Impact of Gellan Incorporation on Properties of Fish Gelatin and Surimi Gels

Tanyamon Petcharat

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

Prince of Songkla University

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Author Ms. Tanyamon Petcharat

Major Program Food Science and Technology

Major Advisor

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

Examining Committee:

….……...…….....Chairperson
(Asst. Prof. Dr. Kongkarn Kijroongrojana)

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

…….…….....Committee
(Asst. Prof. Dr. Phanat Kittiphattanabawon)

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

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

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

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

……..…Signature
(Ms. Tanyamon Petcharat)
Candidate

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

……Signature
(Ms. Tanyamon Petcharat)
Candidate

ชื่อวิทยานิพนธ์ ผลของการเติมเจลแลนต่อสมบัติเจลของเจลาตินจากปลาและเจลซูริมิ
ผู้เขียน นางสาวตันยมน เพชรรัตน์
สาขาวิชา วิทยาศาสตร์และเทคโนโลยีอาหาร
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บทคัดย่อ

จากการศึกษาผลของเจลแลน (GL) ต่อสมบัติของเจลาตินจากปลา (FG) โดยการเติม GL ที่ระดับต่างๆ (ร้อยละ 5-20 ทดแทนปริมาณ FG) ลงใน FG พบว่า ค่าความแข็งแรงเจลและค่าความแข็งของเจลผสม FG/GL เพิ่มขึ้นในขณะที่ค่าความยืดหยุ่นและค่าการยึดเกาะภายในตัวอย่างลดลงเมื่อระดับของ GL เพิ่มขึ้น ($p < 0.05$) อุณหภูมิการเกิดเจลและอุณหภูมิที่เจลเกิดการหลอมละลายเพิ่มขึ้นเมื่อเติม GL ที่ระดับเพิ่มขึ้น ($p < 0.05$) ค่าความสว่าง และค่าความเป็นสีเหลืองของเจลผสม FG/GL ลดลง ในขณะที่ค่าความแตกต่างของสีทั้งหมดเพิ่มขึ้น เมื่อระดับของ GL เพิ่มขึ้น การเติม GL ทำให้โครงข่ายเจลผสมมีความหนาแน่นและมีช่องว่างขนาดเล็ก GL ที่ระดับต่ำ (ร้อยละ 5) ไม่มีผลต่อการยอมรับจากผู้บริโภค และซินเนอรีซิสของเจลผสม FG/GL

เมื่อเติมแคลเซียมคลอไรด์ ($CaCl_2$) ที่ความเข้มข้นต่างๆ (3-9 มิลลิโมลาร์) ร่วมกับ GL (ร้อยละ 2.5-7.5 ทดแทนปริมาณ FG) ในเจล FG พบว่า ค่าความแข็งแรงเจลและค่าความแข็งของเจลผสม FG/GL เพิ่มขึ้นเมื่อระดับของ GL และ $CaCl_2$ เพิ่มขึ้น ($p < 0.05$) ในทางตรงกันข้ามการเพิ่มขึ้นของระดับ GL และ $CaCl_2$ ทำให้ค่าความยืดหยุ่นลดลง แต่เพิ่มซินเนอรีซิส อุณหภูมิการเกิดเจลและอุณหภูมิที่เจลเกิดการหลอมละลายเพิ่มขึ้นเมื่อระดับของ GL และ $CaCl_2$ เพิ่มขึ้น ค่าความสว่างและค่าความเป็นสีเหลืองของเจลผสม FG/GL ลดลง ในขณะที่ค่าความแตกต่างของสีทั้งหมดเพิ่มขึ้นเมื่อระดับการเติม GL และ $CaCl_2$ เพิ่มขึ้น เจลผสมในสภาวะที่มี $CaCl_2$ สูงมีโครงข่ายเจลที่หนาแน่นและมีช่องว่างขนาดเล็ก ดังนั้นการเติม GL ร้อยละ 5 เพียงอย่างเดียวหรือการเติม GL ร้อยละ 2.5 ร่วมกับ $CaCl_2$ เข้มข้น 6 มิลลิโมลาร์สามารถใช้ในการปรับปรุงสมบัติเจลของ FG โดยเพิ่มค่าความแข็งแรงของเจลและอุณหภูมิการเกิดเจล โดยไม่มีผลต่อการยอมรับจากผู้บริโภค

จากการศึกษาผลของการเติม GL ในรูปแบบที่แตกต่างกัน 2 แบบ ได้แก่ แบบผง (GLP) และแบบแขวนลอย (GLS) ต่อสมบัติเจลของซูริมิจากปลาตาหวาน พบว่า เจลซูริมิที่เติม GLP หรือ GLS มีค่าแรงเจาะทะลุและค่าความแข็งของเจลเพิ่มขึ้นเมื่อระดับ GL (ร้อยละ 2-8 ของปริมาณของแข็งในซูริมิ) เพิ่มขึ้น ($p < 0.05$) การเติม GLS ร้อยละ 8 ทำให้ค่าแรงเจาะทะลุและค่าความแข็งของเจลสูงสุด ($p < 0.05$) ความสามารถในการอุ้มน้ำและค่าความขาวของเจลเพิ่มขึ้นเมื่อระดับของ GLP และ GLS เพิ่มขึ้น การเติม GL ทุกระดับไม่มีผลกระทบต่อการพอลิเมอไรเซชันของ

ไมโอซินสายหนัก (MHC) การเติม GLP และ GLS สามารถเพิ่มการเชื่อมต่อระหว่างสายโปรตีนขณะที่ให้ความร้อนซึ่งบ่งชี้ได้จากการเพิ่มขึ้นของค่าโมดูลัสสะสม (G') ทั้ง GLP และ GLS สามารถเพิ่มการยอมรับจากผู้บริโภค เมื่อระดับของ GL เพิ่มขึ้นจนถึงร้อยละ 6 เจลซูริมิที่มี GLS ร้อยละ 6 มีโครงข่ายเจลที่ละเอียดและหนาแน่นมากกว่าเจลซูริมิชุดควบคุม

เมื่อศึกษาการเติม GL ที่ระดับต่างๆ (ร้อยละ 2-8 ของปริมาณของแข็งในซูริมิ) และ $CaCl_2$ ที่ความเข้มข้นต่างๆ (25-75 มิลลิโมลต่อกิโลกรัม) เพื่อปรับปรุงสมบัติเจลของซูริมิจากปลาตาหวาน พบว่าค่าแรงเจาะทะลุ ค่าความแข็ง และค่าความขาวของเจลเพิ่มขึ้น แต่ปริมาณของเหลวจากการบีบอัดลดลง เมื่อระดับของ GL และ $CaCl_2$ เพิ่มขึ้น ($p < 0.05$) การเติม GL และ$CaCl_2$ ทุกระดับความเข้มข้นไม่มีผลกระทบต่อการพอลิเมอไรเซชันของไมโอซินสายหนัก เจลที่เติม GL ร้อยละ 4 ร่วมกับ 75 มิลลิโมล $CaCl_2$ ต่อกิโลกรัม มีคะแนนความชอบโดยรวมสูงสุด การเติม GL และ $CaCl_2$ ยังสามารถเพิ่มค่าแรงเจาะทะลุ ความสามารถในการอุ้มน้ำ และค่าความขาวของเจลซูริมิที่ไม่มีเอนไซม์ทรานส์กลูตามิเนส (TGase) จากการศึกษาทางรีโอโลยี พบว่า GL และ $CaCl_2$ สามารถเพิ่มการเชื่อมต่อระหว่างสายโปรตีนขณะที่ให้ความร้อนซึ่งบ่งชี้ได้จากค่า G' ที่สูงขึ้น ซูริมิมีแนวโน้มให้กราฟ G' ที่แตกต่างกันในสภาวะที่มีและไม่มี TGase โครงข่ายเจลมีความละเอียดและหนาแน่นสูงสำหรับเจลซูริมิที่ประกอบไปด้วย GL ร้อยละ 4 และ 75 มิลลิโมล $CaCl_2$ ต่อกิโลกรัม ในสภาวะที่มี TGase ดังนั้นการเติม GL แบบแขวนลอยในระดับที่เหมาะสม (ร้อยละ 6) สามารถเพิ่มความแข็งแรงของเจลของซูริมิจากปลาตาหวาน ทั้งยังสามารถเพิ่มการยอมรับจากผู้บริโภค นอกจากนี้การเติม GL ร้อยละ 4 ร่วมกับ 75 มิลลิโมล $CaCl_2$ ต่อกิโลกรัม สามารถปรับปรุงสมบัติเจลของซูริมิที่มีความสามารถในการเซ็ตตัวต่ำและสูง ซึ่งกำกับโดยเอนไซม์ทรานส์กลูตามิเนส

โดยภาพรวม GL เป็นไฮโดรคอลลอยด์ที่สามารถปรับปรุงสมบัติของทั้งเจล FG และซูริมิ

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Author Miss Tanyamon Petcharat

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ABSTRACT

Effect of gellan (GL) on properties of fish gelatin (FG) gel was investigated. GL at different levels (5-20 % of FG substitution) was incorporated into FG. Gel strength and hardness of FG/GL mixed gel increased, while springiness and cohesiveness decreased as the levels of GL were increased ($p < 0.05$). Gelling and melting temperatures also increased with increasing levels of GL incorporated ($p < 0.05$). L^*- and b^*-values of FG/GL mixed gel decreased, whereas E^*-value increased with increasing GL levels. The denser structure with very small voids in gel network was observed with GL addition. GL at a low level (5%) had no adverse effect on acceptance, and no effect on syneresis of FG/GL mixed gels.

When calcium chloride ($CaCl_2$) at various concentrations (3-9 mM) was used in combination with GL (2.5-7.5% FG substitution) in FG gel, gel strength and hardness of FG/GL mixed gel increased as the levels of GL and $CaCl_2$ increased ($p < 0.05$). Conversely, the increasing GL and $CaCl_2$ levels caused a decrease in springiness but an increase in syneresis of mixed gels. Gelling and melting temperatures were increased as levels of both GL and $CaCl_2$ increased. L^*- and b^*-values of mixed gels decreased, whereas ΔE^*-value increased with increasing GL and $CaCl_2$ levels. Mixed gel in the presence of $CaCl_2$ at higher levels had denser structure with smaller voids in gel network. Therefore, either 5% alone GL or 2.5% GL in conjunction with 6 mM $CaCl_2$ could be used to improve gelling property of FG via increasing gel strength and gelling point without affecting acceptance of the resulting gels.

Influence of GL with two different forms, powder (GLP) and suspension (GLS), on gel properties of bigeye snapper surimi was studied. Surimi gels added with GLP or GLS had the increases in breaking force and hardness as the levels (2-8% based on surimi solid content) were increased ($p < 0.05$). The highest breaking

force and hardness were observed in surimi gel containing 8% GLS ($p < 0.05$). Water holding capacity and whiteness of resulting gels were increased as levels of GLP and GLS increased. GL at all levels had no effect on polymerization of myosin heavy chain (MHC). Addition of GLP and GLS could enhance the interconnection between protein chains during heating as indicated by the higher G'. Both GLP and GLS increased acceptability of surimi gel as the level of gellan increased up to 6%. Finer and denser network was observed in surimi gel containing 6% GLS in comparison with that of the control.

GL at varying levels (2-6% based on surimi solid content) and $CaCl_2$ at various concentrations (25-75 mmol/kg) were used to improve gel property of surimi from bigeye snapper. Breaking force, hardness and whiteness of surimi gel increased but expressible moisture content decreased as the levels of GL and $CaCl_2$ increased ($p < 0.05$). GL and $CaCl_2$ at all levels had no effect on polymerization of MHC. The highest overall likeness score was found in gel containing 4% GL and 75 mmol $CaCl_2$/kg. Both GL and $CaCl_2$ also increased breaking force, water holding capacity and whiteness of transglutaminase (TGase) free surimi gels. Based on rheological study, GL and $CaCl_2$ could enhance the interconnection between protein chains during heating as indicated by the higher G'. Different G' curves were obtained between surimi in the presence and absence of TGase. Finer and denser network was observed in surimi gel containing 4% GL and 75 mmol $CaCl_2$/kg in the presence of TGase. Therefore, gellan, prepared as suspension, at an appropriate level (6%) could increase gel strength of bigeye snapper surimi with an increased acceptability. In addition, 4% GL in combination with 75 mmol $CaCl_2$/kg could improve gel properties of surimi having low and high setting phenomenon mediated by endogenous TGase.

Overall, GL was shown as a promising hydrocolloid, which was able to improve properties of both FG and surimi gels.

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CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

1.1 Introduction

Gelatin is a protein derived from collagen by thermal denaturation or partial hydrolysis (Benjakul *et al.*, 2012; Johnston-Banks, 1990). Fish gelatin has become the essential alternative and mainly produced from fish processing by-products such as skin, scale, etc. (Sinthusamran *et al.*, 2015). Facing with poor gelling property with low gelling temperature and longer setting time, fish gelatin still has the limited applications (Liu *et al.*, 2008). In general, fish gelatin is dissolved in water at temperatures of 40-60 °C, and forms a gel when cooled down. Fish gelatin possessed the lower gelling temperature (8-25 °C) and melting temperature (11-28 °C), compared with mammalian gelatins having the temperature range of 20-25 °C and 28-31 °C, respectively (Karim and Bhat, 2009). To alleviate such a drawback, cross-linking enzymes such as microbial transglutaminase (Jongjareonrak *et al.*, 2006) and various hydrocolloids including agar, κ-carrageenan, pectin and gellan have been used. As a consequences, gelling properties of fish gelatin could be modified (Huang *et al.*, 2017; Lau *et al.*, 2001; Lee *et al.*, 2003; Sinthusamran *et al.*, 2016; Sinthusamran *et al.*, 2017).

Surimi is minced fish washed with water, rinsed and dewatered to remove sarcoplasmic proteins and to concentrate myofibrillar proteins (Eymard *et al.*, 2005). The washing process has been used to refine fish myofibrillar proteins and undesirable material such as fat, blood, pigment and odorous substances. Surimi is a useful ingredient for producing various kinds of processed foods with the unique textural property. Surimi-based products such as fish ball, fish sausage, breaded fish stick and paupiette, etc. have gained increasing attention due to the preferred textural properties and high nutritional value (Zhou *et al.*, 2017). To improve the properties of surimi gel, a number of additives with different functions have been used. Some additives are used to retard the proteolysis (Singh and Benjakul, 2017), while protein cross-linkers such as microbial transglutaminase (Chanarat *et al.*, 2012) or phenolic

compound (Buamard and Benjakul, 2015) have been added to strengthen the gel matrix. Moreover, some hydrocolloids such as curdlan, pullulan, carrageenan and pectin, etc. have been reported to improve the properties of surimi gels (Barrera *et al.*, 2002; Hunt and Park, 2013; Wei *et al.*, 2017; Wu, 2016).

Gellan is a bacterially derived polymer, produced by *Pseudomonas elodea* (lately referred to as *Sphingomonas paucimobilis*). Gellan, a linear anionic heteropolysaccharide, forms a double helix in aqueous solution, yielding high viscosity. After heating for solubilization, gellan can gel upon cooling (Matricardi *et al.*, 2009). Addition of gellan was reported to provide a wide range of textures for various food applications, especially in protein gel system. Wolf *et al.* (1989) suggested that incorporation of small amounts of gellan into porcine gelatin could greatly improve properties of resulting gel. Gelation temperature and gelation rate of the mixed gels were significantly affected by the ratio of gellan to bovine gelatin (Lau *et al.*, 2001). Addition of gellan into bovine gelatin gel could increase the hardness of the mixed gels (Lau *et al.*, 2000). Furthermore, gellan was used to improve quality of reduced-fat frankfurters. When mixed gels of konjac (1% and 2%) and gellan (0.25% and 0.5%) were incorporated into reduced fat (18%) frankfurters, its sensory property was equivalent to the control frankfurters (without konjac and gellan addition) (Lin and Huang, 2003). Gellan can be another alternative additive, which is able to modify the gelling behavior, particularly increasing setting temperature and increasing gel strength of fish gelatin. As a consequence, more acceptable gelatin can be obtained. Additionally, surimi added with gellan at an appropriate level can be improved in term of textural property, especially gel strength. This can result in the increased acceptability and price of surimi after addition with gellan. To our knowledge, no information on the effect of gellan on gelling property of fish gelatin and surimi exists. Therefore, the present study aims to investigate the impact of gellan incorporation at various levels on the properties of fish gelatin and surimi gels.

1.2 Review of literature

1.2.1 Gelatin

Gelatin is biopolymer manufactured via partial denaturation or hydrolysis of collagen. It has a numerous applications in food and non-food (photographic, cosmetic, and pharmaceutical) industries (Regenstein and Zhou, 2007). Mammalian gelatins, especially pig and cow skins and bones, are commonly available. Recently, outbreaks of mad cow disease (bovine spongiform encephalopathy, BSE) brings about the anxiety for customers. Furthermore, the gelatin from pig skin and pig bone is prohibited in Kosher and Halal foods due to religious constraints. Health concern by consumers has been associated with a high demand of fish gelatin (Kittiphattanabawon *et al.*, 2016). Fish gelatin production, especially from skin, bone, and scale from fish processing by-products, has gained increasing interest due to their abundance and low cost. However, gelatins from these sources have the limited applications due to lower gel strength and lower setting temperature, compared with those from mammalian origins. Fish gelatin is different from gelatin because of the different molecular weight distribution and amino acid content, especially imino acid (proline and hydroxyproline) content (Johnston-Banks, 1990).

1.2.1.1 Fish gelatin

1.2.1.1.1 Composition of gelatin

Gelatin is a heterogeneous mixture of water-soluble proteins with high molecular weight (Budavari, 1996). On a dry weight basis, gelatin has 98 to 99% protein content. The molecular weight of gelatin ranges between 20,000 and 250,000 Da (Keenan, 2000) having amino acid sequence of Gly-Pro-Hyp (Poppe, 1997). Gelatin has high levels of glycine (Gly) 26-34%; proline (Pro) 10-18% and Hydroxyproline (Hyp) 7-15% (Poppe, 1997). Alanine (Ala) 8-11%; arginine (Arg) 8-9%; aspartic (Asp) 6-7% and glutamic acid (Glu) 10-12% are also found in gelatin (Hudson, 1993; Poppe, 1997). Gelatin is not nutritionally complete protein, owing to the lack of tryptophan, isoleucine, threonine and methionine (Moskowitz, 2000). The other sulfur-containing amino acids, cysteine and cystine, are also deficient or absent. In general, mammalian gelatin contains the large amounts of hydroxyproline and hydroxylycine, and the total imino acid (proline and hydroxyproline) content is high.

Table 1 shows the typical amino acid composition of fish gelatin from different sources in comparison with gelatin from porcine skin (Karim and Bhat, 2009). Water varies between 6 and 9%, whereas ash content ranges from 0.1 to 3.25% (Cheow *et al.*, 2007; Jongjareonrak *et al.*, 2006).

1.2.1.1.2 Gelation mechanism and gel properties

An aqueous gelatin solution turns to be viscous at temperature above its melting temperature. Upon cooling, transparent elastic thermoreversible gels is formed when the temperature is below the setting temperature (Babin and Dickinson, 2001; Normand *et al.*, 2000). The interaction initiates a disorder-to-order transition, as the random coil gelatin molecules seek to return to the ordered triple helix conformation (Benjakul *et al.*, 2012). Gelatin forms reversible network stabilized by hydrogen-bonded junction zones. Hydrophobic and ionic interactions are also involved in the gelation of gelatin (Benjakul *et al.*, 2012). The gelation of gelatin is governed by several factors e.g. source of raw material, endogenous protease in raw material, and the conditions for gelatin extraction, which includes temperature (Kittiphattanabawon *et al.*, 2016; Nalinanon *et al.*, 2008; Sinthusamran *et al.*, 2014). The interactions or bonds between the chains that make up the gel are typically van der Waal's interactions and hydrogen bonds. Due to the weak bonds, gelatin is capable of forming thermoreversible gels. Gel strength and gel melting point are considered as the important properties of gelatin gels. These are related with molecular weight, as well as by complex interactions determined by the amino acid composition and the ratio of α/β-chains of gelatin (Cho *et al.*, 2005).

According to Schrieber and Gareis (2007), the gel strength is determined by proportion of fractions having a molecular weight of approximately 100,000 g/mol. A strong correlation between gel strength and the α-chain content in gelatin. Gelatin having more α-chains generally shows higher gel strength. Conversely, a high ratio of peptides with molecular weights higher or lower than the α-chains could lower gel strength (Liu *et al.*, 2008). However, Sinthusamran *et al.* (2014) found that gelatin from seabass skin with higher proportion of β-chain had the higher gel strength than those with lower amount of dimer or trimer.

Table 1 Amino acid content in some fish gelatins compared to pork gelatin (residues/1000 total amino acid residues)

Amino acids	Cod skin[a]	Alaska pollock skin[b]	Hake[a]	Megrim[a]	Tilapia skin[c]	Pork skin[d]
Ala	96	108	119	123	123	112
Arg	56	51	54	54	47	49
Asx	52	51	49	48	48	46
Cys	0	0	–	–	0	0
Glx	78	74	74	72	69	72
Gly	344	358	331	350	347	330
His	8	8	10	8	6	4
Hyl	6	6	5	5	8	6
Hyp	50	55	59	60	79	91
Ile	11	11	9	8	8	10
Leu	22	20	23	21	23	24
Lys	29	26	28	27	25	27
Met	17	16	15	13	9	4
Phe	16	12	15	14	13	14
Pro	106	95	114	115	119	132
Ser	64	63	49	41	35	35
Thr	25	25	22	20	24	18
Trp	0	0	–	–	0	0
Tyr	3	3	4	3	2	3
Val	18	18	19	18	15	26
Imino acids	156	150	173	175	198	223

Source: [a] Gómez-Guillén *et al.* (2002); [b] Zhou *et al.* (2006); [c] Sarabia *et al.* (2000); [d] Eastoe and Leach (1977)

Fish gelatins have lower gelling and melting temperatures than mammalian gelatins (Leuenberger, 1991). Typical gelling and melting points for porcine and bovine gelatins range from 20 to 25 °C and 28 to 31 °C, respectively. Typical gelling and melting points for fish gelatins are in the range of 8-25 °C and 11-28 °C, respectively (Table 2). Gelling temperatures is influenced by the raw material used for gelatin extraction. Gilsenan and Ross-Murphy (2000) compared the rheological properties and melting points of gelatins from different fish with mammalian gelatin. Gelatins from cold-water fish have a much higher critical concentration and lower melting point than mammalian counterpart, because of the lower imino acid contents. As a result, the propensity for intermolecular helix formation is reduced. Warm water fish gelatins, have properties that are quite similar to mammalian samples. The melting temperatures of gelatins derived from the skins of cold-water fish are lower than those of collagens and gelatins from the skins of mammals and fish living in warm waters, caused by the lower imino acid contents and less proline hydroxylation (Gómez-Guillén *et al.*, 2002; Norland, 1990; Yamaguchi *et al.*, 1976). Consequently, cold-water fish gelatins is viscous in nature at room temperature, thus limiting their applications (Avena-Bustillos *et al.*, 2006).

1.2.1.1.3 Improvement of properties of fish gelatin gel

Due to the poor gel-forming ability of fish gelatin to mammalian counterpart, fish gelatin can be modified for gel improvement or alternatively mixed with other gelling agent or additives to strengthen the gel. Several methods or the addition of selected compounds have been used to improve the gelling properties of fish gelatin.

1.2.1.1.3.1 Use of protein cross-linkers

- **Phenolic Compounds**

Phenolic compounds cover than 6000 identified substances, known as the largest group of secondary metabolites in plant foods (Rawel *et al.*, 2007). Those possess an aromatic ring bearing one or more hydroxy substituents (Robards *et al.*, 1999) and also bind to sugars as glycosides (Hollman and Arts, 2000). Phenolic compounds are able to interact with proteins via two different fashions: via

Table 2 Gel strength, gelling and melting temperatures of various fish gelatins

Type of fish	Bloom strength (g)	Gelling temperature (°C)	Melting temperature (°C)
Cod	~90	11-12	13.8
Hake	~110	11-12	14
Sole	350	18-19	19.4
Megrim	340	18-19	18.8
Sin croaker	124.9	7.1	18.5
Shortfin scad	176.9	9.9	24.5
Tilapia spp.	Not reported	18.2	25.8
Young Nile perch	222	13.8	21.4
Adult Nile perch	229	19.5	26.3
Grass carp	267	19.5	26.8
Yellowfin tuna skin	426	18.7	24.3
Catfish	243-256	15-18	23-27

Source: Karim and Bhat (2009)

noncovalent (reversible) interactions and via covalent interactions, which in most cases are irreversible (Prigent, 2005). Interactions between hydroxyl groups of phenolic compounds and the nitrogen or oxygen of lysine, arginine, histidine, asparagine, glutamine, serine, threonine, aspartic acid, glutamic acid, tyrosine, cysteine, and tryptophan via hydrogen bonds occur. Hydrophobic interactions may form between phenolic compounds and amino acids such as valine, isoleucine, leucine, methionine, tyrosine, tryptophan, alanine, phenylalanine, cysteine and glycine

residues (Prigent, 2005). Covalent interactions between phenolic compounds and proteins can occur via oxidation of phenolic compounds to radicals or quinones (Balange and Benjakul, 2009a). Monophenol and polyphenol can be oxidized to ortho-quinone, either enzymatically as in plant tissues, or by molecular oxygen (Benjakul *et al.*, 2012). The quinone forms a dimer through a secondary reaction, or reacts with amino or sulfhydryl side chains of polypeptides to form covalent C-N or C-S bonds with the phenolic ring (Strauss and Gibson, 2004). Nevertheless, gelatin contains a negligible content of cysteine. Lysine of gelatin could provide ε-NH_3 as the binding site in this reaction. Fish gelatin had lysine with the range of 12 to 32 residues/1000 residues (Benjakul *et al.*, 2012). Polyphenols can be reoxidized and bind the second polypeptide. Consequence, protein cross-links can be formed (Benjakul *et al.*, 2012).

Plant phenolic can be used as gelatin cross-linker, thereby increasing gel strength of gelatin. Kaewdang and Benjakul (2015) reported that ethanolic extracts from coconut husk (EECH) at an appropriate level served as the potential cross-linkers to increase the strength of gel of yellowfin tuna swim bladder gelatin. EECH (0.5 mg/g) enhanced gel strength most effectively. EECH at an excessive amount (5 mg/g) had the negative impact on gel strength. Strauss and Gibson (2004) studied the effect of plant phenolics as the cross-linker of gelatin gels and gelatin-based coacervates. Polyphenols react under oxidizing conditions with gelatin side chains and covalent cross-link are formed. Yan *et al.* (2011) studied physicochemical properties of gelatin gels from walleye pollock (*Theragra chalcogramma*) skin cross-linked by gallic acid (10-40 mg/g gelatin) and rutin (2-8 mg/g gelatin). Gel strength increased with increasing gallic acid concentration up to 20 mg/g gelatin, and then decreased at further elevated gallic acid concentration, while it continuously increased with increasing levels of rutin. Both phenolics could enhance the elastic modulus (G') and the viscous modulus (G") of hydrogels, but had no impact on gelling and melting points. X-ray diffraction revealed that both gallic acid and rutin could enter the spacing of polypeptide chains of gelatin to reinforce the intermolecular interaction. FTIR spectra verified that gallic acid and rutin molecules mainly interacted with skeletal C-N-C group and carboxyl group of gelatin molecules in the formation of gels. Temdee and Benjakul (2014) revealed that ethanolic kiam wood extract (EKWE)

and ethanolic cashew bark extract (ECBE) oxygenated at different pHs (8, 9 and 10) could increase gel strength of gelatin from cuttlefish (*Sepia pharaonis*) skins as the pH for oxygenation of the extract increased. Cuttlefish skin gelatin gels added with EKWE or ECBE oxygenated at pH 9 had the lowest free amino groups, suggesting the highest cross-linking of gelatin via amino groups. An increase in gel strength was observed when the concentration of extracts (1-8%, w/w) increased. At the same concentration of extract, gelatins with EKWE had a higher gel strength than those with added ECBE. The larger strands and larger voids in the gel matrix were observed with gels with added oxygenated EKWE or ECBE, compared with the control gel (Temdee and Benjakul, 2014). When different phenolic compounds (catechin, ferulic acid, tannic acid and gallic acid) or the extracts including ethanolic kiam wood extract (EKWE) and ethanolic cashew bark extract (ECBE) oxidized by laccase (20 U/mL) were added in gelatin from cuttlefish skin, gel containing oxidized gallic acid had the highest gel strength, followed by that containing oxidized catechin. Both oxidized gallic acid and catechin exhibited the cross-linking activity shown by the decreases in free amino group contents of gelatin (Temdee and Benjakul, 2015).

Seaweed extract was employed as the natural protein cross-linker, that is capable of modifying the properties of film from gelatin or other proteins (Rattaya *et al.*, 2009). Films added with seaweed extract at pH 9 and 10 exhibited the higher elongation at break (EAB) than the control film. There was no difference in tensile strength (TS) and transparency between films without and with seaweed extract. Formation of non-disulfide covalent bond in the film matrix, induced by the interaction between oxidized phenols in seaweed extract and gelatin molecules contributed to the changes in properties (Rattaya *et al.*, 2009).

- **Transglutaminase**

Transglutaminase (TGase; protein-glutamine-glutamyltransferase, EC 2.3.2.13) catalyzes an acyl-transfer reaction between the γ-carboxamide group of peptide-bound glutamine residues (acyl donors) and primary amines (acyl acceptors), which include ε-amino group of lysine residues (Figure 1). This reaction causes polymerization and inter- or intramolecular cross-linking of protein via formation of ε-(γ-glutamyl)lysine linkages (Ashie and Lanier, 2000; Motoki and Seguro, 1998).

Without amine substrates, water may act as the acyl acceptor. As a result, deamidation of γ-carboxamide group of glutamine to form glutamic acid occurs. Formation of covalent cross-links between proteins modifies the physical properties of food proteins (Ashie and Lanier, 2000).

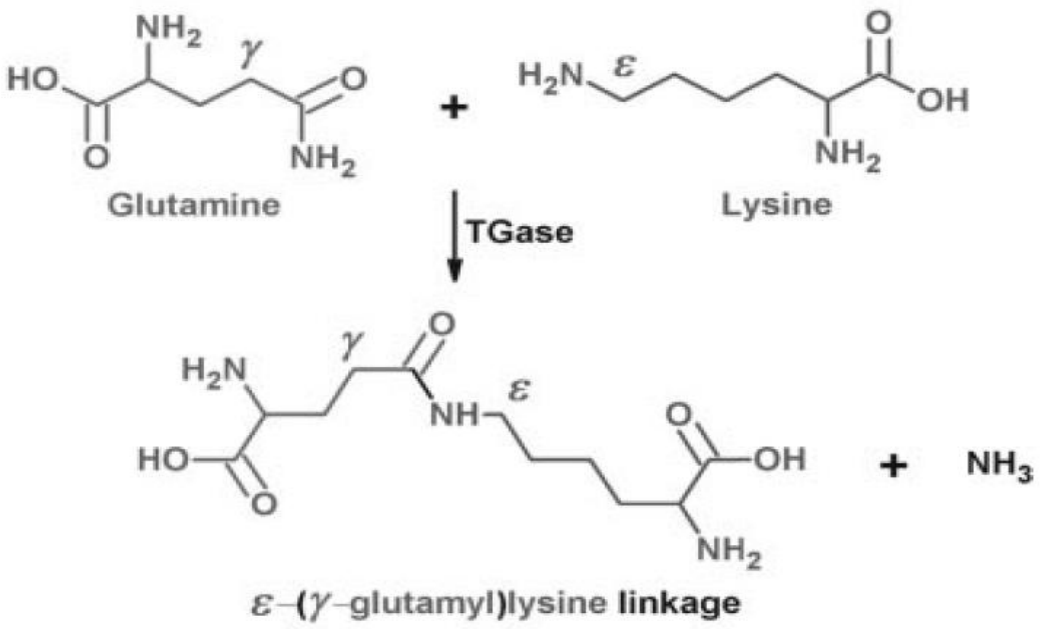

Figure 1 Cross-linking reaction between glutamine and lysine residues induced by transglutaminase.
Source: Benjakul *et al.* (2012)

Microbial transglutaminase (MTGase) has been widely used due to its efficacy in protein-cross-linking. It is widely used as the gel strengthening agent in gelatin. Jongjareonrak *et al.* (2006) studied the effect of MTGase on the gel properties of gelatin from bigeye snapper skin and brownstrip red snapper skin. The addition of MTGase at concentrations up to 0.005% and 0.01% (w/v) increased the bloom strength of gelatin gel from bigeye snapper and brownstripe red snapper, respectively. However, the bloom strength of skin gelatin gel from both fish species decreased with further increase in MTGase concentration. Gelatin gel added with MTGase showed the decrease in band intensity of protein components, especially, β- and

γ- components, suggesting the cross-linking of these components induced by MTGase. Norziah *et al.* (2009) studied the effects of transglutaminase on *Tenualosa ilisha* fish gelatin in terms of melting, gelling temperature, gel strength and pH. MTGase had the significant effect on gel strength when used at an appropriate amount. It caused an increase in G' and G" values, compared to untreated gels. However, the resulting gelatin gel still had low melting and gelling temperatures and gel strength even when transglutaminase was added, compared to both commercial fish and commercial halal bovine gelatin gels. The modified gels had the strengths of 101.1 g and 90.56 g when MTGase at levels of 1.0 and 3.0 mg/g was added, respectively (Norziah *et al.*, 2009). Wangtueai *et al.* (2010) reported that the addition of MTGase up to 0.5% (w/v) generally increased the gel strength of lizardfish (*Saurida* spp.) scale gelatin gels. The addition of MTGase decreased the band intensity of β- and α-components, especially with increasing concentrations of enzyme. In addition, Mohtar *et al.* (2013) optimized the preparation of New Zealand hoki (*Macruronus novaezelandiae*) gelatin gels using MTGase. The addition of MTGase to hoki gelatin at the optimum concentration (3.33 mg/g) increased the gel strength from 197±5 g to 278.2±0.19 g and the melting point from 21.4±0.8 °C to 25.9±0.1 °C. The increase in the G' values with the addition of MTGase indicated the formation of firmer gels (Mohtar *et al.*, 2013).

Furthermore, MTGase has been also employed to improve the property of protein based films. Films cast from lizardfish scale gelatin with and without 0.5% MTGase and bovine gelatin films were compared. Films from of lizardfish scale gelatin containing 0.5% MTGase showed higher TS than the films without enzyme added. The water vapor permeability of films from lizardfish scale gelatin with and without 0.5% MTGase and bovine gelatin films were 21.0±0.17, 26.3±0.79, and 25.8±0.09 g·mm/m^2·d·kPa, respectively, while the oxygen transmission rate of all 3 types of films were less than 50 cc O_2/m^2·d (Wangtueai *et al.*, 2010).

1.2.1.1.3.2 Use of hydrocolloids

Food products are composed of a wide range of ingredients including proteins and carbohydrate-based polysaccharides (Ye, 2008). Complex formation between proteins and polysaccharides generally occurs at pH values below the

isoelectric point (IEP) of the proteins and at low ionic strength, usually <0.3 (Ye, 2008). Protein molecules possess a net positive charge and behave as polycations at pH values below the IEP (de Kruif *et al.*, 2004). At mildly acidic and neutral pH values, which are typical of most foods, carboxyl-containing polysaccharides act as polyanions (Ye, 2008). Electrostatic complex formation between proteins and anionic polysaccharides take place in the pH range between the pKa value of the anionic groups (carboxyl groups) on the polysaccharide and the IEP of the protein (Tolstoguzov, 1997). The interaction between proteins and polysaccharides directly determine the properties of the food complexes.

The mixtures of fish gelatin and *κ*-carrageenan yielded the gels with different degrees of turbidity. The concentration of polymers, pH, ionic strength, and the nature of the added salt were the factors determining turbidity (Haug *et al.*, 2004). Gel strength was highest as 20 mM KCl and 20 mM NaCl were incorporated into the mixtures of 1% (w/v) *κ*-carrageenan containing 2% and 5% (w/v) fish gelatin, respectively. However, these mixtures yielded more turbid gel than *κ*-carrageenan or fish gelatin alone. The system undergoes associative phase separation promoted by the release of counter ions (Piculell, 1995). Complexes of fish gelatin and *κ*-carrageenan at 60 °C were probably stabilized by electrostatic interactions. The solutions became highly turbid, and at 4 °C, the gel was strongest (Haug *et al.*, 2004). Liu *et al.* (2007) determined the hardness of gelatin/pectin mixed gels with different ratios of pectin and gelatin at 0-1.5% (w/w) and 2-8% (w/w), respectively. The addition of pectin increased gel hardness. Two polymers in the mixed solution showed a synergistic effect. At the same gelatin content, the lower pectin content added to the system yielded a harder gel. The excessive pectin content could increase the repulsive forces in the junction zones. Therefore, the formation of linkages between the aggregated helices, leading to weakened gel structures (Liu *et al.*, 2007). The rheological behavior, gel properties and nanostructure of complex modified fish scales gelatin (FSG) from bighead carp (*Hypophthalmichthys nobilis*) by pectin (0.1-1.6%, w/v) and MTGase (0.06%, w/v) were investigated. MTGase and pectin have positive effect on gelling point, melting point, apparent viscosity and gel properties of FSG. Gel strength and melting temperature became highest when pectin at 0.8% (w/v) was added. Nevertheless, at highest pectin concentration (1.6% w/v),

the gel strength and melting temperature of complex modified gelatin gels decreased. Nanostructure and microstructure revealed that MTGase catalyzed cross-links among soluble fish scale gelatin-pectin complexes. This was responsible for the increase in rheological behavior, gel strength and melting temperature of modified complex gels (Huang *et al.*, 2017). Sinthusamran *et al.* (2016) reported that incorporation of agar extracted from *Gracilaria tenuistipitata* and commercial agars into fish gelatin at different various levels (0-20% gelatin substitution) affected textural properties and sensory characteristics differently. Both low strain modulus and failure stress of agar/gelatin mixed gels increased when the levels of agar increased. Nevertheless, commercial agar had a higher gel strengthening impact than *G. tenuistipitata* agar. Both agars decreased the failure strain (springiness) of agar/gelatin mixed gels. However, the incorporation of agar, especially at higher levels, had the detrimental effect on sensory characteristics (Sinthusamran *et al.*, 2016). Furthermore, Bang *et al.* (2014) reported that the addition of hydroxypropyl methylcellulose phthalate (HPMCP) to gelatin at mass ratios ranging from 1:32 to 1:2 generated a composite gel with enhanced rheological and mechanical properties. The maturation process reinforced the composite gel properties. The viscosity values of the composite solutions were retained during the stability test, whereas a significant reduction was indicated in the gelatin-only solution.

1.2.2 Surimi

Surimi refers to as the concentrated myofibrillar protein extracted from fish flesh by washing minced meat, separated from bones, skin, and guts. During washing with cold water, fat and other water-soluble contents are removed, whereas insoluble myofibrillar protein is concentrated (Okada, 1992). High quality surimi is naturally odorless and colorless (Park, 2013). Surimi can be used as raw material for preparing traditional fish gel product, such as kamaboko, tempura, and chikuwa, which are very popular in Japan as well as Asia markets (Jiang *et al.*, 2000).

1.2.2.1 Gelation of surimi

Gel formation is one of the most important functional properties of food protein. Protein gelation is the process of forming a three-dimensional network structure, in which water is entrapped after protein denaturation and aggregation (Guo

et al., 2017). Myofibrillar proteins, especially myosin, are well known to be primarily responsible for gelation associated with textural properties of comminuted muscle foods (Sun and Holley, 2011). The differences in cross-linking of MHC contribute to the differences in gel forming ability among the muscles of various fish (Chanarat *et al.*, 2012). Gelation of surimi consists of two-step processes (Ziegler and Acton, 1984) as follows:

1. Protein denaturation: Addition of salt in combination of heating are two major factors involved in denaturation and gelation of muscle protein (Park and Lanier, 1989). The first step is usually a dissociation of the quaternary structure of the protein and unfolding of the molecule. Salt (NaCl) at 2-3% is typically added during manufacturing of surimi seafood (Tahergorabi and Jaczynski, 2012). As solubility is the key factor governing gelation, the environment conditions must enhance solubility of myofibrillar proteins (Xiong, 1994). The heat induced denaturation of muscle proteins, which consists of actin, tropomyosin, troponin and myosin, with subsequent aggregation, result in the formation of three dimensional network of surimi gel (Zhang and Zhao, 2013).

Conformational changes may occur during the thermal denaturation of natural actomyosin (NAM) as described by Ziegler and Acton (1984). Different myofibrillar proteins undergo major conformational changes upon heating. Around 38 °C, F-actin, that has a double-stranded helical structure, dissociates into single chains. The myosin light chains disconnect from the heavy chains at approximately 40 °C, inducing conformational changes in the head and hinge region of the myosin molecules (Ziegler and Acton, 1984). Around 50 °C, the actomyosin complex dissociates, followed by a helix-coil transition of the light meromyosin chains in the temperature range between 50 and 55 °C. The denaturation of G-actin occurred at temperature greater than 70 °C (Ziegler and Acton, 1984). Dynamic viscoelastic behavior analysis revealed that the gelation of NAM from haddock occurred in two stages: at temperature range of 36-45 °C and 59-90 °C (Leelapongwattana *et al.*, 2008), whilst surimi from Alaska pollock showed the gelation at temperature range of 37-40 °C and 47-75 °C (Yin and Park, 2014). Zhu *et al.* (2016) reported that the incubation temperature used for gel formation affected surimi gel from yellowtail

seabream. The gel incubated at 40 °C, followed by cooking at 90 °C (suwari) showed higher gel strength than that incubated at 67 °C, followed by 90 °C (modori).

2. Aggregation: During heating of surimi pastes, the proteins unfold, exposing reactive surfaces of neighboring protein molecules, which then interact to form intermolecular bonds (Lanier *et al.*, 2000). The first stage of gelation was due to myofibrillar proteins in surimi, which undergo aggregation or entanglement. The second stage was attributed to the formation of irreversible gel network (Rawdkuen *et al.*, 2007). Sano *et al.* (1990) proposed that gel development was due to interactions among the tail portions of myosin molecules and attributed to hydrophobic interactions among the head portions of myosin. In contrast, Samejima *et al.* (1981) revealed that the heat-induced gelation of surimi consists of two reactions as follows: (1) aggregation of the globular head segments of the myosin molecules, which is associated with the oxidation of sulfhydryl groups and (2) network formation resulting from the unfolding of the helical tail segment. Chan *et al.* (1993) reported that both HMM and LMM are involved in thermal aggregation of cod and herring myosins. Thermal aggregation may be initiated by the unfolding and interaction of HMM S-2, and further aggregation may be mediated through the interaction of LMM to form clusters of aggregates at higher temperatures (Figure 2).

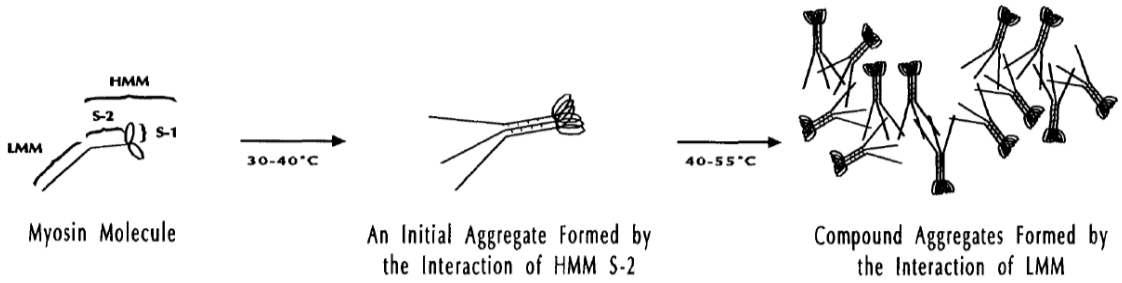

Figure 2 Schematic representation of the thermal aggregation of fish myosin.
Source: Chan *et al.* (1993)

Basically, there are mainly four types of bonds, which contribute to the building of a network structure during the gelation of a surimi paste: hydrogen bonds, salt linkages, hydrophobic interactions and covalent bonds (Lanier *et al.*, 2000). Hydrogen bonds are weaker dipole bonds, not responsible for the gelation of

myofibrillar proteins but are important in the stabilization of bound water within the hydrogel (Park, 2013). A large amount of water molecules is hydrogen bonded to the polar amino acid residues, which are abundantly exposed on the molecular surface of the proteins (Niwa *et al.*, 1992). Calcium ions can form salt linkages between negatively charge localized on two adjacent proteins (Wan *et al.*, 1994). The addition of calcium ions may contribute to the strengthening of surimi gel due to their effect on TGase in the muscle proteins. Addition of calcium ions in surimi resulted the increased gel strength of surimi (Benjakul *et al.*, 2004; Ding *et al.*, 2011; Yin and Park, 2014). Furthermore, Zn^{2+} was able to induce the formation of salt bridge and ionic interaction in gel network of surimi from yellow stripe (Arfat and Benjakul, 2013).

During the slow-setting upon incubation near 40 °C, hydrophobic groups are introduced onto their molecular surface. Hydrophobic interactions take place and play an essential role in the setting phenomenon (Niwa *et al.*, 1981). Benjakul *et al.* (2001) reported the increase in surface hydrophobicity of actomyosin from bigeye snapper actomyosin during thermal gelation, suggesting that hydrophobic interaction is involved in gelation. Covalent bonds such as disulfide bonds are dominant when heating at high temperatures (>40 °C) is introduced (Lanier *et al.*, 2000). The formation of disulfide bonds is more pronounced for carp (Itoh *et al.*, 1979) and Atlantic croaker actomyosin (Lanier *et al.*, 1982) at the higher temperatures (80 °C or above) than at the lower temperatures, at which setting occurs. Benjakul *et al.* (2001) found disulfide bonds in bigeye snapper actomyosin during thermal gelation process, especially at temperature above 35 °C. However, a variety of crosslinks, including ε-(γ-glutamyl)lysine [ε-(γ-Glu)Lys] crosslinks and crosslink through aldol condensation, contributed to texture (Sakamoto *et al.*, 1995). The formation of ε-(γ-Glu)Lys crosslinks by the catalytic action of transglutaminase (TGase) has been intensively reported (Benjakul and Visessanguan, 2003; Chanarat *et al.*, 2012; Jiang *et al.*, 2000). Endogenous TGase plays a role in setting by inducing the formation of isopeptide. Benjakul and Visessanguan (2003) confirmed that TGase was involved in gel enhancement of surimi from two species of bigeye snapper (*Priacanthus tayenus* and *Priacanthus macracanthus*). Setting at either 25 or 40 °C, prior to heating at 90 °C, caused the increase in both breaking force and deformation of surimi from both

species, particularly as setting time increased. A decrease in solubility of surimi gels in a mixture of sodium dodecyl-sulfate, urea and β-mercaptoethanol suggested the increased formation of non-disulfide covalent bonding. This coincided with the increased gel strength and the decrease in myosin heavy chain (MHC) band intensity. The optimum conditions for setting of surimi sol were 40 °C for 2 h for *P. tayenus* and 25 °C for 3 h for *P. macracanthus*. Based on monodancylcadaverine (MDC)-incorporation assay, TGase from *P. tayenus* and *P. macracanthus* exhibited an optimal temperature at 40 and 25 °C, respectively. Breaking force and deformation of surimi from both species increased when calcium chloride was added, but decreased considerably in the presence of EDTA, *N*-methylmaleimide (NBM) and ammonium chloride (Benjakul and Visessanguan, 2003). Textural properties and cross-linking of myofibrillar proteins in suwari gel of surimi from four fish species, including bigeye snapper, threadfin bream, barracuda and bigeye croaker as influenced by transglutaminase activator and inhibitors was studied by Benjakul *et al.* (2004). Breaking force and deformation of suwari increased as the amount of calcium chloride increased. Nevertheless, a slight decrease was found with an excessive amount of calcium chloride. Concomitant decrease in solubility of suwari gel, in a mixture of sodium dodecyl sulfate, urea and β-mercaptoethanol was found, suggesting the increased non-disulfide covalent bond formation. TGase inhibitors, involving NEM, ammonium chloride and EDTA, especially at higher concentrations, resulted in a marked decrease in breaking force and deformation. The gel-forming ability was decreased with lowered associated with the decrease in non-disulfide covalent cross-linking, as indicate by an increase in solubility and more band intensity of myosin heavy chain (MHC) retained (Benjakul *et al.*, 2004).

1.2.2.2 Improvement of properties of surimi gel

1.2.2.2.1 Use of microbial transglutaminase

MTGase induces the formation of ε-(γ-glutamyl) lysine cross-link in the proteins via acyl transfer between the ε-amino groups of a lysine residue and γ-amide group of a glutamine residue (Chanarat *et al.*, 2012). However, efficiency of MTGase in improving gel property of surimi depends on many factors, e.g. amount of MTGase, type of fish, fat content (Zaghbib *et al.*, 2016). The properties of surimi gels

from threadfin bream and pollack surimi added with MTGase from *Streptoverticillium ladakanum* set at 30 °C or 45 °C were determined. The optimal amounts of MTGase and setting conditions were: 0.3 unit/g surimi either at 30 °C for 90 min or at 45 °C for 20 min for threadfin bream, and 0.2 unit/g surimi at 30 °C for 60 min for pollack. The strength of golden threadfin bream surimi gels with 0.35 unit MTGase set at 30 °C for 90 min or 45 °C for 20 min was 3400g·cm, almost 3-fold of the control. SDS-PAGE analyses indicated that inter- and/or intramolecular cross-linking of myosin heavy chain was formed in MTGase-containing surimi gels (Jiang *et al.*, 2000). Ramírez *et al.* (2000) optimized the concentration of MTGase, temperature and time for improvement of the mechanical properties of surimi gels from striped mullet (*Mugil cephalus*). Shear stress was strongly affected by the variables studied, while shear strain was moderately affected. Maximum shear stress (156 kPa) was found with the following setting conditions: a concentration of MTGase of 9.3 g/kg of surimi, a temperature of 37 °C and a time of 3.9 h. Under these conditions the shear strain was 1.34. Maximum shear strain (1.57) was attained with the following conditions: a concentration of MTGase of 5 g/kg of surimi, a temperature of 34.5°C and a time of 1 h. Under these conditions, the shear stress was 123 kPa (Ramírez *et al.*, 2000). Properties of kamaboko gels, produced from sardine surimi in the absence or presence of MTGase at 4 g/kg and addition of $CaCl_2$, $MgCl_2$, and NH_4Cl at 2 g/kg were also evaluated by Karayannakidis *et al.* (2008). The addition of MTGase had a beneficial effect on the *L**-values and whiteness index as well as on firmness and cohesiveness of kamaboko gels. Fish gels containing $CaCl_2$ or $MgCl_2$ were lighter and firmer, compared to those containing NH_4Cl. Both $CaCl_2$ and $MgCl_2$ ions may also bind to the negatively charged residues of fish proteins and form ionic inter-molecular linkages (Karayannakidis *et al.*, 2008). Benjakul *et al.* (2008) studied the effects of MTGase at different levels (0 to 0.8 units/g sample) on the properties of gels from lizardfish (*Saurida undosquamis*) mince set at 25 °C for 2 h or 40 °C for 30 min prior to heating at 90 °C for 20 min. MTGase showed the gel strengthening effect on lizardfish mince, particularly when high amounts of MTGase were used. For the gel added with MTGase at 0.8 units/g and set at 25 °C and 40 °C, the increases in breaking force were 93.1% and 90.7% were observed, respectively. Moreover, the

improved textural property was concomitant with cross-linked myosin heavy chain and tropomyosin, but not actin (Benjakul *et al.*, 2008).

MTGase showed the gel strengthening effect on surimi from red tilapia (*O. niloticus* × *O. placidus*), particularly when high amounts of MTGase were used. The addition of MTGase (2 g/kg) in red tilapia surimi gel gave the highest breaking force, in which the increase by 240% was obtained, compared to the control (Duangmal and Taluengphol, 2010). Chanarat *et al.* (2012) found that the addition of MTGase (0-0.6 units/g) in surimi from threadfin bream (*Nemipterus furcosus*), Indian mackerel (*Rastrelliger kanagurta*) and sardine (*Sardinella gibbosa*) enhanced the gel-forming ability of surimi from three fish species. Surimi gels had the decreases in expressible moisture content when MTGase was added. Gel from threadfin bream, which had the highest ε-amino group content, showed the highest gel strength, when MTGase (0.2-0.6 units/g) was added, followed by Indian mackerel and sardine, respectively. The reactivity of muscle proteins toward MTGase-induced cross-linking and MHC proportion play a major role in the gel strength of surimi or fish muscle proteins (Chanarat *et al.*, 2012). Seighalani *et al.* (2017) studied thermal and physicochemical properties of red tilapia (*Oreochromis niloticus*) surimi gel incorporated with different levels of MTGase (0.1-0.50 units/g surimi). Surimi samples mixed with various concentrations of MTGase were subjected to two-step heating (at 45 °C followed by 90 °C) to prepare surimi gel. Samples formulated with MTGase (0.30 units/g surimi) showed the highest breaking force and deformation, and lowest expressible water content among treatments. Highest storage modulus was found in the gels mixed with 0.30 MTGase (units/g surimi). Compared with control surimi gel, the addition of MTGase at levels of 0.10, 0.20 and 0.30 units/g surimi increased the enthalpy and maximum transition temperature of myosin (Seighalani *et al.*, 2017).

1.2.2.2.2 Use of divalent cations

Divalent cations, e.g. calcium, magnesium and zinc are known to alter the functionality of protein during gelation. At pH values sufficiently far from the isoelectric point of proteins, divalent cations such as Ca^{2+}, Zn^{2+}, Mg^{2+} induce protein cross-linking via the salt bridges between negatively charged carboxyl groups (Thrash *et al.*, 1991). Arfat and Benjakul (2013) reported that gel properties of surimi from

yellow stripe trevally were governed by type and concentration of zinc salts. Kamaboko gel incorporated with 60 μmol/kg $ZnSO_4$ yielded the gel with increased breaking force, deformation and whiteness. Zn^{2+} mainly induced the formation of salt bridge and ionic interaction in gel network. Furthermore, the addition of calcium salt to improve gelling properties of surimi may be due to their activation effects on TGase in the muscle. Addition of calcium salt in the surimi resulted in the increased gel strength of surimi (Benjakul *et al.*, 2004). Optimal conditions for the setting phenomenon in surimi, prepared from striped mullet (*Mugil cephalus*) by adding calcium chloride, were determined. Concentrations of calcium chloride (0-0.4%), setting temperature (25-45 °C), and time (30-90 min) were optimized to improve the shear stress and shear strain properties of fish gels. After setting under the selected conditions, all gels were cooked at 90 °C for 15 min. Shear stress was mainly influenced by the calcium concentration, while shear strain was moderately affected. Shear stress (89.6 kPa) was maximized with addition of 0.4% calcium, a temperature of 39.3 °C and a time of 1 h. Under these conditions, a shear strain of 1.47 was obtained. Thus, mechanical properties of surimi gels can be improved simply by adjusting the level of calcium to induce activity of the endogenous transglutaminase (Ramírez *et al.*, 2003). Ding *et al.* (2011) studied the effects of $CaCl_2$ on chemical interactions, textural properties and expressible moisture content of suwari and kamaboko gels from yellowcheek carp and grass carp. Suwari and kamaboko gels from yellowcheek carp exhibited higher breaking force, deformation and gel strength than these from grass carp. Surimi gels (suwari and kamaboko gels) from yellowcheek carp and grass carp had their maximum gel strength as 40 mmol/kg and 100 mmol/kg $CaCl_2$ was added, respectively. Low water holding capacity of surimi gels was found with addition of $CaCl_2$ at higher concentrations.

Furthermore, calcium from fish bone, which is mainly in the form of hydroxyapatite (HA), could use to improve the gelling properties of surimi while, achieving calcium enhancement. Properties of Alaska pollock surimi gel as affected by addition of nano-scaled fish bone (NFB) at different levels (0.1-2%) were investigated by Yin and Park (2014). Breaking force and penetration distance of surimi gels after setting increased when NFB concentration increased up to 1%. The first peak temperature and value of storage modulus (G'), indicating the unfolding and

aggregation of light meromyosin, respectively, increased as NFB concentration increased. In addition, 1% NFB treatment demonstrated the highest G' after gelation was completed. The activity of endogenous transglutaminase in Alaska pollock surimi increased with increasing NFB calcium concentration. Myosin heavy chain underwent more cross-linking as NFB concentration increased, indicating the formation of more ε-(ɣ-glutamyl)lysine covalent bond (Yin and Park, 2014). Yin *et al.* (2014) also investigated the effects of adding fish bone with two different particle sizes (micro and nano) on Alaska pollock surimi gels prepared by two heating procedures. Heating procedures (with or without setting) resulted in significantly different gel texture values. The addition of nano-scaled fish bone (NFB) (up to 1 g/100 g) increased gel breaking force and penetration distance, while no effect of micro-scaled fish bone (MFB) was noticeable. With MFB, TGase activity increased slightly, while TGase was activated with NFB. Microstructure revealed that NFB imbedded in the gel matrices without interfering myofibrillar gel network.

1.2.2.2.3 Use of phenolic compounds

Phenolic compounds can interact with side chain of amino acid group of proteins and can also affect protein functionality. Interaction of protein with phenolic compounds, especially in their oxidized form, leads to the formation of protein cross-links. This phenomenon affects functionality of modified protein, including gelatin. Phenolic compounds can interact with protein via non-covalent interactions and via covalent interactions (Prigent *et al.*, 2003). Two types of complexation mechanisms can be distinguished, monodentate and multidentate mechanisms (Haslam *et al.*, 1988). The multidentate mechanism generally requires a much lower phenolic compound/protein molar ratio and thus a lower concentration of phenolic compound is needed. For the monodentate mechanism, a phenolic compound interacts with only one protein site and a higher concentration of phenolic compound is required. In addition, the size of phenolic compound is observed to be an important parameter in protein-phenolic compound interaction (Frazier *et al.*, 2003).

Balange and Benjakul (2009b) studied the impact of various oxidized phenolic compounds (caffeic acid, OCF; catechin, OCT; tannic acid, OTA and ferulic acid, OFA) at different levels (0-0.60% of protein content) on the properties of gels from

mackerel (*Rastrelliger kanagurta*) surimi. Gels added with 0.40% OFA, 0.50% OTA, 0.50% OCF or 0.10% OCT had the increases in breaking force by 45%, 115%, 46.1% and 70.3% and in deformation by 12.2, 27.5, 28.1 and 28.4%, respectively, compared with the control (without addition of oxidized phenolics). Expressible moisture content was decreased without any change in the whiteness of resulting gels. Slightly lower band intensity of MHC in gels added with oxidized phenolics at the optimal level was found, compared with that of the control. The addition of oxidized phenolic compounds had no negative impact on the color and taste of the resulting gels. Gels with addition of all oxidized phenolics had a finer matrix with smaller strands. Moreover, the extract from coconut husk could be used as a natural additive to improve the gel properties of surimi manufactured from dark-fleshed fish. According to Buamard and Benjakul (2015), the addition of ethanolic coconut husk extract yielded the surimi gel from sardine (*Sardinella albella*) with the increased breaking force and sensory characteristics. Surimi gel added with ethanolic coconut extracts had highly interconnected network and their microstructure was finer and denser than that of the control. Thus, ethanolic coconut husk extracts at an appropriate level could improve gel strength of sardine surimi with an increased acceptability. Majumdar *et al.* (2015) evaluated the effect of garlic's aqueous extract (GAE) (0.5-2.0%) on the gel property of Pangasius (*Pangasianodon hypophthalmus*). Increase of water holding capacity in GAE added gels indicated stronger protein network formation, whereas decrease of protein solubility suggested formation of protein aggregates during gelation process. Shitole and Balange (2014) suggested that the brown seaweed (*Sargassum tenerrimum*) extract could be used as surimi gel enhancer without affecting its sensory properties. Gel of Japanese threadfin bream (*Nemipterus japonicus*) surimi added with 0.02% seaweed extract showed 30% increase in gel strength, when compared with control. Lower expressible moisture content was observed in surimi gels incorporated with 0.02% seaweed extract.

1.2.2.2.4 Use of hydrocolloids

Hydrocolloids are natural carbohydrate polymers of high molecular weight. They have been used in the food industry to obtain the required functional properties,

in which gel formation and textural improvement can be achieved. Pectin, carrageenan, curdlan, xanthan gum, locust bean gum and pullulan have been used as the gelling agent in foods. Besides the application in the manufacture of modified food, they are used to produce particles for retention of enzymes, microorganisms or aromatic substances (Mammarella and Rubiolo, 2003). Hydrocolloids are ordinarily added to myosystems in the form of an unhydrated dry powder. Addition of prehydrated or thermally-activated hydrocolloids may structurally interfere with the cross-linking required for the protein gel network formation, giving rise to gel weakening (Pérez-Mateos *et al.*, 2001).

Addition of amidated low methoxyl pectin (ALM pectin) at different levels on functionality of Mexican flounder (*Cyclopsetta chittendenii*) mince was studied. ALM pectin at 1% decreased firmness and work of extrusion of fish pastes but increased hardness, chewiness and cohesiveness of the gels. The addition of ALM pectin slightly increased the whiteness and yellowness of mince gels. Therefore ALM pectin at 1% could be employed to modify the textural properties of fish mince (Uresti *et al.*, 2003). Increased gel forming capability of Alaska pollack surimi was obtained, when carrageenan was added. This was due to the interaction of carrageenan's sulphate groups with myofibrillar proteins (Bullens *et al.*, 1990). Carrageenan has been widely used in surimi and its impact depends on the forms used. Montero *et al.* (2000) showed that heat induced gel of blue whiting surimi with 2% ι-carrageenan had small and round cavities and those with κ-carrageenan showed the large and elongated cavities. Brigham *et al.* (1994) also observed a homogenous structure of thin and thick fibers in κ-carrageenan gels, whereas thinner fibers were found in the case of ι-carrageenan. Gómez-Guillón and Montera (1996) suggested that ι-carrageenan forms a fine three-dimensional network with some points of connection with the protein matrix. Eom *et al.* (2013) also studied the influence of carrageenan addition on gelation of salt-based surimi. κ-carrageenan at 0%, 0.2%, and 1.0% and NaCl (2% w/w), KCl (1.5% w/w), and a mixture of NaCl (2% w/w) and KCl (1.5% w/w), were added to Alaska pollock surimi. The gelling property of κ-carrageenan-induced surimi gel was significantly increased by the incorporation of KCl rather than NaCl. The addition of κ-carrageenan increased the breaking force and gel strength of surimi gels. Gels with 1% κ-carrageenan and KCl had the highest breaking force and gel strength.

The addition of κ-carrageenan caused an increase in the whiteness of the surimi gels. Moreover, Hunt and Park (2013) also studied the interactions of refined ι- and κ-carrageenan (0.25-1.0%) with Alaska pollock fish proteins as affected by various salts (NaCl, KCl or $CaCl_2$, at 2%). KCl and NaCl in combination with κ- and ι-carrageenan could increase gel strength and water retention ability of fish proteins gel. Hu *et al.* (2015) studied the effects of curdlan in combination with MTGase on the gelling properties of hairtail muscle protein. When curdlan at a level of 4 g/100 g paste was combined with MTGase at a concentration of 0.4 units/g meat paste, the gel strength, water holding capacity and the whiteness of the heated gel were improved. Textural profiles, such as hardness, springiness, cohesiveness, gumminess and chewiness, were also increased. The increased band intensity of cross-linked proteins, accompanied by lower band intensity of myosin heavy chain, was observed. Thus, curdlan probably activated the formation of more ε-(γ-glutamyl)lysine cross-links induced by MTGase, especially at the level of 0.4 units/g paste, leading to a denser gel matrix.

Addition of locust bean gum in low fat meatballs resulted in the improved sensory properties (Hsu and Chung, 1999). Xanthan gum affected textural properties of low fat frankfurters (Mittal and Barbut, 1994) and low fat sausages (Solheim and Ellekjær, 1993). Locust bean gum and guar gum can be located inside large round cavities evenly and distributed throughout the protein matrix of blue whiting mince gel (Montero *et al.*, 2000). However, xanthan gum caused a decrease in the gel forming capacity of the myofibrillar protein. Ramírez *et al.* (2002) studied the effect of xanthan (X) and locust bean (LB) gums on the gelling ability of myofibrillar proteins at different levels of calcium addition. Locust bean alone negatively affected the shear stress of gels. Xanthan gum at levels of 0.75 and 1.00 had a disruptive effect on the gelling forming ability. This negative effect was partially inhibited with addition of 0.4% of calcium chloride. A profitable effect was found when a X/LB ratio of 0.25/0.75 was added. Furthermore, the addition of pullulan increased the gel properties of *Scomberomorus niphonius* surimi gel. Wu (2016) suggested that the addition of pullulan at level of 0-2% increased the water holding capacity, adhesiveness and cohesiveness and formed a more stable and ordered three-dimensional gel complex.

1.2.3 Gellan

1.2.3.1 Source and production

Gellan is an extracellular polysaccharide produced by *Sphingomonas elodea* (ATCC 31461) previously known as *Pseudomonas elodea*. The medium consists of a carbon source such as glucose, a nitrogen source and a number of inorganic salts. The fermentation is proceeded under sterile conditions with strict control of aeration, agitation, temperature and pH. After fermentation, the viscous broth is pasteurized to kill the viable cells. (Gibson and Sanderson, 1997). The polysaccharide is recovered in several ways. Alcohol precipitation yields the substituted native, or high acyl (HA), form. Alternatively, treatment of the broth with alkali prior to alcohol precipitation results in deacylation and yields the unsubstituted, or low acyl (LA) form (Sworn, 2000).

1.2.3.2 Structure

Gellan consists of repeat units of β-D-glucose (DGlc), β-D-glucuronic acid (D-GlcA) and α-L-rhamnose (L-Rha) in a molar ratio of 2:1:1 with L-glyceric ester at O-2 of the 3-linked D-glucose and acetic esters at the O-6 of the same residue (Figure 3) (Bajaj *et al.*, 2006; Sanderson, 1990). Gellan, a linear anionic heteropolysaccharide, forms a double helix in aqueous solution, yielding high viscosity. At high temperatures (>90 °C), gellan dispersed in water is in a disordered random coil state. Upon cooling to gelling temperature, gellan forms double helices which aggregate to form junction zones, and finally forms three-dimensional gel networks with monovalent or divalent cations (Chandrasekaran and Radha, 1995; Sanderson and Clark, 1983)

1.2.3.3 Gelation of gellan

During gelation, gellan undergoes a disordered (i.e. random coil conformation) to ordered transition. When hot solutions are cooled, double helix structures are formed, primarily through intramolecular interactions (Nickerson *et al.*, 2008) The three-dimensional network is developed by the aggregation of these structures, due to attractive forces and cooperative interactions, in the presence of cations (Grasdalen and Smidsrød, 1987). Cations are screening the carboxyl groups along the gellan backbone, thus reducing charge repulsion. It should be noted that low

acyl gellan is produced as mixed salt, predominantly in the potassium form but also containing divalent ions such as calcium. Weak gels can be formed even without other cation (Sanderson, 1990). The mechanical properties of the resulting gels depend on the concentration of the polymer along with the concentration and the valency of the cations (Grasdalen and Smidsrød, 1987; Sanderson, 1990). Divalent cations are more effective in promoting gelation than monovalent. Monovalent cations (e.g. K^+) promote the aggregation process by site-binding to the helices, thus suppressing the electrostatic repulsion between them (Morris *et al.*, 1996; Robinson and Manning, 1991), Divalent cations (e.g. Ca^{2+}) act by site-binding between pairs of carboxyl groups on neighboring helices (Morris *et al.*, 1996), to give structures analogous to the 'eggbox' junctions (Grant *et al.*, 1973). The latter are much stronger, explaining why divalent cations are more effective in promoting gelation.

Basically, gellan molecules undergo a coil to double-helix transition with decreasing temperature, which may lead to gel formation, depending on the ionic strength and pH of the solution. The gelation process consists of two steps (Milas and Rinaudo, 1996). Firstly the gellan coil molecules form double helices with the reduction in temperature, and secondly, these helices aggregate and form the junction zones, resulting in gelation (Yuguchi *et al.*, 1997). In water, at low ionic strength and neutral pH, aggregation of the helices is impeded by the electrostatic repulsion between negatively charged carboxylic groups on the gellan. The addition of salt or reduction in pH decreases intermolecular repulsion between the helices enhancing junction zone formation associated with gel strength (Noda *et al.*, 2008; Yamamoto and Cunha, 2007). The role of salt ions in gel formation has been widely studied (Fukada *et al.*, 2002; Milas and Rinaudo, 1996; Miyoshi *et al.*, 1996; Moritaka *et al.*, 1992; Moritaka *et al.*, 1995; Noda *et al.*, 2008). In the presence of salt ions, the gelling and melting temperatures of gellan gels shift to higher temperatures and the number of junction zones is enhanced. This makes the gel more heat resistant and leads to an increase in the gel elastic modulus and rigidity (Picone and Cunha, 2011).

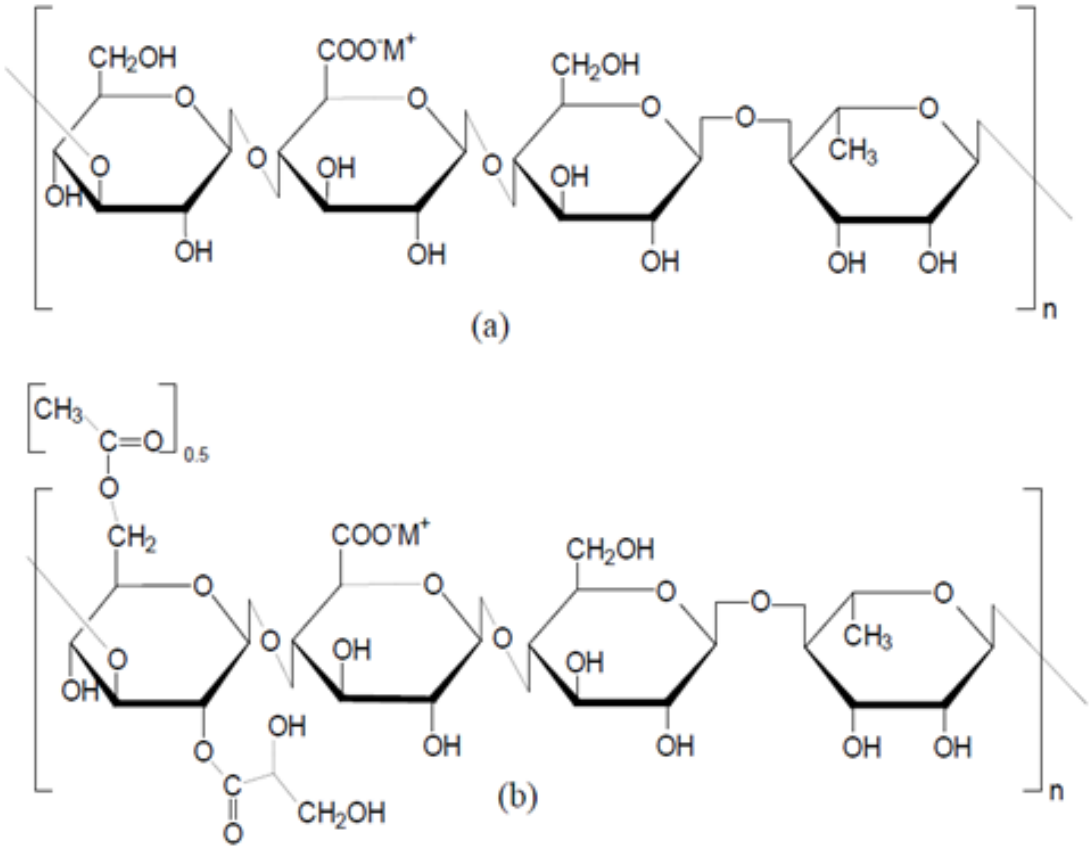

Figure 3 Chemical structure of (a) low and (b) high acyl gellan.
Source: Sworn (2000)

1.2.3.4 Application of gellan in protein gels

Gellan is used in a variety of foods including water-based gels, confectionery, jams and marmalades, pie fillings and puddings, fabricated foods and dairy products (Sanderson and Clark, 1983)Addition of gellan into protein gels such as gelatin and myofibrillar proteins was studied. Wolf *et al.* (1989) suggested that incorporation of small amounts of gellan into porcine gelatin could greatly improve properties of gelatin gel. Gelation temperature and gelation rate of the mixed gels were significantly affected by the ratio of gellan to bovine gelatin (Lau *et al.*, 2001). Addition of gellan into bovine gelatin gel could increase the hardness of the mixed gels (Lau *et al.*, 2000). The mixture of gellan and gelatin could be useful for a wide

range of applications such as processed foods (i.e. water/milk based dessert gels, dessert syrup/topping, and pet foods (Morris *et al.*, 1996). Gellan was used to improve mechanical properties of fish gelatin based film by increasing tensile strength and barrier against water vapor (Pranoto *et al.*, 2007).

Zhou *et al.* (2008) studied the effect of fat pork as well as carrageenan and gellan on properties of silver carp surimi-protein soybean composite product. The addition of carrageenan or gellan improved the properties of silver carp surimi-protein soybean composite product added with fat pork by enhancing the gel strength, decreasing the water loss rate and increasing the folding performance. Moreover, gellan was used to improve quality of reduced-fat frankfurters. Mixed gels of konjac (1% and 2%) and gellan (0.25% and 0.5%) were incorporated into reduced fat (18%) frankfurters and compared with reduced-fat controls (without konjac and gellan addition), it appeared that konjac/gellan mixed gel at current levels yielded the reduced-fat frankfurter with acceptable sensory merits with reasonable shelf (Lin and Huang, 2003).

1.2.3.5 Regulatory status of gellan

In Japan, gellan has been considered as a `natural' food additive since 1988. It is now approved for food use in the USA and the European Union as well as Canada, South Africa, Australia, most of South East Asia and Latin America. Gellan appears as E418 in the European Community Directive EC/95/2 in Annex 1. Both the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the European Community Scientific Committee for Food has given gellan an Acceptable Daily Intake (ADI) of `not specified'. Combinations of HA and LA gellan have one name. A manufacturer may label a product made with a combination of both types of gellan simply, `E418' or `gellan gum' (Sworn, 2000).

1.2.4 Ultrasound

1.2.4.1 Principle of ultrasound technology

Ultrasonic waves are mechanical waves at frequencies between 20 kHz and 100 MHz (Tao and Sun, 2015). An acoustic wave is a propagation of pressure oscillation in a given medium (gas, liquid or solid), with the velocity of sound producing both the rarefaction and compression phases (Nieves-Soto *et al.*,

2012). Sound waves are often disclosed as a series of vertical lines or shaded colors, where line separation or color depth represent the intensity or amplitude of the sin wave; the pitch of the sound depends upon the frequency of the wave (Figure 4). From the sound spectrum, an ultrasonic wave is an acoustic wave with frequency above 20 kHz. It is not audible to human. When a liquid is irradiated by a strong ultrasonic wave, the pressure at some regions in the liquid becomes negative (expansion) because the acoustic amplitude of the wave is larger than the ambient pressure. When the pressure wave propagates through a liquid and has enough intensity, vapor bubbles may form because the gas dissolved in the liquid can no longer be dissolved. In general, the gas solubility is proportional to the pressure is known as the cavitation phenomenon (Yasui *et al.*, 2004). Cavitation bubble collapse is a remarkable phenomenon induced throughout the liquid by the power of sound. In aqueous systems, at an ultrasonic frequency of 20 kHz, each cavitation bubble collapse acts as a localized "hotspot". Bubble collapse induced by cavitation produces the intense local heating and high pressures. These hot spots have temperatures about 4,000 K, pressures in excess of 1000 atmospheres and heating and cooling rates are greater than 10^9 K/s (Suslick, 1990).

1.2.4.2 The use of ultrasound for polysaccharide dispersion

Ultrasound, one of the most efficient and powerful techniques among various equipment used for polysaccharide dispersion, can disperse various polysaccharides with a wide range of particle sizes in dilute solution (Koda *et al.*, 2011; Taghizadeh and Asadpour, 2009; Xiaodong *et al.*, 1998). Moreover, ultrasound is widely used to modify their physical and chemical properties such as the particle size, molecular weight, viscosity, etc. (Bang and Suslick, 2007; Boldyrev, 1995; Camino *et al.*, 2009). Application of high intensity ultrasound for dispersion of carbohydrates can lead to depolymerization due to the intense mechanical and chemical effects associated with cavitation (Tiwari *et al.*, 2010). The use of high intensity ultrasonication, with a frequency of 20 kHz for different water-soluble polymers such as carboxymethylcellulose, pectin, guar, xanthan, pullulan, carrageenan and gellan etc., have been studied over the last several decades. Grönroos *et al.* (2004) reported that

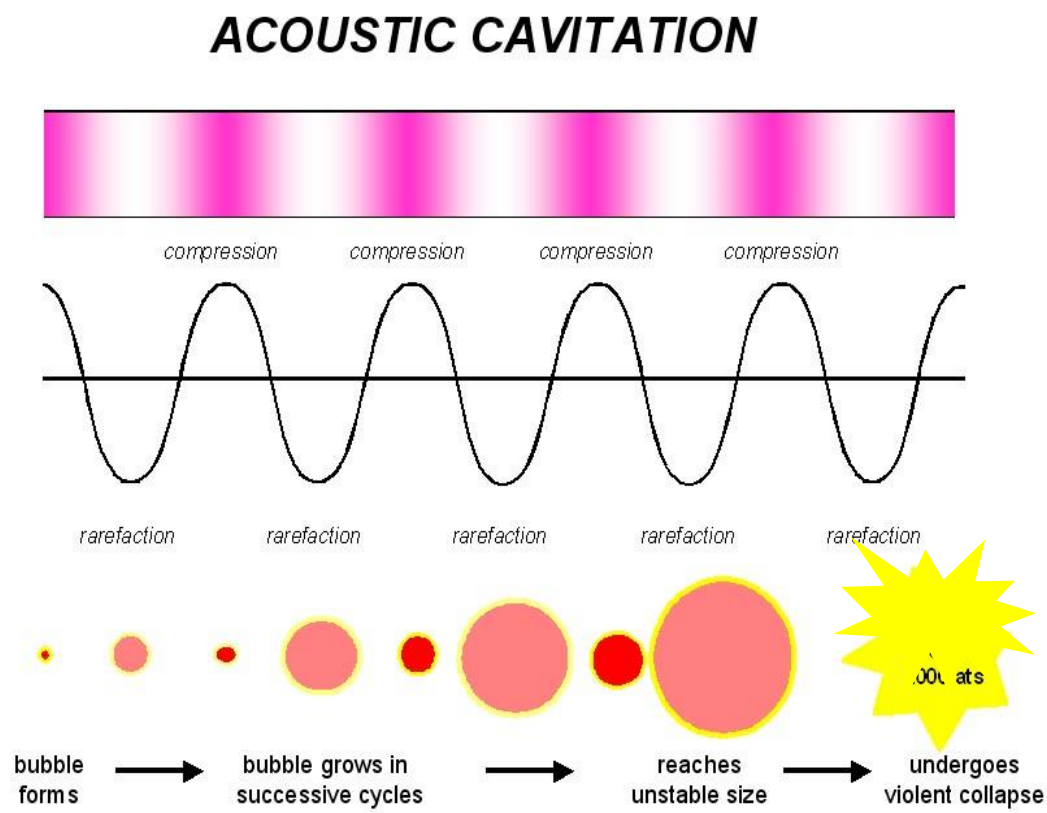

Figure 4 Acoustic cavitation.
Source: Nieves-Soto *et al.* (2012).

ultrasound degraded preferentially large carboxymethylcellulose (CMC) molecules and the cleavage took place roughly at the center of the CMC molecules, mainly due to the disruption of covalent bonds in the polymer chains. Ultrasonication treatment caused a reduction in the weight-average molecular weight of high methoxyl pectin (Seshadri *et al.*, 2003). Goh *et al.* (2015) reported that the molar mass of 1% gellan was decreased from 9.7×10^5 to 2.8×10^3 g/mol as ultrasonication time increased from 0 to 40 min (50% cycle). Wang *et al.* (2010) applied high-power ultrasound (20 kHz) to modify the physicochemical properties of a high-molecular weight (MW) exopolysaccharide (EPS) from mycelial culture of a medicinal fungus. At 35 W/cm^2 or higher ultrasound power, the apparent and intrinsic viscosities of EPS solution was decreased by 85% within 10 min, and the water solubility was increased by more than

fourfold. The ultrasonic treatment caused the marked reduction of the maximum MW and a more uniform MW distribution. Nevertheless, no significant change in the primary structure of the EPS molecules was found (Wang *et al.*, 2010). Furthermore, the influence of high intensity ultrasound on the rheological characteristics of guar, xanthan, and pectin dispersions was investigated. Guar 1%, xanthan 1% and pectin 2% (w/v) dispersions were sonicated at varying intensity levels (3.7, 6.3, 8.1, and 10.1 W/cm^2) for 5 min at 25 $^{\circ}C$. Significant differences were observed in the rheological characteristics of each sonicated hydrocolloid dispersion studied. No recovery was observed in the structure breakdown after storage for 24 h. Changes in the rheological properties of guar and pectin dispersions were significantly higher than that of xanthan dispersions (Tiwari *et al.*, 2010). Polymer degradation is attributed to cavitation. The rapid formation and collapse of bubbles within the liquids, generates intense stress, thus causing irreversible chain scission (Lorimer *et al.*, 1995; Tayal and Khan, 2000).

Ultrasonication has been known to affect the functionality of polysaccharides. Azizi and Farahnaky (2013) suggested that power ultrasound processing could be used to induce gelation of κ-carrageenan dispersions without using heat. As sonication time increased, gel hardness increased up to a certain level (in absence or presence of K^+ during ultrasound treatment). Further ultrasound application showed a negative effect. Ultrasound waves cause the disruption of carrageenan macromolecules. Thus allowed helix to helix associations within strands to be replaced by new associations between strands, leading to the formation of a continuous network. Moreover, the mechanical properties of the gels, in which K^+ ions were added before ultrasound were weaker than the samples when K^+ ions were added after sonication (Azizi and Farahnaky, 2013). Same phenomenon was found in κ-carrageenan gels prepared using ultrasound in the presence or absence of Na^+ from NaCl (Farahnaky *et al.*, 2013). On the other hand, the decrease in gel properties of gellan treated with ultrasound treatment was reported by Goh *et al.* (2015). Low acylated (LA) gellan (1% w/w) was modified by ultrasonicating at 50 °C (above the sol-gel transition temperature) for different durations (5, 10, 20, 30 and 40 min). Hardness and springiness of gellan gel decreased almost linearly with ultrasonication time but the gel cohesiveness and gumminess were drastically decreased, only after 5

min of ultrasonication. The reduction in gel strength was attributed to the reduced chain length of the gellan molecules and the gel network may include dangling chains, which do not contribute to elasticity (Goh *et al.*, 2015).

1.2.5 References

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

Ashie, I. N. and Lanier, T. C. 2000. Transglutaminases in seafood processing. *In* Food science and technology. p. 147-166. Marcel Dekker. New York, USA.

Avena-Bustillos, R. J., Olsen, C. W., Olson, D. A., Chiou, B., Yee, E., Bechtel, P. J. and McHugh, T. H. 2006. Water vapor permeability of mammalian and fish gelatin films. Journal of Food Science. 71: 202-207.

Azizi, R. and Farahnaky, A. 2013. Ultrasound assisted cold gelation of kappa carrageenan dispersions. Carbohydrate Polymers. 95: 522-529.

Babin, H. and Dickinson, E. 2001. Influence of transglutaminase treatment on the thermoreversible gelation of gelatin. Food Hydrocolloids. 15: 271-276.

Bajaj, I. B., Saudagar, P. S., Singhal, R. S. and Pandey, A. 2006. Statistical approach to optimization of fermentative production of gellan gum from *Sphingomonas paucimobilis* ATCC 31461. Journal of Bioscience and Bioengineering. 102: 150-156.

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

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

Bang, J. H. and Suslick, K. S. 2007. Sonochemical synthesis of nanosized hollow hematite. Journal of the American Chemical Society. 129: 2242-2243.

Bang, S. H., Son, J. R., Lee, S. Y. and Park, H. J. 2014. Preparation and characterization of composite gels and films containing gelatin and

hydroxypropyl methylcellulose phthalate. Journal of Applied Polymer Science. 131: doi: 10.1002/app.39597.

Barrera, A. M., Ramírez, J. A., González-Cabriales, J. J. and Vázquez, M. 2002. Effect of pectins on the gelling properties of surimi from silver carp. Food Hydrocolloids. 16: 441-447.

Benjakul, S., Kittiphattanabawon, P. and Regenstein, J. M. 2012. Fish gelatin. *In* Food Biochemistry and Food Processing. (Simpson, K. B., ed.). p. 388-405. John Wiley & Sons Inc. LA, USA.

Benjakul, S., Phatcharat, S., Tammatinna, A., Visessanguan, W. and Kishimura, H. 2008. Improvement of gelling properties of lizardfish mince as influenced by microbial transglutaminase and fish freshness. Journal of Food Science. 73: 239-246.

Benjakul, S. and Visessanguan, W. 2003. Transglutaminase-mediated setting in bigeye snapper Surimi. Food Research International. 36: 253-266.

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

Benjakul, S., Visessanguan, W. and Srivilai, C. 2001. Gel properties of bigeye snapper (*Priacanthus tayenus*) surimi as affected by setting and porcine plasma proteins. Journal of Food Quality. 24: 453-471.

Boldyrev, V. V. 1995. Mechanochemistry and sonochemistry. Ultrasonics Sonochemistry. 2: 143-145.

Brigham, J. E., Gidley, M. J., Hoffmann, R. A. and Smith, C. G. 1994. Microscopic imaging of network strands in agar, carrageenan, locust bean gum and kappa carrageenan/locust bean gum gels. Food Hydrocolloids. 8: 331-344.

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

Budavari, S. 1996. The mack index. An encyclopedia of chemicals, drugs, and biologicals. 12th Ed. Merck & Co., Inc. Whitehouse Station, Germany.

Bullens, C. W., Llanto, M. G., Lee, C. M. and Modliszewski, J. J. 1990. The function o carrageonanbased stabilizers to improve quality in fabricated seafood

products. *In* Advances in Fisheries Technology and Biotechnology for Increased Profitability. p. 313-323. Technomic Pub. Co., Inc. Lancaster, PA.

Camino, N. A., Pérez, O. E. and Pilosof, A. M. R. 2009. Molecular and functional modification of hydroxypropylmethylcellulose by high-intensity ultrasound. Food Hydrocolloids. 23: 1089-1095.

Chan, J. K., Gill, T. A. and Paulson, A. T. 1993. Thermal aggregation of myosin subfragments from cod and herring. Journal of Food Science. 58: 1057-1061.

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

Chandrasekaran, R. and Radha, A. 1995. Molecular architectures and functional properties of gellan gum and related polysaccharides. Trends in Food Science & Technology. 6: 143-148.

Cheow, C. S., Norizah, M. S., Kyaw, Z. Y. and Howell, N. K. 2007. Preparation and characterisation of gelatins from the skins of sin croaker (*Johnius dussumieri*) and shortfin scad (*Decapterus macrosoma*). Food Chemistry. 101: 386-391.

Cho, S. M., Gu, Y. S. and Kim, S. B. 2005. Extracting optimization and physical properties of yellowfin tuna (*Thunnus albacares*) skin gelatin compared to mammalian gelatins. Food Hydrocolloids. 19: 221-229.

de Kruif, C. G., Weinbreck, F. and de Vries, R. 2004. Complex coacervation of proteins and anionic polysaccharides. Current Opinion in Colloid & Interface Science. 9: 340-349.

Ding, Y., Liu, Y., Yang, H., Liu, R., Rong, J., Zhao, S. and Xiong, S. 2011. Effects of $CaCl_2$ on chemical interactions and gel properties of surimi gels from two species of carps. European Food Research and Technology. 233: 569.

Duangmal, K. and Taluengphol, A. 2010. Effect of protein additives, sodium ascorbate, and microbial transglutaminase on the texture and colour of red tilapia surimi gel. International Journal of Food Science & Technology. 45: 48-55.

Eastoe, J. E. and Leach, A. A. 1977. Chemical constitution of gelatin. *In* The science and technology of gelatin. (Ward, A. G. and Courts, A., eds.). p. 73-107. New York. Academic Press.

Eom, S.-H., Kim, J., Son, B.-Y., You, D. H., Han, J. M., Oh, J.-H., Kim, B.-Y. and Kong, C.-S. 2013. Effects of carrageenan on the gelatinization of salt-based surimi gels. Fisheries and aquatic sciences. 16: 143-147.

Eymard, S., Carcouët, E., Rochet, M. J., Dumay, J., Chopin, C. and Genot, C. 2005. Development of lipid oxidation during manufacturing of horse mackerel surimi. Journal of the Science of Food and Agriculture. 85: 1750-1756.

Farahnaky, A., Azizi, R., Majzoobi, M., Mesbahi, G. and Maftoonazad, N. 2013. Using power ultrasound for cold gelation of kappa-carrageenan in presence of sodium ions. Innovative Food Science & Emerging Technologies. 20: 173-181.

Frazier, R. A., Papadopoulou, A., Mueller-Harvey, I., Kissoon, D. and Green, R. J. 2003. Probing protein-tannin interactions by isothermal titration microcalorimetry. Journal of Agricultural and Food Chemistry. 51: 5189-5195.

Fukada, H., Takahashi, K., Kitamura, S., Yuguchi, Y., Urakawa, H. and Kajiwara, K. 2002. Thermodynamics and structural aspect of the gelling process in the gellan gum/metal salt aqueous solutions. Journal of Thermal Analysis and Calorimetry. 70: 797-806.

Gibson, W. and Sanderson, G. R. 1997. Gellan gum. *In* Thickening and Gelling Agents for Food. (Imeson, A. P., ed.). p. 119-143. Springer US. Boston, MA.

Gilsenan, P. M. and Ross-Murphy, S. B. 2000. Rheological characterisation of gelatins from mammalian and marine sources. Food Hydrocolloids. 14: 191-195.

Goh, K. K. T., Yuliarti, O., Yeo, G. T. T. and Or, C. C. 2015. Effect of ultrasonication on low-acetylated gellan gum gel properties. Food Hydrocolloids. 49: 240-247.

Gómez-Guillén, M. C., Turnay, J., Fernández-Díaz, M. D., Ulmo, N., Lizarbe, M. A. and Montero, P. 2002. Structural and physical properties of gelatin extracted

from different marine species: a comparative study. Food Hydrocolloids. 16: 25-34.

Gómez-Guillón, M. C. and Montera, P. 1996. Addition of hydrocolloids and non-muscle proteins to sardine (*Sardina pilchardus*) mince gels. Food Chemistry. 56: 421-427.

Grant, G. T., Morris, E. R., Rees, D. A., Smith, P. J. C. and Thom, D. 1973. Biological interactions between polysaccharides and divalent cations: The egg-box model. FEBS Letters. 32: 195-198.

Grasdalen, H. and Smidsrød, O. 1987. Gelation of gellan gum. Carbohydrate Polymers. 7: 371-393.

Grönroos, A., Pirkonen, P. and Ruppert, O. 2004. Ultrasonic depolymerization of aqueous carboxymethylcellulose. Ultrasonics Sonochemistry. 11: 9-12.

Guo, M., Liu, S., Ismail, M., Farid, M. M., Ji, H., Mao, W., Gao, J. and Li, C. 2017. Changes in the myosin secondary structure and shrimp surimi gel strength induced by dense phase carbon dioxide. Food Chemistry. 227: 219-226.

Haslam, E., Lilley, T. H. and Butler, L. G. 1988. Natural astringency in foodstuffs - A molecular interpretation. Critical Reviews in Food Science and Nutrition. 27: 1-40.

Haug, I. J., Draget, K. I. and Smidsrød, O. 2004. Physical behaviour of fish gelatin-κ-carrageenan mixtures. Carbohydrate Polymers. 56: 11-19.

Hollman, P. C. H. and Arts, I. C. W. 2000. Flavonols, flavones and flavanols – nature, occurrence and dietary burden. Journal of the Science of Food and Agriculture. 80: 1081-1093.

Hsu, S. Y. and Chung, H. Y. 1999. Comparisons of 13 edible gum-hydrate fat substitutes for low fat Kung-wan (an emulsified meatball). Journal of Food Engineering. 40: 279-285.

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

Huang, T., Tu, Z. C., Wang, H., Shangguan, X., Zhang, L., Zhang, N. H. and Bansal, N. 2017. Pectin and enzyme complex modified fish scales gelatin: Rheological

behavior, gel properties and nanostructure. Carbohydrate Polymers. 156: 294-302.

Hudson, C. B. 1993. Gelatine - relating structure and chemistry to functionality. *In* Food hydrocolloids: structures, properties, and functions. (Nishinari, K. and Doi, E., eds.). p. 347-354. Springer. Boston, MA.

Hunt, A. and Park, J. W. 2013. Alaska pollock fish protein gels as affected by refined carrageenan and various salts. Journal of Food Quality. 36: 51-58.

Itoh, Y., Yoshinaka, R. and Ikeda, S. 1979. Effects of sulfhydryl reagents on gel formation of carp actomyosin by heating. Bulletin of the Japanese Society of Scientific Fisheries. 45: 1023-1025.

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

Johnston-Banks, F. A. 1990. Gelatin. *In* Food gels. (Harris, P., ed.). p. 233-289. Elsevier Science Publishing Co. New York.

Jongjareonrak, A., Benjakul, S., Visessanguan, W. and Tanaka, M. 2006. Skin gelatin from bigeye snapper and brownstripe red snapper: Chemical compositions and effect of microbial transglutaminase on gel properties. Food Hydrocolloids. 20: 1216-1222.

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

Karayannakidis, P. D., Zotos, A., Petridis, D. and Taylor, K. D. A. 2008. The effect of washing, microbial transglutaminase, salts and starch addition on the functional properties of sardine (*Sardina Pilchardus*) kamaboko gels. Food Science and Technology International. 14: 167-177.

Karim, A. A. and Bhat, R. 2009. Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins. Food Hydrocolloids. 23: 563-576.

Keenan, T. R. 2000. Gelatin. *In* Kirk-othmer encyclopedia of chemical technology. John Wiley & Sons, Inc.

Kittiphattanabawon, P., Benjakul, S., Sinthusamran, S. and Kishimura, H. 2016. Gelatin from clown featherback skin: Extraction conditions. LWT - Food Science and Technology. 66: 186-192.

Koda, S., Taguchi, K. and Futamura, K. 2011. Effects of frequency and a radical scavenger on ultrasonic degradation of water-soluble polymers. Ultrasonics Sonochemistry. 18: 276-281.

Lanier, T. C., Carvajal, P. and Yongsawatdigul, J. 2000. Surimi gelation chemistry. *In* Surimi and surimi seafood. Marcel Dekker. New York, USA.

Lanier, T. C., Lin, T. S., Liu, Y. M. and Hamann, D. D. 1982. Heat gelation properties of actomyosin and surimi prepared from Atlantic croaker. Journal of Food Science. 47: 1921-1925.

Lau, M. H., Tang, J. and Paulson, A. T. 2000. Texture profile and turbidity of gellan/gelatin mixed gels. Food Research International. 33: 665-671.

Lau, M. H., Tang, J. and Paulson, A. T. 2001. Effect of polymer ratio and calcium concentration on gelation properties of gellan/gelatin mixed gels. Food Research International. 34: 879-886.

Lee, K. Y., Shim, J., Bae, I. Y., Cha, J., Park, C. S. and Lee, H. G. 2003. Characterization of gellan/gelatin mixed solutions and gels. LWT - Food Science and Technology. 36: 795-802.

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

Leuenberger, B. H. 1991. Investigation of viscosity and gelation properties of different mammalian and fish gelatins. Food Hydrocolloids. 5: 353-361.

Lin, K.-W. and Huang, H.-Y. 2003. Konjac/gellan gum mixed gels improve the quality of reduced-fat frankfurters. Meat Science. 65: 749-755.

Liu, H., Li, D. and Guo, S. 2008. Rheological properties of channel catfish (*Ictalurus punctaus*) gelatine from fish skins preserved by different methods. LWT - Food Science and Technology. 41: 1425-1430.

Liu, H., Zhu, D. S., Xu, X. M., Guo, S. D. and Jin, Z. Y. 2007. Rheological, textural and microstructure analysis of the high-methoxy pectin/gelatin mixed systems. Journal of Texture Studies. 38: 577-600.

Lorimer, J. P., Mason, T. J., Cuthbert, T. C. and Brookfield, E. A. 1995. Effect of ultrasound on the degradation of aqueous native dextran. Ultrasonics Sonochemistry. 2: 55-57.

Majumdar, R. K., Saha, A., Dhar, B., Maurya, P. K., Roy, D., Shitole, S. and Balange, A. K. 2015. Effect of garlic extract on physical, oxidative and microbial changes during refrigerated storage of restructured product from Thai pangas (*Pangasianodon hypophthalmus*) surimi. Journal of Food Science and Technology. 52: 7994-8003.

Mammarella, E. J. and Rubiolo, A. C. 2003. Crosslinking kinetics of cation-hydrocolloid gels. Chemical Engineering Journal. 94: 73-77.

Matricardi, P., Cencetti, C., Ria, R., Alhaique, F. and Coviello, T. 2009. Preparation and characterization of novel gellan gum hydrogels suitable for modified drug release. Molecules. 14: 3376-3391.

Milas, M. and Rinaudo, M. 1996. The gellan sol-gel transition. Carbohydrate Polymers. 30: 177-184.

Mittal, G. S. and Barbut, S. 1994. Effects of fat reduction on frankfurters' physical and sensory characteristics. Food Research International. 27: 425-431.

Miyoshi, E., Takaya, T. and Nishinari, K. 1996. Rheological and thermal studies of gel-sol transition in gellan gum aqueous solutions. Carbohydrate Polymers. 30: 109-119.

Mohtar, N. F., Perera, C. O., Quek, S.-Y. and Hemar, Y. 2013. Optimization of gelatine gel preparation from New Zealand hoki (*Macruronus novaezelandiae*) skins and the effect of transglutaminase enzyme on the gel properties. Food Hydrocolloids. 31: 204-209.

Montero, P., Hurtado, J. L. and Pérez-Mateos, M. 2000. Microstructural behaviour and gelling characteristics of myosystem protein gels interacting with hydrocolloids. Food Hydrocolloids. 14: 455-461.

Moritaka, H., Nishinari, K., Nakahama, N. and Fukuba, H. 1992. Effects of potassium chloride and sodium chloride on the thermal properties of gellan gum gels. Bioscience, Biotechnology, and Biochemistry. 56: 595-599.

Moritaka, H., Nishinari, K., Taki, M. and Fukuba, H. 1995. Effects of pH, potassium chloride, and sodium chloride on the thermal and rheological properties of gellan gum gels. Journal of Agricultural and Food Chemistry. 43: 1685-1689.

Morris, E. R., Gothard, M. G. E., Hember, M. W. N., Manning, C. E. and Robinson, G. 1996. Conformational and rheological transitions of welan, rhamsan and acylated gellan. Carbohydrate Polymers. 30: 165-175.

Moskowitz, R. W. 2000. Role of collagen hydrolysate in bone and joint disease. Seminars in Arthritis and Rheumatism. 30: 87-99.

Motoki, M. and Seguro, K. 1998. Transglutaminase and its use for food processing. Trends in Food Science & Technology. 9: 204-210.

Nalinanon, S., Benjakul, S., Visessanguan, W. and Kishimura, H. 2008. Improvement of gelatin extraction from bigeye snapper skin using pepsin-aided process in combination with protease inhibitor. Food Hydrocolloids. 22: 615-622.

Nickerson, M. T., Paulson, A. T. and Hallett, F. R. 2008. Pre-gel solution properties of gellan polysaccharides: Effect of potassium and calcium ions on chain associations. Food Research International. 41: 462-471.

Nieves-Soto, M., Hernández-Calderón, O. M., Guerrero-Fajardo, C. A., Sánchez-Castillo, M. A., Viveros-García, T. and Contreras-Andrade, I. 2012. Biodiesel current technology: Ultrasonic process a realistic industrial application. *In* Biodiesel - feedstocks, production and applications. (Fang, Z., ed.). InTech. Rijeka, Croatia.

Niwa, E., Nakayama, T. and Hamada, I. 1981. Effect of arylation for setting of muscle proteins. Agricultural and Biological Chemistry. 45: 341-344.

Niwa, E., Ueno, S. and Kanoh, S. 1992. Mechanism for the gelation of unheated surimi by vinegar curing. Bioscience, biotechnology, and biochemistry. 56: 58-61.

Noda, S., Funami, T., Nakauma, M., Asai, I., Takahashi, R., Al-Assaf, S., Ikeda, S., Nishinari, K. and Phillips, G. O. 2008. Molecular structures of gellan gum imaged with atomic force microscopy in relation to the rheological behavior in

aqueous systems. 1. Gellan gum with various acyl contents in the presence and absence of potassium. Food Hydrocolloids. 22: 1148-1159.

Norland, R. E. 1990. Fish gelatin. Advances in fisheries technology and biotechnology for increased profitability. 325-333.

Normand, V., Muller, S., Ravey, J.-C. and Parker, A. 2000. Gelation kinetics of gelatin: A master curve and network modeling. Macromolecules. 33: 1063-1071.

Norziah, M. H., Al-Hassan, A., Khairulnizam, A. B., Mordi, M. N. and Norita, M. 2009. Characterization of fish gelatin from surimi processing wastes: Thermal analysis and effect of transglutaminase on gel properties. Food Hydrocolloids. 23: 1610-1616.

Okada, M. 1992. History of surimi technology in Japan. Surimi Technology.

Park, J. W. 2013. Surimi seafood. *In* Surimi and surimi seafood. 3rd Ed. (Park, J. W., ed.). p. 375-433. CRC Press. Florida.

Park, J. W. and Lanier, T. C. 1989. Scanning calorimetric behavior of tilapia myosin and actin due to processing of muscle and protein purification. Journal of Food Science. 54: 49-51.

Pérez-Mateos, M., Hurtado, J. L., Montero, P. and Fernández-Martín, F. 2001. Interactions of κ-carrageenan plus other hydrocolloids in fish myosystem gels. Journal of Food Science. 66: 838-843.

Picone, C. S. F. and Cunha, R. L. 2011. Influence of pH on formation and properties of gellan gels. Carbohydrate Polymers. 84: 662-668.

Piculell, L. 1995. Gelling carrageenans. *In* Food science and technology. p. 205-205. Marcel Dekker. New York, USA.

Poppe, J. 1997. Gelatin. *In* Thickening and gelling agents for food. (Imeson, A. P., ed.). p. 144-168. Springer. Boston, MA.

Pranoto, Y., Lee, C. M. and Park, H. J. 2007. Characterizations of fish gelatin films added with gellan and κ-carrageenan. LWT - Food Science and Technology. 40: 766-774.

Prigent, S. V. E. 2005. Interactions of phenolic compounds with globular proteins and their effects on food-related functional properties.

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

Ramírez, J. A., Barrera, M., Morales, O. G. and Vázquez, M. 2002. Effect of xanthan and locust bean gums on the gelling properties of myofibrillar protein. Food Hydrocolloids. 16: 11-16.

Ramírez, J. A., Rodríguez-Sosa, R., Morales, O. G. and Vázquez, M. 2000. Surimi gels from striped mullet (*Mugil cephalus*) employing microbial transglutaminase. Food Chemistry. 70: 443-449.

Ramírez, J. A., Rodríguez-Sosa, R., Morales, O. G. and Vázquez, M. 2003. Preparation of surimi gels from striped mullet (*Mugil cephalus*) using an optimal level of calcium chloride. Food Chemistry. 82: 417-423.

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

Rawdkuen, S., Benjakul, S., Visessanguan, W. and Lanier, T. C. 2007. Effect of chicken plasma protein additives on proteolysis and gel-forming ability of sardine (*Sardinella gibbosa*) surimi. Journal of Food Processing and Preservation. 31: 492-516.

Rawel, H. M., Kroll, J. and Kulling, S. 2007. Effect of non-protein components on the degradability of proteins. Biotechnology Advances. 25: 611-613.

Regenstein, J. M. and Zhou, P. 2007. Collagen and gelatin from marine by-products. *In* Maximising the value of marine by-products. (Shahidi, F., ed.). p. 279-303. Woodhead Publishing Limited. Cambridge, UK.

Robards, K., Prenzler, P. D., Tucker, G., Swatsitang, P. and Glover, W. 1999. Phenolic compounds and their role in oxidative processes in fruits. Food Chemistry. 66: 401-436.

Robinson, G. and Manning, C. E. 1991. Conformation and physical properties of the bacterial polysaccharides gellan, welan, and rhamsan. Food polymers, gels and colloids. 22-33.

Sakamoto, H., Kumazawa, Y., Toiguchi, S., Seguro, K., Soeda, T. and Motoki, M. 1995. Gel strength enhancement by addition of microbial transglutaminase during on shore surimi manufacture. Journal of Food Science. 60: 300-304.

Samejima, K., Ishioroshi, M. and Yasui, T. 1981. Relative roles of the head and tail portions of the molecule in heat-induced gelation of myosin. Journal of Food Science. 46: 1412-1418.

Sanderson, G. R. 1990. Gellan gum. *In* Food gels. (Harris, P., ed.). p. 201-232. Springer. Dordrecht, Netherlands.

Sanderson, G. R. and Clark, R. C. 1983. Gellan gum. Food Technology. 37: 62-70.

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

Sarabia, A. I., Gómez-Guillén, M. C. and Montero, P. 2000. The effect of added salts on the viscoelastic properties of fish skin gelatin. Food Chemistry. 70: 71-76.

Schrieber, R. and Gareis, H. 2007. From collagen to gelatine. *In* Gelatine Handbook. p. 45-117. Wiley-VCH Verlag GmbH & Co. KGaA. Weinheim, Germany.

Seighalani, F. Z. B., Bakar, J., Saari, N. and Khoddami, A. 2017. Thermal and physicochemical properties of red tilapia (*Oreochromis niloticus*) surimi gel as affected by microbial transglutaminase. Animal Production Science. 57: 993-1000.

Seshadri, R., Weiss, J., Hulbert, G. J. and Mount, J. 2003. Ultrasonic processing influences rheological and optical properties of high-methoxyl pectin dispersions. Food Hydrocolloids. 17: 191-197.

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

Singh, A. and Benjakul, S. 2017. Serine protease inhibitors from squid ovary: extraction and its effect on proteolysis and gel properties of surimi. Journal of Food Science and Technology. 54: 267-275.

Sinthusamran, S., Benjakul, S. and Hemar, Y. 2016. Rheological and sensory properties of fish gelatin gels as influenced by agar from *Gracilaria*

tenuistipitata. International Journal of Food Science & Technology. 51: 1530-1536.

Sinthusamran, S., Benjakul, S. and Hemar, Y. 2017. Physical and sensory properties of gelatin from seabass (*Lates calcarifer*) as affected by agar and κ-carrageenan. Journal of Texture Studies. 1-9.

Sinthusamran, S., Benjakul, S. and Kishimura, H. 2014. Characteristics and gel properties of gelatin from skin of seabass (*Lates calcarifer*) as influenced by extraction conditions. Food Chemistry. 152: 276-284.

Sinthusamran, S., Benjakul, S. and Kishimura, H. 2015. Molecular characteristics and properties of gelatin from skin of seabass with different sizes. International Journal of Biological Macromolecules. 73: 146-153.

Solheim, R. and Ellekjær, M. R. 1993. Sensory quality of low-fat sausages affected by fat substitutes. Food Quality and Preference. 4: 127-131.

Strauss, G. and Gibson, S. M. 2004. Plant phenolics as cross-linkers of gelatin gels and gelatin-based coacervates for use as food ingredients. Food Hydrocolloids. 18: 81-89.

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

Suslick, K. S. 1990. Sonochemistry. Science. 247: 1439-1446.

Sworn, G. 2000. Gellan gum. Handbook of hydrocolloids. 117-135.

Taghizadeh, M. T. and Asadpour, T. 2009. Effect of molecular weight on the ultrasonic degradation of poly(vinyl-pyrrolidone). Ultrasonics Sonochemistry. 16: 280-286.

Tahergorabi, R. and Jaczynski, J. 2012. Physicochemical changes in surimi with salt substitute. Food Chemistry. 132: 1281-1286.

Tao, Y. and Sun, D.-W. 2015. Enhancement of food processes by ultrasound: A review. Critical Reviews in Food Science and Nutrition. 55: 570-594.

Tayal, A. and Khan, S. A. 2000. Degradation of a water-soluble polymer: molecular weight changes and chain scission characteristics. Macromolecules. 33: 9488-9493.

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

Temdee, W. and Benjakul, S. 2015. Effect of phenolic compounds and bark/wood extracts oxidised by laccase on properties of cuttlefish (*Sepia pharaonis*) skin gelatin gel. International Food Research Journal. 22: 246-253.

Thrash, S. L., Otto, J. C. and Deits, T. L. 1991. Effect of divalent ions on protein precipitation with polyethylene glycol: Mechanism of action and applications. Protein Expression and Purification. 2: 83-89.

Tiwari, B. K., Muthukumarappan, K., O'Donnell, C. P. and Cullen, P. J. 2010. Rheological properties of sonicated guar, xanthan and pectin dispersions. International Journal of Food Properties. 13: 223-233.

Tolstoguzov, V. B. 1997. Protein-polysaccharide interactions. *In* Food science and technology. p. 171-198. Marcel Dekker. New York, USA.

Uresti, R. M., López-Arias, N., Ramirez, J. A. and Vazquez, M. 2003. Effect of amidated low methoxyl pectin on the mechanical properties and colour attributes of fish mince. Food Technology and Biotechnology. 41: 131-136.

Wan, J., Kimura, I., Satake, M. and Seki, N. 1994. Effect of calcium ion concentration on the gelling properties and transglutaminase activity of walleye pollack surimi paste. Fisheries science. 60: 107-113.

Wang, Z.-M., Cheung, Y.-C., Leung, P.-H. and Wu, J.-Y. 2010. Ultrasonic treatment for improved solution properties of a high-molecular weight exopolysaccharide produced by a medicinal fungus. Bioresource Technology. 101: 5517-5522.

Wangtueai, S., Noomhorm, A. and Regenstein, J. M. 2010. Effect of microbial transglutaminase on gel properties and film characteristics of gelatin from lizardfish (*Saurida* spp.) scales. Journal of Food Science. 75: 731-739.

Wei, Y., Zhang, T., Yu, F., Xue, Y., Li, Z., Wang, Y. and Xue, C. 2017. Effects of curdlan on the texture and structure of Alaska pollock surimi gels treated at 120 °C. International Journal of Food Properties. 10.1080/10942912.10942017.11306557.

Wolf, C. L., LaVelle, W. M. and Clark, R. C. 1989. Gellan gum/gelatin blends. US 4876105.

Wu, S. 2016. Effect of pullulan on gel properties of *Scomberomorus niphonius* surimi. International Journal of Biological Macromolecules. 93: 1118-1120.

Xiaodong, Z., Qunfang, L., Gance, D. and Faxiang, J. 1998. Ultrasonic degradation of polysilane polymers. Polymer Degradation and Stability. 60: 409-413.

Xiong, Y. L. 1994. Myofibrillar protein from different muscle fiber types: Implications of biochemical and functional properties in meat processing. Critical Reviews in Food Science and Nutrition. 34: 293-320.

Yamaguchi, K., LavÉTy, J. and Love, R. M. 1976. The connective tissues of fish VIII. Comparative studies on hake, cod and catfish collagens. International Journal of Food Science & Technology. 11: 389-399.

Yamamoto, F. and Cunha, R. L. 2007. Acid gelation of gellan: Effect of final pH and heat treatment conditions. Carbohydrate Polymers. 68: 517-527.

Yan, M., Li, B., Zhao, X. and Yi, J. 2011. Physicochemical properties of gelatin gels from walleye pollock (*Theragra chalcogramma*) skin cross-linked by gallic acid and rutin. Food Hydrocolloids. 25: 907-914.

Yasui, K., Tuziuti, T., Sivakumar, M. and Iida, Y. 2004. Sonoluminescence. Applied Spectroscopy Reviews. 39: 399-436.

Ye, A. 2008. Complexation between milk proteins and polysaccharides via electrostatic interaction: principles and applications - A review. International Journal of Food Science & Technology. 43: 406-415.

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

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

Yuguchi, Y., Urakawa, H. and Kajiwara, K. 1997. Structural characteristics of crosslinking domain in gellan gum gel. Macromolecular Symposia. 120: 77-89.

Zaghbib, I., Arafa, S., Félix, M., Hassouna, M. and Romero, A. 2016. Enhancement of the gelling properties of sardine surimi with transglutaminase and

optimization of its activity using response surface methodology. Journal of Food Processing and Technology. 7: doi: 10.4172/2157-7110.1000594.

Zhang, Y. N. and Zhao, X. H. 2013. Study on the functional properties of soybean protein isolate cross-linked with gelatin by microbial transglutaminase. International Journal of Food Properties. 16: 1257-1270.

Zhou, P., Mulvaney, S. J. and Regenstein, J. M. 2006. Properties of Alaska pollock skin gelatin: A comparison with tilapia and pork skin gelatins. Journal of Food Science. 71: 313-321.

Zhou, X., Jiang, S., Zhao, D., Zhang, J., Gu, S., Pan, Z. and Ding, Y. 2017. Changes in physicochemical properties and protein structure of surimi enhanced with camellia tea oil. LWT - Food Science and Technology. 84: 562-571.

Zhou, Y.-Y., Cai, S.-J., Xu, X.-P. and Chen, R.-W. 2008. Study on improvement effects of carrageenan and gellan gum on properties of silver carp surimi-protein soybean compound. Food Science. 10: 071.

Zhu, J., Fan, D., Zhao, J., Zhang, H., Huang, J., Zhou, W., Zhang, W. and Chen, W. 2016. Enhancement of the gelation properties of surimi from yellowtail seabream (*Parargyrops edita, Sparidae*) with Chinese oak silkworm pupa, *Antheraea pernyi*. Journal of Food Science. 81: 396-403.

Ziegler, G. R. and Acton, J. C. 1984. Mechanisms of gel formation by proteins of muscle tissue. Food Technology. 38: 77-82.

1.3 Objectives

1. To investigate the effect of gellan at different levels on properties of fish gelatin gel

2. To study the effect of gellan in combination with divalent cation (Ca^{2+}) on properties of fish gelatin gel

3. To examine the impact of gellan with different preparations at various levels on properties of surimi gel

4. To evaluate the effect of gellan in combination with calcium chloride on the gel properties of surimi gel from bigeye snapper in the absence and presence of TGase.

CHAPTER 2

IMPROVEMENT OF GEL PROPERTIES OF FISH GELATIN USING GELLAN

2.1 Abstract

The impact of gellan (GL) at different levels (5-20% of total solid) on the properties of fish gelatin (FG) gels was studied. Gel strength and hardness of FG/GL mixed gel increased, while springiness and cohesiveness decreased as the levels of GL were increased ($p < 0.05$). Gelling and melting temperatures also increased with increasing levels of GL incorporated ($p < 0.05$). L^*- and b^*-values of FG/GL mixed gel decreased, whereas ΔE^*-value increased with increasing GL levels ($p < 0.05$). Microstructure studies revealed that denser structure with very small voids in gel network was observed upon GL addition. The addition of gellan at a low level (5%) had no adverse effect on acceptance, and no effect on syneresis of FG/GL mixed gels. Therefore, the addition of 5% GL can be used to improve gelling property of FG via increasing gelling points without affecting acceptance of the resulting gel.

2.2 Introduction

Gelatin is a protein derived from collagen by thermal denaturation or partial hydrolysis (Benjakul *et al.*, 2012; Johnston-Banks, 1990). Worldwide gelatin consumption is forecasted to reach 395.84 thousand metric tons by the year 2017. It has a wide range of applications in food (51%), pharmaceutical (31%), photographic and technical products (8%) and others (2%) (Vergauwen *et al.*, 2016). Most of the currently available beef and pork gelatins do not meet mainstream kosher (Jewish) and halal (Muslim) standards. In addition, the bovine spongiform encephalopathy (BSE) became an issue of concern for the consumption of cow-derived products (Gudmundsson, 2002). These limitations and concerns have drawn interests in alternative source of gelatins.

Fish gelatin (FG) has become the essential alternative and is mainly produced from fish processing by-products such as skin, scale, etc. (Sinthusamran *et al.*, 2015). Faced with poor gelling property due to low gelling temperature and longer setting time, FG applications are limited. In general, gelatin is dissolved in water at

temperatures of 40-60 °C, and gelifies when cooled down. However, FG possesses a low gelling temperature (8-25 °C) and melting temperature (11-28 °C), compared with those of mammalian counterpart with temperature of 20-25 °C for gelling and 28-31 °C for melting (Karim and Bhat, 2009). To alleviate such a drawback, cross-linking enzymes such as microbial transglutaminase (Jongjareonrak *et al.*, 2006) and various hydrocolloids including agar, κ-carrageenan, pectin and gellan (GL) have been used to modify the gelling properties of FG (Haug *et al.*, 2004; Lau *et al.*, 2001; Liu *et al.*, 2007; Sinthusamran *et al.*, 2016).

GL is a polymer produced by *Pseudomonas elodea* (lately referred to as *Sphingomonas paucimobilis*). It consists of repeat units of β-D-glucose (DGlc), β-D-glucuronic acid (D-GlcA) and α-L-rhamnose (L-Rha) in molar ratio of 2:1:1 with L-glyceric ester at O-2 of the 3-linked D-glucose and acetic esters at the O-6 of the same residue (Bajaj *et al.*, 2006; Sanderson, 1990). GL, a linear anionic heteropolysaccharide, which forms a double helix in aqueous solution, yielding high viscosity. At high temperatures (>90 ^{o}C), GL in water is in a disordered random coil state. Upon cooling to the gelling temperature (50-70 ^{o}C), GL forms double helices, which aggregate to form junction zones, and finally a three-dimensional gel network is developed with monovalent or divalent cations (Chandrasekaran and Radha, 1995; Sanderson and Clark, 1983). In general, gelation of GL is enhanced in the presence of divalent cations. Those cations act by site-binding between pairs of carboxyl groups on neighboring helices, thus giving the structures analogous to the ‘eggbox’ junctions (Evageliou *et al.*, 2011). Wolf *et al.* (1989) suggested that incorporation of small amounts of GL into porcine gelatin could greatly improve the properties of the gelatin gel. The gelation temperature and the gelation rate of the mixed gels a significantly affected by the ratio of GL to bovine gelatin (Lau *et al.*, 2001). The addition of GL into bovine gelatin could increase the hardness of the mixed gels (Lau *et al.*, 2000). The mixture of GL and gelatin could be used in several foods i.e. water/milk based dessert gels, dessert syrup/topping, and pet foods (Kasapis *et al.*, 1993). Furthermore, GL was used to improve the mechanical properties of FG-based film by increasing its tensile strength and its water vapor barrier property (Pranoto *et al.*, 2007). To strengthen the gel of gellan-bovine gelatin mixture, calcium chloride was employed (Lau *et al.*, 2001). Nevertheless, gelatin and gellan might undergo aggregation via

ionic interaction between the charged domains in the absence of cations. FG added with gellan (without divalent cations) at an appropriate level might possess the modified gelling behavior, e.g. increased setting temperature and gel strength. In the presence of cations, the gel more likely becomes very rigid or brittle with the loss in elasticity, resulting in the lower acceptability. To the best of our knowledge, no information on the effect of gellan on the gelling property of FG in the absence of divalent cations exists in the published literature. Therefore, the present study aims to investigate the effect of gellan at various levels on the gelling, acceptability and microstructural properties of FG gels.

2.3 Materials and methods

2.3.1 Materials

Food-grade low acyl GL was purchased from CP Kelco (Atlanta, GA, USA). FG produced from tilapia skin with gel strength of approximately 240 bloom was procured from Lapi Gelatine S.p.A (Empoli, Italy).

2.3.2 Preparation of FG/GL mixed solutions and gels

GL and FG powders were firstly dispersed individually in deionized water to obtain a concentration of 6.67% (w/v). Therefore, the mixtures were heated at 90 °C and 60 °C for GL and FG, respectively. GL solution was then added into FG solution at different levels (0, 5, 10, 15 and 20% (w/w), as a FG substitution). The solutions were stirred for 10 min until they became homogeneous. Thereafter, the solutions were incubated at 60 °C until used for gel formation.

For gel preparation, the prepared solutions were transferred into cylindrical molds with 3 cm diameter and 2.5 cm height. All samples were incubated at refrigerated temperature (4 °C) for 18 h prior to analyses.

2.3.3 Analyses

2.3.3.1 Textural and physical properties

2.3.3.1.1 Determination of gel strength

Gel strength was determined at 8-10 °C using a texture analyzer (Stable Micro System, Surrey, UK) with a load cell of 5 kg and cross-head speed of 1 mm/s. A 1.27 cm diameter flat-faced cylindrical Teflon® plunger was used. The maximum

force (gram), was recorded as the force reached, when the plunger had penetrated 4 mm into the gelatin gels.

2.3.3.1.2 Texture profile analysis

Texture profile analysis (TPA) was performed using a TA-XT2 texture analyzer (Stable Micro Systems, Godalming, Surrey, UK) with a load cell of 50 kg, fitted with a cylindrical aluminium probe (50 mm diameter). The samples were placed on the instrument's base, and the tests were run with two compression cycles. TPA textural parameters were measured at 8-10 °C with the following testing conditions: crosshead speed of 0.5 mm/s, 50% compression of the original sample height, with a time interval between the first and second compression of 10 s. For each sample, hardness, cohesiveness, springiness, chewiness, gumminess and adhesiveness were calculated from the force-time curves (Lau *et al.*, 2000).

2.3.3.1.3 Determination of gelling and melting temperatures

All sample solutions (6.67%, w/v, and 60 °C) were prepared as described previously. The gelling and melting temperatures of mixed FG/GL solutions and FG solution alone were determined using a controlled stress rheometer (Rheo-Stress 1, HAAKE, Karlsruhe, Germany) as per the method of Sinthusamran *et al.* (2016). The measuring geometry included a 3.5 cm parallel plate and the gap was set at 1.0 mm. A sample was transferred to the rheometer, and the measurement was performed at a scan rate of 1 °C/min, frequency of 1 Hz, oscillating applied stress of 1 Pa during cooling from 60 to 5 °C and heating from 5 to 90 °C. The elastic modulus G′ and the loss (viscous) modulus G″ were recorded. Finally, the gelling and melting temperatures were determined as the temperatures, at which tan δ (=G″ / G′) became 1 (or $\delta=45^{o}$).

2.3.3.1.4 Determination of color

The color of FG and FG/GL mixed gels (6.67% w/v) were measured with a Hunter lab colorimeter (Color Flex, Hunter Lab Inc., Reston, VA, USA). The *L**, *a** and *b** values indicating lightness/brightness, redness/greenness and yellowness/blueness, respectively, were recorded. The colorimeter was warmed for 10 min and calibrated with a white standard. The total difference in color (ΔE^{*}) was calculated as follows (Gennadios *et al.*, 1996):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

where ΔL^*, Δa^* and Δb^* are the difference between the corresponding color parameter of the sample and that of the white standard (L^* = 93.6, a^* = -0.94 and b^* = 0.50).

2.3.3.1.5 Determination of syneresis

The syneresis of FG and FG/GL mixed gels were determined as described by Banerjee and Bhattacharya (2011). Hot solution samples (30 mL) were poured into 50 mL graduated centrifuge tubes and their masses (m_1) were recorded. Gels were incubated at the refrigerated temperature (4 °C) for 18 h. Before measurement, gels were equilibrated at room temperature for 3 h. These samples were subsequently centrifuged at 2150 xg at 25 °C for 10 min using a refrigerated centrifuge (Beckman Coulter, Palo Alto, CA, USA). After centrifugation, the separated water was discarded and the remaining gels with the tubes were weighed (m_2). The percentage of syneresis was calculated as follows:

$$\text{Syneresis (\%)} = \frac{m_1 - m_2}{m_1} \times 100 \quad (2)$$

2.3.3.2 Microstructure

Microstructures of FG and FG/GL mixed gels were visualized using scanning electron microscopy (SEM) following the method of Kaewdang and Benjakul (2015). All gels having a thickness of ~2-3 mm were fixed with 2.5% (v/v) glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 12 h. The samples were then rinsed with distilled water for 1 h and dehydrated in ethanol with a serial concentration of 25%, 50%, 70%, 80%, 90% and 100% (v/v). Thereafter, the samples were critical point dried using CO_2 as transition fluid. Dried samples were mounted on a bronze stub and sputter-coated with gold (Sputter coater SPI-Module, West Chester, PA, USA). The specimens were observed with a scanning electron microscope (Quanta 400; FEI, Eindhoven, Netherlands) at an acceleration voltage of 20 kV.

2.3.3.3 Acceptance test

Gel samples were cut into bite-size (1 cm thick and 2.5 cm in diameter), coded with 3-digit random numbers. The gel samples were kept at 8-10 °C until the acceptance test was performed. The 50 non-trained panelists (aged between 20 and 45), were students and staffs from the Department of Food Technology. All panelists, who were accustomed with gelatin products, were asked to assess appearance, color, odor, firmness, springiness, mouth feel and overall liking of the gel samples using a 9-point hedonic scale (Meilgaard *et al.*, 2006). Gel samples were served on a covered white plastic cup under fluorescent daylight-type illumination. Between samples, panelists were asked to rinse their mouth with drinking water at room temperature.

2.3.4 Statistical analysis

All experiments were run in triplicate. For experimental designs, completely randomized design (CRD) was used for textural and physical properties and randomized complete block design (RCBD) was used for acceptance test. Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by the Duncan's multiple range test (Steel and Torrie, 1980). Statistical analysis was performed using SPSS for Windows (SPSS Inc., Chicago, IL, USA). Data with $p < 0.05$ were considered to be statistically significant.

2.4 Results and discussion

2.4.1 Textural and physical properties of FG gel as affected by GL addition

2.4.1.1 Gel strength

Gel strength of FG gels without and with GL addition at different levels (0-20%) are shown in Table 3. FG gel exhibited the lowest gel strength (~198.92 g), compared with FG/GL mixed gels ($p < 0.05$). The gel strength of FG/GL mixed gels increased as the level of GL increased ($p < 0.05$). The increase in gel strength of FG gel containing GL might be caused by interactions between FG and GL, resulting in a stronger network. GL could form 'new heterolytic junction zones' networks with the gelatin molecules (Pranoto *et al.*, 2007). Anionic domains of GL and cationic domain of FG might undergo ionic interaction, thereby leading to the increased gel strength. When GL was incorporated, FG gel became more resistant to applied force as shown by the increased gel strength. In general, GL forms double helices, which further

aggregate to form junction zones, and finally forms three-dimensional gel networks with monovalent or divalent cations (Chandrasekaran and Radha, 1995; Sanderson and Clark, 1983). In the absence of cations, the increase in gel strength of FG containing GL was most likely due to the interaction or aggregation between both biopolymers. Negatively charged domain of gellan might undergo interaction with positively charged residues of gelatin via ionic interaction. Therefore, the incorporation of GL could increase the strength of FG gel in a dose-dependent manner. Based on a plot of the gel strength (g) as a function of GL concentration in percent (GL%), using the values reported in Table 3, a linear curve was obtained; g = 211.2+65.26×GL[%] with $R^2 = 0.9986$. This shows clearly a linear increase in the gel strength with the addition of gellan for up to 20%.

2.4.1.2 Textural properties

Hardness of FG gels generally increased with increasing GL content as shown in Table 4. The hardness of FG gel was ~14.84 N. Hardness is the peak force during the first compression cycle, and is related to the strength of the gel structure under compression (Yang *et al.*, 2007). With addition of 20% GL, the resulting mixed gel showed the highest hardness (141.67 N) ($p < 0.05$). Increasing hardness was in agreement with the increase in gel strength as the levels of incorporated GL increased. In fact, plotting the data obtained for the gel strength in Table 3 against the hardness data reported in Table 4, yields a linear relationship with an $R^2 = 0.9962$. Lau *et al.* (2000) reported that hardness of GL/bovine gelatin mixed gels increased when the ratio of GL to gelatin was increased. Thus, GL played a positive role in the hardness of FG/GL mixed gels. FG had the highest springiness (0.96 cm), while FG gel containing 20% GL had the lowest springiness (0.85 cm) ($p < 0.05$). The springiness of the mixed gels generally decreased with increasing levels of GL ($p < 0.05$). However, there was no difference in springiness between FG gel containing 10% GL and that added with 15% GL ($p > 0.05$). Springiness (also called “elasticity”) is a perception of gel “rubberiness” in the mouth, and is a measure of the degree of destruction of the gel during the first compression (Souissi *et al.*, 2016). It was noted that FG gel became more brittle (lower springiness) when GL was added, especially at higher levels. It has been known that GL forms hard and brittle gels. On the other hand, gelatin forms soft, flexible and elastic gels (Nussinovitch, 1997). The rigidity of

Table 3 Gel strength, syneresis, gelling and melting temperatures of fish gelatin as affected by the addition of gellan at different levels

Gellan (% FG substitution)	Gel strength (g)	Syneresis (%)	Gelling temperature (^{o}C)	Melting temperature (^{o}C)
0	198.92±7.85^{e}	0.11±0.02^{d}	18.44±0.06^{d}	27.08 ±0.14^{d}
5	531.59±11.92^{d}	0.14±0.04^{d}	36.27±0.17^{c}	49.81±0.05^{c}
10	888.68±12.28^{c}	0.26±0.04^{c}	36.51±0.15^{c}	53.77±0.10^{b}
15	1205.29±19.52^{b}	0.37±0.03^{b}	36.91±0.01^{b}	55.29±0.74^{a}
20	1493.70±18.04^{a}	0.47±0.04^{a}	38.30±0.19^{a}	>90.00

Values are presented as mean ± SD (n = 3).

Different lowercase superscripts within the same column indicate significant differences ($p < 0.05$).

GL gel contributed to the increased rigidity or brittleness. Thus, the addition of GL at a high level negatively affected the textural characteristic of FG/GL mixed gels as indicated by the loss in springiness. The cohesiveness of FG gel was 0.85. Cohesiveness of FG gels decreased as the level of GL increased ($p < 0.05$). The lowest cohesiveness was obtained as GL at a level of 20% was added ($p < 0.05$). Cohesiveness is a measure of the degree of difficulty in breaking down the internal structure of gel (Sanderson, 1990; Wolf *et al.*, 1989). Lee et al. (Lee *et al.*, 2003) reported that cohesiveness of GL/porcine gelatin mixed gels depended on the ratio of GL in the mixed gel. The results suggested that the internal structure of FG gels might be more difficult to break during the first compression, in comparison with those added with GL. Both gumminess and chewiness of FG gel increased when the GL content was increased ($p < 0.05$). FG gel had the lowest gumminess and chewiness (12.23 N and 11.77 N × cm, respectively), and FG gel containing 20% GL had the highest values (90.89 N and 81.75 N × cm, respectively) ($p < 0.05$). Gumminess represents the energy required to breakdown a semi-solid food ready for swallowing and chewiness represents the required energy to chew the sample to the point required for swallowing it. Furthermore, the addition of GL markedly increased the adhesiveness of FG gel ($p < 0.05$). However, there were no differences in adhesiveness between mixed gels containing 5, 10 and 15% GL ($p > 0.05$). Therefore, GL in mixed gel directly affected the textural properties of the resulting FG/GL mixed gel. Furthermore, the level of GL was the major factor affecting the texture of resulting FG/GL mixed gels.

For all the TPA attributes, except for adhesiveness, when plotted as a function of the GL concentration, a linear relationship with different coefficient of determination (R^2) was observed (Figure 5). Positive correlations were found for hardness ($R^2 = 0.9912$), gumminess ($R^2 = 0.9689$) and chewiness ($R^2 = 0.9754$), while negative correlations were attained for springiness ($R^2 = 0.9778$) and cohesiveness ($R^2 = 0.9521$). Furthermore, the standard errors of the regressions for all plots were low, indicating the accuracy of linear regression. These linear relationships, obtained for both the TPA measurements and for the gel strength, can be exploited to tailor accurately the textural properties of FG/GL mixtures when the amount of GL added was lower than 20%.

Table 4 Texture profile analysis (TPA) of fish gelatin gel as affected by the addition of gellan at different levels

Gellan (% FG substitution)	Hardness (N)	Springiness (cm)	Cohesiveness	Adhesiveness (N × s)	Gumminess (N)	Chewiness (N × cm)
0	14.84±0.50^{e}	0.96±0.01^{a}	0.85±0.02^{a}	-0.72±0.02^{c}	12.23±0.59^{e}	11.77±0.63^{e}
5	46.13±1.56^{d}	0.92±0.01^{b}	0.75±0.01^{b}	-0.07±0.02^{a}	33.76±1.66^{d}	31.71±1.56^{d}
10	85.62±3.35^{c}	0.89±0.00^{c}	0.71±0.02^{c}	-0.08±0.01^{a}	62.75±4.02^{c}	56.87±3.30^{c}
15	120.81±2.21^{b}	0.88±0.00^{c}	0.66±0.01^{d}	-0.07±0.02^{a}	83.26±3.84^{b}	71.79±3.73^{b}
20	141.67±3.26^{a}	0.85±0.02^{d}	0.52±0.00^{e}	-0.12±0.01^{b}	90.89±1.72^{a}	81.75±1.17^{a}

Values are presented as mean ± SD (n = 3).

Different lowercase superscripts within the same column indicate significant differences ($p < 0.05$).

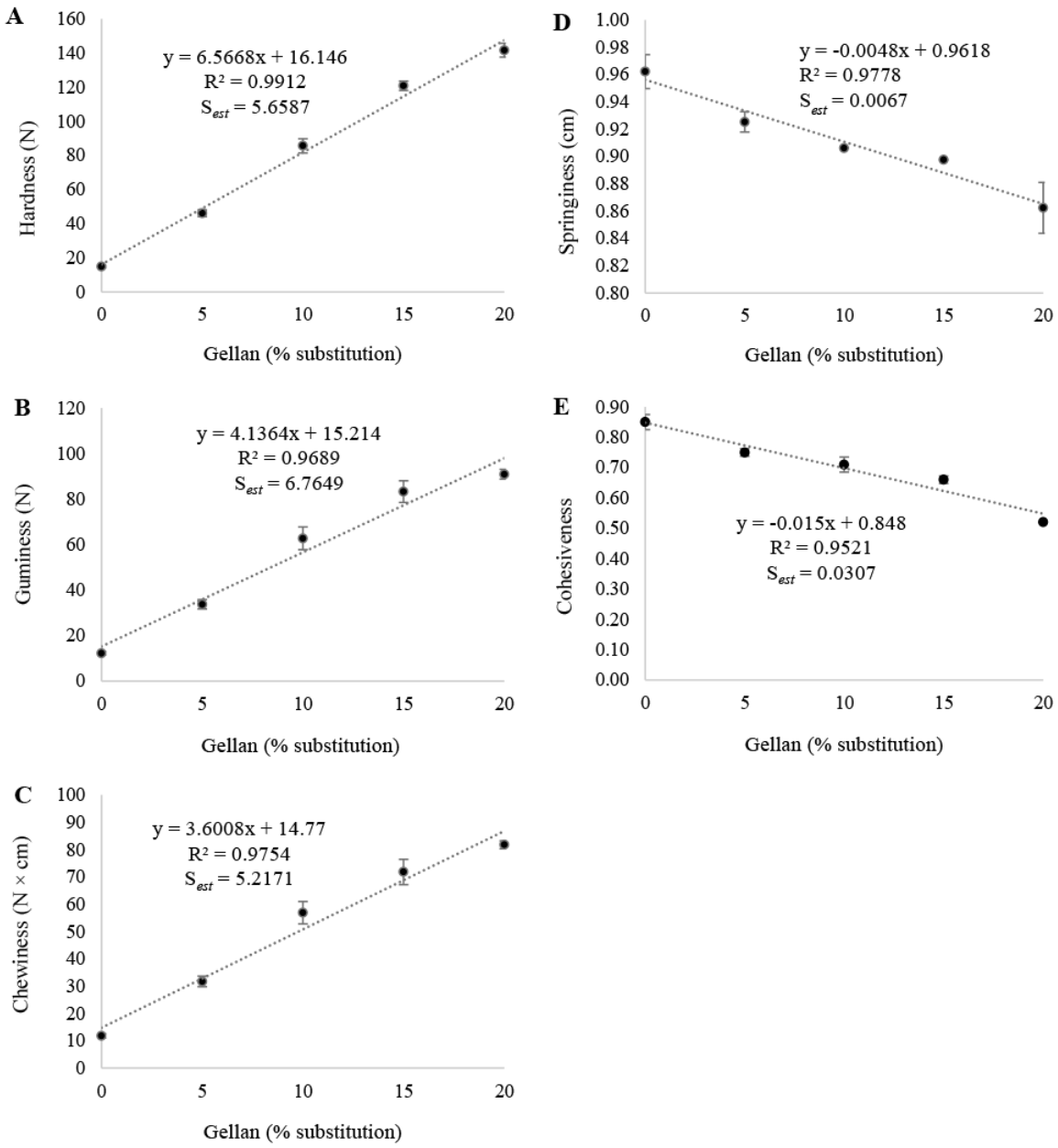

Figure 5 Correlation between the levels of gellan added and TPA attributes of the fish gelatin gels. Hardness (A), gumminess (B), chewiness (C), springiness (D) and cohesiveness (E). Error bars indicate 95% confidence intervals based on the Student T distribution.

2.4.1.3 Gelling and melting temperatures

Gelling and melting temperatures of FG gel and FG/GL mixed gels are shown in Table 3. Gelling temperatures of FG was 18.44 °C. This means that FG could not form gel at room temperature. Gelling temperature of FG/GL mixed gel increased as levels of GL were increased ($p < 0.05$). All FG/GL mixed gels were able to set at room temperature (>25 °C). However, no differences between the mixed gels containing 5 and 10% GL were observed ($p > 0.05$). Gelation of biopolymer normally occurs by physical cross-linking, leading to the formation of junction zone and ultimately a three-dimensional branched network with entrapped water (Gilsenan and Ross-Murphy, 2000). In the absence of cations, carboxyl group of GL might interact with fish gelatin, which has positively charged domain, leading to the enhancement of network formation. The gelation temperature and gelation rate of the mixed GL/bovine gelatin gels were previously reported to be significantly affected by the ratio of GL to bovine gelatin as well as the concentration of calcium used (Lau *et al.*, 2001).

Melting temperature of FG/GL mixed gels was increased with increasing GL content ($p < 0.05$). Increasing melting temperature of mixed gels containing GL at higher levels was in accordance with increasing gelling temperature of mixed FG/GL solutions. Among all mixed gels, the highest melting temperature was found for FG gel containing 20% GL (>90 °C). The result suggested that GL directly contributes to the melting behavior of a mixed gel. GL more likely increased the stability of the mixed gel network, as evidenced by the increased melting temperatures.

2.4.1.4 Color

Color of FG gels and FG/GL mixed gels expressed as L^*, a^*, b^* and ΔE^* is shown in Table 5. Among all gel samples, FG gel had the highest lightness (L^*-value), compared with other gels ($p < 0.05$). The L^*-values of FG/GL mixed gels decreased with increasing levels of GL added ($p < 0.05$). The lowest L^*-value (56.44) was obtained for FG gel containing 20% GL. In GL/porcine gelatin mixed gels, coacervation between the two biopolymers was suggested to lower the L^*-value (Chilvers and Morris, 1987). Furthermore, the addition of GL increased the redness (a^*-value) of the FG/GL mixed gel.

Gel containing 5% GL had the highest *a**-value among all the gels tested ($p < 0.05$). On the other hand, yellowness (*b**-value) of gel decreased as GL content was increased ($p < 0.05$). Overall, the increases in ΔE*-value were in agreement with the decreases in *L**- and *b**-values. FG gel showed the lowest ΔE*-value (21.72) with the highest *L**- and *b**-values. This results also suggested that the color of FG/GL mixed gels was influenced by the color of individual hydrocolloid and the way both hydrocolloids structured (e.g. alignment and aggregation) in the gel network.

Table 5 Color values of fish gelatin gel as affected by the addition of gellan at different levels

Gellan (% FG substitution)	*L**	*a**	*b**	ΔE^*
0	76.20±1.05^{a}	-2.14±0.06^{e}	13.77±0.21^{a}	21.72±0.65^{e}
5	62.21±0.47^{b}	1.75±0.13^{a}	13.15±0.09^{b}	34.18±0.27^{d}
10	59.28±0.32^{c}	1.30±0.05^{b}	-0.54±0.58^{c}	35.46±0.33^{c}
15	58.10±0.51^{d}	0.15±0.03^{c}	-3.79±0.14^{d}	36.58±0.11^{b}
20	56.44±0.91^{e}	-0.25±0.16^{d}	-4.43±0.33^{e}	37.28±0.47^{a}

Values are presented as mean ± SD (n = 3).
Different lowercase superscripts within the same column indicate significant differences ($p < 0.05$).

2.4.1.5 Syneresis

Syneresis of FG gels and FG/GL mixed gels at different levels is shown in Table 3. Among all gel samples, FG gel had the lowest syneresis, which was 0.11%. The highest syneresis (0.47%) was found in the FG gel containing 20% GL ($p < 0.05$). Syneresis of FG/GL mixed gel increased linearly ($R^2 = 0.9746$) with increasing GL levels ($p < 0.05$), although FG gel mixed with 5% GL showed no significant differences in syneresis, compared with FG gel ($p > 0.05$). Syneresis is a natural phenomenon, indicating unbound excess water released from the gel matrix (Banerjee and Bhattacharya, 2011). The interaction between FG and GL, especially in the presence of GL at high level, might yield more compact structure, thereby having less

room for water holding. Sinthusamran et al. (Sinthusamran *et al.*, 2016) also reported that syneresis of agar/gelatin mixed gel increased with increasing agar levels.

2.4.2 Microstructures of FG gel as affected by GL addition

The microstructures of FG and FG/GL mixed gels are illustrated in Figure 6. The FG gel (without GL addition) had a coarser network with larger void or cavities (Figure 6(A)). With the addition of GL above 5%, the mixed gels showed a finer and denser strands and smaller voids. When GL was added at higher level (>5%), a denser structure with small voids in the gel network was observed (Figure 6(B-E)). Ionic interaction between negative charge of GL from carboxyl group (Morris *et al.*, 2012) and positive charge of FG, especially from basic amino acids including arginine, lysine and histidine (Sae-leaw *et al.*, 2016; Sarabia *et al.*, 2000) were postulated. In general, the structure of gel matrix can be directly linked to the gel strength of gelatin (Benjakul *et al.*, 2009); the more compact and denser the gel network structure, the higher gel strength and higher hardness of FG/GL mixed gel are expected, especially when GL content was higher than 5% (Table 3 and 4). The same phenomenon was found in pectin/gelatin mixed system. When pectin was added into the gelatin, the cavities were partially covered by pectin and seemed more compact. Electrostatic interaction of the pectin and gelatin in the mixed system might be because of the adsorbed force from the pectin and gelatin molecules (Liu *et al.*, 2007).

2.4.3 Acceptability of FG gel as affected by GL addition

Likeness score of FG gel without and with GL addition at different levels is shown in Table 6. Addition of GL decreased appearance and color likeness of mixed gels, especially when the level of GL was increased ($p < 0.05$). These results correlated with the decreases in lightness and with the increased ΔE^*-value (Table 5). It was noted that FG/GL mixed gels showed lower mouth feel likeness score than FG gel alone ($p < 0.05$). These might be caused by the increased melting temperature as shown in Table 3. All mixed gels could not melt in the mouth as did FG gel alone. This melt-in-mouth property is one of the most important characteristics of gelatin gels (Karim and Bhat, 2009). The addition of GL at all tested levels did not significantly affect the odor likeness score ($p > 0.05$). Firmness and springiness

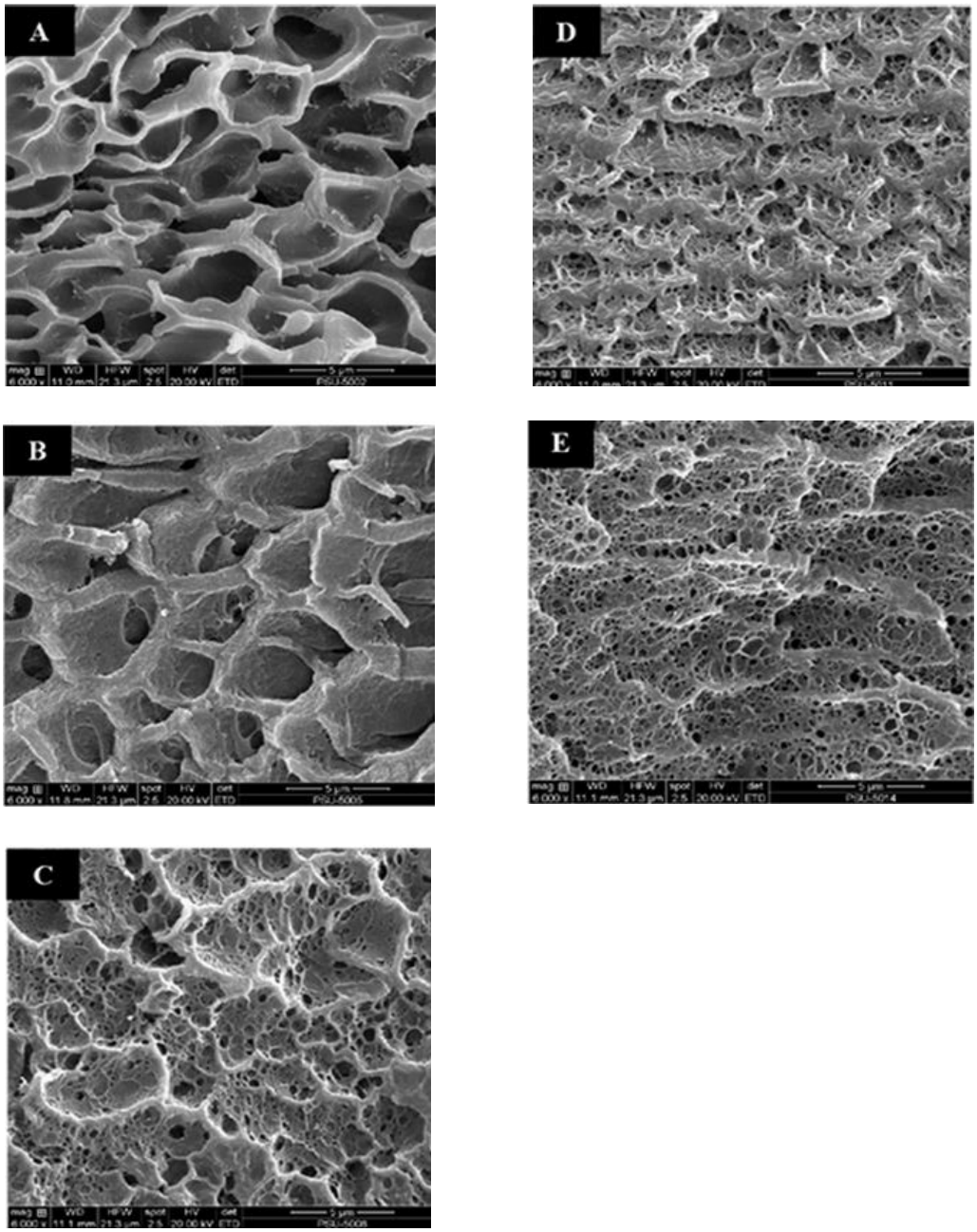

Figure 6 Microstructures of fish gelatin gel as affected by the addition of gellan at different levels. 0% gellan (A), 5% gellan (B), 10% gellan (C), 15% gellan (D) and 20% gellan (E). Magnification: 6000X.

Table 6 Acceptability of fish gelatin gel as affected by the addition of gellan at different levels

Gellan (% FG substitution)	Appearance	Color	Odor	Firmness	Springiness	Mouth feel	Overall
0	8.56±0.54^{a}	8.10±0.81^{a}	7.06±1.60^{a}	7.94±1.00^{a}	7.66 ±1.02^{a}	7.14±1.68^{a}	7.16±1.80^{a}
5	7.56 ±0.81^{b}	7.62±0.83^{b}	6.80±1.76^{a}	7.80±1.03^{a}	7.32±1.20^{ab}	6.76±1.55^{b}	7.00±1.27^{a}
10	7.18 ±1.00^{b}	7.10±0.89^{c}	7.24±1.25^{a}	7.32±1.00^{b}	6.90±1.27^{bc}	6.62±1.21^{b}	6.66±1.12^{b}
15	6.74 ±1.23^{c}	6.76±1.08^{d}	6.80±1.48^{a}	6.88±0.92^{c}	6.60±1.39^{c}	6.64±1.45^{b}	6.68±1.02^{b}
20	6.84 ±1.28^{c}	6.24±1.39^{e}	6.94±1.41^{a}	6.72±1.41^{c}	6.26±1.44^{d}	6.44±1.50^{b}	6.62±1.07^{b}

Values are presented as mean ± SD.

Different lowercase superscripts within the same column indicate significant differences ($p < 0.05$).

likeness scores of FG gel generally decreased as the levels of GL were increased ($p < 0.05$), although there were no differences in firmness and springiness likeness scores between FG gel and FG containing 5% GL ($p > 0.05$). This is similar to the result of overall likeness, in which FG gel showed no difference in likeness score with FG gel containing 5% GL ($p > 0.05$). Therefore, the addition of GL at low level (≤5%) had no adverse effect on the acceptance of FG/GL mixed gels. It was noted that the values of springiness obtained through the TPA measurements and likeness score for springiness correlated extremely well ($R^2 = 0.9841$).

2.5 Conclusions

The addition of GL to FG in the absence of cations could improve the properties of FG/GL mixed gels. Textural properties of FG/GL mixed gels were affected by the added GL levels. Gel strength and hardness increased, but springiness and cohesiveness of the mixed gels decreased as the levels of GL increased. Gelling and melting temperatures of FG/GL mixed gels increased when GL levels were increased. L^*- and b^*-values of FG/GL mixed gel decreased, while ΔE^*-value increased as the levels of GL increased. Syneresis of FG/GL mixed gels increased as the levels of GL increased, except for 5% GL addition. Electron microscopy showed that a finer network was obtained when the GL concentration was added. With higher levels of GL added, the acceptance, namely appearance, color, firmness, springiness and mouth feel likeness, decreased. However, the addition of GL at low level (5%) had no adverse effect on the acceptance of resulting mixed gels. Thus, the addition of GL at low levels could improve the textural properties as well as increasing the gelling temperature, to achieve FG solutions gel-setting at room temperature. Since linear relationships between the gel strength, texture profile analysis (TPA) parameters, and likeness scores as a function of the GL concentrations are obtained, these might help tailor FG/GL mixtures with suitable attributes.

2.6 References

Bajaj, I. B., Saudagar, P. S., Singhal, R. S. and Pandey, A. 2006. Statistical approach to optimization of fermentative production of gellan gum from *Sphingomonas paucimobilis* ATCC 31461. Journal of Bioscience and Bioengineering. 102: 150-156.

Banerjee, S. and Bhattacharya, S. 2011. Compressive textural attributes, opacity and syneresis of gels prepared from gellan, agar and their mixtures. Journal of Food Engineering. 102: 287-292.

Benjakul, S., Kittiphattanabawon, P. and Regenstein, J. M. 2012. Fish gelatin. *In* Food Biochemistry and Food Processing. (Simpson, K. B., ed.). p. 388-405. John Wiley & Sons Inc. LA, USA.

Benjakul, S., Oungbho, K., Visessanguan, W., Thiansilakul, Y. and Roytrakul, S. 2009. Characteristics of gelatin from the skins of bigeye snapper, *Priacanthus tayenus* and *Priacanthus macracanthus*. Food Chemistry. 116: 445-451.

Chandrasekaran, R. and Radha, A. 1995. Molecular architectures and functional properties of gellan gum and related polysaccharides. Trends in Food Science & Technology. 6: 143-148.

Chilvers, G. R. and Morris, V. J. 1987. Coacervation of gelatin-gellan gum mixtures and their use in microencapsulation. Carbohydrate Polymers. 7: 111-120.

Evageliou, V., Gerolemou, A., Zikas, A., Basios, A. and Komaitis, M. 2011. Effect of salts and sugars on the clarity of gellan gels. International Journal of Food Science and Technology. 46: 1001-1006.

Gennadios, A., Weller, C. L., Hanna, M. A. and Froning, G. W. 1996. Mechanical and barrier properties of egg albumen films. Journal of Food Science. 61: 585-589.

Gilsenan, P. M. and Ross-Murphy, S. B. 2000. Rheological characterisation of gelatins from mammalian and marine sources. Food Hydrocolloids. 14: 191-195.

Gudmundsson, M. 2002. Rheological Properties of Fish Gelatins. Journal of Food Science. 67: 2172-2176.

Haug, I. J., Draget, K. I. and Smidsrød, O. 2004. Physical behaviour of fish gelatin-κ-carrageenan mixtures. Carbohydrate Polymers. 56: 11-19.

Johnston-Banks, F. A. 1990. Gelatin. *In* Food gels. (Harris, P., ed.). p. 233-289. Elsevier Science Publishing Co. New York.

Jongjareonrak, A., Benjakul, S., Visessanguan, W. and Tanaka, M. 2006. Skin gelatin from bigeye snapper and brownstripe red snapper: Chemical compositions and effect of microbial transglutaminase on gel properties. Food Hydrocolloids. 20: 1216-1222.

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

Karim, A. A. and Bhat, R. 2009. Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins. Food Hydrocolloids. 23: 563-576.

Kasapis, S., Morris, E. R., Norton, I. T. and Clark, A. H. 1993. Phase equilibria and gelation in gelatin/maltodextrin systems-part I: gelation of individual components. Carbohydrate Polymers. 21: 243-248.

Lau, M. H., Tang, J. and Paulson, A. T. 2000. Texture profile and turbidity of gellan/gelatin mixed gels. Food Research International. 33: 665-671.

Lau, M. H., Tang, J. and Paulson, A. T. 2001. Effect of polymer ratio and calcium concentration on gelation properties of gellan/gelatin mixed gels. Food Research International. 34: 879-886.

Lee, K. Y., Shim, J., Bae, I. Y., Cha, J., Park, C. S. and Lee, H. G. 2003. Characterization of gellan/gelatin mixed solutions and gels. LWT - Food Science and Technology. 36: 795-802.

Liu, H., Zhu, D. S., Xu, X. M., Guo, S. D. and Jin, Z. Y. 2007. Rheological, textural and microstructure analysis of the high-methoxy pectin/gelatin mixed systems. Journal of Texture Studies. 38: 577-600.

Meilgaard, M. C., Carr, B. T. and Civille, G. V. 2006. Sensory evaluation techniques. CRC press. Florida.

Morris, E. R., Nishinari, K. and Rinaudo, M. 2012. Gelation of gellan – A review. Food Hydrocolloids. 28: 373-411.

Nussinovitch, A. 1997. Gellan gum. *In* Hydrocolloid Applications: Gum technology in the food and other industries. (Nussinovitch, A., ed.). p. 63-82. Springer. Boston, US.

Pranoto, Y., Lee, C. M. and Park, H. J. 2007. Characterizations of fish gelatin films added with gellan and κ-carrageenan. LWT - Food Science and Technology. 40: 766-774.

Sae-leaw, T., Benjakul, S., O'Brien, N. M. and Kishimura, H. 2016. Characteristics and functional properties of gelatin from seabass skin as influenced by defatting. International Journal of Food Science & Technology. 51: 1204-1211.

Sanderson, G. R. 1990. Gellan Gum. *In* Food Gels. (Harris, P., ed.). p. 201-232. Springer. Dordrecht, Netherlands.

Sanderson, G. R. and Clark, R. C. 1983. Gellan gum. Food Technology. 37: 62-70.

Sarabia, A. I., Gómez-Guillén, M. C. and Montero, P. 2000. The effect of added salts on the viscoelastic properties of fish skin gelatin. Food Chemistry. 70: 71-76.

Sinthusamran, S., Benjakul, S. and Hemar, Y. 2016. Rheological and sensory properties of fish gelatin gels as influenced by agar from *Gracilaria tenuistipitata*. International Journal of Food Science & Technology. 51: 1530-1536.

Sinthusamran, S., Benjakul, S. and Kishimura, H. 2015. Molecular characteristics and properties of gelatin from skin of seabass with different sizes. International Journal of Biological Macromolecules. 73: 146-153.

Souissi, N., Jridi, M., Nasri, R., Ben Slama, R., Njeh, M. and Nasri, M. 2016. Effects of the edible cuttlefish gelatin on textural, sensorial and physicochemical quality of octopus sausage. LWT - Food Science and Technology. 65: 18-24.

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

Vergauwen, B., Stevens, P., Prawitt, J., Olijve, J., Brouns, E., Babel, W., Schulz, D., Giesen-Wiese, M., Seybold, U., Gareis, H., Dick, E., Schrieber, R., Schott, A. and Stein, W. 2016. Ullmann's Encyclopedia of Industrial Chemistry. Wiley-VCH Verlag GmbH & Co. Weinheim, German.

Wolf, C. L., LaVelle, W. M. and Clark, R. C. 1989. Gellan gum/gelatin blends. US 4876105.

Yang, H., Wang, Y., Jiang, M., Oh, J.-H., Herring, J. and Zhou, P. 2007. 2-step optimization of the extraction and subsequent physical properties of channel catfish (*Ictalurus punctatus*) skin gelatin. Journal of Food Science. 72: 188-195.

CHAPTER 3

PROPERTY OF FISH GELATIN GEL AS AFFECTED BY THE INCORPORATION OF GELLAN AND CALCIUM CHLORIDE

3.1 Abstract

Properties of fish gelatin (FG) gel as affected by gellan (GL) at different levels (2.5-7.5% FG substitution) in combination with calcium chloride ($CaCl_2$) at various concentrations (3-9 mM) were studied. Gel strength and hardness of FG/GL mixed gel increased as the levels of GL increased ($p < 0.05$). Increasing $CaCl_2$ concentration also resulted in the increases in both gel strength and hardness of mixed gel ($p < 0.05$). Conversely, the increasing GL and $CaCl_2$ levels caused a decrease in springiness but an increase in syneresis of mixed gels ($p < 0.05$). Gelling and melting temperatures were increased in the mixed gel as levels of GL and $CaCl_2$ increased. L^*- and b^*-values of mixed gels decreased, whereas ΔE^*-value increased with increasing GL and $CaCl_2$ levels ($p < 0.05$). Microstructure studies revealed that denser structure with smaller voids in gel network was observed in the mixed gel in the presence of $CaCl_2$ at higher levels. However, mixed gels incorporated with GL above 5%, regardless of $CaCl_2$ levels, yielded the lower likeness score than FG gel (control) ($p < 0.05$). The addition of GL at low level (2.5%) with $CaCl_2$ (up to 6 mM) had no adverse effect on acceptance of mixed gels but could improve gelling property of FG via increasing gel strength and gelling point.

3.2 Introduction

Gelatin is a water soluble protein obtained by partial hydrolysis or thermal denaturation of collagen (Mohtar *et al.*, 2010). Food and pharmaceutical industries throughout the world have a growing demand of gelatin (Sheela, 2014). Among gelatins, that from mammals (bovine and porcine) have been widely used. However, restrictions and skepticism among consumers by socio-cultural and health concerns have led to the alternative gelatin (Yasin *et al.*, 2017). Fish gelatin has attracted

attention for kosher and halal markets and it is mainly produced from fish processing by-products such as skin, scale, etc. (Sinthusamran *et al.*, 2015). Facing with poor gelling property, e.g. low gel strength and low gelling temperature, fish gelatin still has the limited applications (Liu *et al.*, 2008). To overcome such a drawback, cross-linking enzymes such as microbial transglutaminase (Jongjareonrak *et al.*, 2006; Mohtar *et al.*, 2013), natural cross-linkers, particularly plant phenolic compounds, etc. have been used to strengthen fish gelatin gels (Kaewdang and Benjakul, 2015). Furthermore, various hydrocolloids including agar, κ-carrageenan, pectin and gellan have been reported to modify the gelling properties of fish gelatin (Haug *et al.*, 2004; Huang *et al.*, 2017; Lee *et al.*, 2003; Sinthusamran *et al.*, 2016).

Gellan, a microbial polysaccharide secreted by the bacterium *Sphingomonas elodea* (formerly referred to as *Pseudomonas elodea*), has the increasing use in the food industry as a texturizing and gelling agents. Deacylated gellan polymer consists of a linear tetrasaccharide repeating unit of glucose, glucuronic acid and rhamnose in the molar ratio of 2:1:1. It forms gel in the presence of cations (Yang and Paulson, 2000). During gelation, gellan undergoes the conformational change from disordered random coil conformation to the ordered form. When hot solutions are cooled, double helix structures are developed, primarily through intramolecular interactions (Nickerson *et al.*, 2008). Divalent cations act as a binder between pairs of carboxyl groups on neighboring helices, thus giving structures analogous to the 'eggbox' junctions (Evageliou *et al.*, 2011). According to Lau *et al.* (2001), gelling temperature and gelation rate of bovine gelatin/gellan mixed gels were significantly affected by the ratio of gellan to bovine gelatin as well as concentration of calcium. Hardness of mixed gels increased with increasing gellan levels and calcium ions (Lau *et al.*, 2001). From previous study, gellan could improve gelling property of fish gelatin by addition of gellan at a low level (5%), in which gelling point was increased without affecting acceptance of the resulting mixed gel. The incorporation of divalent cation into fish gelatin in the presence of gellan could be a promising means to improve the gelling property of fish gelatin. Nevertheless, no information on the effect of gellan in combination with divalent cation, especially Ca^{2+}, on properties of fish gelatin exists. Therefore, the present study aimed to investigate the effect of gellan in combination

with Ca^{2+} on the gelling, acceptability and microstructural properties of fish gelatin gels.

3.3 Materials and methods

3.3.1 Materials

Food-grade low acyl gellan was purchased from CP Kelco (Atlanta, GA, USA). Fish gelatin produced from tilapia skin with gel strength of approximately 240 bloom was procured from Lapi Gelatine S.p.A (Empoli, Italy).

3.3.2 Preparation of FG/GL mixed solutions and gels

Gellan (GL) and fish gelatin (FG) powders were individually solubilized in deionized water at 90°C and 60°C, respectively. GL solution was mixed with FG solution at different levels (0, 2.5, 5 and 7.5% (w/w) FG substitution) and the final solid concentration of 6.67% (w/v) in the obtained solution was fixed. $CaCl_2$ at different concentrations (0, 3, 6 and 9 mM) was added into the prepared solutions. The solutions were stirred using a magnetic stirrer until the homogeneity was attained. Subsequently, the solutions were incubated at 60 °C until used for gel formation.

To prepare the gel, the prepared solutions were transferred into cylindrical molds with 3 cm diameter and 2.5 cm height. All samples were incubated at refrigerated temperature (4 °C) for 18 h prior to analyses.

3.3.3 Analyses

3.3.3.1 Textural and gelling properties

3.3.3.1.1 Determination of gel strength

Gel strength was determined at 8-10 °C using a texture analyzer (Stable Micro System, Surrey, UK) with a load cell of 5 kg and cross-head speed of 1 mm/s. Flat-faced cylindrical Teflon® plunger with diameter of 1.27 cm was used. The maximum force (gram) was recorded when the plunger had penetrated 4 mm into the gels.

3.3.3.1.2 Texture profile analysis

Texture profile analysis (TPA) was performed using a TA-XT2 texture analyzer (Stable Micro Systems, Godalming, Surrey, UK) with a load cell of 50 kg, using a cylindrical aluminium probe (50 mm diameter). The samples were placed on the instrument's base and the tests were run with two compression cycles. TPA textural parameters were measured at 8-10 °C with the following testing conditions: crosshead speed of 0.5 mm/s, 50% compression of the original sample height, with a time interval between the first and second compression of 10 s. Hardness, cohesiveness, springiness, chewiness, gumminess and adhesiveness were calculated from the force-time curves (Lau *et al.*, 2000).

3.3.3.1.3 Determination of gelling and melting temperatures

All sample solutions (6.67%, w/v, 60 °C) were prepared as described previously. The gelling and melting temperatures of all samples were determined using a controlled stress rheometer (Rheo-Stress 1, HAAKE, Karlsruhe, Germany) as per the method of Sinthusamran *et al.* (2016). The measuring geometry included a 3.5 cm parallel plate and the gap was set at 1.0 mm. A sample was transferred to the rheometer, and the measurement was performed at a scan rate of 1 °C/min, frequency of 1 Hz, oscillating applied strain of 1% during cooling from 60 to 5 °C and heating from 5 to 90 °C. The elastic modulus G′ and the loss (viscous) modulus G″ were recorded. Finally, the gelling and melting temperatures were determined as the temperatures, at which tan δ (=G″ / G′) became 1 (or $\delta=45^{o}$).

3.3.3.2 Color

The color of gel samples (6.67% w/v) was measured with a Hunter lab colorimeter (Color Flex, Hunter Lab Inc., Reston, VA, USA). The *L**, *a** and *b** values indicating lightness/brightness, redness/greenness and yellowness/blueness, respectively, were recorded. The colorimeter was warmed for 10 min and calibrated with a white standard. The total difference in color (ΔE^{*}) was calculated as follows (Gennadios *et al.*, 1996):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

where ΔL^*, Δa^* and Δb^* are the difference between the corresponding color parameter of the sample and that of the white standard (L^* = 93.62, a^* = -0.91 and b^* = 0.50).

3.3.3.3 Syneresis

The syneresis of all gel samples were determined as described by Banerjee and Bhattacharya (2011). Hot solutions (30 mL) were poured into 50 mL graduated centrifuge tubes and their masses (m_1) were recorded. Mass of initial sample (m_3) was calculated by subtracting the mass of graduated centrifuge tube from m_1. Gels were incubated at the refrigerated temperature (4 °C) for 18 h. Before measurement, gels were equilibrated at room temperature for 3 h. Then, these samples were centrifuged at 2150 x g at 25 °C for 10 min using a refrigerated centrifuge (Beckman Coulter, Palo Alto, CA, USA). After centrifugation, gels along with the tubes were weighed (m_2) again after discarding the separated water. The syneresis was calculated as follows:

$$\text{Syneresis (\%)} = \frac{m_1 - m_2}{m_3} \times 100 \quad (2)$$

3.3.3.4 Acceptance test

Gel samples were cut into a bite-size (1 cm thickness and 2.5 cm diameter) and coded with 3-digit random numbers. The gel samples were kept at 8-10 °C until the acceptance test was performed. The 50 non-trained panelists (aged between 20 and 45) were students and staffs from the Department of Food Technology. All panelists, who were accustomed with gelatin products, were asked to assess for appearance, color, odor, firmness, springiness, mouth feel and overall liking of the gel samples using a 9-point hedonic scale (Meilgaard *et al.*, 2006). Gel samples were served on a covered white plastic cup under fluorescent daylight-type illumination. Between samples, panelists were asked to rinse their mouth with distilled water at room temperature.

3.3.3.5 Microstructure

Microstructures of FG and the selected FG/GL mixed gels were visualized using a scanning electron microscopy (SEM) following the method of Kaewdang and Benjakul (2015). All gels having a thickness of ~2-3 mm were fixed with 2.5% (v/v) glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 12 h. The samples were then rinsed with distilled water for 1 h and dehydrated in ethanol with a serial concentration of 25%, 50%, 70%, 80%, 90% and 100% (v/v). Thereafter, the samples were critical point dried using CO_2 as transition fluid. Dried samples were mounted on a bronze stub and sputter-coated with gold (Sputter coater SPI-Module, West Chester, PA, USA). The specimens were observed with a scanning electron microscope (Quanta 400; FEI, Eindhoven, Netherlands) at an acceleration voltage of 20 kV.

3.3.4 Statistical analysis

All experiments were run in triplicate. For experimental designs including control, completely randomized design (CRD) was used for textural and physical properties and randomized complete block design (RCBD) was used for acceptance test. Without control, factorial design with 2 factors (3 gellan levels × 4 $CaCl_2$ concentrations) was used for experimental design. Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by the Duncan's multiple range test (Steel and Torrie, 1980). Statistical analysis was performed using SPSS for Windows version 17 (SPSS Inc., Chicago, IL, USA). Data with $p < 0.05$ were considered to be statistically significant.

3.4 Results and discussion

3.4.1 Textural and gelling properties of FG gel as affected by GL and $CaCl_2$ at different levels

3.4.1.1 Gel strength

Gel strength of FG gel and mixed gels containing GL at 2.5-7.5% without and with $CaCl_2$ at different concentrations is presented in Table 7. Gel strength is one of the most important properties of gelatin gels, and the specific application of gel is

governed by the range of gel strength values (Cho *et al.*, 2005). When comparing the gel strength of all samples, FG gel had the lowest gel strength (194 g) ($p < 0.05$). The gel strength of FG/GL mixed gels increased as the level of GL increased ($p < 0.05$). The increase in gel strength of mixed gels might be caused by interactions between FG and GL, resulting in a stronger network. GL could form 'new heterolytic junction zones' networks with the gelatin molecules (Pranoto *et al.*, 2007). Anionic domains of GL might undergo ionic interaction with the positively charged residues of FG, thereby leading to the increased gel strength. Gel strength of mixed gels containing GL increased when the concentration of $CaCl_2$ increased ($p < 0.05$). GL itself has been known to yield the strong gel (Lee *et al.*, 2003). Generally, GL forms double helices, which further aggregate to form junction zones, and finally forms three-dimensional gel networks in the presence of monovalent or divalent cations (Sanderson and Clark, 1983). $CaCl_2$ could provide Ca^{2+}, which formed the bridges between pairs of carboxyl groups on neighbouring helices (Evageliou *et al.*, 2011). When $CaCl_2$ was incorporated, FG/GL mixed gel became more resistant to applied force as shown by the increased gel strength. Therefore, the incorporation of GL and $CaCl_2$ could increase the strength of FG gel in a dose-dependent manner.

3.4.1.2 Textural properties

TPA parameters of FG gel and the mixed gels having various GL levels in the absence and presence of $CaCl_2$ at different concentrations are shown in Table 8. Hardness of FG gel generally increased with increasing GL content ($p < 0.05$), regardless of $CaCl_2$ levels. The hardness of FG gel was ~14.76 N. Hardness of mixed gels increased as the concentration of $CaCl_2$ increased ($p < 0.05$). The highest hardness (173.17 N) was found in the mixed gel containing 7.5% GL and 9 mM $CaCl_2$ ($p < 0.05$). Increasing hardness was in agreement with the increase in gel strength (Table 7). Lau *et al.* (2000) reported that hardness of GL/bovine gelatin mixed gels increased when the ratio of GL to gelatin was increased. Hardness of GL gels generally increased with increasing calcium ion until calcium concentration reached a critical level (Tang *et al.*, 1995). Thus, GL and $CaCl_2$ played a role in

Table 7 Gel strength, gelling, melting temperatures and syneresis of FG gel as affected by the addition of GL and $CaCl_2$ at different levels

Gellan (% FG substitution)	$CaCl_2$ (mM)	Gel strength (g)	Gelling temperature (°C)	Melting temperature (°C)	Syneresis (%)
0	0	194±6 ^{m}	18.48±0.16 ^{l}	27.08±0.14 ^{i}	0.11±0.02 ^{i}
2.5	0	387±25 ^{lCz}	25.98±0.04 ^{kCz}	36.51±0.20 ^{hCx}	0.10±0.01 ^{iCz}
	3	592±25 ^{jCy}	26.44±0.13 ^{jCy}	36.89±0.03 ^{gCw}	0.12±0.01 ^{iCy}
	6	685±25 ^{iCx}	27.72±0.09 ^{iCx}	37.18±0.06 ^{f*}	0.15±0.03 ^{hCx}
	9	766±23 ^{gCw}	29.22±0.02 ^{hCw}	37.76±0.14 ^{e*}	0.20±0.01 ^{gCw}
5	0	537±16 ^{kBz}	36.27±0.07 ^{gBz}	49.81±0.05 ^{dBx}	0.14±0.04 ^{hiBz}
	3	808±28 ^{fBy}	37.69±0.09 ^{fBy}	63.15±0.09 ^{cBw}	0.22±0.02 ^{fBy}
	6	956±32 ^{eBx}	38.36±0.11 ^{eBx}	>90*	0.25±0.03 ^{eBx}
	9	1082±19 ^{dBw}	40.82±0.12 ^{cBw}	>90*	0.34±0.01 ^{dBw}
7.5	0	743±36 ^{hAz}	36.42±0.21 ^{gAz}	52.70±0.19 ^{bAx}	0.26±0.04 ^{eAz}
	3	1235±30 ^{cAy}	39.33±0.11 ^{dAy}	75.25±0.15 ^{aAw}	0.39±0.01 ^{cAy}
	6	1430±30 ^{bAx}	41.08±0.08 ^{bAx}	>90*	0.46±0.02 ^{bAx}
	9	1659±33 ^{aAw}	42.48±0.22 ^{aAw}	>90*	0.53±0.02 ^{aAw}

Values are presented as mean ± SD (n = 3). *No comparison was done.

Different lowercase (a-p) superscripts within the same column indicate significant differences including control ($p < 0.05$).

Different uppercase (A-C) superscripts in the same column indicate significant differences between GL levels ($p < 0.05$).

Different lowercase (w-z) superscripts in the same column indicate significant differences between $CaCl_2$ concentrations ($p < 0.05$).

increasing the hardness of FG/GL mixed gels. FG gel had the highest springiness (0.98 cm), while FG gel containing 7.5% GL and 9 mM $CaCl_2$ had the lowest springiness (0.84 cm) ($p < 0.05$). With the addition of 2.5% GL, springiness of the mixed gels slightly decreased with increasing concentrations of $CaCl_2$ ($p < 0.05$). However, there was no difference in springiness between FG gel containing 2.5% GL in the presence of 3, 6 and 9 mM $CaCl_2$ ($p > 0.05$). Gel structure being broken into a few large pieces during the first TPA compression yields high springiness. Low springiness is attributed to the gel breaking into many small pieces (Yang *et al.*, 2007). Less springy gels, such as low-methoxy pectin, carrageenan and agar gels would break down more easily during mastication than a springy gelatin gel (Lau *et al.*, 2000). Generally, GL forms hard and brittle gels. On the other hand, gelatin forms soft, flexible and elastic gels (Nussinovitch, 1997). It was noted that FG gel became more brittle (lower springiness) when GL was added, especially in combination with $CaCl_2$ at higher concentrations. The addition of GL contributed to the increased rigidity or brittleness of FG gel. Thus, the addition of GL and $CaCl_2$ at high level negatively affected the textural characteristic of FG/GL mixed gels as indicated by the loss in springiness. The cohesiveness of FG gel was 0.85. Cohesiveness of FG gels decreased as the level of GL increased ($p < 0.05$). Increasing $CaCl_2$ concentration also resulted in the decreases in cohesiveness of mixed gel ($p < 0.05$). Enhanced interaction between FG and GL via the calcium bridge more likely contributed to the stronger gel as shown by the increased chewiness and gumminess. Gumminess and chewiness were increased in the mixed gel containing GL and $CaCl_2$ in a dose dependent manner ($p < 0.05$). FG gel had the lowest gumminess and chewiness (12.49 N and 12.02 N × cm, respectively), and FG gel containing 7.5% GL and 9 mM $CaCl_2$ had the highest values (84.82 N and 77.45 N × cm, respectively) ($p < 0.05$). Moreover, the addition of GL markedly increased the adhesiveness of FG gel ($p < 0.05$). However, there were no differences in adhesiveness between mixed gels containing 2.5 and 5% GL, regardless of $CaCl_2$ concentrations ($p > 0.05$). Therefore, the levels of GL and $CaCl_2$ were the major factors affecting the texture of the resulting mixed gels.

Table 8 Texture profile analysis (TPA) of FG gel as affected by the addition of GL and $CaCl_2$ at different levels

Gellan (% FG substitution)	$CaCl_2$ (mM)	Hardness (N)	Springiness (cm)	Cohesiveness	Adhesiveness (N × s)	Gumminess (N)	Chewiness (N × cm)
0	0	14.76±0.47 ^{m}	0.98±0.01 ^{a}	0.85±0.02 ^{a}	-0.71±0.09 ^{g}	12.49±0.28 ^{m}	12.02±0.27 ^{m}
2.5	0	32.07±2.67 ^{lCz}	0.96±0.00 ^{bAw}	0.83±0.01 ^{bAw}	-0.09±0.01 ^{fBy}	26.47±2.33 ^{lCz}	25.37±2.18 ^{lCz}
	3	53.63±1.08 ^{jCy}	0.94±0.01 ^{cAx}	0.66±0.02 ^{dAx}	-0.08±0.00 ^{eBx}	35.55±0.76 ^{kCy}	33.51±0.75 ^{kCy}
	6	61.20±1.52 ^{iCx}	0.94±0.01 ^{cAx}	0.62±0.03 ^{fAy}	-0.08±0.01 ^{eBx}	37.82±0.94 ^{jCx}	35.59±0.65 ^{jCx}
	9	70.89±2.59 ^{hCw}	0.94±0.00 ^{cAy}	0.60±0.02 ^{fAz}	-0.08±0.02 ^{eBw}	42.87±1.67 ^{hCw}	40.42±1.68 ^{hCw}
5	0	50.38±1.39 ^{kBz}	0.93±0.00 ^{dBw}	0.78±0.01 ^{cBw}	-0.09±0.01 ^{fBy}	39.12±1.08 ^{iBz}	36.71±1.20 ^{iBz}
	3	95.43±1.11 ^{fBy}	0.89±0.01 $^{f Bx}$	0.57±0.01 ^{gBx}	-0.08±0.00 ^{eBx}	54.68±0.63 ^{fBy}	48.69±0.72 ^{fBy}
	6	112.28±2.98 ^{eBx}	0.89±0.01 ^{fBx}	0.54±0.02 ^{iBy}	-0.08±0.02 ^{eBx}	60.99±1.69 ^{eBx}	54.52±1.53 ^{eBx}
	9	127.83±2.72 ^{dBw}	0.87±0.01 ^{hBy}	0.53±0.00 ^{jBz}	-0.07±0.01 ^{dBw}	67.20±1.76 ^{dBw}	58.47±3.47 ^{dBw}
7.5	0	78.97±0.39 ^{gAz}	0.91±0.00 ^{eCw}	0.65±0.01 ^{eCw}	-0.07±0.05 ^{dAy}	51.06±0.33 ^{gAz}	46.72±0.86 ^{gAz}
	3	152.01±1.67 ^{cAy}	0.88±0.01 ^{gCx}	0.55±0.02 ^{hCx}	-0.06±0.02 ^{cAx}	82.85±1.24 ^{cAy}	73.04±1.17 ^{cAy}
	6	165.94±0.63 ^{bAx}	0.86±0.00 ^{iCx}	0.50±0.00 ^{kCy}	-0.05±0.01 ^{bAx}	83.53±1.12 ^{bAx}	75.15±1.20 ^{bAx}
	9	173.17±2.57 ^{aAw}	0.84±0.01 ^{jCy}	0.49±0.01 ^{lCz}	-0.04±0.00 ^{aAw}	84.82±1.26 ^{aAx}	77.45±1.11 ^{aAw}

Values are presented as mean ± SD (n = 3).

Different lowercase (a-p) superscripts within the same column indicate significant differences including control ($p < 0.05$).

Different uppercase (A-C) superscripts in the same column indicate significant differences between GL levels ($p < 0.05$).

Different lowercase (w-z) superscripts in the same column indicate significant differences between $CaCl_2$ concentrations ($p < 0.05$).

3.4.1.3 Gelling and melting temperatures

Gelling and melting temperatures of FG gel, mixed gels having various GL levels with and without $CaCl_2$ are shown in Table 7. Apart from gel strength, gelling and melting temperatures are important functional properties of gelatin since they determine physical characteristic in actual application in food products (Pranoto *et al.*, 2007). Gelling temperatures of FG was 18.48 °C. This indicated that FG could not form gel at room temperature. Gelling temperature of FG/GL mixed gels increased as levels of GL were increased ($p < 0.05$). All mixed gels were able to set at room temperature (>25 °C). However, no differences in gelling temperature between the mixed gels containing 5 and 7.5% GL were observed ($p > 0.05$), in the absence of Ca^{2+}. The result suggested that the interaction between GL and FG via ionic interaction might favor the gel formation of the mixture (Pranoto *et al.*, 2007). When $CaCl_2$ concentrations were increased, the increases in gelling temperatures of gel containing GL were attained ($p < 0.05$). The highest gelling temperatures (42.48 °C) was obtained for FG gel containing 7.5% GL in combination with 9 mM $CaCl_2$ ($p < 0.05$). In general, gelling temperature of GL increased when the level of GL and Ca^{2+} increased (Tang *et al.*, 1997). The results reconfirmed that the presence of Ca^{2+} could promote the formation of junction zone in the mixed gels via the formation of bridges between biopolymers. A similar phenomenon was also observed by Lau *et al.* (2001) who reported that gelling temperature and gelation rate of the GL/bovine mixed gels were significantly affected by the ratio of GL to bovine gelatin as well as the concentration of calcium ion used.

All the mixed gels showed higher melting temperature than FG gel ($p < 0.05$). Melting temperature of FG/GL mixed gels was increased with increasing GL content ($p < 0.05$). Increasing $CaCl_2$ concentration caused an increase in melting temperature of mixed gel ($p < 0.05$). Nevertheless, the mixed gels containing 5 and 7.5% GL in the presence of 6 and 9 mM $CaCl_2$ had melting temperatures above 90 °C. Those gels were not molten at the range of temperature tested (5-90 °C). Increasing melting temperature of mixed gels was in accordance with increasing gelling temperature of FG/GL mixed solutions. In general, the increases in gelling and melting temperatures are due to the increasing number of chemical junctions

responsible for the formation of three-dimensional gel networks (Saito *et al.*, 2007). The result suggested that GL and $CaCl_2$ directly contributed to the melting behavior of a mixed gel. Incorporation of GL along with $CaCl_2$ played a role in increasing the stability of the FG/GL mixed gel network, as evidenced by the increased melting temperatures. Furthermore, $CaCl_2$ addition could induce the gel formation. Gel was formed suddenly after $CaCl_2$ was added. Thus, $CaCl_2$ could help in setting gel (data not shown).

3.4.2 Color of FG gel as affected by GL and $CaCl_2$ at different levels

Color of FG gel and all mixed gels expressed as L^*, a^*, b^* and ΔE^* is shown in Table 9. Among all gel samples, FG gel had the higher lightness (L^*-value), compared with mixed gels ($p < 0.05$). The L^*-values of mixed gels continuously decreased with increasing levels of GL and $CaCl_2$ added ($p < 0.05$). The lowest L^*-value (56.30) was obtained for FG gel containing 7.5% GL in the presence of 9 mM $CaCl_2$ ($p < 0.05$). The decreases in L^*-values of FG gel added with GL, especially at higher levels, were shown by the development of dull/opaque gels (Figure 7). A similar phenomenon was also observed by Pranoto *et al.* (2007) who reported that L^*-value was significantly decreased by the addition of 2% GL into FG based film. Moreover, the addition of GL increased the redness (a^*-value) of the mixed gels. On the other hand, increasing $CaCl_2$ concentration caused a decrease in a^*-value of gel containing GL ($p < 0.05$). Yellowness (b^*-value) of gel decreased as GL content was increased ($p < 0.05$). However, no differences in b^*-value between the mixed gels containing 2.5% GL and FG gel were found ($p > 0.05$). The b^*-value of mixed gels decreased as the concentrations of $CaCl_2$ increased ($p < 0.05$). Nevertheless, no differences in b^*-value between the mixed gels containing 2.5% GL without and with 3 mM $CaCl_2$ were observed ($p > 0.05$). Similar gels were obtained (Figure 7). Overall, the increases in ΔE^*-value were in agreement with the decreases in L^*- and b^*-values. FG gel showed the lowest ΔE^*-value (23.45) with the highest L^*- and b^*-values. The results also suggested that the color of mixed gels was influenced by the color of GL and also determined the appearance (Figure 7). Additionally, the way the hydrocolloids were dispersed and interacted with each other

in the gel network also had the impact on color of mixed gel, particularly those incorporated with $CaCl_2$ at high levels.

Table 9 Color values of FG gel as affected by the addition of GL and $CaCl_2$ at different levels

Gellan (% FG substitution)	$CaCl_2$ (mM)	L^*	a^*	b^*	ΔE^*
0	0	75.42±0.07 ^{a}	-1.92±0.02 ^{m}	14.86±0.06 ^{a}	23.45±0.06 ^{m}
2.5	0	68.18±0.01 ^{bAw}	2.02±0.03 ^{aAw}	14.80±0.08 ^{aAw}	29.71±0.04 ^{lCz}
	3	63.28±0.09 ^{cAx}	1.90±0.06 ^{bAx}	14.76±0.04 ^{aAx}	33.65±0.05 ^{kCy}
	6	63.13±0.03 ^{dAy}	1.30±0.02 ^{gAy}	14.51±0.22 ^{bAy}	33.81±0.11 ^{jCx}
	9	63.03±0.05 ^{eAz}	1.04±0.03 ^{jAz}	14.07±0.03 ^{cAz}	34.09±0.05 ^{iCw}
5	0	62.37±0.03 ^{fBw}	1.65±0.10 ^{cBw}	13.81±0.06 ^{dBw}	34.58±0.02 ^{hBz}
	3	61.01±0.02 ^{gBx}	1.56±0.05 ^{dBx}	11.76±0.05 ^{eBx}	34.68±0.03 ^{gBy}
	6	59.40±0.01 ^{iBy}	1.17±0.10 ^{hBy}	11.57±0.06 ^{fBy}	36.11±0.03 ^{fBx}
	9	57.66±0.02 ^{kBz}	0.72±0.02 ^{kBz}	8.73±0.18 ^{gBz}	37.04±0.04 ^{dbw}
7.5	0	59.67±0.24 ^{hCw}	1.44±0.02 ^{eCw}	8.04±0.10 ^{hCw}	36.78±0.02 ^{eAz}
	3	57.94±0.02 ^{jCx}	1.35±0.04 ^{fCx}	7.49±0.04 ^{iCx}	37.47±0.01 ^{cAy}
	6	57.37±0.02 ^{lCy}	1.13±0.10 ^{iCy}	5.71±0.13 ^{jCy}	38.21±0.03 ^{bAx}
	9	56.30±0.01 ^{mCz}	0.55±0.02 ^{lCz}	2.43±0.07 ^{kCz}	38.78±0.09 ^{aAw}

Values are presented as mean ± SD (n = 3). Different lowercase (a-p) superscripts within the same column indicate significant differences including control ($p < 0.05$). Different uppercase (A-C) superscripts in the same column indicate significant differences between GL levels ($p < 0.05$). Different lowercase (w-z) superscripts in the same column indicate significant differences between $CaCl_2$ concentrations ($p < 0.05$).

3.4.3 Syneresis of FG gel as affected by GL and $CaCl_2$ at different levels

Syneresis of FG gel and mixed gels is shown in Table 7. Among all gel samples, the highest syneresis (0.53%) was found in the FG gel containing 7.5% GL with 9 mM $CaCl_2$ ($p < 0.05$). Syneresis of FG/GL mixed gel generally increased with increasing GL levels ($p < 0.05$). It was noted that FG gel mixed with 2.5 and 5% GL showed no differences in syneresis, compared with FG gel alone ($p > 0.05$). When $CaCl_2$ was added, the increases in syneresis were attained in a dose-dependent manner ($p < 0.05$). Syneresis is a natural phenomenon, indicating unbound excess water released from the gel matrix (Banerjee and Bhattacharya, 2011). Sinthusamran *et al.*

(2016) also found that syneresis of agar/FG mixed gel increased with increasing agar levels. The interaction between FG and GL, especially in the presence of $CaCl_2$, might yield more compact structure, thereby having less space for water entrapment. As a result, free or unbound water was released to a higher extent in the presence of both GL and $CaCl_2$.

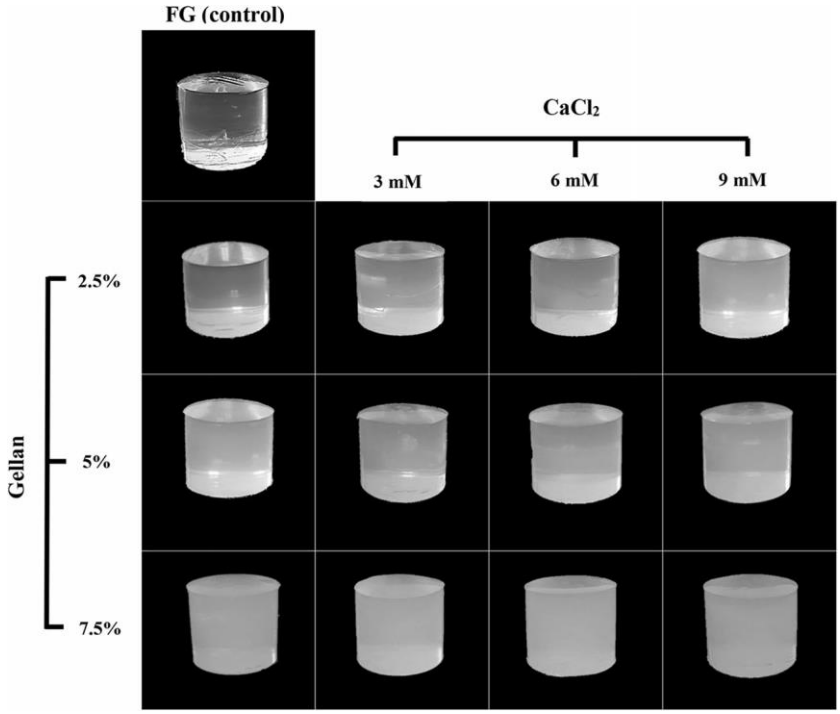

Figure 7 The photographs of FG gel as affected by the addition of GL and $CaCl_2$ at different levels.

3.4.4 Acceptability of FG gel as affected by GL and $CaCl_2$ at different levels

Likeness scores of FG gel and mixed gels containing GL and $CaCl_2$ at various levels are shown in Table 10. Addition of GL decreased appearance and color likeness score of FG/GL mixed gels, especially when the level of GL was increased ($p < 0.05$). Increasing $CaCl_2$ concentration also caused the decrease in appearance and color likeness score of mixed gel ($p < 0.05$). These results correlated with the decreases in lightness and the increased ΔE^*-value (Table 9). However, the addition of GL and $CaCl_2$ at all levels tested did not affect the odor likeness score ($p > 0.05$). Firmness and springiness likeness scores of FG gel were generally decreased as the levels of GL were increased ($p < 0.05$). Nevertheless, there were no differences in firmness and

springiness likeness scores between FG gel and FG containing 2.5% GL ($p > 0.05$). The increases in $CaCl_2$ decreased firmness and springiness likeness scores of mixed gels ($p < 0.05$). Mixed gels with higher hardness and lower springiness generally had lower firmness and springiness likeness scores (Table 8). Gel with higher brittleness and lower elasticity in texture was undesirable for gelatin gel product (Sinthusamran *et al.*, 2016). Mixed gels showed lower mouth feel likeness score than FG gel alone ($p < 0.05$), except for those with 2.5% GL addition, which showed similar score ($p > 0.05$). Mouth feel likeness score of mixed gels decreased as the concentrations of $CaCl_2$ increased ($p < 0.05$). However, it was noted there was no difference in mouth feel likeness score of mixed gels containing 2.5% GL, regardless of $CaCl_2$ concentrations, compared to that of FG gel ($p > 0.05$). The decreased mouth feel likeness score was most likely caused by the increased melting temperature as shown in Table 7. Gels containing 2.5% GL with and without $CaCl_2$ had melting temperature of approximately 37 °C. As a consequence, those gels could be molten in the mouth, which was similar to FG gel alone. This melt-in-mouth property is one of the most important characteristics of gelatin gels (Karim and Bhat, 2009). The addition of GL in combination with $CaCl_2$ also decreased overall likeness of the resulting gels ($p < 0.05$). However, no difference between FG gel and mixed gel containing 2.5% GL in the presence of $CaCl_2$ up to 6 mM ($p > 0.05$). It was concluded that the addition of 2.5% GL in combination with $CaCl_2$ up to 6 mM had no detrimental effect on the acceptance of the resulting mixed gels.

3.4.5 Microstructures of FG gel as affected by GL and $CaCl_2$ at different levels

Microstructures of mixed gels containing 2.5% GL in the presence of $CaCl_2$ at different concentrations in comparison with that of FG gel are illustrated in Figure 8. FG gel (control) had a coarser network with larger void or cavities (Figure 8a). With the addition of 2.5% GL, the mixed gels showed the slightly larger strands with larger voids (Figure 8b). The similar phenomenon was found in the sample containing 2.5% GL and 3 mM $CaCl_2$ (Figure 8c). With increasing levels of $CaCl_2$ from 6 to 9 mM, the finer network with smaller voids was observed (Figure 8d, e). The interconnectivity was more pronounced when 9 mM $CaCl_2$ was incorporated. This

was in accordance with the increased gel strength and gelling point of the mixed gel added with higher $CaCl_2$ concentration. In general, microstructure of gel networks is

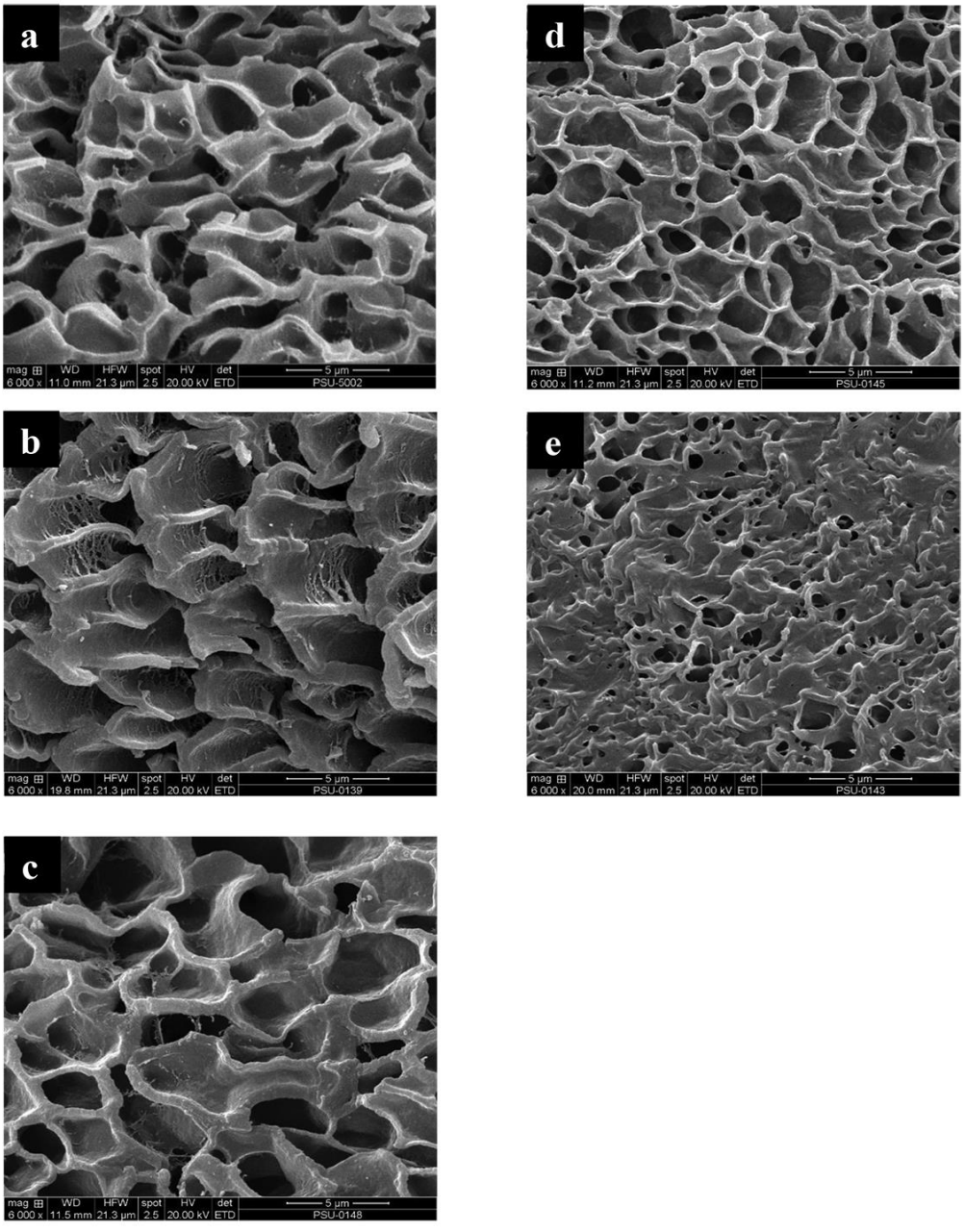

Figure 8 Microstructures of FG gel (a) and mixed gels containing 2.5% GL in the presence of $CaCl_2$ at 0 mM (b), 3 mM (c), 6 mM (d) and 9 mM (e). Magnification: 6000X.

Table 10 Acceptability of FG gel as affected by the addition of GL and $CaCl_2$ at different levels

Gellan (% substitution)	$CaCl_2$ (mM)	Appearance	Color	Odor	Firmness	Springiness	Mouth feel	Overall
0	0	8.65±1.02 ^{a}	8.24±0.77 ^{a}	6.98±1.50 ^{aAw}	7.96±1.17 ^{a}	7.96±1.11 ^{a}	7.80±1.05 ^{a}	7.94±1.13 ^{a}
2.5	0	7.78±0.95 ^{bAw}	7.96±0.88 ^{bAw}	7.06±1.44 ^{aAw}	7.86±1.03 ^{aAw}	7.80±0.81 ^{aAw}	7.82±0.95 ^{aAw}	7.80±0.81 ^{aAw}
	3	7.54±1.05 ^{cAx}	7.72±0.78 ^{cAx}	7.04±1.33 ^{aAw}	7.32±1.70 ^{bAx}	7.56±1.12 ^{aAx}	7.80±1.18 ^{aAx}	7.48±1.08 ^{abAx}
	6	7.42±1.01 ^{dAy}	7.54±1.55 ^{dAy}	6.95±1.57 ^{aAw}	7.38±1.05 ^{bAy}	7.30±1.05 ^{bAy}	7.84±1.00 ^{aAy}	7.49±1.25 ^{abAy}
	9	7.38±1.05 ^{eAz}	7.54±1.48 ^{dAz}	7.13±1.74 ^{aAw}	7.35±1.19 ^{bAz}	7.24±1.18 ^{bAz}	7.74±0.98 ^{aAz}	7.20±1.09 ^{bAz}
5	0	7.16±1.17 ^{fBw}	7.06±1.13 ^{eBw}	7.09±1.46 ^{aAw}	7.71±0.86 ^{aBw}	7.38±1.97 ^{abBw}	7.19±1.00 ^{bBw}	7.52±1.05 ^{abBw}
	3	7.00±1.12 ^{gBx}	6.92±0.94 ^{fBx}	6.95±1.38 ^{aAw}	6.82±1.06 ^{cBx}	6.48±1.03 ^{cBx}	6.48±0.49 ^{eBx}	6.60±1.03 ^{cBx}
	6	6.84±1.13 ^{hBy}	6.82±1.05 ^{gBy}	7.21±1.30 ^{aAw}	6.56±0.91 ^{eBy}	6.20±1.16 ^{eBy}	6.32±1.00 ^{fBy}	6.40±1.03 ^{eBy}
	9	6.54±1.07 ^{jBz}	6.60±1.04 ^{hBz}	7.16±1.36 ^{aAw}	6.38±0.59 ^{fBz}	6.06±1.01 ^{fBz}	5.90±0.83 ^{hBz}	6.16±1.13 ^{fBz}
7.5	0	6.72±0.52 ^{icw}	5.86±1.18 ^{iCw}	6.97±1.43 ^{aAw}	6.72±1.23 ^{dCw}	6.34±0.72 ^{dCw}	6.72±1.05 ^{cCw}	6.56±1.07 ^{dCw}
	3	6.68±0.40 ^{kCx}	5.78±0.52 ^{jCx}	7.11±1.44 ^{aAw}	5.92±1.16 ^{gCx}	5.90±0.45 ^{gCx}	6.61±1.13 ^{dCx}	6.14±0.22 ^{gCx}
	6	6.31±1.18 ^{lCy}	5.60±1.25 ^{jCy}	7.19±1.30 ^{aAw}	5.64±1.04 ^{hCy}	5.52±1.13 ^{hCy}	6.22±1.05 ^{gCy}	6.00±0.49 ^{hCy}
	9	6.00±0.99 ^{mCz}	5.46±1.16 ^{kCz}	7.23±1.56 ^{aAw}	5.12±1.13 ^{iCz}	5.48±0.67 ^{iCz}	6.02±0.95 ^{hCz}	5.64±0.13 ^{iCz}

Values are presented as mean ± SD.

Different lowercase (a-p) superscripts within the same column indicate significant differences including control ($p < 0.05$).

Different uppercase (A-C) superscripts in the same column indicate significant differences between GL levels ($p < 0.05$).

Different lowercase (w-z) superscripts in the same column indicate significant differences between $CaCl_2$ concentrations ($p < 0.05$).

considered as an important factor influencing the physical properties and viscoelastic behavior of gel (Sinthusamran *et al.*, 2014; Tang *et al.*, 1998). The structure of gel matrix is related with the gel strength of gelatin (Benjakul *et al.*, 2009). The more compact and denser gel network was related with higher gel strength of mixed gel (Table 7). Basically, stress relaxation characteristics of GL gel was governed by microstructure (Tang *et al.*, 1998). The result suggested that GL and $CaCl_2$ could provide reinforcement for the gelatin network, in which interconnection via Ca^{2+}-bridges could be augmented. Thus, the levels of GL and the concentrations of $CaCl_2$ had the marked influence on the gel network associated with the textural properties of gel.

3.5 Conclusions

The addition of GL to FG in the presence of $CaCl_2$ directly affected the properties of mixed gels. Gel strength of FG/GL mixed gels was strongly influenced by the level of added GL. Increasing $CaCl_2$ concentration caused an increase in gel strength of FG/GL mixed gels. Hardness generally increased, but springiness and cohesiveness of the mixed gels decreased as the levels of GL and $CaCl_2$ increased. With increasing levels of GL and $CaCl_2$, L^*- and b^*-values of mixed gel decreased, while ΔE^*-value increased. Both gelling and melting temperatures as well as syneresis of the mixed gels increased with increasing levels of GL and $CaCl_2$. Mixed gel had the denser structure with very small voids in the presence of $CaCl_2$ at high concentration. With higher levels of GL and $CaCl_2$ added, the acceptance, namely appearance, color, firmness, springiness and mouth feel likeness, decreased. This was more likely due to the changes in mouth feel associated with the increased brittleness and lower melting of resulting gel. However, the addition of GL at low level (2.5%) with $CaCl_2$ up to 6 mM had no negative effect on acceptance of mixed gels but could improve gelling property of FG via increasing gel strength and gelling points.

3.6 References

Banerjee, S. and Bhattacharya, S. 2011. Compressive textural attributes, opacity and syneresis of gels prepared from gellan, agar and their mixtures. Journal of Food Engineering. 102: 287-292.

Benjakul, S., Oungbho, K., Visessanguan, W., Thiansilakul, Y. and Roytrakul, S. 2009. Characteristics of gelatin from the skins of bigeye snapper, *Priacanthus tayenus* and *Priacanthus macracanthus*. Food Chemistry. 116: 445-451.

Cho, S. M., Gu, Y. S. and Kim, S. B. 2005. Extracting optimization and physical properties of yellowfin tuna (*Thunnus albacares*) skin gelatin compared to mammalian gelatins. Food Hydrocolloids. 19: 221-229.

Evageliou, V., Gerolemou, A., Zikas, A., Basios, A. and Komaitis, M. 2011. Effect of salts and sugars on the clarity of gellan gels. International Journal of Food Science and Technology. 46: 1001-1006.

Gennadios, A., Weller, C. L., Hanna, M. A. and Froning, G. W. 1996. Mechanical and barrier properties of egg albumen films. Journal of Food Science. 61: 585-589.

Haug, I. J., Draget, K. I. and Smidsrød, O. 2004. Physical behaviour of fish gelatin-κ-carrageenan mixtures. Carbohydrate Polymers. 56: 11-19.

Huang, T., Tu, Z. C., Wang, H., Shangguan, X., Zhang, L., Zhang, N. H. and Bansal, N. 2017. Pectin and enzyme complex modified fish scales gelatin: Rheological behavior, gel properties and nanostructure. Carbohydrate Polymers. 156: 294-302.

Jongjareonrak, A., Benjakul, S., Visessanguan, W. and Tanaka, M. 2006. Skin gelatin from bigeye snapper and brownstripe red snapper: Chemical compositions and effect of microbial transglutaminase on gel properties. Food Hydrocolloids. 20: 1216-1222.

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

Karim, A. A. and Bhat, R. 2009. Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins. Food Hydrocolloids. 23: 563-576.

Lau, M. H., Tang, J. and Paulson, A. T. 2000. Texture profile and turbidity of gellan/gelatin mixed gels. Food Research International. 33: 665-671.

Lau, M. H., Tang, J. and Paulson, A. T. 2001. Effect of polymer ratio and calcium concentration on gelation properties of gellan/gelatin mixed gels. Food Research International. 34: 879-886.

Lee, K. Y., Shim, J., Bae, I. Y., Cha, J., Park, C. S. and Lee, H. G. 2003. Characterization of gellan/gelatin mixed solutions and gels. LWT - Food Science and Technology. 36: 795-802.

Liu, H., Li, D. and Guo, S. 2008. Rheological properties of channel catfish (*Ictalurus punctaus*) gelatine from fish skins preserved by different methods. LWT - Food Science and Technology. 41: 1425-1430.

Meilgaard, M. C., Carr, B. T. and Civille, G. V. 2006. Sensory evaluation techniques. CRC press. Florida.

Mohtar, N. F., Perera, C. and Quek, S. Y. 2010. Optimisation of gelatine extraction from hoki (*Macruronus novaezelandiae*) skins and measurement of gel strength and SDS-PAGE. Food Chemistry. 122: 307-313.

Mohtar, N. F., Perera, C. O., Quek, S. Y. and Hemar, Y. 2013. Optimization of gelatine gel preparation from New Zealand hoki (*Macruronus novaezelandiae*) skins and the effect of transglutaminase enzyme on the gel properties. Food Hydrocolloids. 31: 204-209.

Nickerson, M. T., Paulson, A. T. and Hallett, F. R. 2008. Pre-gel solution properties of gellan polysaccharides: Effect of potassium and calcium ions on chain associations. Food Research International. 41: 462-471.

Nussinovitch, A. 1997. Gellan gum. *In* Hydrocolloid Applications: Gum technology in the food and other industries. (Nussinovitch, A., ed.). p. 63-82. Springer. Boston, US.

Pranoto, Y., Lee, C. M. and Park, H. J. 2007. Characterizations of fish gelatin films added with gellan and κ-carrageenan. LWT - Food Science and Technology. 40: 766-774.

Saito, H., Taguchi, T., Aoki, H., Murabayashi, S., Mitamura, Y., Tanaka, J. and Tateishi, T. 2007. pH-responsive swelling behavior of collagen gels prepared

by novel crosslinkers based on naturally derived di- or tricarboxylic acids. Acta Biomaterialia. 3: 89-94.

Sanderson, G. R. and Clark, R. C. 1983. Gellan gum. Food Technology. 37: 62-70.

Transparency market research Web. 2014. Gelatin Market - global industry analysis, size, share, growth, trends and fsorecast, 2012-2018 (online). Available: http://www.transparencymarketresearch.com/gelatin.html. (01 Fabruary 2017)

Sinthusamran, S., Benjakul, S. and Hemar, Y. 2016. Rheological and sensory properties of fish gelatin gels as influenced by agar from *Gracilaria tenuistipitata*. International Journal of Food Science and Technology. 51: 1530-1536.

Sinthusamran, S., Benjakul, S. and Kishimura, H. 2014. Characteristics and gel properties of gelatin from skin of seabass (*Lates calcarifer*) as influenced by extraction conditions. Food Chemistry. 152: 276-284.

Sinthusamran, S., Benjakul, S. and Kishimura, H. 2015. Molecular characteristics and properties of gelatin from skin of seabass with different sizes. International Journal of Biological Macromolecules. 73: 146-153.

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

Tang, J., Tung, M. A. and Zeng, Y. 1995. Mechanical properties of gellan gels in relation to divalent cations. Journal of Food Science. 60: 748-752.

Tang, J., Tung, M. A. and Zeng, Y. 1997. Gelling temperature of gellan solutions containing calcium ions. Journal of Food Science. 62: 276-280.

Tang, J., Tung, M. A. and Zeng, Y. 1998. Characterization of gellan gels using stress relaxation. Journal of Food Engineering. 38: 279-295.

Yang, H., Wang, Y., Jiang, M., Oh, J. H., Herring, J. and Zhou, P. 2007. 2-step optimization of the extraction and subsequent physical properties of channel catfish (*Ictalurus punctatus*) skin gelatin. Journal of Food Science. 72: 188-195.

Yang, L. and Paulson, A. T. 2000. Mechanical and water vapour barrier properties of edible gellan films. Food Research International. 33: 563-570.

Yasin, H., Babji, A. S. and Norrakiah, A. S. 2017. Modification of chicken feet gelatin with aqueous sweet basil and lemongrass extract. LWT - Food Science and Technology. 77: 72-79.

CHAPTER 4

EFFECT OF GELLAN INCORPORATION ON GEL PROPERTIES OF BIGEYE SNAPPER SURIMI

4.1 Abstract

Effects of gellan on gelling properties of surimi from bigeye snapper (*Priacanthus macranthus*) were investigated. Two forms of gellan, powder (GLP) and suspension (GLS), were incorporated into surimi to obtain different final concentrations (2-8%, based on surimi solid content). Gels added with GLP or GLS had the increases in breaking force and hardness as the levels were increased ($p < 0.05$). The highest breaking force and hardness were observed in surimi gel containing 8% GLS ($p < 0.05$). Water holding capacity and whiteness of resulting gels were increased as levels of GLP and GLS increased ($p < 0.05$). Electrophoretic studies showed that gellan at all levels had no effect on polymerization of myosin heavy chain. Addition of GLP and GLS could enhance the interconnection between chains during heating as indicated by the higher G'. Both GLP and GLS increased acceptability of surimi gel as the level of gellan increased up to 6% ($p < 0.05$). However, the decrease in overall likeness score was found in gel added with 8% GLS. Microstructure studies revealed that finer and denser network was observed in surimi gel containing 6% GLS in comparison with that of the control. Therefore, gellan, prepared as suspension, at an appropriate level could improve gel strength of bigeye snapper surimi with an increased acceptability.

4.2 Introduction

Surimi is a concentrated myofibrillar proteins obtained by successive washing of minced fish, in which sarcoplasmic proteins and undesirable substances such as fat, blood, pigment and odorous substances are removed (Eymard *et al.*, 2005). Surimi is a useful ingredient for producing various kinds of processed foods with the unique textural property. Surimi-based products such as fish ball, fish

sausage, breaded fish stick and paupiette, etc. have gained increasing attention due to the preferred textural properties and high nutritional value (Zhou *et al.*, 2017). However, some surimi, particularly from poor quality material, may render the weak gel with low acceptability. To improve the properties of surimi gel, a number of additives with different functions have been employed. Some additives are used to retard proteolysis (Singh and Benjakul, 2017a; Singh and Benjakul, 2017b), while protein cross-linkers such as microbial transglutaminase (Chanarat *et al.*, 2012; Seighalani *et al.*, 2016) or phenolic compounds (Buamard and Benjakul, 2015; Buamard *et al.*, 2017) have been added to strengthen the gel of surimi. Moreover, some hydrocolloids such as curdlan, pullulan, carrageenan and pectin, etc. have been used to improve the properties of surimi gels (Barrera *et al.*, 2002; Hunt and Park, 2013; Wei *et al.*, 2017; Wu, 2016).

Gellan is an anionic microbial polysaccharide, secreted from *Sphingomonas elodea*, consisting of repeating tetrasaccharide units of glucose, glucuronic acid and rhamnose residues at a ratio of 2:1:1 (Matricardi *et al.*, 2009). At high temperatures (>90 °C), gellan is dispersed in water in a disordered random coil state. Upon cooling to gelling temperature, gellan forms double helices, in which junction zones are formed, and finally three-dimensional gel networks are developed in the presence of monovalent or divalent cations (Evageliou *et al.*, 2011). Owing to its gelling ability, it has been used in protein gels such as gelatin and myofibrillar proteins. Petcharat and Benjakul (2017) reported that the addition of 2.5% gellan along with calcium chloride (up to 6 mM) could improve property of fish gelatin gel without affecting acceptance of the resulting mixed gel. Gellan was able to improve the properties of silver carp surimi-protein soybean composite product added with fat pork by increasing gel strength and decreasing the water loss (Zhou *et al.*, 2008). Moreover, gellan was used to improve quality of reduced-fat frankfurters. Konjac (1% and 2%) incombination with gellan (0.25% and 0.5%) were incorporated into reduced fat (18%) frankfurters, yielding the finished product with acceptability (Lin and Huang, 2003). Since gellan is not water-soluble, the distribution in surimi paste at low temperature is poor and may impede the development of ordered gel network. Thus, the appropriate preparation of gellan prior to incorporation into surimi could be another means to

better exploit gellan as the gel strengthener of surimi. Additionally, the level of gellan can be another factor affecting the property of surimi gel. Therefore, the present study aimed to investigate the effect of gellan with different preparations at various levels on properties of bigeye snapper surimi gel.

4.3 Materials and methods

4.3.1 Materials

Food-grade low acyl gellan was purchased from CP Kelco (Atlanta, GA, USA). Grade A frozen surimi from bigeye snapper (*Priacanthus macranthus*) was obtained from Man A Frozen Foods Co., Ltd. (Songkhla, Thailand) and kept at -20 °C until use but not longer than 2 months. Surimi had a moisture content of 75% (w/w).

4.3.2 Preparation of gellan suspension

To prepare gellan suspension (GLS), gellan (1, 2, 3 and 4 g) were dispersed in distilled water at a gellan/water ratio of 1:15 (w/v). Ultrasound was applied using an ultrasonic processor (Sonics, Model VC750, Sonica & Materials, Inc., Newtown, CT, USA) at the amplitude of 40% for 4 min with a constant frequency of 20 kHz ± 50 Hz and high intensity power of 750 W.

4.3.3 Preparation of surimi gel added with gellan

Frozen surimi (200 g) was partially thawed at 4 °C for 2-3 h until the core temperature reached 0-2 °C. The surimi was chopped into small pieces and mixed with 2.5% salt in a mixer (Panasonic, Model MK-5087M, Selangor, Malaysia) for 2 min. During chopping, the temperature was maintained below 10 °C. The paste was either added with gellan powder (GLP) or GLS to obtain the final gellan levels of 0, 2, 4, 6 and 8% (based on surimi solid content). The moisture content of all surimi paste was adjusted to 80% with cold distilled water. Then, the mixture was chopped for another 3 min and the paste was stuffed into a polyvinylidene chloride casing with a diameter of 2.5 cm. The resulting paste was sealed tightly and subjected to the incubation at 40 °C for 30 min, followed by heating at 90 °C for 20 min.

Subsequently, all gels were cooled in iced water for 30 min and stored at 4 °C for 24 h prior to analyses.

4.3.4 Analyses

4.3.4.1 Breaking force and deformation

Breaking force (gel strength) and deformation (deformability) of surimi gels were determined using a texture analyzer (Model TA-XT2, Stable MicroSystems, Surrey, UK) as described by Benjakul *et al.* (2001a). Cylindrical gel samples (2.5 cm in height) were prepared and equilibrated at room temperature (28-30 °C) for 30 min before analyses. A spherical plunger (diameter 5 mm) was pressed into the cut surface of a gel sample perpendicularly at a constant depression speed (60 mm/min). The force to puncture into the gel (breaking force) and the distance at which the plunger punctured into the gel (deformation) were recorded.

4.3.4.2 Texture profile analysis

Gel samples were subjected to texture profile analysis (TPA) following the method of Buamard and Benjakul (2015). A texture analyzer (Model TA-XT2, Stable MicroSystems, Surrey, UK) with a load cell of 50 kg and a cylindrical aluminium probe (diameter 50 mm) was used. The samples were placed on the instrument's base and the tests were run with two compression cycles. TPA textural parameters were measured with the following testing conditions: crosshead speed of 0.5 mm/s, 50% compression of the original sample height, and a time interval between the first and second compression of 10 s. Hardness, cohesiveness, springiness, chewiness and gumminess were calculated from the force-time curves.

4.3.4.3 Expressible moisture content

Gel samples were measured for expressible moisture content according to the method of Chanarat *et al.* (2012). Cylindrical gel samples were cut into a thickness of 5 mm, weighed accurately (X) and placed between three pieces of Whatman filter paper No.1 (Whatman International Ltd., Maidstone, UK) at the bottom and two pieces on the top of the sample. A standard weight of 5 kg was placed on the top of

the sample for 2 min. The sample was then removed from the papers and weighed again (*Y*). Expressible moisture content was calculated with the following equation and expressed as percentage of sample weight:

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

4.3.4.4 Whiteness

Whiteness of gel samples was determined using a colorimeter (HunterLab, Colorflex, Hunter Associates Laboratory, VA, USA). CIE L^*, a^* and b^* values were measured and whiteness was then calculated using the following equation (Benjakul *et al.*, 2004):

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

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

4.3.4.5 SDS-polyacrylamide gel electrophoresis

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

4.3.4.6 Dynamic rheology

Surimi pastes were prepared as previously described and further subjected to dynamic rheological measurements following the method of Singh and Benjakul

(2017a). A rheometer (HAAKE RheoStress1, ThermoFisher Scientific, Karlsruhe, Germany) with 35 mm parallel plate geometry was used for monitoring the changes in storage or elastic modulus (G'). An oscillation of 1 Hz with 1% deformation was used for testing. This condition yielded a linear response in the viscoelastic region. The temperature sweep was recorded during heating from 10 to 90 °C with heating rate of 1 °C/min. To minimize water evaporation of surimi pastes during measurement, silicon oil was applied to cover the samples.

4.3.4.7 Acceptance test

Gel samples were cut into a bite-size (1 cm thickness and 2.5 cm diameter), equilibrated at room temperature (28-30 °C) for 30 min and coded with 3-digit random numbers. Samples were kept in plastic cup with the cover before the acceptance test. Fifty non-trained panelists (aged between 20 and 45), who were the students and staffs at Department of Food Technology and were accustomed with surimi products, were asked to evaluate for appearance, color, odor, firmness, springiness, taste and overall liking of gel samples using 9-point hedonic scale (Meilgaard *et al.*, 2007). Gel samples were served on a covered white plastic cup at room temperature under the fluorescent daylight-type illumination. Between samples, panelists were asked to rinse their mouth with drinking water at room temperature.

4.3.4.8 Microstructure

Microstructures of control surimi gel and those containing 6% GLP or 6% GLS were examined using a scanning electron microscope (SEM). The samples with a thickness of 2-3 mm were fixed with 2.5% (v/v) glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 3 h at room temperature, followed by rinsing with distilled water. Fixed specimens were dehydrated in ethanol with serial concentrations of 25, 50, 70, 80, 90, and 100%. Samples were critical point dried using CO_2 as transition fluid. The prepared samples were mounted on a bronze stub and sputter-coated with gold. The specimens were visualized using SEM (Quanta 400, FEI, Eindhoven, the Netherlands).

4.3.5 Statistical analysis

All experiments were run in triplicate. For experimental designs including control, completely randomized design (CRD) was used for textural and physical properties and randomized complete block design (RCBD) was used for acceptance test. Without control, factorial design with 2 factors (2 gellan forms × 4 gellan levels) was used for experimental design. Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by the Duncan's multiple range test (Steel and Torrie, 1980). Statistical analysis was performed using SPSS for Windows (SPSS Inc., Chicago, IL, USA). Data with $p < 0.05$ were considered to be statistically significant.

4.4 Results and discussion

4.4.1 Textural and physical properties of surimi gel as affected by gellan addition

4.4.1.1 Breaking force and deformation

Breaking force and deformation of surimi gels from bigeye snapper without and with GLP and GLS addition at different levels are shown in Figure 9. Generally, breaking force is a key parameter used to determine the quality of fish meat gel-based products (Wei *et al.*, 2017). The control gel (without gellan) had the lowest breaking force, compared with those added with gellan ($p < 0.05$). For both gels incorporated with GLP or GLS, breaking force increased with increasing levels of gellan added up to 8% ($p < 0.05$). The result indicated that gellan more likely acted as active fillers, which were able to interact with surimi proteins, thus strengthening the gel matrix. In general, protein-hydrocolloid interactions mainly occur via electrostatic interaction; between the anionic groups on the hydrocolloid and the positively charged groups on the protein (Montero *et al.*, 2000). Negatively charged domain of carboxyl group in gellan might undergo interaction with positively charged residues of myofibrillar proteins in surimi via ionic interaction. This was evidenced by the increased gel strength of resulting surimi. Gels added with GLS showed the higher breaking force than those containing GLP ($p < 0.05$). Among all gel samples, surimi gel incorporated with 8% GLS had the highest breaking force (637 g) ($p < 0.05$), in which breaking force was increased by 99%, compared to that of control. With addition of 8% GLP,

breaking force of 433 g was obtained. The result indicated that the form of gellan had the profound impact on gel property of surimi gel containing gellan. Application of high intensity ultrasound could lead to the increased dispersion and partial depolymerization of gellan. This was associated with cavitation effect (Tiwari *et al.*, 2010). Goh *et al.* (2015) reported that ultrasonication treatment resulted in a reduction in polymer chain lengths of gellan. When GLS with shorter chain length was incorporated, the thorough dispersion of gellan throughout gel matrix more likely favored the interaction between gellan and myofribrillar proteins. This led to the strengthened gel network as indicated by the increased breaking force. On the other hand, the powder of gellan was not dispersed uniformly, especially at low temperature of surimi paste. Therefore, gellan could form agglomerate and the aggregation of surimi proteins was subsequently disrupted. According to Zhou *et al*. (2008), the addition of carrageenan or gellan improved the properties of silver carp surimi-protein soybean composite product added with fat pork by enhancing the gel strength, decreasing the water loss and increasing the folding performance. The results showed that gellan forms had no effect on deformation of surimi gel ($p > 0.05$). However, gel containing 8%GLP had higher deformation than those containing 8%GLS ($p < 0.05$). When gellan level was increased, slight decrease in deformation of resulting gel was observed. The lowest deformation was found in gel added with 8%GLS ($p < 0.05$). Breaking force is positively correlated with gel strength, while deformation represents the elasticity of the gels (Chanarat and Benjakul, 2013a). Gellan generally forms hard and brittle gels (Nussinovitch, 1997). It was noted that surimi gel became low elastic when higher level of gellan, especially GLS, was added as shown by the decreased deformation. Since surimi protein-gellan interaction was governed by ionic bonds and hydrogen bonds, the deformation of resulting gel was not much affected by incorporation of gellan. Therefore, gellan at an appropriate level could be used as a gel strengthener in surimi.

4.4.1.2 Textural properties

Textural profiles of surimi gels added with GLP and GLS at different levels are depicted in Table 11. Gels had the increases in hardness as the levels of GLP or GLS increased ($p < 0.05$). Hardness represents the maximum force generated in

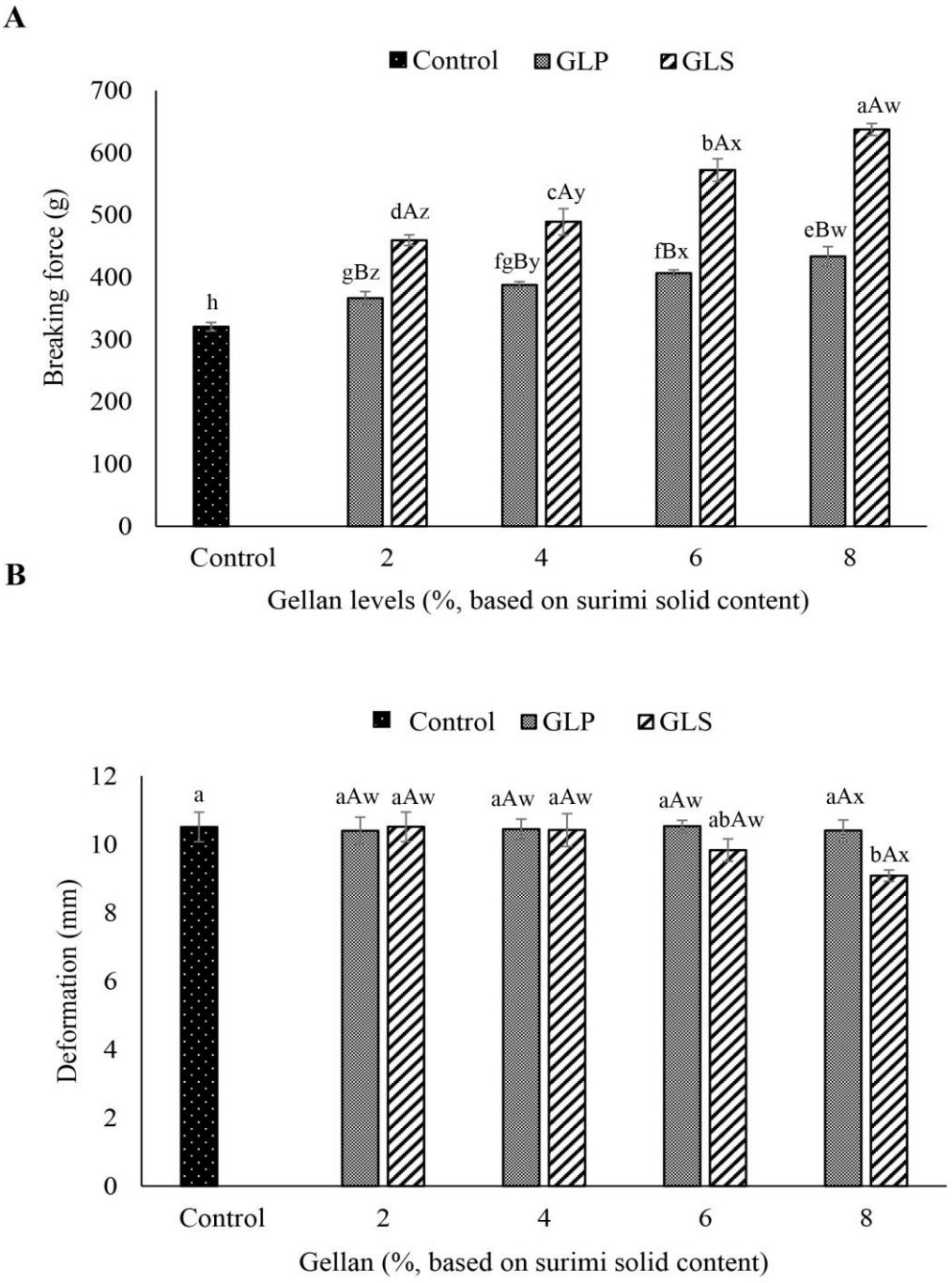

Figure 9 Breaking force (A) and deformation (B) of gels from bigeye snapper surimi without and with GLP or GLS at different levels. Bars represent the standard deviation (n=3). Lowercase letters (a-h) on the bar including the control indicate significant differences ($p < 0.05$). Uppercase (A, B) and lowercase (w-z) letters on the bar indicate significant differences ($p < 0.05$) within GL forms and within levels, respectively.

in resistance to the first compression. At the same level of gellan added, surimi gels containing GLS showed the higher hardness than those added with GLP ($p < 0.05$). The highest hardness was obtained for gel added with 8% GLS (65.74 N) ($p < 0.05$). This was coincidental with the highest breaking force (Figure 9A). Furthermore, GLS showed the higher gel strengthening effect than GLP. These results reconfirmed that the addition of gellan into surimi could increase gel strength of surimi. Additionally, the form of gellan also played a role in property of gel. Gellan more likely undergo polymerization and aggregation together with myofibrils during either setting or heating, resulting in the increases in both breaking force and hardness. Normally, the textural properties of protein gels containing fillers depend on the molecular structure of the fillers that can either depress or reinforce the primary gel structure. Benjakul *et al.* (2001b) reported that the addition of chitosan at higher amount into surimi from barred garfish (*Hemiramphus far*) diluted the concentration of myofibrils, resulting in the decrease in breaking force of resulting gel (Benjakul *et al.*, 2001b). However, high level of gellan added had no negative effect on gel strength or hardness of resulting gels. Similar results were observed for gumminess, the energy required to breakdown a semi-solid food ready for swallowing and chewiness, the required energy to chew the sample to the point required for swallowing it. Both gumminess and chewiness of surimi gel increased noticeably when added with GLP or GLS in a dose dependent manner ($p < 0.05$). Superior gelation of gellan directly contributed to the stronger network of surimi gel. Nevertheless, there were no differences in springiness and cohesiveness between surimi gel containing GLP and GLS ($p > 0.05$). The increase in gellan levels caused the decrease in both springiness and cohesiveness, especially at higher level added ($p < 0.05$). The lowest springiness and cohesiveness was found in gel added with 8%GLS ($p < 0.05$). This was in accordance with the lowest deformation (Figure 9B). It was noted that GLS increased hardness, gumminess and chewiness more effectively than GLP. Nevertheless, the addition of either

Table 11 Texture profile analysis (TPA) of bigeye snapper surimi gel as affected by gellan with different forms at various levels

Gellan forms	Gellan levels (%)*	Hardness (N)	Springiness (cm)	Cohesiveness	Gumminess (N)	Chewiness (N × cm)
Control	-	39.66±0.57 ^{h}	0.92±0.01 ^{a}	0.78±0.01 ^{a}	30.79±0.92 ^{h}	28.23±1.01 ^{h}
GLP	2	46.93±1.05 ^{gBz}	0.92±0.01 ^{aAw}	0.76±0.01 ^{abAw}	35.77±0.68 ^{gBz}	32.88±0.75 ^{gBz}
	4	49.74±1.19 ^{fgBy}	0.92±0.00 ^{aAw}	0.76±0.00 ^{abAw}	37.88±0.89 ^{fBy}	34.94±0.85 ^{fgBy}
	6	51.68±0.76 ^{fBx}	0.92±0.02 ^{aAw}	0.76±0.01 ^{bAw}	39.26±0.67 ^{efBx}	36.30±0.88 ^{efBx}
	8	54.73±0.82 ^{eBw}	0.92±0.00 ^{aAx}	0.75±0.00 ^{bAx}	40.93±0.49 ^{eBw}	37.75±0.26 ^{eBw}
GLS	2	57.25±0.22 ^{dAz}	0.92±0.01 ^{aAw}	0.75±0.00 ^{bAw}	43.13±0.24 ^{dAz}	39.58±0.40 ^{dAz}
	4	59.03±1.78 ^{cAy}	0.92±0.01 ^{aAw}	0.75±0.02 ^{bAw}	44.19±0.79 ^{cAy}	40.47±0.67 ^{cAy}
	6	63.05±1.11 ^{bAx}	0.91±0.02 ^{abAw}	0.74±0.01 ^{bcAw}	45.86±0.51 ^{bAx}	42.09±0.95 ^{bAx}
	8	65.74±0.36 ^{aAw}	0.89±0.00 ^{bAx}	0.73±0.00 ^{cAx}	48.03±0.13 ^{aAw}	43.41±0.28 ^{aAw}

* Based on surimi solid content. Values are presented as mean ± SD (n = 3). GLP, gellan powder; GLS, gellan suspension.

Different lowercase (a-h) superscripts within the same column indicate significant differences including control ($p < 0.05$).

Different uppercase (A, B) superscripts in the same column indicate significant differences between gellan forms ($p < 0.05$).

Different lowercase (w-z) superscripts in the same column indicate significant differences between gellan levels ($p < 0.05$).

GLP or GLS could improve textural property of surimi gel. The increase in gel strength of surimi gel was possibly caused by the filler or binder effect of gellan with myofibrillar proteins via electrostatic interaction. Moreover, with suspension form, gellan could be dispersed more uniformly throughout gel matrix and reinforced gel network more effectively. Petcharat *et al.* (2017) reported that the increase in gel strength of fish gelatin containing gellan was most likely due to the interaction or aggregation between both biopolymers via ionic interaction. It can be inferred that the level and form of gellan added could play an essential role in textural properties of surimi gel from bigeye snapper.

4.4.1.3 Expressible moisture content

Expressible moisture content of gel from bigeye snapper surimi added with GLP or GLS at different levels is shown in Table 12. The expressible moisture content of surimi gel decreased when the levels of gellan increased ($p < 0.05$). The highest expressible moisture content was found in the control gel (without gellan) (3.5%) ($p < 0.05$). Normally, the expressible moisture content is indicative for the water holding capacity of gel (Rawdkuen *et al.*, 2004). The decreased expressible moisture content of gels suggested that more water was bound or retained in the gel network (Chanarat and Benjakul, 2013b). Gels added with GLS exhibited the lower expressible moisture content than those containing GLP ($p < 0.05$). Addition of gellan, especially GLS into surimi, could induce the formation of stronger network, in which more water could be held in the gel network. Moreover, gellan might absorb water during the heating process via charged domains. GLS had more surface area, thus having higher ability in binding water. As a result, surimi gel containing GLS had the improved water holding capacity. In general, gums or hydrocolloids are added in the dry state, and the water in myofibrillar proteins is limited. Hence the gums or hydrocolloids are less exposed to available water in comparison with aqueous system (Pérez-Mateos and Montero, 2002). The lower expressible moisture content of gels added with GLS might be caused by the greater surface exposed to water in gel than GLP. Consequently, gels containing GLS, especially at high level, could entrap water in the network more potentially than those incorporated with GLP. Therefore, the

addition of gellan, especially GLS at high level, was able to increase water holding capacity of surimi gels.

Table 12 Expressible moisture content and whiteness of bigeye snapper surimi gel as affected by gellan with different forms at various levels

Gellan forms	Gellan levels (%)*	Expressible moisture content (%)	Whiteness
Control	-	3.50± 0.27 ^{a}	78.36±0.03 ^{f}
GLP	2	2.99±0.28 ^{bAw}	78.42±0.03 ^{eBw}
	4	2.56±0.09 ^{cAx}	78.44±0.04 ^{eBx}
	6	2.37±0.08 ^{cdAy}	78.88±0.01 ^{cBy}
	8	2.33±0.15 ^{cdAz}	79.43±0.06 ^{bBz}
GLS	2	2.13±0.15 ^{dBw}	78.77±0.02 ^{dAw}
	4	1.33±0.02 ^{eBx}	78.91±0.06 ^{cAx}
	6	1.14±0.06 ^{efBy}	79.41±0.02 ^{bAy}
	8	1.04±0.07 ^{fBz}	79.92±0.02 ^{aAz}

* Based on surimi solid content. Values are presented as mean ± SD (n = 3). GLP, gellan powder; GLS, gellan suspension. Different lowercase (a-h) superscripts within the same column indicate significant differences including control ($p < 0.05$). Different uppercase (A, B) superscripts in the same column indicate significant differences between gellan forms ($p < 0.05$). Different lowercase (w-z) superscripts in the same column indicate significant differences between gellan levels ($p < 0.05$).

4.4.1.4 Whiteness

Whiteness of gels from bigeye snapper surimi as affected by gellan with different forms at various levels is shown in Table 12. Among all gel samples, surimi gel without gellan had the lowest whiteness (78.36), compared with others ($p < 0.05$). Both GLP and GLS increased whiteness of surimi gels in a dose dependent manner ($p < 0.05$). Whiteness is another important quality index for surimi products (Park,

2013). The color characteristics of gels from fish muscle protein are largely dependent on the types and amounts of additives added (Rawdkuen *et al.,* 2007). Hu *et al.* (2015) found that the addition of curdlan at levels lower than 4% paste could increase the whiteness of hairtail surimi. The light scattering effect of curdlan contributed to the increased whiteness of gels (Hu *et al.,* 2015). Moreover, Zhang *et al.* (2015) reported that the addition of deacetylated konjac glucomannan into Alaska pollock surimi, which subjected to a high temperature (120 °C) treatment, increased the whiteness of surimi gels. Based on the result, the addition of GLP and GLS could increase whiteness of surimi gel. GLS rendered gel with higher whiteness, compared to GLP. Higher distribution of GLS was plausibly associated with higher light scattering, related with increased whiteness.

4.4.1.5 Dynamic rheology

Changes in elastic modulus (G') of bigeye snapper surimi paste added without and with GLP or GLS at different levels during transition from sol to gel as a function of temperature are depicted in Figure 10A. Similar elastic modulus curves were observed for surimi pastes incorporated without and with gellan. Gel samples added with GLP or GLS showed the higher G' than the control (without GLP or GLS). In general, G′ is used to measure the amount of energy stored as mechanical energy after a deforming force is applied and relates to changes of sample elasticity during gelation (Hunt and Park, 2013). G' of control gel increased continuously and reached the highest value at approximately 35 ˚C. This indicated the formation of protein network via weak bonds, e.g. hydrogen bonds between protein molecules (Buamard *et al.,* 2017). Thereafter, G' rapidly decreased and the lowest value was obtained at about 50 ˚C. Degradation mediated by endogenous proteolytic enzymes in the temperature range of 50-60 ˚C (Buamard and Benjakul, 2015) and disaggregation of actin-myosin network structure more likely enhanced mobility of protein, resulting in a decreased G' (Zhang *et al.,* 2015). G' was subsequently increased again when heated up to 65 ˚C. This probably resulted from an increase in the number of cross-links between dissociated protein molecules and the denaturation of myosin heavy

chain and actomyosin, leading to a formation of a thermo-irreversible gel network (Mleko and Foegeding, 2000). Unfolded proteins might favor the aggregation via reactive groups or domains. Hydrophobic domains plausibly underwent interaction via hydrophobic-hydrophobic interaction, while sulfhydryl groups were oxidized, in which disulfide bond could be formed (Buamard and Benjakul, 2015). Thereafter, G' decreased again until the temperature reached 80 ˚C. Weak bonds such as hydrogen bonds might be destroyed during heating process at high temperature.

Samples had the increases in G' as the levels of GLP or GLS increased. At the same level of gellan added, G′ of pastes incorporated with GLS was higher than that of GLP. The highest G′ was observed in surimi paste added with 8% GLS. These results were in accordance with the highest breaking force of gel containing 8% GLS (Figure 9A). For all gellan levels tested, the initial G' of sample containing GLS was higher than that added with GLP. This might be caused by the formation of hydrogen bond or ionic interaction between GLS and myofibrils in surimi. GLS was pre-hydrated and had the enhanced the dispersion with ultrasonication treatment before added into surimi. For active fillers, a strong interaction exists between individual filler particles and the gel matrix, and the elastic modulus increases with increasing volume fraction of filler. On the other hand, there is little or no interaction between inactive filler particles and gel matrix and the modulus decreases with increasing volume fraction of the inactive filler (Rawdkuen *et al.*, 2004). The result suggested that gellan might act as an active filler, which increased G' of surimi samples, especially when gellan with increasing levels was incorporated.

4.4.1.6 Protein patterns

Protein patterns of surimi gels without and with the addition of GLP or GLS at various levels are depicted in Figure 10B. Surimi paste contained myosin heavy chain (MHC) and actin as the major proteins, respectively. In the control gels, MHC completely disappeared. Cross-linking of proteins mediated by indigenous transglutaminase played a major role in the disappearance of MHC band (Singh and Benjakul, 2017a). Bigeye snapper surimi was reported to have the pronounced setting phenomenon (Benjakul and Visessanguan, 2003). Nevertheless, degradation also

occurred during gelation. When GLP or GLS was added, no MHC band was observed at all the levels of gellan used. This result suggested that inter- and intra-molecular cross-linking of MHC via non-disulfide covalent bonds still took place without interfering effect by gellan, regardless of its forms. Gellan most likely interacted with muscle proteins by weak bonds, such as ionic bonds, hydrogen bonds and hydrophobic interactions, which could be destroyed by SDS used for solubilization. Therefore, the addition of gellan had no effect on setting mediated by endogenous transglutaminase, in which isopeptide could be formed. ε-(γ-glutamyl)lysine isopeptide is reported to be formed as induced by transglutaminase(Benjakul and Visessanguan, 2003). It was noted that no changes in actin band intensity were noticeable for all samples, irrespective of amount of gellan or forms used. Thus, actin was not served as the preferable substrate for transglutaminase. Additionally, actin was resistant to proteolysis (Buamard and Benjakul, 2015).

4.4.2 Acceptability of surimi gel as affected by gellan addition

Likeness score of gels from bigeye snapper surimi without and with GLP or GLS at various levels is shown in Table 13. Addition of GLS increased appearance likeness score of gel higher than GLP ($p < 0.05$). Gel containing 8%GLS had the highest appearance likeness score ($p < 0.05$). GLS effectively increased firmness likeness score of resulting gel than GLP ($p < 0.05$). When the levels of gellan increased up to 6%, firmness likeness score was increased ($p < 0.05$). However, the score was decreased when gellan at 8% was added ($p < 0.05$). The incorporation of excessive gellan into surimi led to the rigid and brittle texture, which was not desirable for consumers. Similar phenomenon was found in fish gelatin gel added with gellan at high level (>5% gellan), in which the decrease in overall likeness score was found (Petcharat *et al.*, 2017). The incorporation of GLP or GLS into surimi had no impact on springiness, color and odor likeness score of resulting gels ($p > 0.05$). However, gel added with 8% GLS showed the lowest springiness likeness score ($p > 0.05$). This was coincidental with the lowest deformation of gel added with 8% GLS (Figure 9B). Taste likeness score of surimi gel was generally decreased as the levels of gellan were increased ($p < 0.05$). Nevertheless, there were no differences

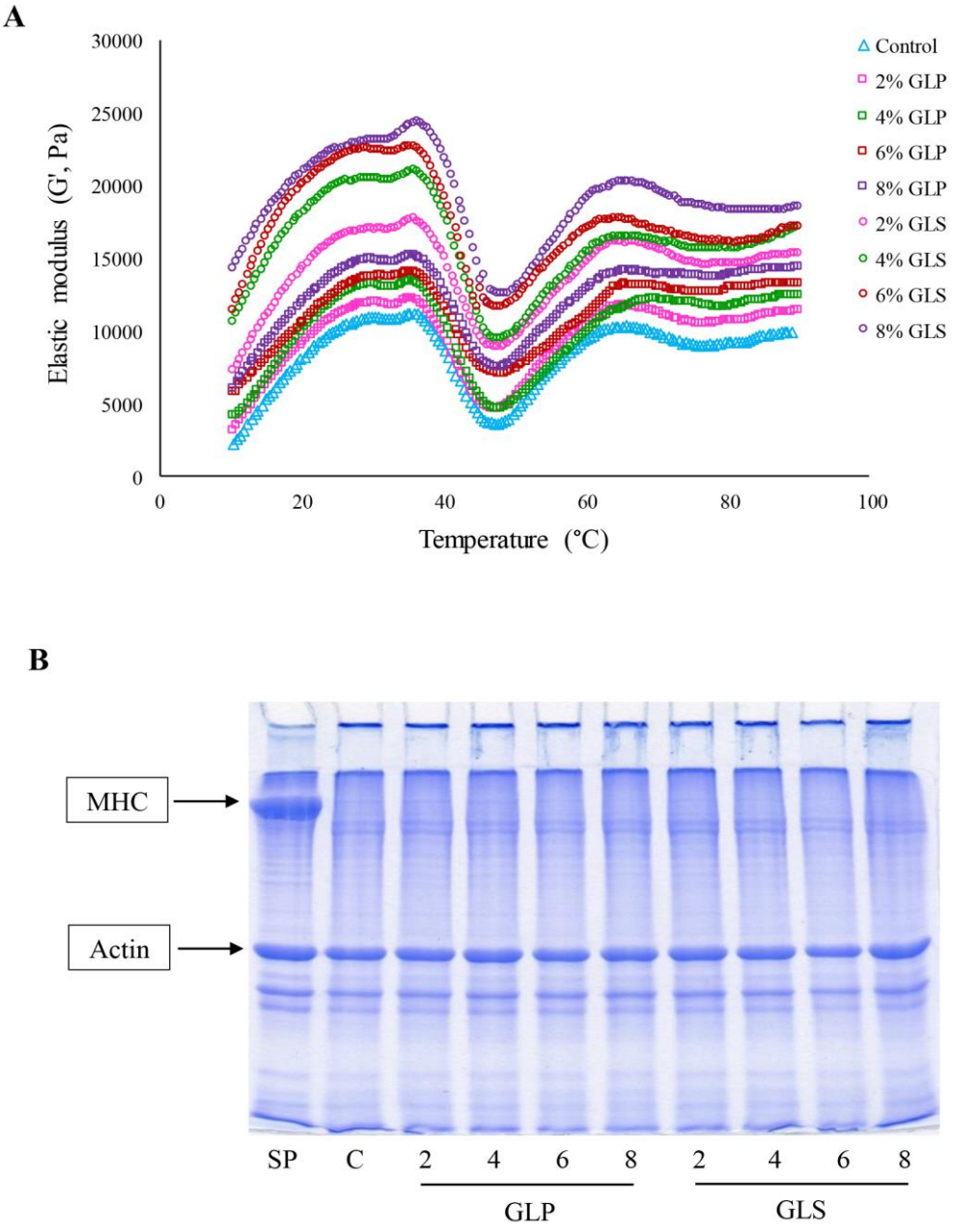

Figure 10 Elastic modulus (G') during heating of bigeye snapper surimi paste (A) and protein patterns (B) of bigeye snapper surimi gel without and with GLP or GLS at different levels. MHC, myosin heavy chain; SP, surimi paste; and C, the control gel. Numbers denote the levels of gellan (%, based on surimi solid content).

in taste likeness scores between gel containing GLP and GLS ($p > 0.05$). This was owing to the dilution effect of gellan toward surimi. Surimi contained several amino acids such as glutamic acid or aspartic acid, which are known to provide umami taste (Dewi *et al.*, 2016). Increasing GLP and GLS yielded surimi gel with increased overall likeness. However, overall likeness score was decreased as GLS level was above 6%. Among all samples, the highest overall likeness score was obtained for gel containing 6% GLS ($p < 0.05$). This was in agreement with the highest score for firmness likeness for the sample containing 6% GLS ($p < 0.05$). Lin and Huang (2003) found that partial replacement of fat with konjac/gellan gum mixed gel improved the overall sensory quality of reduced-fat frankfurter. As a result, the addition of GLS at an appropriate level (6%) could improve property and acceptability of bigeye snapper surimi gel. At high level (8%), panelists gave the comment that the gel had the decreases in surimi taste/flavor. This led to lowered acceptance.

4.4.3 Microstructure of surimi gel as affected by gellan addition

Microstructures of bigeye snapper surimi gels prepared without and with 6% GLP and 6% GLS were visualized by SEM as shown in Figure 11. The control gel (without GLP or GLS addition) had a coarser network with the larger void or cavities, compared with those added with gellan. This was in agreement with the lowest breaking force (Figure 9A) and the highest expressible moisture content (Table 12). Surimi gel network became finer and denser with the addition of 6% GLP, as compared to the control gel. The most ordered microstructure with the highest interconnection was observed in gel containing 6% GLS. The results reconfirmed that gellan might act as the active filler, which could interact with myofibrillar proteins of surimi. Ionic interaction between negative charge of gellan from carboxyl group (Morris *et al.*, 2012) and positive charge of myofibrillar protein constructed the network of mixed gel. Gellan suspension treated with ultrasound could be used to yield the finer dispersion of gellan throughout the surimi gel matrix, in which the

Table 13 Likeness score of bigeye snapper surimi gel as affected by gellan with different forms at various levels

Gellan forms	Gellan levels (%)*	Appearance	Color	Odor	Firmness	Springiness	Taste	Overall
Control	-	8.10±1.19^{c}	8.12±0.97 ^{a}	7.29±1.53 ^{a}	7.60±0.76 ^{e}	7.44±0.84 ^{a}	7.58±0.91 ^{a}	7.68±0.62 ^{g}
GLP	2	8.02±0.98^{cBy}	8.12±0.92 ^{aAw}	7.14±1.25 ^{aAw}	7.70±0.21 ^{eBy}	7.50±1.12 ^{aAw}	7.56±1.07 ^{aAw}	7.60±0.93 ^{gBy}
	4	8.04±1.26^{cBx}	8.04±0.99 ^{aAw}	7.18±1.47 ^{aAw}	7.76±1.16 ^{eBx}	7.46±1.26 ^{aAw}	7.59±1.30 ^{aAw}	7.72±0.97 ^{fBx}
	6	8.02±1.11^{cBw}	8.09±1.01 ^{aAw}	7.04±1.31 ^{aAw}	7.82±0.62 ^{dBw}	7.52±1.08 ^{aAw}	7.44±0.89 ^{abAx}	7.75±0.83 ^{efBw}
	8	7.99±1.08^{cBw}	8.14±0.81 ^{aAw}	7.22±1.66 ^{aAw}	7.85±1.20 ^{cdBz}	7.48±1.12 ^{aAx}	7.38±0.56 ^{bAy}	7.82±0.41 ^{dBz}
GLS	2	8.06±0.94^{cAy}	8.11±1.21 ^{aAw}	7.08±1.26 ^{aAw}	7.88±0.36 ^{cAy}	7.50±1.15 ^{aAw}	7.58±0.42 ^{aAw}	7.94±0.90 ^{cAy}
	4	8.37±0.53^{bAx}	8.18±1.00 ^{aAw}	7.02±1.41 ^{aAw}	7.96±0.59 ^{bAx}	7.48±0.95 ^{aAw}	7.55±0.77 ^{aAw}	8.04±0.67 ^{bAx}
	6	8.58±0.28^{abAw}	8.17±0.96 ^{aAw}	7.20±1.27 ^{aAw}	8.68±0.40 ^{aAw}	7.40±0.97 ^{abAw}	7.41±0.86 ^{abAx}	8.52±0.68 ^{aAw}
	8	8.62±0.30^{aAw}	8.06±0.89 ^{aAw}	7.16±1.23 ^{aAw}	7.08±0.52 ^{fAz}	7.10±0.74 ^{bAx}	7.34±0.69 ^{bAy}	7.43±0.76 ^{hAz}

* Based on surimi solid content. Values are presented as mean ± SD. GLP, gellan powder; GLS, gellan suspension.

Different lowercase (a-h) superscripts within the same column indicate significant differences including control ($p < 0.05$).

Different uppercase (A, B) superscripts in the same column indicate significant differences between gellan forms ($p < 0.05$).

Different lowercase (w-z) superscripts in the same column indicate significant differences between gellan levels ($p < 0.05$).

interconnectivity of proteins in the resulting gel was more pronounced when 6% GLS was added. Fine and ordered gel network more likely imbibed water (Buamard *et al.*, 2017). This might result in the high water holding capacity of gel added with gellan, especially when GLS was incorporated. Therefore, GLS at a level of 6% could strengthen gel network of surimi.

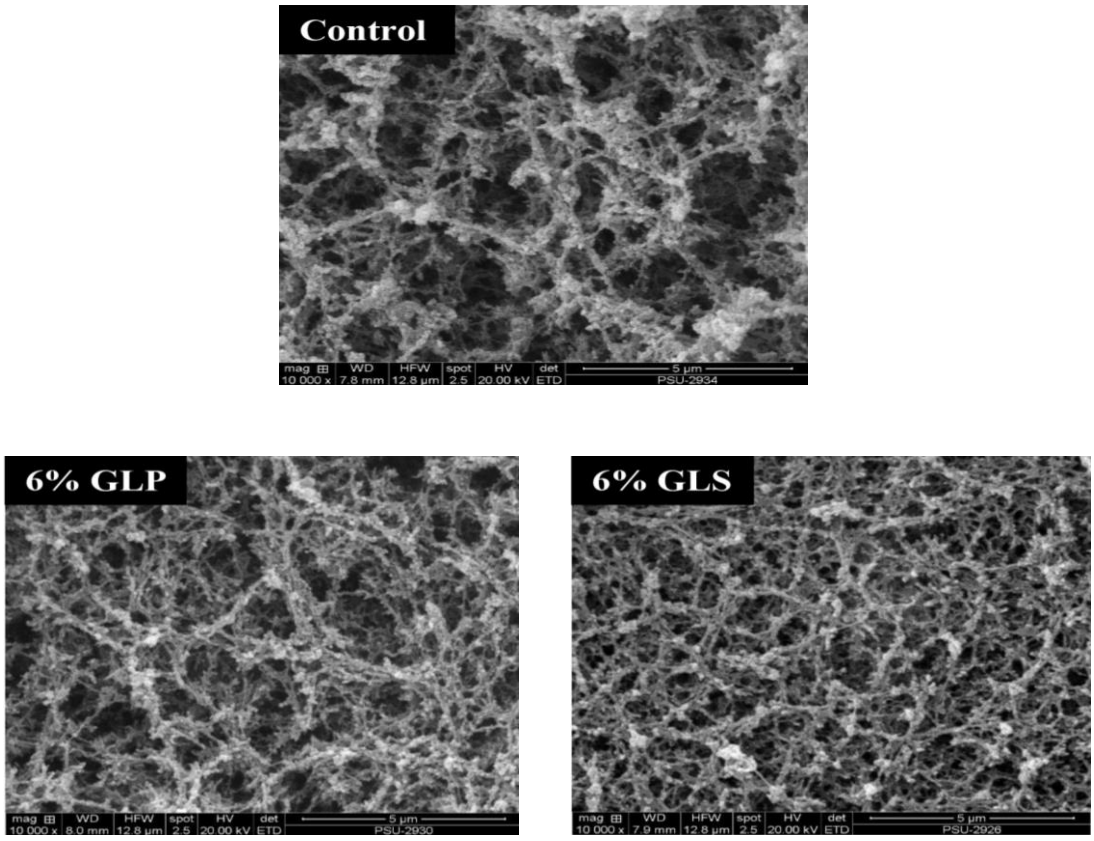

Figure 11 Electron microscopic images of surimi gel without and with 6% GLP or 6% GLS. Magnification: 10,000X.

4.5 Conclusions

Gel properties of surimi from bigeye snapper were governed by form and level of gellan. The addition of GLP and GLS increased breaking force, hardness, water holding capacity and whiteness of surimi gel as the levels of gellan increased. Both GLP and GLS could enhance viscoelastic property of surimi paste during heating as indicated by the increased G'. Gellan level had no effect on polymerization of myosin heavy chain. Based on acceptance test, gels added with 6% GLS showed the highest overall likeness score. Finer and denser network was observed in surimi gel containing 6% GLS. The addition of 6% GLS could therefore improve textural properties of bigeye snapper surimi with the highest acceptability. Thus, gellan could be used as alternative additive for improving the gelling properties of surimi from bigeye snapper.

4.6 References

Barrera, A. M., Ramírez, J. A., González-Cabriales, J. J. and Vázquez, M. 2002. Effect of pectins on the gelling properties of surimi from silver carp. Food Hydrocolloids. 16: 441-447.

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

Benjakul, S., Visessanguan, W. and Chantarasuwan, C. 2004. Effect of porcine plasma protein and setting on gel properties of surimi produced from fish caught in Thailand. LWT - Food Science and Technology. 37: 177-185.

Benjakul, S., Visessanguan, W. and Srivilai, C. 2001a. Gel properties of bigeye snapper (*Priacanthus tayenus*) surimi as affected by setting and porcine plasma proteins. Journal of Food Quality. 24: 453-471.

Benjakul, S., Visessanguan, W., Tanaka, M., Ishizaki, S., Suthidham, R. and Sungpech, O. 2001b. Effect of chitin and chitosan on gelling properties of surimi from barred garfish (*Hemiramphus far*). Journal of the Science of Food and Agriculture. 81: 102-108.

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

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

Chanarat, S. and Benjakul, S. 2013a. Effect of formaldehyde on protein cross-linking and gel forming ability of surimi from lizardfish induced by microbial transglutaminase. Food Hydrocolloids. 30: 704-711.

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

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

Dewi, E. N., Amalia, U. and Mel, M. 2016. The effect of different treatments to the amino acid contents of mcicro algae *Spirulina* sp. Aquatic Procedia. 7: 59-65.

Evageliou, V., Gerolemou, A., Zikas, A., Basios, A. and Komaitis, M. 2011. Effect of salts and sugars on the clarity of gellan gels. International Journal of Food Science & Technology. 46: 1001-1006.

Eymard, S., Carcouët, E., Rochet, M. J., Dumay, J., Chopin, C. and Genot, C. 2005. Development of lipid oxidation during manufacturing of horse mackerel surimi. Journal of the Science of Food and Agriculture. 85: 1750-1756.

Goh, K. K. T., Yuliarti, O., Yeo, G. T. T. and Or, C. C. 2015. Effect of ultrasonication on low-acetylated gellan gum gel properties. Food Hydrocolloids. 49: 240-247.

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

Hunt, A. and Park, J. W. 2013. Alaska pollock fish protein gels as affected by refined carrageenan and various salts. Journal of Food Quality. 36: 51-58.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature. 227: 680-685.

Lin, K. W. and Huang, H. Y. 2003. Konjac/gellan gum mixed gels improve the quality of reduced-fat frankfurters. Meat Science. 65: 749-755.

Matricardi, P., Cencetti, C., Ria, R., Alhaique, F. and Coviello, T. 2009. Preparation and characterization of novel gellan gum hydrogels suitable for modified drug release. Molecules. 14: 3376-3391.

Meilgaard, M. C., Carr, B. T. and Civille, G. V. 2007. Sensory evaluation techniques. 4th Ed. CRC press. Florida.

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

Montero, P., Hurtado, J. L. and Pérez-Mateos, M. 2000. Microstructural behaviour and gelling characteristics of myosystem protein gels interacting with hydrocolloids. Food Hydrocolloids. 14: 455-461.

Morris, E. R., Nishinari, K. and Rinaudo, M. 2012. Gelation of gellan - A review. Food Hydrocolloids. 28: 373-411.

Nussinovitch, A. 1997. Gellan gum. *In* Hydrocolloid applications: Gum technology in the food and other industries. (Nussinovitch, A., ed.). p. 63-82. Springer. Boston.

Park, J. W. 2013. Surimi seafood. *In* Surimi and surimi seafood. 3rd Ed. (Park, J. W., ed.). p. 375-433. CRC Press. Florida.

Pérez-Mateos, M. and Montero, P. 2002. Effects of cations on the gelling characteristics of fish mince with added nonionic and ionic gums. Food Hydrocolloids. 16: 363-373.

Petcharat, T. and Benjakul, S. 2017. Property of fish gelatin gel as affected by the inorporation of gellan and calcium chloride. Food Biophysics. 1-9.

Petcharat, T., Benjakul, S. and Hemar, Y. 2017. Improvement of gel properties of fish gelatin using gellan. International Journal of Food Engineering. (accepted: DGIJFE.2016.0410.R2):

Rawdkuen, S., Benjakul, S., Visessanguan, W. and Lanier, T. C. 2004. Chicken plasma protein affects gelation of surimi from bigeye snapper (*Priacanthus tayenus*). Food Hydrocolloids. 18: 259-270.

Rawdkuen, S., Benjakul, S., Visessanguan, W. and Lanier, T. C. 2007. Effect of chicken plasma protein additives on proteolysis and gel-forming ability of sardine (*Sardinella gibbosa*) surimi. Journal of Food Processing and Preservation. 31: 492-516.

Robinson, H. W. and Hogden, C. G. 1940. The biuret reaction in the determination of serum proteins. 1. A study of the conditions necessary for the production of a stable color which bears a quantitative relationship to the protein concentration. Journal of Biological Chemistry. 135: 707-725.

Seighalani, F. Z. B., Bakar, J., Saari, N. and Khoddami, A. 2016. Thermal and physicochemical properties of red tilapia (*Oreochromis niloticus*) surimi gel as affected by microbial transglutaminase. Animal Production Science. 57: 993-1000.

Singh, A. and Benjakul, S. 2017a. Effect of serine protease inhibitor from squid ovary on gel properties of surimi from Indian mackerel. Journal of Texture Studies. 10.1111/jtxs.12262.

Singh, A. and Benjakul, S. 2017b. Serine protease inhibitors from squid ovary: extraction and its effect on proteolysis and gel properties of surimi. Journal of Food Science and Technology. 54: 267-275.

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

Tiwari, B. K., Muthukumarappan, K., O'Donnell, C. P. and Cullen, P. J. 2010. Rheological properties of sonicated guar, xanthan and pectin dispersions. International Journal of Food Properties. 13: 223-233.

Wei, Y., Zhang, T., Yu, F., Xue, Y., Li, Z., Wang, Y. and Xue, C. 2017. Effects of curdlan on the texture and structure of Alaska pollock surimi gels treated at 120 °C. International Journal of Food Properties. 10.1080/10942912.10942017.11306557.

Wu, S. 2016. Effect of pullulan on gel properties of *Scomberomorus niphonius* surimi. International Journal of Biological Macromolecules. 93: 1118-1120.

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

Zhou, X., Jiang, S., Zhao, D., Zhang, J., Gu, S., Pan, Z. and Ding, Y. 2017. Changes in physicochemical properties and protein structure of surimi enhanced with camellia tea oil. LWT - Food Science and Technology. 84: 562-571.

Zhou, Y. Y., Cai, S. J., Xu, X. P. and Chen, R. W. 2008. Study on improvement effects of carrageenan and gellan gum on properties of silver carp surimi-protein soybean compound. Food Science. 29: 313-319.

CHAPTER 5

EFFECT OF GELLAN AND CALCIUM CHLORIDE ON PROPERTIES OF SURIMI GEL WITH LOW AND HIGH SETTING PHENOMENON

5.1 Abstract

Properties of bigeye snapper surimi gel as affected by gellan (GL) at different levels (2-6% based on surimi solid content) in combination with calcium chloride ($CaCl_2$) at various concentrations (25-75 mmol/kg) were examined. Breaking force, hardness and whiteness of surimi gel increased but expressible moisture content decreased as both levels of GL and $CaCl_2$ increased ($p < 0.05$). Electrophor etic studies showed that GL and $CaCl_2$ at all levels had no effect on polymerization of myosin heavy chain (MHC). The highest overall likeness score was found in gel containing 4% GL and 75 mmol $CaCl_2$/kg ($p < 0.05$). Effects of GL and $CaCl_2$ in the absence of endogenous transglutaminase (TGase) on surimi gel properties were also evaluated. Both GL and $CaCl_2$ increased breaking force, water holding capacity and whiteness of TGase free surimi gels ($p < 0.05$). Based on rheological study, GL and $CaCl_2$ could enhance the interconnection between chains during heating as indicated by the higher G'. Different G' curves were obtained between surimi in the presence and absence of TGase. Protein pattern revealed an important role of TGase in MHC cross-linking of surimi gel. Microstructure studies demonstrated that finer and denser network was observed in surimi gel containing 4% GL and 75 mmol $CaCl_2$/kg in the presence of TGase, whereas the coarser network was obtained in gels free of TGase. Therefore, GL and $CaCl_2$ at an appropriate level could be used to improve gel properties of surimi having either low or high setting phenomenon mediated by endogenous TGase.

5.2 Introduction

Surimi is a concentrated myofibrillar protein, mainly produced from washed fish mince, in which cryoprotectants are commonly added to stabilize proteins during frozen storage (Park, 2005). Surimi products were firstly produced in Japan, but they

have become popular in many parts of the world. Those products include fish balls, fish cake, crab meat analogues, etc. (Seighalani *et al.*, 2017). Textural property is the most important attribute of surimi-based products. The prime factor in achieving the desired texture is the gel-forming ability of fish myofibrillar proteins (Sun and Holley, 2011). A number of additives with different functions have been used in surimi to improve properties of resulting gels. Some additives are used to retard proteolysis (Singh and Benjakul, 2017a; Singh and Benjakul, 2017b), while protein cross-linkers such as microbial transglutaminase (Chanarat *et al.*, 2012) or phenolic compounds (Buamard and Benjakul, 2015; Buamard *et al.*, 2017) have been added to strengthen the gel network of surimi. Moreover, some hydrocolloids such as curdlan, pullulan, carrageenan and pectin, etc. have been employed to strengthen surimi gels (Barrera *et al.*, 2002; Hunt and Park, 2013; Wei *et al.*, 2017; Wu, 2016).

Gellan is the latest biopolymer available on the market and it has been used by the food industry as gelling agent (Iurciuc *et al.*, 2016). Gellan is the anionic polysaccharide produced by the microorganism, *Pseudomonas elodea*. It consists of repeat units of β-D-glucose (DGlc), β-D-glucuronic acid (D-GlcA) and α-L-rhamnose (L-Rha) in a molar ratio of 2:1:1 (Banerjee and Bhattacharya, 2012). During gelation, gellan is converted from a disordered i.e. random coil conformation to ordered form. When hot solutions are cooled, double helix structures are formed, primarily through intramolecular interactions (Nickerson *et al.*, 2008). In the presence of gel promoting cations, especially divalent cation (Ca^{2+}), the double helices form cation-mediated junction zones, in which the strong gel networks can be developed (Goh *et al.*, 2006). Due to the gelling ability of gellan, it has been used in protein-based gels including fish gelatin and myofibrillar proteins. The addition of gellan at 2.5% in combination with $CaCl_2$ up to 6 mM into fish gelatin had no negative effect on acceptance of mixed gels but could increase gel strength and gelling points (Petcharat and Benjakul, 2017b). In addition, gellan was used to improve quality of reduced-fat frankfurters. Konjac (1% and 2%) in combination with gellan (0.25% and 0.5%) were incorporated into reduced fat (18%) frankfurters, yielding the finished product with acceptability (Lin and Huang, 2003). Recently, Petcharat and Benjakul (2017a) has reported that gellan, prepared as suspension, at 6% could improve gel strength of bigeye snapper surimi with the highest acceptability.

Setting phenomenon has been known to enhance gel strength of surimi. TGase play an important role in formation of ε-(γ-glutamyl) lysine linkage formation during setting (Hu *et al.*, 2017). Nevertheless, surimi from some fish species has poor setting, due to the lower levels of TGase (Park, 2005). To tackle such a problem, microbial TGase (MTGase) has been used (Chanarat *et al.*, 2012). Incorporation of divalent cation into surimi in the presence of gellan could be a promising means to improve the gelling property of surimi. Gellan can gel as induced by Ca^{2+} (Morris *et al.*, 2012). Simultaneously both gellan gel formed and Ca^{2+} are able to strengthen the surimi network. Ca^{2+} can induce protein cross-linking mediated by TGase and via Ca^{2+} bridge (Ding *et al.*, 2011). The use of gellan, particularly in the presence of Ca^{2+}, could be a promising means to improve the property of surimi gel, especially those with poor gelling property associated with low setting phenomenon. Therefore, the present study aimed to investigate the effect of gellan in combination with calcium chloride on the gel properties of surimi gel from bigeye snapper in the absence and presence of TGase.

5.3 Materials and methods

5.3.1 Materials

Food-grade low acyl gellan was purchased from CP Kelco (Atlanta, GA, USA). Frozen surimi from bigeye snapper (*Priacanthus macranthus*), Grade A, was obtained from Man A Frozen Foods Co., Ltd. (Songkhla, Thailand) and kept at -20 °C until use but not longer than 2 months. Surimi had a moisture content of 75% (w/w) as determined by an oven method (AOAC, 2000).

5.3.2 Preparation of gellan (GL) suspension

GL (1, 2 and 3 g) was dispersed in distilled water at a GL/water ratio of 1:15 (w/v). Ultrasound was applied using an ultrasonic processor (Sonics, Model VC750, Sonica & Materials, Inc., Newtown, CT, USA) at an amplitude of 40% for 4 min with a constant frequency of 20 kHz ± 50 Hz and high intensity power of 750 W.

5.3.3 Study on impact of GL and $CaCl_2$ at different levels of on properties of surimi gel

Frozen surimi (200 g) was partially thawed at 4 °C for 2-3 h until the core temperature reached 0-2 °C. The surimi was chopped into small pieces and mixed with 2.5% salt in a mixer (Panasonic, Model MK-5087M, Selangor, Malaysia) for 2 min. During chopping, the temperature was maintained below 10 °C. The paste was added with gellan suspension to obtain the final GL levels of 0, 2, 4 and 6% (based on surimi solid content). The moisture content of all surimi paste was adjusted to 80% with cold distilled water. Calcium chloride ($CaCl_2$) was added to the paste to obtain the final different concentrations (0, 25, 50 and 75 mmol/kg paste). Subsequently, the mixture was chopped for another 3 min and the paste was stuffed into a polyvinylidene chloride casing with a diameter of 2.5 cm. The resulting paste was sealed tightly and subjected to the incubation at 40 °C for 30 min, followed by heating at 90 °C for 20 min. Thereafter, all gels were cooled in iced water for 30 min and stored at 4 °C for 24 h prior to analyses. Gel samples, added with the same level of $CaCl_2$, were also prepared and referred to as the controls.

5.3.4 Analyses

5.3.4.1 Breaking force and deformation

Breaking force (gel strength) and deformation (deformability) of surimi gels were determined using a texture analyzer (Model TA-XT2, Stable MicroSystems, Surrey, UK) as described by Benjakul *et al.* (2001). Cylindrical gel samples (2.5 cm in height) were prepared and equilibrated at room temperature (28-30 °C) for 30 min before analyses. A spherical plunger (diameter 5 mm) was pressed into the cut surface of a gel sample perpendicularly at a constant depression speed (60 mm/min). The force to puncture into the gel (breaking force) and the distance at which the plunger punctured into the gel (deformation) were recorded.

5.3.4.2 Texture profile analysis

Gel samples were subjected to texture profile analysis (TPA) following the method of Buamard and Benjakul (2015). A texture analyzer (Model TA-XT2, Stable MicroSystems, Surrey, UK) with a load cell of 50 kg and a cylindrical aluminium

probe (diameter 50 mm) was used. The samples were placed on the instrument's base and the tests were run with two compression cycles. TPA textural parameters were measured with the following testing conditions: crosshead speed of 0.5 mm/s, 50% compression of the original sample height, and a time interval between the first and second compression of 10 s. Hardness, cohesiveness, springiness, chewiness and gumminess were calculated from the force-time curves.

5.3.4.3 Expressible moisture content

Gel samples were measured for expressible moisture content according to the method of Chanarat *et al*. (2012). Cylindrical gel samples were cut into a thickness of 5 mm, weighed accurately (X) and placed between three pieces of Whatman filter paper No.1 (Whatman International Ltd., Maidstone, UK) at the bottom and two pieces on the top of the sample. A standard weight of 5 kg was placed on the top of the sample for 2 min. The sample was then removed from the papers and weighed again (Y). Expressible moisture content was calculated with the following equation and expressed as percentage of sample weight:

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

5.3.4.4 Whiteness

Whiteness of gel samples was determined using a colorimeter (HunterLab, Colorflex, Hunter Associates Laboratory, VA, USA). CIE L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness) values were measured and whiteness was then calculated using the following equation (Benjakul *et al.*, 2004a):

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

5.3.4.5 SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

Protein patterns of surimi gels were analyzed by SDS-PAGE under the reducing condition according to the method of Laemmli (1970). To the finely chopped gel samples (3 g), 27 mL of heated SDS solution (85 °C) were added. The mixture was then homogenized at a speed of 11,000 rpm for 2 min using a homogenizer (IKA, Labortechnik homogenizer Selangor, Malaysia). The homogenate was incubated at 85 °C for 1 h to dissolve total proteins. The mixtures

were centrifuged at 3,500 *x*g for 20 min to remove undissolved matters. Protein concentration of the supernatant was determined by the Biuret method (Robinson and Hogden, 1940) using bovine serum albumin as standard. SDS-PAGE gel consisted of 10% running gel and 4% stacking gel. After separation, the proteins were stained with 0.02% (w/v) Coomassie Brilliant Blue R-250 in 50% (v/v) methanol and 7.5% (v/v) acetic acid and destained with 50% methanol and 7.5% (v/v) acetic acid, followed by 5% methanol (v/v) and 7.5% (v/v) acetic acid.

5.3.4.6 Acceptability test

Surimi gels added with GL at 0, 2, 4 and 6% in the absence or presence of $CaCl_2$ at a level of 75 mmol/kg were examined. Gel samples were cut into a bite-size (1 cm thickness and 2.5 cm diameter), equilibrated at room temperature (28-30 °C) for 30 min and coded with 3-digit random numbers. Samples were kept in plastic cup with the cover before the acceptability test. Fifty non-trained panelists (aged between 20 and 45), who were the students and staffs at Department of Food Technology and were accustomed with surimi products, were asked to evaluate for appearance, color, odor, firmness, springiness, taste and overall liking of gel samples using 9-point hedonic scale (Meilgaard *et al.*, 2007). Gel samples were served at room temperature under the fluorescent daylight-type illumination. Between samples, panelists were asked to rinse their mouth with drinking water at room temperature.

5.3.5 Study on the effect of GL and $CaCl_2$ at the selected concentration on properties of surimi without and with TGase

To prepare surimi without endogenous TGase, NH_4Cl at a level of 1 mol/kg was added into surimi paste (Benjakul *et al.*, 2004c). Surimi paste (without NH_4Cl) was also used to represent that containing TGase. For each surimi (without and with TGase), different samples were prepared. Those included 1) surimi gel (without GL and $CaCl_2$), 2) gel added with 75 mmol $CaCl_2$/kg, 3) gel added with 4% GL and 4) gel added with 4% GL in combination with 75 mmol $CaCl_2$/kg. The preparation of surimi gels was performed as previously described. Gels were subjected to analyses of breaking force, deformation, expressible moisture content, whiteness and protein pattern as described previously. Additional analyses including dynamic rheology and

microstructure were also performed. A completely randomized design (CRD) under a factorial design with 2 factors (2 TGase conditions × 4 selected concentration of gellan and $CaCl_2$ concentrations) was used for statistical analysis.

5.3.5.1 Dynamic rheology

Surimi pastes were prepared as previously described and then subjected to dynamic rheological measurements as per the method of Singh and Benjakul (2017a). A rheometer (HAAKE RheoStress1, ThermoFisher Scientific, Karlsruhe, Germany) with 35 mm parallel plate geometry was used for monitoring the changes in storage or elastic modulus (G'). An oscillation of 1 Hz with 1% deformation was used for testing. This condition yielded a linear response in the viscoelastic region. The temperature sweep was recorded during heating from 10 to 90 °C with heating rate of 1 °C/min. To minimize water evaporation of surimi pastes during measurement, silicon oil was applied to cover the samples.

5.3.5.2 Microstructure

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

5.3.6 Statistical analysis

All experiments were run in triplicate. For textural and physical properties, a completely randomized design (CRD) under a factorial design with 2 factors (4 gellan levels × 4 $CaCl_2$ concentrations) was used. For acceptance test, a randomized complete block design (RCBD) under a factorial design with 2 factors (4 gellan levels × 2 $CaCl_2$ concentrations) was used. Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by the Duncan’s multiple range test

(Steel and Torrie, 1980). Statistical analysis was performed using SPSS for Windows (SPSS Inc., Chicago, IL, USA). Data with $p < 0.05$ were considered to be statistically significant.

5.4 Results and discussion

5.4.1 Textural and physical properties of surimi gel as affected by gellan and $CaCl_2$ at various concentrations

5.4.1.1 Breaking force and deformation

Breaking force is one of the most important properties of surimi gels and the application of gel is generally governed by this value. When comparing the breaking force of all samples, the control gel (without GL and $CaCl_2$) had the lowest breaking force (317 g) ($p < 0.05$). In the absence of $CaCl_2$, breaking force of resulting gels increased as the level of GL increased ($p < 0.05$) (Figure 12A). Gellan plausibly acted as an active filler, which was able to interact with surimi proteins to form the stronger network as evidenced by the increased breaking force. Protein-hydrocolloids interactions mainly occur via electrostatic interaction between opposite charges between proteins and hydrocolloids (Montero *et al.*, 2000). Carboxyl group of GL might undergo ionic interaction with the positively charged residues of myofibrillar proteins, thereby leading to the strengthened gel network. Without GL added, breaking force of surimi gels increased when the concentration of $CaCl_2$ increased ($p < 0.05$). Ca^{2+} could form salt linkages between negatively charge localized on two adjacent proteins (Ding *et al.*, 2011). In addition, Ca^{2+} could activate endogenous transglutaminase (TGase) in surimi. TGase can catalyze the acyl transfer reaction between γ-carboxyamide groups of glutamine and ε-amino groups of lysine, resulting in the formation of ε-(γ-glutamyl) lysine cross-linkings and stronger gels (Benjakul *et al.*, 2004c). Similar trend was observed for all surimi gel added with GL at all levels, in which the higher breaking force was obtained with increasing $CaCl_2$ levels ($p < 0.05$). However, at higher level of GL, breaking force of resulting gel was higher ($p < 0.05$). Among all gel samples, surimi gel containing 6% GL and 75 mmol $CaCl_2$/kg had the highest breaking force (706 g) ($p < 0.05$), in which breaking force was increased by 122%, compared to that of control (without GL and $CaCl_2$). The

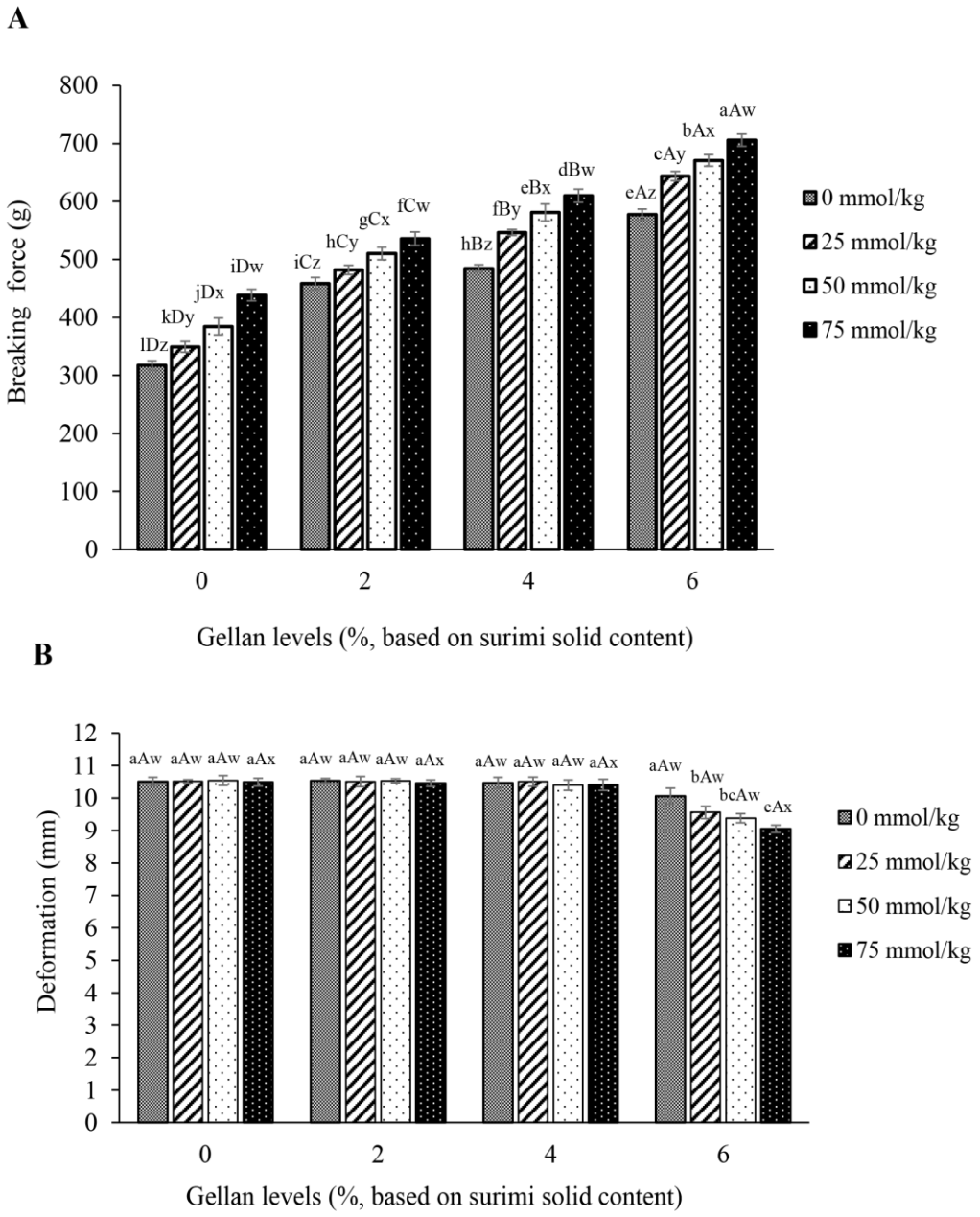

Figure 12 Breaking force (A) and deformation (B) of gels from bigeye snapper surimi as affected by GL and $CaCl_2$ at different levels. Bars represent the standard deviation (n=3). Lowercase letters (a-h) on the bar including the control indicate significant differences ($p < 0.05$). Uppercase (A-D) or lowercase (w-z) letters on the bar indicate significant differences ($p < 0.05$) within GL or $CaCl_2$ levels, respectively.

result indicated that the concentration of $CaCl_2$ had the profound impact on gel property of surimi gel containing GL. Theoretically, gelation of gellan was induced by cations. Calcium ions promote the aggregation process by site-binding between pairs of carboxyl groups on neighboring helices to give structures analogous to the "egg box" junctions as proposed for calcium induced gelation of alginate or pectin (Evageliou *et al.*, 2011). It was noted that the increase in GL levels had no effect on deformation of resulting gel ($p > 0.05$), while the increase in the concentrations of $CaCl_2$ slightly decreased deformation of resulting gel ($p < 0.05$) (Figure 12B). Thus, deformation of resulting gel was not much affected by incorporation of GL and $CaCl_2$ when the appropriate levels of both additives were used. Excessive strong bonding or interaction in network could lead to the loss in flexibility of gel, as indicated by the decreased deformation. Some hydrocolloids have been employed in surimi in conjunction with salts. Eom *et al.* (2013) reported that the addition of κ-carrageenan into Alaska pollock surimi increased breaking force and gel strength of surimi gels. Gels with 1% κ-carrageenan and KCl had the highest breaking force and gel strength. Furthermore, Hunt and Park (2013) also studied the impact of refined ι- and κ-carrageenan (0.25-1.0%) in Alaska pollock fish proteins as affected by various salts (NaCl, KCl or $CaCl_2$, at 2%). KCl and NaCl in combination with κ- and ι-carrageenan could increase gel strength and water retention ability of fish protein gel (Hunt and Park, 2013).

5.4.1.2 Textural properties

TPA parameters of surimi gels incorporated with GL at various levels in the absence and presence of $CaCl_2$ at different concentrations are depicted in Table 14. Hardness of surimi gel generally increased with increasing GL content ($p < 0.05$), regardless of $CaCl_2$ levels. Hardness of resulting gels increased as the concentration of $CaCl_2$ increased ($p < 0.05$). The highest hardness (68.16 N) was found in gel containing 6% GL and 75 mmol $CaCl_2$/kg ($p < 0.05$). This was related to the highest breaking force (Figure 12A). These results revealed that GL showed gel strengthening effect on surimi gel from bigeye snapper by acting as filler or binder in surimi gel network. Ca^{2+} could promote the formation of junction zone in the resulting gels via

Table 14 Texture profile analysis (TPA) of bigeye snapper surimi gel as affected by the addition of GL and $CaCl_2$ at different levels

Gellan (%)*	$CaCl_2$ (mmol/kg)	Hardness (N)	Springiness (cm)	Cohesiveness	Gumminess (N)	Chewiness (N × cm)
0	-	39.69±0.63 ^{pDz}	0.92±0.01 ^{aAw}	0.78±0.00 ^{aAw}	31.00±0.47 ^{pDz}	28.49±0.69 ^{oDz}
	25	40.74±0.52 ^{oDy}	0.91±0.02 ^{aAw}	0.78±0.01 ^{aAw}	31.64±0.35 ^{oDy}	28.88±0.79 ^{nDy}
	50	42.38±0.09 ^{nDx}	0.91±0.02 ^{aAw}	0.78±0.01 ^{aAw}	33.13±0.30 ^{nDx}	30.28±0.54 ^{mDx}
	75	43.01±0.10 ^{mDw}	0.92±0.01 ^{aAx}	0.77±0.01 ^{aAx}	33.28±0.34 ^{mDw}	30.64±0.38 ^{lDw}
2	-	57.39±0.32 ^{lCz}	0.92±0.00 ^{aAw}	0.75±0.01 ^{bABw}	42.99±0.24 ^{lCz}	39.75±0.34 ^{kCz}
	25	58.29±0.46 ^{kCy}	0.91±0.01 ^{aAw}	0.74±0.01 ^{bABw}	43.24±0.27 ^{kCy}	39.95±0.51 ^{jCy}
	50	60.14±0.26 ^{iCx}	0.92±0.01 ^{aAw}	0.75±0.01 ^{bABw}	45.02±0.79 ^{iCx}	41.51±0.84 ^{hCx}
	75	61.46±0.11 ^{hCw}	0.91±0.03 ^{aAx}	0.75±0.01 ^{bABx}	46.03±0.68 ^{hCw}	42.09±0.51 ^{gCw}
4	-	59.11±0.22 ^{jBz}	0.91±0.01 ^{aAw}	0.75±0.01 ^{bABw}	44.22±0.74 ^{jBz}	40.29±0.55 ^{iBz}
	25	62.61±0.21 ^{gBy}	0.92±0.00 ^{aAw}	0.75±0.03 ^{bABw}	47.06±0.89 ^{fBy}	43.65±0.79 ^{eBy}
	50	63.74±0.04 ^{eBx}	0.92±0.01 ^{aAw}	0.75±0.02 ^{bABw}	47.56±0.29 ^{dBx}	43.72±0.46 ^{dBx}
	75	65.38±0.15 ^{cBw}	0.92±0.01 ^{aAx}	0.75±0.01 ^{bABx}	48.15±0.47 ^{bBw}	45.15±0.83 ^{bBw}
6	-	63.00±0.16 ^{fAz}	0.92±0.01 ^{aAw}	0.75±0.01 ^{bcBw}	46.99±0.43 ^{gAz}	42.12±0.59 ^{fAz}
	25	64.28±0.22 ^{dAy}	0.90±0.01 ^{abAw}	0.73±0.01 ^{cBw}	47.26±0.92 ^{eAy}	43.78±0.34 ^{dAy}
	50	65.45±0.21 ^{bAx}	0.88±0.00 ^{bcAw}	0.73±0.00 ^{cBw}	47.73±0.29 ^{cAx}	44.05±0.37 ^{cAx}
	75	68.16±0.39 ^{aAw}	0.87±0.01 ^{cAx}	0.71±0.01 ^{dBx}	48.55±0.11 ^{aAw}	45.47±0.63 ^{aAw}

* Based on surimi solid content. Values are presented as mean ± SD (n = 3).

Different lowercase (a-h) superscripts within the same column indicate significant differences ($p < 0.05$).

Different uppercase (A-D) superscripts in the same column indicate significant differences between gellan levels ($p < 0.05$).

Different lowercase (w-z) superscripts in the same column indicate significant differences between $CaCl_2$ concentrations ($p < 0.05$).

the formation of bridges between both biopolymers, resulting in the increases in both breaking force and hardness. The increases in hardness of surimi gel gel without and with GL were observed as the concentrations of $CaCl_2$ increased. The results indicated the role of endogenous TGase in cross-linking of proteins via the formation of ε-(γ-glutamyl)lysine linkages and protein-gellan conjugates. Similar results were observed for gumminess and chewiness. Both gumminess and chewiness of surimi gel increased noticeably when added with GL and $CaCl_2$ in a dose dependent manner ($p < 0.05$). There were no differences in springiness when the levels of GL increased ($p > 0.05$). However, the springiness was decreased when the concentrations of $CaCl_2$ increased ($p < 0.05$). Cohesiveness of surimi gels slightly decreased when GL was added. However, there was no difference in cohesiveness between surimi gel containing 2, 4 and 6% GL, regardless of $CaCl_2$ levels ($p > 0.05$). Increasing $CaCl_2$ concentration resulted in the decreases in cohesiveness of surimi gel ($p < 0.05$). This was in accordance with the lowed deformation (Figure 12B). In the presence of cations, the gellan more likely becomes very rigid or brittle, associated with the loss in elasticity (Petcharat *et al.*, 2017). In general, the addition of hydrocolloids into surimi, especially in the presence of cation, have been proposed to improve the textural properties of gel (Ramírez *et al.*, 2011). The result suggested the filler or binder effect of GL via electrostatic interaction, which was triggered by calcium ion. Addition of GL could therefore play an essential role in textural properties of surimi gel from bigeye snapper.

5.4.1.3 Expressible moisture content

Expressible moisture content of gel from bigeye snapper surimi added with GL and $CaCl_2$ at different levels is shown in Table 15. For surimi gel without $CaCl_2$ addition, the expressible moisture content of surimi gel decreased when the levels of GL increased ($p < 0.05$), suggesting the increased water holding capacity. Decrease in expressible moisture content of gel containing $CaCl_2$ was in agreement with the increase in breaking force (Figure 12A). It has been known that the addition of divalent cation into surimi enhanced the cross-linking of proteins in the ordered fashion, resulting in the formation of stronger and fine network with greater water holding capacity of gels (Arfat and Benjakul, 2013).

Generally, the expressible moisture content is indicative for the water holding capacity of gel (Rawdkuen *et al.*, 2004) been used in restructured products for improving the water holding capacity (Ramírez *et al.*, 2011). The result suggested that GL could induce the formation of stronger network, in which more water could be held in the gel network. Moreover, GL might absorb water during the heating process via charged domains. Nevertheless, the increasing $CaCl_2$ concentrations caused an increase in expressible moisture content of gels ($p < 0.05$). This might be caused by the interaction between GL and myofibrillar proteins, especially in the presence of $CaCl_2$ via Ca^{2+}-bridges. As a consequence, more compact structure had less space for water entrapment. Additionally, free charged residues became less, resulting in the decreased number of sites for water binding. Therefore, GL and $CaCl_2$ played an important role in water holding capacity of surimi gels from bigeye snapper.

5.4.1.4 Whiteness

Whiteness of gels from bigeye snapper surimi as affected by GL at different levels and $CaCl_2$ at various concentrations is shown in Table 15. Surimi gel without GL had the lowest whiteness (78.35), compared with others ($p < 0.05$). Both GL and $CaCl_2$ increased whiteness of surimi gels in a dose dependent manner ($p < 0.05$). In general, whiteness is used as one of the quality indices for surimi quality (Duangmal and Taluengphol, 2010). The color characteristics of gels from fish muscle protein are largely dependent on the types and amounts of additives used (Rawdkuen *et al.*, 2007). GL gel itself is opaque in appearance (data not shown). Light scattering effect of GL might contribute to the increased whiteness of surimi gels, especially when the higher level of GL was incorporated. Without GL addition, $CaCl_2$ might form complex with some anion of muscle proteins. The formation of insoluble particles plausibly contributed to the light scattering in resulting gels (Benjakul *et al.*, 2004b). With the addition of GL, incorporation of $CaCl_2$ at higher levels could increase whiteness of resulting gel. The same phenomenon was found in fish gelatin/gellan mixed system as reported by Petcharat and Benjakul (2017b). Fish gelatin gel became more dull/opaque, particularly when $CaCl_2$ at higher levels was added. Furthermore, the increase in whiteness of surimi gel added with GL, especially in combination with

$CaCl_2$ at higher levels, was in accordance with the increased expressible moisture content. Free water or released water could cause light scattering or reflection on the surface of gel. This was associated with the increased whiteness. Thus, the use of GL in combination with $CaCl_2$ is a potential approach for increasing gel strength and exhibited the positive effect on whiteness of resulting gel.

Table 15 Expressible moisture content and whiteness of bigeye snapper surimi gel as affected by the addition of GL and $CaCl_2$ at different levels

Gellan (%)*	$CaCl_2$ (mmol/kg)	Expressible moisture content (%)	Whiteness
0	-	3.57± 0.15 ^{bAz}	78.35± 0.01 ^{nDz}
	25	3.34±0.07 ^{cAy}	78.44±0.04 ^{mDy}
	50	3.21±0.18 ^{dAx}	78.63±0.09 ^{lDx}
	75	3.05±0.11 ^{eAw}	78.77±0.05 ^{kDw}
2	-	2.15±0.12 ^{kBz}	78.76±0.02 ^{kCz}
	25	2.51±0.16 ^{hBy}	78.86±0.14 ^{jCy}
	50	2.79±0.10 ^{fBx}	79.49±0.08 ^{hCx}
	75	3.73±0.23 ^{aBw}	79.77±0.04 ^{gCw}
4	-	1.34±0.15 ^{nCz}	78.90±0.03 ^{iBz}
	25	1.92±0.08 ^{lCy}	79.79±0.06 $^{f B y}$
	50	2.28±0.04 ^{jCx}	80.97±0.01 ^{dBx}
	75	2.53±0.07 ^{gCw}	81.15±0.02 ^{cBw}
6	-	1.14±0.09 ^{oDz}	79.40±0.05 ^{hAz}
	25	1.44±0.08 ^{mDy}	80.28±0.07 ^{eAy}
	50	2.14±0.06 ^{klDx}	81.39±0.03 ^{bAx}
	75	2.35±0.08 ^{iDw}	81.73±0.03 ^{aAw}

* Based on surimi solid content. Values are presented as mean ± SD (n = 3).

Different lowercase (a-h) superscripts within the same column indicate significant differences ($p < 0.05$). Different uppercase (A-D) superscripts in the same column indicate significant differences between gellan levels ($p < 0.05$). Different lowercase (w-z) superscripts in the same column indicate significant differences between $CaCl_2$ concentrations ($p < 0.05$).

5.4.1.5 Protein patterns

Protein patterns of surimi gels without and with the addition of GL and $CaCl_2$ at various levels are depicted in Figure 13. Surimi paste contained myosin heavy chain (MHC) and actin as the major proteins, respectively. In the control gel (without GL and $CaCl_2$), MHC band intensity was markedly decreased, compared to that of surimi paste. Cross-linking of proteins mediated by indigenous TGase played a major role in the disappearance of MHC band (Singh and Benjakul, 2017a). For surimi gel without GL addition, the band intensity of MHC was decreased to higher extent when $CaCl_2$ at higher levels was incorporated. Bigeye snapper surimi was reported to have the pronounced setting phenomenon (Benjakul and Visessanguan, 2003).

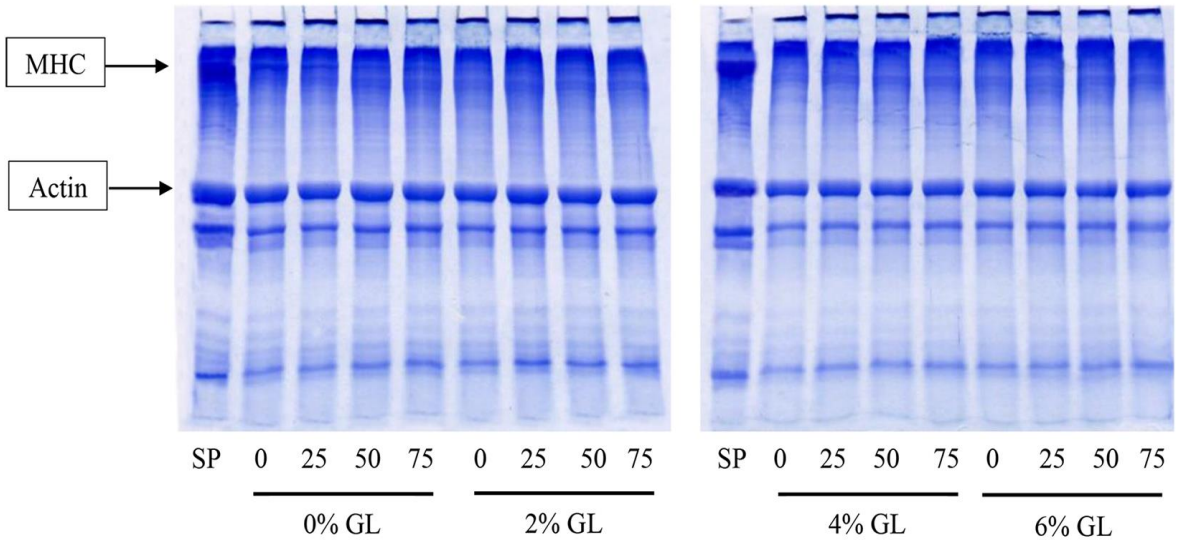

Figure 13 Protein patterns of bigeye snapper surimi gel without and with GL and $CaCl_2$ at different levels. MHC, myosin heavy chain; SP, surimi paste; and C, the control gel. Numbers (0, 25, 50, 75) denote the concentrations of $CaCl_2$ (mmol/kg).

Nevertheless, degradation also occurred during gelation. When GL at all levels was added, no MHC band was observed. This result suggested that inter- and intra-molecular cross-linking of MHC via non-disulfide covalent bonds still took place without interfering effect by GL. GL most likely interacted with muscle proteins

by weak bonds, such as ionic bonds, hydrogen bonds and hydrophobic interactions, which could be destroyed by SDS used for solubilization. Therefore, the addition of GL had no effect on setting mediated by endogenous TGase, in which isopeptides could be formed. Formation of ε-(γ-glutamyl)lysine isopeptide is induced by TGase (Benjakul and Visessanguan, 2003). When $CaCl_2$ was incorporated, no MHC band was found in all gels, regardless of GL levels. It was noted that no changes in actin and tropomyosin band intensity were noticeable for all samples, irrespective of amounts of GL and $CaCl_2$ used. Thus, actin was not served as the preferable substrate for TGase (Rawdkuen and Benjakul, 2008). In addition, actin was resistant to proteolysis (Buamard and Benjakul, 2015). The result suggested that GL had no negative effect on surimi protein cross-linking, while $CaCl_2$ could promote the polymerization of MHC.

5.4.2 Acceptability of surimi gel as affected by gellan and $CaCl_2$ at the selected concentration

$CaCl_2$ at higher concentrations yielded the gel with higher gel strength. $CaCl_2$ at 75 mmol/kg was selected to incorporate into surimi gel containing GL at various levels. Addition of GL at higher levels increased the appearance likeness score of gel ($p < 0.05$) (Table 16). The presence of $CaCl_2$ also caused the increase in appearance likeness score of gel, especially those containing GL at higher levels ($p < 0.05$). Surimi gel containing 6% GL, incorporated with $CaCl_2$, exhibited the highest appearance likeness score ($p < 0.05$). Regardless of $CaCl_2$ added, the addition of GL increased firmness likeness score of resulting gel ($p < 0.05$). When $CaCl_2$ was incorporated, the firmness likeness score of gel was increased. Nevertheless, the lowest in firmness likeness score of gel containing 6% GL incorporated with 75 mmol$CaCl_2$/kg was observed ($p < 0.05$). This might due to the interaction effect of GL and $CaCl_2$. The incorporation of GL and $CaCl_2$, especially at higher level, into surimi led to the rigid and brittle texture, which was not desirable for consumers. Petcharat and Benjakul (2017b) also reported that fish gelatin gel containing GL at high level (>5% GL) had the decrease in overall likeness score of resulting gel when $CaCl_2$ was incorporated. Addition of GL into surimi had no impact on springiness,

Table 16 Likeness score of bigeye snapper surimi gel as affected by the addition of GL and $CaCl_2$ at different levels

Gellan (%)*	$CaCl_2$ (mmol/kg)	Appearance	Color	Odor	Firmness	Springiness	Taste	Overall
0	-	8.11±1.08^{eCy}	8.02±1.01 ^{aAx}	7.39±1.53 ^{aAx}	7.64±0.82 ^{eDy}	7.53±1.51 ^{aAx}	7.59±0.93 ^{aAx}	7.68±0.62 ^{fDy}
	75	8.09±1.40^{eCx}	8.14±1.21 ^{aAx}	7.20±2.20 ^{aAx}	7.72±0.55 ^{dDx}	7.51±1.12 ^{aAy}	7.50±0.66 ^{aAy}	7.81±0.71 ^{eDx}
2	-	8.06±0.89^{eCy}	8.16±1.06 ^{aAx}	7.34±1.21 ^{aAx}	7.80±0.56 ^{cCy}	7.45±1.26 ^{aAx}	7.58±0.42 ^{aAx}	7.94±0.90 ^{dCy}
	75	8.13±0.65^{eCx}	8.07±0.96 ^{aAx}	7.28±1.47 ^{aAx}	7.90±0.76 ^{bCx}	7.52±1.08 ^{aAy}	7.48±0.43 ^{bAy}	8.32±0.86 ^{bCx}
4	-	8.37±0.43^{dBy}	8.09±0.49 ^{aAx}	7.24±1.31 ^{aAx}	7.98±0.59 ^{bAy}	7.48±1.12 ^{aAx}	7.60±0.77 ^{aAx}	8.04±0.67 ^{cAy}
	75	8.45±0.32^{cBx}	8.18±1.20 ^{aAx}	7.22±1.66 ^{aAx}	8.58±0.32 ^{aAx}	7.50±1.15 ^{aAy}	7.49±0.36 ^{bAy}	8.71±0.43 ^{aAx}
6	-	8.58±0.71^{bAy}	8.11±0.96 ^{aAx}	7.18±1.26 ^{aAx}	8.60±0.78 ^{aBy}	7.48±0.95 ^{aAx}	7.38±0.86 ^{bBx}	8.52±0.68 ^{abBy}
	75	8.64±0.63^{aAx}	8.04±1.22 ^{aAx}	7.21±1.47 ^{aAx}	7.24±0.61 ^{fBx}	7.03±0.56 ^{bAy}	7.27±0.90 ^{cBy}	7.22±0.56 ^{gBx}

* Based on surimi solid content. Values are presented as mean ± SD.

Different lowercase (a-h) superscripts within the same column indicate significant differences ($p < 0.05$).

Different uppercase (A-D) superscripts in the same column indicate significant differences between gellan levels ($p < 0.05$).

Different lowercase (x, y) superscripts in the same column indicate significant differences between $CaCl_2$ concentrations ($p < 0.05$).

color and odor likeness score of resulting gels ($p > 0.05$), while slight decrease in springiness likeness score was found when $CaCl_2$ was added ($p < 0.05$). Taste likeness score of surimi gel was generally decreased as the levels of GL and $CaCl_2$ were increased ($p < 0.05$). High amount of $CaCl_2$ could provide bitter and salty taste (Lawless *et al.*, 2003). As a result, slightly bitter and salty might reduce taste likeness scores of resulting gel. The lower taste likeness score was found in surimi gel added with GL at higher levels. This probably due to the dilution effect of GL on myofibrillar proteins, especially when GL at higher proportion was present. Surimi contained several amino acids such as glutamic acid or aspartic acid, which are known to provide umami taste (Dewi *et al.*, 2016). For the gel containing GL (without $CaCl_2$), surimi added with 6% GL had the highest overall likeness score. When $CaCl_2$ was incorporated, the highest overall likeness score was obtained for gel containing 4% GL ($p < 0.05$). This result suggested that GL at less amount (4%) in combination with $CaCl_2$ (75 mmol/kg) could improve property and acceptability of bigeye snapper surimi gel. Additionally, gel could maintain the deformability as indicated by the unchanged deformation (Figure 12) and springiness (Table 14).

5.4.3 Properties of surimi gel as affected by gellan and $CaCl_2$ in the presence and absence of endogenous TGase

5.4.3.1 Breaking force and deformation

Breaking force and deformation of surimi gels added with 4% GL and 75 mmol $CaCl_2$/kg in the presence and absence of endogenous TGase are shown in Table 17. For both gels (with and without endogenous TGase), breaking force increased when GL or $CaCl_2$ were added ($p < 0.05$). The results reconfirmed that the increase in breaking force of surimi gel was owing to electrostatic interaction between the carboxyl groups of GL and the positively charged domains of myofibrillar protein which could be enhanced by Ca^{2+} added. Thus, the addition of GL and $CaCl_2$ could be used to reinforce surimi gel from bigeye snapper in the presence and absence of endogenous TGase. Gels containing TGase showed the higher breaking force than those without TGase ($p < 0.05$). Marked increase in breaking force was found in the presence of endogenous TGase, especially in gel containing 4% GL and 75 mmol $CaCl_2$/kg ($p < 0.05$), in which the increase in breaking force by 92.25% was

Table 17 Gel property of bigeye snapper surimi gel as affected by gellan and $CaCl_2$ in the presence and absence of TGase

Gellan (%)*	$CaCl_2$ (mmol/kg)	Breaking force (g)	Deformation (mm)	Expressible moisture content (%)	Whiteness
With TGase					
0	-	317.28±8.04 ^{fAz}	10.51±0.43 ^{aAw}	3.51±0.16 ^{dBw}	78.32±0.10 ^{eAw}
	75	438.67±10.01 ^{dAy}	10.49±0.12 ^{aAw}	3.05±0.18 ^{eBx}	78.74±0.05 ^{dAx}
4	-	484.37±6.55 ^{bAx}	10.47±0.17 ^{aAw}	1.35±0.17 ^{hBz}	78.91±0.01 ^{cAy}
	75	609.96±11.36 ^{aAw}	10.38±0.17 ^{aAx}	2.59±0.13 ^{gBy}	81.14±0.03 ^{aAz}
Without TGase					
0	-	182.99±19.52 ^{hBz}	6.18±0.23 ^{bBw}	4.48±0.30 ^{aAw}	77.44±0.10 ^{gBw}
	75	242.96±12.13 ^{gBy}	6.23±0.61 ^{bBw}	4.11±0.17 ^{bAx}	77.72±0.05 ^{fBx}
4	-	369.23±8.11 ^{eBx}	5.33±0.31 ^{cBw}	2.77±0.23 ^{fAz}	78.40±0.10 ^{eBy}
	75	473.27±14.83 ^{cBw}	5.34±0.47 ^{cBx}	3.72±0.19 ^{cAy}	80.68±0.02 ^{bBz}

* Based on surimi solid content**.** Values are presented as mean ± SD.

Different lowercase (a-h) superscripts within the same column indicate significant differences ($p < 0.05$).

Different uppercase (A-D) superscripts in the same column indicate significant differences between TGase conditions ($p < 0.05$).

Different lowercase (w-z) superscripts in the same column indicate significant differences between treatments ($p < 0.05$).

achieved, compared with the control. Furthermore, the addition of GL and $CaCl_2$ had no effect on deformation of surimi gels in the presence of TGase ($p > 0.05$) (Table 17). It was noted that the decrease in deformation was observed in surimi gel in the absence of TGase. Thus, TGase played an essential role in deformation of surimi gel. In the present study, NH_4Cl was used to suppress the formation of the ε-(γ-glutamyl) lysine isopeptide via generated ammonia during the acyl transfer reaction between γ-carboxyamide groups of glutamine residues and primary amines and the excess amount of ammonium ion prevents further progress of the reaction (Benjakul *et al.*, 2004c). The decrease in deformation of surimi gel containing no TGase when GL was added was more likely due to the dilution effect of GL toward myofibrillar proteins in surimi. Therefore, both GL and $CaCl_2$ were able to improve the textural property of surimi gels in the presence and absence of TGase.

5.4.3.2 Expressible moisture content

Expressible moisture content of gel from bigeye snapper surimi added with GL and $CaCl_2$ in the presence and absence of TGase is shown in Table 17. Addition of GL and $CaCl_2$ into surimi decreased expressible moisture content of the resulting gels, compared to the control ($p < 0.05$). The decreased expressible moisture content indicated that more favorable physical entrapment of water occurred in the protein matrix. In general, polysaccharides namely starch, carboxymethylcellulose, locust bean gum, xanthan gum, konjacglucomannan, curdlan and sodium alginate could be used to improve protein network and increase water holding capacity of protein gel (Ramírez *et al.*, 2011). Ding *et al.* (2011) suggested that the addition of $CaCl_2$ (10 mmol/kg) could improve water holding capacity of suwari and kamaboko gels from yellowcheek carp and grass carp surimi. The incorporation of GL and $CaCl_2$ thus played an essential role in gel strengthening as evidenced by the highest breaking force of gel containing 4% GL and 75 mmol $CaCl_2$/kg. However, the expressible moisture content of gel containing 4% GL was lower than that of gel incorporated with both 4% GL and 75 mmol $CaCl_2$/kg. More compact structure, having less space for water entrapment, presumably contributed to the lower water holding capacity of gel added with both GL and $CaCl_2$. It was noted that all samples containing TGase showed higher water holding capacity than those without TGase ($p < 0.05$) as

indicated by the higher expressible moisture content of the latter. These results revealed that higher endogenous TGase was required for development of myofibrillar protein network in conjunction with GL addition, which could hold or entrap water in the gel.

5.4.3.3 Whiteness

Whiteness of gels from bigeye snapper surimi in the presence and absence of TGase as affected by GL and $CaCl_2$ is shown in Table 17. The increases in whiteness were found in surimi gels added with both GL and $CaCl_2$, irrespective of endogenous TGase ($p < 0.05$). The increase in whiteness of surimi gels containing both GL and $CaCl_2$ more likely due to light scattering effect of gellan and released water. Hydrocolloids generally have no significant effect on color of restructured products (Ding *et al.*, 2011). Nevertheless, Hu *et al.* (2015) found that the addition of curdlan at levels lower than 4% paste could increase the whiteness of hairtail surimi. Moreover, Zhang *et al.* (2015) reported that the addition of deacetylated konjac glucomannan into Alaska pollock surimi, which subjected to a high temperature (120 °C) treatment, increased the whiteness of surimi gels. When both GL and $CaCl_2$ were added, surimi gels had higher whiteness than those containing GL or $CaCl_2$ alone ($p < 0.05$). Benjakul *et al.* (2010) found that the addition of whey protein concentrate in combination with $CaCl_2$ (50 mmol/kg) into surimi from goatfish (*Mulloidichthys martinicus*) resulted in the increased whiteness of both kamaboko and modori gels. In the presence of TGase, the addition of both GL and $CaCl_2$ increased whiteness of gels more slightly than those without TGase ($p < 0.05$). Cross-links mediated by TGase was another factor governing the whiteness of surimi gel, regardless of additive used. Thus, the use of GL in combination with $CaCl_2$ had the positive effect on whiteness of resulting gel, especially in the presence of TGase.

5.4.3.4 Dynamic rheology

Viscoelasticity determines quality of surimi products and the ingredients used in their preparation are significant factors influencing the rheological properties of surimi (Chen and Xue, 2009). Elastic modulus (G') is a good index for heat induced gel formation of food proteins (Pietrowski *et al.*, 2012). Changes in G' of bigeye

snapper surimi paste added with GL and $CaCl_2$ alone or in combination in the presence and absence of endogenous TGase during transition from sol to gel as a function of temperature are depicted in Figure 14A. Similar G' curves were observed for all surimi pastes in the presence of TGase. Gel samples added with GL or $CaCl_2$ or both showed the higher G' than the control (without GL and $CaCl_2$). The highest G′ was observed in surimi paste containing 4% GL in combination with $CaCl_2$ at 75 mmol/kg. These results were in accordance with the highest breaking force of gel added with both 4% GL and 75 mmol $CaCl_2$/kg (Table 17). In the presence of TGase, G' of control gel increased continuously and reached the highest value at approximately 35 ˚C. This indicated the formation of protein network via weak bonds, e.g. hydrogen bonds between protein molecules (Buamard *et al.*, 2017). Thereafter, G' rapidly decreased and the lowest value was obtained at about 50 ˚C. Degradation mediated by endogenous proteolytic enzymes in the temperature range of 50-60 ˚C (Buamard and Benjakul, 2015) and disaggregation of actin-myosin network structure more likely enhanced mobility of proteins, resulting in a decreased G' (Zhang *et al.*, 2015). G' was subsequently increased again when heated up to 65 ˚C. This probably resulted from an increase in the number of cross-links between dissociated protein molecules and the further denaturation of MHC and actomyosin, leading to a formation of a thermo-irreversible gel network (Mleko and Foegeding, 2000). Unfolded proteins might undergo the aggregation via reactive groups or domains. Hydrophobic domains plausibly interacted each other via hydrophobic-hydrophobic interaction, while sulfhydryl groups were oxidized, in which disulfide bond could be formed (Buamard and Benjakul, 2015). Thereafter, G' slightly decreased until the temperature reached 80 ˚C. Weak bonds such as hydrogen bonds might be destroyed during heating at high temperature. Slight increase in G' was found after further heating up to 90 ˚C, suggesting the continuous aggregation of unfold proteins as induced by high temperature.

G' curves of surimi pastes in the absence of TGase were different from those observed for pastes with TGase. Within the temperature range of 10-50 ˚C, G' continuously increased and reached the constant value at approximately 35-45 ˚C. No sharp decrease in G' was found at the aforementioned temperature range. It was assumed that constant increase in G' in the range of 10-50 ˚C was associated with the

gradual interaction of muscle proteins in the absence of TGase, in which no cross-linking mediated by non-disulfide covalent bonds was involved. As a consequence, no drastic increase in G' was found within the temperature range of 10-35 ˚C. TGase was a potential protein cross-linking enzyme, which could introduce the strong bond to stabilize protein network. During 50-90 ˚C, the similar trend was noticeable to those of paste samples containing TGase. At the same level of GL and $CaCl_2$ added, G′ of pastes in the presence of TGase was higher than that in the absence of TGase. Nevertheless, the addition of GL and $CaCl_2$ alone or in combination also increased G' of surimi pastes in the absence of TGase when compared with the corresponding control. The highest G′ was found in paste added with 4% GL and $CaCl_2$, followed by the paste containing 4% GL and paste added with $CaCl_2$, respectively. The result reconfirmed that GL acted as an active filler, which could increase the elastic modulus via ionic interaction. Furthermore, the addition of $CaCl_2$ could promote the formation of junction zone in the gel matrix via salt bridge.

5.4.3.5 Protein patterns

Protein patterns of surimi gels without and with the addition of GL and $CaCl_2$ alone or in combination in the presence and absence of TGase are shown in Figure 14B. MHC and actin were found as the major proteins in surimi paste. In the presence of TGase, MHC of control gels completely disappeared. The disappearance of MHC band was mostly mediated by endogenous TGase (Buamard and Benjakul, 2015). When GL and/or $CaCl_2$ were added, no differences in protein patterns were observed. This result reconfirmed that both GL and $CaCl_2$ had no effect on inter- and intra-molecular cross-linking of MHC via non-disulfide covalent bonds mediated by endogenous TGase. Surimi used in the presence study might contain the sufficient indigenous Ca^{2+}, which was enough for activation of TGase. In the absence of TGase, MHC was more retained when compared with that found in surimi gel containing TGase. Nevertheless, no difference in MHC band intensity of gel as affected by GL and/or $CaCl_2$ incorporated was observed. The result reflected the role of endogenous TGase in cross-linking of myofibrillar proteins, particularly MHC during setting of surimi. The addition of NH_4Cl could inhibit TGase reaction in surimi. Moreover, actin and tropomyosin band density in all samples remained unchanged, regardless of TGase. It

could be inferred that GL and $CaCl_2$ at the level tested had no effect on polymerization of myosin heavy chain both in the presence and absence of TGase.

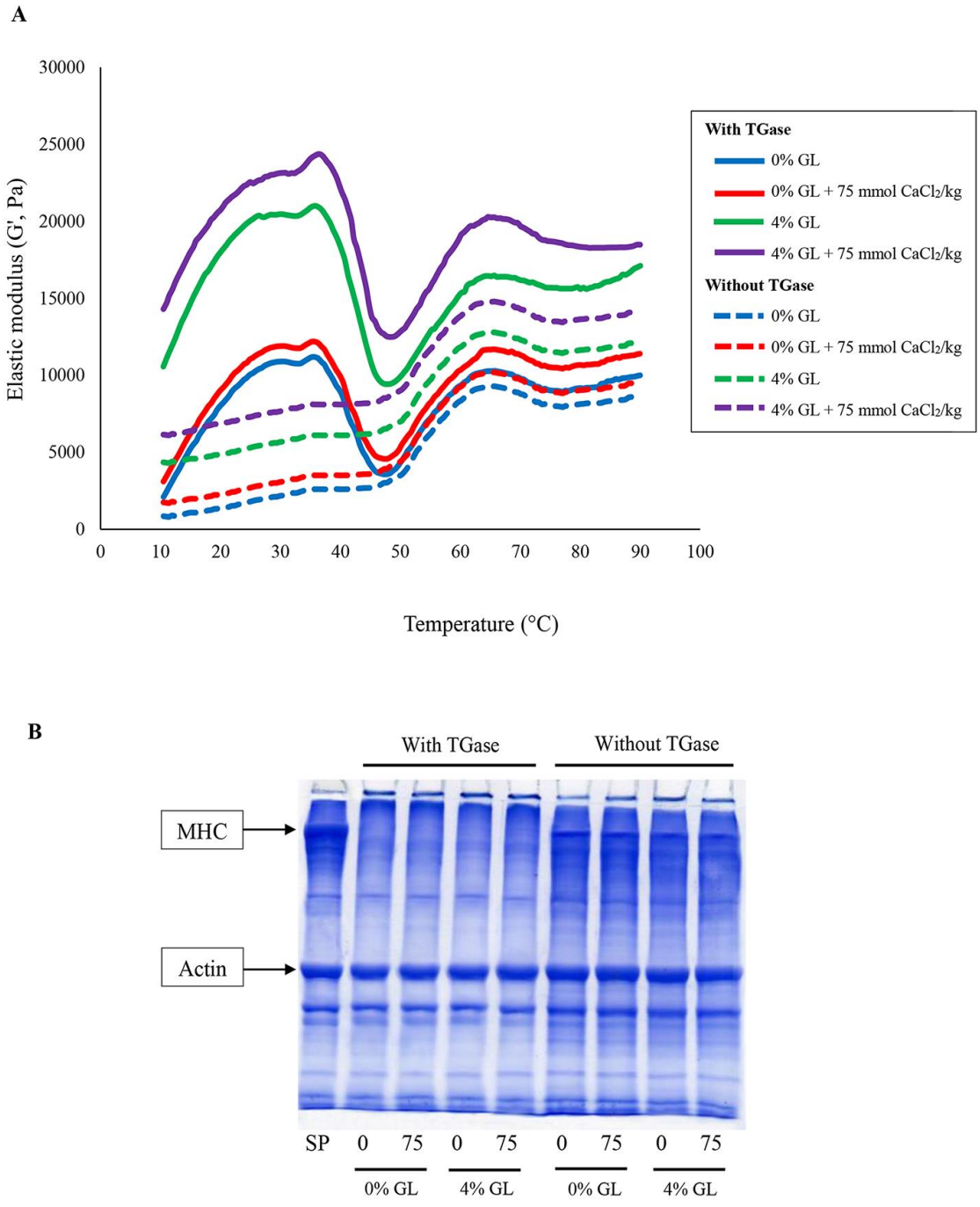

Figure 14 Elastic modulus (G') during heating of bigeye snapper surimi paste (A) and protein patterns (B) of bigeye snapper surimi gel without and with GL and $CaCl_2$ at different levels in the presence and absence of TGase. MHC, myosin heavy chain; SP, surimi paste. Numbers (0, 75) denote the concentrations of $CaCl_2$ (mmol/kg).

5.4.3.6 Microstructures

Microstructure of the selected surimi gels from bigeye snapper including 1) control gel (without GL and $CaCl_2$), 2) gel added with 75 mmol $CaCl_2$/kg, 3) gel added with 4% GL and 4) gel added with 4% GL in combination with 75 mmol $CaCl_2$/kg in the presence and absence of TGase were visualized by SEM as shown in Figure 15. In the presence of TGase, a coarser network with the larger void or cavities was found in the control gel (without GL or $CaCl_2$ addition). This was in accordance with the lowest breaking force and water holding capacity (Table 17). Finer and denser structure was observed in gel added with GL or $CaCl_2$. Nevertheless, the highest compactness with the highest interconnectivity of proteins was observed in gel containing both 4% GL and 75 mmol $CaCl_2$/kg. These observations suggested that GL might distribute uniformly as the active filler in the ordered network and was able to interact with myofibrillar proteins of surimi via ionic interaction. The results also confirmed the presumption that Ca^{2+} could promote interconnection of protein-protein and protein-gellan via salt bridge. The control gels in the absence of TGase showed more discontinuous network with larger grooves and holes, compared with the control gel containing TGase. Similar trend of microstructure was also found in surimi gel without TGase. Nevertheless, surimi gels in the presence of TGase had finer and denser structure than those without TGase when both GL and $CaCl_2$ was added. This indicated the essential role of endogenous TGase in gel strengthening of surimi. Therefore, GL and $CaCl_2$ at appropriate levels could strengthen gel network of surimi in the presence and absence of TGase as evidenced by finer and more ordered network with increased compactness.

5.5 Conclusions

Gel properties of surimi from bigeye snapper were governed by level of GL and $CaCl_2$. The addition of GL and $CaCl_2$ increased breaking force, hardness and whiteness of surimi gel in a dose dependent manner. Water holding capacity of resulting gels increased when GL and $CaCl_2$ were added. GL and $CaCl_2$ at all levels had no effect on polymerization of MHC. Overall likeness score of resulting gels was increased as the level of GL was increased. Gel containing 4% GL in the presence of

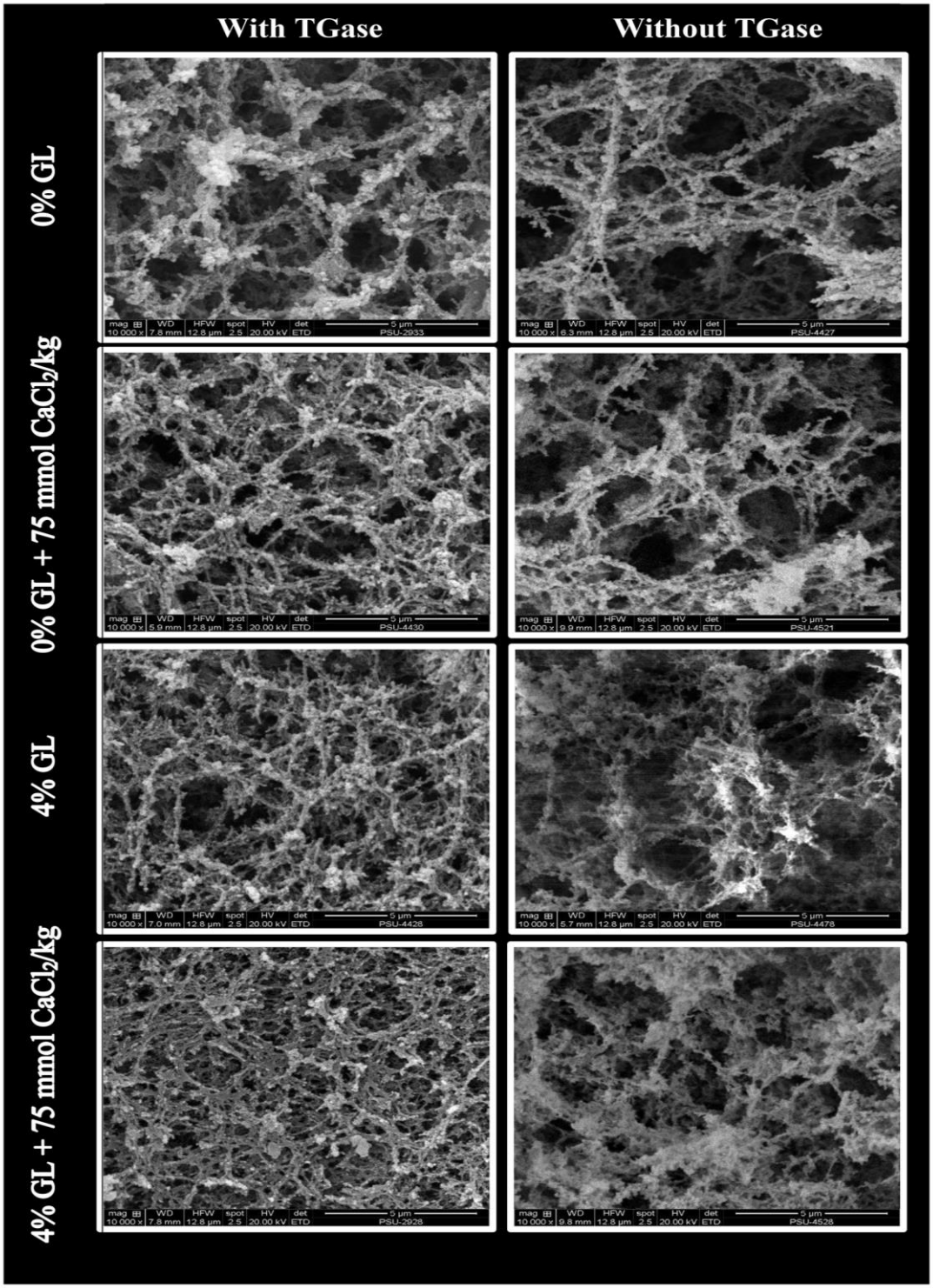

Figure 15 Electron microscopic images of surimi gel as affected by the addition of GL and $CaCl_2$ in the presence and absence of TGase. Magnification: 10,000X.

75 mmol $CaCl_2$/kg had the highest acceptability. In general, gels with TGase exhibited the higher gel strength and water holding capacity than those without TGase. Both GL and $CaCl_2$ could improve gel property, namely breaking force, water holding capacity and whiteness of surimi gels without TGase. The addition of both GL and $CaCl_2$ could enhance viscoelastic property of surimi paste during heating as indicated by the increased G'. Finer and denser network was observed in surimi gel containing both GL and $CaCl_2$ in the presence of TGase, compared to those without TGase. Therefore, GL at 4% in combination with 75 mmol $CaCl_2$/kg could improve gel strength of bigeye snapper surimi with low or high setting phenomenon regulated by endogenous TGase.

5.6 References

AOAC. 2000. Official methods of analysis of AOAC International. 17th Ed. USA: AOAC International. Gaithersburg, MD.

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

Banerjee, S. and Bhattacharya, S. 2012. Food gels: gelling process and new applications. Critical reviews in food science and nutrition. 52: 334-346.

Barrera, A. M., Ramírez, J. A., González-Cabriales, J. J. and Vázquez, M. 2002. Effect of pectins on the gelling properties of surimi from silver carp. Food Hydrocolloids. 16: 441-447.

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

Benjakul, S., Visessanguan, W. and Chantarasuwan, C. 2004a. Effect of porcine plasma protein and setting on gel properties of surimi produced from fish caught in Thailand. LWT - Food Science and Technology. 37: 177-185.

Benjakul, S., Visessanguan, W. and Kwalumtharn, Y. 2004b. The effect of whitening agents on the gel-forming ability and whiteness of surimi. International Journal of Food Science & Technology. 39: 773-781.

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

Benjakul, S., Visessanguan, W. and Srivilai, C. 2001. Gel properties of bigeye snapper (*Priacanthus tayenus*) surimi as affected by setting and porcine plasma proteins. Journal of Food Quality. 24: 453-471.

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

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

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

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

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

Chen, H. and Xue, C. 2009. Effect of hydrocolloids on the gel properties of horse-mackerel surimi. Transactions of the Chinese Society for Agricultural Machinery. 40: 119-125.

Dewi, E. N., Amalia, U. and Mel, M. 2016. The effect of different treatments to the amino acid contents of mcicro algae *Spirulina* sp. Aquatic Procedia. 7: 59-65.

Ding, Y., Liu, Y., Yang, H., Liu, R., Rong, J., Zhao, S. and Xiong, S. 2011. Effects of $CaCl_2$ on chemical interactions and gel properties of surimi gels from two species of carps. European Food Research and Technology. 233: 569-576.

Duangmal, K. and Taluengphol, A. 2010. Effect of protein additives, sodium ascorbate, and microbial transglutaminase on the texture and colour of red tilapia surimi gel. International Journal of Food Science & Technology. 45: 48-55.

Eom, S.-H., Kim, J., Son, B.-Y., You, D. H., Han, J. M., Oh, J.-H., Kim, B.-Y. and Kong, C.-S. 2013. Effects of carrageenan on the gelatinization of salt-based surimi gels. Fisheries and aquatic sciences. 16: 143-147.

Evageliou, V., Gerolemou, A., Zikas, A., Basios, A. and Komaitis, M. 2011. Effect of salts and sugars on the clarity of gellan gels. International Journal of Food Science & Technology. 46: 1001-1006.

Goh, K. K. T., Haisman, D. R. and Singh, H. 2006. Characterisation of a high acyl gellan polysaccharide using light scattering and rheological techniques. Food Hydrocolloids. 20: 176-183.

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

Hu, Y., Shao, Y., Wu, C., Yuan, C., Ishimura, G., Liu, W. and Chen, S. 2017. γ-PGA and MTGase improve the formation of ε-(γ-glutamyl) lysine cross-links within hairtail (*Trichiurus haumela*) surimi protein. Food Chemistry. https://doi.org/10.1016/j.foodchem.2017.08.087:

Hunt, A. and Park, J. W. 2013. Alaska pollock fish protein gels as affected by refined carrageenan and various salts. Journal of Food Quality. 36: 51-58.

Iurciuc, C. E., Savin, A., Lungu, C., Martin, P. and Popa, M. 2016. Gellan. Food applications. Cellulose Chemistry and Technology. 50: 1-13.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature. 227: 680-685.

Lawless, H. T., Rapacki, F., Horne, J. and Hayes, A. 2003. The taste of calcium and magnesium salts and anionic modifications. Food Quality and Preference. 14: 319-325.

Lin, K. W. and Huang, H. Y. 2003. Konjac/gellan gum mixed gels improve the quality of reduced-fat frankfurters. Meat Science. 65: 749-755.

Meilgaard, M. C., Carr, B. T. and Civille, G. V. 2007. Sensory evaluation techniques. 4th Ed. CRC press. Florida.

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

Montero, P., Hurtado, J. L. and Pérez-Mateos, M. 2000. Microstructural behaviour and gelling characteristics of myosystem protein gels interacting with hydrocolloids. Food Hydrocolloids. 14: 455-461.

Morris, E. R., Nishinari, K. and Rinaudo, M. 2012. Gelation of gellan - A review. Food Hydrocolloids. 28: 373-411.

Nickerson, M. T., Paulson, A. T. and Hallett, F. R. 2008. Pre-gel solution properties of gellan polysaccharides: Effect of potassium and calcium ions on chain associations. Food Research International. 41: 462-471.

Park, J. W. 2005. Surimi and surimi seafood. 2 Ed. Taylor and Francis/CRC Press. Boca Raton, FL, USA.

Petcharat, T. and Benjakul, S. 2017a. Effect of gellan incorporation on gel properties of bigeye snapper surimi. Food Hydrocolloids (Submitted).

Petcharat, T. and Benjakul, S. 2017b. Property of fish gelatin gel as affected by the inorporation of gellan and calcium chloride. Food Biophysics. 12: 339-347.

Petcharat, T., Benjakul, S. and Hemar, Y. 2017. Improvement of gel properties of fish gelatin using gellan. International Journal of Food Engineering. 13: https://doi.org/10.1515/ijfe-2016-0410.

Pietrowski, B. N., Tahergorabi, R. and Jaczynski, J. 2012. Dynamic rheology and thermal transitions of surimi seafood enhanced with ω-3-rich oils. Food Hydrocolloids. 27: 384-389.

Ramírez, J. A., Uresti, R. M., Velazquez, G. and Vázquez, M. 2011. Food hydrocolloids as additives to improve the mechanical and functional properties of fish products: A review. Food Hydrocolloids. 25: 1842-1852.

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

Rawdkuen, S., Benjakul, S., Visessanguan, W. and Lanier, T. C. 2004. Chicken plasma protein affects gelation of surimi from bigeye snapper (*Priacanthus tayenus*). Food Hydrocolloids. 18: 259-270.

Rawdkuen, S., Benjakul, S., Visessanguan, W. and Lanier, T. C. 2007. Effect of chicken plasma protein additives on proteolysis and gel-forming ability of

sardine (*Sardinella gibbosa*) surimi. Journal of Food Processing and Preservation. 31: 492-516.

Robinson, H. W. and Hogden, C. G. 1940. The biuret reaction in the determination of serum proteins. 1. A study of the conditions necessary for the production of a stable color which bears a quantitative relationship to the protein concentration. Journal of Biological Chemistry. 135: 707-725.

Seighalani, F. Z. B., Bakar, J., Saari, N. and Khoddami, A. 2017. Thermal and physicochemical properties of red tilapia (*Oreochromis niloticus*) surimi gel as affected by microbial transglutaminase. Animal Production Science. 57: 993-1000.

Singh, A. and Benjakul, S. 2017a. Effect of serine protease inhibitor from squid ovary on gel properties of surimi from Indian mackerel. Journal of Texture Studies. 1-9.

Singh, A. and Benjakul, S. 2017b. Serine protease inhibitors from squid ovary: extraction and its effect on proteolysis and gel properties of surimi. Journal of Food Science and Technology. 54: 267-275.

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

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

Wei, Y., Zhang, T., Yu, F., Xue, Y., Li, Z., Wang, Y. and Xue, C. 2017. Effects of curdlan on the texture and structure of Alaska pollock surimi gels treated at 120 °C. International Journal of Food Properties. 10.1080/10942912.10942017.11306557.

Wu, S. 2016. Effect of pullulan on gel properties of *Scomberomorus niphonius* surimi. International Journal of Biological Macromolecules. 93: 1118-1120.

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

CHAPTER 6

SUMMARY AND FUTURE WORKS

6.1 Summary

1. Gellan (GL) at 5% could improve gelling property of fish gelatin (FG) via increasing gel strength and gelling points without affecting sensory property of resulting FG/GL mixed gel.

2. The addition of GL (2.5%) and $CaCl_2$ up to 6 mM was able to strengthen FG gel. FG/GL mixture could form gel at room temperature, while melt-in-mouth characteristic of gel was still maintained.

3. Form and level of GL affected gel properties of bigeye snapper surimi. GL prepared as suspension (GLS) using ultrasound treatment at an appropriate level (6%) could improve textural property of bigeye snapper surimi with an increased acceptability.

4. Incorporation of GL and $CaCl_2$ could strengthen bigeye snapper surimi gel. The addition of 4% GL in combination with 75 mmol $CaCl_2$/kg into surimi was recommended, in which the resulting gel had the increased gel strength and acceptability.

5. Both GL and $CaCl_2$ could improve gel property of bigeye snapper surimi with low or high setting phenomenon mediated by endogenous transglutaminase.

6.2 Future works

1. Application of FG/GL mixed gel, especially with the incorporation of $CaCl_2$, in some food product should be studied.
2. The use of other divalent cations in combination with GL in other food systems should be investigated.

VITAE

Name Miss Tanyamon Petcharat

Student ID 5811020021

Education Attainment

Degree	Name of Institution	Year of Graduation
Bachelor of Science (Agro-Industry, First Class Honor)	Prince of Songkla University	2015

List of Publication and Proceedings

Publication

1. Petcharat, T., Benjakul, S. and Hemar, Y. 2017. Improvement of gel properties of fish gelatin using gellan. International Journal of Food Engineering. DOI: 10.1515/ijfe-2016-0410.

2. Petcharat, T. and Benjakul, S. 2017. Property of fish gelatin gel as affected by the inorporation of gellan and calcium chloride. Food Biophysics. 12: 339-347.

3. Petcharat, T. and Benjakul, S. 2017. Effect of gellan incorporation on gel properties of bigeye snapper surimi. Food Hydrocolloids (Under revised).

4. Petcharat, T. and Benjakul, S. 2017. Effect of gellan and calcium chloride on properties of surimi gel with low and high setting phenomenon. RSC Advances (Under revised).

Proceeding

1. Petcharat, T. and Benjakul, S. 2017. Property of fish gelatin gel as affected by gellan in combination with calcium chloride. The 19th Food Innovation Asia Conference 2017. BITEC Bangna, Bangkok, Thailand. 15-17th June 2017. Poster presentation.