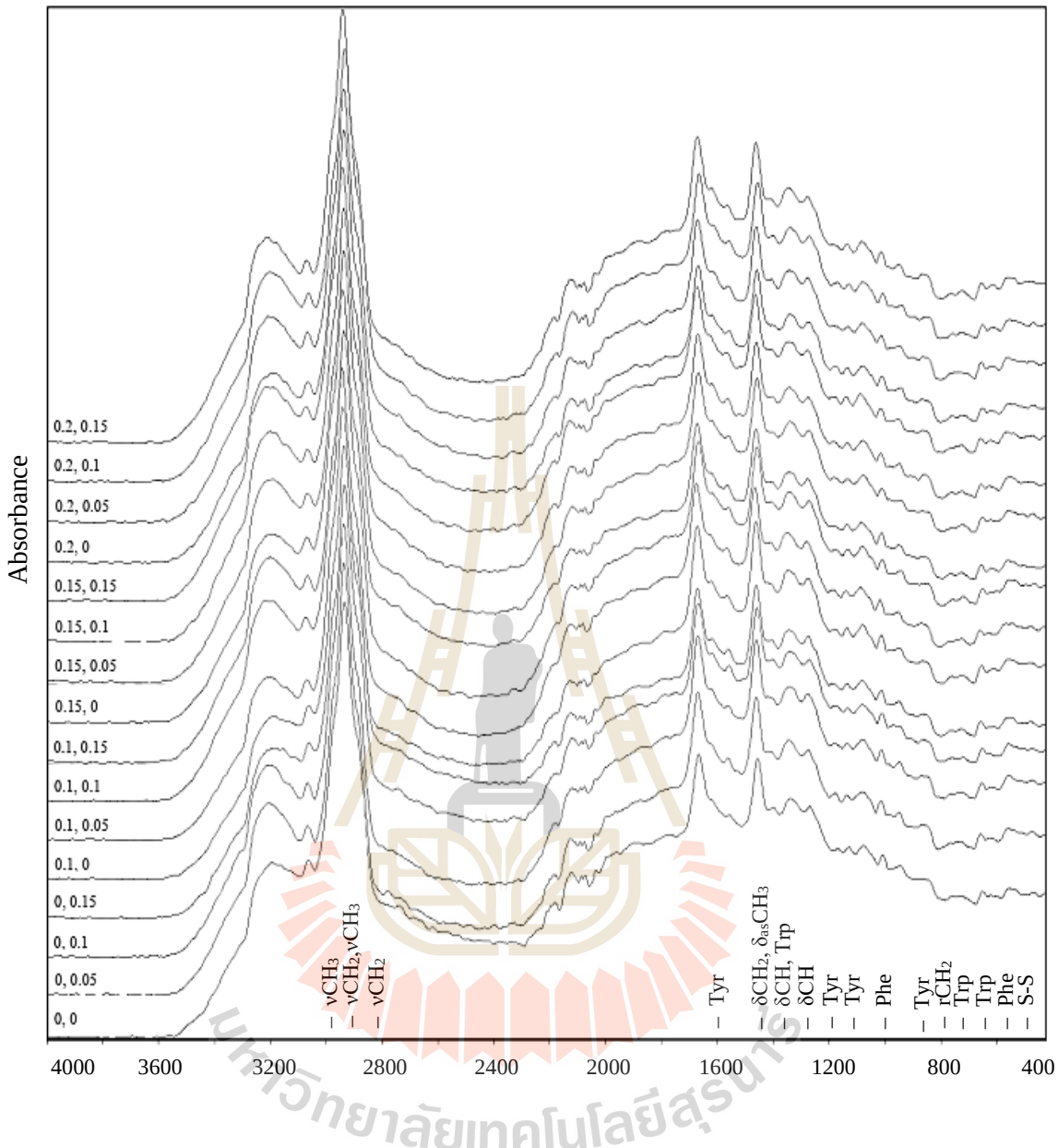

Figure 17 Raman spectra of SD directly cooked gels added various concentrations of AsA, H_2O_2.

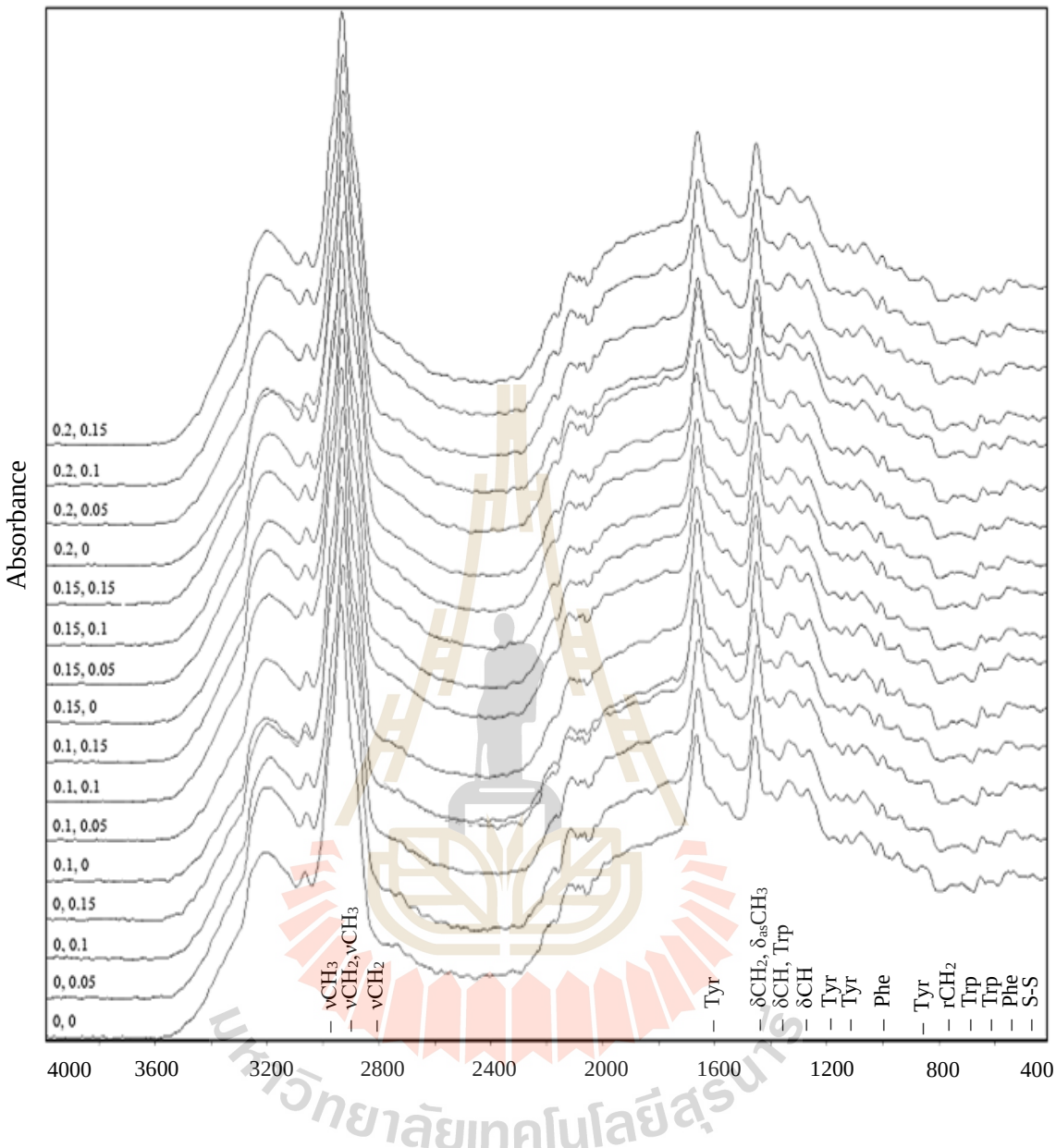

Figure 18 Raman spectra of SD set gels added various concentrations of AsA, H_2O_2.

Table 1 Integral area ($\times 10^{-3}$) at selected regions of Raman spectra of LZ paste as affected by various conditions of AsA and H_2O_2.

Wavenumber (cm^{-1})	Band assignment	Additives	LZ surimi paste															
		AsA (%)	0				0.1				0.15				0.2			
		H_2O_2 (%)	0	0.05	0.1	015	0	0.05	0.1	015	0	0.05	0.1	015	0	0.05	0.1	015
Aliphatic residues		**Total**	**7.29** ^{a}	**6.50** ^{b}	**6.31** ^{bc}	**5.67** ^{def}	**6.13** ^{bcd}	**5.77** ^{def}	**5.95** ^{def}	**5.89** ^{def}	**5.89** ^{def}	**6.06** ^{cde}	**5.60** ^{def}	**5.66** ^{def}	**5.14** ^{f}	**5.21** ^{def}	**5.12** ^{ef}	**5.12** ^{ef}
2980	νCH_3		0.55 ^{ab}	0.44 ^{def}	0.30 ^{g}	0.18 ^{h}	0.53 ^{abc}	0.43 ^{def}	0.41 ^{def}	0.52 ^{bcd}	0.43 ^{def}	0.56 ^{a}	0.48 ^{cde}	0.46 ^{def}	0.35 ^{fg}	0.42 ^{def}	0.46 ^{def}	0.39 ^{efg}
2936	νCH_2, νCH_3		3.00 ^{a}	2.85 ^{ab}	2.96 ^{a}	2.71 ^{bcd}	2.72 ^{bcd}	2.45 ^{de}	2.72 ^{bcd}	2.57 ^{cde}	2.77 ^{abc}	2.74 ^{bcd}	2.60 ^{cde}	2.54 ^{bcd}	2.61 ^{cde}	2.47 ^{de}	2.48 ^{cde}	2.32 ^{e}
2874	νCH_2		0.97	0.74	0.76	0.80	0.73	0.67	0.77	0.78	0.78	0.76	0.69	0.75	0.69	0.84	0.70	0.74
1450	δCH_2, $\delta_{as}CH_3$		1.91 ^{a}	1.78 ^{ab}	1.72 ^{abc}	1.65 ^{bcd}	1.55 ^{cde}	1.48 ^{def}	1.48 ^{def}	1.40 ^{def}	1.41 ^{def}	1.47 ^{def}	1.34 ^{ef}	1.34 ^{ef}	1.24 ^{f}	1.43 ^{def}	1.35 ^{ef}	1.44 ^{def}
1340	δCH, Trp		0.44	0.38	0.36	0.16	0.33	0.44	0.31	0.38	0.31	0.34	0.28	0.38	0.39	0.29	0.33	0.39
1320	δCH		0.28 ^{a}	0.16 ^{bc}	0.15 ^{bc}	0.14 ^{bc}	0.13 ^{bc}	0.19 ^{b}	0.12 ^{bc}	0.08 ^{c}	0.15 ^{bc}	0.15 ^{bc}	0.13 ^{bc}	0.12 ^{bc}	0.16 ^{bc}	0.15 ^{bc}	0.14 ^{bc}	0.14 ^{bc}
720	rCH_2		0.13	0.15	0.07	0.03	0.14	0.11	0.14	0.16	0.05	0.04	0.09	0.06	0.06	0.03	0.12	0.09
Aromatic residue		**Total**	**2.36**	**1.91**	**1.86**	**1.82**	**2.20**	**2.00**	**2.19**	**1.81**	**1.67**	**2.03**	**2.20**	**1.76**	**1.83**	**1.81**	**2.09**	**1.86**
1605	Tyr		0.18 ^{bc}	0.11 ^{cde}	0.13 ^{cde}	0.17 ^{bc}	0.21 ^{ab}	0.16 ^{bcd}	0.16 ^{bcd}	0.06 ^{de}	0.06 ^{de}	0.19 ^{bc}	0.30 ^{a}	0.29 ^{a}	0.04 ^{e}	0.14 ^{cde}	0.13 ^{cde}	0.13 ^{cde}
1208	Tyr		0.21	0.17	0.19	0.07	0.13	0.17	0.25	0.19	0.18	0.17	0.10	0.13	0.21	0.14	0.20	0.16
1173	Tyr		0.20	0.05	0.09	0.14	0.13	0.12	0.14	0.10	0.06	0.24	0.25	0.07	0.13	0.04	0.10	0.14
1003	Phe		0.63 ^{cde}	0.59 ^{de}	0.63 ^{cde}	0.63 ^{cde}	0.85 ^{a}	0.77 ^{ab}	0.77 ^{ab}	0.70 ^{bc}	0.73 ^{bc}	0.53 ^{e}	0.59 ^{de}	0.59 ^{de}	0.63 ^{cde}	0.65 ^{cde}	0.72 ^{bc}	0.56 ^{de}
850	Tyr		0.27 ^{a}	0.22 ^{ab}	0.20 ^{bc}	0.13 ^{cde}	0.12 ^{de}	0.13 ^{cde}	0.17 ^{cde}	0.13 ^{cde}	0.13 ^{cde}	0.11 ^{e}	0.13 ^{cde}	0.15 ^{cde}	0.15 ^{cde}	0.11 ^{e}	0.13 ^{cde}	0.18 ^{bcd}
830	Tyr		0.28 ^{cde}	0.36 ^{abc}	0.22 ^{e}	0.32 ^{cde}	0.36 ^{abc}	0.28 ^{cde}	0.32 ^{cde}	0.33 ^{cde}	0.24 ^{de}	0.42 ^{ab}	0.33 ^{cde}	0.23 ^{de}	0.28 ^{cde}	0.26 ^{cde}	0.44 ^{a}	0.34 ^{bcd}
757	Trp		0.22 ^{a}	0.14 ^{abc}	0.09 ^{cde}	0.06 ^{de}	0.08 ^{cde}	0.10 ^{cde}	0.11 ^{cde}	0.06 ^{de}	0.04 ^{e}	0.06 ^{de}	0.11 ^{cde}	0.08 ^{cde}	0.06 ^{de}	0.17 ^{ab}	0.14 ^{bcd}	0.11 ^{cde}
644	Tyr		0.20	0.15	0.20	0.17	0.23	0.23	0.17	0.19	0.21	0.22	0.25	0.19	0.27	0.30	0.16	0.22
620	Phe		0.17 ^{a}	0.13 ^{abc}	0.13 ^{abc}	0.13 ^{abc}	0.09 ^{bcd}	0.04 ^{de}	0.09 ^{bcd}	0.04 ^{de}	0.04 ^{de}	0.08 ^{cde}	0.15 ^{ab}	0.02 ^{e}	0.06 ^{de}	0.01 ^{e}	0.07 ^{cde}	0.02 ^{e}

Different letters within the same row indicate significant differences ($P < 0.05$) between mean values.

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Table 2 Integral area ($\times 10^{-3}$) at selected regions of Raman spectra of LZ directly cooked gel as affected by various conditions of AsA and H_2O_2.

Wavenumber (cm^{-1})	Band assignment	Additives	LZ gel (Direct heating)															
		AsA (%)	0				0.1				0.15				0.2			
		H_2O_2 (%)	0	0.05	0.1	015	0	0.05	0.1	015	0	0.05	0.1	015	0	0.05	0.1	015
Aliphatic residues		Total	8.68^{a}	7.97^{b}	7.69^{cde}	7.98^{b}	7.84^{bc}	7.72^{bcd}	7.78^{bcd}	7.51^{def}	7.72^{bcd}	7.75^{cde}	7.95^{b}	7.18^{f}	7.44^{def}	7.60^{cde}	7.63^{cde}	7.30^{ef}
2980	νCH_3		1.25^{a}	0.83^{bcd}	0.86^{bcd}	0.81^{bcd}	0.98^{b}	0.82^{bcd}	0.88^{bc}	0.71^{de}	0.89^{bc}	0.84^{bcd}	0.84^{bcd}	0.65^{e}	0.81^{bcd}	0.62^{e}	0.63^{e}	0.59^{e}
2936	νCH_2, νCH_3		3.45^{a}	3.42^{ab}	3.39^{abc}	3.33^{bcd}	3.22^{de}	3.34^{bcd}	3.32^{bcd}	3.27^{bcd}	3.24^{cd}	3.34^{bcd}	3.28^{bcd}	3.34^{bcd}	3.24^{cd}	3.39^{abc}	3.21^{de}	3.09^{e}
2874	νCH_2		0.97^{bcd}	0.95^{bcd}	0.98^{bcd}	1.00^{abc}	1.07^{ab}	0.85^{d}	0.89^{cd}	0.94^{bcd}	1.02^{abc}	1.06^{ab}	1.10^{a}	0.89^{cd}	0.98^{bcd}	1.04^{ab}	1.07^{ab}	1.06^{ab}
1450	δCH_2, $\delta_{as}CH_3$		2.04	2.05	2.04	2.09	1.84	2.00	1.96	1.82	1.89	1.91	1.95	1.81	1.87	1.81	2.05	1.96
1340	δCH, Trp		0.49^{a}	0.41^{b}	0.20^{e}	0.35^{bc}	0.31^{bc}	0.36^{bc}	0.33^{bc}	0.36^{bc}	0.34^{bc}	0.34^{bc}	0.39^{b}	0.23^{de}	0.23^{de}	0.35^{bc}	0.30^{cd}	0.35^{bc}
1320	δCH		0.37^{a}	0.22^{cde}	0.11^{f}	0.26^{cde}	0.27^{b}	0.22^{cde}	0.27^{b}	0.23^{cde}	0.13^{f}	0.16^{def}	0.24^{bcd}	0.15^{ef}	0.17^{def}	0.29^{b}	0.18^{def}	0.16^{def}
720	rCH_2		0.12^{bcd}	0.09^{e}	0.12^{cde}	0.14^{bcd}	0.16^{abc}	0.14^{bcd}	0.13^{bcd}	0.19^{ab}	0.22^{a}	0.11^{de}	0.17^{abc}	0.11^{de}	0.14^{bcd}	0.11^{de}	0.19^{ab}	0.10^{e}
Aromatic residue		Total	2.50^{bc}	2.22^{de}	2.18^{de}	2.12^{e}	2.34^{cde}	2.87^{a}	2.41^{bcd}	2.88^{a}	2.63^{ab}	2.35^{cde}	2.46^{bcd}	2.14^{e}	2.36^{bcd}	2.23^{cde}	2.42^{bcd}	2.20^{de}
1605	Tyr		0.27	0.17	0.20	0.24	0.31	0.32	0.14	0.26	0.24	0.14	0.21	0.24	0.20	0.25	0.26	0.19
1208	Tyr		0.18	0.08	0.15	0.06	0.13	0.26	0.15	0.10	0.24	0.17	0.17	0.18	0.16	0.11	0.10	0.12
1173	Tyr		0.11	0.15	0.12	0.16	0.24	0.23	0.17	0.11	0.15	0.10	0.16	0.13	0.12	0.10	0.12	0.11
1003	Phe		0.73^{cd}	0.77^{cd}	0.67^{de}	0.66^{de}	0.75^{cd}	1.01^{a}	0.77^{cd}	1.03^{a}	0.72^{d}	0.73^{cd}	0.90^{b}	0.89^{b}	0.84^{bc}	0.69^{de}	0.84^{bc}	0.59^{e}
850	Tyr		0.25^{ab}	0.13^{g}	0.13^{g}	0.15^{efg}	0.20^{abc}	0.23^{abc}	0.15^{efg}	0.21^{abc}	0.26^{a}	0.18^{cde}	0.24^{ab}	0.18^{cde}	0.18^{cde}	0.26^{a}	0.18^{def}	0.16^{def}
830	Tyr		0.33^{bcd}	0.43^{b}	0.42^{b}	0.42^{b}	0.27^{ef}	0.29^{def}	0.42^{b}	0.53^{a}	0.43^{b}	0.42^{b}	0.37^{bc}	0.22^{f}	0.42^{bc}	0.40^{bc}	0.42^{b}	0.37^{bcd}
757	Trp		0.19	0.14	0.15	0.16	0.16	0.20	0.18	0.17	0.15	0.17	0.09	0.09	0.10	0.13	0.16	0.14
644	Tyr		0.31^{bc}	0.23^{cde}	0.16^{e}	0.15^{e}	0.16^{e}	0.27^{bcd}	0.36^{ab}	0.33^{abc}	0.27^{bcd}	0.25^{cde}	0.17^{de}	0.18^{de}	0.25^{cde}	0.17^{e}	0.26^{bcd}	0.42^{a}
620	Phe		0.14	0.11	0.18	0.12	0.13	0.08	0.08	0.15	0.16	0.19	0.15	0.04	0.11	0.12	0.08	0.11

Different letters within the same row indicate significant differences ($P < 0.05$) between mean values.

Table 3 Integral area ($\times 10^{-3}$) at selected regions of Raman spectra of LZ set gel as affected by various conditions of AsA and H_2O_2.

Wavenumber (cm^{-1})	Band assignment	Additives	LZ gel (Setting)															
		AsA (%)	0				0.1				0.15				0.2			
		H_2O_2 (%)	0	0.05	0.1	015	0	0.05	0.1	015	0	0.05	0.1	015	0	0.05	0.1	015
Aliphatic residues		**Total**	**8.64** ^{a}	**8.21** ^{ab}	**8.03** ^{bc}	**8.29** ^{ab}	**7.55** ^{cd}	**7.63** ^{cd}	**7.63** ^{cd}	**7.45** ^{cd}	**7.58** ^{cd}	**7.34** ^{de}	**7.31** ^{de}	**6.84** ^{e}	**7.29** ^{de}	**7.47** ^{cd}	**7.45** ^{cd}	**7.10** ^{de}
2980	νCH_3		0.91 ^{a}	0.86 ^{abc}	0.86 ^{abc}	0.88 ^{abc}	0.90 ^{ab}	0.83 ^{abc}	0.81 ^{abc}	0.62 ^{ade}	0.75 ^{c}	0.78 ^{bc}	0.78 ^{bc}	0.58 ^{e}	0.57 ^{e}	0.74 ^{cd}	0.55 ^{e}	0.58 ^{e}
2936	νCH_2, νCH_3		3.82 ^{a}	3.54 ^{abc}	3.41 ^{bcd}	3.62 ^{ab}	3.19 ^{d}	3.25 ^{cd}	3.28 ^{cd}	3.17 ^{d}	3.22 ^{cd}	3.16 ^{d}	3.06 ^{d}	3.07 ^{d}	3.26 ^{cd}	3.16 ^{d}	3.18 ^{d}	3.08 ^{d}
2874	νCH_2		1.13 ^{ab}	1.00 ^{ab}	1.00 ^{ab}	1.08 ^{ab}	1.04 ^{ab}	1.17 ^{a}	1.13 ^{ab}	1.13 ^{ad}	1.06 ^{ad}	0.79 ^{c}	0.96 ^{b}	1.02 ^{ab}	1.06 ^{ab}	1.03 ^{ab}	1.04 ^{ab}	0.96 ^{b}
1450	δCH_2, $\delta_{as} CH_3$		2.00	1.93	1.92	1.92	1.72	1.60	1.77	1.78	1.85	1.93	1.88	1.78	1.72	1.78	1.95	1.85
1340	δCH, Trp		0.43 ^{abc}	0.43 ^{ab}	0.46 ^{a}	0.41 ^{ab}	0.42 ^{abc}	0.42 ^{abc}	0.32 ^{cd}	0.43 ^{acd}	0.38 ^{acd}	0.37 ^{acd}	0.33 ^{bcd}	0.24 ^{d}	0.36 ^{abc}	0.39 ^{abc}	0.45 ^{abc}	0.32 ^{cd}
1320	δCH		0.22	0.21	0.24	0.25	0.19	0.26	0.17	0.22	0.18	0.18	0.18	0.12	0.22	0.23	0.23	0.17
720	rCH_2		0.13	0.24	0.14	0.14	0.09	0.09	0.15	0.11	0.13	0.14	0.13	0.03	0.09	0.16	0.05	0.14
Aromatic residue		**Total**	**2.81** ^{a}	**2.63** ^{abc}	**2.45** ^{bcd}	**2.67** ^{ab}	**2.60** ^{abc}	**2.80** ^{a}	**2.67** ^{ab}	**2.49** ^{bcd}	**2.51** ^{bcd}	**2.06** ^{f}	**2.10** ^{f}	**2.06** ^{f}	**2.28** ^{def}	**2.37** ^{cde}	**2.40** ^{bcd}	**2.22** ^{ef}
1605	Tyr		0.25 ^{bcd}	0.23 ^{bcd}	0.35 ^{a}	0.24 ^{bcd}	0.25 ^{bcd}	0.30 ^{ab}	0.30 ^{ab}	0.22 ^{bcd}	0.26 ^{abc}	0.13 ^{g}	0.21 ^{bcd}	0.14 ^{fg}	0.24 ^{bcd}	0.20 ^{cde}	0.17 ^{def}	0.16 ^{efg}
1208	Tyr		0.22	0.16	0.19	0.19	0.20	0.23	0.12	0.17	0.13	0.15	0.16	0.17	0.12	0.24	0.17	0.14
1173	Tyr		0.25 ^{b}	0.31 ^{a}	0.12 ^{de}	0.19 ^{bc}	0.24 ^{b}	0.19 ^{bc}	0.12 ^{de}	0.11 ^{e}	0.13 ^{cde}	0.11 ^{e}	0.14 ^{cde}	0.10 ^{e}	0.13 ^{cde}	0.14 ^{cde}	0.15 ^{cde}	0.16 ^{cde}
1003	Phe		0.86 ^{b}	0.68 ^{c}	0.69 ^{c}	1.00 ^{a}	0.85 ^{b}	0.91 ^{ab}	0.83 ^{b}	0.86 ^{b}	0.86 ^{b}	0.64 ^{c}	0.68 ^{c}	0.72 ^{c}	0.85 ^{d}	0.84 ^{d}	0.94 ^{ab}	0.85 ^{b}
850	Tyr		0.21	0.21	0.21	0.20	0.18	0.21	0.19	0.21	0.16	0.16	0.15	0.15	0.16	0.12	0.20	0.17
830	Tyr		0.40 ^{bcd}	0.40 ^{bcd}	0.31 ^{de}	0.32 ^{de}	0.33 ^{de}	0.44 ^{abc}	0.52 ^{a}	0.46 ^{ab}	0.44 ^{abc}	0.40 ^{bcd}	0.35 ^{cde}	0.38 ^{bcd}	0.27 ^{e}	0.40 ^{bcd}	0.32 ^{de}	0.36 ^{bcd}
757	Trp		0.31 ^{a}	0.28 ^{a}	0.23 ^{ab}	0.15 ^{bcd}	0.23 ^{ab}	0.24 ^{ab}	0.28 ^{a}	0.18 ^{bcd}	0.18 ^{bcd}	0.16 ^{bcd}	0.15 ^{bcd}	0.12 ^{d}	0.14 ^{de}	0.12 ^{d}	0.14 ^{de}	0.12 ^{e}
644	Tyr		0.16	0.23	0.25	0.24	0.17	0.15	0.18	0.15	0.19	0.19	0.21	0.21	0.23	0.18	0.20	0.17
620	Phe		0.15	0.13	0.12	0.13	0.13	0.14	0.13	0.13	0.17	0.12	0.07	0.06	0.15	0.12	0.12	0.08

Different letters within the same row indicate significant differences ($P < 0.05$) between mean values.

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Table 4 Integral area ($\times 10^{-3}$) at selected regions of Raman spectra of TB paste as affected by various conditions of AsA and H_2O_2.

Wavenumber (cm^{-1})	Band assignment	Additives	TB surimi paste															
		AsA (%)	0				0.1				0.15				0.2			
		H_2O_2 (%)	0	0.05	0.1	015	0	0.05	0.1	015	0	0.05	0.1	015	0	0.05	0.1	015
Aliphatic residues		Total	6.29 ^{a}	5.71 ^{cde}	5.58 ^{bcd}	5.49 ^{ef}	5.99 ^{abc}	5.92 ^{abc}	6.18 ^{ab}	5.73 ^{cde}	6.13 ^{abc}	5.80 ^{bcd}	5.78 ^{bcd}	5.77 ^{bcd}	5.90 ^{abc}	5.89 ^{abc}	5.58 ^{def}	5.26 ^{f}
2980	νCH_3		0.55 ^{a}	0.45 ^{b}	0.43 ^{bc}	0.41 ^{bcd}	0.37 ^{bcd}	0.36 ^{cde}	0.36 ^{cde}	0.20 ^{g}	0.34 ^{efg}	0.37 ^{bcd}	0.39 ^{bcd}	0.33 ^{def}	0.28 ^{ef}	0.34 ^{def}	0.35 ^{def}	0.26 ^{fg}
2936	νCH_2, νCH_3		2.56 ^{abc}	2.32 ^{abc}	2.22 ^{cd}	2.18 ^{d}	2.55 ^{bc}	2.48 ^{abc}	2.47 ^{abc}	2.36 ^{abc}	2.62 ^{ab}	2.63 ^{a}	2.55 ^{abc}	2.47 ^{abc}	2.65 ^{a}	2.37 ^{abc}	2.30 ^{abc}	2.28 ^{bcd}
2874	νCH_2		0.65 ^{bc}	0.60 ^{cd}	0.56 ^{de}	0.53 ^{e}	0.62 ^{cd}	0.68 ^{bc}	0.72 ^{b}	0.67 ^{bc}	0.64 ^{bc}	0.63 ^{cd}	0.61 ^{cd}	0.65 ^{bc}	0.63 ^{cd}	0.83 ^{a}	0.63 ^{cd}	0.65 ^{bc}
1450	δCH_2, $\delta_{as}CH_3$		1.66	1.63	1.63	1.64	1.64	1.79	1.85	1.80	1.80	1.60	1.66	1.70	1.78	1.79	1.70	1.63
1340	δCH, Trp		0.46 ^{a}	0.40 ^{abc}	0.39 ^{abc}	0.42 ^{abc}	0.42 ^{abc}	0.33 ^{bcd}	0.45 ^{ab}	0.40 ^{abc}	0.35 ^{abc}	0.25 ^{e}	0.32 ^{cde}	0.37 ^{abc}	0.31 ^{cde}	0.27 ^{de}	0.35 ^{abc}	0.33 ^{bcd}
1320	δCH		0.22 ^{abc}	0.16 ^{bcd}	0.22 ^{abc}	0.16 ^{bcd}	0.26 ^{a}	0.18 ^{bcd}	0.18 ^{bcd}	0.14 ^{ef}	0.24 ^{ab}	0.18 ^{bcd}	0.14 ^{ef}	0.12 ^{ef}	0.15 ^{de}	0.15 ^{de}	0.15 ^{de}	0.06 ^{f}
720	rCH_2		0.18 ^{a}	0.14 ^{ab}	0.14 ^{ab}	0.14 ^{ab}	0.15 ^{ab}	0.12 ^{b}	0.17 ^{a}	0.16 ^{ab}	0.14 ^{ab}	0.14 ^{ab}	0.12 ^{b}	0.14 ^{ab}	0.12 ^{b}	0.14 ^{ab}	0.10 ^{bc}	0.05 ^{c}
Aromatic residue		Total	1.97	1.64	1.73	1.73	1.99	2.30	2.23	1.85	1.90	1.53	1.75	1.77	1.79	1.95	2.16	2.00
1605	Tyr		0.21 ^{a}	0.14 ^{bc}	0.19 ^{ab}	0.14 ^{bc}	0.13 ^{bc}	0.17 ^{abc}	0.12 ^{c}	0.17 ^{abc}	0.13 ^{bc}	0.16 ^{abc}	0.14 ^{bc}	0.13 ^{bc}	0.13 ^{bc}	0.11 ^{c}	0.14 ^{bc}	0.14 ^{bc}
1208	Tyr		0.15	0.18	0.20	0.14	0.16	0.19	0.24	0.15	0.15	0.11	0.06	0.16	0.13	0.18	0.18	0.14
1173	Tyr		0.19	0.11	0.15	0.12	0.16	0.17	0.15	0.10	0.13	0.07	0.11	0.10	0.13	0.05	0.11	0.14
1003	Phe		0.56 ^{cd}	0.45 ^{e}	0.54 ^{cd}	0.53 ^{d}	0.57 ^{cd}	0.73 ^{a}	0.82 ^{a}	0.55 ^{cd}	0.61 ^{c}	0.45 ^{e}	0.55 ^{cd}	0.55 ^{cd}	0.58 ^{cd}	0.68 ^{ab}	0.70 ^{a}	0.57 ^{cd}
850	Tyr		0.18 ^{a}	0.08 ^{e}	0.11 ^{bcd}	0.12 ^{abc}	0.14 ^{abc}	0.17 ^{abc}	0.15 ^{abc}	0.14 ^{abc}	0.14 ^{abc}	0.16 ^{abc}	0.11 ^{bcd}	0.09 ^{de}	0.11 ^{cde}	0.12 ^{abc}	0.16 ^{abc}	0.17 ^{ab}
830	Tyr		0.22 ^{d}	0.23 ^{d}	0.22 ^{d}	0.25 ^{cd}	0.36 ^{a}	0.35 ^{a}	0.34 ^{a}	0.31 ^{abc}	0.36 ^{a}	0.27 ^{bcd}	0.33 ^{ab}	0.33 ^{ab}	0.37 ^{a}	0.35 ^{a}	0.27 ^{bcd}	0.35 ^{a}
757	Trp		0.10 ^{bcd}	0.10 ^{bcd}	0.06 ^{def}	0.13 ^{abc}	0.14 ^{ab}	0.19 ^{a}	0.13 ^{abc}	0.13 ^{abc}	0.07 ^{cde}	0.02 ^{f}	0.12 ^{bcd}	0.10 ^{bcd}	0.05 ^{ef}	0.13 ^{abc}	0.12 ^{bc}	0.10 ^{bcd}
644	Tyr		0.28	0.26	0.23	0.22	0.24	0.24	0.22	0.21	0.21	0.27	0.22	0.19	0.21	0.23	0.34	0.28
620	Phe		0.08	0.09	0.03	0.09	0.08	0.10	0.07	0.09	0.10	0.02	0.11	0.13	0.09	0.10	0.15	0.11

Different letters within the same row indicate significant differences ($P < 0.05$) between mean values.

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Table 5 Integral area ($\times 10^{-3}$) at selected regions of Raman spectra of TB directly cooked gel as affected by various conditions of AsA and H_2O_2.

Wavenumber (cm^{-1})	Band assignment	Additives	TB gel (Direct heating)															
		AsA (%)	0				0.1				0.15				0.2			
		H_2O_2 (%)	0	0.05	0.1	015	0	0.05	0.1	015	0	0.05	0.1	015	0	0.05	0.1	015
Aliphatic residues		Total	7.63 ^{a}	7.55 ^{ab}	7.05 ^{cde}	6.52 ^{h}	7.42 ^{abc}	7.21 ^{bcd}	7.57 ^{ab}	7.31 ^{abc}	6.83 ^{fgh}	6.84 ^{efg}	6.80 ^{fgh}	6.90 ^{efg}	6.64 ^{gh}	7.07 ^{cde}	7.12 ^{cde}	6.99 ^{def}
2980	νCH_3		0.76 ^{b}	0.71 ^{b}	0.61 ^{cd}	0.61 ^{cd}	0.76 ^{b}	0.72 ^{b}	0.84 ^{a}	0.76 ^{b}	0.64 ^{c}	0.60 ^{bc}	0.56 ^{de}	0.52 ^{e}	0.55 ^{de}	0.49 ^{e}	0.52 ^{e}	0.51 ^{e}
2936	νCH_2, νCH_3		3.03 ^{a}	2.96 ^{ab}	2.74 ^{bc}	2.61 ^{c}	3.04 ^{a}	2.91 ^{ab}	3.06 ^{a}	3.04 ^{a}	3.05 ^{a}	3.03 ^{a}	3.05 ^{a}	3.08 ^{a}	2.74 ^{bc}	3.09 ^{a}	3.04 ^{a}	3.00 ^{ab}
2874	νCH_2		0.95	0.89	0.83	0.84	0.83	0.74	0.89	0.70	0.83	0.83	0.86	0.76	0.83	0.79	0.84	0.85
1450	δCH_2, $\delta_{as}CH_3$		2.18 ^{ab}	2.26 ^{a}	2.14 ^{b}	1.76 ^{f}	2.11 ^{bc}	2.08 ^{bc}	2.05 ^{bc}	2.12 ^{bc}	1.79 ^{ef}	1.74 ^{f}	1.69 ^{f}	1.99 ^{cd}	1.88 ^{de}	2.11 ^{bc}	2.10 ^{bc}	2.05 ^{bc}
1340	δCH, Trp		0.35	0.46	0.37	0.38	0.32	0.36	0.43	0.34	0.32	0.36	0.44	0.35	0.36	0.30	0.35	0.33
1320	δCH		0.25 ^{ab}	0.20 ^{abc}	0.24 ^{ab}	0.18 ^{abc}	0.25 ^{a}	0.26 ^{a}	0.21 ^{abc}	0.25 ^{a}	0.14 ^{d}	0.14 ^{d}	0.16 ^{bcd}	0.13 ^{d}	0.15 ^{cd}	0.15 ^{cd}	0.17 ^{abc}	0.13 ^{d}
720	rCH_2		0.12 ^{abc}	0.08 ^{cde}	0.13 ^{abc}	0.13 ^{abc}	0.11 ^{abc}	0.15 ^{ab}	0.09 ^{bcd}	0.12 ^{abc}	0.07 ^{de}	0.14 ^{abc}	0.04 ^{e}	0.08 ^{cde}	0.13 ^{abc}	0.15 ^{a}	0.11 ^{abc}	0.12 ^{abc}
Aromatic residue		Total	2.81 ^{a}	2.60 ^{abc}	2.71 ^{a}	2.35 ^{cd}	2.64 ^{ab}	2.43 ^{bcd}	2.25 ^{cd}	2.32 ^{d}	2.28 ^{d}	2.24 ^{d}	2.32 ^{d}	2.37 ^{cd}	2.64 ^{abc}	2.28 ^{d}	2.26 ^{d}	2.22 ^{d}
1605	Tyr		0.18	0.13	0.22	0.25	0.24	0.25	0.24	0.24	0.24	0.18	0.18	0.24	0.22	0.17	0.16	0.23
1208	Tyr		0.21 ^{abc}	0.26 ^{a}	0.23 ^{ab}	0.18 ^{bcd}	0.16 ^{bcd}	0.15 ^{cde}	0.10 ^{e}	0.16 ^{bcd}	0.12 ^{de}	0.10 ^{e}	0.17 ^{bcd}	0.14 ^{de}	0.18 ^{bcd}	0.16 ^{bcd}	0.16 ^{bcd}	0.12 ^{de}
1173	Tyr		0.13 ^{bcd}	0.19 ^{a}	0.11 ^{cde}	0.16 ^{abc}	0.15 ^{abc}	0.09 ^{efg}	0.05 ^{g}	0.11 ^{cde}	0.11 ^{cde}	0.19 ^{a}	0.15 ^{abc}	0.09 ^{efg}	0.16 ^{abc}	0.16 ^{abc}	0.13 ^{cde}	0.14 ^{abc}
1003	Phe		0.97 ^{a}	0.67 ^{f}	0.91 ^{ab}	0.82 ^{abc}	0.93 ^{ab}	0.85 ^{abc}	0.82 ^{abc}	0.84 ^{abc}	0.89 ^{abc}	0.76 ^{cde}	0.83 ^{abc}	0.83 ^{abc}	0.87 ^{abc}	0.69 ^{def}	0.68 ^{ef}	0.85 ^{abc}
850	Tyr		0.22 ^{bc}	0.34 ^{a}	0.17 ^{bcd}	0.11 ^{cd}	0.20 ^{bcd}	0.21 ^{bc}	0.14 ^{cd}	0.12 ^{cd}	0.15 ^{bcd}	0.15 ^{bcd}	0.15 ^{bcd}	0.17 ^{bcd}	0.26 ^{ab}	0.20 ^{bcd}	0.19 ^{bcd}	0.10 ^{cd}
830	Tyr		0.59 ^{a}	0.47 ^{bc}	0.58 ^{a}	0.33 ^{e}	0.43 ^{bcd}	0.44 ^{bcd}	0.45 ^{bc}	0.41 ^{cde}	0.43 ^{bcd}	0.41 ^{cde}	0.37 ^{de}	0.45 ^{bc}	0.46 ^{bc}	0.51 ^{ab}	0.47 ^{cd}	0.43 ^{bcd}
757	Trp		0.15	0.17	0.15	0.19	0.17	0.07	0.14	0.10	0.04	0.14	0.11	0.15	0.15	0.09	0.26	0.11
644	Tyr		0.24 ^{b}	0.34 ^{a}	0.26 ^{b}	0.24 ^{b}	0.25 ^{b}	0.27 ^{ab}	0.28 ^{ab}	0.22 ^{bc}	0.23 ^{bc}	0.23 ^{bc}	0.28 ^{ab}	0.25 ^{b}	0.23 ^{bc}	0.26 ^{b}	0.15 ^{d}	0.17 ^{cd}
620	Phe		0.12	0.03	0.09	0.08	0.12	0.11	0.03	0.12	0.07	0.09	0.08	0.07	0.13	0.04	0.06	0.07

Different letters within the same row indicate significant differences ($P < 0.05$) between mean values.

Table 6 Integral area ($\times 10^{-3}$) at selected regions of Raman spectra of TB set gel as affected by various conditions of AsA and H_2O_2.

Wavenumber (cm^{-1})	Band assignment	Additives	TB gel (setting)															
		AsA (%)	0				0.1				0.15				0.2			
		H_2O_2 (%)	0	0.05	0.1	015	0	0.05	0.1	015	0	0.05	0.1	015	0	0.05	0.1	015
Aliphatic residues		Total	8.42 ^{a}	7.16 ^{cde}	7.28 ^{cde}	6.79 ^{f}	7.70 ^{b}	7.36 ^{bc}	7.42 ^{bc}	7.32 ^{cd}	7.20 ^{cde}	7.14 ^{cde}	7.15 ^{cde}	6.95 ^{def}	6.91 ^{ef}	7.10 ^{cde}	7.20 ^{cde}	6.95 ^{def}
2980	νCH_3		0.77 ^{a}	0.66 ^{abc}	0.63 ^{bcd}	0.57 ^{de}	0.74 ^{ab}	0.73 ^{ab}	0.72 ^{abc}	0.68 ^{abc}	0.62 ^{bcd}	0.63 ^{bcd}	0.66 ^{abc}	0.58 ^{de}	0.58 ^{de}	0.57 ^{de}	0.56 ^{de}	0.49 ^{e}
2936	νCH_2, νCH_3		3.23 ^{a}	2.93 ^{cd}	2.99 ^{bcd}	2.92 ^{cd}	3.08 ^{bc}	3.02 ^{bcd}	3.05 ^{bc}	3.07 ^{bc}	3.06 ^{bc}	3.00 ^{bcd}	3.01 ^{bcd}	3.04 ^{bc}	3.05 ^{bc}	2.87 ^{d}	3.03 ^{bc}	3.05 ^{bc}
2874	νCH_2		1.06 ^{a}	0.84 ^{bc}	0.85 ^{bc}	0.78 ^{c}	0.85 ^{bc}	0.84 ^{bc}	0.87 ^{bc}	0.89 ^{bc}	0.83 ^{bc}	0.93 ^{b}	0.80 ^{bc}	0.78 ^{c}	0.81 ^{bc}	0.81 ^{bc}	0.88 ^{bc}	0.87 ^{bc}
1450	δCH_2, $\delta_{as}CH_3$		2.30 ^{a}	2.02 ^{bcd}	2.08 ^{abc}	1.98 ^{cde}	2.24 ^{ab}	2.11 ^{abc}	2.13 ^{abc}	2.08 ^{abc}	2.02 ^{bcd}	1.96 ^{cde}	2.04 ^{bcd}	1.96 ^{cde}	1.87 ^{cde}	2.06 ^{abc}	1.97 ^{cde}	1.84 ^{e}
1340	δCH, Trp		0.53 ^{a}	0.44 ^{abc}	0.39 ^{bcd}	0.30 ^{e}	0.34 ^{bcd}	0.34 ^{bcd}	0.35 ^{bcd}	0.32 ^{cde}	0.35 ^{bcd}	0.38 ^{bcd}	0.34 ^{bcd}	0.31 ^{de}	0.39 ^{bcd}	0.43 ^{abc}	0.44 ^{abc}	0.44 ^{abc}
1320	δCH		0.35 ^{a}	0.17 ^{cde}	0.22 ^{bcd}	0.14 ^{ef}	0.30 ^{ab}	0.19 ^{cde}	0.19 ^{cde}	0.26 ^{bc}	0.18 ^{cde}	0.08 ^{f}	0.16 ^{def}	0.18 ^{cde}	0.16 ^{def}	0.23 ^{bcd}	0.18 ^{cde}	0.15 ^{def}
720	rCH_2		0.18 ^{a}	0.11 ^{ab}	0.12 ^{ab}	0.10 ^{bc}	0.14 ^{ab}	0.14 ^{ab}	0.11 ^{ab}	0.02 ^{d}	0.15 ^{ab}	0.17 ^{ab}	0.16 ^{ab}	0.10 ^{bc}	0.04 ^{cd}	0.12 ^{ab}	0.13 ^{ab}	0.12 ^{ab}
Aromatic residue		Total	3.26 ^{a}	2.59 ^{bc}	2.46 ^{cd}	2.10 ^{f}	2.74 ^{b}	2.29 ^{def}	2.30 ^{def}	2.36 ^{cde}	2.38 ^{cde}	2.32 ^{def}	2.22 ^{def}	2.15 ^{ef}	2.17 ^{ef}	2.47 ^{cde}	2.32 ^{def}	2.24 ^{def}
1605	Tyr		0.34 ^{a}	0.21 ^{bcd}	0.17 ^{cd}	0.15 ^{d}	0.17 ^{cd}	0.17 ^{cd}	0.27 ^{ab}	0.15 ^{d}	0.26 ^{abc}	0.24 ^{bcd}	0.17 ^{cd}	0.16 ^{d}	0.17 ^{cd}	0.34 ^{a}	0.17 ^{cd}	0.15 ^{d}
1208	Tyr		0.23 ^{ab}	0.15 ^{cde}	0.18 ^{abc}	0.18 ^{abc}	0.19 ^{abc}	0.09 ^{de}	0.15 ^{cde}	0.15 ^{cde}	0.10 ^{de}	0.14 ^{cde}	0.12 ^{cde}	0.10 ^{de}	0.13 ^{cde}	0.15 ^{bcd}	0.25 ^{a}	0.19 ^{abc}
1173	Tyr		0.32 ^{a}	0.15 ^{cd}	0.10 ^{cde}	0.14 ^{cd}	0.24 ^{b}	0.13 ^{cd}	0.05 ^{e}	0.08 ^{de}	0.10 ^{cde}	0.11 ^{cde}	0.17 ^{bc}	0.16 ^{cd}	0.16 ^{bcd}	0.12 ^{cde}	0.17 ^{bc}	0.13 ^{cd}
1003	Phe		0.93 ^{ab}	0.89 ^{abc}	0.81 ^{cde}	0.77 ^{efg}	0.95 ^{a}	0.83 ^{bcd}	0.73 ^{fg}	0.88 ^{abc}	0.93 ^{ab}	0.81 ^{cde}	0.72 ^{g}	0.86 ^{abc}	0.80 ^{def}	0.85 ^{abc}	0.91 ^{abc}	0.84 ^{abc}
850	Tyr		0.21 ^{abc}	0.12 ^{de}	0.24 ^{a}	0.15 ^{bcd}	0.22 ^{ab}	0.17 ^{abc}	0.16 ^{abc}	0.16 ^{abc}	0.21 ^{abc}	0.19 ^{abc}	0.16 ^{abc}	0.11 ^{e}	0.16 ^{abc}	0.17 ^{abc}	0.12 ^{de}	0.19 ^{abc}
830	Tyr		0.39 ^{abc}	0.38 ^{abc}	0.38 ^{abc}	0.32 ^{cde}	0.44 ^{abc}	0.45 ^{abc}	0.53 ^{a}	0.39 ^{abc}	0.44 ^{abc}	0.47 ^{ab}	0.42 ^{abc}	0.41 ^{abc}	0.39 ^{abc}	0.39 ^{abc}	0.28 ^{de}	0.25 ^{e}
757	Trp		0.32 ^{a}	0.26 ^{a}	0.17 ^{b}	0.14 ^{bc}	0.17 ^{b}	0.12 ^{bc}	0.05 ^{d}	0.14 ^{bc}	0.09 ^{cd}	0.12 ^{bc}	0.13 ^{bc}	0.15 ^{bc}	0.12 ^{bc}	0.15 ^{bc}	0.13 ^{bc}	0.15 ^{bc}
644	Tyr		0.37 ^{a}	0.32 ^{abc}	0.27 ^{bcd}	0.19 ^{def}	0.31 ^{abc}	0.24 ^{cde}	0.31 ^{abc}	0.34 ^{ab}	0.21 ^{def}	0.16 ^{ef}	0.21 ^{def}	0.18 ^{def}	0.18 ^{def}	0.25 ^{bcd}	0.13 ^{f}	0.25 ^{bcd}
620	Phe		0.16 ^{a}	0.11 ^{abc}	0.14 ^{abc}	0.08 ^{bcd}	0.07 ^{bcd}	0.09 ^{abc}	0.05 ^{def}	0.07 ^{bcd}	0.05 ^{ef}	0.08 ^{bcd}	0.13 ^{abc}	0.01 ^{f}	0.06 ^{cde}	0.06 ^{cde}	0.14 ^{abc}	0.10 ^{abc}

Different letters within the same row indicate significant differences ($P < 0.05$) between mean values.

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Table 7 Integral area ($\times 10^{-3}$) at selected regions of Raman spectra of SD paste as affected by various conditions of AsA and H_2O_2.

Wavenumber (cm^{-1})	Band assignment	Additives	SD surimi paste															
		AsA (%)	0				0.1				0.15				0.2			
		H_2O_2 (%)	0	0.05	0.1	015	0	0.05	0.1	015	0	0.05	0.1	015	0	0.05	0.1	015
Aliphatic residues		Total	6.59 ^{a}	5.72 ^{b}	5.36 ^{c}	5.19 ^{cde}	5.36 ^{c}	5.11 ^{cde}	4.75 ^{fg}	4.45 ^{h}	5.28 ^{cd}	5.04 ^{de}	5.04 ^{de}	5.00 ^{ef}	5.17 ^{cde}	5.22 ^{cde}	4.99 ^{ef}	4.64 ^{gh}
2980	νCH_3		0.35	0.30	0.29	0.26	0.33	0.34	0.30	0.25	0.32	0.32	0.35	0.30	0.30	0.28	0.27	0.21
2936	νCH_2, νCH_3		2.79 ^{a}	2.53 ^{b}	2.47 ^{bc}	2.40 ^{cd}	2.22 ^{e}	2.19 ^{ef}	1.99 ^{g}	1.87 ^{h}	2.32 ^{de}	2.20 ^{ef}	2.21 ^{ef}	2.20 ^{ef}	2.26 ^{e}	2.25 ^{e}	2.31 ^{de}	2.09 ^{fg}
2874	νCH_2		1.04 ^{a}	0.69 ^{cde}	0.68 ^{de}	0.67 ^{def}	0.77 ^{c}	0.67 ^{def}	0.61 ^{efg}	0.61 ^{efg}	0.63 ^{efg}	0.63 ^{efg}	0.62 ^{efg}	0.62 ^{efg}	0.75 ^{cd}	0.89 ^{b}	0.58 ^{fg}	0.56 ^{g}
1450	δCH_2, $\delta_{as}CH_3$		1.51 ^{a}	1.50 ^{ab}	1.38 ^{abc}	1.43 ^{abc}	1.42 ^{abc}	1.43 ^{abc}	1.37 ^{bcd}	1.27 ^{e}	1.43 ^{abc}	1.34 ^{cde}	1.36 ^{cde}	1.39 ^{abc}	1.35 ^{cde}	1.34 ^{cde}	1.36 ^{cde}	1.28 ^{de}
1340	δCH, Trp		0.50 ^{a}	0.37 ^{b}	0.30 ^{bc}	0.22 ^{c}	0.25 ^{c}	0.22 ^{c}	0.23 ^{c}	0.22 ^{c}	0.26 ^{c}	0.26 ^{c}	0.23 ^{c}	0.25 ^{c}	0.27 ^{c}	0.22 ^{c}	0.25 ^{c}	0.25 ^{c}
1320	δCH		0.26 ^{a}	0.17 ^{cde}	0.10 ^{e}	0.11 ^{de}	0.24 ^{a}	0.15 ^{cde}	0.14 ^{cde}	0.14 ^{cde}	0.20 ^{bc}	0.18 ^{bcd}	0.17 ^{cde}	0.15 ^{cde}	0.12 ^{de}	0.13 ^{cde}	0.12 ^{de}	0.14 ^{cde}
720	rCH_2		0.15	0.16	0.14	0.09	0.13	0.11	0.11	0.10	0.12	0.11	0.11	0.08	0.12	0.11	0.11	0.12
Aromatic residue		Total	2.57 ^{a}	2.15 ^{bc}	1.88 ^{fg}	1.96 ^{cde}	2.31 ^{b}	2.09 ^{cde}	1.86 ^{fg}	1.76 ^{g}	2.11 ^{cd}	2.01 ^{cde}	1.89 ^{fg}	1.94 ^{def}	1.77 ^{g}	1.91 ^{efg}	1.82 ^{fg}	1.80 ^{g}
1605	Tyr		0.25 ^{a}	0.12 ^{de}	0.12 ^{de}	0.13 ^{cde}	0.22 ^{ab}	0.17 ^{cd}	0.13 ^{cde}	0.11 ^{e}	0.14 ^{cde}	0.13 ^{cde}	0.15 ^{cde}	0.18 ^{bc}	0.12 ^{de}	0.12 ^{de}	0.15 ^{cde}	0.12 ^{de}
1208	Tyr		0.13 ^{a}	0.11 ^{a}	0.11 ^{ab}	0.12 ^{a}	0.15 ^{a}	0.13 ^{a}	0.12 ^{a}	0.05 ^{c}	0.13 ^{a}	0.13 ^{a}	0.13 ^{a}	0.16 ^{a}	0.12 ^{a}	0.10 ^{ab}	0.03 ^{c}	0.06 ^{bc}
1173	Tyr		0.11	0.11	0.10	0.09	0.11	0.11	0.10	0.10	0.15	0.15	0.12	0.12	0.13	0.11	0.10	0.11
1003	Phe		0.66 ^{a}	0.62 ^{abc}	0.63 ^{ab}	0.63 ^{ab}	0.67 ^{a}	0.66 ^{a}	0.55 ^{bcd}	0.62 ^{abc}	0.62 ^{abc}	0.59 ^{abc}	0.53 ^{def}	0.49 ^{efg}	0.43 ^{g}	0.55 ^{bcd}	0.46 ^{fg}	0.46 ^{fg}
850	Tyr		0.24 ^{a}	0.16 ^{b}	0.12 ^{b}	0.13 ^{b}	0.16 ^{b}	0.16 ^{b}	0.12 ^{b}	0.10 ^{b}	0.16 ^{b}	0.14 ^{b}	0.12 ^{b}	0.12 ^{b}	0.14 ^{b}	0.14 ^{b}	0.12 ^{b}	0.13 ^{b}
830	Tyr		0.26	0.23	0.22	0.21	0.26	0.24	0.24	0.23	0.24	0.24	0.22	0.23	0.25	0.28	0.24	0.24
757	Trp		0.15 ^{a}	0.14 ^{a}	0.10 ^{abc}	0.12 ^{abc}	0.14 ^{a}	0.10 ^{abc}	0.10 ^{abc}	0.07 ^{bcd}	0.15 ^{a}	0.13 ^{a}	0.14 ^{a}	0.14 ^{a}	0.04 ^{d}	0.06 ^{cd}	0.12 ^{ab}	0.13 ^{ab}
644	Tyr		0.59 ^{a}	0.52 ^{abc}	0.39 ^{e}	0.45 ^{bcd}	0.49 ^{bcd}	0.42 ^{de}	0.42 ^{de}	0.42 ^{de}	0.43 ^{de}	0.45 ^{bcd}	0.44 ^{cde}	0.42 ^{de}	0.46 ^{bcd}	0.46 ^{bcd}	0.53 ^{ab}	0.45 ^{bcd}
620	Phe		0.17 ^{a}	0.15 ^{ab}	0.09 ^{bcd}	0.09 ^{bcd}	0.11 ^{bc}	0.12 ^{abc}	0.08 ^{cd}	0.06 ^{cd}	0.09 ^{bcd}	0.06 ^{cd}	0.04 ^{d}	0.09 ^{bcd}	0.08 ^{cd}	0.08 ^{cd}	0.08 ^{cd}	0.11 ^{bc}

Different letters within the same row indicate significant differences ($P < 0.05$) between mean values.

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Table 8 Integral area ($\times 10^{-3}$) at selected regions of Raman spectra of SD directly cooked gel as affected by various conditions of AsA and H_2O_2.

Wavenumber (cm^{-1})	Band assignment	Additives	SD gel (direct heating)															
		AsA (%)	0				0.1				0.15				0.2			
		H_2O_2 (%)	0	0.05	0.1	015	0	0.05	0.1	015	0	0.05	0.1	015	0	0.05	0.1	015
Aliphatic residues		**Total**	**7.01** ^{a}	**6.65** ^{b}	**6.02** ^{fg}	**6.25** ^{def}	**6.67** ^{b}	**6.54** ^{bc}	**6.07** ^{ef}	**6.20** ^{def}	**6.33** ^{cde}	**6.14** ^{def}	**6.17** ^{def}	**6.09** ^{def}	**6.35** ^{cd}	**6.04** ^{fg}	**6.04** ^{fg}	**5.80** ^{g}
2980	νCH_3		0.70 ^{a}	0.64 ^{ab}	0.60 ^{bc}	0.60 ^{bc}	0.65 ^{ab}	0.64 ^{ab}	0.63 ^{abc}	0.62 ^{abc}	0.55 ^{cd}	0.61 ^{abc}	0.55 ^{cd}	0.55 ^{cd}	0.59 ^{bc}	0.63 ^{abc}	0.56 ^{cd}	0.48 ^{d}
2936	νCH_2, νCH_3		3.06 ^{a}	2.84 ^{ab}	2.41 ^{cd}	2.76 ^{b}	2.88 ^{ab}	2.68 ^{bc}	2.46 ^{cd}	2.72 ^{b}	2.85 ^{ab}	2.84 ^{ab}	2.84 ^{ab}	2.78 ^{b}	2.90 ^{ab}	2.68 ^{bc}	2.77 ^{b}	2.73 ^{b}
2874	νCH_2		0.86 ^{a}	0.86 ^{a}	0.80 ^{abc}	0.75 ^{bc}	0.84 ^{ab}	0.83 ^{ab}	0.83 ^{ab}	0.78 ^{abc}	0.84 ^{ab}	0.70 ^{c}	0.75 ^{bc}	0.75 ^{bc}	0.80 ^{abc}	0.75 ^{bc}	0.83 ^{ab}	0.73 ^{bc}
1450	δCH_2, $\delta_{as}CH_3$		1.67 ^{a}	1.63 ^{a}	1.64 ^{a}	1.63 ^{a}	1.67 ^{a}	1.71 ^{a}	1.64 ^{a}	1.50 ^{b}	1.45 ^{bc}	1.39 ^{bc}	1.48 ^{bc}	1.50 ^{b}	1.49 ^{bc}	1.48 ^{bc}	1.38 ^{c}	1.39 ^{bc}
1340	δCH, Trp		0.35 ^{a}	0.35 ^{a}	0.31 ^{ab}	0.25 ^{bc}	0.30 ^{abc}	0.35 ^{a}	0.27 ^{abc}	0.25 ^{bc}	0.30 ^{abc}	0.25 ^{bc}	0.23 ^{bc}	0.25 ^{bc}	0.26 ^{bc}	0.23 ^{bc}	0.24 ^{bc}	0.21 ^{c}
1320	δCH		0.15	0.16	0.15	0.15	0.17	0.18	0.10	0.17	0.21	0.21	0.18	0.16	0.19	0.13	0.16	0.15
720	rCH_2		0.23 ^{a}	0.18 ^{b}	0.12 ^{bc}	0.12 ^{bc}	0.15 ^{bc}	0.13 ^{bc}	0.12 ^{bc}	0.15 ^{bc}	0.13 ^{bc}	0.14 ^{bc}	0.14 ^{bc}	0.10 ^{b}	0.13 ^{bc}	0.13 ^{bc}	0.11 ^{c}	0.11 ^{c}
Aromatic residue		**Total**	**2.53** ^{a}	**2.37** ^{abc}	**2.17** ^{cde}	**2.16** ^{cde}	**2.25** ^{bcd}	**2.42** ^{ab}	**2.00** ^{ef}	**2.00** ^{ef}	**2.20** ^{cde}	**2.00** ^{ef}	**2.00** ^{ef}	**1.93** ^{f}	**2.06** ^{def}	**2.17** ^{cde}	**2.25** ^{bcd}	**2.05** ^{def}
1605	Tyr		0.24 ^{a}	0.24 ^{a}	0.23 ^{a}	0.22 ^{ab}	0.25 ^{a}	0.25 ^{a}	0.24 ^{a}	0.17 ^{abc}	0.21 ^{ab}	0.18 ^{abc}	0.19 ^{abc}	0.13 ^{bc}	0.22 ^{ab}	0.25 ^{a}	0.19 ^{abc}	0.12 ^{c}
1208	Tyr		0.11	0.08	0.11	0.09	0.13	0.11	0.07	0.09	0.10	0.08	0.11	0.12	0.08	0.07	0.14	0.09
1173	Tyr		0.17 ^{a}	0.17 ^{a}	0.13 ^{abc}	0.15 ^{abc}	0.12 ^{bcd}	0.11 ^{cde}	0.12 ^{bcd}	0.14 ^{abc}	0.07 ^{e}	0.17 ^{a}	0.13 ^{abc}	0.09 ^{de}	0.11 ^{cde}	0.11 ^{cde}	0.11 ^{cde}	0.10 ^{cde}
1003	Phe		0.80 ^{ab}	0.73 ^{bcd}	0.69 ^{cde}	0.64 ^{efg}	0.71 ^{cde}	0.82 ^{a}	0.67 ^{def}	0.60 ^{fg}	0.77 ^{abc}	0.56 ^{gh}	0.53 ^{h}	0.64 ^{efg}	0.63 ^{efg}	0.63 ^{efg}	0.63 ^{efg}	0.64 ^{efg}
850	Tyr		0.18	0.19	0.14	0.13	0.14	0.14	0.15	0.12	0.14	0.14	0.16	0.12	0.13	0.15	0.16	0.15
830	Tyr		0.28	0.29	0.29	0.28	0.24	0.24	0.24	0.23	0.24	0.20	0.24	0.22	0.31	0.35	0.37	0.32
757	Trp		0.15	0.13	0.10	0.12	0.15	0.11	0.09	0.12	0.11	0.11	0.11	0.10	0.11	0.09	0.11	0.11
644	Tyr		0.48	0.46	0.45	0.44	0.45	0.55	0.34	0.45	0.49	0.45	0.42	0.42	0.41	0.45	0.44	0.42
620	Phe		0.12 ^{a}	0.08 ^{abc}	0.03 ^{d}	0.11 ^{abc}	0.07 ^{cd}	0.09 ^{abc}	0.10 ^{abc}	0.08 ^{abc}	0.07 ^{bcd}	0.11 ^{abc}	0.11 ^{abc}	0.10 ^{abc}	0.06 ^{cd}	0.08 ^{abc}	0.10 ^{abc}	0.10 ^{abc}

Different letters within the same row indicate significant differences ($P < 0.05$) between mean values.

Table 9 Integral area ($\times 10^{-3}$) at selected regions of Raman spectra of SD set gel as affected by various conditions of AsA and H_2O_2.

Wavenumber (cm^{-1})	Band assignment	Additives	SD gel (setting)															
		AsA (%)	0				0.1				0.15				0.2			
		H_2O_2 (%)	0	0.05	0.1	015	0	0.05	0.1	015	0	0.05	0.1	015	0	0.05	0.1	015
Aliphatic residues		**Total**	**6.94** ^{b}	**6.58** ^{cd}	**6.15** ^{e}	**5.73** ^{hi}	**7.34** ^{a}	**6.74** ^{c}	**5.82** ^{fgh}	**5.37** ^{j}	**5.91** ^{fgh}	**5.99** ^{efg}	**5.98** ^{efg}	**5.62** ^{i}	**6.51** ^{d}	**6.01** ^{ef}	**5.99** ^{efg}	**5.80** ^{ghi}
2980	νCH_3		0.77 ^{a}	0.63 ^{bcd}	0.64 ^{bc}	0.63 ^{bcd}	0.69 ^{b}	0.65 ^{bc}	0.51 ^{efg}	0.46 ^{i}	0.60 ^{cde}	0.56 ^{def}	0.60 ^{cde}	0.58 ^{cde}	0.61 ^{cde}	0.55 ^{efg}	0.54 ^{fgh}	0.49 ^{hi}
2936	νCH_2, νCH_3		2.75 ^{b}	2.74 ^{b}	2.64 ^{c}	2.62 ^{c}	3.02 ^{a}	2.95 ^{a}	2.49 ^{de}	2.26 ^{f}	2.52 ^{de}	2.56 ^{cd}	2.52 ^{de}	2.45 ^{e}	2.96 ^{a}	2.74 ^{b}	2.74 ^{b}	2.76 ^{b}
2874	νCH_2		0.98 ^{a}	0.86 ^{bcd}	0.72 ^{fg}	0.69 ^{g}	0.94 ^{ab}	0.85 ^{bcd}	0.74 ^{efg}	0.72 ^{fg}	0.82 ^{cde}	0.83 ^{cde}	0.89 ^{abc}	0.71 ^{g}	0.85 ^{bcd}	0.76 ^{def}	0.74 ^{efg}	0.74 ^{efg}
1450	δCH_2, $\delta_{as}CH_3$		1.77 ^{b}	1.66 ^{bc}	1.53 ^{cd}	1.27 ^{e}	2.06 ^{a}	1.77 ^{b}	1.54 ^{cd}	1.49 ^{d}	1.46 ^{d}	1.54 ^{cd}	1.45 ^{d}	1.43 ^{d}	1.63 ^{c}	1.48 ^{d}	1.50 ^{d}	1.45 ^{d}
1340	δCH, Trp		0.30 ^{ab}	0.33 ^{a}	0.27 ^{abc}	0.26 ^{abc}	0.29 ^{ab}	0.22 ^{bcd}	0.22 ^{bcd}	0.19 ^{cd}	0.26 ^{abc}	0.26 ^{abc}	0.27 ^{abc}	0.27 ^{abc}	0.26 ^{abc}	0.28 ^{ab}	0.24 ^{bc}	0.16 ^{d}
1320	δCH		0.22 ^{a}	0.22 ^{a}	0.21 ^{ab}	0.14 ^{ef}	0.22 ^{a}	0.19 ^{abc}	0.21 ^{ab}	0.14 ^{def}	0.17 ^{abc}	0.14 ^{def}	0.15 ^{bcd}	0.12 ^{f}	0.12 ^{f}	0.14 ^{def}	0.18 ^{abc}	0.11 ^{f}
720	rCH_2		0.15	0.14	0.14	0.13	0.12	0.11	0.11	0.12	0.08	0.10	0.11	0.07	0.08	0.07	0.06	0.09
Aromatic residue		**Total**	**2.87** ^{a}	**2.59** ^{b}	**2.36** ^{cd}	**2.10** ^{g}	**2.63** ^{b}	**2.48** ^{bc}	**2.17** ^{efg}	**2.07** ^{g}	**2.46** ^{bc}	**2.28** ^{def}	**2.10** ^{g}	**1.87** ^{h}	**2.34** ^{cde}	**2.33** ^{cde}	**2.12** ^{fg}	**2.14** ^{fg}
1605	Tyr		0.24 ^{a}	0.21 ^{abc}	0.23 ^{ab}	0.14 ^{de}	0.20 ^{abc}	0.16 ^{cde}	0.16 ^{cde}	0.14 ^{de}	0.15 ^{cde}	0.14 ^{de}	0.16 ^{cde}	0.18 ^{bcd}	0.13 ^{de}	0.15 ^{cde}	0.15 ^{cde}	0.15 ^{cde}
1208	Tyr		0.18 ^{a}	0.13 ^{abc}	0.14 ^{abc}	0.17 ^{ab}	0.16 ^{ab}	0.12 ^{bcd}	0.12 ^{bcd}	0.10 ^{cde}	0.09 ^{de}	0.09 ^{de}	0.05 ^{ef}	0.05 ^{ef}	0.08 ^{de}	0.07 ^{def}	0.03 ^{f}	0.09 ^{de}
1173	Tyr		0.16 ^{ab}	0.15 ^{abc}	0.13 ^{bcd}	0.14 ^{bc}	0.15 ^{abc}	0.19 ^{a}	0.16 ^{ab}	0.12 ^{bcd}	0.14 ^{abc}	0.14 ^{abc}	0.14 ^{abc}	0.10 ^{cd}	0.12 ^{bcd}	0.11 ^{bcd}	0.11 ^{bcd}	0.08 ^{d}
1003	Phe		0.66 ^{ab}	0.64 ^{abc}	0.54 ^{e}	0.57 ^{cde}	0.64 ^{abc}	0.67 ^{a}	0.64 ^{abc}	0.64 ^{abc}	0.61 ^{abc}	0.67 ^{a}	0.66 ^{ab}	0.56 ^{de}	0.58 ^{bcd}	0.62 ^{abc}	0.63 ^{abc}	0.65 ^{ab}
850	Tyr		0.28 ^{a}	0.22 ^{abc}	0.21 ^{abc}	0.15 ^{cde}	0.24 ^{ab}	0.18 ^{bcd}	0.13 ^{de}	0.17 ^{bcd}	0.17 ^{bcd}	0.16 ^{bcd}	0.14 ^{de}	0.08 ^{e}	0.21 ^{abc}	0.18 ^{bcd}	0.17 ^{bcd}	0.16 ^{bcd}
830	Tyr		0.39 ^{a}	0.34 ^{abc}	0.27 ^{cde}	0.23 ^{ef}	0.26 ^{cde}	0.24 ^{def}	0.22 ^{f}	0.21 ^{f}	0.38 ^{ab}	0.38 ^{ab}	0.33 ^{abc}	0.32 ^{abc}	0.40 ^{a}	0.33 ^{abc}	0.34 ^{abc}	0.29 ^{bcd}
757	Trp		0.24 ^{a}	0.25 ^{a}	0.18 ^{bc}	0.14 ^{cd}	0.22 ^{ab}	0.24 ^{a}	0.15 ^{cd}	0.14 ^{cd}	0.18 ^{bc}	0.13 ^{cde}	0.13 ^{cde}	0.07 ^{f}	0.12 ^{def}	0.13 ^{cde}	0.08 ^{ef}	0.15 ^{cd}
644	Tyr		0.62 ^{abc}	0.55 ^{cde}	0.56 ^{bcd}	0.45 ^{g}	0.64 ^{ab}	0.57 ^{abc}	0.52 ^{def}	0.49 ^{efg}	0.64 ^{ab}	0.48 ^{fg}	0.44 ^{g}	0.45 ^{g}	0.63 ^{abc}	0.64 ^{ab}	0.53 ^{def}	0.50 ^{def}
620	Phe		0.11	0.10	0.10	0.11	0.12	0.11	0.08	0.08	0.11	0.11	0.05	0.06	0.07	0.10	0.10	0.08

Different letters within the same row indicate significant differences ($P < 0.05$) between mean values.

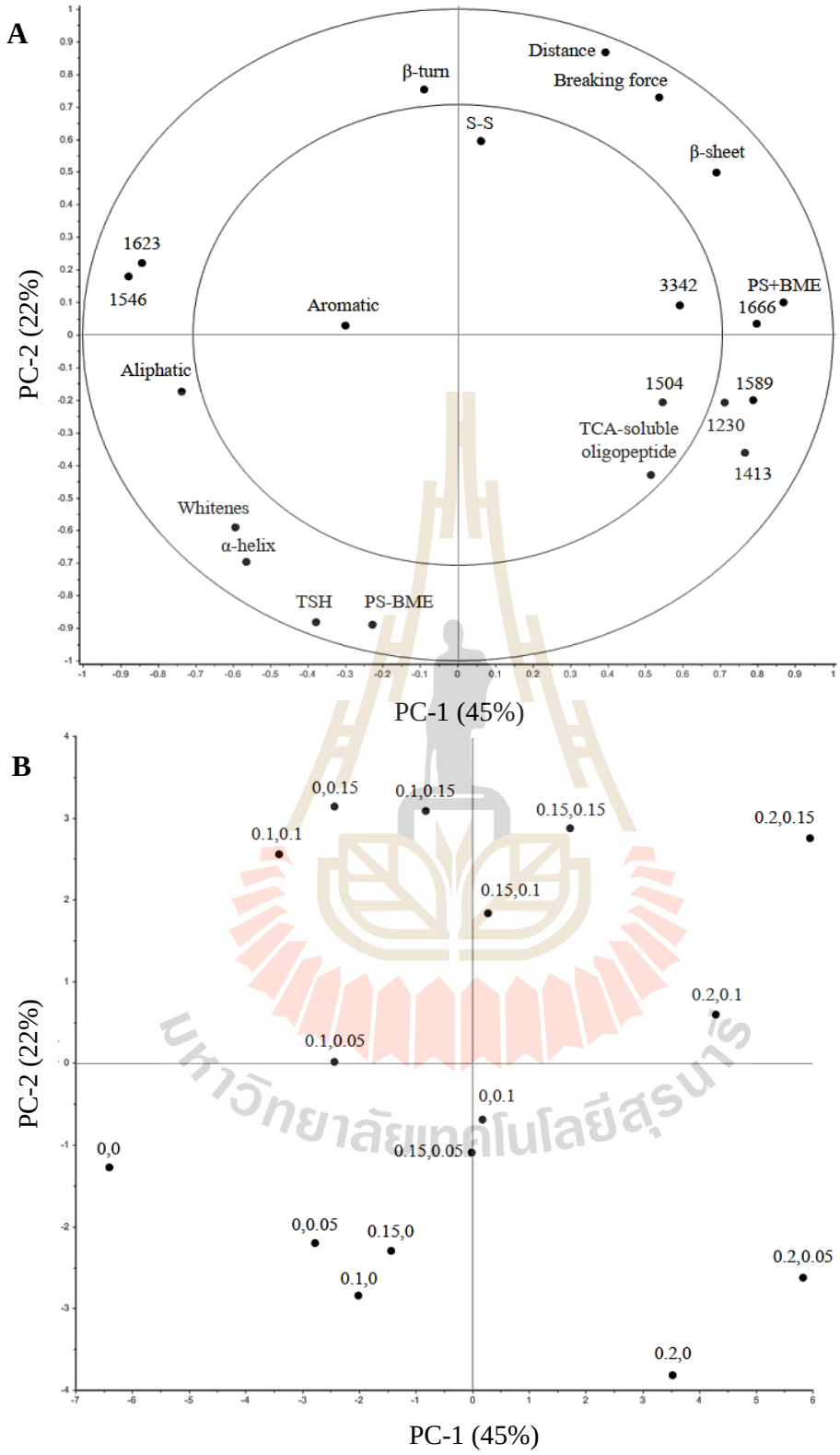

Figure 19 PCA correlation loading plots (A) and score plots (B) of LZ surimi gels cooked by direct heating.

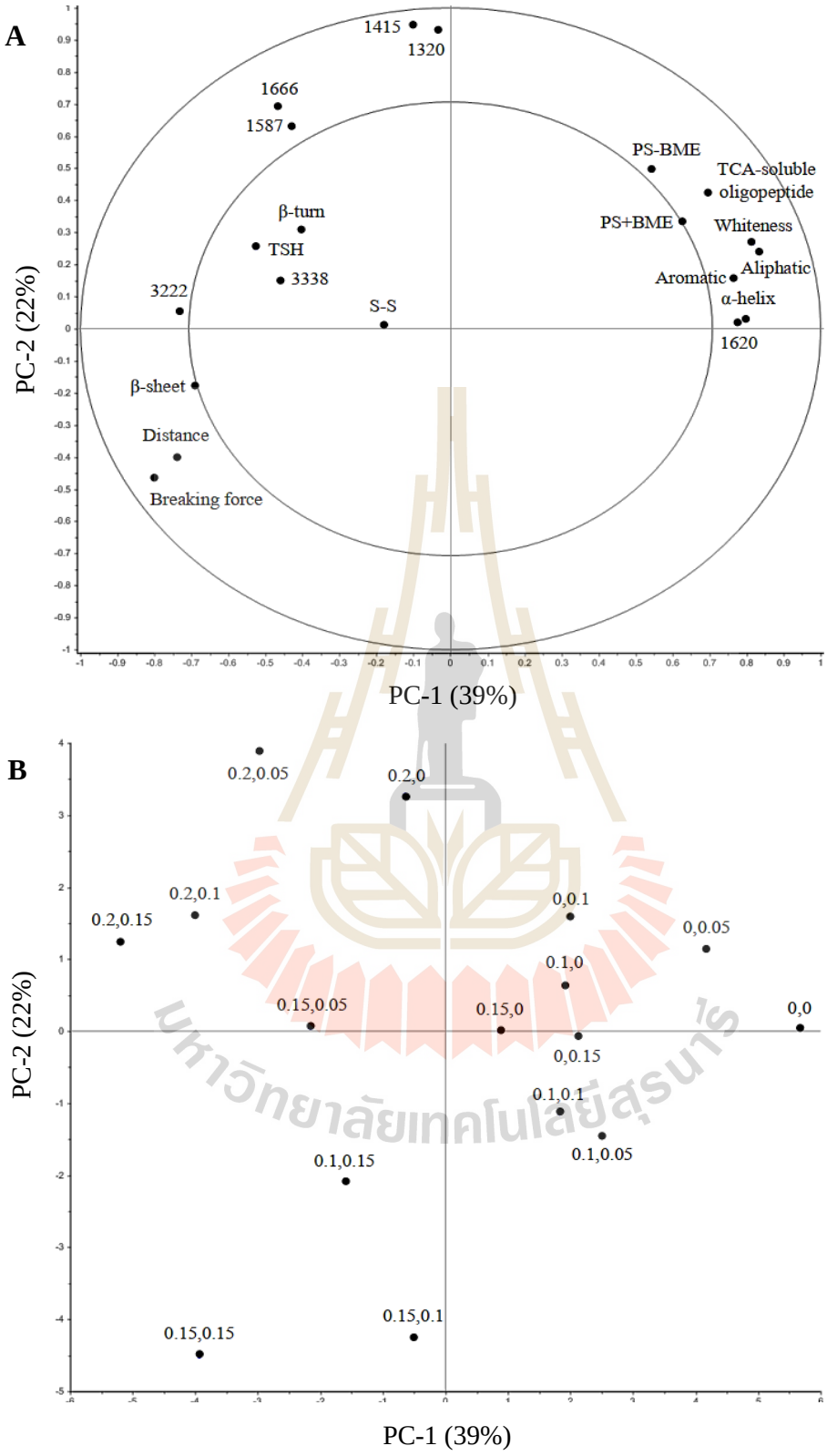

Figure 20 PCA correlation loading plots (A) and score plots (B) of LZ surimi gels cooked by setting.

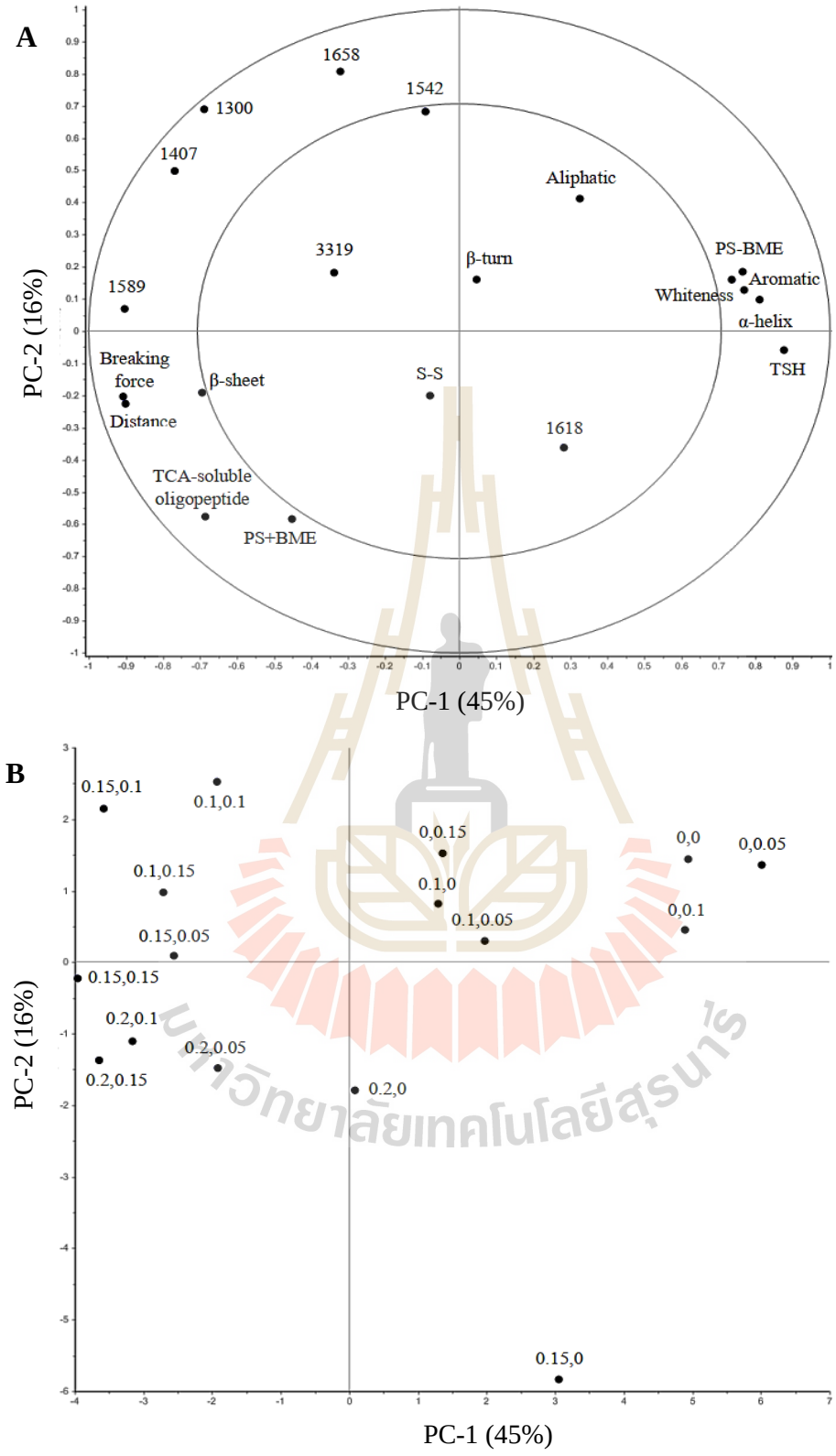

Figure 21 PCA correlation loading plots (A) and score plots (B) of TB surimi gels cooked by direct heating.

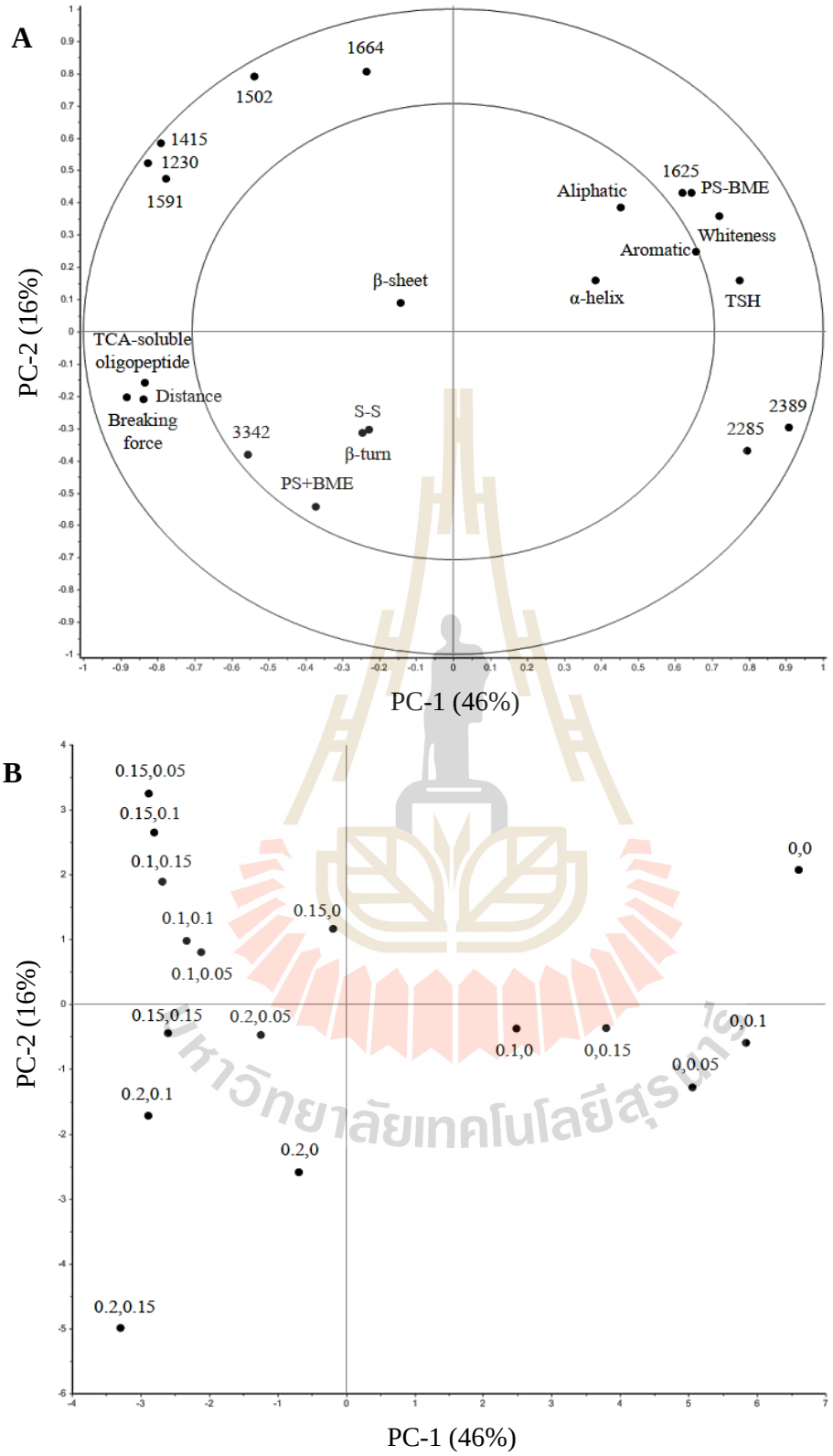

Figure 22 PCA correlation loading plots (A) and score plots (B) of TB surimi gels cooked by setting.

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Awards The Oral Presentation Award from Agro-industry Academic Council Association (AIAC) in Food Innovation Asia Conference 2019.

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Position and Place of Work None.