ผลของไคโตซานและโปรตีนไฮโดรไลเซตต่อโคลเซตเจลเลชันของโปรตีนละลายได้ในน้ำเกลือและเนื้อไก่ดิบขึ้นรูป

นางสาว ทานตะวัน คเชนทร์ชัย

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาเทคโนโลยีทางอาหาร ภาควิชาเทคโนโลยีทางอาหาร
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2549

EFFECTS OF CHITOSAN AND PROTEIN HYDROLYSATES ON COLD-SET GELATION OF SALT SOLUBLE PROTEINS AND RAW RESTRUCTURED CHICKEN MEAT

Miss Tantawan Kachanechai

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Food Technology
Department of Food Technology
Faculty of Science
Chulalongkorn University
Academic Year 2006

Thesis Title	EFFECTS OF CHITOSAN AND PROTEIN HYDROLYSATES ON COLD-SET GELATION OF SALT SOLUBLE PROTEINS AND RAW RESTRUCTURED CHICKEN MEAT
By	Miss Tantawan Kachanechai
Filed of study	Food Technology
Thesis Advisor	Associate Professor Pantipa Jantawat, Ph.D.
Thesis Co-advisor	Rath Pichyangkura, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Doctoral Degree

..... Dean of the Faculty of Science
(Professor Piamsak Menasveta, Ph.D.)

THESIS COMMITTEE

..... Chairman
(Assistant Professor Romanee Sanguandeekul, Ph.D.)

..... Thesis Advisor
(Associate Professor Pantipa Jantawat, Ph.D.)

..... Thesis Co-advisor
(Rath Pichyangkura, Ph.D.)

..... Member
(Associate Professor Ninnart Chinprahast, Ph.D.)

ทานตะวัน คเชนทร์ชัย: ผลของไคโตซานและโปรตีนไฮโดรไลเซตต่อโคลเซตเจลเลชันของโปรตีนละลายได้ในน้ำเกลือและเนื้อไก่ดิบขึ้นรูป (EFFECTS OF CHITOSAN AND PROTEIN HYDROLYSATES ON COLD-SET GELATION OF SALT SOLUBLE PROTEINS AND RAW RESTRUCTURED CHICKEN MEAT), อ. ที่ปรึกษา: รศ.ดร. พันธิพา จันทวัฒน์, อ.ที่ปรึกษาร่วม: อ.ดร. รัฐ พิชญางกูร, 234 หน้า.

งานวิจัยนี้ทำขึ้นเพื่อศึกษาคุณลักษณะที่เหมาะสมของไคโตซาน (chitosan) และโปรตีนไฮโดรไลเซตจากเนื้อไก่ (protein hydrolysate) ในการเป็นสารเชื่อมที่อุณหภูมิต่ำหรือโคลเซตบายเดอร์ (cold-set binder) ในเจลของโปรตีนเนื้อไก่ที่ละลายได้ในน้ำเกลือและเนื้อไก่ดิบขึ้นรูป ปัจจัยที่ศึกษาสำหรับไคโตซานได้แก่ น้ำหนักโมเลกุล ปริมาณหมู่อะเซตทิล (degree of deacetylation or DD) และความเข้มข้นของไคโตซาน โดยในแต่ละปัจจัยแบ่งเป็น 3 ระดับย่อยได้แก่ ต่ำ กลาง และสูง ปัจจัยที่ศึกษาสำหรับโปรตีนไฮโดรไลเซตได้แก่ ค่าปริมาณการตัดสายโปรตีน (degree of hydrolysis or DH) 4 ระดับ และความเข้มข้น 3 ระดับ โคลเซตเจลเตรียมโดยผสมโปรตีนเนื้อไก่ที่ละลายได้ในน้ำเกลือที่ความเข้มข้น 8% โดยน้ำหนักต่อปริมาตรกับโคลเซตบายเดอร์ จากนั้นทำให้เกิดเจลที่ 4 องศาเซลเซียส เป็นเวลา 24 ชม. แล้วตรวจวัดสมบัติทางเคมีกายภาพซึ่งประกอบด้วย ค่าพีเอช สี สมบัติด้านการไหล เนื้อสัมผัส ปริมาณพันธะไดซัลไฟด์ (disulfide content) เจลอิเลคโตรโฟเรซิสแบบไม่ทำให้โปรตีนเสียสภาพ (non-denaturing gel-electrophoresis) และลักษณะโครงสร้างของเจล ผลการทดลองแสดงว่าไคโตซานเป็นโคลเซตบายเดอร์ที่ดี เมื่อใช้ในรูปของไคโตซานที่ผ่านการละลายและตกตะกอนด้วยด่าง น้ำหนักโมเลกุล ปริมาณหมู่อะเซตทิล และความเข้มข้นของไคโตซานมีผลต่อสมบัติทางเคมีกายภาพที่ตรวจวัดทั้งหมด ($p\leq0.05$) ไคโตซานในรูปแบบดังกล่าวช่วยให้เนื้อสัมผัสและสมบัติด้านการไหลของเจลดีขึ้น ($p\leq0.05$) ปริมาณพันธะไดซัลไฟด์เพิ่มขึ้น ($p\leq0.05$) โครงสร้างของเจลดีขึ้น แต่ไม่พบการเปลี่ยนแปลงของแถบโปรตีนในเจลอิเลคโตรโฟเรซิส ไคโตซานตัวอย่างที่ดีที่สุดสำหรับใช้เป็นโคลเซตบายเดอร์ มีน้ำหนักโมเลกุล 1.84×10^5 ดาลตัน ปริมาณหมู่อะเซตทิล 94% ที่ความเข้มข้น 1.5% โดยน้ำหนัก โปรตีนไฮโดรไลเซตจากเนื้อไก่ที่มีค่า DH 6.42-35.80% ทำให้โปรตีนเนื้อไก่ที่ละลายได้ในน้ำเกลือมีความสามารถในการเกิดเจลที่อุณหภูมิต่ำดีขึ้น ($p\leq0.05$) ค่า DH และความเข้มข้นของโปรตีนไฮโดรไลเซตมีผลต่อสมบัติทางเคมีกายภาพที่ตรวจวัดทั้งหมด ($p\leq0.05$) โดยทำให้ปริมาณพันธะไดซัลไฟด์ เนื้อสัมผัส สมบัติด้านการไหล และโครงสร้างของเจลดีขึ้น ($p\leq0.05$) และพบว่ามีแถบโปรตีนที่ติดสีเข้มขึ้นกว่าตัวอย่างควบคุมในแผ่นเจลอิเลคโตรโฟเรซิส ซึ่งแสดงถึงการเกิดเจลที่อุณหภูมิต่ำที่คงตัวอยู่ได้ด้วยพันธะไดซัลไฟด์ โปรตีนเนื้อไก่ที่ละลายได้ในน้ำเกลือตัวอย่างที่ผสมกับโปรตีนไฮโดรไลเซตที่มีค่า DH 22.38% และใช้ที่ความเข้มข้น 3% โดยน้ำหนัก เกิดเจลที่คุณภาพดีที่สุด เมื่อนำไคโตซานและโปรตีนไฮโดรไลเซตตัวอย่างที่ดีที่สุดมาใช้ร่วมกันเป็นโคลเซตบายเดอร์ในเจลโปรตีนเนื้อไก่ที่ละลายได้ในน้ำเกลือ พบว่าให้ผลในการปรับปรุงสมบัติทางเคมีกายภาพที่ดีมากที่สุด ($p\leq0.05$) การใช้ไคโตซานที่ความเข้มข้น 1.5% โดยน้ำหนัก ร่วมกับโปรตีนไฮโดรไลเซตที่ความเข้มข้น 3% โดยน้ำหนัก ให้ผลดีมากที่สุด

เนื้อไก่ดิบขึ้นรูปที่เตรียมจากเนื้อไก่ส่วนอกกับเกลือที่ความเข้มข้น 1% โดยน้ำหนัก และไคโตซานหรือโปรตีนไฮโดรไลเซตตัวอย่างที่ดีที่สุดเพียงชนิดเดียว หรือใช้ทั้งสองชนิดร่วมกัน โดยทำให้เกิดเจลที่ 4 องศาเซลเซียส เป็นเวลา 24 ชม. แล้วตรวจวัดสมบัติทางเคมีกายภาพซึ่งประกอบด้วย ค่าพีเอช สี สมบัติด้านการไหล เนื้อสัมผัส สมบัติด้านการจับกับน้ำและลักษณะโครงสร้าง ผลการทดลองแสดงว่าไคโตซานและโปรตีนไฮโดรไลเซตเป็นโคลเซตบายเดอร์ที่ดีในผลิตภัณฑ์นี้ไม่ว่าจะใช้เพียงชนิดเดียวหรือใช้ทั้งสองชนิดร่วมกัน การเปลี่ยนแปลงลักษณะทางคุณภาพที่พบในผลิตภัณฑ์ เป็นไปในรูปแบบเดียวกันกับที่พบในระบบจำลองจากโปรตีนเนื้อไก่ที่ละลายได้ในน้ำเกลือ

4473811123 : MAJOR FOOD TECHNOLOGY
KEY WORD: CHITOSAN / CHICKEN PROTEIN HYDROLYSATES / CHICKEN SALT-SOLUBLE PROTEINS / RAW RESTRUCTURED CHICKEN MEAT / COLD-SET BINDER/ COLD-SET GELATION.

TANWATAN KACHANECHAI: EFFECTS OF CHITOSAN AND PROTEIN HYDROLYSATES ON COLD-SET GELATION OF SALT SOLUBLE PROTEINS AND RAW RESTRUCTURED CHICKEN MEAT. THESIS ADVISOR: ASSOC. PROF. PANTIPA JANTAWAT, Ph.D. THESIS COADVISOR: RATH PICHYANGKURA, Ph.D., 234 pp.

This research was aimed at investigating the proper characteristics of chitosan, chicken protein hydrolysates and their combinations as cold-set binders in chicken salt-soluble protein (SSP) gel and raw restructured chicken meat. The studying factors for chitosan in the SSP model were molecular weight (MW), degree of deacetylation (DD), and concentration (C), each at 3 levels (low, medium, and high), while those of the chicken protein hydrolysates were degree of hydrolysis (DH) at 4 levels and C at 3 levels. The cold-set gels were formed by setting the mixture of 8%w/v SSP and binders at 4°C for 24 hrs The physico-chemical properties of the formed gels, including pH, color, rheological characteristics, texture, disulfide content, non-denaturing gel-electrophoresis, and gel structure, were determined. The experimental results indicated that chitosan acted as good cold-set binder when using in alkali-precipitated form. The MW, DD and C affected all of the characteristics measured ($p \leq 0.05$). The inclusion of chitosan resulted in improvement of texture and rheological characteristics ($p \leq 0.05$), increasing of disulfide content ($p \leq 0.05$), and improvement of structure of the cold-set SSP gel, while the pattern on non-denaturing gel-electrophoresis remained unchanged. The most appropriate chitosan sample found was that with 1.84×10^5 Dalton MW, 94% DD and 1.5% by weight. Chicken protein hydrolysates at 6.42-35.80% DH enhanced cold-set gelation ability of SSP ($p \leq 0.05$). Both DH and C affected all parameters determined ($p \leq 0.05$). The increase of disulfide content ($p \leq 0.05$) and the improvement of texture ($p \leq 0.05$), rheological characteristic and structure of the developed gels were observed. Results from the non-denaturing gel-electrophoretic patterns indicated the formation of cold-set gels which were partially stabilized with disulfide bonds. The highest quality improvement was observed in the gel sample containing 3% by weight of the 22.38% DH hydrolysates. When combination of the selected chitosan and hydrolysate samples were used as cold-set binder in SSP gel, the highest quality improvement of the gel was obtained. The most appropriate combination found were 1.5% by weight chitosan and 3% by weight chicken protein hydrolysates.

Raw restructured chicken meats were prepared from ground chicken broiler tenderloin meats, 1% sodium chloride and the selected chitosan, hydrolysates or their combinations, and set at 4°C for 24 hrs before determining physico-chemical properties, including pH, color, rheological characteristics, texture, water binding properties and structure profiles. The experimental results indicated that the selected chitosan, hydrolysates and their combinations functioned as good cold-set binder in raw restructured chicken meats. The improving pattern of all quality characteristics of the raw restructured

ACKNOWLEDGEMENTS

First of all, I would like to express my respect and sincerely thank to my advisor, Assoc. Prof. Dr.Pantapa Jantawat. Her contributions to my dissertation were the greatest thing in my graduate study. Her supports and valuable suggestions in various aspects, such as skills for handling research problems, a vision for the future research, and how to become a good researcher, have never been faded from my memory forever.

The next person who I would like to express my gratitude to is my co-advisor, Dr.Rath Pichyangkura. His valuable suggestions to my research and his supports on chitosan guided me to pass through my Ph.D. journey. His contributions and supports will always be retained in my memory.

I would like to extend my gratitude to Assist. Prof. Dr.Romanee Sanguandeekul, Assoc. Prof. Dr.Ninnart Chinprahast, and Assist. Prof. Dr.Jiraporn Runglerdkriangkrai for being my dissertation examination commitees. Their suggestions as well as insightful discussions greatly helped me to improve the quality of this dissertation.

I would like to acknowledge the grant from Commission of Higher Education, Ministry of Education, Royal Thai Government in supporting me for persuing my research. I would also like to acknowledge the grant from Chulalongkorn University in providing me the scholarship to attend the International Hydrocolloids Conference at Norway. Without these fundings, this dissertation could never be completed.

I would like to acknowledge all labolatories in Food Technology Department, Faculty of Science, Chulalongkorn University for providing me the facilities throughout my Ph.D. research.

Finally, I would like to express my deep gratitude to my dearest mum and dad, and my dearest grandmum who passed away, for supporting me throughout my Ph.D. journey. Their encouragement, warmth, and love were the great power for me to overcome all troubles I had faced. I would also like to express my warm thank to Dr.Chaiyod Pirak for his gentle assistance, kind encouragement and being my will power, throughout my Ph.D. program. I would like to extend my thank to my colleagues and friends at Food Technology Department, Chulalongkorn University, for their encouragement and assistance. Without them, I could not pass this Ph.D. journey with the success.

TABLE OF CONTENTS

Page

CHAPTER Page

CHAPTER Page

CHAPTER Page

CHAPTER Page

LIST OF TABLES

TABLE Page

TABLE Page

LIST OF FIGURES

FIGURE Page

FIGURE Page

FIGURE Page

FIGURE Page

FIGURE Page

FIGURE Page

FIGURE Page

FIGURE Page

FIGURE Page

FIGURE Page

FIGURE Page

FIGURE Page

FIGURE Page

FIGURE Page

CHAPTER I

INTRODUCTION

Restructuring of meat is the process for producing meat products by binding small pieces of meat and reforming into a large single piece. This process provides many advantages to both of consumer and meat industries. For consumer, meat restructuring can meet their demand in producing the products with variety of size, shape, and nutritional value at the reasonable price. For meat manufacturer, the expansion of market share can be resulted from the production of new restructured meat products. Many types of meat, such as beef, pork, chicken, turkey and lamb, can be used to produce the products alone or in combination. Furthermore, the production cost is also reduced because less valuable meats, including trimmings and small particles, can be used to produce restructured meat products with desirable texture and palatability.

The method for restructuring of meat can be divided into two techniques comprising heat-set gelation and cold-set gelation. In heat-set gelation, the meats are bonded together via the auto adhesion of the extracted myofibrillar proteins occurred after heating to 60-70˚C. The major binder proteins are myosin, actin and actomyosin which coat around the meat pieces (Macfarlane, Schmidt, and Turner, 1977). Restructured meats produce with this mechanism have very low binding strength in raw state, thus, this product can be sold only in precooked state. After reheating by consumer, a dried and tough meat could be resulted. Furthermore, the obtained products frequently have the serious problem in oxidative rancidity accelerated by the high amount of salt and phosphate in the products (Gray and Pearson, 1987).

Cold-set gelation which is a novel method for producing restructured meats in raw state provides the products with similar eating characteristics to meat cuts from intact muscle. This method reduces the rancidity problem which often occurs in heat-set gelation products. The formation of cold-set gelation is based on the ability to form chemical bonds between muscle proteins, especially myofibrillar proteins, cold-set binder and other components in the system at low temperature. It initiates upon the reduction of electrostatic repulsion between such macromolecules during mixing and gel-setting period (Bryant and McClements, 2000). The chemical bonds, including salt bridge, hydrogen bond, electrostatic interactions and some covalent bonds, occur to stabilize the gel structure. These bonds are induced by the addition of binders which has the unique characteristic in forming gel at low temperature in the proper gelling time. Thus, the addition of suitable cold-set binder in an adequate concentration is necessary to produce raw products with desirable texture and binding strength. Few of polysaccharides and proteins can be used for this purpose. There are some differences in cold-set gelation of restructured meat products incorporated with polysaccharide binders and protein binders. With polysaccharides, the gel formation is stabilized by covalent bonds, ionic bonds and electrostatic interactions, including salt-bridges and hydrogen bonds, between charges of polysaccharide monomers and myofibrillar proteins in mild acidic condition. Thus, the suitable polysaccharide binders for this system must be able to ionize and form chemical bonds in such condition. Alginate and κ-carrageenan are the reported cold-set binders in this group. For the system with protein binders, the binders will expose their thiol groups inside the protein chain after suitable modification, e.g. heating, acidic and enzymatic modification. Then, the formation of chemical bonds, mostly disulfide bonds, occurs and results in protein aggregation and cold-set gelation (Nashef *et al*., 1977). Proteins

that were previously reported to be used as cold-set binder were preheated whey and soy proteins, plasma compounds and transglutaminase enzyme (Pyne, 2001).

Although some previous reports have already revealed the use of unique proteins and polysaccharides as cold-set binders, the investigation into the mechanism of proper cold-set binder in providing a high quality raw restructured meat product is still scarce. Truthfully, each binder has different basic requirement for completing its cold-set gelation process. For example, whey proteins require the partial denaturation by heating to expose the thiol groups inside their side chain. Alginate requires the calcium salt (calcium chloride) to form salt bridges stabilized cold-set gel, while cold-set gelation of κ-carrageenan, preheated soy proteins and plasma compound require the addition of transglutaminase enzyme to crosslink between their molecules and myofibrillar proteins. However, restructured meats produced with transglutaminase had microbiological problem from the long gel-setting period as reported by Serrano, Cofrades and Jiménez-Colmenero (2003). Thus, the investigation of cold-set binder, which can be used instantaneously without prerequisite step and the addition of transglutaminase, is still needed.

Chitosan is a linear-cationic biopolymer derived from deacetylation of chitin, which is the second most abundant biopolymer in the world. The major source of chitosan is shrimp shells which are underutilized waste from seafood industries in Thailand. Because of the availability, non-toxic, unique structure and physico-chemical properties (Li *et al.*, 1997), chitosan becomes more applicable for consumable products. Chitosan can be considered as one of the promising cold-set binders since it has the unique structure containing reactive hydroxyl and amino groups, and can ionize in mild acidic condition. However, chitosan can dissolve only in some organic acid-water solvent such as formic, acetic and lactic acid. Some

modification to make it suitable as cold-set binder in restructured meat products is indispensable.

The enzymatic hydrolysis of muscle proteins with endopeptidase such as papain can reduce the length of protein chain into different sizes by varying the degree of hydrolysis. As a result, the thiol and disulfide groups which are required for cold-set gelation could further be exposed; therefore, the formation of cold-set gelation should be enhanced. However, further study in the abilities of these cold-set binders for using in model systems with salt-soluble proteins and raw restructured meat are still required. Moreover, the effect of using protein and polysaccharide in combination as cold-set binders on the cold-set gelation abilities, gel structures and physico-chemical properties of raw restructured meat products, especially in chicken meat which is fairly susceptible to water loss, has never been reported by any researcher. This research is therefore aimed at investigating the proper characteristics of cold-set binders which can enhance the cold-set gelation ability of raw restructured chicken meat using chitosan and chicken protein hydrolysates as representatives for polysaccharides and proteins, respectively. Furthermore, the minimization of water loss is also expected by using these cold-set binders to replace polyphosphates. The presence of excessive amounts of polyphosphates in diets, influence calcium, iron and magnesium balance in human body and increases of bone diseases (Shahidi and Synowieki, 1997). Thus, polyphosphates has been prohibited in raw meat products which grind or restructure before packing by the declaration of EU white paper on Food Safety in 2002 by European Parliament and Council.

1.1 Objectives

1) Study the effects of chitosan at different degree of deacetylation, molecular weight and concentration on cold-set gelation abilities, structures and physico-chemical properties of chicken salt-soluble protein gels and raw restructured chicken meat.
2) Study the effects of native and hydrolyzed chicken proteins on cold-set gelation abilities, structures and physico-chemical properties of chicken salt-soluble protein gels and raw restructured chicken meat.
3) Study the effects of interactions between chitosan and chicken protein hydrolysates on cold-set gelation abilities, structures and physico-chemical properties of chicken salt-soluble protein gels and raw restructured chicken meat.

CHAPTER II

LITERATURE REVIEW

2.1 Restructured Meat Products

During the past few decades, a variety of restructured meat products have come onto the market because the demand has grown up from consumers and fast food industries (Gagliardi and Eugene, 1988). These products are produced by binding and reforming the small pieces of meat into the large single piece. The restructuring of meat requires the achievement of binding between pieces of muscle while retaining desired textural characteristics associated with intact muscle. This process changes meat in its form but does not signify to dehydration, addition or removal step (King and Macfarlane, 1987). Restructured meats offer many advantages for consumer and meat industries. For consumer, restructuring of the meat provides the variety and flexibility in meat products. They can select the products which have desirable portion size, shape and nutritional value in the reasonable price, and these products are convenience and also easy to prepared. Restructuring of meats also provides many advantages for meat manufacturers. This product is enable to use of less valuable meat components to produce palatable meat products at reduced cost. Large cuts can be conformed to the desired container without large voids or unattached pieces. Cooking losses are substantially reduced. Efficiency is improved because virtually all of the meat can be utilized, since trimming and smaller particles can be included. Mechanical working has the ability to improve tenderness which makes it possible to utilize lower grade cuts of meat without reducing product quality. Furthermore, the market value also increases from the use of low-value meats and the

market share of new products also expands (Secrist, 1987; Tsai *et al*., 1998; Serrano *et al*., 2003).

The major quality attributes of restructured meat products needed by consumer are palatability, texture and binding properties. To achieve the best quality of restructured meat products, various ingredients, mainly proteins and polysaccharides, have been applied as binders, fillers and extenders to improve the quality (Pearson and Tauber, 1984). These ingredients are used to improve the binding ability and/or texture modification so that the restructured meats with desirable quality are achieved.

2.2 Manufacturing Process

The restructured meat products can be produced through the formation of gel which thermally-set (heat-set) or chemically-set (cold-set). Conventional restructured meat products depended on heat-set binding of myofibrillar proteins that were extracted from meat proteins with the combined effects of salt (NaCl), phosphate and mechanical actions (Boles and Shand, 1998). With this process, the products had to be sold in precooked, cooked or frozen state because the product binding in raw state was not strong. For cold-set binding systems, the finished products were sold in chilled or raw state and had eating characteristics similar to cuts from intact muscles (Means and Schmidt, 1986). These two processes are different in mechanism of meat binding and type of binders. Huffman (1981) divided the process of restructured meat products into 4 steps comprising raw material preparation, mixing, restructuring and freezing.

Raw material preparation: Raw materials for producing restructured meat products are meats, salt, binders and water. The preparation of meats must be done readily after obtaining and kept in freezer for further use (Mandigo, 1986). Only fresh

meat with microbiologically sounds and free from flavor defects should be utilized in producing restructured meat. After trimming, the meats were either chunking, dicing, slicing, flaking or grinding to reduce particle size (Pearson and Tauber, 1984). Salt was added for extracting myofibrillar proteins, improving flavor and inhibiting microorganisms (Trout and Schmidt, 1987). Binders acted like glue for binding the meat pieces together. Many types of proteins and polysaccharides were used as heat-set binders while cold-set binders are very restricted. Some of heat-set binders are xanthan gum, starch and its derivatives, methylcellulose, soy flour, hydrolyzed vegetable proteins, gluten, albumin, sodium caseinate and non-fat dry milk. The addition of these binders was required for texturing, and stabilizing water and emulsion of the products, while the major binder in heat-set gelation is salt-soluble proteins (Booren and Mandigo, 1987). The reported cold-set binders were some of special polysaccharides and proteins such as alginate, κ-carrageenan, preheated globular proteins and cross-linking enzyme, transglutaminase. The addition of cold-set binder provided raw meat products with desirable eating quality; however, some modifications before use were required (Alting *et al.*, 2003). The binders were added in the mixing step in the form of dried powder or water suspension. Water was added mainly to dissolve the ingredients in the mixing step. The addition of water was done in the form of liquid water or ice. Even though water was added primarily as a processing aid, it affected both functional and sensory properties, especially juiciness of the products (Pearson and Tauber, 1984).

Mixing: Mechanical agitation was necessary for disrupting muscle fibers and releasing the myofibrillar proteins by salt extraction. This step was essential for developing the sticky matrix on the surface of the meat pieces and was responsible for

binding. The temperature during mixing step was controlled at below 4˚C since this temperature was suitable for salt extraction of myofibrillar proteins. The suitable mixing equipments were blender, tumbler or massager. Mixing time must be long enough to create a uniform meat mass and to cause solubilization of proteins through the action of impact and frictional energies (Booren and Mandigo, 1987).

Restructuring: Reforming or restructuring was the step in which the blended mixture was pressed into the desired shape using appropriate type of meat pressing equipment. It was the most important step for restructured meats produced with cold-set binding system. After pressing the product into the desired shape, the meat gel was allowed to set at low temperature in proper time. The chemical bonds between meat proteins and cold-set binders occurred and resulted in cold-set gelation of the restructured meat (Schmidt and Means, 1986; Schmidt and Means, 1991).

Freezing: Freezing was necessary for restructuring process using heat-set gelation mechanism because the binding strength of the finished product is very low at raw state. Thus, freezing was performed in order to stabilize the shape of products and make it easy to cut or slice (Huffman, 1981; Pearson and Tauber, 1984). In addition, some of patented processes suggested that heating of the shaped products before freezing was performed in order to denature meat proteins and stabilize the chemical bonds and structure of the products (Shapiro and Peck, 1983; Okada, 1985; O'Connell, 1985; Cohen, 1990; Fradin, 1991). In this case, freezing was the storage method which extended the shelf-life of the finished products (Booren and Mandigo, 1987). The temperature in this step should be below -18˚C to protect the oxidation of myoglobin (Pearson and Tauber, 1984). The suitable freezing methods were air blast and plate freezing. For products produced with cold-set gelation, freezing was not

required in the process. The shaped products were allowed to set gel at low temperature and kept in refrigerator in order to complete the gelation process. The suitable temperature range was 0-5˚C and the product could be stored and sold in raw refrigerated state (Means and Schmidt, 1987).

2.3 Mechanism of Meat Binding in Restructured Meat Products

The mechanism of meat binding in restructured meat products were categorized into two categories which were conventional heat-set gelation and cold-set gelation.

Mechanism of conventional heat-set gelation was based on binding properties of extracted myofibrillar proteins on surface of meat pieces. According to Jolley and Purslow (1988), meat binding occurred via autoadhesion. The major binder proteins were myosin, actin and actomyosin. These proteins coated around the meat pieces and functioned as the binder. The meat pieces bound together with low binding strength of adhesive force. When heat was applied, these myofibrillar proteins was unfolded and formed the protein-protein association at 36-40˚C. After heating to 45-50˚C, the partial denatured myofibrillar proteins was aggregated and formed gel network. The complete heat-set gel was achieved after the temperature reached 60-70˚C (Tornberg, 2005). During heat-set gelation, the changes of physico-chemical properties occurred and resulted in changing of adhesive structure to cohesive structure which had higher binding strength.

Cold-set gelation was the ability of some proteins and polysaccharides to form gel at low temperature. The cold-gels initiated upon reduction of the electrostatic repulsion between their molecules (Bryant and McClements, 2000). The mechanism of cold-set binding system was based on the chemical bond formation between meat

proteins, cold-set binders and other components in the system. The addition of cold-set binder was necessary to produce raw products with desirable texture and binding strength. These binders could either be proteins or polysaccharides and the ability to form chemical bonds, especially covalent and ionic bonds, was required (Bryant and McClements, 1998; Alting *et al.*, 2003). Other chemical bonds and interactions, such as hydrophobic interactions, electrostatic interactions including salt bridge and dipole-dipole interaction, and hydrogen bonds, were also occurred to stabilize the cold-set gels (Wong, 1989). After mixing with cold-set binders, the reaction between thiol and other reactive groups of meat proteins with active groups of cold-set binders was achieved. The formation of disulfide bonds, salt bridges, and other interactions occurred during gelation period at low temperature and resulted in protein aggregation and cold-set gelation, respectively (Means and Schmidt, 1986).

2.4 Cold-Set Binders

The addition of cold-set binders is essential for cold-set gelation and the cold-set binders can be polysaccharides or proteins which have the ability to form gel at low temperature. Some of polysaccharides, i.e. alginates and κ-carrageenanan, and proteins, i.e., preheated globular proteins, plasma factors and cross-linking enzymes, were reported as the effective cold-set binders (Pyne, 2001).

Alginate: Alginate is the one of the abundant complex natural hydrocolloid which occurred as a structure component in marine brown algae (*Phaeophyceae*) and capsular polysaccharides in soil bacteria. Alginate is in a family of unbranched binary copolymers of (1,4)-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues. Its sequence is concluded that alginate is a true block copolymer composed of homopolymeric regions of M and G block. This polysaccharide is the most

abundant component in brown algae. It comprises up to 40% of the dry matter (Moe and Draget, 1995) and locates in the intercellular matrix as a gel containing sodium, calcium, magnesium, strontium and barium ions which function as a skeletal structure.

Alginate has widely been used industrially, especially in the water soluble form of sodium alginate, because of its ability to retain water and its gelling, viscosifying and stabilizing properties. Alginates reacted with soluble calcium salt such as calcium carbonate and form cold-set insoluble calcium alginate gels. This phenomenon was enhanced by the inclusion of an ionizing compound of calcium such as weak acid in common solution of sodium alginate (Schmidt and Means, 1986).

Since 1986, many researches reported the use of alginates in restructured meat products. The restructured meat products produced with alginates/calcium system could form the strong gel at low temperature using cold-set binding system and could be treated and sold as the raw products. Schmidt and Means (1986) reported the procedure for preparation of raw-restructured meat products by mixing comminuted meat with sodium alginate and calcium carbonate as binders. They explained that a slow released acid such as glucono-delta lactone, citric acid and lactic acid could be added to lower the pH of the meat and improve its flavor. These acids produced enough hydrogen ions for stimulating the release of sufficient calcium ions from the calcium salt to accelerate formation of an alginate/calcium gel. After the mixing and forming steps in the prepared process, the meat was usually stored under refrigeration, from one to four days to allow the alginate/calcium gel to set. Means and Schmidt (1986) studied the effect of sodium alginate and calcium carbonate level on raw bind, cooked bind and sensory evaluation of restructured beef steaks by using several levels

of sodium alginate and calcium carbonate. They found that higher concentration of sodium alginate required more calcium carbonate to form a strong gel. Similar results were concluded in the report of Means *et al*. (1987). They found that alginate/calcium-treated products exhibited better binding and color in raw state but had lower palatability scores in cooked state than salt/phosphate controls. The addition of glucono-delta-lactone offered no benefit to binding in raw state. Chen and Trout (1991) studied the effect of various binders comprising calcium alginate, salt/tripolyphosphate, crude myosin extracts, whey protein, wheat gluten and soy protein isolate on sensory, texture and cooking properties of restructured beef steaks. They concluded that steak made with calcium alginate had superior binding. The hardness and fracturability of steaks were improved while the springiness was not. Furthermore, the study of Devatkal and Mendiratta (2001) also confirmed the ability of alginate-calcium binding in raw state. They studied the effects of calcium lactate with sodium alginate-calcium carbonate system in restructured pork rolls compared with the traditional salt-phosphate system. They found that the addition of calcium lactate promoted the cold-set gel with desirable firmness, cohesiveness, sensory characteristics, and shelf life, and had the synergistic effects with calcium carbonate. The raw binding strength, cooking yield, and cooking loss, of treatments with alginate and calcium carbonate were significantly ($p<0.05$) higher than treatment with salt and phosphate.

Carrageenan: Carrageenan is linear sulfated food grade polysaccharide obtained from various species of the red seaweeds (*Rhodophyta*). The unique gelation ability of carrageenan depends on branches which are named kappa, iota and lambda. They are well differentiated by their gelling properties and protein reactivity.

κ-carrageenan was repoted to improve texture, water binding and gelling properties of meat gels (Pietrasik and Li-Chan, 2002; Pietrasik and Jamoluk, 2003). According to Pietrasik (2003), the effect of κ-carrageenan on texture of comminuted meat products was not due to molecular interaction between proteins and hydrocolloids, but related to physical rearrangement of κ-carrageenan and meat protein molecules. Pietrasik and Jarmoluk (2003) investigated effects of sodium caseinate, microbial transglutaminase and κ-carrageenan on water binding, texture and color characteristics of pork gels. They found that higher κ-carrageenan content favored hydration properties and thermal stability, yielding lower cooking loss and higher water holding capacity. Its addition also increased hardness of pork gel but was unable to improve springiness and cohesiveness. These results could be obtained when using κ-carrageenan alone and in combination with sodium caseinate and microbial transglutaminase. They explained that the improvement of water holding capacity could be achieved from the synergistic interactions exist through balanced electrostatic repulsion of like charges and attraction between polar or negatively charged groups and positively charged groups on proteins. Pietrasik and Li-Chan (2002) and Pietrasik (2003) reported similar findings in beef gels.

Preheated globular proteins: Globular proteins can be used as the effective cold-set binder in the gel paste form only when the pretreatment by partial denaturing with heat or pressure is applied. The preparation of this cold-set binder was studied and its mechanism was clearly elucidated by many researches (Alting *et al*., 2000; Marangoni *et al*., 2000; Alting *et al*., 2003). Wang and Damodaran (1990) investigated the effect of heat treatment of globular proteins comprising bovine serum albumin, soy protein isolate and phaseolin on their gelation properties. They found

that the breakage of disulfide bonds occurred during heat treatment and resulted in protein unfolding and exposing of hydrophobic parts. Consequently, the thiol groups in their side chains were liberated. At this step, the preheated globular proteins consisted of many free thiol groups which were reactive and ready to be used as cold-set binder. These thiol groups could form the disulfide bonds with other protein component in the food systems and resulted in cold-set gelation (Alting *et al*., 2003). For food application, whey proteins were the most useful due to its availability and ability to form cold-set gels after heating and inducing with the addition of salt or acid (Alting *et al*., 2000; Alting *et al*., 2003). Preheated whey proteins could widely be used in many types of food such as surimi, mayonnaise, gelatin-like desserts and restructured meat products.

Whey proteins were major globular proteins in the supernatant of milk that were precipitated at pH 4.6. These proteins were more water soluble than casein. Native whey proteins had good gelling and whipping properties. Denaturation increased their water holding capacity. The major fractions of whey proteins was composed of β-lactoglobulin, α-lactalbumin, bovine serum albumin (BSA) and immunoglobulins (Ig) (Oakenfull, Pearce and Burley, 1997). Barbut and Drake (1997) reported that the sufficient heating time and temperature for producing cold-set binder from whey protein isolates were 30 minutes at 80˚C. According to Hongsprabhas and Barbut (1997, 1999a, 1999b) the addition of calcium chloride into preheated whey protein solution resulted in self-stable cold-set gel which was not suitable for using as cold-set binder. The pretreatment of whey protein for using in this purpose was only heating the suspension of whey protein in deionized distilled water at appropriate time and temperature. The preheated whey protein could be used to improve water holding

capacity and enhance cold-set gelation abilities of poultry meat batters. They concluded that the addition of preheated whey protein isolates to replace part of the poultry meat proteins at appropriate concentration resulted in improving of texture and water holding capacity of the obtained cold-set batter.

Plasma factors: Plasma factors were blood-based ingredients that used the clotting mechanism of fibrinogen and thrombin to form gel matrix. The plasma components could aggregate, cross-link, and form cold-set gel matrix. Boles and Shand (1998) studied the effect of raw binder systems comprising alginate and Fibrimex™ on raw bind, cook yield and sensory scores of restructured beef. They found that beef steaks made with alginate had significantly higher raw bind values than steaks made with Fibrimex™. Similar result was found in their continue study in 1999. However, restructured beef steaks made with Fibrimex™ had greater binding between meat pieces after cooking.

Cross-Linking Enzymes: Transglutaminase (TGase, glutaminyl-peptide: amine λ-glutamyltranferase, E.C. 2.3.2.13) was capable in cross-linking proteins, peptides and some other primary amines. The reaction of TGase occurred by catalyzing acyl transfer between a λ-carboxylamide of glutamine and lysine and forming an ε-(λ-glutamyl) lysine cross-link. The cross-linking resulted in the polymerization of protein and peptide molecules with a subsequent increasing of molecular mass and cold-set gelation (Walsh *et al*., 2003).

Many researches studied the use of TGase as cold-set binder in restructured meat products. When using of TGase and sodium caseinate as cold-set binder, the restructured beef steaks presented strong meat particle binding for handling in raw state (Nielsen, Pedersen and Møller, 1995). The report of Carballo, Ayo and Jiménez-

Colmenero (2006) also revealed that the hardness and chewiness of both raw and cooked restructured pork, chicken, and lamb steaks were improved by the addition of microbial TGase and sodium caseinate. However, the restructured meats added with TGase without salt exhibited less water and fat binding. The good activity of TGase could be enhanced in the presence of sodium salt, especially sodium caseinate (Kiliç, 2003). When cross-linking reaction of TGase occurred, reducing of cooking loss was resulted. However, the effect of transglutaminase on water-binding properties depended on the level and type of transglutaminase as well as the conditions used to produce restructured meats. The addition of TGase also improved the cohesiveness, hardness and elasticity of cold-set gel in raw state. Kolle and Savell (2003) studied the effect of TGase (Activa^{TM} TG-RM) along with sodium caseinate on binding of ribeye roll and top blade roast prepared from beef muscle. They found that raw and cooked binding strengths of the obtained products were acceptable. Serrano, Cofrades and Jiménez-Colmenero (2003) reported that the suitable time for producing restructured beef steaks with TGase was 6 days at 3˚C.

Hydrolyzed soy proteins with protease or acid at proper degree of hydrolysis were suitable substrates for TGase (Walsh *et al*., 2003). Their functional properties were improved after treating with TGase (Fadil *et al*., 1996). The hydrolyzed or preheated soy proteins were used to improve water binding and cold-set gelling properties of restructured meat products. Ramírez-Suárez and Xiong (2003) studied the cross-linking reaction and gelation of myofibrillar/soy protein mixtures induced by microbial TGase. They reported that the cross-linking reaction occurred via ε-(γ-glutamyl) lysyl isopeptide bond, both in inter- and intra-molecular protein chain. The addition of salts gradually changed the conformation of proteins, especially soy

proteins, due to the increase of ionic strength. This phenomenon modified soy globulin complex and increased the susceptibility of the reactive side chain groups (Lys-NH_2 and Gln-$CONH_2$) to microbial TGase attack. However, at high ionic strength conditions, the changes in myofibrillar proteins structure also occurred and resulted in minimizing the interactions with soy proteins. Ramírez-Suárez, Addo and Xiong (2005) reported a similar finding; they also suggested the existing of interactions between myofibrillar proteins and non meat proteins, such as wheat gluten, when treating with TGase.

2.5 Factors Affecting Raw Bind

Factors that influenced meat binding in raw state were divided into two major categories including raw material characteristics and production factors.

Raw Material Characteristics: All of the ingredients used for restructured meat production had to be in high quality grade. The meat used could either be trims or low value cuts, but it had to be in the good microbiological condition and free from flavor defects. The use of meat with high initial microorganisms affected shelf-life of finished products since raw binding products were not exposed to heat before sale. Thus, the use of fresh meat was essential to produce high quality products (Pearson and Tauber, 1984). Furthermore, lean meat substantially free from fat, connective tissue, gristle and sinew was preferred since high level of fat, connective tissue, gristle and sinew directly inhibited the extraction of myofibrillar proteins and resulted in lower raw binding strength of the final products (O'Connell, 1985).

Production Factors: Booren and Mandigo (1987) reported that the major production factors affecting raw binding were salt, temperature, mixing time, vacuum, and pressure. Salt was very important ingredient which was used to extract

myofibrillar proteins. The addition of salt markedly influenced water holding capacity, shear force, raw bind, texture, and juiciness of restructured meats since it was the major ingredient for dissolving myofibrillar proteins. The temperature during processing should be controlled at near freezing point since myofibrillar proteins were readily extracted at this temprerature. The mixing time affected the subsequent texture of the restructured meat. Mixing time had to be sufficient for a complete extraction of myofibrillar proteins. The vacuum and pressure were also applied into the process to enhance the binding ability and improve the binding strength of the resultant products. The addition of cold-set binder was reported to be the most important step in cold-set gelation. Ingredients for binding were incorporated by various equipments such as mixer and tumbler. When using cold-set binders, it was important to mix at a slow speed for the minimum time required to get good dispersion of the product. Cold-set products needed to be handled rapidly because if the matrix started to form before finish handling, the binding strength would be reduced. Suklim *et al*. (2004) reported that setting time was the major factor affecting raw binding strength. The appropriate setting time depended on type of cold-set binder. Boles and Shand (1998) reported that the production method and the instrument used affected the binding strength and sensory characteristics of the final products. They concluded that the production method using high pressure in the restructuring step improved raw binding strength, water holding capacity and juiciness of the finished products. Boles and Shand (1999) also showed that the method of size reduction, such as grinding or chunking, significantly influenced binding system and processing properties of restructured beef steak since the higher binding strength was only with high amount of extracted myofibrillar proteins.

2.6 Rheological Properties and Structure of Muscle Protein Gels

Muscle Proteins and Their Gel Formation: In fresh meat, muscle proteins were the major component next to water. Its content was 19-20%. This protein was roughly classified into three groups on the basis of solubility, including salt-soluble myofibrillar proteins (50-55%), water-soluble sarcoplasmic proteins (30-35%), and connective tissue or stromal proteins (10-15%) (Wong, 1989; Greaser and Pearson, 1999). Myofibrillar protein was the most abundant protein in meats. The major constituents were myosin (48%), actin (22%), titin (8%), tropomyosin (5%), troponin (5%), nebulin (3%), C-protein (2%), M-protein (2%) and α-actinin (2%). The protein in this group dissolved in high ionic strength buffer such as 0.58 M saline buffer (Camou, Sebranek and Olson, 1989; DeFreitas *et al.*, 1997). Many researches documented that this group of proteins, especially myosin and actin, played the major roles in muscle protein gelation and accounted for most of the gel-forming capacity (Wong, 1989; Xiong, 1997; Zayas, 1997; Xiong, 2004). For sarcoplasmic proteins, the major constituents were enzymes in glycolytic cycles while the rest were enzymes needed for cell metabolism and myoglobin (Greaser and Pearson, 1999). This group of protein could function as meat binder in the heat-set gelation of muscle proteins and also affected the gel color (Xiong, 2004). The connective tissue proteins consisted of five major protein fractions comprising collagen (type I, III, IV, V, VI and VII), elastin, laminin, fibronectin and decorin. In fresh meat, the main function of these proteins was to link and support muscle cells (Greaser and Pearson, 1999). Collagen which was the major constituent of connective tissue proteins also affected texture of muscle protein gel by dissolving and forming gelatin inside meat gel matrix at temperature over 70˚C (Xiong, 2004; Tornburg, 2005).

In order to form muscle protein gels, myofibrillar proteins must first be extracted, and this was usually initiated by mixing with salt and/or phosphates. The heating was performed subsequently in order to obtain muscle protein gels via heat-set gelation mechanism (Xiong, 2004). During heating, the myosin heads started to aggregate at the relatively low temperature (36-40˚C). This aggregation involved disulfide exchange and intermolecular association of the side chains. Consequently, the head-to-head aggregation occurred and provided a junction zone linking the myosin rods to form gel network (Wong, 1989; Xiong, 2004; Tornberg, 2005). The presence of actin enhanced gel forming ability of myosin. The gel network was further stabilized by noncovalent bonding among the binding sites obtained from the unfolding of myofibrillar proteins (Wong, 1989).

Rheological Properties of Muscle Protein Gels: As previously stated, the heat-induced gelation was the multi-step thermodynamic process that involved denaturation, aggregation and formation of a three dimensional network structure. The multiple transitions of the storage modulus (G') was used to indicate changes in the rate of forming elastic gel network and reflect the different stages of structure unfolding and subsequent aggregation (Lesiów and Xiong, 2001; Lesiów and Xiong, 2003). After complete gelation, the rigid gel was obtained and could be detected by the changes of rheological properties including G', complex modulus (G*), and phase angle (δ). G' was the parameter which implies the elastic solid property of the gels. The gels with high and steady G' values along the frequency range in oscillation frequency sweep test exhibited the property of very strong gel network. G* was the ratio of stress amplitude to the strain amplitude and calculated from G' and viscous modulus (G'') ($G^* = (G'^2 + G''^2)^{1/2}$). η* was the dynamic or complex viscosity

calculated by dividing G* with the applied frequency in the test (ω) (Shoemaker, 1992). The G′ and G* of the rigid gel increased and reached the highest point at temperature around 70˚C, indicating the formation of high elastic gel network, while the reduction of δ indicated the formation of firm and rigid gel (Verbeken *et al*., 2005). Muscle protein gels with good textural quality should possess high and steady G′ and G* values along the frequency range and the G′ curve should shift up higher than G′′ which was the viscous response curve, while the δ value should be nearly reached to 0˚ (Shoemaker, 1992).

Structure of Muscle Protein Gels: The structure of muscle protein gels was formed during gelation period from the entanglement and aggregation of muscle protein fibers especially myofibrillar proteins. The resulting 3-dimensional gel network structure observed by scanning electron microscopy was micro-connected myofibrillar protein filaments with various micro porous. Various chemical interactions including covalent disulfide bonds, electrostatic interactions, hydrogen bonding and hydrophobic interactions, were found to occur and stabilized the gel network (Wong, 1989). The amount of extracted myobrillar proteins was the key factor affecting microstructure of the resulting gels. The system with high amount of myofibrillar proteins possessed the dense fibrous protein gel network that was composed of many micro-connected pores inside and water was efficiently hold by capillary force (Tornberg, 2005). The microstructure of the gel was also related to the dispersion of the aggregating state of the protein prior to gelation and the existence of other components in the system like meat fibers, connective tissues and fat (Hung and Smith, 1993; Verbeken *et al*., 2005).

2.7 Quality Attributes of Meat Gels and Determination Methods

Gelation of muscle proteins was crucial to the formation of desired textural quality in meat products. Restructuring of meat was based on the ability of muscle protein gelation which induced numerous levels of hardness, cohesiveness, springiness, gumminess, chewiness and influenced the quality of the end product (Bourne, 1978). The important quality attribute of meat gel was composed of textural properties, rheological properties, structure, water binding properties, color, and the formation of chemical bonds and interactions.

Textural Properties: The textural characteristic of meat gel was a function of structure and composition which was grouped into two main classes including mechanical and geometrical characteristics (Larmond, 1976; Stanley, 1976). The mechanical characteristics were manifested by the reaction of stress and could be expressed in term of hardness, cohesiveness, springiness, adhesiveness, fracturability, chewiness, gumminess and viscosity. The geometrical characteristics conformed to the arrangement of protein gels and related to particle size, shape, and network formation (Larmond, 1976). Both instrumental and sensory measurement can be used to characterize the texture of meat gels (Stanley, 1976).

The instrumental methods were divided according to the motion used to deform the gels, into linear, rotary and combined linear and rotary. To measure textural properties, the gels must be deformed by applying compression, tension, shear or a combination of these operations, and the reaction of the sample to the applied force was measured (Voisey, 1976). Various methods such as shear devices, biting devices, compression methods, tensile methods, and penetration devices, were used for this purpose (Greaser and Pearson, 1999). These methods were composed of

three basic components that are deformation device, recorder and texture test cell (Voisey, 1976). The obtained results can be expressed in term of gel strength, texture profiles and maximum compression force depended on the testing method.

Sensory analysis can also be used to measure the gel texture. However, training of panelist is required for correct and acceptable results. Cross, Moen and Stanfield (1978) suggested the training and testing method for sensory analysis of meat products. In order to obtain a reliable data, the panelist had to be selected and screened using four steps including personal interview, screening, training and performance evaluation. The sensory procedures used for texture assessment are chew count and panel scores, e.g. softness, tenderness, hardness and juiciness (Honikel, 1998, Greaser and Pearson, 1999).

Rheological Properties: For better understanding of meat gels, the rheological properties are assessed to elucidate the type and behavior of such gels. According to Hermansson (1994), the rheological measurement of meat gels was divided into small and large deformation tests which gave complementary information. The small deformation test determined viscoelastic parameters and commonly derived by dynamic oscillatory testing in the linear viscoelastic region of the gels. Two dependent parameters were obtained including G' which described the amount of the energy that was stored elastically in the structure and G'' which was a measure of energy loss or the viscous response. The G* which calculated from G' and G'', $(G'^2 + G''^2)^{1/2}$, was also detected and further calculated to η* by dividing G* with the applied frequency. The δ which was the measure of how much stress was out of phase with strain was 0˚ for a completely elastic material and 90˚ for a purely viscous fluid. The rheometer used was normally equipped with concentric cylinder, cone and

plate or parallel plate and the test performed at a constant strain or stress rate (Vliet, 1999). The large deformation measured stress, strain and failure properties of a gel using universal testing machine, which performed in either a compression or tensile test. The compression test was commonly used because it was easy to perform. However, the tensile test provided the fracture properties precisely with high sensitivity (Hermansson, 1994).

Gel Structure: Gel structure was responsible for many physical properties such as water binding, texture and rheological properties (Hermansson, 1994). The microstructure of meat gels was characterized by using microscopic techniques, including light microscopy, scanning electron microscopy, transmission electron microscopy, immuno-electron microscopy, confocal scanning laser microscopy and phase contrast microscopy. *Light microscopy* was suitable for multiphase systems and mixed gels such as starch gels. Its principle was based on the emission of light beam from the light source on base of microscope through the specimen, thus the piece of specimen had to be thin enough. The image was magnified by objective and optical convex lens. The 2-dimensional gel structure was elucidated by this technique. *Scanning electron microscopy* was suitable for non-transparent gels. It was primarily used for imaging the surface structure of gel. This microscope employed a finely focused electron beam to scan across the sample surface. The structure of interest had to be stable after exposure to the electron beam and vacuum, as well as reflected sufficient back-scattered electrons and emitted sufficient secondary electron needed for visualization of the image. Thus for biological and food gels, the samples had to be coated with metal such as gold and drying before visualizing. *Transmission electron microscopy* was used in transparent gels and could be visualized at higher

resolution and magnification. The principle of this technique was similar to that of the scanning electron microscope; however, the difference was the preparation of sample. The sample had to be transparent, thin and dried (Hoppert, 2003). The remaining aforesaid methods were used to visualize gel structure at molecular level since they provided better resolution. The principle of *immuno-electron microscopy* was based on the principle of electron microscopy which detected the emitted electron from samples to visualize the gel structure. In *confocal scanning laser microscopy*, an image composed of defined points of a sample were produced and exited by a focused beam of light. The confocal image was a reflected light image. The laser light was the common source for confocal scanning laser microscopy. *Phase contrast microscopy* was the technique which obtained from switching the mode of illumination in laboratory microscopes from bright field to phase contrast. In this mode, the contrast was brought about by the different refractive indices between sample structures (Hoppert, 2003). However, the technique used and the operating condition were rather complicated and the instruments were very expensive (Verbeken *et al*., 2005; Iwasaki *et al*., 2006; Trespalacios and Pla, 2007).

Water Binding Properties: Water binding ability of meat is the most concerning parameter in meat processing from slaughtering to final cooking procedures. The qualities of meat and meat products were usually judged by the amount of the remained water (Barbanti and Pasquini, 2005). Water binding properties affected texture, taste and color of meat gels. These properties were expressed in term of water holding capacity, expressible moisture, and cooking loss. The water holding capacity referred to the ability to absorb and retain water during mechanical treatment such as chopping, coarse grinding, comminuting and stuffing,

thermal treatment and subsequent transportation and storage (Zayas, 1997). Expressible moisture was the release of moisture from unheated gels during application of external forces such as pressing, centrifugation or suction. Cooking loss was the release of fluid after cooking either with or without the application of external forces such as centrifugation or pressing (Honikel and Hamm, 1994). The measuring of water holding capacity was carried out in many different ways such as filter paper press, centrifugation, capillary volumeter and imbibing method. The *filter paper press* has widely been used because it was easy to apply and very rapid. However, the results were depended on the amount of applied pressure, the time used and the plasticity of meat. *Centrifugation method* was applicable and suitable for meat gels. Both high and low speed centrifuge were used, but the length of centrifugal time should be adequate. This method also widely used because it was easy to determine. *Capillary volumeter method* was faster than filter paper press method; however, the specific instrument was required. The *imbibing method* was the fastest method which provided rather acceptable result for drip loss measurement and was suitable for fresh meat rather than meat gel. In this method, the filter paper was placed on the cut muscle surface for 1-2 seconds and immediate read for the wetness score. The *expressible moisture* could be determined with or without applying pressure but enough length of time should be regulated in order to obtain the reliable results. The *cooking loss* of gels depended on the end point temperature, time and the rate of heating. The condition used had to be controlled. The containing vessel used needed to be a thin-walled bag with waterproof and withstanding at over 75˚C (Honikel and Hamm, 1994).

Gel Color: Color is known to play an important role in the acceptability of meat products. The instrumental measurement of meat gel color was based on human color perception which contained correction factors for both lighting and human visual response (Macdougall, 1994). Two different color scales widely used were CIE-L*, a*, b* and Hunter-L, a, b. The L* or L value represented gel lightness. The a* or a value represented redness and the b* or b value was yellowness of gel. The instruments used were the trichromatic colorimeters and spectrophotometers. The trichromatic colorimeters constructed by Hunter in 1940 (Macdougall, 1994). This instrument comprised a stable light source and three wide band red, green and blue filters, which approximates CIE standard illuminant C and the 2˚ observer. The obtained tristimulus values were transformed into the Hunter-L a b color space to approximate visual spacing. Such instruments were less expensive than the more precise spectrophotometers but were sufficiently accurate at measuring color differences for industrial process control. Furthermore, a wide range of foods could be measured even though their surface structure varied (Cornforth, 1994; Macdougall, 1994). The spectrophotometers were the most accurate type of color measuring and usually fitted with an integrating sphere and a choice of reflectance geometries. Inclusion or exclusion of the specular or gloss component depended on which geometry was appropriate for the particular foods application. The CIE recommended that color measurement of opaque materials, including meat gels, should be obtained with one of the following illumination conditions including 45˚/0˚ or 0˚/45˚ with exclusion of spectral component of the reflected light from uniform flat surfaces, or diffuse/0˚ or 0˚/diffuse with inclusion or exclusion of spectral components

(Macdougall, 1994). All angles were in degrees and giving the illumination angle first followed by refraction angle.

The Formation of Chemical Bonds and Interactions: The formation of chemical interaction in meat gel was detected and quantified by techniques such as sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), native or non denaturing gel electrophoresis (native-PAGE) and differential scanning calorimetry (DSC) (Fernández-Martín *et al*., 2002; Aktaş and Kiliç, 2005; Westphalen, Briggs and Lonergan, 2005; Xiong, 2005). Both SDS- and native-PAGE elucidated the formation of large macromolecules which occur from protein-protein interaction in gels. The SDS-PAGE based on the separation of charged proteins in an electric field, while the native-PAGE separated the protein only with their molecular weight or size of molecule. Either rod or slab polyacrylamide gel was used depending on instrument. The gel sample was prepared by dissolving in sample buffer before applying into the polyacrylamide gel. However, this method was laborious and require experienced technician (Iwasaki *et al*., 2006). DSC was part of a group of techniques called Thermal Analysis (TA) and based upon the detection of changes in the heat content (enthalpy) or the specific heat of a sample with temperature. In DSC, the measuring principle was to compare the rate of heat flow to the sample and to an inert material which was heated and cooled at the same rate. Changes in the sample which were associated with absorption or evolution of heat caused a change in the differential heat flow which was then recorded as a peak. The area under the peak was directly proportional to the enthalpic change and its direction indicated whether the thermal event was endothermic or exothermic. For proteins, the thermally induced process detectable by DSC was the structural melting or unfolding of the molecule.

The transition of protein from native to denatured conformation was accompanied by the rupture of inter- and intra-molecular bonds, and the process had to occur in a cooperative manner to be discerned by DSC (Ma and Harwalkar, 1991). This method provided accurate results, but the techniques used and the instrumental operation were complicated. The cost of this method was also very expensive (Aktaş and Kiliç, 2005).

2.8 Chitosan

Chitosan is the polysaccharide derived from chitin. It is the deacetylated form of chitin. Chitin is the most abundant biopolymer after cellulose. It is the major component in shells of crustacea such as crab, shrimp, and crawfish. According to the citation of Winterowd and Sandford (1995) they reported that chitin was discovered in 1811 by the French scientist, Henri Braconnot, who isolated this substance from mushrooms. In 1823, Odier found the same compound in cuticles of insects and named it chitin which was derived from the Greek word “chiton”, meaning the coat of mail or envelope. In 1859, Rouget published his discovering substance which could be prepared by boiling chitin in concentrated aqueous solution of potassium hydroxide and named “modified chitin”. Modified chitin was soluble in dilute organic acid-water solution and this was different to chitin which was not soluble in such solution. The modified chitin was renamed to chitosan by Hoppe-Seiler in 1894.

Structure and Chemical Properties: Winterowd and Sandford (1995) described that chitosan was a linear copolymer composed of approximately 5-25% *N*-acetyl-D-glucosamine and 75-95% D-glucosamine units which connected through (1,4)-linked β-glycisidic linkages. The molecular weight of chitosan varied widely and could be as higher as 10^6 Dalton. The lower molecular weight of chitosan was

received by some degraded treatment using enzyme, alkali, or acid. Chitosan had the reactive hydroxyl and high primary amine groups in its structure and usually had less crystalline than chitin. This made chitosan more accessible to reagent. Chitosan was insoluble in neutral or alkaline aqueous solutions but was dissolved in some proper acidic/water solutions such as formic acid, acetic acid, and lactic acid. When subjected to acid/water mixtures, a proportion of the primary amine groups on the chitosan molecule became protonated and acquired a positive charge. The solution of chitosan was highly viscous even at low temperature. The solvated chitosan molecules were polycationic and coagulated if a particle or molecule carrying multiple negative charges was added. For example, sodium alginate, most bivalent anions such as sulfate and phosphate, or heavily charged metal polyoxyanionic, and many proteins could form the ionic complexes with chitosan. Furthermore, the solubility of chitosan was directly depended on its internal parameters that were degree of deacetylation and molecular weight. Chitosans with high degree of deacetylation and low molecular weight were dissolved easily.

Preparation of Chitosan: According to No *et al*. (2003), chitosan was prepared from deacetylation of chitin. Isolation of chitin from crustacean shell generally consisted of three basic steps: demineralization using dilute hydrochloric acid, deproteinization using dilute sodium hydroxide solution, and decoloration using 70-90% ethanol. The conversion of chitin to chitosan was achieved by treatment using concentrated sodium hydroxide (40-50 % by weight), usually at high temperature (90-120˚C). The soaking time and temperature in deacetylation step was important and had to be carried out carefully because it regulated the degree of deacetylation and influenced the solubility of the obtained chitosan.

The studies of Lertsuriwong *et al*. (2002), No *et al*. (2003), and Tolaimate *et al*. (2003) revealed that different order and condition of chemical treatments in the production of chitin and chitosan directly affected characteristics of the obtained chitosan. The strong conditions in the isolation step of chitin affected the degree of deacetylation and the size chain of chitosan. Furthermore, they reported that the production step and the soaking time and temperature in the demineralization and the deproteinization, also affected the properties of the obtained chitin. Lertsuriwong *et al*. (2002) reported that the process started with demineralization gave chitin with higher ash contents and yield than the process started with deproteinization. When the demineralization was done first, the chitin was protected by adhering protein, resulting in less hydrolysis of the backbone and higher yield. On the contrary, if deproteinization was done first, the protective layer of protein was removed and unprotected chitin was exposed to the demineralizing acid. This might lead to efficient demineralization but also to more hydrolysis and loss of material in the solid chitin fraction. Thus, all of the parameters in each step had to be regulated in order to receive chitosan with desirable properties.

Characterization of Chitosan: According to Tolaimate *et al*. (2003), the degree of deacetylation and molecular weight were the important inherent properties of chitosan. The determination of these properties had to be done before use in food applications. Muzzarelli and Rocchetti (1997) cited many methods for determination of degree of deacetylation including the first derivative ultraviolet spectrophotometry, infrared spectrophotometry, enzymatic determination and chromatographic determination. The *first derivative ultraviolet spectrophotometric technique* was widely used because it was the easiest method that provided accurate and precise

results for highly decetylated chitosan. For *infrared spectrophotometric method*, the technique used was very difficult and requires the prerequisite preparation of chitosan samples. For *enzymatic determination*, exo-β-D-glucosaminidase and β-*N*-acetylhexosaminidase from *Nocardia orientalis*, and chitosanase from *Bacillus pumilus* were used. This method was complicated but gave the reproducible results for a full range of chitosan samples having different degree of *N*-acetylation. For *chromatographic determination*, the acid hydrolysis with acetic acid and the detection of methyl group by high performance liquid chromatography (HPLC) was used. This method required the complicate technique and the cost of the instrument was quite expensive.

According to Terbojevich and Cosani (1997), various methods were used to evaluate the molecular weight of chitosan. The wide-spread methods were *viscometric and gel permeation chromatographic techniques*, which were easy to perform and not time consuming. On the other hand, they were empirically related to molecular weight, because the measurement depended upon the hydrodynamic volume of the macromolecule, which was a function of molecular weight, conformational properties and polymer-solvent interactions. As a result, the calibration curve was required. On the contrary, the *light scattering method* gave the absolute values for molecular weight, but the technique was more difficult and sometimes the data was not easy to interpret in the presence of aggregation and/or association.

Applications of Chitosan in Foods: Both chitin and chitosan were center of interest in the past few decades due to their potential broad range of industrial applications. In foods, there were many reported applications including the use of chitosan as antimicrobial agent, food additive and nutritional quality enhancer.

Many studies using chitosan as antimicrobial agent in foods were reported. Roller and Covill (1999) studied the antimicrobial properties of chitosan glutamate in apple juice. They reported the growth inhibition of all spoilage yeasts at 25˚C. The initial effect of chitosan in apple juice was biocidal with viable numbers reduced by up to 3 log cycles. The most sensitive strain was *Zygosaccharomyces bailii* while the most resistant strain was *Saccharomycodes ludwigii*. Devlieghere *et al*. (2004a) studied the antimicrobial activity of chitosan in fruit and vegetable coatings. They found that gram-negative bacteria seemed to be very sensitive to chitosan, while the sensitivity of gram-positive bacteria was highly variable and that of yeast was intermediary. Chitosan was coated on strawberries and lettuce by dipping in a chitosan–lactic acid/sodium lactate solution at pH similar to each product. Chitosan coating on strawberries was applicable, but not on the mixed lettuce due to the development of a bitter taste. The microbiological load on the chitosan-dipped samples was lower for both products. The antimicrobial effect of chitosan on lettuce disappeared after 4 days of storage, while it maintained longer on strawberries. Another report of Devlieghere *et al*. (2004b) presented the new preservative techonologies using natural compounds, such as essential oils, chitosan, nisin or lysozyme in food products which were called ‘green label’ products.

Darmadji and Izumimoto (1994) studied the effect of chitosan in meat preservation. The use of chitosan in minced beef inhibited the growth of spoilage bacteria (*Bacillus subtilis, Escherichai coli, Pseudomonas fragi,* and *Staphylococcus aureus*), reduced lipid oxidation and putrefaction, and improved the sensory attributes. Furthermore, chitosan also had a good effect on the development of the red color during storage. In 2002, Roller *et al*. and Sagoo *et al*. tried to developed the

novel preservation systems for chilled, comminuted pork products by using chitosan. According to Roller *et al*. (2002), the novel preservation system for fresh pork sausages using the combinations of chitosan, carnocin (a bacteriocin produced by *Carnobacterium piscicola*) and low concentrations of sulphite was obtained. They found that chitosan combined with sulphite at low level effectively retarded the growth of spoilage organisms. Microbial counts for frozen sausages showed that the preservative efficacy of the chitosan/sulphite combination was maintained following frozen storage. Sagoo *et al*. (2002a) also reported the effective result from dipping skinless pork sausage in chitosan solution. The native microflora (total viable counts, yeasts and moulds, and lactic acid bacteria) was reduced by approximately 1-3 log cfu/g. Moreover, the addition of chitosan to an unseasoned minced pork mixture reduced total viable counts, yeasts and moulds, and lactic acid bacteria by up to 3 log cfu/g compared with that found in the untreated control. Thus, chitosan can successfully be used as effective inhibitor of microbial growth in chilled comminuted pork products. The additional work by the same researchers in 2002 also showed that chitosan glutamate inhibited spoilage yeast (*Saccharomyces exiguus, Saccharomycodes ludwigii and Torulaspora delbrueckii*) in saline solution and had synergistic effect with sodium benzoate (Sagoo *et al*., 2002b). Tsai *et al*. (2002) reported the effects of DD and preparation methods of chitosan on antimicrobial activity in *Oncorhynchus nereka* fillet. Chitin was prepared by chemical and microbiological methods and further chemically deacetylated to obtain various DD chitosan. The antimicrobial activity of chitosan increased with increasing DD, and was stronger against bacteria (*Bacillus cereus, Escherichia coli, Listeria monocytogenes, Pseudomonas aeruginosa, Shigella dysenteriae, Staphylococcus*

aureus, *Vibrio cholerae*, and *V. parahaemolyticus*) than fungi (*Candida albicans*, *Fusarium oxysporum*, *Aspergillus fumigatus* and *A. parasiticus*). They also found that pretreatment of fish fillets with high DD chitosan solution retarded the increase in volatile basic nitrogen content, as well as the counts for mesophiles, psychrotrophs, coliforms, *Aeromonas spp*., and *Vibrio spp*.

Qin *et al*. (2006) studied the feasilibity of using chitosan in water soluble form as antimicrobial agent and found the positive action on the growth of *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. The water-soluble half *N*-acetylated chitosans and chitooligomers had no significant antimicrobial activity. However, the water-insoluble chitosan in acidic medium resulted in inhibitory effect against these microorganisms. The antimicrobial mechanism was hypothesized as the forming of an impervious layer around the microbial cell.

Various researchers investigated the use of chitosan as texture modifying agent in food products. Jo *et al*. (2001) reported that emulsion type sausage with the addition of chitosan oligomer had desirable texture and acceptable sensory characteristics. The report of Chang, Lin, and Chen (2003) showed the use of chitosan as texture modifier in tofu (soybean curd). The addition of chitosan increased the gel strength and shelf life of tofu and these parameters increased with the increased DD. The tofu prepared with chitosan and acetic acid as curdling agent showed the highest improvement of gel strength.

The use of chitosan as texture modifying agent or gel enhancer cooperated with tranglutaminase enzyme in surimi and fishery products were already documented (Benjakul *et al*., 2003; Gómez-Guillén *et al*., 2005). Chitosan was not required for tranglutaminase activity, but gel formation was faster and the resulting gels were

stronger in the presence of chitosan (Chen *et al*., 2003). The report of Benjakul *et al*. (2003) showed the synergistic effect of chitosan and endogenous transglutaminase during gel setting period of surimi which resulted in the formation of protein-protein and protein-chitosan conjugates. The increase in breaking force of surimi gel added with chitosan indicated the gel enhancing effect of chitosan on the heat-induced gelation of fish myofibrillar proteins. However, no synergistic effect on gel strengthening was observed when chitosan was added in combination with microbial tranglutaminase. This result suggested that different type of transglutaminase exhibited different specificity to chitosan, particularly in term of the formation of chitosan-protein conjugate. The study of Somjit *et al*. (2005) revealed that the addition of chitin, chitosan and their hydrolysates retarded the effects on freeze-induced denaturation of myofibrillar protein in surimi by stabilizing the hydrating water molecules that surrounded the proteins. As a result, the fish myofibrillar proteins were retained in their native conformation and provided the surimi gel with desirable appearance, texture and water holding capacity during frozen storage. The addition of chitosan in combination with transglutaminase was studied by Gómez-Guillén *et al*. (2005). The high pressure treatment was applied in order to enhance the gel formation of mackerel mince at low temperature. They reported that the high pressure treated mackerel mince, containing chitosan and microbial transglutaminase had higher hardness, but lower elasticity. The rheological and microstructural properties of gels were not modified by the addition of chitosan and no synergistic effect of chitosan and microbial transglutaminase was observed.

Some reports also showed the use of chitosan as the clarifying agent in fruit juices. Chatterjee *et al*. (2004) reported that chitosan prepared from shrimp shell

partially hydrolyzed with acetic acid was the effective clarifying agent for apple, lemon, grape and orange juices when use at low concentration. Rungsardthong *et al*. (2006) reported the use of fungal chitosan extracted from mycelia of *Absidia glauca* var. *paradoaxa* IFO 4007 for clarifying apple juice. They found that the fungal chitosan proved highly effective in reducing the apple juice turbidity and gave lighter juices than the sample treated with commercial shrimp chitosan.

The anti-oxidation effect of chitosan was clearly reported in some food products. Darmadji and Izumimoto (1994) cited the anti-oxidation effect of chitosan in beef mince. Kamil, Jeon and Shahidi (2002) studied the antioxidant efficacy of chitosans in cooked, comminuted flesh of herring (*Clupea harengus*). They concluded that chitosans exhibited good antioxidant activities by reducing the formation of hydroperoxides and thiobarbituric reactive substance (TBARS) in a fish flesh model system. Kanatt, Chander and Sharma (2004) reported that pretreatment of chitosan by irradiation of gamma radiation resulted in a six-fold increase in its antioxidant activity and could be used as the novel natural antioxidant for minimizing lipid peroxidation of radiation-processed lamb meat. Furthermore, the study of Chen *et al*. (2003) showed the ability of chitosan in emulsion stabilization. They revealed that chitosan modified by coupling with linoleic acid through the 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide mediated the reaction to increase the amphipathicity and improved emulsification effect.

Chitosan was repoted to have potential as food supplements since it exhibited anticholesterolemic, antiulcer and antiuricemic properties when orally administered (Muzzarelli, 1996; Trautwein *et al*., 1997; Koide, 1998). These properties were from the capacity to bind specifically with fatty acids, bile acids, phospholipids, uric acid

and the toxic gliadin fraction. The antitumor activity of chitosan in water soluble form was also discovered (Qin *et al*., 2002). The review of Borderías *et al*. (2005) showed the potential of using chitosan as dietary fiber in restructured fish products with the benefit in enhancing gel formation and anti-oxidation. Muzzarelli (1996) reported that chitosan met the criteria of dietary fiber which had the hypocholesterolemic potential including high viscosity, polymeric nature, high water-binding properties and nondigestibility in the upper gastrointestinal tract, together with low water binding in the lower gastrointestinal tract. When acting as dietary fiber, chitosan could bind anions such as bile acids or free fatty acids at low pH by ionic bonds from its amino group. Koide (1998) reported the benefits and risks of using chitosan as food supplement. The benefit was the reduction of plasma cholesterol and triglyceride due to the ability to bind dietary lipid. Chitosan acted by forming gels in the intestinal tract which entrapped lipids and other nutrients, including fat soluble vitamins and minerals, thus interfering with their absorption. However, dietary chitosan might influence calcium metabolism by accelerating its urinary excretion, decreasing plasma vitamin E level, reducing in bone mineral content. Chitosan had a protective effect on *Candida* infection. The antibacterial and antiyeast activities of chitosan were desirable and useful in promoting wound healing, increasing immune response, and possessing antitumor activity. The mechanism of chitosan in decreasing cholesterol levels was not clearly understood (Winterowd and Sandford, 1995). However, it was known that the ingestion of chitosan had effect on the bile acids used by body to emulsify water-insoluble contents of stomach, such as cholesterol, which was not able to transport across the small intestinal wall without being emulsified. Chitosan was shown to reduce the concentration of emulsified cholesterol in the intestines by entrapping

some of the cholesterol-containing micelles as it precipitated in the small intestine. Chitosan blocked the conversion of cholesterol to coprostanol in the colon, increased cholesterol level of feces and decreased the level in serum and liver. The benefit of chitosan over the hypocholesterolemic drugs was the effect in non-increase of bile acid in feces. Thus, chitosan had the high feasibility for using in this purpose (Winterowd and Sandford, 1995; Muzzarelli, 1996).

2.9 Chicken Protein Hydrolysates

Hydrolysis of food proteins is carried out for many reasons, including improving nutritional characteristics, retarding deterioration, imparting texture, increasing or decreasing solubility, adding foaming or coagulation properties, adding emulsifying capacity, preventing undesired interactions, removing off-flavors or odors, and removing toxic or inhibitory ingredients (Lahl and Braun, 1994). It was reported that the first commercially available protein hydrolysates for food use appeared during and after World War II in two completely different contexts: as the casein hydrolysates for use in dietetic feeding, and as pepsin-modified soya protein for use as egg white replacer in confectionary industry (Adler-Nissen, 1986).

Preparation of Protein Hydrolysates: Protein hydrolysates was prepared by hydrolysis reaction with acid, alkali, or enzyme. However, the enzymatic hydrolysis was strongly preferred and widely used over strictly chemical methods because it provided products with well-defined peptide profiles suited for using in nutritional applications. Acid and alkali hydrolysis destroyed L-form amino acids which resulted in the formation of D-form amino acids and toxic substances like lysino-alanine (Lahl and Braun, 1994).

The selection of suitable protease enzymes and the optimum conditions were important factor which regulated the quality of the enzymic hydrolysates (Adler-Nissen, 1986). The commercial food grade enzymes were generally used either in liquid or dry pellets form. In practical, either exopeptidases or endopeptidases was used with different pathway of hydrolysis. Proteolysis with exopeptidases sequentially hydrolyzed peptide chain from the outer side and released one peptide at a time, while endopeptidases hydrolyzed inside the peptide chain and released two different size peptide chains (Panyam and Kilara, 1996). At optimum condition of the selected enzyme, the catalytic mechanisms were driven in three consecutive reactions including formation of complex between the original peptide chain and enzyme, cleavage of the peptide bond to liberate one of the two resulting peptides, and a nucleophilic attack on the remains of the complex to split off the other peptide and the reconstituted free enzyme (Adler-Nissen, 1986; Lahl and Braun, 1994). During hydrolysis reaction, the degree of hydrolysis (DH) was monitored in order to produce protein hydrolysates with desirable properties and the process was terminated by adjusting the pH or temperature to inactivate the enzyme, or filtering through ultrafiltration membrane to remove the enzyme (Lahl and Braun, 1994). After terminating the reaction, the obtained slurry was centrifuge and the supernatant was collected for further drying by spray, drum or freeze drying (Adler-Nissen, 1986; Lahl and Braun, 1994).

Various factors affected reaction of enzymatic hydrolysis. These factors were enzyme specificity, substrate and enzyme concentration and ratio, pH, ionic strength, tempertature and the existence of inhibitory substances (Adler-Nissen, 1986; Lahl and Braun, 1994; Panyam and Kilara, 1996; Simpson *et al*., 1998; Šližytė *et al*., 2005).

Optimum condition for a specific protease had to be regulated in order to receive the highest enzyme activity (Adler-Nissen, 1986).

Determination of DH: DH was reported to be the most important parameter that had to be characterized and monitored along the hydrolysis reaction. It was defined as the percentage of the cleaved peptide bonds. Several methods for determining DH have been documented; for example, pH-stat, osmometry, soluble nitrogen content, trinitrobenzenesulfonic acid (TNBS) and o-phthaldialdehyde (OPA) (Adler-Nissen, 1986; Nielsen *et al*., 2001). The favorable methods used in most researches today are pH-stat, TNBS and OPA. The *pH-stat* was suitable for monitoring the DH during hydrolysis because it provided the fast result which was calculated suddenly from the consumption of base used. However, this method was not economically feasible to carry out at the DH above 30% and might produce the protein hydrolysates with high amount of base (Nielsen *et al*., 2001). *TNBS* provided the accurate and precise result for protein hydrolysis produced with enzymatic reaction. However, it had the drawbacks in laborious method, toxic reagent and complexity of the slow rate reaction (Adler-Nissen, 1986). Thus, the OPA method was developed. This method did not required toxic reagent but provided the accurate and precise result similar to TNBS (Nielsen *et al*., 2001). *OPA method* was based on the reaction between amino groups of protein hydrolysates which liberated during hydrolysis and the OPA reagent in the presence of β-mercaptoethanol or dithiothereitol. This reaction resulted in the formation of a colored compound detectable by reading the absorbance at 340 nm from spectrophotometer. Since the reaction was time dependent, the reaction time needed to be strictly controlled in order to receive the correct results (Nielsen *et al*., 2001).

Functional Properties and Applications of Protein Hydrolysates: Various reports showed the advantage of enzymatic hydrolysis in the improvement of functional properties of food proteins including solubility, water holding capacity, gelation and coagulation, emulsification, foaming and hydrophobicity. Panyam and Kilara (1996) reported that enzymatic modification of food proteins by controlled proteolysis enhanced their functional properties over a wide pH range, and other processing conditions. Diniz and Martin (1997) reported the effect of enzymatic hydrolysis on shark proteins that the nitrogen solubility and foaming stability of the shark protein hydrolysates were improved, especially in treatment with 6.5% DH. Jeon *et al*. (1999) studied the recovery of cod fish processing by-product using enzymatic hydrolysis with crude proteinase extracted from tuna pyloric caeca. They reported that the fractionation of the resultant protein hydrolysates through a series of ultrafiltration (UF) membranes with several molecular weight cut-off enhanced various functional properties such as emulsifying and foaming properties. Similar result was confirmed by Pouliot *et* al. (1999). They reported the possibility to separate peptides from tryptic hydrolysates of whey proteins with charged UF membranes. The report of Chiang, Shih, and Chu (1999) also showed the functional enhancement comprising solubility, water binding capacity and antioxidant activity of soy protein hydrolysates after fractionation with continuous membrane reactor. Clemente *et al*. (1999) revealed that, with alcalase and neutrase hydrolysis, the solubility of chickpea hydrolysates was increased and stable over the wide pH range. Maehashi *et al*. (1999) also showed that chicken protein hydrolysates from bromelain hydrolysis possessed the most favorable umami flavor. Similar result was reported by the study of Pinto E. Silva, Mazilli, and Cusin (1999). Tsumura *et al*. (2005) also reported the improvement

of solubility, emulsifying activity, whip ability, and gel-forming ability of soy protein hydrolysates produced from enzymatic hydrolysis with pepsin and papain.

The application of protein hydrolysates in foods including the uses as water binding agent, cryoprotectant, antioxidant and nutritional diet supplement were reported. Shahidi and Synowiecki (1997) revealed the feasibility of protein hydrolysates from mechanically separated seal meat as phosphate alternatives in meat products to improve water-binding capacity. The addition of seal protein hydrolysate improved the water binding capacity of seal meat similar to that of phosphate. The drip volume from thermally processed mechanically separated seal meat protein hydrolysate was reduced and the cooking loss also decreased. Shahidi *et al*. (1997) also showed the potential of seal protein hydrolysate as water binding agent in salami products. Tsumura *et al*. (2005) reported that the mixing of conglycinin protein hydrolysates in meat proteins resulted in increasing gel-forming ability and water retention.

Some reports revealed the cryoprotecting effect of protein hydrolysate on myofibrillar protein during frozen storage. Yamashita, Zhang, and Nozaki (2003) studied the effect of chitin hydrolysate made from crustacean shells and cephalopod cartilage on denaturation and state of water in lizard fish myofibrillar protein during frozen storage. They found that the addition of chitin hydrolysate markedly increased the amount of unfrozen water in the protein by inactivating the enzyme activity. Another report of Hossain *et al*. (2004) also revealed the effect of proteolytic squid protein hydrolysates produced with protease from *Bacillus subtilis* on the state of water and the denaturation of myofibrillar protein extracted from frozen lizard fish (*Saurida wanieso*). According to their findings, the myofibrillar protein with squid

protein hydrolysates contained higher levels of monolayer and multilayer sorption water, resulting in decreased water activity and Ca-ATPase inactivation. The amount of unfrozen water in that lizard fish myofibrillar protein with squid protein hydrolysates increased significantly. This suggested that the peptides of squid protein hydrolysates stabilized water molecules on the hydration sphere of lizard fish myofibrillar protein, which maintained the structural stability of lizard fish myofibrillar protein, and therefore suppressed dehydration-induced denaturation. The surimi with higher water holding capacity was obtained. The work of Ruttanapornvareesakul *et al*. (2006) on the effect of protein hydrolysate from shrimp head on dehydration-induced denaturation of lizard fish myofibrils also indicated a similar result to previous reports. After the addition of shrimp head protein hydrolysate, the amount of unfrozen water significantly increased and the Ca-ATPase inactivation rate decreased. The water molecules around lizard fish myofibrillar proteins were stabilized and resulted in retention of the native protein molecules which had the ability to bind effectively with water. The surimi with desirable water binding property was obtained.

Many studies on application of protein hydrolysates as antioxidant were carried out. Shahidi, Han, and Synowiecki (1995) reported that incorporation of capelin protein hydrolysates in meat model systems resulted in an increase in cooking yield and inhibition of lipid oxidation. Aida Penã-Ramos and Xiong (2003) found that the oxidation of pork patties was significantly inhibited by the addition of hydrolyzed whey protein isolate or soy protein isolate. The study of Wang and Xiong (2005) also showed the inhibition of lipid oxidation by hydrolyzed potato protein in cooked beef patties. Flaczyk *et al*. (2006) reported the effect of acid pig cracklings hydrolysates

addition on the oxidative stability of fat and cholesterol in meatballs under refrigerated storage. According to their findings, the obtained hydrolysates exhibited a superior inhibition activity against fat oxidation and the formation of cholesterol oxidation products in stored meatballs comparing to that of commercial antioxidants. Sakanaka *et al*. (2004) studied the antioxidant activities of fat-free egg-yolk protein hydrolysates prepared by hydrolysis with protease from *Bacillus* sp. in a linoleic acid system. The egg-yolk protein hydrolysates showed strong antioxidant activities in this system and in cookies containing linoleic acid. The continue research of Sakanaka and Tachibana (2006) also revealed the active oxygen scavenging activity of egg-yolk protein hydrolysates prepared from enzymic hydrolysis of fat-free egg-yolk protein that it exhibited superoxide-scavenging activity and showed the reduction of 1,1-diphenyl-2-picrylhydrazyl radical and hydroxyl radical-scavenging activity. This particular hydrolysate also effectively inhibited thiobarbituric acid reactive substances formation in ground beef and tuna homogenates.

Protein hydrolysates have also been used as nutritional diet supplementation. Zhang *et al*. (2003) revealed the preparation technology of semi-fluid high-energy food from meat and soy protein hydrolysates produced by enzymatic hydrolysis. Manninen (2004) reported the production of sports drinks containing protein hydrolysates and claimed that this drinks might have great value because protein hydrolysates containing mostly di- and tripeptides which were absorbed more rapidly than free form amino acids and much more rapidly than intact proteins. In addition, protein hydrolysate ingestion had strong effect on insulin response by activating its response to glucose molecules (insulinotropic effect). The oral intake of protein hydrolysates and amino acids in combination with carbohydrates resulted in an

insulinotropic effect as much as 100% greater than with the intake of carbohydrates alone. Duarte *et al*. (2006) revealed that fish protein hydrolysates was an immunomodulating agent which had the capacity to enhance non-specific host defense mechanisms. They tested for the mucosal immune response of fish protein hydrolysates supplementation on murine model which characterized by cytokine analysis and found that the formation of immunoglobulin A was increased and phagocytic activity of peritoneal macrophages was enhanced. They concluded that the immune response of murine was successfully stimulated by dietary fish protein hydrolysates.

2.10 Protein-Polysaccharide Complexes

A better understanding of protein-polysaccharide interactions is currently become very important. A proper understanding and control of these different interactions should enable food scientists to design products with desirable structure and texture. In many food systems, proteins and polysaccharides were usually present together in a heterogeneous mixture with different chemical nature, conformation, chain rigidity, size and shape of molecules, degree of hydrolysis, denaturation, dissociation and aggregation. These macromolecules interacted and contributed the structure, texture and stability of food through their thickening, gelling behavior and surface properties (Tolstoguzov, 1997). However, even though the investigation of protein-polysaccharide interactions has begun since 1896, the understanding about the role of their interactions in relation to the functionality in multiphasic systems, such as food mixed solutions, emulsions or gels, was still needed (Tolstoguzov, 1997; Schmitt *et al.*, 1998; Doublier *et al.*, 2000; Turgeon *et al.*, 2003).

Interaction and Formation: The interactions of proteins and polysaccharides occurred during and after mixing. Their interactions in water system were repulsive and unstable. The destabilization forces were greater than the stabilization forces, so that the separation into two phases was generally observed (Schmitt *et al.*, 1998). The phase separation was achieved in two distinct ways which were thermodynamically incompatability or segregative phase separation and complex coacervation or associative phase separation.

The thermodynamically incompatability often occurred in ternary system containing neutral charged proteins (pH of the system was similar to isoelectric pH of protein) with neutral charged polysaccharides, or proteins and polysaccharides with similar charges, in aqueous medium (Schmitt *et al.*, 1998). In this system, the Gibbs free energy of mixing and the net repulsion of two biopolymer interactions were positive, indicating the detriment of favorable interaction between biopolymer/biopolymer and solvent/solvent. Consequently, the system finally separated into two phases, each containing one of the two biopolymers and this separation was called segregative phase separation (Tolstoguzov, 1997; Schmitt *et al.*, 1998; Turgeon *et al*., 2003). This phenomenon was experienced when the proteins were particle-like, such as micellar casein or large aggregates of heat-denatured proteins. Doublier *et al*. (2000) reported that the system of whey protein-xanthan gum, bovine serum albumin-sodium alginate, galactomannan-casein, and high methoxy pectin-casein were categorized within this phenomenon.

The complex coacervation occurred in the system with two highly compatible biopolymers, such as two oppositely charged proteins and polysaccharides, in solvent (Schmitt *et al.*, 1998). In this system, the Gibbs free energy of mixing was negative,

indicating the spontaneous phase separation called associative phase separation. This phenomenon implied the formation of soluble or insoluble protein-polysaccharide complexes stabilized by electrostatic interactions between charged (acidic and basic) side groups of macromolecules and/or non-electrostatic interactions including hydrophobic interactions, dipole-dipole interactions, covalent bond and hydrogen bonds. These complexes were ultimately settled to form the coacervated phase containing both biopolymers separated from the solvent phase (Tolstoguzov, 1997; Doublier *et al.*, 2000). Turgeon *et al*. (2003) clearly demonstrated that milk proteins-low methoxyl pectin, milk proteins-high methoxyl pectin, and milk proteins-carrageenan formed the complex coacervation. Furthermore, the report of Doublier *et al*. (2000) also found the complex coacervation in the mixed systems of whey proteins with pectin, κ-carrageenan, sodium alginate, carboxymethy1cellulose or xanthan gum; ovalbumin with dextran sulfate or carrageenan; and gelatin with ι-carrageenan or κ-carrageenan.

The complex coacervation of protein and polysaccharide was prepared by three different method including microprecipitation, microparticulation and electrosynthesis. Among these three methods, microprecipitation was the easiest method. With this method, the protein-polysaccharide complexes were formed totally by electrostatic interactions (Tolstoguzov, 1997). The selected protein and polysaccharide was dissolved in suitable solvent, mixed, adjusted the pH to precipitate the complexes and then post-treated with shearing and/or drying in order to obtain the protein-polysaccharide complex powder (Schmitt *et al.*, 1998). For microparticulation, the pH of protein and polysaccharide mixture was adjusted and the solution heated and homogenized before post-treatment with shearing and/or drying

(Schmitt *et al*., 1998). The electrosynthesis method was the modern method which was used to effectively prepare the protein-polysaccharide complexes. The selected protein and polysaccharide were dissolved in the suitable solvent, mixed in an electrolytic cell equipped with stainless steel electrode, and the electricity at suitable voltage applied. After completing the synthesis, the resulting protein-polysaccharide complexes were isolated and dried (Zaleska, Ring and Tomasik, 2001; Lii *et al.*, 2002; Zaleska, Tomasik and Lii, 2002).

Parameters Influencing the Formation of Protein-Polysaccharide Complexes: Since the complex coacervation between proteins and polysaccharides was mainly driven by electrostatic interactions, the physicochemical parameters affecting such interaction (e.g. pH, ionic strength, polysaccharide charge density, polysaccharide concentration, and protein-polysaccharide ratio) strongly influenced the formation of complexes. Moreover, the external parameters or processing factors such as temperature, shear rate, shear time and pressure also exhibited influence on the development of the coacervation phenomenon (Tolstoguzov, 1997; Schmitt *et al.*, 1998). The review of Schmitt *et al.* (1998) shown in the following paragraphs clearly elucidated the effect of each parameter.

The pH played a key role in the formation of complexes because of its effect on the ionization degree of the functional side groups carried by the biopolymers (i.e., amino and carboxylic groups). For this reason, when considering a mixture containing an anionic polysaccharide and a protein, the maximum coacervation yield was obtained below the pI of the protein, more precisely at the electrical equivalent pH. At this pH value, the two biopolymers carried exactly opposite net charges, resulting in a maximum electrostatic attraction.

The number of microions or ionic strength of the biopolymer solution was the important factor affecting complex coacervation. As the formation of protein-polysaccharide complexes was mediated by electrostatic attractions between the biopolymers, the charge neutralization by microions could hinder it. At low ionic strength, the microion concentration had a small effect on coacervation. The number of charges present on the biopolymers was sufficient to allow electrostatic interactions. Conversely, at high salt concentration, the net charge carried by the biopolymers was reduced by interaction with the microions, resulting in a decrease of the electrostatic attraction between the macromolecules. Moreover, the counterions associated with the biopolymers before dissolution were not liberated in the medium because of the high salt concentration. In this case the coacervation was suppressed.

The charge density of the biopolymer was defined as the number of charges on the polymers per unit length. This factor had to be controlled in order to achieve the complex coacervation phenomenon because no coacervation occurred under a critical charge density values. To conduct a higher yield of complex coacervation, the suitable pH and ionic strength of each protein-polysaccharide system, which directly affect the charge density of biopolymer, had to be controlled.

Molecular weight of biopolymers was another factor affecting complex formation of proteins and polysaccharide. The increase in molecular weight was expected to lower the biopolymer compatability in solution by lowering the combinatorial entropy of mixing and thereby resulted in increasing of complex coacervation. This phenomenon was explained by an increase of the space filled with polysaccharide molecules which resulted in the increase of the accessible junction for proteins.

The protein-polysaccharide ratio was considered as the important factor. The maximum complex coacervation yield was obtained at the specific ratio for each protein-polysaccharide system. For ratios where one of the biopolymers was in excess, soluble complexes were obtained because of the presence of non-neutralized charges. Otherwise, when both of polysaccharide and protein concentrations were excess in the solution, no coacervation occurred because of the low energetic interest of concentrating the biopolymers into coacervates if the concentration in solution is already high. At high biopolymer concentrations, the system exhibited phase separation through thermodynamic incompatibility because of the competition for the solvent between the macromolecules.

The coacervation temperature influenced the complex coacervation phenomenon through a number of effects. The decrease of temperature favored biopolymer/biopolymer interactions. The non-coulombic interactions such as hydrophobic interactions and hydrogen bonds were influenced by temperature. Low temperature was conducive to hydrogen bond formation, whereas hydrophobic interactions and covalent bonding were enhanced by increasing the temperature, because of the exposure of reactive sites by thermal denaturation of globular proteins and conformational changes of polysaccharide structure. Thus, the suitable temperature for each system had to be regulated in order to obtain the highest complex yield.

Increasing of the shear rate resulted in smaller protein-polysaccharide complex size. Increasing shear time resulted in lowering the complex formation and the larger complex size occurred from the coalescence of the small complexes through the depletion-flocculation mechanism.

The effect of high pressure on the formation of protein-polysaccharide complexes was documented. Two kinds of high pressure treatments applied to the biopolymer solutions were high hydrostatic pressure treatment and microfluidization treatment. In the high hydrostatic pressure treatment, high pressure was applied to water or any fluid contained protein-polysaccharide complexes which transmits static weight or pressure to the complexes equally in all directions. The microfluidization treatment combined high pneumatic pressure with turbulence, cavitation, and shear phenomena. At high pressure, the complex coacervation occurred rapidly as induced by stronger electrostatic interactions between protein and polysaccharide and resulted in formation of fibrous protein-polysaccharide complexes (Laneuville, Paquin and Turgeon, 2000).

Studying of structure of protein-polysaccharide complexes was of paramount importance for many reasons. On a fundamental approach, structure of the complexes gave more information on involvements of different regions of biopolymers in the interactions. The structure of the protein-polysaccharide complex was greatly responsible of its physicochemical properties. Some of these properties could clearly be elucidated if the structure of the complexes was well known (Schmitt *et al.*, 1998). Since the structure of each complex was stabilized by different chemical bonds and interactions, the difference in size and shape was generally observed (Tolstoguzov, 1997). Many techniques were used to investigate the structure of the complexes including confocal scanning laser microscopy (CSLM), light scattering, scanning electron microscopy (SEM), differential scanning calorimetry (DSC), infrared spectroscopy (IR) and nuclear magnetic resonance spectroscopy (NMR). These techniques could be used either alone or in combination, depended on the

characteristics of samples. The combinations of these techniques were recently used in many researches for better characterization. Laneuville *et al.* (2000) successfully elucidated the fibrous structure and mornitoring the structural changes during microfluidization of whey protein-xanthan complexes using optical microscopy and computer-aided image analysis. These combination techniques provided clear result with high resolution. Schmitt *et al*. (2001) used the diffusing-wave spectroscopy and CSLM to characterize the structure of β-lactoglobulin-acacia gum complex and the precise results in size and shape of complexes were obtained. Zaleska, Tomasik, and Lii (2002) disclosed the 2-dimensional fibrous structure of carboxymethyl cellulose-casein complexes by the use of combination techniques of SEM and powder X-ray diffractometry. Sanchez and Renard (2002) were successful in using only CSLM to elucidate the globular complexes of β-lactoglobulin-acacia gum and similar results were also found in the latter investigation of Turgeon *et al*. (2003). CSLM has the ability to deliver the small optical sections through the large and thick specimen and provided the 3-dimensional structure. Peng and Zhang (2003) elucidated the composition and fibrous structure of water-soluble polysaccharide-protein complex extracted from the mycelium of *Ganoderma tsugae* using size-exclusion chromatography combined with laser light scattering (SEC-LLS).

Functional Properties of Protein-Polysaccharide Complexes: The protein and polysaccharide functions including viscosity, gelation, foaming properties, and emulsification properties, were improved after formation of protein-polysaccharide complex (Tolstoguzov, 1997; Schmitt *et al.*, 1998; Mishra, Mann and Joshi, 2001). These functional properties were modulated by controlling the conditions of

formation of the complexes, that is, pH, ionic strength, polysaccharide concentration, biopolymer ratio, heat and mechanical treatments (Schmitt *et al.*, 1998).

The addition of protein-polysaccharide complexes in food systems provided a sharp increase in the system viscosity. This additional resistance to flow occurred from the dispersion of macromolecules complexes into the system. The interaction of the added complexes with water and other macromolecules and the swelling of such biopolymers could occur and resulted in increasing the viscosity of the system. Moreover, viscosity of the system was depended on volume fraction, surface charge density, and thickness of the hydration layer, size, shape, and deformability of the complexes (Schmitt *et al.*, 1998). The volume fraction of the system must be suitable for each food system to produce the desirable viscosity. The surface charge density regulated the solubility of protein-polysaccharide complexes. The complexes with low surface charge density could only swelled when dissolve in food system with water hence the increase of viscosity was obtained. Thickness of hydration layer depended on type and solubility of each biopolymer. The complexes produced with thick hydration layer biopolymers exhibited high water solubility thus the increase of viscosity was inhibited. Protein-polysaccharide complexes produced from high molecular weight biopolymer had large complex size. When adding these complexes into food system, the high system viscosity was usually obtained. The shape and deformability of the complexes depended on the structure of biopolymers and the process used in complexation. The complexes with large size, non-spherical shape and small biopolymer deformation exhibited better result for using as viscosifying agent in food (Schmitt *et al.*, 1998).

The thermal gelation of protein-polysaccharide mixtures was a well-known texturing treatment in food processing. However, most of the mixed gels were obtained from thermal treatment of protein-polysaccharide dispersions under thermodynamic incompatibility conditions. In some cases, the complex coacervation of biopolymers was used, because it allowed the thermal gelation, under specific conditions of pH and ionic strength, of proteins and polysaccharides that do not form gels alone. The major forces involved in the gelation by complex formation are nonspecific electrostatic interactions, hydrophobic and hydrogen bonds, rather than specific covalent disulfide bonds (Tolstoguzov, 1986). The addition of protein-polysaccharide complexes into food system, such as casein-κ-carrageenan complexes in milk chocolate, remarkably modified and improved gelation properties. The coupled networks of the existent protein and polysaccharide were induced and resulted in the formation of stronger gel network (Oakenfull, Pearce and Burley, 1997). Mishra, Mann and Joshi (2001) observed the better gelation of whey protein-pectin complexes as comparing with whey protein alone.

The foaming property of protein was its ability to decrease the interfacial tension appearing at the water-air interface of air bubbles. Polysaccharides also exhibited foam-stabilization properties, but these were mainly due to their ability to thicken the aqueous medium. Combining the foaming properties and stabilization effects of these two classes of biopolymers through the complex formation, an enhancement of the surface properties was occurred (Rabiskova *et al*., 1994). The study of Dickinson and Izgi (1996) revealed that the complexation of protein and polysaccharide through covalently-linked bonds resulted in improvement of foaming ability and stability when compared to protein or polysaccharide alone. In their study,

the covalent complexes of lysosyme and nonionic polysaccharide dextran produced a very substantial enhancement in foaming properties and this improvement exhibited because of the increase in molecular weight of the complex occurred by covalent complexation with polysaccharide. Mishra, Mann and Joshi (2001) also reported a similar finding that foaming properties of whey protein-pectin complexes were substantially improved comparing with that belonged to each biopolymer.

From the review of Schmitt *et al.* in 1998, many studies on the emulsification properties of protein-polysaccharide complex were reported. Both soluble and insoluble complexes such as whey protein-dextran complexes were used to stabilize emulsion. The improvement of emulsion stability was mainly driven by protein fractions in the complex and this stability was increased by increasing protein concentration in the complex formation step. The explanation given was that the protein parts of the complexes adsorbed oil droplets and resulted in increasing of viscosity at the oil/water interface, forming a gel like structure around the oil droplets and improving emulsification properties. The report of Mishra, Mann and Joshi (2001) showed that the emulsifying properties including emulsion activity and emulsion stability of whey protein-pectin complexes was higher than emulsifying activity of whey protein or pectin alone. The similar results were found in the study of Akhtar and Dickinson (2003) that whey protein-dextran complexes gave much better emulsion stability than gum arabic, whey protein or dextran alone under similar conditions. Their explanations were that whey protein on complexing with dextran enhanced the steric stabilization provided by the bulky hydrophilic polysaccharide moiety.

Applications in Food Products: As previously described, the complex coacervation of proteins with polysaccharides was a mean of improving the functional property of each biopolymer. Thus, research documents and patents on application of protein-polysaccharide complexes as food preserving agent, emulsion stabilizing agent, fat replacer, and meat replacer in meat analog were available.

The application of protein-polysaccharide complexes as food preserving agent was already patented by Blaise, and Freedman and Blaise in 1997. Their patents claimed that protein-polysaccharide complex could be used as the novel preservative. The preservation against deterioration of organoleptic properties during storage of foods, such as seafood, fruits, vegetables and meat, was obtained by contacting such foods with an aqueous solution of a stabilizing composition containing one stabilizing acid and a protein-polysaccharide complex composition including at least one of water-soluble polysaccharide and substantially water-insoluble protein at the weight ratio of 99:1. The water-soluble polysaccharide used for this purpose were alginate, carrageenan, gum arabic, tragacanth gum, guar gum, pectin, ghatti gum, xanthan gum and mixtures there of, while the substantially water-insoluble protein was a prolamine, zein, hordein or gliadin. The process for preparing their complexes started with preparing the solvent for dissolving biopolymers. One of citric acid, malic acid, adipic acid, tannic acid, lactic acid, ascorbic acid, acetic acid or fumaric acid was selected only one type to dissolve in water and then ethyl alcohol was added and mixed homogeneously. The obtained solvent was used to dissolve the selected proteins. After receiving the protein solution, the water soluble polysaccharide was added and mixed. The final solution was dried and the protein-polysaccharide

complexes were obtained. These complexes were claimed as the effective preserving agent when using in the form of dipping solution.

Some of proteins and polysaccharides were used as emulsion stabilizing agent. Their emulsifying properties were improved by producing protein-polysaccharide complex via the suitable method (Johannes, Cornelis and Leendert, 1977; Bishay and Clark, 1996; Eric and Christophe, 2004). Johannes, Cornelis and Leendert (1977) reported the use of protein-polysaccharide complexes produced from globular proteins such as whey proteins, blood serum proteins, egg white and egg yolk proteins, soy proteins, and yeast proteins, and algal-anionic polysaccharides such as carrageenan and alginate, as an effective emulsion stabilizing agent. The isolated complexes were applicable in food compositions both oil-in-water emulsion such as mayonnaise, liquid pancake butter, creams, and dairy products, and water-in-oil emulsions, such as spreads like butter and margarine. The study of Eric and Christophe (2004) disclosed the emulsion stability improvement from the addition of protein-polysaccharide complexes. The preferable proteins were β-lactoglobulin, gelatin, α-lactalbumin, bovin serum albumin, soy globulin, wheat protein, whey protein and soy protein, and the polysaccharide was taken from the group consisting of charged natural or synthetic polysaccharides. Most preferably, the polysaccharide was acacia gum, carboxy-methyl-cellulose, chitosan, xanthan, alginate, propyleneglycol alginate, carrageenans, low or high methoxylated pectins, arabinogalactans, rye arabinoxylans and wheat arabinoxylans. The method for preparing the emulsion stabilized by protein-polysaccharide complexes was performed by mixing the protein stabilized emulsion with the polysaccharide stabilized emulsion at a 1:1 weight ratio using a mixer or a high shear pump.

Some patents were already specified the use of protein-polysaccharide complexes as fat replacer (Tarr and Bixby, 1995; Zolper, 1995; Adriaan, Maria, and Johannes, 1999). The suitable complexes for this purpose were in fibrous, elliptical or spherical shape and could be produced by applying pressure into the solution of protein-polysaccharide mixture before complexation. Tarr and Bixby in 1995 reported novel fat substitutes produced from defatted oat protein and oat or barley starch complexes. These spherical complexes were used as ingredient in the formulation of non-fat ice cream, soft butter-like spread, artificial butter, fat free salad dressing, sour cream substitute, and non-fat chocolate filling. Another patent of Zolper (1995) described the process for producing fat substitutes from undenatured whole milk proteins-carrageenan complexes and their application in food products. The process for the production of the fat substitute consisted of five essential steps. The carrageenan and undenatured whole milk proteins were mixed into water, heated at an elevated temperature to dissolve the biopolymers, added calcium chloride to the solution, cooled to room temperature and subjected to shear to produce small size complex coacervates. The obtained complexes were in gel paste form and could be applied as fat substitutes in frozen desserts, dairy based spreads such as butter, cream cheese and dips, mayonnaise and other salad dressings, low fat ground and emulsion meats, candy and icing, non-fat coffee creamer, low fat milk, non-fat cheese, non-fat whipped topping, and some bakery items such as cheese cake. The report of Adriaan, Maria, and Johannes (1999) clearly demonstrated the use of protein-polysaccharide complexes as fat replacer in salad dressings, creams, toppings, cheese, sauces, spreads, margarines and ice-cream. The biopolymer combinations that were successfully used were gelatin and alginate, gelatin and pectin, gelatin and acacia

gum, gelatin and lecithin, gelatin and carrageenan, gelatin and xanthan, and gelatin and gellan gum. The resulting complex coacervate particles were generally in elliptical shape and could be prepared by dissolving of the selected biopolymer materials in water at slightly elevated temperature. Consequently, the biopolymer mixture was allowed to form the complex by adjusting the pH of the solution closed to the isoelectric point of the coacervates and applying the pressure while mixing. The obtained protein-polysaccharide mixture solution was cooled to the desired temperature. The complex coacervate particles was isolated by centrifuging, filtration or (freeze) drying.

The application of protein-polysaccharide complexes as meat replacer was reported. Tolstoguzov *et al*. (1974) cited that the suitable proteins for making complexes used for this purpose, were soy protein, peanut protein, whey protein, casein, egg protein, cotton seed protein, pea protein and gelatin, while suitable polysaccharides were xanthan gum, alginate and pectin. The heat-stable meat analog from protein-polysaccharide complexes produced by preparing protein and polysaccharide solution, mixing, heating to 50˚C, adjusting the pH below isoelectric point of proteins, keeping at 4˚C, mixing with other ingredients, homogenizing, packing and then cooking. They concluded that the resulting meat analog had desirable texture and flavor which was acceptable by consumer. The patent of Chen and Soucie (1985) revealed that the edible protein-polysaccharide fibrous complexes produced from whey protein and xanthan gum was the most effective meat replacer. The method for manufacturing such complexes were started by making whey protein solution before adding xanthan gum, mixing in Waring blender with medium speed, and then adjusting the pH to the isoelectric point of the coacervates. The obtained

fibrous complexes of whey protein and xanthan gum were separated from the remaining aqueous phase component by filtration or centrifugation and then washed with water. Such fibers were pressed in a cheese-press and/or dried to provide meat-like fibers which suitable for use in chewable meat-like products. The continue study of Soucie and Chen in 1986 showed the successful production of meat-like fibrous complexes from soy or egg proteins with xanthan gum using similar method to their patent in 1985.

From the past until now, there is no patent or research studied on the use of protein-polysaccharide complexes as cold-set binder in meat products. Thus, the study in this research area is still needed.

CHAPTER III

METHODOLOGY

3.1 Preparation and Characterization of Chitosans and Chicken Protein Hydrolysates

3.1.1 Chitosan

Preparation: Chitosan was prepared from dried and cleaned shrimp shells supplied by Ta Ming Enterprises (Samutsakorn, Thailand). The preparation method was composed of the following four consecutive steps: deproteinization (soaking 2 times in 1.5 M sodium hydroxide for 12 hrs), demineralization (soaking 2 times in 1.5 M hydrochloric acid for 12 hrs), decoloration (soaking for 1 hr in 90% ethanol at 90˚C), and deacetylation. The deacetylation steps were carried out by soaking the dried chitin samples in 50% by weight sodium hydroxide for 72 hrs at room temperature, or 72 hrs at room temperature plus 24 hrs at 90˚C, or 72 hrs at room temperature plus 48 hrs at 90˚C, in order to obtain chitosan with 70-80, 80-90, and 90-100% degree of deacetylation, respectively (Tolaimate *et al*., 2003).

The chemical reagents used in all experiments were analytical grade and were obtained from USB (USB Corporation, OH, USA), Merck (Merck KGaA, Darmstadt, Germany), and Sigma Aldrish (St Louis, MO). The caustic soda for chitosan preparation was commercial grade and obtained from Thasco Chemical, Bangkok, Thailand.

Degree of deacetylation (DD): DD of chitosan was determined using the first derivative ultraviolet spectrophotometry described by Muzzarelli and Rocchetti (1997). The scanning spectrophotometer was used to detect the zero order

absorption spectra of free amino group in chitosan chain and then calculated into the first derivative spectra. The analytical procedure was described as follows:

Three solution of acetic acid at 0.01, 0.02, and 0.03 M were prepared and their first derivative spectra from 240-190 nm were recorded against deionized distilled water. The superposition of the three spectra showed the zero crossing point (ZCP) of the acid. Then, eight reference solutions of *N*-acetyl-D-glucosamine at 0, 0.005, 0.010, 0.015, 0.020, 0.025, 0.030, 0.035 g/L in 0.01 M acetic acid were prepared and the spectra were recorded. All spectra were superimposed and the distance or height (mm) for each reference concentration above the zero crossing point was measured. The calibration curve of height versus concentration of *N*-acetyl-D-glucosamine was drawn.

The 0.2 g each, of dried powder chitosan samples were dissolved in 100 ml of 0.2 M acetic acid and diluted to 0.01%w/v with deionized distilled water. The resulting solutions were transferred into a 10 mm cuvette and the spectra recorded. The height of each sample obtained from superimposition of the spectra were then used to read the concentration of *N*-acetyl-D-glucosamine from the calibration curve. DD of chitosan samples were calculated.

$$DD(\%) = \left\{ \frac{(1-A)}{\left[(10W - 204A) + \left(\frac{A}{161} \right) \right]} \right\} x100$$

Where DD = Degree of deacetylation of chitosan samples

A = Real concentration of *N*-acetyl-D-glucosamine (g/L) obtained from calibration curve divided with 204

W = Weight of chitosan (g)

204 = Molecular weight of *N*-acetyl-D-glucosamine

161 = Molecular weight of D-glucosamine

Molecular weight (MW): Chitosans at various MW were prepared. The original samples obtained from the previous step (deacetylation step) were used as such, as the high MW chitosans. The medium and low MW samples were obtained by partial hydrolyzing the original samples with chitosanase from *Bacillus sp.* PP8 at 50˚C, pH 4.5 for 10 and 24 hrs, respectively.

MW of the resultant chitosan samples were determined by gel permeation chromatography (GPC) (Waters 600E, MA, USA) with ultrahydrogel linear column set and refractive index detector (Terbojevich and Cosani, 1997). Chitosan samples were prepared by dissolving dried chitosans (0.2%w/v) in 0.5 M sodium acetate buffer and filtering through the microfilter membrane with pore diameter at 0.45 μm before injecting into the GPC column. The 20 μl of chitosan solution was injected into the column at 30˚C. The eluent was 0.5 M sodium acetate buffer with 0.6 ml/min flow rate. The MW of chitosan was calculated using pullulans as standard.

3.1.2 Chicken Protein Hydrolysates

Preparation: Chicken protein hydrolysates were prepared from chicken broiler tenderloin meats purchased from Better Foods poultry processing plant (Samutsakorn, Thailand). The meats were packed in insulated box and brought to the laboratory within 2 hrs, at 4-10˚C. After trimming and grinding through 4 mm plate of a meat grinder, they were vacuum packed (1 kg/pack), and kept at -18˚C for further use. Moisture, protein (total N x 6.25), ash, and fat contents were determined according to the procedures outlined by Association of Official Analytical Chemists (AOAC, 1995). Papain from papaya latex at 3.3 units/mg activity was obtained from Sigma Aldrish (St Louis, MO). The weight ratio of ground chicken broiler tenderloin

to water during hydrolysis was 1:2. The weight ratio of the meat to enzyme was 250:1. The hydrolysis reaction was carried out at pH 7 and the temperature controlled at 60˚C. The incubation time was varied from 0-120 mins to obtain chicken protein hydrolysates with different degree of hydrolysis. The hydrolysate samples were freeze-dried prior to further use. Figure 3.1 showed a flow diagram for chicken protein hydrolysates preparation.

Degree of hydrolysis (DH): The DH of the chicken protein hydrolysates was determined by o-phthaldialdehyde method reported by Neilsen *et al.* (2001). Protein content (%) by Kjeldahl method (total N x 6.25) was determined (AOAC, 1995). The DH was defined as the percentage of cleaved peptide bonds and calculated using the following equation:

$$\mathbf{DH\ (\%)\ =\ h/h_{tot}\ *\ 100}$$

Where **h** is the number of hydrolyzed bonds, and

h = [(serine-NH_2)-(β)/α] meqv/g protein**,**

$\mathbf{h_{tot}}$ is the number of total peptide bonds per protein equivalent which is related to amino acids in protein, and

$\mathbf{h_{tot}}$ = 7.6 (according to the protein equivalent of meat),

$$\mathbf{serine\text{-}NH_2} = \frac{(OD_{340}\text{sample} - OD_{340}\text{blank})}{(OD_{340}\text{standard} - OD_{340}\text{blank})} * (0.9516)(0.1)(100/(X)(P))$$

X = g protein used in sample preparation

P = % protein content from Kjeldahl method

β = 0.4

α = 1.0

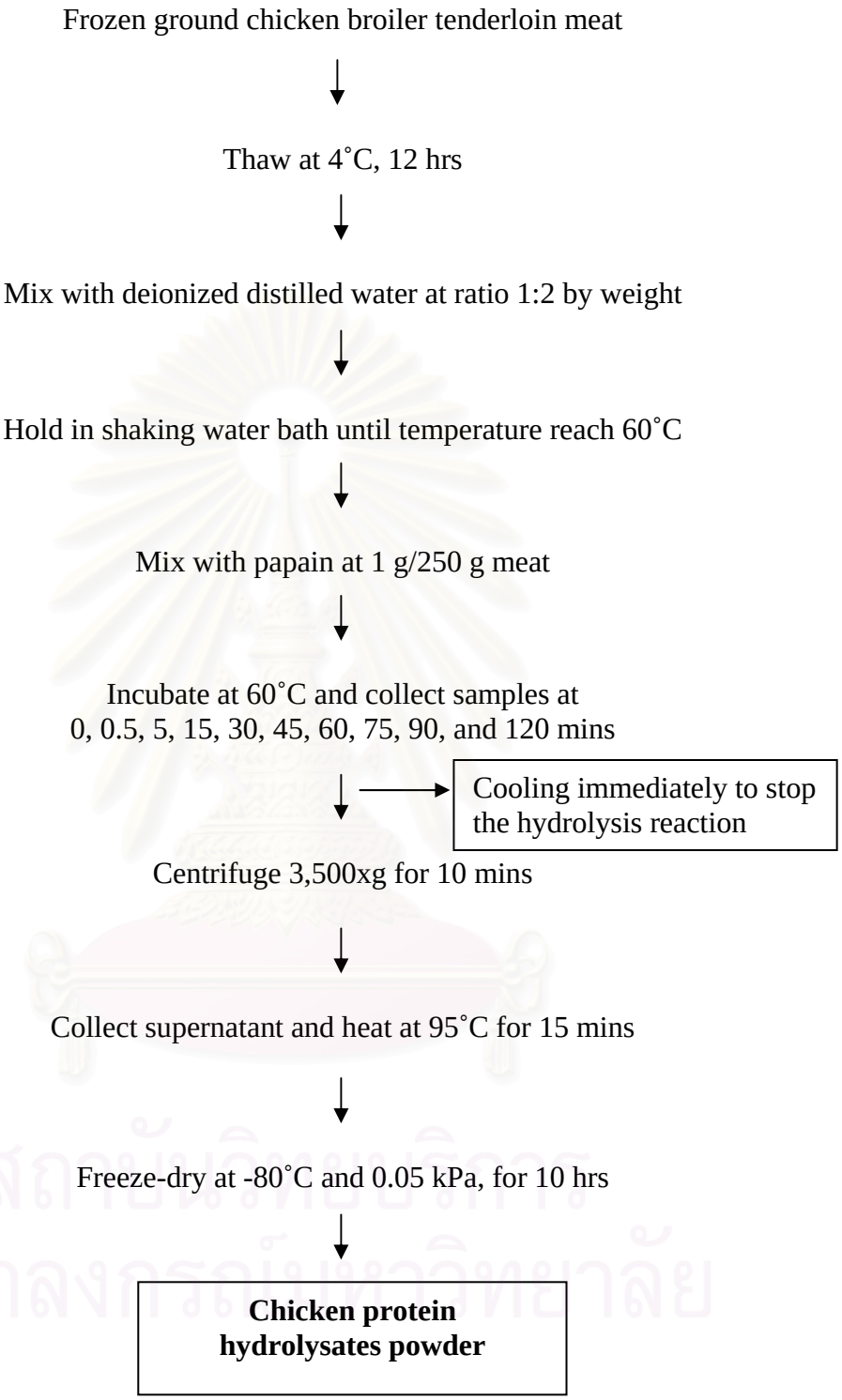


Figure 3.1 Process for chicken protein hydrolysates preparation

3.2 Effect of Chitosan at Different DD, MW and Concentration (C) on Cold-Set Gelation Abilities, Structures and Physico-chemical Properties of Chicken Salt-Soluble Protein (SSP) Gels

3.2.1 Cold-Set Gel

Preparation of SSP: Frozen chicken broiler tenderloin meats were thawed in refrigerator (4˚C) for 12 hrs before use. The SSP were extracted with 0.58 M saline buffer (0.49 M sodium chloride, 17.8 mM pentasodium triphosphate and 1 mM sodium azide), based on the method of Camou, Sebranek and Olson (1989), and DeFreitas *et al.* (1997) with slight modifications in mixing time and instrument used. One part of ground chicken broiler tenderloin meat and two parts of saline buffer were blended for 20 sec in Waring blender at low speed (Waring Commercial, Connecticut, USA). The slurry was kept at 4˚C for 1 hr and centrifuged (9,500 x g at 4˚C) for 2 hrs (Hettich Cetrifugen model Universal 32R, Andreas Hettich GmbH & Co KG, Tuttlingen, Germany). The pH (pH meter model M 360, QIS, Netherland), salt content (AOAC, 1995), and protein concentration (Biuret's method, Copeland, 1994) were determined. Consequently, the protein concentration was adjusted to exactly 8%w/v with the saline buffer and the SSP sample was kept at 4˚C for 12 hrs before use in order to complete the protein dispersion and solubilization in saline solution, and used within 2 weeks.

Preparation of alkali-precipitated chitosan: The alkali-precipitated chitosans were prepared by dissolving dried powder chitosan in 1% acetic acid at various concentrations (0.50, 0.75, and 1.00% for high MW, 1.00, 1.25, and 1.50% for low and medium MW chitosan samples). The obtained chitosan solutions were precipitated with 10 M sodium hydroxide and rinsed with deionized distilled

water until the pH reach to 6.54-6.57. The resulting alkali-precipitated chitosans were suspended in 5 ml deionized distilled water for further use.

Preparation of cold-set gels: The cold-set gels were formed by mixing SSP with various preparations of alkali-precipitated chitosan. Figure 3.2 showed a flow diagram for cold-set gel preparation.

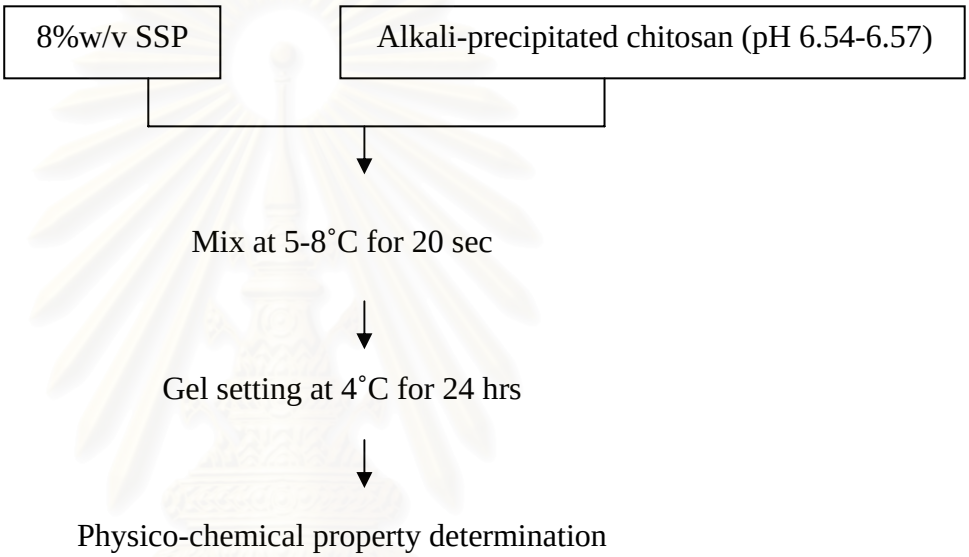


Figure 3.2 Process for preparation of cold-set gel of alkali-precipitated chitosan and SSP

3.2.2 Physico-chemical Properties of the Developed Cold-Set Gels

The physico-chemical properties of the developed cold-set gels comprising color, rheological characteristics, texture, disulfide content, non-denaturing gel-electrophoretic pattern, and structure profile, were determined.

Color: 20 g of each developed cold-set gel sample was placed in plastic cell and assessed for color using Hunter Lab Color Flex spectrophotometer 45˚/0˚ (Hunter Associates Laboratory Inc., Reston, VA). This assessment was

performed in 3 replicates for each sample and each sample was scanned for at least 6 spots. The results expressed in term of L* (lightness), a* (redness), and b* (yellowness) were used to calculate the color difference value (ΔE*) using the following formula and the control color was used as standard color (Gilchrist and Nobbs, 1999).

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

Where $\Delta L^* = L^*_{trt} - L^*_{std}$

$\Delta a^* = a^*_{trt} - a^*_{std}$

$\Delta b^* = b^*_{trt} - b^*_{std}$

Rheological characteristics: The oscillation frequency sweep test with parallel plate was performed to determine the rheological characteristics of the developed cold-set gels, using Bohlin rheometer model CVOR 150 (Bohlin Instruments, Worcestershire, UK). The gap setting between samples and plate was 0.15 mm. The target strain was selected in the linear viscoelastic range of the SSP at 0.5%. The temperature used was isothermal at 4˚C and the frequency range was varied from 0.001-30.000 Hz. Each sample was determined for 3 times. The rheological characteristics of the developed cold-set gel gels were expressed in term of elastic modulus (G´), viscous modulus (G´´), complex modulus (G*), complex viscosity (η*), and phase angle (δ) (Rao, 1992).

Textural characteristics: The compression analysis was performed to determine the texture characteristic of the developed cold-set gels in 3 replicates and each sample was determined for 6 times. The gel diameter and thickness were 4 and 5 cm, respectively. Texture analyzer model TAXT 2i (Stable Micro Systems, Surrey, England) equipped with hemispherical plastic probe (1 cm

diameter) was used. The probe was penetrated into the gel at the speed of 0.80 mm/s, while pre- and post-test speed was controlled at 1.00 mm/s. The distance of the compression was set at 10 mm. The texture expert software system (Stable Micro Systems, Surrey, England) was used to detect the maximum compression force (g).

Disulfide content: The disulfide content of the developed cold-set gels was determined according to the method of Beveridge, Toma and Nakai (1974), and LeBlanc and LeBlanc (1992) with some modifications in sample size and preparation. The available thiol (SH) and total thiol content were determined and used for calculating the disulfide content. The samples for determining available thiol content were prepared by mixing 1.5 g of cold-set gels with 10 ml of 1%w/v sodium chloride in TRIS-Glycine buffer (pH 8). The available thiol content was determined by mixing 0.1 ml of sample solution with 2.9 ml of 0.5%w/v sodium dodecyl sulfate (SDS) in TRIS-Glycine buffer, and 20 µl of Ellman's reagent (5,5′, dithio-bis-2-nitrobenzoic acid). After standing for exactly 2 mins at room temperature, the absorbance at 412 nm was read concurrently with the determination of reagent blank. For total thiol content, the sample solutions were prepared by mixing 0.1 g of cold-set gel with buffer solution containing 2%w/v SDS and EDTA at 0.50 mg/ml in 0.08 M sodium phosphate (pH 8). The total thiol content was determined by mixing exactly 3 ml of sample solution with 20 µl of Ellman's reagent. The absorbance at 412 nm was read after standing for 15 mins. The available and total thiol contents were determined 4 times per each sample and calculated using the equation below. The disulfide content was later calculated by subtracting the available thiol content from the total thiol content.

Thiol content (μM/g) = (73.53)(A412)(D)/(C)

Where A412 = Absorbance at 412 nm

D = Dilution factor which is equal to **30.2 and 150** for available and total disulfide content, respectively

C = Concentration of sample (mg/ml)

Non-denaturing gel-electrophoresis: The non-denaturing gel-electrophoresis was performed according to Laemmli (1970) with some modifications in which all of the reagents used were prepared without the addition of dithiotheritol (DTT) and sodium dodecyl sulfate (SDS). The slab gel of 10% resolving gel concentration and 4% stacking gel concentration with vertical electrophoresis system was used. The developed cold-set gel sample solution was prepared by dissolving 10 μl of such gel in 200 μl of treatment buffer without boiling and applied into slab gel at 10 μl/well, and 2 well/sample. After separation, the proteins were fixed and stained with Coomassie blue R-250 and destained in solution containing 10% methanol and 10% acetic acid in deionized distilled water.

Structure profiles: The scanning electron microscope (SEM) model JEOL JSM-5410 LV scanning microscope (JEOL, Japan) was used to investigate the structural characteristics of the developed cold-set gels. The samples were prepared by conventional-chemical fixation method, 3 pieces per sample (Gómez-Guillén *et al*., 2005). After coating with gold, the samples were analyzed for the structure and scanning electron micrographs were collected at 1,000, 5,000, and 15,000 magnifications.

3.2.3 Experimental Design and Statistical Analysis

The factorial design with 3 factors, each at 3 levels was used for this experiment. The factors studied were DD of chitosan at 70-80, 80-90, and 90-

100%, MW of chitosan at <100,000, 100,000-1,000,000, and >1,000,000 Da, and concentration at 0.50, 0.75, and 1.00% for high MW, 1.00, 1.25, and 1.50% for low and medium MW chitosan samples. The experiment was carried out in duplicate. Statistical analysis was performed by using ANOVA and general linear model (GLM) in SPSS version 11.5 (SPSS, 2002). Duncan's new multiple range test ($p \leq 0.05$) was used to detect differences among treatment means.

3.3 Effect of Chicken Protein Hydrolysates at Different DH and Concentration (C) on Cold-Set Gelation Abilities, Structures and Physico-chemical Properties of SSP Gels

3.3.1 Cold-Set Gel

The chicken protein hydrolysates with proper degree of hydrolysis were selected to be used as the cold-set binder in this experiment. From the preliminary study, it was found that the highest quality gels were obtained in treatments with chicken protein hydrolysates at 6.42, 15.12, 22.38, and 35.80% degree of hydrolysis. Thus, these hydrolysate samples were selected as the cold-set binders for this experiment. The developed cold-set gel samples were prepared by mixing the selected chicken protein hydrolysates with 8%w/v SSP and the developed gel was allowed to set at 4˚C for 24 hrs.

3.3.2 Physico-chemical Properties of the Developed Cold-Set Gels

The physico-chemical properties of the developed cold-set gels including color, rheological characteristics, texture, disulfide content, non-denaturing gel-electrophoretic pattern, and structure profile, were determined using the methods similar to those used in 3.2.2, while the pH was measured using the pH meter model

M 360 (QIS, Netherland). The measurement was performed in 3 replicates and each sample was measured for 5 times.

3.3.3 Experimental Design and Statistical Analysis

A completely randomized 4 x 3 factorial set of DH (6.42, 15.12, 22.38, and 35.80%) and concentration (1, 2, and 3% by weight) were used. The experiment was carried out in duplicate. Statistical analysis was performed by using ANOVA and general linear model (GLM) in SPSS version 11.5 (SPSS, 2002). Duncan's new multiple range test ($p \leq 0.05$) was used to detect differences among treatment means.

3.4 Combination Effects of Chitosan and Chicken Protein Hydrolysates on Cold-Set Gelation Abilities, Structures and Physico-chemical Properties of SSP Gels

The most efficient chitosan samples from 3.2 and chicken protein hydrolysates from 3.3 were concomitantly used as binders in the cold-set SSP gels. The SSP was extracted with the method similar to that used in 3.2.1. The selected alkali-precipitated chitosan and chicken protein hydrolysate samples were mixed before adding to 8%w/v SSP at the weight ratio of 0.5:1, 0.5:2, 0.5:3, 1:1, 1:2, 1:3, 1.5:1, 1.5:2, and 1.5:3 and the cold-set gel was formed at 4˚C for 24 hrs.

3.4.1 Physico-chemical Properties of the Developed Cold-Set Gels

The physico-chemical properties of the developed gels including pH, color, rheological characteristics, texture, disulfide content, non-denaturing gel-electrophoresis pattern, and structure profile, were determined by the methods similar to those used in 3.3.2.

3.4.2 Experimental Design and Statistical Analysis

A completely randomized design of alkali-precipitated chitosan concentration (0.5, 1, and 1.5% by weight) and chicken protein hydrolysates concentration (1, 2, and 3% by weight) were used. The experiment was carried out in duplicate. Statistical analysis was performed by using ANOVA and general linear model (GLM) in SPSS version 11.5 (SPSS, 2002). Duncan's new multiple range test ($p \leq 0.05$) was used to detect differences among treatment means.

3.5 Application of Chitosan, Chicken Protein Hydrolysates and Their Combinations in Raw Restructured Chicken Meat

3.5.1 Raw Restructured Chicken Meat

Frozen ground chicken broiler tenderloin meats were thawed in refrigerator (4˚C) for 12 hrs before use. The selected alkali-precipitated chitosan and chicken protein hydrolysates from 3.4 were used to produce raw restructured chicken meat both alone and in combination.

The process for producing raw restructured chicken meat was based on the method of Huffman (1981). Ground chicken broiler tenderloin meats were mixed for 1 min with 1% by weight sodium chloride using Kenwood mixer (Kenwood LTD., Havant Hants, UK), at low speed. The selected alkali-precipitated chitosan (medium MW chitosan with 94% DD at 1 and 1.5% by weight), chicken protein hydrolysates (22% DH at 2 and 3% by weight) and their combinations (weight ratio of alkali precipitated chitosan and chicken protein hydrolysate at 1:2 and 1.5:3) were added and the mixing continued for 2 mins. The control was chicken broiler tenderloin meats with 1% salt only. The obtained batters were stuffed into 2.5 cm diameter collagen casings, each with 10 cm length, tied with the string, and allowed to

set at 4˚C for 24 hrs. The obtained raw restructured chicken samples were sliced to 1.5 cm thickness with sharp-thin knife before determining the physico-chemical properties.

3.5.2 Physico-chemical Properties of Raw Restructured Chicken Meat

The physico-chemical properties of the raw restructured chicken meat, including pH, color, rheological characteristics, texture, water binding properties and structure profile, were determined.

pH: The pH of raw restructured chicken meats were determined by pH meter model M 360 (QIS, Netherland) with pH electrode (QP 123X) for meat, bread and cheese. This probe was able to pierce into the sample pieces and the pH was measured readily. The determination was carried out in triplicate.

Color: Each piece of raw restructured chicken meat was placed in plastic cell and assessed for color using HunterLab ColorFlex spectrophotometer 45˚/0˚ (Hunter Associates Laboratory Inc., Reston, VA). The results were expressed in term of ΔE^*_{ab} value which calculated from the average Hunter L*, a* and b* values of each sample (5 points/sample).

Rheological characteristics: The rheological characteristics of raw restructured chicken meat were determined using two different tests: the oscillation frequency sweep test (isothermal mode and parallel plate probe) and the oscillation temperature sweep test (temperature gradient mode and cone and plate probe). Bohlin rheometer model CVOR 150 (Bohlin Instruments, Worcestershire, UK) was used. The condition of oscillation frequency sweep test was similar to that used in 3.2.2. Each sample was determined for 3 times. The rheological characteristics

of this test were expressed in term of elastic modulus (G′), viscous modulus (G′′), complex modulus (G*), complex viscosity (η*), and phase angle (δ).

For the oscillation temperature sweep test, the conditions used were frequency at 1 Hz, strain at 0.5%, and delay time at 1 second with continuous oscillation. The temperature profile was started from 25˚C with the heating rate at 5˚C/min to 90˚C, and held at this temperature for 30 sec before cooling to 50˚C at the rate of 5˚C/min. Each sample was determined for 3 times. The rheological characteristic was expressed in term of elastic modulus (G′).

Textural characteristics: The texture characteristics of the raw restructured chicken meat were analyzed using texture profile analysis (TPA) with 40 mm compression plate in Instron Universal Testing Machine model 5565 p8935 (Instron corporation, MA, USA). The casing was peeled out of the sample before analyzing and 5 pieces of each sample were measured. The test speed and the pre- and post-test speed were controlled at 1.50 mm/s, and the distance of the compression was set at 75% of sample thickness. The texture software system (Instron corporation, MA, USA) was used to detect the texture profiles of samples and expressed in terms of hardness (N/cm^2), cohesiveness (ratio), adhesiveness (cm^3), springiness (cm), gumminess (N/cm^2) and chewiness (N/cm).

Water binding properties: Cooking loss and water holding capacity (WHC) were determined according to the method of Honikel and Hamm (1994) and Hongsprabhas and Barbut (1999a) with slight modification in sample size. The cooking loss was measured by placing 10 g of each sample into the thin walled-waterproof bag, then placing the bag in water at 90˚C and heating until the final temperature reached 75˚C. Consequently, the bag was removed and placed in running

tab water until the sample temperature reached room temperature (25˚C), after which the samples were taken from the bag, mopped dry and weighed. The cooking loss was expressed as the percentage of water loss after cooking (g) per initial weight before cooking (g). WHC of raw restructured chicken meats was measured by centrifugation method. 10 g of each sample was put into centrifuge tube and weighed accurately. After balancing the tubes, they were placed in the fixed angle rotor (Hettich Cetrifugen model Universal 32R, Andreas Hettich GmbH & Co KG, Tuttlingen, Germany) and centrifuged for 90 min at 9,500xg. After the centrifugation, the tubes were at once turned upside down on filter paper for 90 min and then the tubes were weighed to record the weight loss of samples. WHC was expressed as percentage of sample weight after centrifugation to the original weight.

Structure profiles: The scanning electron microscope (SEM) model JEOL JSM-5410 LV scanning microscope (JEOL, Japan) was used to investigate the structural characteristics of the raw restructured chicken meat. The samples were prepared by conventional-chemical fixation method for 3 pieces per sample (Gómez-Guillén *et al*., 2005). After coating with gold, the samples were analyzed for the structure and scanning electron micrographs were collected at 500, 1,000, 3,500 and 5,000 magnifications.

3.5.3 Experimental Design and Statistical Analysis

A completely randomized design was used. The experiment was carried out in duplicate. Statistical analysis was performed by using ANOVA in SPSS version 11.5 (SPSS, 2002). Duncan's new multiple range test ($p \leq 0.05$) was used to detect differences among treatment means.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Preparation and Characterization of Chitosan and Chicken Protein Hydrolysates

4.1.1 Chitosan

Clean and white chitosan flakes were obtained from the four consecutive preparing steps proposed in this study. Process with deproteinization before demineralization made chitosan become whiter with high purity (Lertsuriwong *et al.*, 2002). The reacting time and temperature in the deacetylation step was adequate to produce chitosan with desirable degree of deacetylation (DD) as shown in table 4.1. After the preparation, molecular weight (MW) of the obtained chitosan was over 10^6 Dalton. Hence, the high MW chitosan which proposed to have the MW over 10^6 Dalton was automatically obtained. This result indicated that the reagent concentration and condition used in chitin extraction and deacetylation step were suitable to be used in chitosan preparation and provided the chitosan samples with controllable physico-chemical properties. Tolaimate *et al*. (2003) reported that the proper adjustment of the process conditions was the important factor that would make possible to produce chitin very similar to their native form, and to prepare chitosan with the controllable characteristics. Furthermore, their study also showed that the use of sodium hydroxide solution as deacetylating agent for α-chitin from shrimp and crab shells was more suitable than potassium hydroxide. The chitosan with better solubility was achieved.

In preparation of the medium and the low MW chitosans, chitosanase was selected as the hydrolyzing enzyme since it produced chitosans with homogeneous shorter chain length and did not affect DD of the resulting samples (Vårum *et al*., 1996).

The hydrolysis with chitosanase from *Bacillus sp.* PP8 effectively reduced molecular weight of chitosan and the condition used in this study resulted in chitosan samples with homogeneous MW with only slightly variation in the low MW group (table 4.2). Moreover, the polymer dispersibility of medium and low MW chitosan was lower than the high MW chitosan which naturally obtained after preparation. The broad range of MW of high MW chitosan samples was resulted from alkali treatment in the preparation step (Synowiecki and Al-Khateep, 2003). The reduction of polymer dispersibility after chitosanase hydrolysis was also reported by Vårum *et al*. (1996). They explained that chitosanase hydrolyzed chitosan chain specifically and provided chitosan with homogeneous chain length. The obtained chitosan chain consisted mainly of D-glucosamine units and only small amount of chitosan chains with D-glucosamine linked with *N*-acetyl-D-glucosamine was produced.

The colors of the low and the medium MW chitosan were different from that of the non hydrolyzed or the high MW chitosan (table 3). L* significantly decreased, while significant increase of a* and b* was found in both low and medium MW chitosans ($p \leq 0.05$). The light yellow chitosan samples were obtained after hydrolysis, precipitation and drying. These changes might occur due to the liberation of reducing end and free aldehyde groups in chitosan side chain, and the occurrence of some monomer, dimer or trimer of glucosamine units from the hydrolysis of chitosanase.

However, this occurrence was non-homogeneous and occurred in only small amount because the hydrolysis reaction was time limitation. Consequently, these obtained molecules could react in browning reaction during incubation at 50˚C and drying at 40˚C, and form the small amount of browning substances (Chung, Tsai and Li, 2006). Hence, the light yellow hydrolyzed chitosan was resulted. However, this phenomenon did not affect the color of alkali-precipitated chitosan. The white chitosan beads were obtained after the preparation. This result might occur from the reprecipitation of chitosan samples after hydrolysis with concentrated sodium hydroxide solution in which the deproteinization and decoloration of chitosan occurred (Synowiecki and Al-Khateep, 2003).

Table 4.1 Conditions for chitosan preparation and the obtained degree of deacetylation (DD)

Treatment	Soaking time in 50% by weight NaOH (hr)	Soaking time in 50% by weight NaOH at 90 ˚C (hr)	DD (%)
Low DD	**72**	**-**	**78**
Medium DD	**72**	**24**	**88**
High DD	**72**	**48**	**94**

Table 4.2 Molecular weight (MW) of the obtained chitosan samples

DD (%)	MW (Dalton)		
	Low	**Medium**	**High**
Low (**78**%)	**5.43×10^4**	**1.73×10^5**	**1.10×10^6**
Medium (**88**%)	**6.55×10^4**	**1.92×10^5**	**1.02×10^6**
High (**94**%)	**9.86×10^4**	**1.84×10^5**	**1.04×10^6**

Table 4.3 Color (L* a* b*) of the obtained chitosan samples

Treatment		Color		
		L*	**a***	**b***
Low MW	**Low DD**	$67.47^{e} \pm 0.31$	$1.98^{d} \pm 0.04$	$15.80^{h} \pm 0.03$
	Medium DD	$50.56^{d} \pm 0.05$	$3.24^{e} \pm 0.04$	$11.97^{d} \pm 0.02$
	High DD	$40.91^{a} \pm 0.05$	$4.72^{h} \pm 0.03$	$12.31^{e} \pm 0.01$
Medium MW	**Low DD**	$48.50^{c} \pm 0.11$	$4.38^{g} \pm 0.03$	$11.84^{c} \pm 0.03$
	Medium DD	$47.78^{b} \pm 0.02$	$4.17^{f} \pm 0.01$	$12.99^{f} \pm 0.01$
	High DD	$48.72^{c} \pm 0.01$	$4.94^{i} \pm 0.03$	$15.67^{g} \pm 0.02$
High MW	**Low DD**	$87.43^{f} \pm 0.47$	$-0.02^{a} \pm 0.01$	$10.98^{b} \pm 0.18$
	Medium DD	$88.98^{g} \pm 0.12$	$-0.75^{b} \pm 0.05$	$10.72^{b} \pm 0.05$
	High DD	$91.45^{h} \pm 0.06$	$1.11^{c} \pm 0.09$	$8.72^{a} \pm 0.05$

$^{a-i}$ Means followed by different letters within the same column are significantly different ($p \leq 0.05$).

4.1.2 Chicken Protein Hydrolysates

Chicken broiler tenderloin meats were selected for preparing chicken protein hydrolysates because they had low fat content which might not affected the hydrolysis reaction of papain. The proximate compositions obtained from standard methods (AOAC, 1995) of the chicken broiler tenderloin meats were 78.50% moisture, 18.45% protein, 2.01% carbohydrate, 0.98% ash, and 0.16% fat.

Papain was selected to be used in this study because it functioned as good protein hydrolyzing agent. It effectively reduced muscle protein chain length with the low production cost. Furthermore, papain specifically hydrolyzed myofibrillar proteins and revealed the myosin tails from myosin heads. The thiol groups along the myosin tails and inside globular myosin heads should therefore be exposed (Fik and Surówka, 1986). Kominz *et al*. (1965) reported that papain digested myosin molecules better than trypsin. Furthermore, the report of Webster, Ledward and Lawrie (1982) also suggested that papain was the most effective hydrolyzing agent for meat by products and offal when comparing with pepsin, neutrase and alcalase. The light yellow chicken protein hydrolysate powder was obtained from the enzymatic hydrolysis with papain and freeze-drying. The processing yield was obtained at 10-11%. The protein contents of all samples, determined by Kjeldahl method, were 86-89% (table 4.4) which were allotted into protein concentrate category (USDA, 2003). This result was in accord with the report of Fik and Surówka (1986). They also used enzymatic hydrolysis with papain to produce protein concentrate with 85% protein content from broiler chicken heads.

The use of optimum condition for papain in this study resulted in continuous hydrolysis of chicken muscle protein into smaller peptides as detected by the

increase of degree of hydrolysis (DH) shown in table 4.4. The sharp increase of DH was occurred at the beginning (0-45 min) and the final (90-120 min) period of incubation time as shown in figure 4.1. The slight increase of DH was found at incubation time from 45-90 min. The occurrence of hydrolysis curve similar to this study was reported by Webster, Ledward and Lawrie (1982) and Fonkwe and Singh (1996). Furthermore, results from the investigation of Fonkwe and Singh (1996) also agreed with this study in that DH of the final protein hydrolysates (120 min of incubation time) was over 60%.

Table 4.4 Protein content (%) and DH (%) of chicken protein hydrolysates obtained from the hydrolytic reaction of papain at 250 to 1 ratio of substrate to enzyme and 0-120 min. incubations at 60˚C.

Incubation time (min)	Protein content (%)	DH (%)
0	87.72	0.03
0.5	88.48	6.42
5	87.52	15.12
15	88.07	22.38
30	87.19	35.80
45	87.24	36.80
60	89.62	37.74
75	86.28	39.53
90	88.01	51.22
120	87.89	60.12

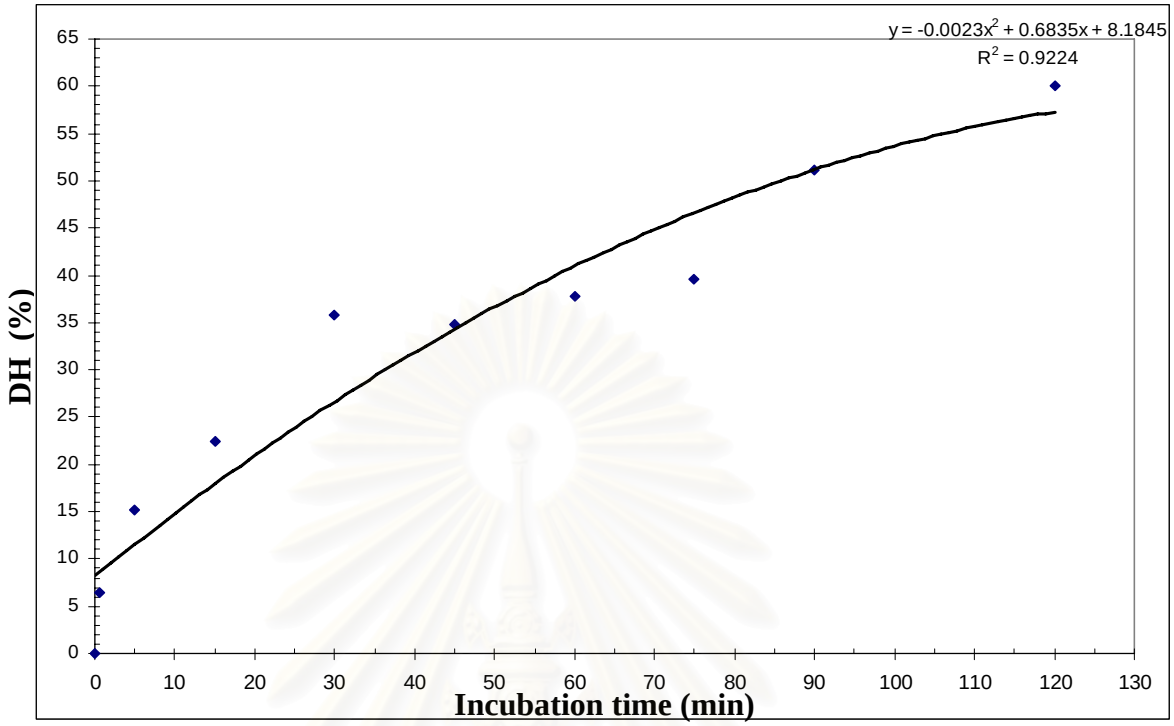


Figure 4.1 Hydrolysis curve of chicken broiler tenderloin meat which was hydrolyzed with papain at the ratio of substrate:enzyme equal to 250:1 and 0-120 min. incubations at 60˚C.

In this study, freeze drying technique was used to separate water from protein hydrolysates. After freeze drying, the obtained chicken protein hydrolysate powder had light yellow color. It can be postulated that the obtained protein did not denature since the color did not turn to brown. This result may due to the low drying temperature used. The color (L* a* b*) of the obtained chicken protein hydrolysates shown in table 4.5 demonstrated that the hydrolysis process and incubation time did not affect this parameter. The lightness (L*), redness (a*) and yellowness (b*) of the resulting chicken protein hydrolysate samples were not different when comparing among themselves ($p>0.05$).

Table 4.5 Color (L* a* b*) of the obtained chicken protein hydrolysate samples

Sample DH (%)	Color [ns]		
	L*	**a***	**b***
0.03	89.25 ± 0.35	-0.92 ± 0.03	14.20 ± 0.89
6.42	89.78 ± 0.58	-1.47 ± 0.09	14.70 ± 0.71
15.12	92.05 ± 1.12	-1.58 ± 0.11	12.87 ± 0.55
22.38	93.48 ± 1.08	-1.34 ± 0.08	14.15 ± 0.22
35.80	92.56 ± 0.45	-1.95 ± 0.07	15.87 ± 0.54
36.80	90.21 ± 0.59	-1.26 ± 0.05	13.87 ± 0.78
37.74	92.11 ± 0.43	-1.61 ± 0.08	13.88 ± 0.71
39.53	91.29 ± 0.69	-1.79 ± 0.14	13.06 ± 0.63
51.22	92.23 ± 0.51	-1.82 ± 0.15	13.21 ± 0.19
60.12	93.78 ± 0.87	-1.98 ± 0.04	14.10 ± 0.95

[ns] Treatments were not significantly different (P>0.05).

4.2 Effect of Chitosan at Different DD, MW and Concentration (C) on Cold-Set Gelation Abilities, Structures and Physico-chemical Properties of Chicken Salt-Soluble Protein (SSP) Gels

The addition of alkali-precipitated chitosan into SSP gels affected various physico-chemical properties of the developed cold-set gels including color, rheological characteristics, texture, disulfide content, non-denaturing gel-electrophoretic pattern, and structure profile.

4.2.1 Effect on Color

The color of the developed cold-set gels comparing with that of the control (8%w/v SSP) sample as shown in figure 4.2, were expressed in term of color difference value (ΔE^*_{ab}). ΔE^*_{ab} was calculated from the color changes of the developed cold-set gels from control. The increase of ΔE^*_{ab} indicated the higher color change from the addition of alkali-precipitated chitosan. It was found that the addition of alkali-precipitated chitosan directly affected color of the gels, especially in treatments with low MW and higher concentration of chitosans. For all MW samples, the more increase of ΔE^*_{ab} was observed when concentration used was increased. This result may due to the color of the chitosan sample itself. After precipitation, white chitosan beads were obtained. After mixing with SSP, the L* of the developed cold-set gels increased, the a* slightly decreased while the b* remained unchanged. This phenomenon was also reported by Jo *et al*. (2001), in which they observed an increase of lightness and reduction of redness on the surface color of pork sausage with chitosan oligomer. Moreover, the DD and MW of chitosan also affected the color of the developed gels. For treatment with high MW at similar concentration, ΔE^*_{ab} was increased in accordance with the increase

of DD. However, the different results were observed in treatment with medium and low MW samples. ΔE^*_{ab} of such treatments were getting higher from low DD (DD_1) to medium DD (DD_2) and then reduced at high DD (DD_3). In addition, the higher color change was observed when lower MW samples were used. The bead size of the alkali-precipitated chitosan, which depends on its MW, may be responsible for this result. High MW chitosan beads are the largest in size and some can still be detected in the developed gel matrix. From this phenomenon, the color of gels with high MW exhibited the lowest ΔE^*_{ab}. For medium MW chitosan, the smaller beads were obtained. During mixing with SSP, these small chitosan beads were able to penetrate and suspend in the gel matrix properly and resulted in higher lightness than SSP samples treated with high MW chitosan. Consequently, higher value was achieved. Similar result was found in low MW chitosan. The highest ΔE^*_{ab} value observed in treatments with low MW, high concentration and medium DD, indicating the more paleness of the gels with homogeneous suspension of fine chitosan beads.

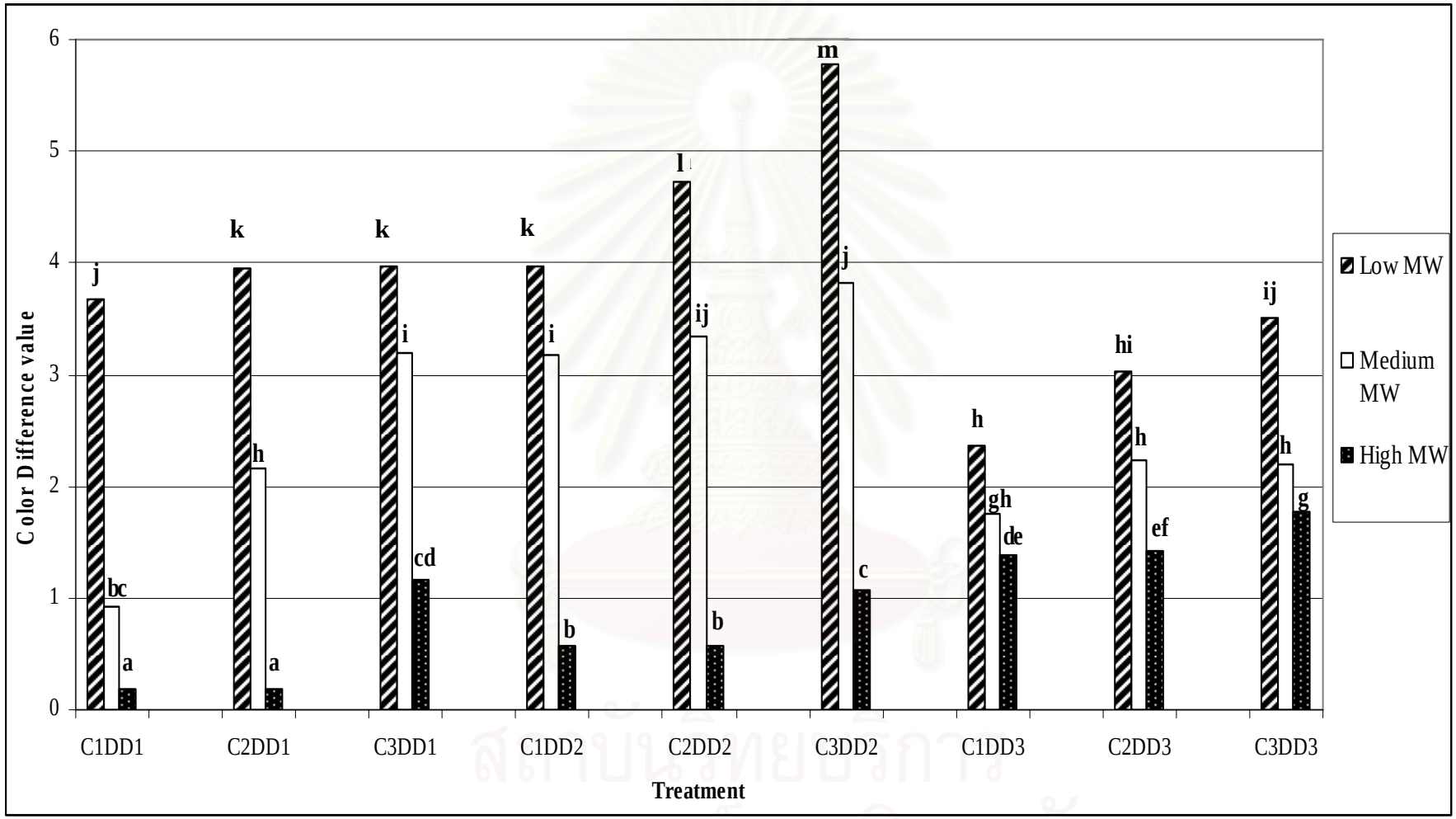


Figure 4.2 Color difference value (ΔE^*_{ab}) of the developed cold-set gels from 8%w/v SSP and various treatments of alkali-precipitated chitosan. C1, 2 and 3 represent concentration of chitosan at 1, 1.25 and 1.5% for low and medium MW and 0.25, 0.5 and 0.75% for high MW chitosan. DD1, 2 and 3 represent degree of deacetylation at 78, 88 and 94%, respectively. Different letters a, b…m indicate significant difference ($p \leq 0.05$) among treatments.

4.2.2 Effect on Rheological Characteristics

Rheological characteristics of the developed cold-set gels compared with that of the control were studied and various parameters were determined. However, the parameters which clearly indicated the changes of rheological characteristics were elastic modulus (G'), complex modulus (G^*) and complex viscosity (η^*). G' is the parameter which implies the elastic solid property of the gels, while G^* is calculated from G' and viscous modulus (G'') ($G^* = (G'^2 + G''^2)^{1/2}$). For η^*, it is the dynamic viscosity which was calculated by dividing G^* with frequency (ω) (Shoemaker, 1992).

The G' lines of the developed cold-set gels as shown in figure 4.3 were shifted up by the addition of alkali-precipitated chitosan. The experimental results in figure 4.3 B and 4.3 C showed the elevated position of G' of the cold-set gels with medium and high MW, while those of the cold-set gels with low MW samples were not significantly different from the control (figure 4.3 A). This result indicated the improper interaction of SSP with short chain chitosan. At high frequency range (1-30 Hz), the increasing of G' slope of the control patterns was observed indicating the deformation of SSP gel. When alkali-precipitated chitosan was added, the steadier elastic modulus patterns resulted. It was also observed that the overall G' of SSP gels with alkali-precipitated chitosan were higher than that of the control SSP gel. These results suggested the improvement of gel network by the addition of chitosan. Moreover, it was obvious that the G' pattern of cold-set gel with medium MW, high DD and high C (figure 4.3 B) was stable over a wide frequency range, and exhibited the highest shift of the elastic

modulus. This particular pattern also indicated the formation of rubber elasticity of the cold-set gel. These experimental results indicated the occurrence of strong gel network between SSP and medium chitosan chain with low acetyl group, which has previously been reported by Sionkowska *et al*. (2004). They explained that chitosan chains with low acetyl group tended to be extended and could easily interact with other macromolecules.

It was observed that most of the G* values of cold-set gels with alkali-precipitated chitosan were higher than those of the control as shown in figure 4.4 A, B and C. The DD, MW and concentration of chitosan influenced this parameter. Regardless of the DD, the more steady slope of G* lines was found when increasing of MW. The G* line with the steadiest slope was detected in treatment with high MW alkali-precipitated chitosan; however, the beginning of this line and its G* values along the frequency range stood lower than those of medium and low MW. These results implied the incidence of weaker SSP gel network from the addition of high MW alkali precipitated chitosan. When considering the effect of MW, it was found that G* lines of treatments with low MW samples were shifting lower when increasing of the chitosan concentration (figure 4.4 A). This result was in accord with the report of Chen *et al*. (2003). They also found that the thermal gelation of porcine SSP was interfered by the addition of low MW chitosan. The increase of chitosan concentration provided stronger interference on SSP gelation and lowered the G* values. The opposite result was found in the treatments with medium MW alkali-precipitated chitosan (figure 4.4 B). The shifting up of G* lines exhibited as the chitosan concentration was increased. This result suggested the formation of electrostatic interactions between charge groups of chitosan and SSP (Chen *et al*., 2003). Moreover, the more entanglement of macromolecules was

normally occurred when mechanical forces such as mixing or stirring was applied to the high MW biopolymers (Morris, 1995). For treatments with high MW samples, the G* lines of cold-set gels with DD_1 and DD_2 were lowered when increasing the chitosan concentration, while those of the cold-set gels with DD_3 samples shifted up as the chitosan concentration increased (figure 4.4 C). The highest improvement of G* which resulted in the highest position and steadiest slope, was detected in treatment with the highest DD, medium MW and the highest C. This occurrence suggested the better improvement of chitosan samples with high DD. The chitosan chains with low acetyl groups were more linear and therefore could interact more readily with other molecules (Synoweicki and Al-Khateep, 2003). Thus, the homogeneous and strong gel networks were obtained.

The results in figure 4.5 show the changes of η* values of all treatments compared with those of the control. The η* values were plotted versus the radiance frequency (ω) in order to indicate the type of biopolymer system in which a concentrated biopolymer solution or a gel was formed. The η* plot of control as shown by the arrowheads in figure 4.5 exhibited the pattern of concentrated biopolymer solution. Unlike the η* patterns of the cold-set gels with alkali-precipitated chitosan, the linear η* lines along the frequency range were observed, especially in treatment with high DD and C, indicated the behavior of strong gel network (Morris, 1995; Ross-Murphy, 1995). The most linear η* line with the highest shifting up from that of the control was observed in treatment with medium MW, high DD and high C. This result along with the other rheological parameters suggested the formation of stronger SSP gel network in the presence of alkali-precipitated chitosan.

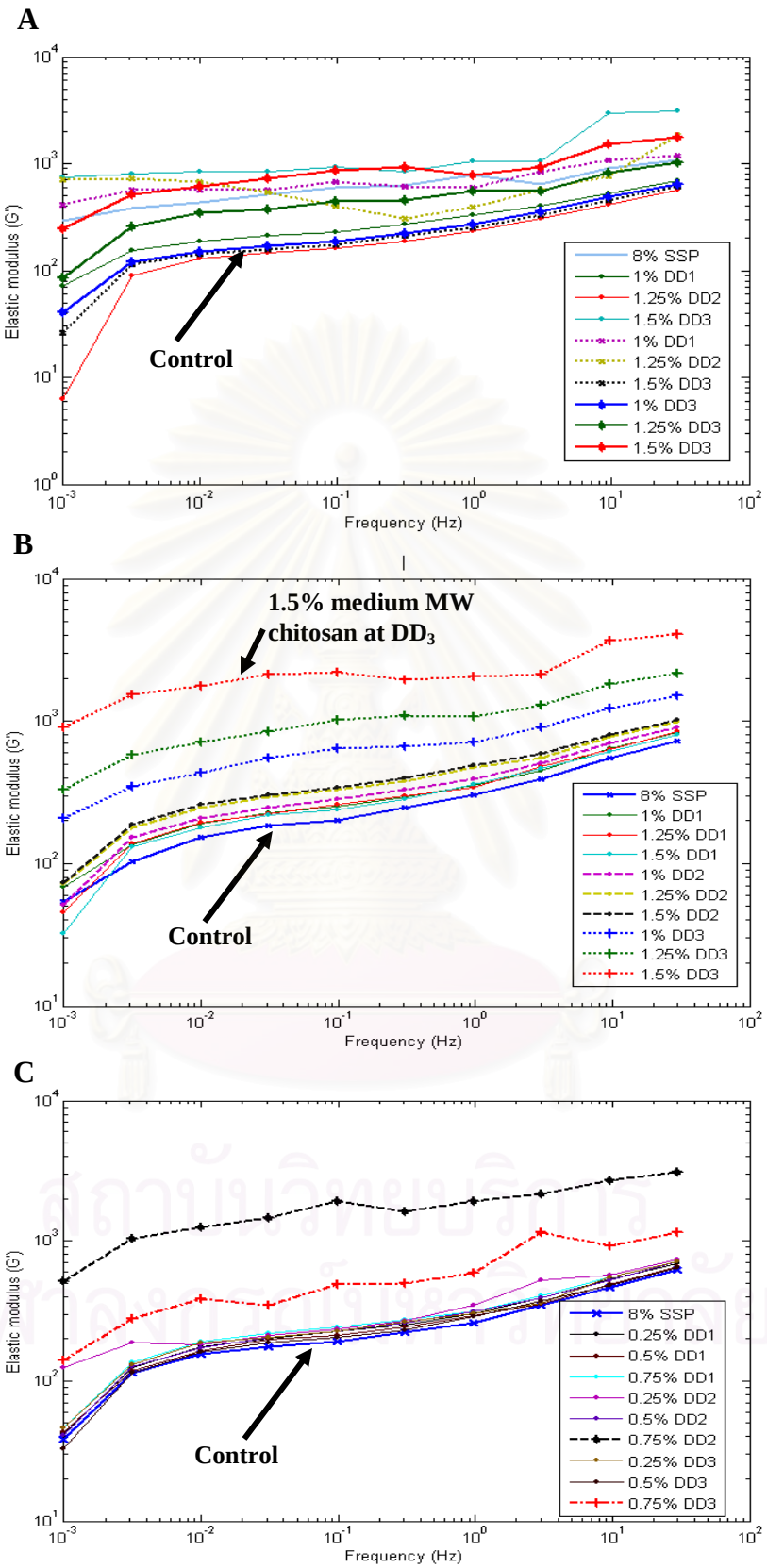


Figure 4.3 Elastic modulus or G' (Pa) of cold-set gels from 8%w/v SSP and various treatments of alkali-precipitated chitosan. A, B and C represent G' of low, medium and high MW alkali-precipitated chitosan. Control is 8% SSP only. DD1, 2 and 3 represent degree of deacetylation at 78, 88 and 94%, respectively.

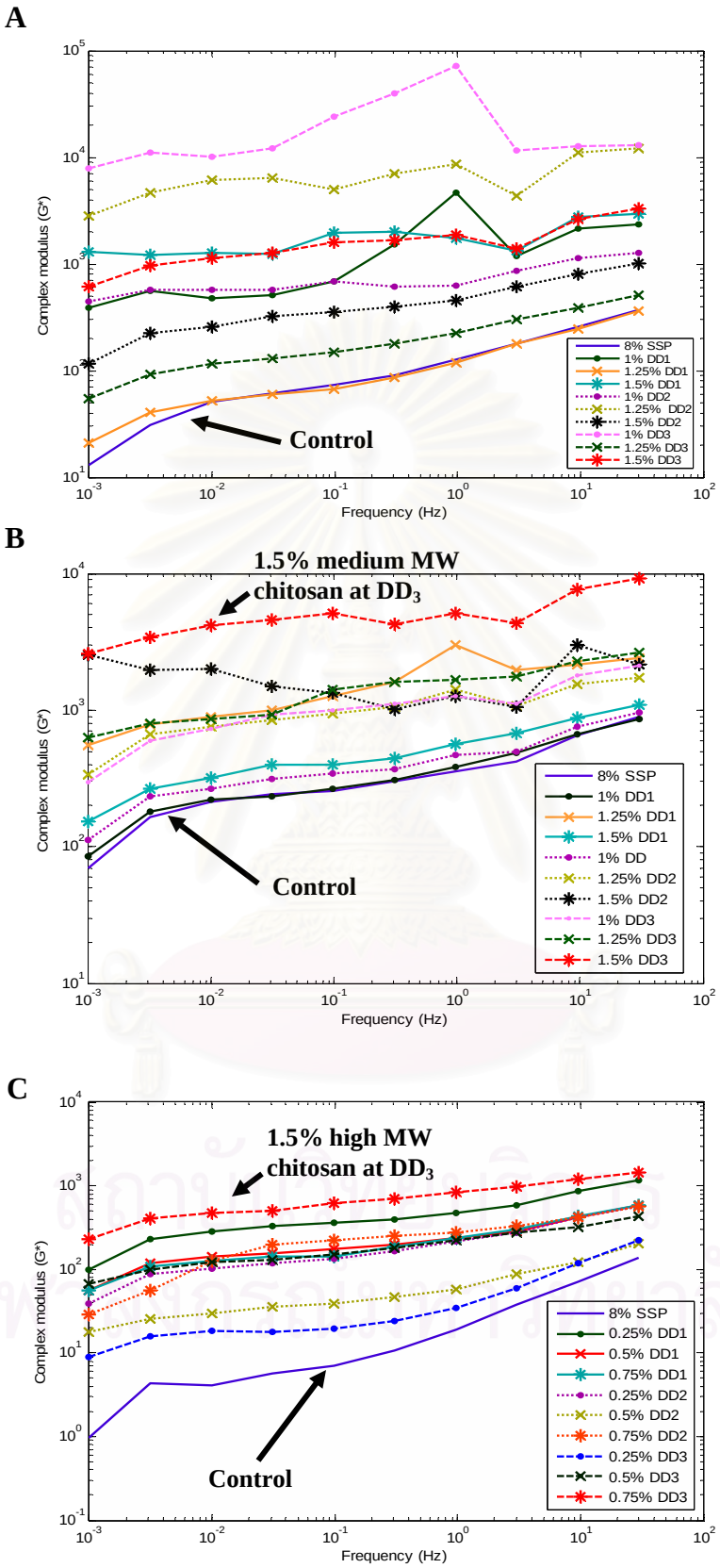


Figure 4.4 Complex modulus or G* (Pa) of cold-set gels from 8%w/v SSP and various treatments of alkali-precipitated chitosan. A, B and C represent G* of low, medium and high MW alkali-precipitated chitosan. Control is 8% SSP only. DD1, 2 and 3 represent degree of deacetylation at 78, 88 and 94%, respectively.

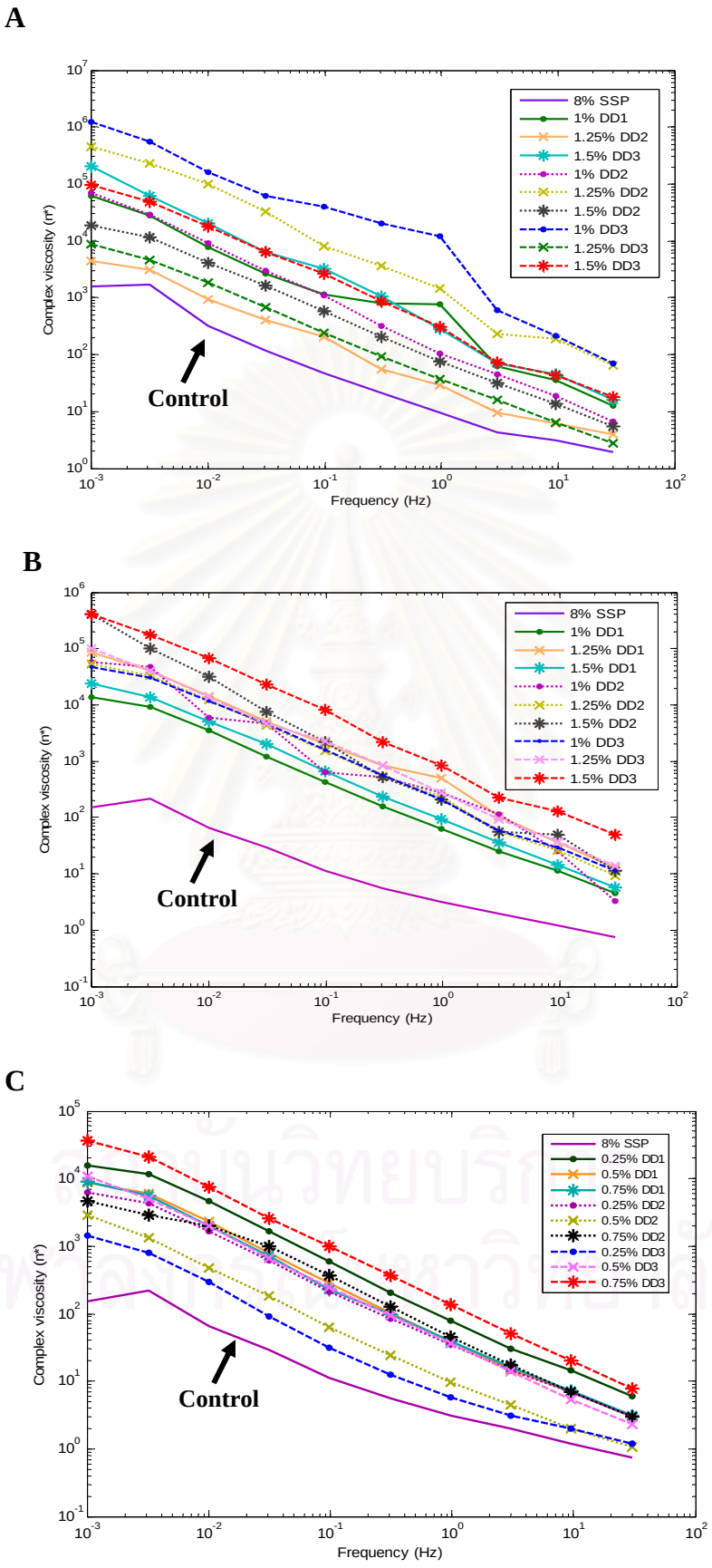


Figure 4.5 Complex viscosity or η* of cold-set gels from 8%w/v SSP and various treatments of alkali-precipitated chitosan. A, B and C represent η* of low, medium and high MW alkali-precipitated chitosan. Control is 8% SSP only. DD1, 2 and 3 represent degree of deacetylation at 78, 88 and 94%, respectively.

4.2.3 Effect on Textural Characteristic

The textural characteristic was studied using compression test in the texture analyzer. The maximum compression force of the developed cold-set gels increased when alkali-precipitated chitosan was added (figure 4.6). The DD, MW and concentration of chitosan significantly affected the texture characteristic of the cold-set gels ($p \leq 0.05$), while the MW displayed the major role. For treatments with low MW chitosan, the increase of concentration did not have any effect on the maximum compression force of the gel. The significant effect of concentration was observed in treatment with medium MW at high DD and high MW at low and medium DD. The cold-set gels with medium MW chitosan at high DD and high C exhibited the highest quality improvement. This result as well as the rheological characteristics indicated the optimal interaction of SSP with low-acetyl, medium-size chain chitosan. The improvement of the texture quality may occur because chitosan can act as both gel filler and gel binder in the continuous network of SSP (Benjakul *et al*., 2003). In the presence of chitosan, the conformational change of SSP may occur during mixing and after the low temperature gel setting, resulting in aggregation and formation of strong gel network. The use of chitosan as gel enhancer was also found in surimi by the study of Benjakul *et al*. in 2003. They reported the improvement of surimi texture when chitosan was added. Previous work from Samant *et al*., (1993) suggested that the presence of some incompatible polysaccharide in protein solution affected the thermodynamic of proteins, hence stimulate the aggregation of protein molecules, promoting gelation process and providing stronger gel network.

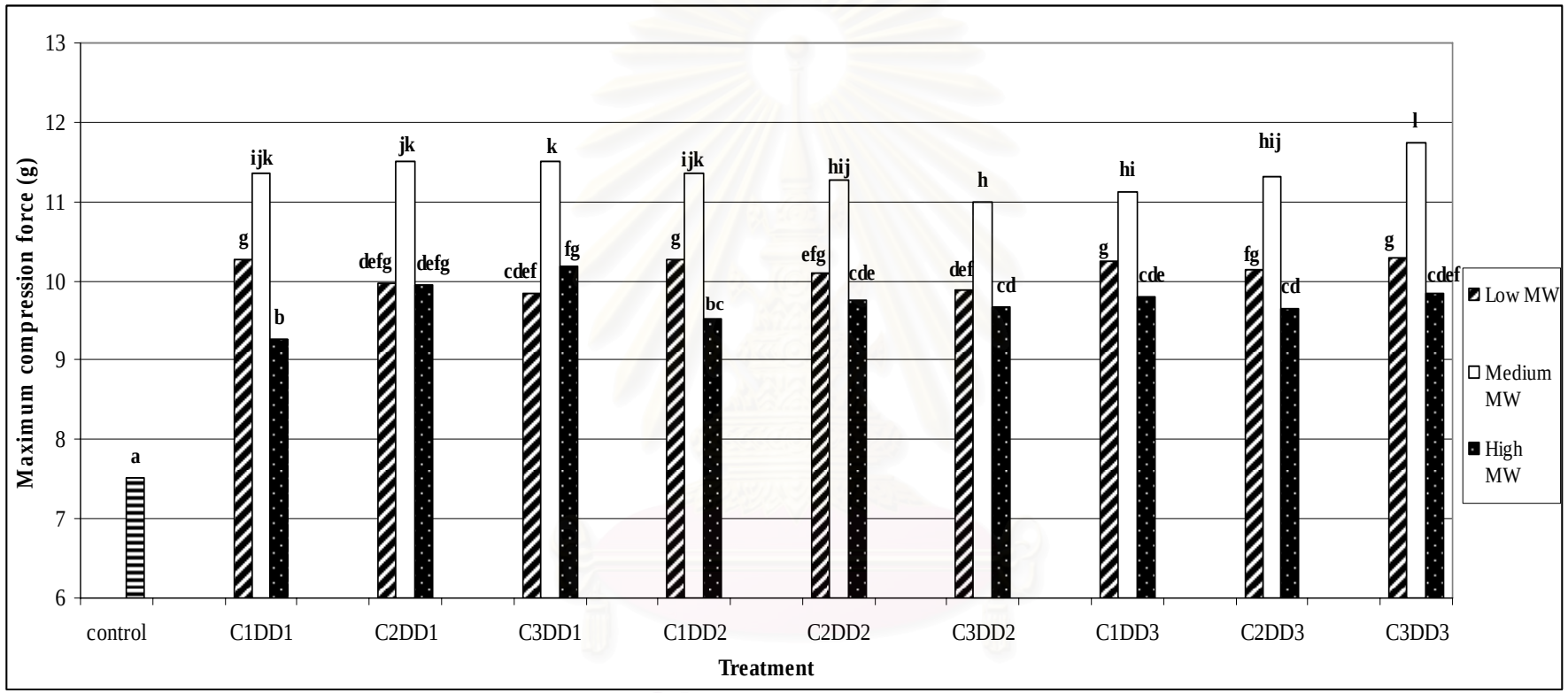


Figure 4.6 Maximum compression force (g) of the developed cold-set gels made from 8%w/v SSP and various treatments of alkali-precipitated chitosan. C1, 2 and 3 represent concentration of chitosan at 1, 1.25 and 1.5% for low and medium MW and 0.25, 0.5 and 0.75% for high MW chitosan. DD1, 2 and 3 represent degree of deacetylation at 78, 88 and 94%, respectively. Different letters a, b… l indicate significant difference ($p \leq 0.05$) among treatments.

4.2.4 Effect on Disulfide Content

The disulfide contents of various treatments compared with that of the control were shown in figure 4.7. The disulfide contents significantly increased in the presence of alkali-precipitated chitosan ($p \leq 0.05$). MW and DD of alkali-precipitated chitosan significantly altered the disulfide contents of the resulting cold-set gels. Regardless of DD, the increase of chitosan concentration did not influence the disulfide content of the obtained cold-set gels, except the treatment with medium MW at high DD. On the other hand, the disulfide content was significantly affected by DD of alkali-precipitated chitosan ($p \leq 0.05$). The significant increase was detected when DD was increased for all MW samples. For effect of MW, it was found that the cold-set gels with medium MW chitosans exhibited the highest disulfide content among the three MW groups. The highest improvement was found in treatment with medium MW, high DD and high C. This result was in accord with rheological and textural characteristics which suggested the strongest gel network from the proper interaction of SSP with medium size-low acetyl group chitosan. Furthermore, the detectable increase of the disulfide content indicated the formation of cold-set gels which partially stabilized by disulfide bonds. The increase of the disulfide bonds in the developed cold-set gels may occur due to the rearrangement of protein molecules by the induction of chitosan during mixing at low temperature. This might lead to the formation of new disulfide bonds (Wong, 1989). In addition, this result was agreed with the report of Chen *et al*. (2003). They explained that the changes of molecular weight and size occurred from the disulfide stabilized SSP and chitosan molecules which were detected by the stack of high MW biopolymers in the top of wells in SDS-PAGE.

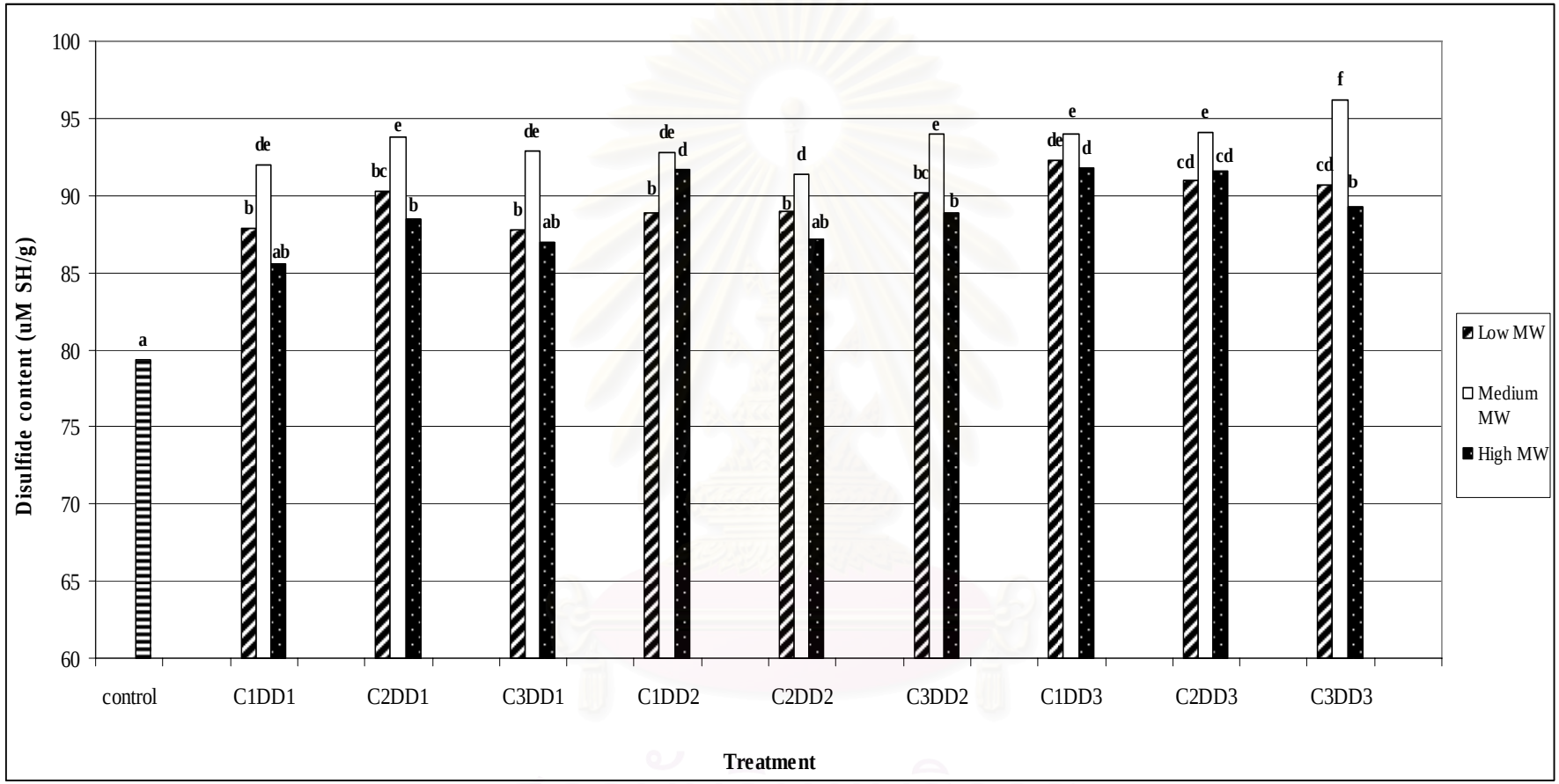
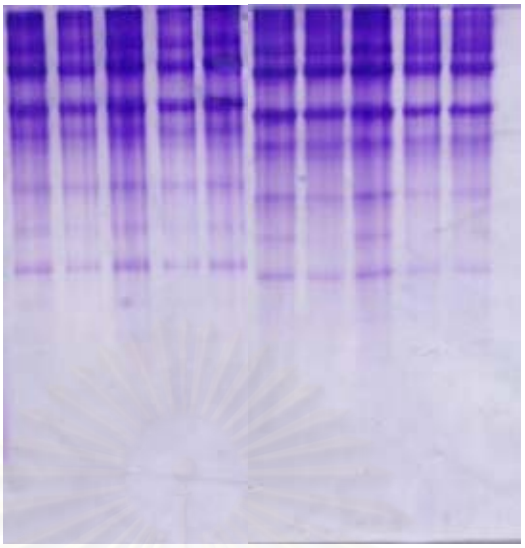


Figure 4.7 Disulfide content (μM SH/g) of the developed cold-set gels made from 8%w/v SSP and various treatments of alkali-precipitated chitosan C1, 2 and 3 represent concentration of chitosan at 1, 1.25 and 1.5% for low and medium MW and 0.25, 0.5 and 0.75% for high MW chitosan. DD1, 2 and 3 represent degree of deacetylation at 78, 88 and 94%, respectively. Different letters a, b… f indicate significant difference ($p \leq 0.05$) among treatments.

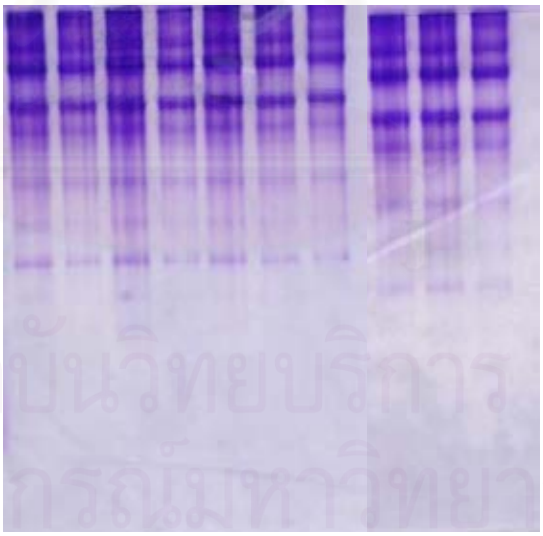
4.2.5 Effect on Non-Denaturing Gel-Electrophoresis

Differences between non-denaturing gel-electrophoretic patterns of all treatments and that of the control sample (lane 1) were not detected (figure 4.8, 4.9 and 4.10). This result indicated that only minor portion of the protein gel was stabilized by disulfide bonds. Thus, most of the chemical interactions that stabilized the developed cold-set gels should be composed of electrostatic interactions, hydrophobic interactions and salt-bridges (Edwards, Lillford and Blanshard, 1987; Wong, 1989). These weak bonds could readily be destroyed when adequate level of force was applied to the gel. The experimental results suggested that shaking or stirring of the cold-set gels resulted in weakening the gel networks. However, when such gels were reset at low temperature, their strengths were restored. In the sample preparation step of this experiment, the gels were scooped and shook vigorously with sample buffer, and this might lead to the breaking of the majority weak bonds between SSP and chitosan. Thus, the distinct difference among the non-denaturing gel-electrophoretic patterns of the chitosan containing samples and the control could not be detected by the non-denaturing gel-electrophoretic technique.



1 2 3 4 5 6 7 8 9 10

Figure 4.8 Non-denaturing gel-electrophoretic patterns (10% resolving gel) of cold-set gels of 8%w/v SSP and high MW alkali-precipitated chitosan: Lane 1 = 8%w/v SSP, Lane 2-10 = 8%w/v SSP with 0.25, 0.5 and 0.75% high MW chitosan at DD_1, DD_2 and DD_3, respectively. DD_1, DD_2 and DD_3 represent degree of deacetylation at 78, 88 and 94%.



1 2 3 4 5 6 7 8 9 10

Figure 4.9 Non-denaturing gel-electrophoretic patterns (10% resolving gel) of cold-set gels of 8%w/v SSP and medium MW alkali-precipitated chitosan: Lane 1 = 8%w/v SSP, Lane 2-10 = 8%w/v SSP with 1, 1.25 and 1.5% medium MW chitosan at DD_1, DD_2 and DD_3, respectively. DD_1, DD_2 and DD_3 represent degree of deacetylation at 78, 88 and 94%.

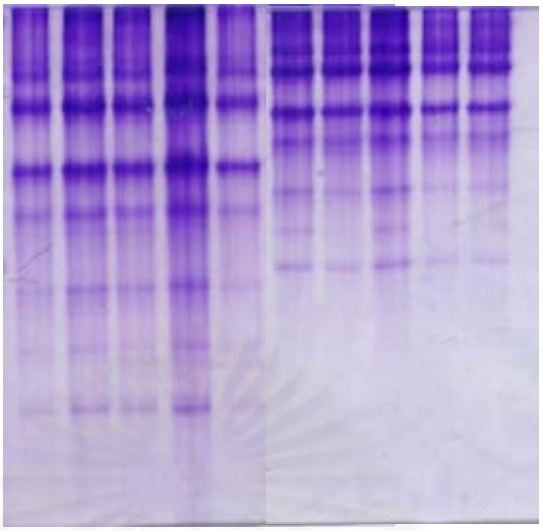


Figure 4.10 Non-denaturing gel-electrophoretic patterns (10% resolving gel) of cold-set gels of 8%w/v SSP and low MW alkali-precipitated chitosan: Lane 1 = 8%w/v SSP, Lane 2-10 = 8%w/v SSP with 1, 1.25 and 1.5% low MW chitosan at DD_1, DD_2 and DD_3, respectively. DD_1, DD_2 and DD_3 represent degree of deacetylation at 78, 88 and 94%.

4.2.6 Effect on Structure Profiles

Since the non-denaturing gel-electrophoretic patterns did not provide any clear information on development of macromolecules, scanning electron microscopy was performed to confirm and elucidate the interaction between alkali-precipitated chitosan and SSP. The scanning electron micrographs (figure 4.11) clearly showed that the addition of chitosan drastically improved and modified microstructures of the developed cold-set gels. Denser and more uniform structures were obtained, especially in treatment with medium and low MW chitosan. The scanning electron micrographs with low magnifications (figure 4.11) and high magnifications (figure 4.12 and 4.13) demonstrated the uniform structures of the gel matrix. These results indicated that alkali-precipitated chitosan molecules could interact and modulate the structure of SSP in the presence of buffer solution containing salt, phosphate and water. The possible interactions, which stabilize the cold-set gels, were hydrophobic interactions, electrostatic interactions including salt bridge and dipole-dipole interaction, hydrogen bonding, and some disulfide bonds (Wong, 1989). These bonds might occur between chitosan-chitosan, chitosan-protein, and protein-protein molecules. These results indicated that better gel structure can be enhanced by the addition of alkali-precipitated chitosan with proper MW, DD and concentration. The synergistic effect of chitosan on structural modulation also reported in surimi and horse mackerel fish muscle (Benjakul *et al*., 2003; Gómez-Guillén *et al*., 2005). They explained that chitosan acted as catalyst in the reaction of endogenous transglutaminase (TGase) in fish muscle which resulted in accelerating of TGase reaction. The faster reaction was obtained and provided the more uniform and denser gel network.

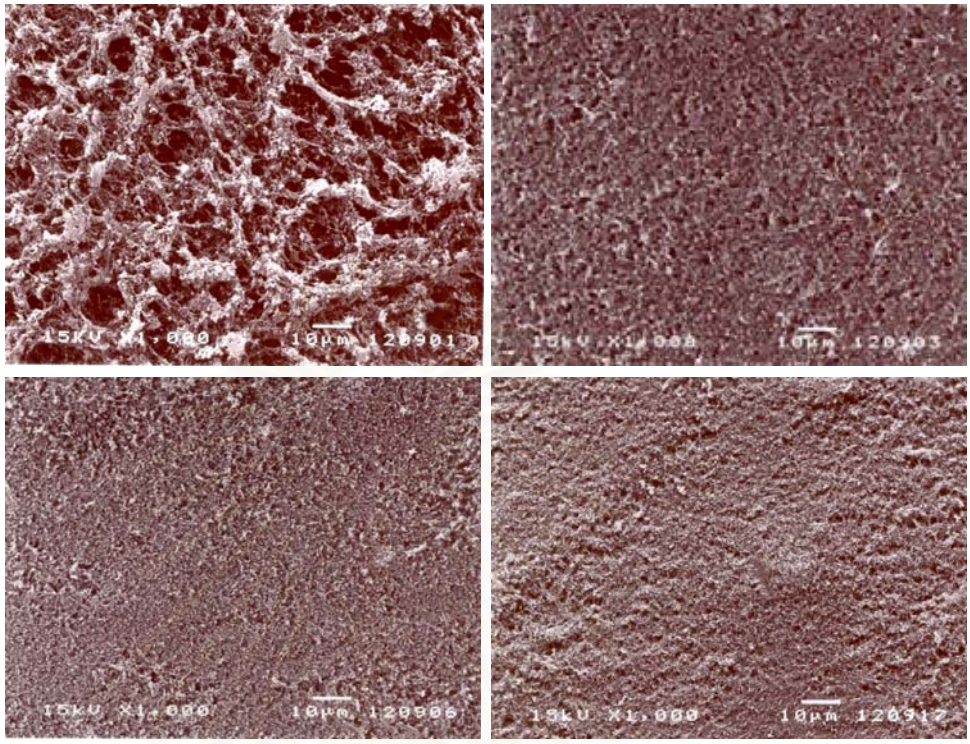


Figure 4.11 Scanning electron micrographs (1000 x magnifications) of cold-set gels made from 8%w/v SSP and various treatments of alkali-precipitated chitosan: **A** = control or 8%w/v SSP only; **B** = 8%w/v SSP with 0.5% high MW alkali-precipitated chitosan; **C** = 8%w/v SSP with 1.5% medium MW alkali-precipitated chitosan; **D** = 8%w/v SSP with 1.25% low MW alkali-precipitated chitosan

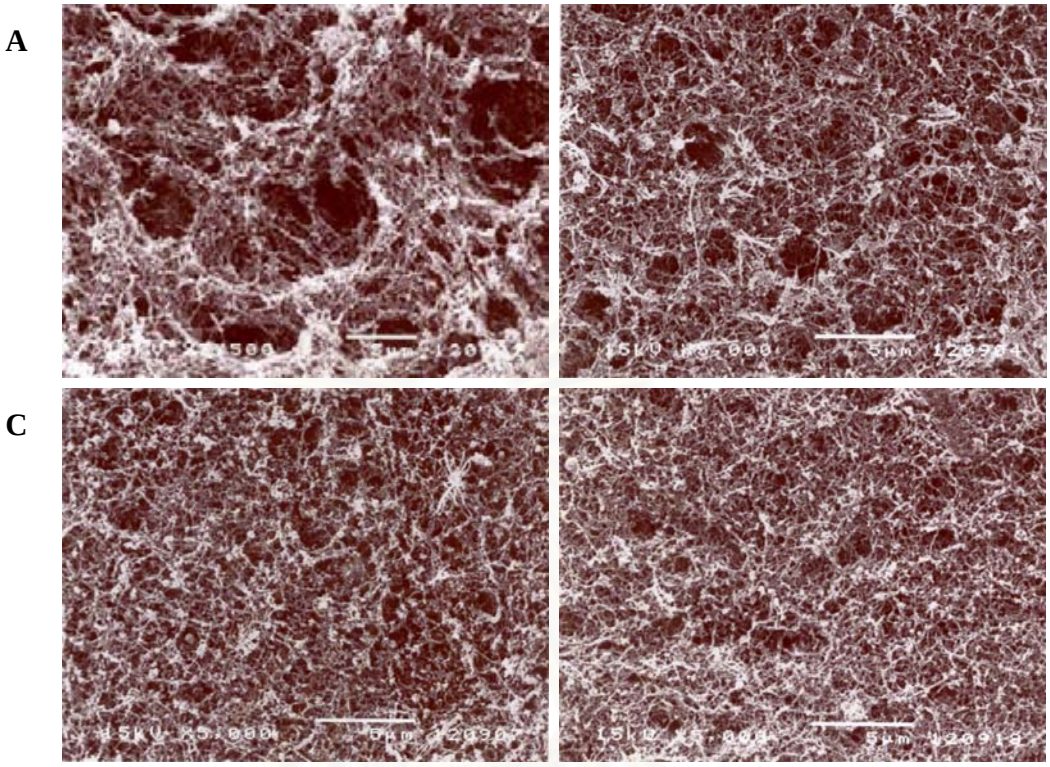


Figure 4.12 Scanning electron micrographs (5000 x magnifications) of cold-set gels made from 8%w/v SSP and various treatments of alkali-precipitated chitosan: **A** = control or 8%w/v SSP only; **B** = 8%w/v SSP with 0.5% high MW alkali-precipitated chitosan; **C** = 8%w/v SSP with 1.5% medium MW alkali-precipitated chitosan; **D** = 8%w/v SSP with 1.25% low MW alkali-precipitated chitosan

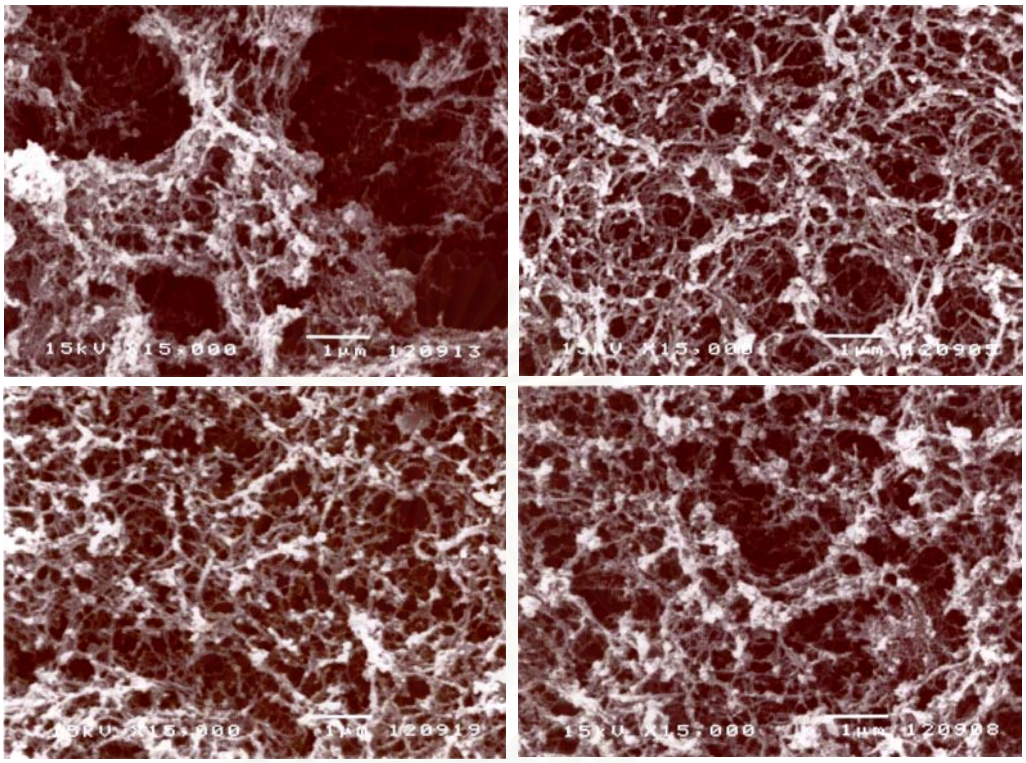


Figure 4.13 Scanning electron micrographs (15,000 x magnifications) of cold-set gels made from 8%w/v SSP and various treatments of alkali-precipitated chitosan: **A** = control or 8%w/v SSP only; **B** = 8%w/v SSP with 0.5% high MW alkali-precipitated chitosan; **C** = 8%w/v SSP with 1.5% medium MW alkali-precipitated chitosan; **D** = 8%w/v SSP with 1.25% low MW alkali-precipitated chitosan

4.2.7 Concluding Remarks

The addition of alkali-precipitated chitosan remarkably improved cold-set gelation ability of SSP. The significant increase of maximum compression force and disulfide content, and the improvement of rheological characteristics and structure profile of the developed cold-set gels were obtained. However, the non-denaturing gel-electrophoretic patterns of all treatments remained unchanged. The color of the developed cold-set gels with alkali-precipitated chitosan was paler than that of the control. DD, MW and C of alkali-precipitated chitosan had the great impact on these physico-chemical properties. The developed cold-set gels with 1.5% by weight alkali-precipitated chitosan at 94% DD and MW of 1.84×10^5 Daltons, exhibited the highest improvement of all parameters determined.

4.3 Effect of Chicken Protein Hydrolysates at Different DH and C on Cold-Set Gelation Abilities, Structures and Physico-Chemical Properties of SSP Gels

In this experiment, the preliminary study was performed to eliminate some treatment combinations which were not significant. The chicken protein hydrolysates with proper DH were selected to be used as the cold-set binder in combination with other parameter. The preliminary experiment was performed by mixing the obtained samples of chicken protein hydrolysates at concentration of 1% by weight with 8%w/v SSP, then setting at 4˚C. The physico-chemical properties of the cold-set gels including texture, disulfide content and color were determined. As shown in table 4.6, it was found that treatments 2 to 5 exhibited better improvement of the maximum compression force and disulfide content when compared with other treatments and control. This result indicated the occurrence of the stronger gel network in cold-set gels with chicken protein hydrolysates at 6.42, 15.12, 22.38, and 35.80 % DH. Thus, these four samples were selected to be used in the consecutive experiments as one of the parameter studied in combination with another which was concentration at 1, 2 and 3%. The physico-chemical properties of the resulting cold-set gels including pH, color, rheological characteristics, texture, disulfide content, non-denaturing gel-electrophoretic pattern, and structure profile, were studied.

4.3.1 Effect on pH

The reduction of pH of the chicken protein hydrolysates occurred when comparing with that of the native protein. This phenomenon arose from the revelation of short chain peptides containing carboxyl group (-COOH). However, the pH of the obtained cold-set gels were not affected by the hydrolysate pH. As shown in table

4.7, the differences among pH of the cold-set gels could not be observed ($p>0.05$). Therefore, should there be any changes occurred among the gel's other physico-chemical properties, that would not be arisen from the effect of the pH.

Table 4.6 Properties of the developed cold-set gels made from 8%w/v SSP only (control) and 8%w/v SSP with 1% chicken protein hydrolysates at DH 0.03-60.13%

Trt.	Protein hydrolysates		Max. force (g)	Disulfide content (mM SH/g)	Color		
	Incubation time (min.)	DH (%)			L*	a*	b*
Control	-	-	6.733^{a}	53.817^{a}	49.710^{a}	-5.713^{cd}	-1.947^{a}
1	0	0.03	7.733^{cde}	55.785^{abc}	52.633^{h}	-5.637^{de}	0.430^{h}
2	0.5	**6.42**	**7.867^{de}**	**59.899^{de}**	53.087^{j}	-5.347^{e}	0.173^{g}
3	5	**15.12**	**8.233^{e}**	**61.588^{e}**	52.720^{i}	-5.680^{cde}	0.970^{i}
4	15	**22.38**	**7.900^{de}**	**59.518^{de}**	51.540^{g}	-5.800^{bc}	-0.127^{f}
5	30	**35.80**	**7.800^{cde}**	**59.896^{de}**	51.107^{f}	-5.630^{de}	-0.353^{cd}
6	45	36.80	7.500^{bcd}	57.259^{bcd}	50.583^{c}	-5.910^{b}	-0.527^{b}
7	60	37.74	7.100^{ab}	57.965^{cd}	50.860^{e}	-5.617^{de}	-0.097^{f}
8	75	39.53	7.200^{abc}	54.936^{abc}	50.663^{d}	-5.747^{cd}	-0.233^{e}
9	90	51.22	7.433^{bcd}	54.377^{ab}	51.493^{g}	-5.550^{d}	-0.317^{de}
10	120	60.13	7.367^{bcd}	55.327^{abc}	50.080^{b}	-6.107^{a}	-0.440^{bc}

$^{a-j}$ Means followed by different letters within the same column are significantly different ($p\leq0.05$).

Table 4.7 Treatment combinations and pH of the developed cold-set gels made from 8% w/v SSP and 1, 2 and 3% chicken protein hydrolysates at DH 6.42-35.80%

Treatment No.	Treatment combination	pH[ns]
Control (C)	8%w/v SSP	6.59 ± 0.11
1	8% SSP + 1% chicken protein hydrolysates at 6.42% DH	6.58 ± 0.05
2	8% SSP + 2% chicken protein hydrolysates at 6.42% DH	6.55 ± 0.08
3	8% SSP + 3% chicken protein hydrolysates at 6.42% DH	6.55 ± 0.16
4	8% SSP + 1% chicken protein hydrolysates at 15.12% DH	6.57 ± 0.14
5	8% SSP + 2% chicken protein hydrolysates at 15.12% DH	6.55 ± 0.12
6	8% SSP + 3% chicken protein hydrolysates at 15.12% DH	6.54 ± 0.17
7	8% SSP + 1% chicken protein hydrolysates at 22.38% DH	6.57 ± 0.16
8	8% SSP + 2% chicken protein hydrolysates at 22.38% DH	6.57 ± 0.09
9	8% SSP + 3% chicken protein hydrolysates at 22.38% DH	6.56 ± 0.15
10	8% SSP + 1% chicken protein hydrolysates at 35.80% DH	6.56 ± 0.18
11	8% SSP + 2% chicken protein hydrolysates at 35.80% DH	6.55 ± 0.06
12	8% SSP + 3% chicken protein hydrolysates at 35.80% DH	6.54 ± 0.08

[ns] Treatments were not significantly different (P>0.05).

4.3.2 Effect on Color

Color changes of the resulting cold-set gels as shown in figure 4.14 were expressed in term of the color difference value (ΔE^*_{ab}) which indicated color changes of the treated samples from control. L*, a* and b* values of each sample were also shown in order to clarify the cause of the changes of ΔE^*_{ab}. It was observed that both DH and C of chicken protein hydrolysates significantly affected color of the resulting cold-set gels ($p \leq 0.05$). The raise of ΔE^*_{ab} was found when increasing of concentration from 1 to 3% by weight while the increase of DH resulted in lowering ΔE^*_{ab} as shown in figure 4.14. ΔE^*_{ab} of treatments with hydrolysate at 35.80% DH had lower ΔE^*_{ab} than that of the treatment with 6.42% DH. The detectable changes of ΔE^*_{ab} were from the increase of L* after the addition of chicken protein hydrolysates (figure 4.15) while b* and a* increased only slightly. These results indicated the paleness of gels with chicken protein hydrolysates and might be due to the light yellow color of the hydrolysates itself. The color changes of chicken protein hydrolysates after hydrolysis with papain occurred from the heat treatment at enzyme inactivation step. The light yellow denatured chicken protein solution was obtained. McWilliams (1999) explained that heating enhanced the light color of fish and poultry by increasing opacity and denaturing the proteins which caused the pigment loss and denaturation of heme pigments. After freeze drying, the color of the obtained products was not change. Thus the light yellow chicken protein hydrolysate was obtained. This well-soluble chicken protein hydrolysate was dissolved readily after mixing with the SSP gels and dispersed into the gel matrix homogeneously. Thus, the changes of color of the resulting cold-set gels were clearly observed. This

occurrence was agreed with the report of Barbut (2006) which found the slightly increasing of L* and b* in poultry meat batters adding with whey protein hydrolysates.

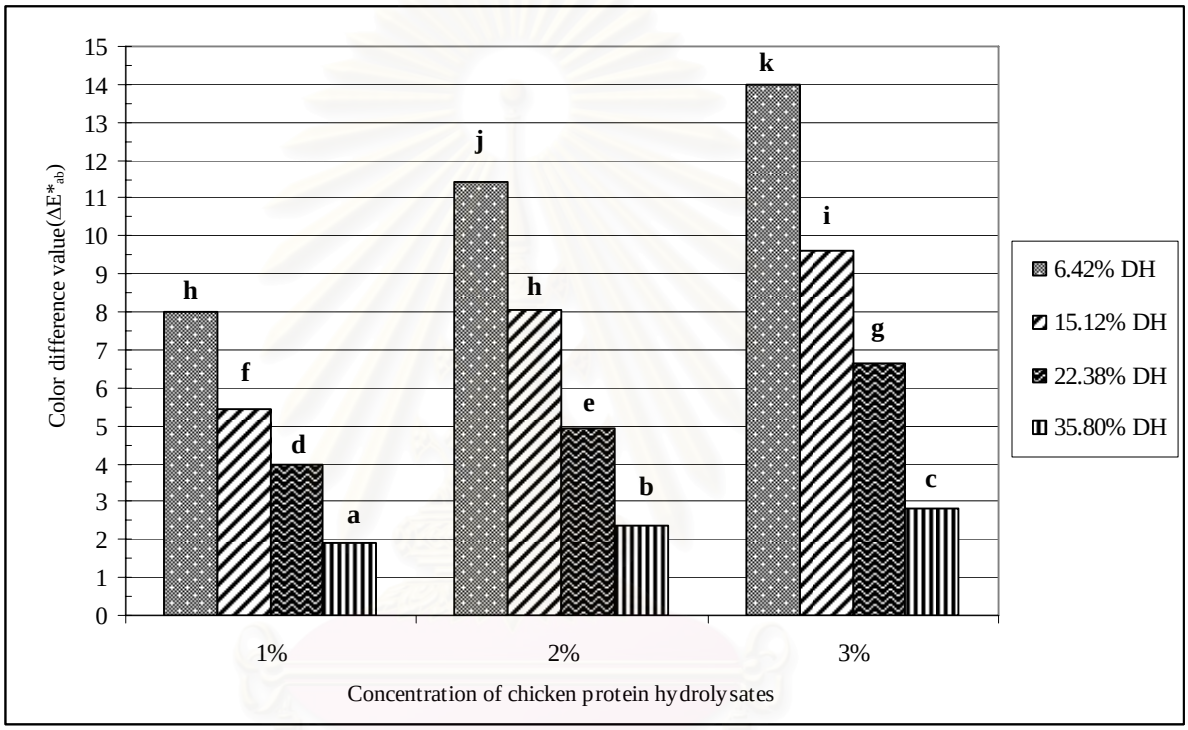


Figure 4.14 Color difference value (ΔE^*_{ab}) of the developed cold-set gels made from 8%w/v SSP and 1, 2, and 3% chicken protein hydrolysates at DH 6.42-35.80 %. Different letters a, b…k indicate significant difference ($p \leq 0.05$) among treatments.

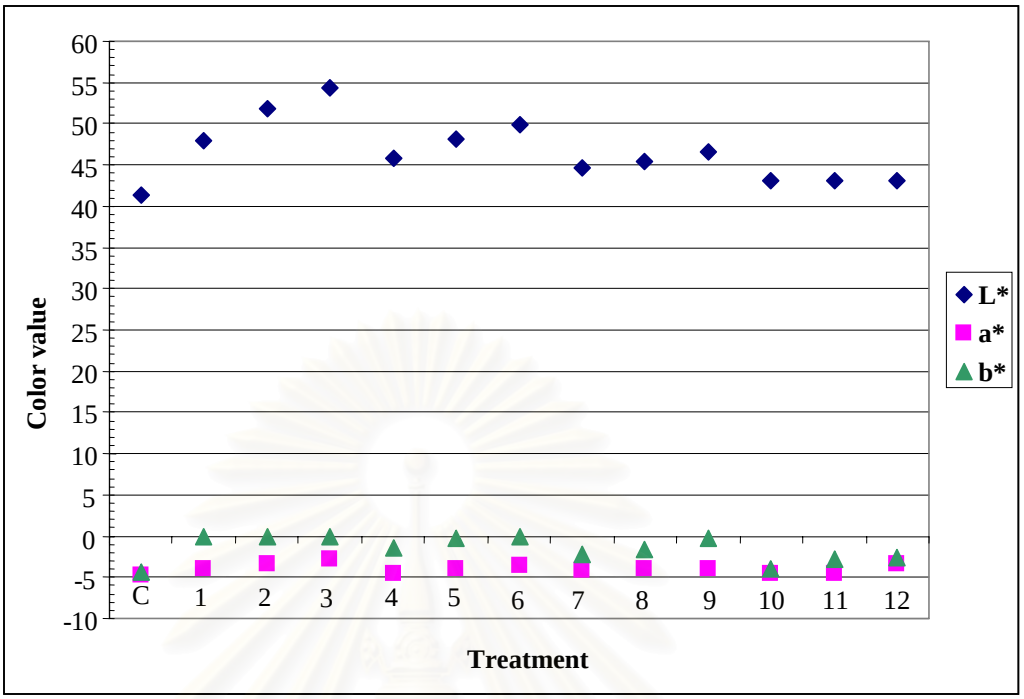


Figure 4.15 Color (L* a* b*) of the developed cold-set gels made from 8%w/v SSP and 1, 2, and 3% chicken protein hydrolysates at DH 6.42-35.80 %.

4.3.3 Effect on Rheological Characteristics

The rheological characteristics of the developed cold-set gels with chicken protein hydrolysates compared with that of the control were investigated and expressed in term of elastic modulus (G'), complex modulus (G^*) and complex viscosity (η^*). The G', patterns which shifted up from control were found only in treatments with chicken protein hydrolysate at 22.38% DH (figure 4.16). This result indicated the suitable chain length of chicken protein hydrolysate which can interact properly with SSP.

The G^* along the frequency range (0.001-30 Hz) of all treatments as demonstrated in figure 4.17 were shifted up from that of the control. At high frequency range (1-30 Hz), the G^* of all treatments including control gradually rose up indicating the deformation of gel network when high radiance frequency was applied. Under this sinusoidal shear, the measurement of the energy recovered per cycle of sinusoidal shear deformation (storage modulus, G') and the estimation of energy dissipated as heat per cycle (loss modulus, G'') were carried out (Tung and Poulson, 1995; Vliet, 1999). The increase of G' and G'' which occurred when this sinusoidal shear increased suggested the deformation of the gel network (Tung and Poulson, 1995). The highest shifted pattern was observed in treatment with 3% chicken protein hydrolysate at 22.38% DH. This result was in accord with the G' pattern and suggested the better interaction of SSP with this chicken protein hydrolysate sample.

The result of η^* (figure 4.18) also showed the similar propensity to G^* patterns. The η^* patterns of all treatments with chicken protein hydrolysate shifted up when comparing with that of the control. The highest shifted pattern was detected in

treatment similar to that of the G*. Moreover, the pattern of η* of treatment with chicken protein hydrolysate exhibited a linear line and continuously decrease along the frequency range. This phenomenon was normally occurred in the gels of biopolymer such as proteins or polysaccharides (Morris, 1995). This result suggested the formation of stronger gel network of SSP from the addition of chicken protein hydrolysates. The improvement of these rheological parameters might occur due to the more entanglement of SSP chains and chicken protein hydrolysates. Various binding mechanisms were reported to occur between these two proteins including van der Waals force, electrostatic interactions, hydrogen bonds and also disulfide bonds. Electrostatic interactions mostly occurred and stabilized the protein gels since proteins contained both positively and negatively charged side chains (Dalgleish and Hunt, 1995).

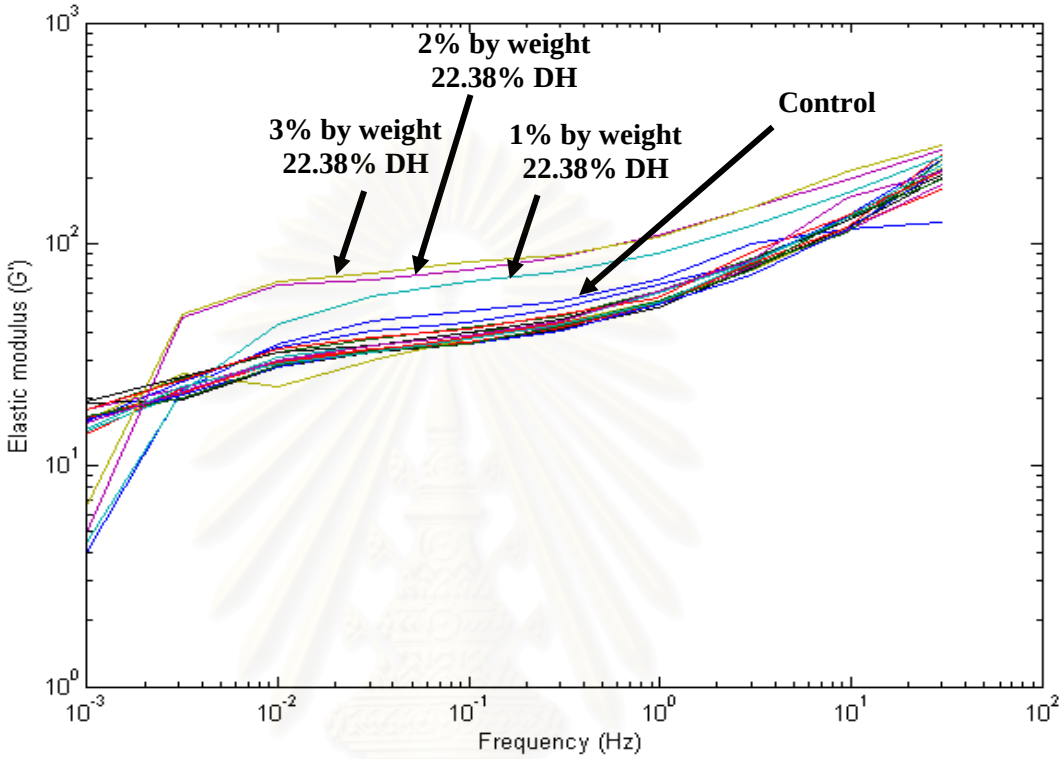


Figure 4.16 Elastic modulus or G′ (Pa) of the developed cold-set gels made from 8%w/v SSP and 1, 2, and 3% chicken protein hydrolysates at DH 6.42-35.80 %. Control is 8%w/v SSP only.

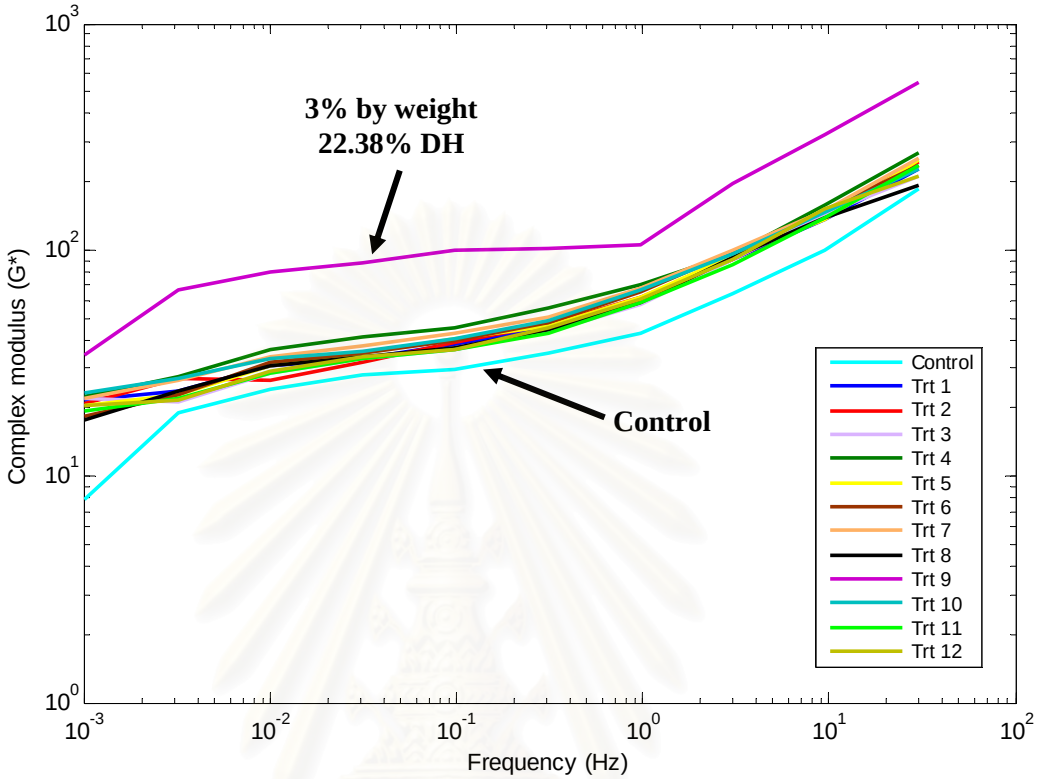


Figure 4.17 Complex modulus or G* (Pa) of the developed cold-set gels made from 8%w/v SSP and 1, 2, and 3% chicken protein hydrolysates at DH 6.42-35.80 %. Control is 8%w/v SSP only.

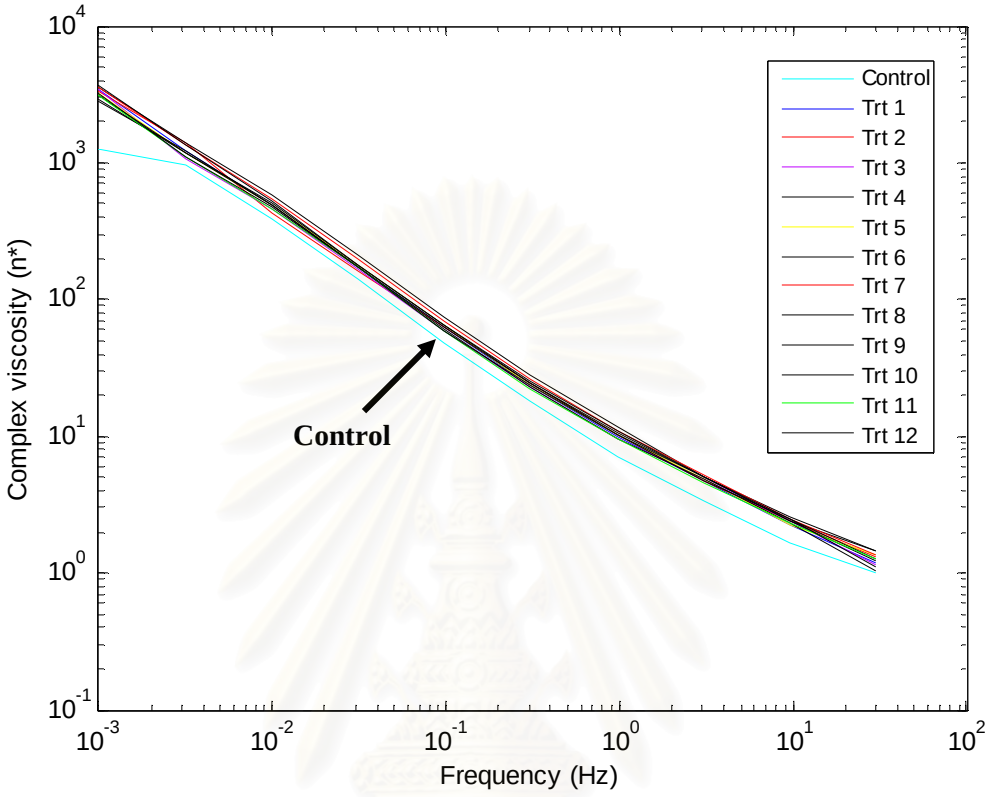


Figure 4.18 Complex viscosity or η* of the developed cold-set gels made from 8%w/v SSP and 1, 2, and 3% chicken protein hydrolysates at DH 6.42-35.80 %. Control is 8%w/v SSP only.

4.3.4 Effect on Textural Characteristic

The textural characteristics of all treatments were expressed in term of maximum compression force (g). As shown in figure 4.19, the maximum compression forces significantly increased when chicken protein hydrolysates was added ($p \leq 0.05$). Both DH and C of chicken protein hydrolysates significantly affected the textural characteristic ($p \leq 0.05$). The heightening of concentration resulted in significant increase of maximum compression force in treatment with chicken protein hydrolysates at 6.42, 15.12 and 22.38% DH ($p \leq 0.05$) while the effect of DH was also observed. The gel network of the developed cold-set gels with chicken protein hydrolysates at 15.12 and 22.38% DH were found to be stronger than treatments with 6.42 and 35.80% DH as revealed by the remarkable improvement of maximum compression forces. The highest improvement detected in treatment with 3% chicken protein hydrolysates at 15.12 and 22.38% DH indicated proper interaction of SSP with chicken protein hydrolysate chains at relatively low DH. This result was in accord with the review of Panyam and Kilara (1996). They cited that the enhancement of gelation ability of food proteins was received after limited hydrolysis with enzyme. In addition, the textural improvement of SSP from the addition of chicken protein hydrolysates may result from the gel filling ability of the hydrolysates in the continuous network of SSP. The enzymatic hydrolysis with papain revealed drastic change in myosin molecules and resulted in the breaking of myosin head from myosin tail and the increase of charged groups on myosin chains was obtained (Samant *et al*., 1993). Moreover, the charged groups of myosin tails were exposed in approximately every third amino acid from the liberation of helical myosin tails (Ockerman, 1977). The helical portions of myosin were unraveled into randomly

organized chains and produced random cross-links via electrostatic interaction, hydrogen and ionic bonds (Pomeranz, 1985). Therefore, the uniform gel network was obtained from the addition of protein hydrolysates (Satterlee, Zachariah and Levin, 1973).

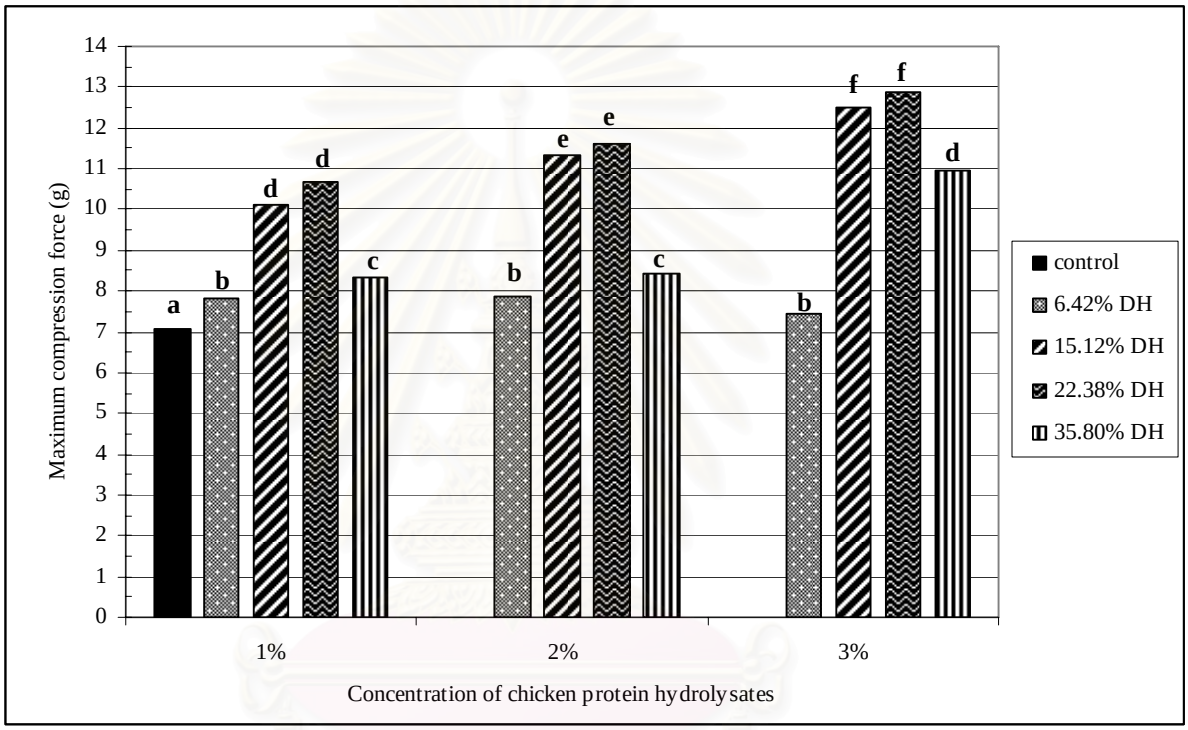


Figure 4.19 Maximum compression force (g) of the developed cold-set gels made from 8%w/v SSP and 1, 2, and 3% chicken protein hydrolysates at DH 6.42-35.80 %. Different letters a, b…f indicate significant difference ($p \leq 0.05$) among treatments.

4.3.5 Effect on Disulfide Content

An almost similar result with that of the textural characteristic was observed in the disulfide content (figure 4.20). The amount of disulfide bond significantly increased in the presence of chicken protein hydrolysates ($p \leq 0.05$). This phenomenon might occur from the enhancement of disulfide bond formation from the addition of chicken protein hydrolysates. During mixing with SSP at low temperature, the attractive van der Waals forces arose and brought the protein molecules together. The protein molecules approached closely to each other and the specific interactions such as disulfide bonds developed (Dalgleigh and Hunt, 1995). Moreover, the increase of disulfide content after the addition of chicken protein hydrolysates might cause by their own expressed disulfide and thiol groups which increased after enzymatic hydrolysis (Panyam and Kilara, 1996). Figure 4.20 revealed that DH and C of chicken protein hydrolysates did not affect the disulfide content ($p > 0.05$). Among treatments with chicken protein hydrolysates, the highest disulfide content was found in that with 3% chicken protein hydrolysate at 22.38% DH. This result was in accord with the textural characteristic and suggested the proper interaction of SSP with these chicken protein hydrolysate chains. The increase of disulfide content indicated the formation of strong cold-set gel network which partially stabilized by disulfide bonds. During mixing and gel setting period, the formation of new-stabilized disulfide bonds could have occurred between SSP chains and the exposed thiol (SH-) groups of myosin globular heads and resulted in the increase of the disulfide contents (Wong, 1989; Zayas, 1997).

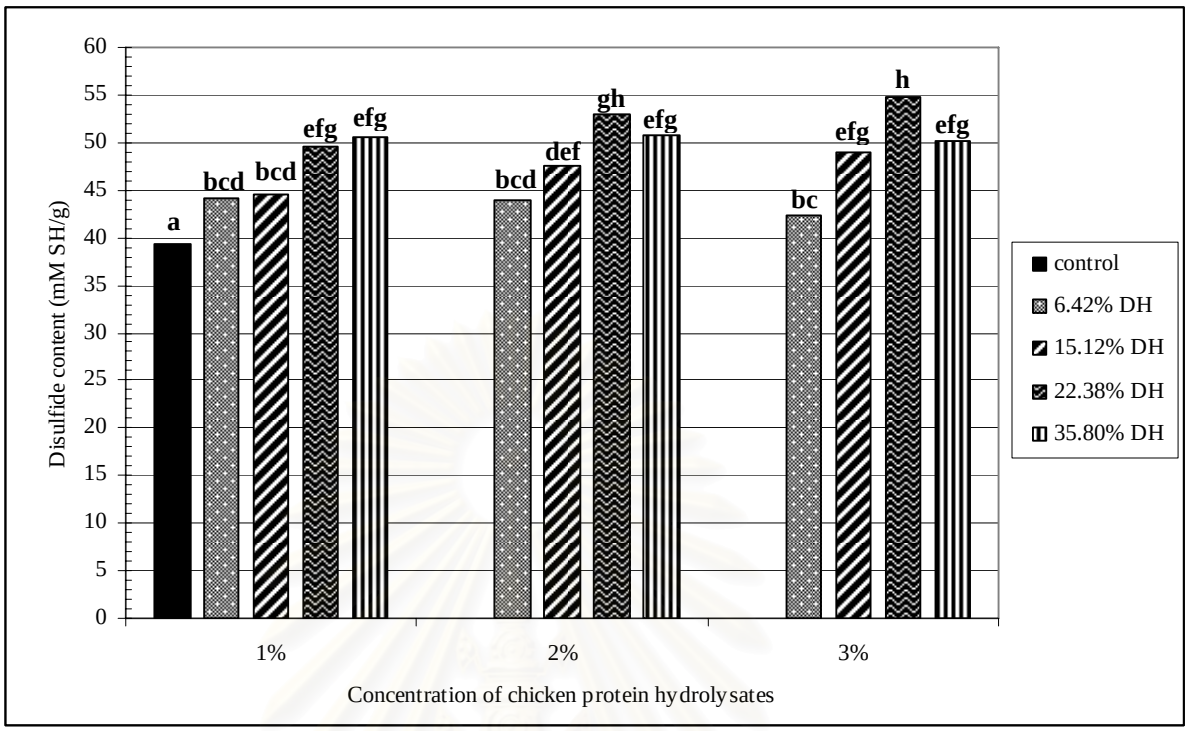


Figure 4.20 Disulfide content (mM SH/g) of the developed cold-set gels made from 8%w/v SSP and 1, 2, and 3% chicken protein hydrolysates at DH 6.42-35.80 %. Different letters a, b…h indicate significant difference ($p \leq 0.05$) among treatments.

4.3.6 Effect on Non-Denaturing Gel-Electrophoresis

The non-denaturing gel-electrophoretic patterns of all treatments compared with that of control were shown in figure 4.21. The intact large protein molecules were observed in all treatments with chicken protein hydrolysates as shown by the intense bands on the top of lane 2-13. This result indicated the increase concentration of high MW protein macromolecules that might occur from various interactions, such as electrostatic interactions, hydrophobic interactions and some disulfide bonds, between SSP chains and the added chicken protein hydrolysates (Wong, 1989). With papain hydrolysis, the modification of myosin structure could have been occurred and the enhancement of the free tail portions from the myosin heads resulted. The formation of stronger gel network was then obtained as a result of non-covalent and some covalent interactions between the extracted SSP and the added chicken protein hydrolysates enriched with myosin fractions (Macfarlane, Schmidt and Turner, 1977). Furthermore, the exposure of tail and head proteins of myosin enhanced the gelation ability. Myosin tails became unfolding and interacted with other molecules easily, while the globular myosin heads with involvement of disulfide bonds formed the disulfide bonds and resulted in aggregation (Zayas, 1997). As a result, the enhancement of cold-set gelation ability of SSP with much higher rigidity and stronger gel network achieved as detected by the formation of high MW macromolecules.

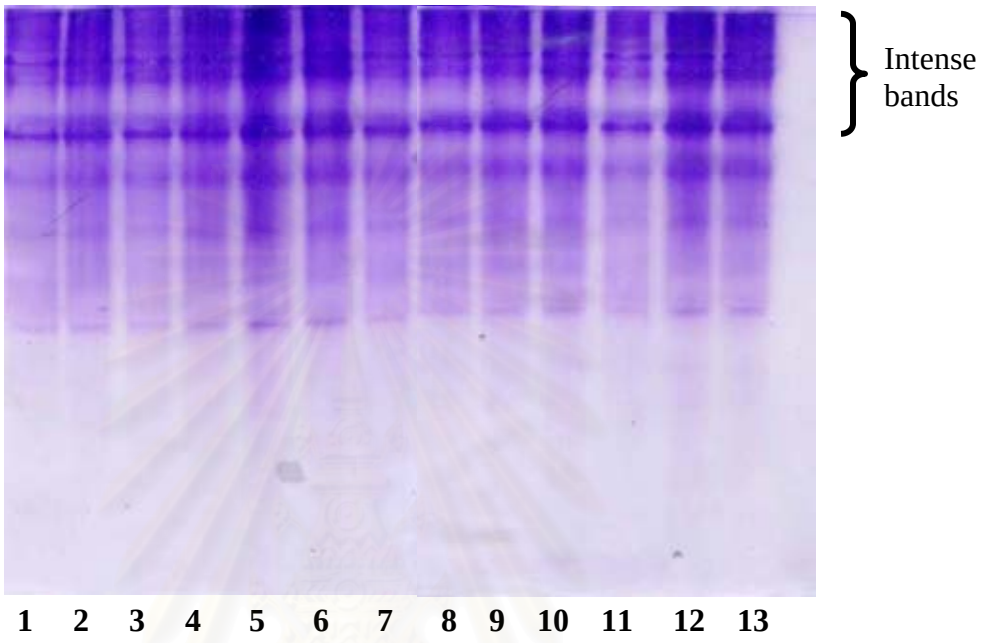


Figure 4.21 Non-denaturing gel-electrophoretic patterns (10% resolving gel) of the developed cold-set gels: Lane1 = Control (8%w/v SSP) and Lane 2-13 = cold-set gels made from 8%w/v SSP and 1, 2 and 3% chicken protein hydrolysates at DH 6.42-35.80%.

4.3.7 Effect on Structure Profiles

The structures of the developed cold-set gels were investigated in order to elucidate the interaction between SSP and chicken protein hydrolysates. Scanning electron micrographs of cold-set gels with 3% chicken protein hydrolysates at 15.12, 22.38 and 35.80% DH compared with that of control were shown in figure 4.22. The cold-set gels with chicken protein hydrolysates at 6.42% DH were not included because such gels exhibited the lowest improvement as indicated by the small increase of rheological and textural characteristics, and disulfide content. Scanning electron micrographs (5,000 x magnifications) revealed the slight improvement of chicken protein hydrolysates on gel structure of the obtained cold-set gels (figure 4.22 B, C and D) when comparing to the control structure (figure 4.22 A). Moreover, the denser and more uniform gel structures were observed in treatments with chicken protein hydrolysates at 15.12 and 22.38% DH. This result was in accord with those found with the maximum compression force and the disulfide content. The occurring of the biopolymer interactions among appropriate chain length macromolecules was again confirmed. During the cold-setting period, the entanglement of these two protein species arose, when temperature of the gels continuously reduced, indicating the formation of interpenetrating network (Oakenfull, Pearce and Burley, 1997; Samant *et al*., 1993). This phenomenon along with the increase of charged groups of chicken protein hydrolysates enhanced by enzymatic hydrolysis with papain, promoted electrostatic interactions and hydrogen bonds between the charge groups of SSP, chicken protein hydrolysates, water and other ingredients in the system. The van der Waals force which naturally occurred when two biopolymers come closer, and salt-bridge were included (Wong, 1989) and stabilized these cold-set

gels structure. Furthermore, disulfide bond was also formed and partially stabilized such gel structure as implied by the increase of disulfide content in 4.3.5.

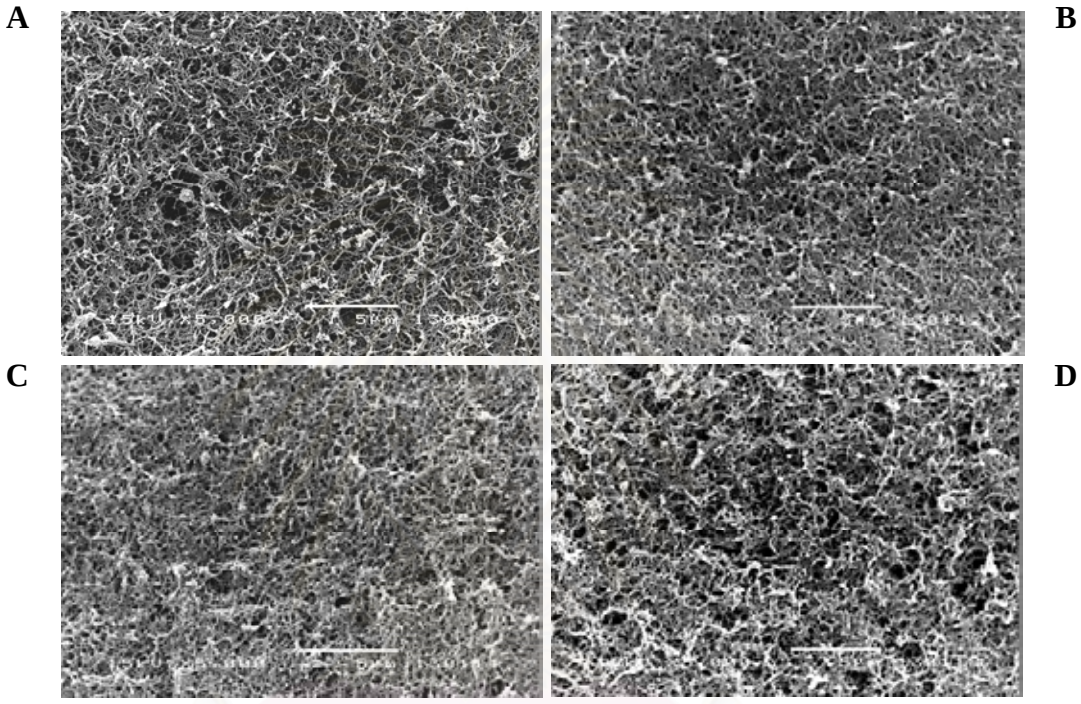


Figure 4.22 Scanning electron micrographs (5000 x magnifications) of control (8% w/v SSP) (A), and the developed cold-set gels made from 8% w/v SSP and 3% chicken protein hydrolysates at 15.12, 22.38 and 35.80% DH (B-D).

4.3.8 Concluding Remarks

The cold-set gelation ability of SSP was enhanced when chicken protein hydrolysates were added. DH and C of chicken protein hydrolysates directly influenced color, rheological characteristics, texture and gel structure of the resulting cold-set gels. Improving of rheological characteristics, gel structure, compression force and disulfide content was observed. The non-denaturing gel-electrophoretic patterns of SSP gels with chicken protein hydrolysates suggested the reinforcement of this protein into the structure of SSP gels. The ΔE^*_{ab} was increased with the increase of C and the decrease of DH. The developed cold-set gels with 3% by weight chicken protein hydrolysates at 22.38% DH exhibited the highest improvement of all parameters determined.

4.4 Combination Effects of Chitosan and Chicken Protein Hydrolysates on Cold-Set Gelation Abilities, Structures and Physico-chemical Properties of SSP Gels

In this experiment, the combination effect of alkali-precipitated chitosan and chicken protein hydrolysates as cold-set binder in SSP gels was estimated. The medium MW (1.84 x 10^5 Dalton) and high DD (94%) chitosan and the 22.38% DH chicken protein hydrolysate were selected to be used concomitantly in this study. The 16 treatment combinations were designed as shown in table 4.8. The physico-chemical properties of the resulting cold-set gels including pH, color, rheological characteristics, texture, disulfide content, non-denaturing gel-electrophoretic pattern, and structure profile, were studied.

4.4.1 Effect on pH

The effect of cold-set binders on pH was presented in table 4.9. According to 4.3.1, the reduction of pH of SSP gels with chicken protein hydrolysates was not observed ($p>0.05$). In this experiment, the different result was found in that the pH of treatments with the mixture of 1 and 1.5% by weight alkali-precipitated chitosan with 3% by weight chicken protein hydrolysates were significantly decreased from that of the control ($p\leq0.05$). This result might occur from the interaction between these two cold-set binders which could react and form the protein-polysaccharide complexes. Tolstoguzov (1986) reported that the liberation of free hydrogen atom from these reactions might occur and result in the decrease of system pH.

Schmitt *et al.* (1998) explained that pH had great impact on the ionization degree of the functional side groups of biopolymers. The net charges of proteins in the system were changed by the changing of pH. At pH over isoelectric point

(pI) of muscle proteins or over 5.4, the net negative charged of these proteins could be formed (Forrest *et al.*, 1975) and interacted electrostatically with the positive charged groups in the system (Samant *et al.*, 1993). In this experiment, the system pH of all treatments with cold-set binders was higher than 5.4. The net charge of SSP and the added chicken protein hydrolysates was negative and might form electrostatic interactions with positive amino groups inside chitosan chains. The electrostatic complexes could be partially formed and reinforced the structure of the SSP gels. Hence, the better gel texture and structure could therefore be obtained as shown in 4.4.7 (figure 4.33 B and C).

Table 4.8 Treatment combinations of the developed cold-set gels made from 8% w/v SSP, 0.5-1.5% alkali-precipitated chitosan, 1-3% chicken protein hydrolysates and their combinations.

Treatment No.	Treatment Combination
Control (C)	8%w/v SSP
1	8% SSP + 1% chicken protein hydrolysates
2	8% SSP + 2% chicken protein hydrolysates
3	8% SSP + 3% chicken protein hydrolysates
4	8% SSP + 0.5% chitosan
5	8% SSP + 1% chitosan
6	8% SSP + 1.5% chitosan
7	8% SSP + 0.5% chitosan + 1% chicken protein hydrolysates
8	8% SSP + 0.5% chitosan + 2% chicken protein hydrolysates
9	8% SSP + 0.5% chitosan + 3% chicken protein hydrolysates
10	8% SSP + 1% chitosan + 1% chicken protein hydrolysates
11	8% SSP + 1% chitosan + 2% chicken protein hydrolysates
12	8% SSP + 1% chitosan + 3% chicken protein hydrolysates
13	8% SSP + 1.5% chitosan + 1% chicken protein hydrolysates
14	8% SSP + 1.5% chitosan + 2% chicken protein hydrolysates
15	8% SSP + 1.5% chitosan + 3% chicken protein hydrolysates

Table 4.9 pH of the developed cold-set gels made from 8% w/v SSP and 8% w/v SSP with medium MW ($1.84x10^5$ Dalton)-high DD (94%) chitosans, 22.38% DH chicken protein hydrolysates, and their combinations.

Treatment No.	pH
Control	$6.63^{bc} \pm 0.010$
1	$6.61^{bc} \pm 0.009$
2	$6.60^{b} \pm 0.010$
3	$6.59^{ab} \pm 0.008$
4	$6.64^{bc} \pm 0.004$
5	$6.63^{bc} \pm 0.044$
6	$6.63^{bc} \pm 0.017$
7	$6.62^{bc} \pm 0.006$
8	$6.59^{ab} \pm 0.012$
9	$6.58^{ab} \pm 0.001$
10	$6.63^{bc} \pm 0.002$
11	$6.61^{bc} \pm 0.013$
12	$6.55^{a} \pm 0.005$
13	$6.61^{bc} \pm 0.010$
14	$6.60^{bc} \pm 0.003$
15	$6.57^{a} \pm 0.005$

$^{a-f}$ Means followed by different letters are significantly different ($p \leq 0.05$).

4.4.2 Effect on Color

The color differences of the developed cold-set gels when comparing with that of the control were expressed in term of the color difference value (ΔE^*_{ab}). The ΔE^*_{ab} was calculated from L*, a* and b* of the cold-set gels and the control. The L*, a* and b* values of all samples and control were also shown to elucidate the cause of ΔE^*_{ab} changes. As demonstrated in figure 4.23, both concentration and type of binders significantly affected this parameter ($p \leq 0.05$). The increase of ΔE^*_{ab} value was observed in all treatments with cold-set binders, especially at high concentration. The color of the obtained cold-set gels was paler than that of the control. The addition of chicken protein hydrolysates significantly increased L* and b* of the developed cold-set gels, while the addition of alkali-precipitated chitosan significantly heightened only L* ($p \leq 0.05$) (figure 4.24). The more increment of these color parameters were observed in treatments with combination of chicken protein hydrolysates and alkali-precipitated chitosan, especially at high concentration. These results occurred from the light color of binders themselves. The significant pH reduction of treatments with 3% by weight chicken protein hydrolysates in combination with alkali-precipitated chitosan at 1 and 1.5% by weight did not have any effect on color of the resulting cold-set gels. This result indicated that there was no correlation between color changes and pH of the developed gels. Hunt and Kroft (1987) and Kranen *et al*. (1999) explained that raw chicken breast meats possessed myoglobin which was their major pigment in only small amount at 0.05 mg/g meat; therefore their loss or denaturation had only small impact on color of such meats. Besides the color changes normally occurred when the system pH changed over 0.1 pH unit due to the change of the charges on heme protein and the change of its native

structure and conformational. These conformational changes affected light reflecting and absorbing and hence, the change of color (Fletcher, 1999; Qiao *et al*., 2002). However, in this experiment, the changes of pH of all treatments with cold-set binders from that of the control were lower than 0.1 pH unit. Hence, the change of color of the heme pigment from the reduction of system pH could not be observed.

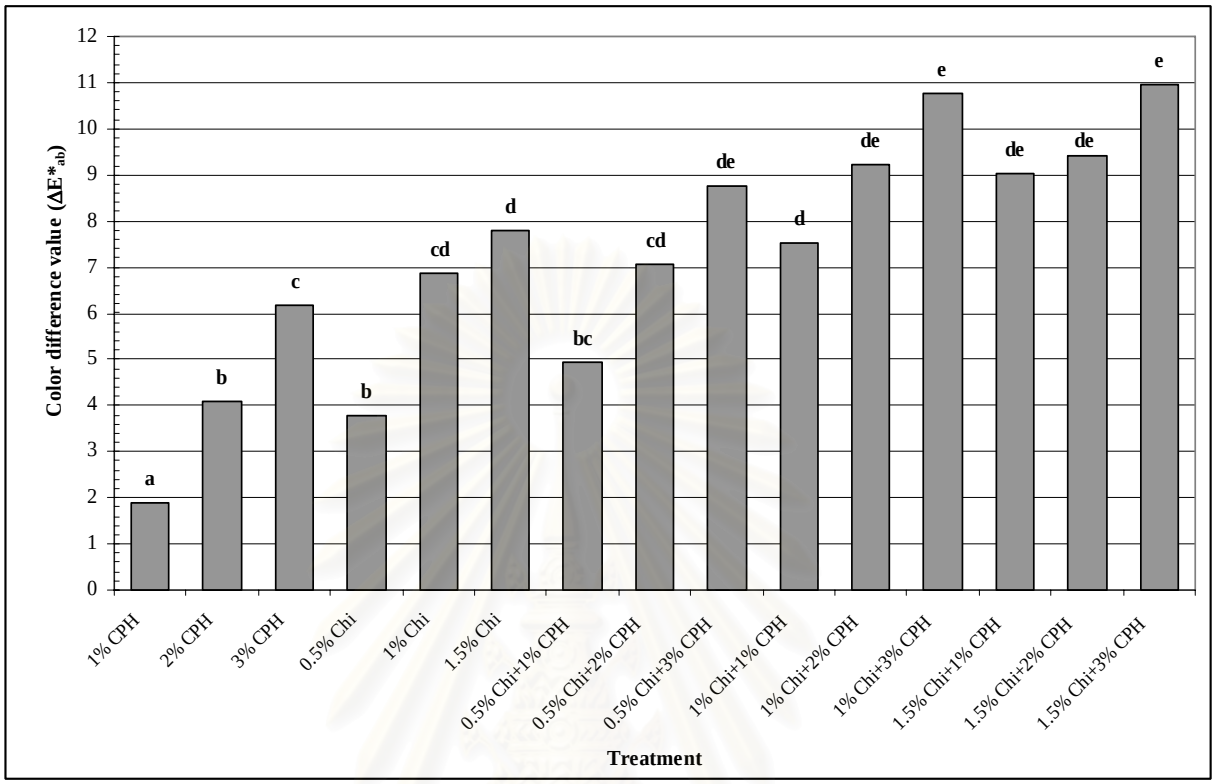


Figure 4.23 Color difference value (ΔE^*_{ab}) of the developed cold-set gels made from 8% w/v SSP with 22.38% DH chicken protein hydrolysates (CPH) at 1, 2 and 3% by weight, medium MW ($1.84x10^5$ Dalton)-high DD (94%) chitosan (Chi) at 0.5, 1 and 1.5% by weight, and their combinations. Different letters a, b…e indicate significant difference ($p \leq 0.05$) among treatments.

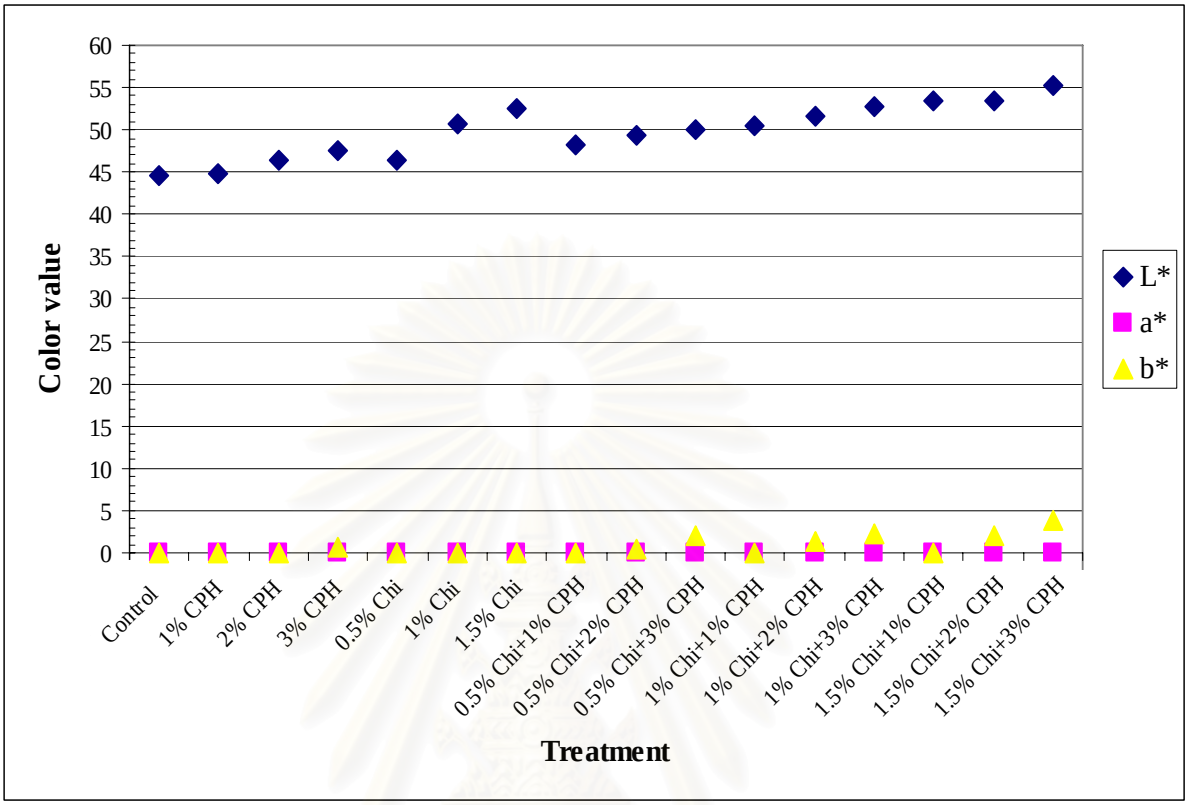


Figure 4.24 Color (L* a* b*) of the developed cold-set gels made from 8% w/v SSP alone and 8% w/v SSP with 22.38% DH chicken protein hydrolysates (CPH) at 1, 2 and 3% by weight, medium MW ($1.84x10^5$ Dalton)-high DD (94%) chitosan (Chi) at 0.5, 1 and 1.5% by weight, and their combinations.

4.4.3 Effect on Rheological Characteristics

The rheological characteristics of all treatments were expressed in term of elastic modulus (G′), complex modulus (G*) and complex viscosity (η*) which indicated the elastic properties of the cold-set gels (figure 4.25, 4.26 and 4.27). The G′ lines of all treatments with cold-set binders were shifted up from those of the controls and more elevation of G′ values occurred when increasing of binder concentration (figure 4.25). This result indicated the occurrence of stronger gel network by the addition of cold-set binders into SSP gels. The highest improvement found when using alkali-precipitated chitosan and chicken protein hydrolysate mixture as cold-set binder, especially in treatment with highest concentration (treatment 15), indicated the synergistic effect of the binders and concentration. At high frequency range (1-30 Hz), the G′ of control was gradually inclined. This indicated the weak gel network of SSP which can not retained its structure under the high frequency. While, steadier G′ lines were observed with the reinforcement of SSP gel with 1.5% alkali-precipitated chitosan and 2 or 3% chicken protein hydrolysates (treatment 14 and 15). The combination of elevated of G′ with the steadiest slope as seen in treatment 15 exhibited the property of strong gel network and indicated the formation of electrostatic interactions between charged groups and other reactive groups of alkali-precipitated chitosan, chicken protein hydrolysates and SSP chains. These interactions formed when segment of linear-charged biopolymers come closer. The association over several chain units was occurred and resulted in the more entanglement of biopolymer chains which give rise to junction zone. When biopolymer chains formed a junction zone at one place along their chain lengths or with other chains, the approaching of such biopolymers distance resulted and formed a

junction zone with other biopolymer chains. Consequently, the chain segments from different polymers became packed and resulted in stronger tridimentional gel network mainly linked by electrostatic interactions and hydrogen bonds (Oakenfull, Pearce and Burley, 1997; Smewing, 1999). Other interactions such as disulfide bonds between protein chains was also reported, hence the gelation ability was promoted (Samant *et al*., 1993).

The G* lines of all treatments with cold-set binders either using alone or in combinations were located higher than that of the control as shown in figure 4.26. The highest improvement of G* was also detected in treatment with mixture of 1.5% alkali-precipitated chitosan and 3% chicken protein hydrolysates similar to the result from G′. This line possessed the steadiest slope along the frequency range which exhibited the behavior of strong gel network. This result might occur due to the synergistic effect of alkali-precipitated chitosan and chicken protein hydrolysates. When using in combination, these cold-set binders could interact with each other and also interacted with SSP as both gel binder and gel filler. As a result, the SSP chains could pack together and formed the stronger gel network (Tolstoguzov, 1997). In the condition with saline buffer, the interaction of SSP and these two cold-set binders was observed as shown by the increase of both G′ and G* values from that of control. Furthermore, the presence of polysaccharides in suitable form contributed to the thermodynamic activity of proteins in buffer solution. It intensified the aggregation of the protein molecules and hence promoted the gelation process (Samant *et al*., 1993).

The η* lines of the developed cold-set gels with cold-set binders was slightly shifted up from that of the control; nevertheless, the more linearity line along

the frequency range was observed (figure 4.27). This result also indicated the changes of weak SSP gels into the stronger gel network obtained by the reinforcement of alkali-precipitated chitosan and chicken protein hydrolysates both using alone and in combination. Accordingly, the most linear η* line was found in treatment with mixture of 1.5% alkali-precipitated chitosan and 3% chicken protein hydrolysates similar to the result from G′ and G*. This result confirmed the characteristic of strong gel network (Morris 1995; Ross-Murphy, 1995) and suggested that cold-set gelation ability of SSP could be enhanced by the addition of alkali-precipitated chitosan in combination with chicken protein hydrolysates at appropriate concentration.

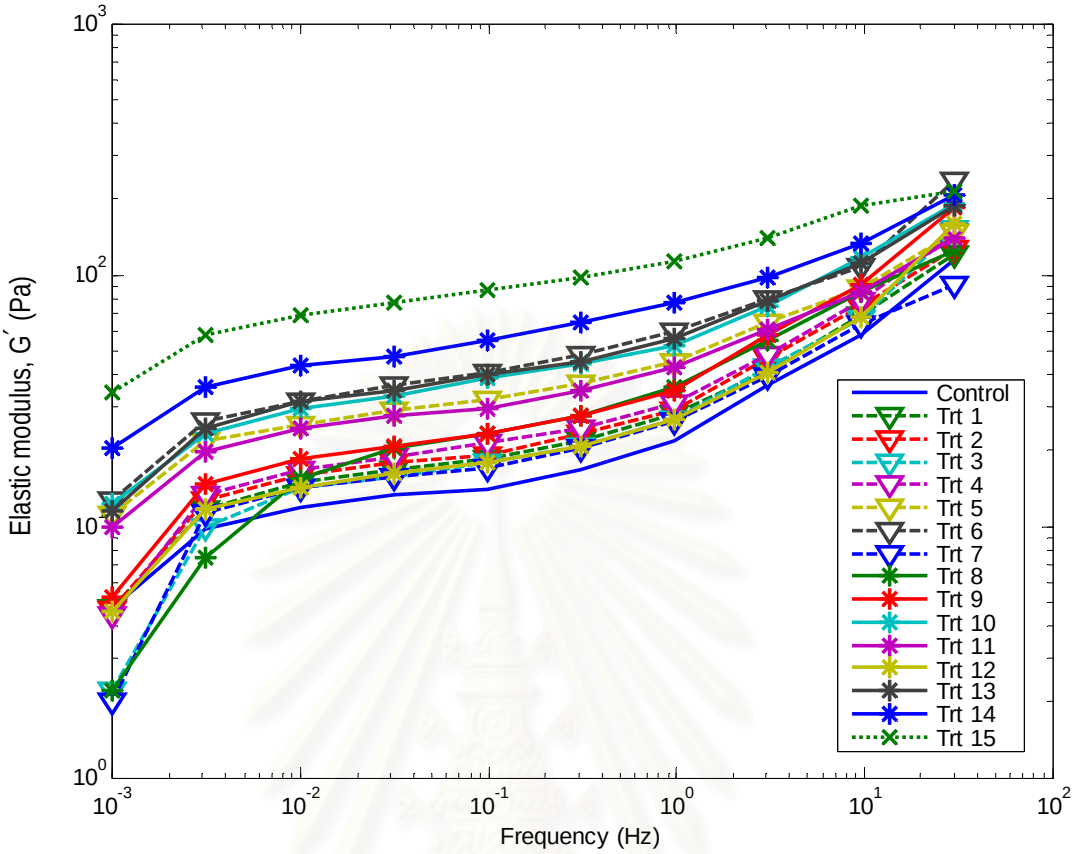


Figure 4.25 Elastic modulus (G´) of the developed cold-set gels made from 8% w/v SSP only and 8% w/v SSP with 22.38% DH chicken protein hydrolysates at 1, 2 and 3% by weight, medium MW (1.84×10^5 Dalton)-high DD (94%) chitosan at 0.5, 1 and 1.5% by weight, and their combinations.

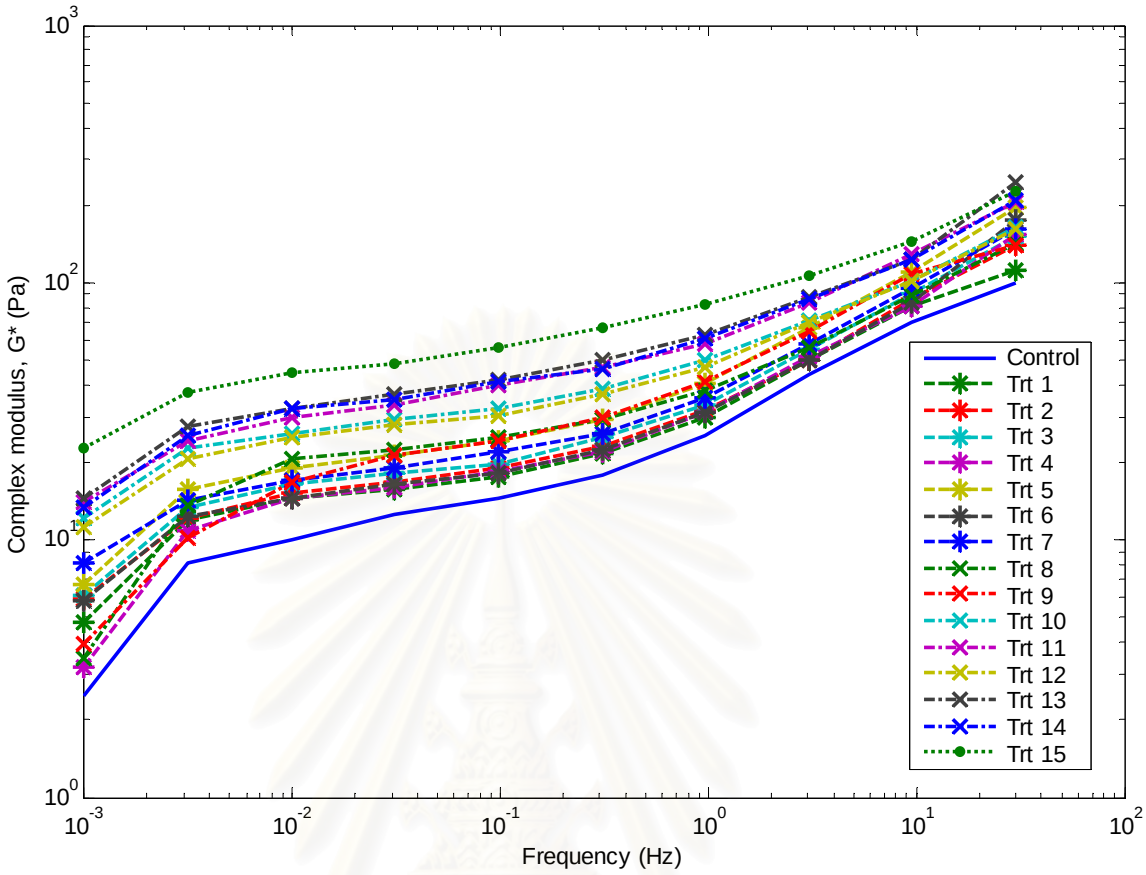


Figure 4.26 Complex modulus (G*) of the developed cold-set gels made from 8% w/v SSP only and 8% w/v SSP with 22.38% DH chicken protein hydrolysates at 1, 2 and 3% by weight, medium MW (1.84×10^5 Dalton)-high DD (94%) chitosan at 0.5, 1 and 1.5% by weight, and their combinations.

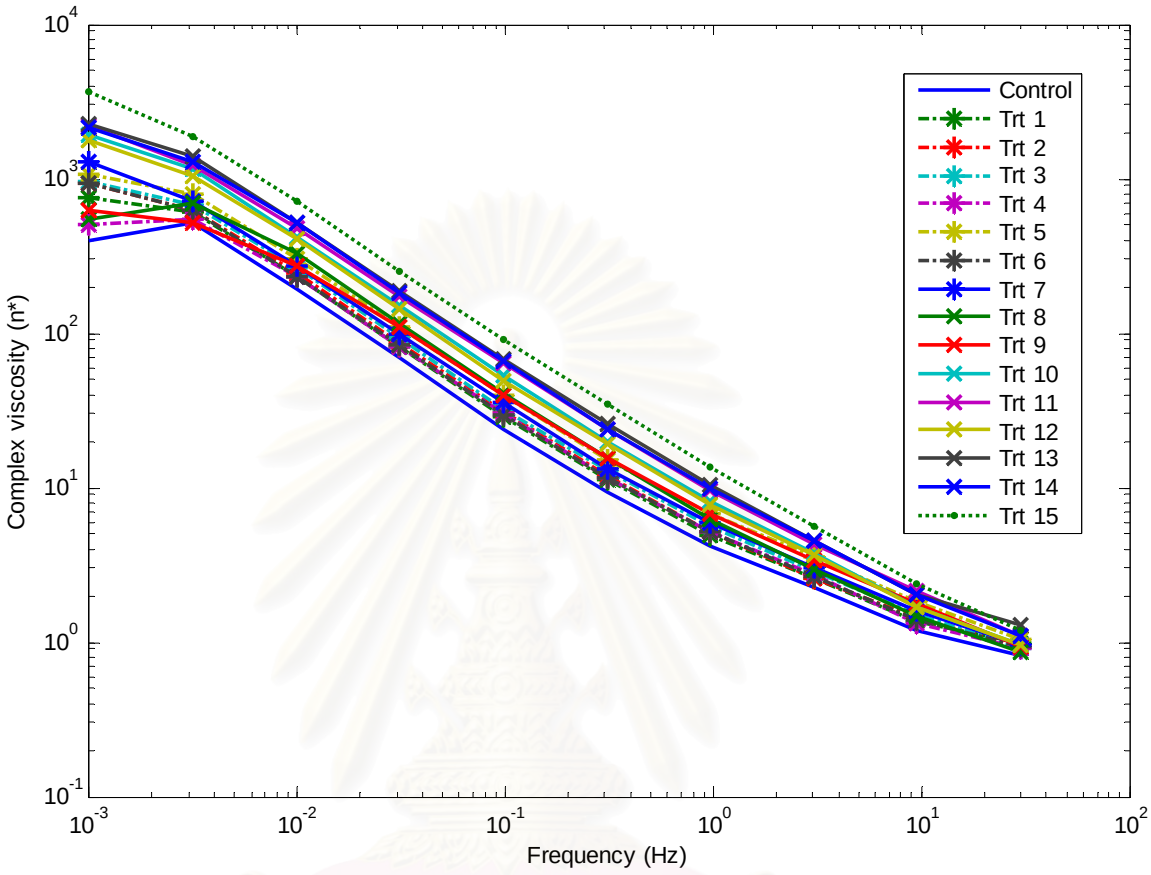


Figure 4.27 Complex viscosity (η*) of the developed cold-set gels made from 8% w/v SSP only and 8% w/v SSP with 22.38% DH chicken protein hydrolysates at 1, 2 and 3% by weight, medium MW (1.84×10^5 Dalton)-high DD (94%) chitosan at 0.5, 1 and 1.5% by weight, and their combinations.

4.4.4 Effect on Textural Characteristic

The textural characteristic of all treatments assessed in term of maximum compression force (g) is shown in figure 4.28. The addition of cold-set binders significantly increased the maximum compression force from control ($p \leq 0.05$). Moreover, the higher improvement was significantly found in treatments with combination of alkali-precipitated chitosan and chicken protein hydrolysates, especially at high concentration. When using each binder alone, the increase of binder concentration significantly heightened the maximum compression force ($p \leq 0.05$). When using both cold-set binders in combination, a similar trend was observed but with remarkable improvement. The best improvement was detected in treatment with mixture of alkali-precipitated chitosan and chicken protein hydrolysates at the highest concentration (treatment 15). This result was in accord with the rheological characteristics including G', G^* and η^* of the gels and accentuated the synergistic effect of these two cold-set binders in enhancing cold-set gelation ability of SSP. Various interactions that could occur to stabilize the cold-set gels including electrostatic interactions, hydrogen bonds and some of ionic bonds which normally occurred when biopolymers come closer at low temperature (Dalgleish and Hunt, 1995). Moreover, the interactions between these cold-set binders could have occurred between charged groups of alkali-precipitated chitosan and chicken protein hydrolysates resulted in electrostatic interpolymer complexes. This electrostatic complex formation was also described by Tolstoguzov (1997) and Schmitt *et al*. (1998). When using these complexes as cold-set binder in SSP gels, the remarkable enhancement of cold-set gelation ability could therefore be obtained. According to Samant *et al*. (1993), the protein gel forming ability could be enhanced by protein and

polysaccharide complexes. The gelation of the obtained complexes could occur even under the conditions that each individual components might not be able to function.

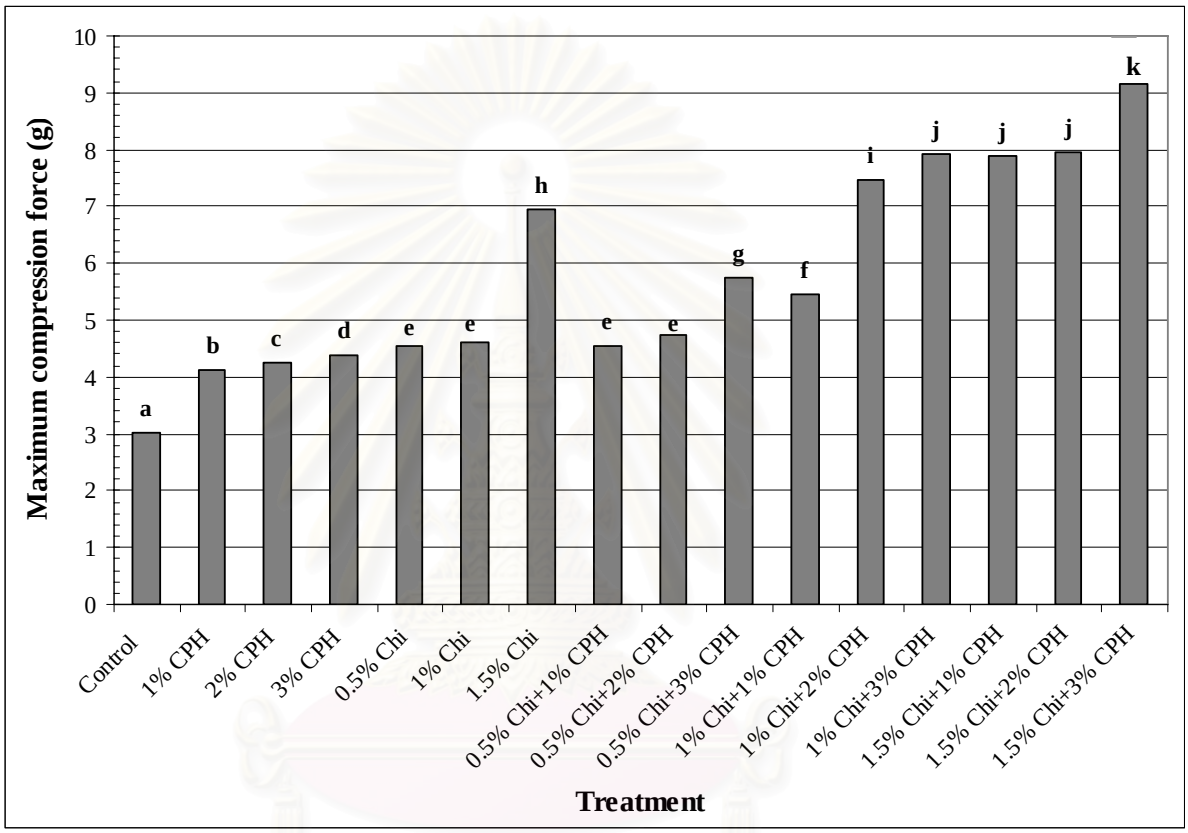


Figure 4.28 Maximum compression force (g) of the developed cold-set gels made from 8% w/v SSP only and 8% w/v SSP with 22.38% DH chicken protein hydrolysates (CPH) at 1, 2 and 3% by weight, medium MW ($1.84x10^5$ Dalton)-high DD (94%) chitosan (Chi) at 0.5, 1 and 1.5% by weight, and their combinations. Different letters a, b…k indicate significant difference ($p \leq 0.05$) among treatments.

4.4.5 Effect on Disulfide Content

The binder type and concentration significantly influenced the disulfide contents of the cold-set gels (figure 4.29). The improvement of disulfide content was significantly detected in the presence of the cold-set binders ($p \leq 0.05$). This result indicated the formation of cold-set gels which partially stabilized by disulfide bonds. For treatments with alkali-precipitated chitosan or chicken protein hydrolysates only, disulfide contents were not increased accordingly with the increase of the binder concentration. The increase of concentration affected the disulfide content only in treatments with combination of alkali-precipitated chitosan and chicken protein hydrolysates and the highest increment was found in treatment with 1.5% alkali-precipitated chitosan and 3% chicken protein hydrolysate (treatment15). This result along with the rheological and textural characteristics suggested the formation of strong and uniform gel network by the addition of alkali-precipitated chitosan and chicken protein hydrolysate mixture. The increase of disulfide content might occur during mixing and cold-setting period. Under low temperature, van der Waals forces and electrostatic interactions were induced. Once these interactions occurred, the macromolecule chains were brought to become closer. The new stabilized disulfide bonds could be induced when those macromolecules approached the close enough distance (Dalgleish and Hunt, 1995). Furthermore, the increase of the detectable disulfide content might occur from the highly exposed thiol contents in chicken protein hydrolysates which were previously enhanced by hydrolyzing effect of papain (Panyam and Kilara, 1996).

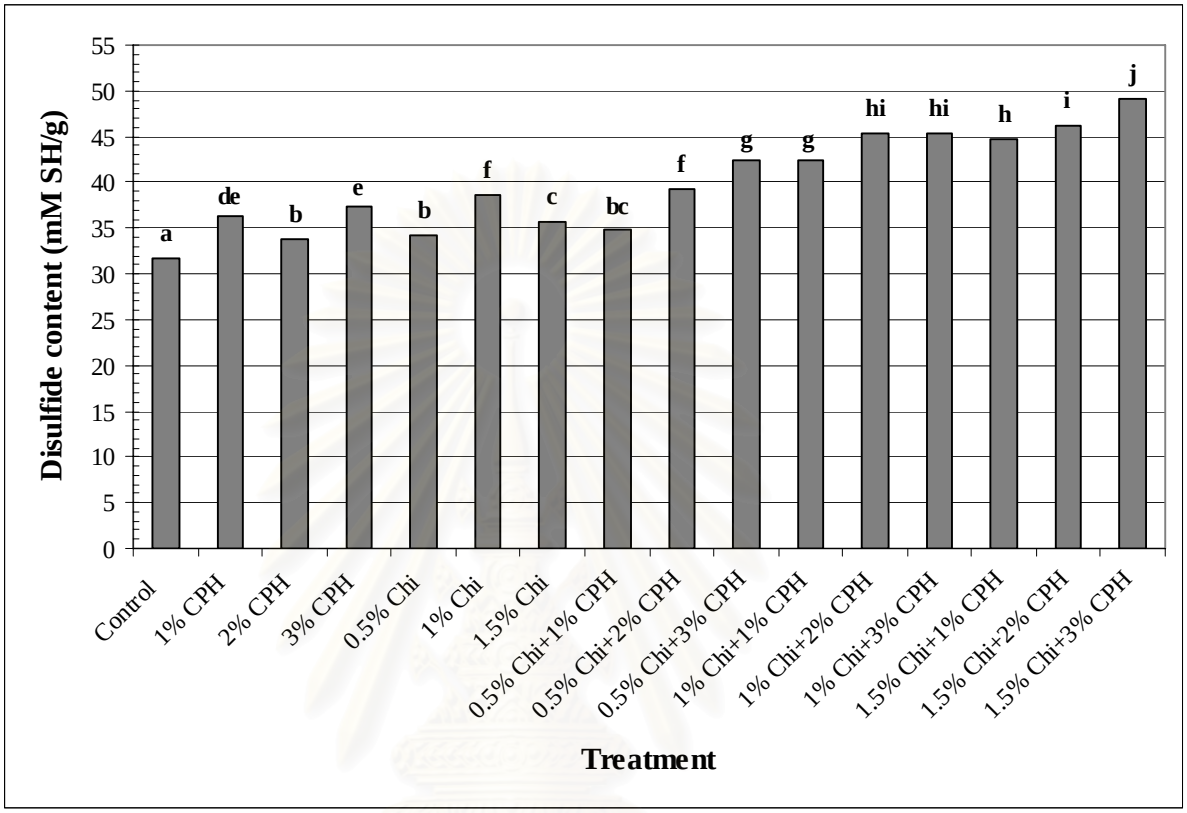


Figure 4.29 Disulfide content (mM SH/g) of the developed cold-set gels made from 8% w/v SSP only and 8% w/v SSP with 22.38% DH chicken protein hydrolysates (CPH) at 1, 2 and 3% by weight, medium MW ($1.84x10^5$ Dalton)-high DD (94%) chitosan (Chi) at 0.5, 1 and 1.5% by weight, and their combinations. Different letters a, b…j indicate significant difference ($p \leq 0.05$) among treatments.

4.4.6 Effect on Non-Denaturing Gel-Electrophoresis

The results from non-denaturing gel-electrophoresis are shown in figure 4.30. When comparing the gel-electrophoretic patterns of all treatments containing various treatment combinations of cold-set binders with the control pattern, differences could clearly be observed. The stacking bands of high MW macromolecules on top of the wells were detected. The obvious results were found in treatments with combination of alkali-precipitated chitosan and chicken protein hydrolysates (Lane 7-15), especially at the top of well No. 15. The thickest stacking band produced with this sample indicated the interactions of SSP with the two cold-set binders which resulted in the formation of high MW macromolecules. These results also suggested the formation of strong gel networks which were partially stabilized by disulfide bonds and particularly stabilized by various interactions including electrostatic interactions, hydrogen bonds, salt bridge and also disulfide content. Wong (1989) also reported that these chemical bonds were usually occurred in the protein gel. The results from rheological, textural characteristics, and disulfide content coincidently supported to the occurrence of macromolecules which were detected by the non-denaturing gel-electrophoretic patterns.

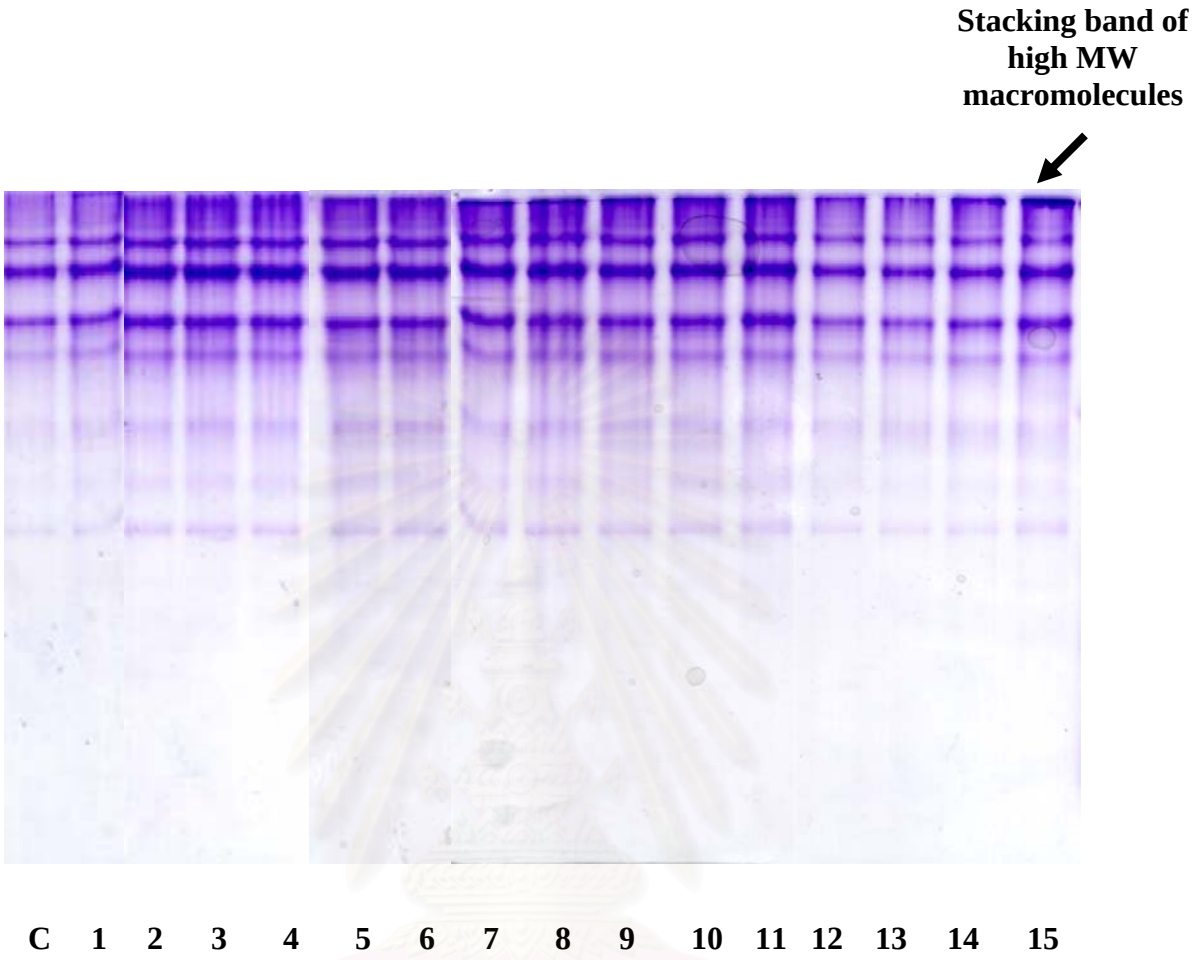


Figure 4.30 Non-denaturing gel-electrophoretic patterns (10% resolving gel) of the developed cold-set gels made from 8% w/v SSP only and 8% w/v SSP with 22.38% DH chicken protein hydrolysates at 1, 2 and 3% by weight, medium MW ($1.84x10^5$ Dalton)-high DD (94%) chitosan at 0.5, 1 and 1.5% by weight and their combinations. Lane C, 1…15 represent treatment combinations explained in table 4.8.

4.4.7 Effect on Structure Profiles

The interactions between SSP and various cold-set binders were confirmed by the structural study. The scanning electron micrographs of various treatments were shown in figure 4.31-4.33. When alkali-precipitated chitosan or chicken protein hydrolysates was added alone, the similar results to that of the previous study (4.2.6 and 4.3.7) were observed as shown in figure 4.31 and 4.32. When these two cold-set binders were combined, the drastic improvement of the gel network was achieved (figure 4.33 B and C). The densest and most uniform structure was observed in treatment with the highest concentration of alkali-precipitated chitosan and chicken protein hydrolysates (figure 4.33 C). This result was in accord with the previous parameters (rheological and textural characteristics, disulfide content and non-denaturing gel-electrophoretic patterns) reported. As already mentioned in the previous topics, these cold-set gels were stabilized by disulfide bonds, electrostatic interactions, hydrogen bonds and also some of ionic bonds (Wong, 1989).

The structure of the mixture of 1.5% by weight alkali-precipitated chiosan and 3% chicken protein hydrolysates was presented in figure 4.34. The fibrous structure occurred from the entanglement of these two binders was observed. When these fibrous complexes were added into the loose and porous structure of SSP gels (figure 4.33 A), the densest and most uniform gel structure was obtained (figure 4.33 C). These results might occur because these binder complexes was dispersed uniformly in the SSP gel matrix and might act as gel filler which could reinforce the strength of the obtained gel structure. Samant *et al*. (1993) explained that the better gelation properties of the biopolymers were increased when subjected them in proper concentration to form the

complex. Furthermore, Schmitt *et al*. (1998) also reported the use of protein-polysaccharide complexes to produce the meat analog with desirable texture and structure in their review.

A

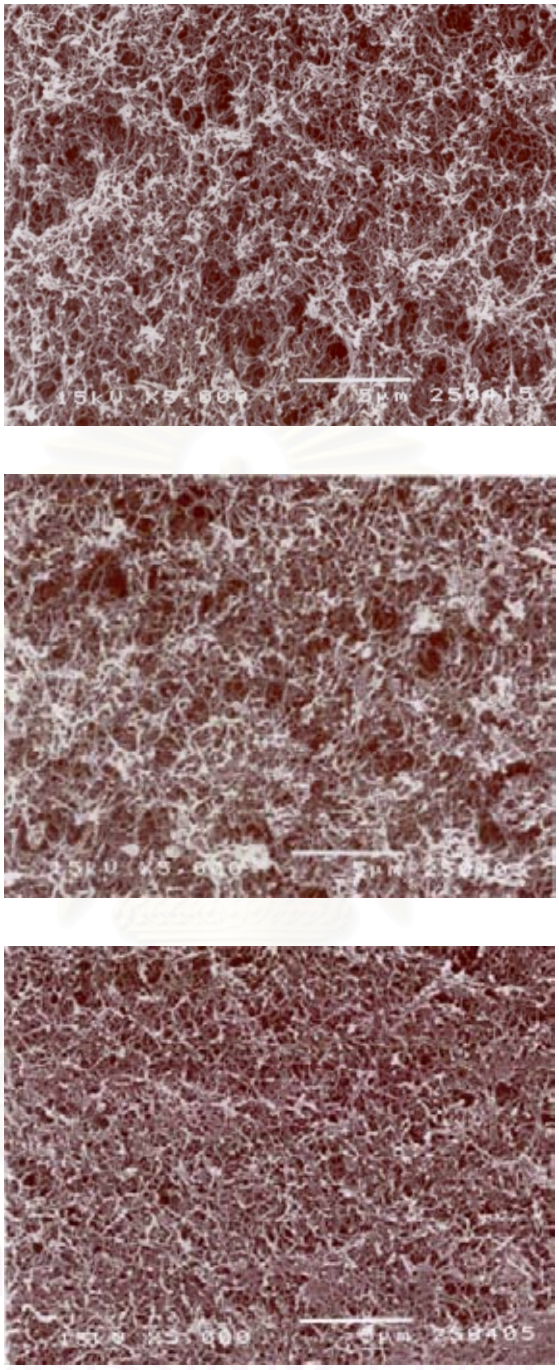


Figure 4.31 Scanning electron micrographs (5,000 x magnifications) of the developed cold-set gels: **A** = 8%w/v SSP only, **B** = 8%w/v SSP with 2% by weight chicken protein hydrolysates, and **C** = 8%w/v SSP with 3% by weight chicken protein hydrolysates.

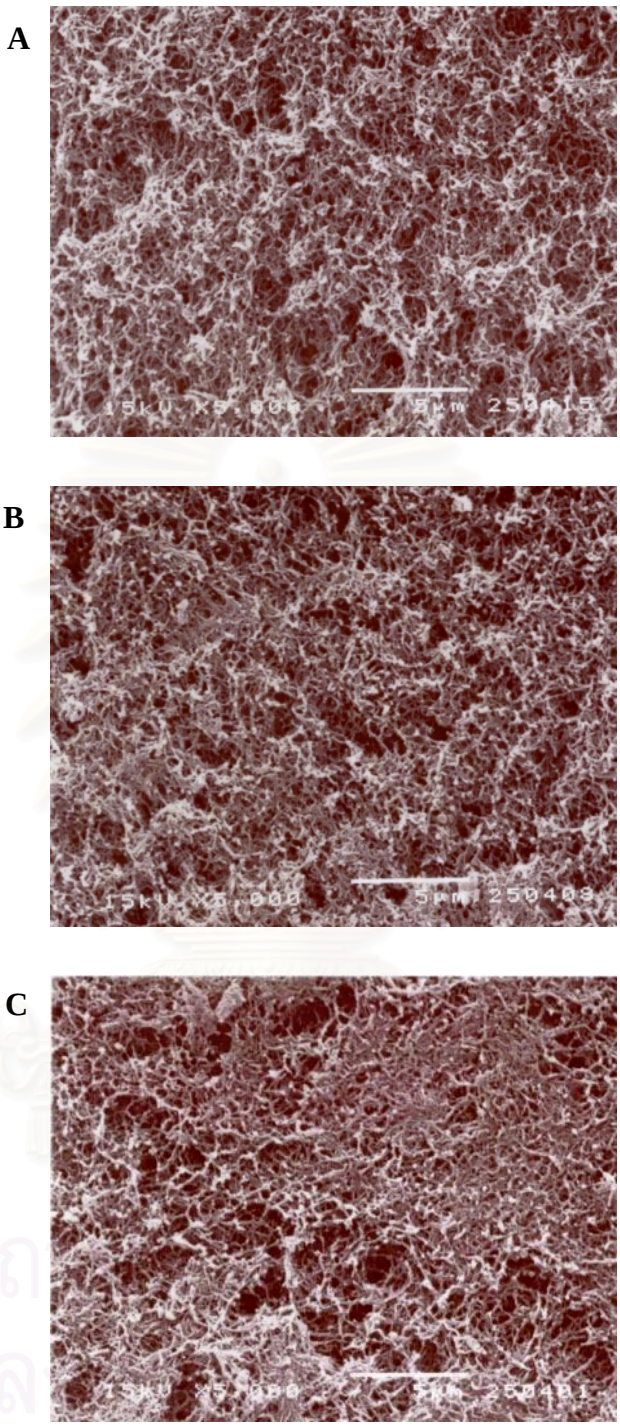


Figure 4.32 Scanning electron micrographs (5,000 x magnifications) of the developed cold-set gels: **A** = 8%w/v SSP only, **B** = 8%w/v SSP with 1% by weight alkali-precipitated chitosan, and **C** = 8%w/v SSP with 1.5% by weight alkali-precipitated chitosan.

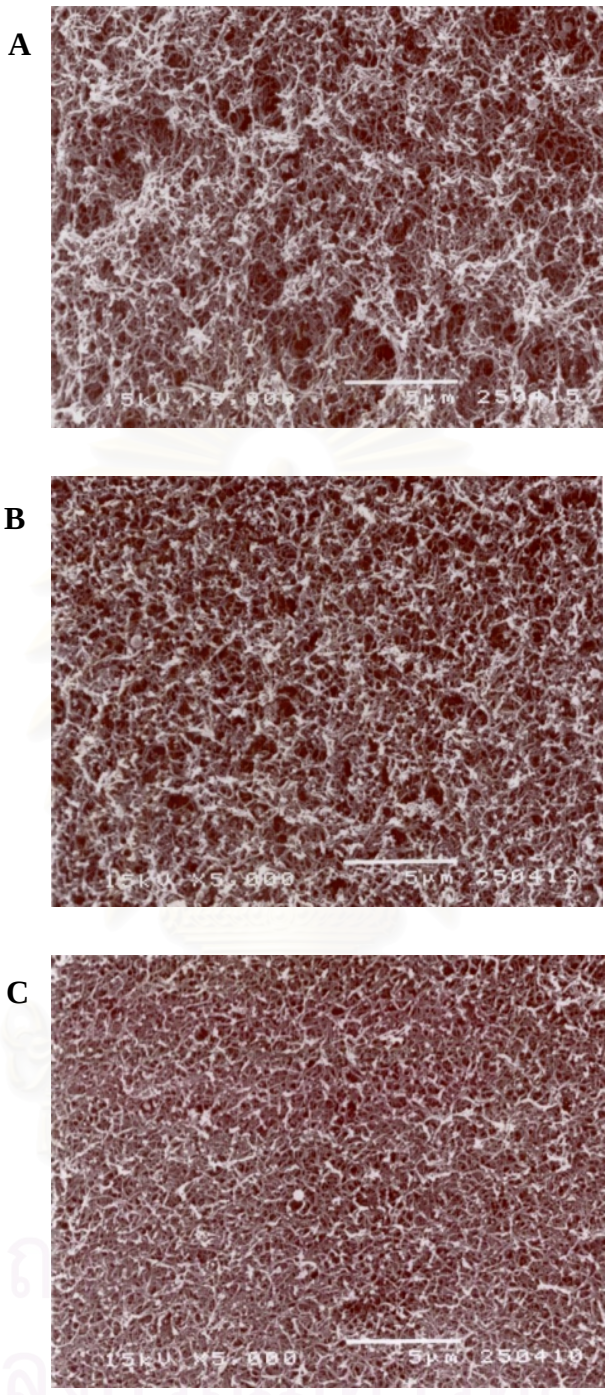


Figure 4.33 Scanning electron micrographs (5,000 x magnifications) of the developed cold-set gels: **A** = 8%w/v SSP only, **B** = 8%w/v SSP with 1% alkali-precipitated chitosan and 2% chicken protein hydrolysates, and **C =** 8%w/v SSP with 1.5% alkali-precipitated chitosan and 3% chicken protein hydrolysates

A **B**

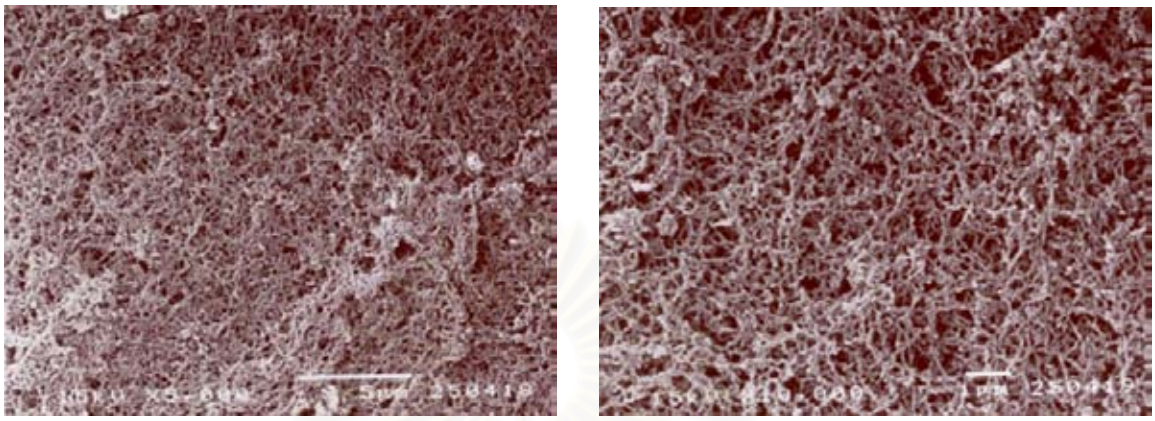


Figure 4.34 Scanning electron micrographs at 5,000 x magnifications (A) and 10,000 x magnifications (B) of the mixture of 1.5% alkali-precipitated chitosan and 3% chicken protein hydrolysates.

4.4.8 Concluding Remarks

The addition of either alkali-precipitated chitosan or chicken protein hydrolysates and their combinations effectively improved rheological characteristics, texture and structural network of the SSP gel when comparing with that of the control. The high disulfide content and stacking bands of high MW macromolecules on the top of the non-denaturing gel-electropheretic wells were obtained only in treatments containing various concentrations of the combined binders. The densest and most uniform gel networks were also seen in treatments containing the combined binders especially at the highest concentration designed for this experiment. It can be concluded that the developed cold-set gel with combination of 1.5% by weight, medium MW ($1.84x10^5$ Dalton)-high DD (94%) alkali-precipitated chitosan and 3% by weight, 22.38% DH chicken protein hydrolysates exhibited the highest quality.

4.5 Application of Chitosan, Chicken Protein Hydrolysates and Their Combinations in Raw Restructured Chicken Meat

The effect of alkali-precipitated chitosan, chicken protein hydrolysates and their combinations as cold-set binders in raw-restructured chicken meats were investigated. Since alkali-precipitated chitosan with medium MW ($1.84x10^5$ Dalton) and high DD (94%) at 1.5%, and chicken protein hydrolysates with 22.38% DH at 3% concentration exhibited the highest binding abilities when using either alone and in combination, they were chosen to be used in this experiment. Moreover, the cold-set SSP gels containing of alkali-precipitated chitosan at 1%, and chicken protein hydrolysates at 2% either alone or in combination also exhibited the high improvement in the textural quality, disulfide content and gel structure when comparing with that of the control and treatments with each binder alone. These samples were therefore selected to be used in this experiment. Raw restructured chicken meats were prepared in 6 different formulas and the effects of cold-set binders were studied in comparison with control. The treatment formulations were shown in table 4.10.

Regarding the structure of the obtained raw restructured chicken meat products, the dense and uniform gel matrix of chicken meat particles and the added cold-set binders was required. This structure should compose of the continuous matrix of chicken meat particles coated around with the added cold-set binders which filled into the SSP gel network, and the dense and uniform gel network-meat particle matrix resulted. However, the good quality raw restructured chicken meat products must not possess only the dense gel-meat particle matrix but sufficient binding strength for handling at low temperature as raw meat cuts with firm texture and high juiciness were also needed (Berry, 1987). Their

color was supposed to be similar to the raw chicken meat color. Their structure must be able to retain the gel network when applying force or frequency in the rheological oscillation frequency sweep test and exhibited the G′ graph over G′′ graph with linear slope along the frequency range. The physico-chemical properties of the prepared raw restructured chicken samples including pH, color, rheological characteristics, texture, water binding properties and structure profile were therefore assessed in order to elucidate whether the required aforesaid characteristics were met or not.

4.5.1 Effect on pH

The pH of the obtained raw restructured chicken meat samples was varied from 6.08-6.11 (table 4.10) in which, significant difference among the designed treatments was not observed ($p>0.05$). This result implied that the addition of cold-set binders at appropriate quantity would not affect pH of the obtained products. Trout and Schmidt (1987) also reported that restructured meats with moderate sodium chloride concentrations (1-1.5%) possessed the pH in the range of 6.0-6.4.

Table 4.10 Treatment combinations and pH of the raw restructured chicken meat samples

Treatment No.	Treatment combination	pH[ns]
Control	500 g chicken broiler tenderloin meats + 1% by weight NaCl	6.08 ± 0.29
1	500 g chicken broiler tenderloin meats + 1% NaCl + 1% by weight alkali-precipitated chitosan	6.09 ± 0.25
2	500 g chicken broiler tenderloin meats + 1% NaCl + 1.5% alkali-precipitated chitosan	6.11 ± 0.27
3	500 g chicken broiler tenderloin meats + 1% NaCl + 2% by weight chicken protein hydrolysates	6.09 ± 0.26
4	500 g chicken broiler tenderloin meats + 1% NaCl + 3% chicken protein hydrolysates	6.08 ± 0.24
5	500 g chicken broiler tenderloin meats + 1% NaCl + 1% alkali-precipitated chitosan+ 2% chicken protein hydrolysates	6.10 ± 0.25
6	500 g chicken broiler tenderloin meats + 1% NaCl + 1.5% alkali-precipitated chitosan+ 3% chicken protein hydrolysates	6.08 ± 0.26

[ns] Treatments were not significantly different ($p>0.05$).

4.5.2 Effect on Color

The results in table 4.11 show the effect of cold-set binders on color of the obtained raw restructured chicken meat products. The color changes of all treatments compared with that of the control were evaluated in term of L*, a*, and b*. The color difference value (ΔE^*_{ab}) was calculated from such measured parameters in order to indicate the changes the meats color from the color of control. The chroma values (C*) and hue angle (h*) were also calculated from a* and b* to elucidate the position of raw restructured chicken meat color in the CIE color space.

Color of the obtained products slightly differed from that of the control (table 4.11). The significant color changes from the addition of alkali-precipitated chitosan and chicken protein hydrolysates either using alone or in combination was observed in L* ($p \leq 0.05$). The L* gradually heightened when using these two cold-set binders at high concentration ($p \leq 0.05$). This result was in accord with the previous studies of the cold-set SSP gels and also agreed with the previous report of Andrés, Zaritzky and Califano (2006). They also reported the increase of L* after the addition of combination binders (white guar gum and cream-colored whey protein powder) into low fat chicken sausages. The increase of L* may occur from the uniform dispersion of white alkali-precipitated chitosan beads and light yellow chicken protein hydrolysates powders in the raw restructured chicken meats matrix. Because of the light color of these binders, their applications should appropriate only in light meat such as chicken, turkey and fish. The a* and b* values of the resulted raw restructured chicken meats with cold-set binders were not different from control which resulted in non-significant difference of C* and h* values of all treatments ($p > 0.05$). The ΔE^*_{ab} increased accordingly with the increase of

L* and significant effect was found in treatment with high binder concentration. When considering the position of raw restructured chicken meat color in the CIE color space, the small shift up in the Y axis of lightness was observed and this color change was not detected in cooked samples.

Table 4.11 Color difference value (ΔE^*_{ab}) and color (lightness, chroma and hue angle) of raw restructured chicken meat samples containing various concentrations of chitosan, chicken protein hydrolysates and their combinations.

Treatment	Color			
	ΔE^*_{ab}	**Lightness (L*)**	**Chroma^{ns} (C*)**	**Hue^{ns} (h*)**
Control	-	$45.38^{a} \pm 1.29$	16.79 ± 1.17	70.42 ± 2.68
1	$2.06^{a} \pm 0.44$	$47.31^{b} \pm 1.17$	17.45 ± 1.17	70.04 ± 3.20
2	$3.70^{bc} \pm 0.41$	$48.94^{cd} \pm 0.93$	17.22 ± 1.17	71.19 ± 2.67
3	$2.33^{a} \pm 0.634$	$47.28^{b} \pm 1.57$	18.08 ± 1.17	70.10 ± 2.27
4	$2.84^{ab} \pm 1.15$	$47.99^{bc} \pm 1.83$	17.91 ± 1.17	70.73 ± 3.67
5	$3.94^{bc} \pm 0.87$	$49.00^{cd} \pm 1.27$	17.93 ± 1.17	71.11 ± 4.29
6	$4.73^{c} \pm 0.50$	$49.89^{d} \pm 0.95$	17.39 ± 1.17	71.29 ± 3.54

ns Treatments were not significantly different ($p>0.05$).
a-d Means followed by different letters within the same column are significantly different ($p \leq 0.05$).

4.5.3 Effect on Rheological Characteristics

The rheological characteristics of raw restructured chicken meat samples obtained from oscillation frequency and temperature sweep test were shown in figure 4.35-4.41. The G′ values from oscillation frequency sweep test of all samples were measured in order to indicate their gel strength. The plot of G′ versus frequency indicated the changes of gel rigidity along the frequency range (0.001-100 Hz). In SSP gel, the interactions among proteins and cold-set binders occurred directly since there were no others interfering components in the system. The entanglement and rearrangement to form the dense and uniform three-dimensional gel network could occur properly (Samant *et al.*, 1993). With this reason, the shift up and steady G′ line along the frequency range was resulted (figure 4.25). In raw restructured chicken meat in this experiment, the cold-set binders were added and homogeneously dispersed in the matrix of meat particles coated around with the extracted SSP gel. In this system, the SSP was also the major binder, while the added cold-set gel could reinforce the gel structure by interacting and forming various interactions with SSP. Thus, the steady G′ line along the frequency range was also expected. However, the obtained G′ values from these two systems (SSP gels and raw restructured chicken meats) were not equal. The raw restructured chicken meat possessed both meat particles and the extracted SSP in the system and its texture was more rigid than that of the SSP gels. Hence, the higher G′ value was expected. Moreover, the G′ patterns of the raw restructured chicken meat were almost similar to that of the SSP patterns. This result might occur from the similar cold-set gel formation mechanism of these two systems which resulted in the formation of filled gel.

The result in figure 4.35 was similar to that of the result from the system of SSP gels (figure 4.25). The G′ lines of all treatments with the cold-set binders were shifted up from the control line. When alkali-precipitated chitosan and chicken protein hydrolysates were added concomitantly, the highest shifting up with the steadiest G′ line was obtained, especially at high concentration (treatment 6). This result was expected and agreed with the previous study in SSP gel system. The obvious result was detected in phase angle (δ) (figure 4.36). The δ values of all treatments with cold-set binders were remarkably shifted down from that of the control to near 0˚. Dublier, Luanney and Cuvelier (1992), and Ross-Murphy (1995) explained that the materials possessed the δ values at 0˚ were exhibited the properties of solid materials. Thus, this result indicated the formation of strong and uniform gel network with high viscoelasticity from the addition of these binders.

The results of G′ obtained from oscillation temperature sweep test of all treatments were shown in figure 4.37. The G′ pattern of control was similar to that of the SSP pattern in which the rheological transitions occurred within 50-60˚C. This result occurred from the dissociation of actomyosin complex into the light meromysin followed by the rapid increase of G′ from the structural change into rapid aggregation and gel network formation (Xiong, 1997; Lesiów and Xiong, 2001). The addition of cold-set binders resulted in structural modification as detected by the significant increase of G′ at 80˚C from that of the control ($p \leq 0.05$) (table 4.12). When alkali-precipitated chitosan was added alone, the obtained G′ patterns were changed. The peak of actomyosin complex dissociation at 50-55˚C was disappeared and the shifting up of G′ patterns from the

control pattern was resulted (figure 4.37 and 4.39). This result implied that the structural modification of the extracted SSP around meat particles could occur by the induction of alkali-precipitated chitosan and the formation of various chemical interactions could occur similar to those of the SSP system. The stronger SSP gel-actomyosin matrix could therefore be obtained. When chicken protein hydrolysates was added into raw restructured chicken meat, the G′ patterns similar to that of the control pattern was observed and the shifting up of these patterns was obvious (figure 4.37 and 4.40). This result indicated the formation of stronger gel network. Dublier, Launay and Cuvelier (1992) explained that the shifting up of G′ pattern occurred when the system was reinforced with the suitable fillers or binders and the more rigid gels were obtained. In this experiment, the extracted SSP around meat particles could form the continuous gel network and the added protein hydrolysates could act as gel filler. The formation of the filled gel was also reported by Tolstoguzov (1986). They also report that the filled gel was formed in the system conditions similar to this experiment in that one of the components could form a continuous network over the entire system and the other polymeric component could disperse into the gel space and served as the gel filler. Correia and Mittal (1992) also reported the rheological improvement of meat emulsion batters from the addition of protein filler such as soy protein hydrolysates and whey protein hydrolysates. When alkali-precipitated chitosan was added concomitantly with chicken protein hydrolysates, an almost similar G′ patterns to that of the control were observed (figure 4.37 and 4.41). However, the patterns with the significant increase of G′ value and the loss of the peak of the dissociation of actomyosin complex at 50-55˚C were resulted. The highest G′ value at 80˚C was also obtained when comparing with treatments

with each binder alone and control ($p \leq 0.05$) (table 4.12). These results suggested that raw restructured chicken meats with the combination of alkali-precipitated chitosan and chicken protein hydrolysates exhibited the stronger gel network. Correia and Mittal (1992) also explained that the shift up of G' patterns indicated the formation of strong and rigid structure of meat emulsion batters. Andrès, Zaritzky and Califano (2006) also reported that textural improvement of chicken sausages from the addition of whey protein concentrate in cooperated with guar or xanthan gum at suitable concentration.

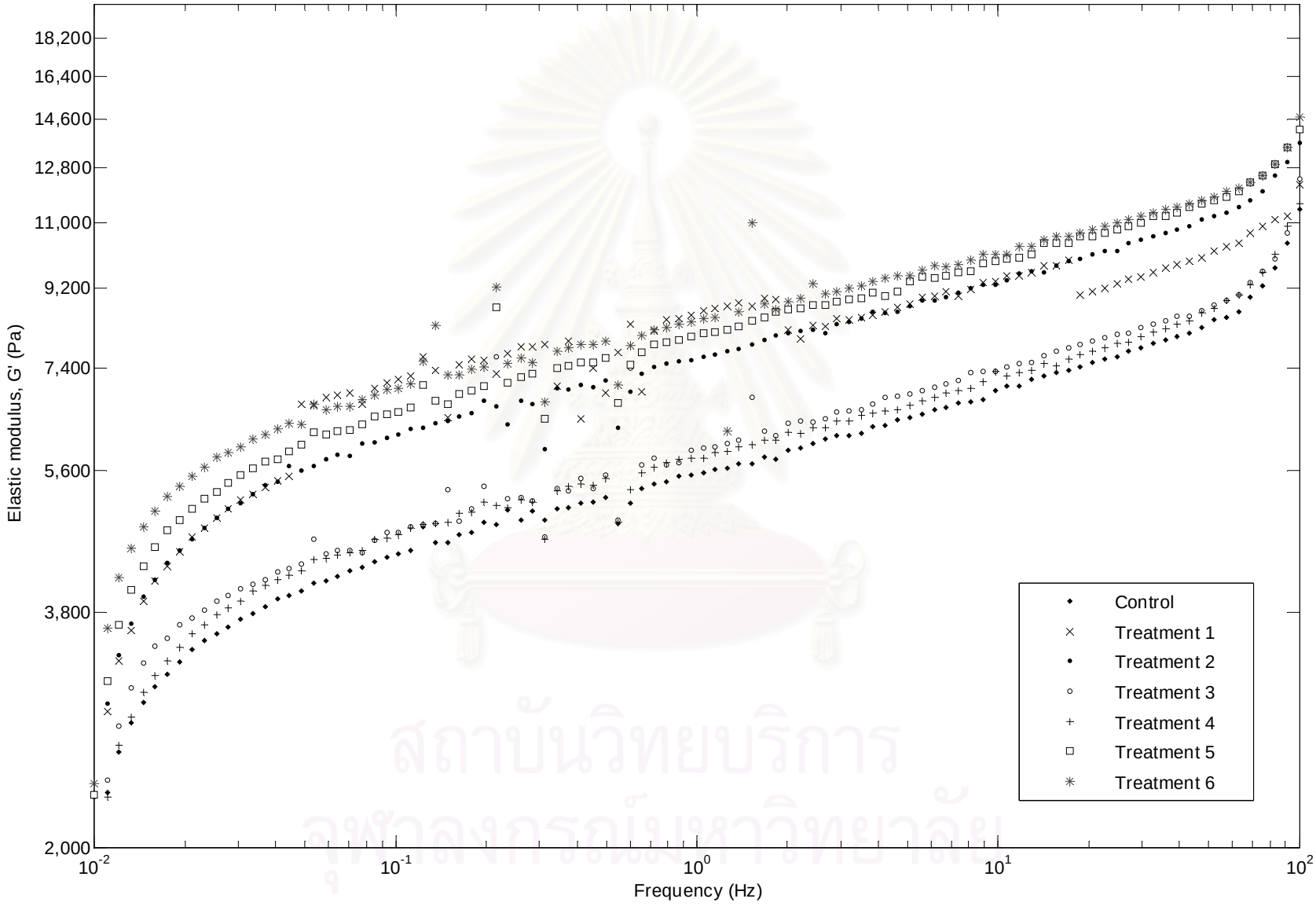


Figure 4.35 Elastic modulus or G′ (Pa) from oscillation frequency sweep test of raw restructured chicken meat samples containing various concentrations of chitosan, chicken protein hydrolysates and their combinations.

164

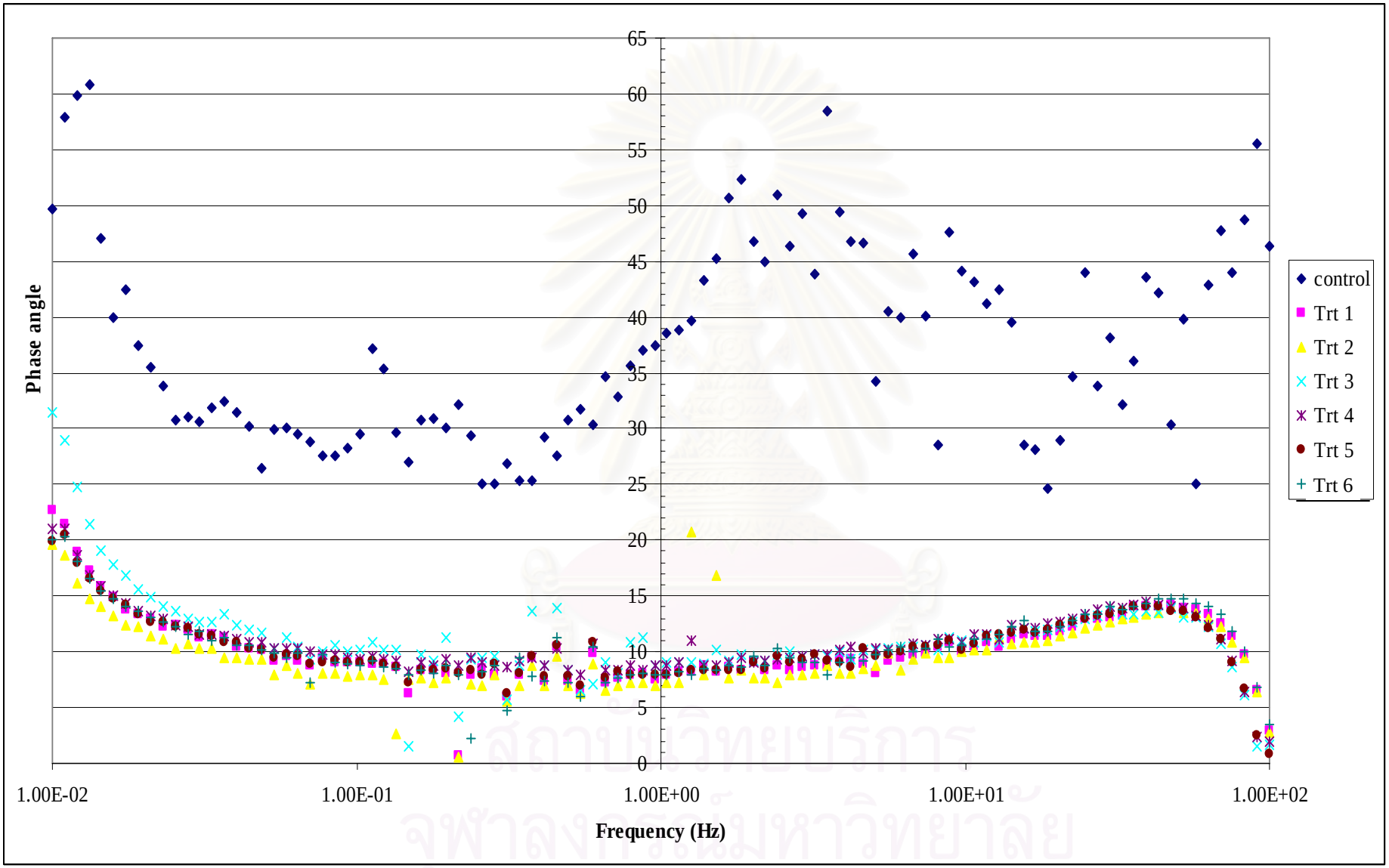


Figure 4.36 Phase angle (δ) from oscillation frequency sweep test of raw restructured chicken meat samples containing various concentrations of chitosan, chicken protein hydrolysates and their combinations.

165

Table 4.12 Storage modulus (G′) before cooking (25˚C) and after cooking (80˚C) of raw restructured chicken meat samples containing various concentrations of chitosan, chicken protein hydrolysates and their combinations.

Treatment No.	**Storage Modulus** (G′, Pa) (cooking)	
	25˚C	80˚C
Control	949.67[a]	17,933.34[a]
1	1,990.12[b]	22,900.25[b]
2	2,758.68[c]	26,933.87[c]
3	2,852.34[cd]	28,239.45[c]
4	3,036.67[d]	29,406.57[d]
5	3,750.06[e]	35,910.29[e]
6	3,926.67[ef]	45,287.65[f]

[a-e] Means followed by different letters within the same column are significantly different ($p \leq 0.05$)

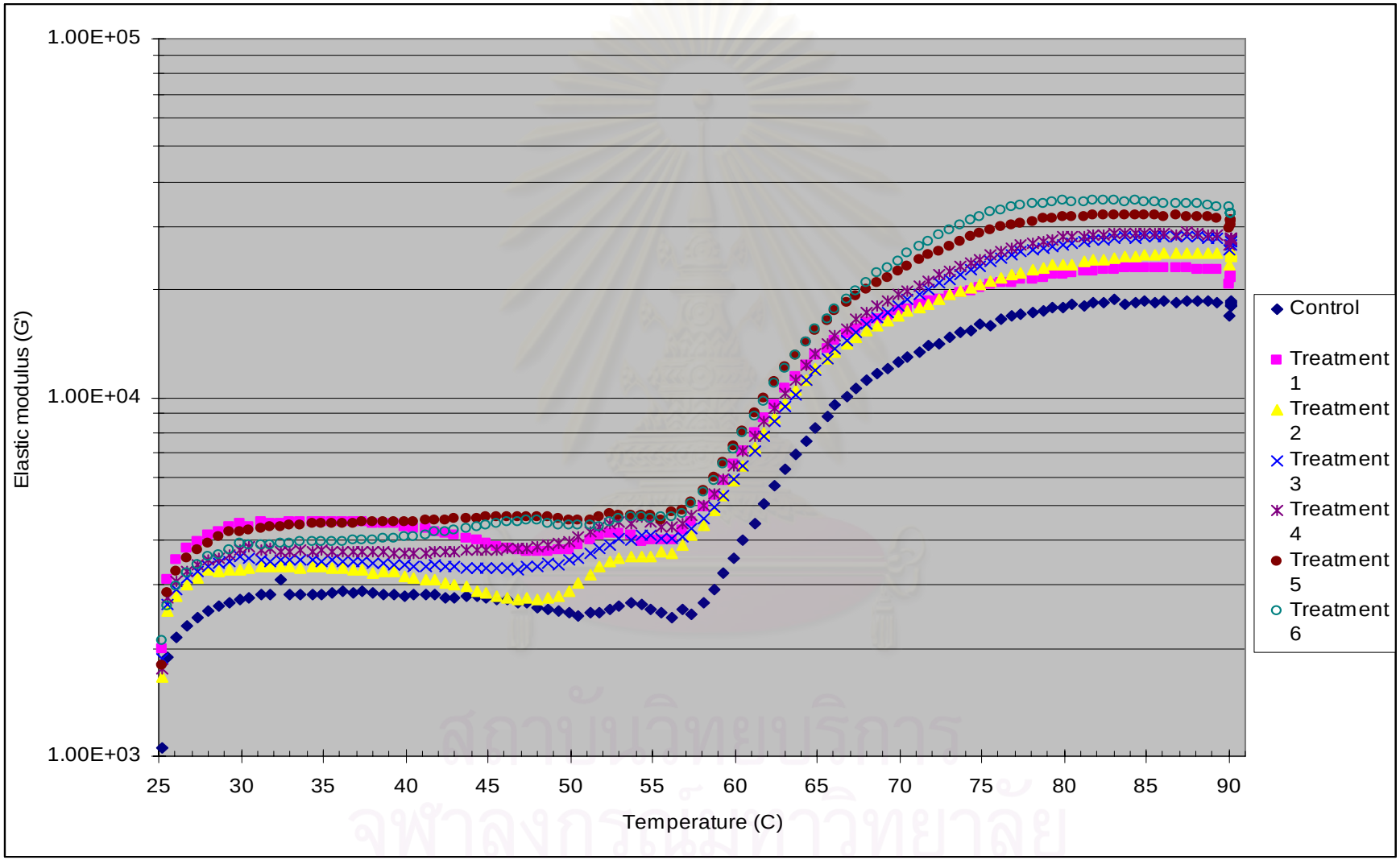


Figure 4.37 Elastic modulus or G′ (Pa) from oscillation temperature sweep test (heating phase) of raw restructured chicken meat samples containing various concentrations of chitosan, chicken protein hydrolysates and their combinations.

167

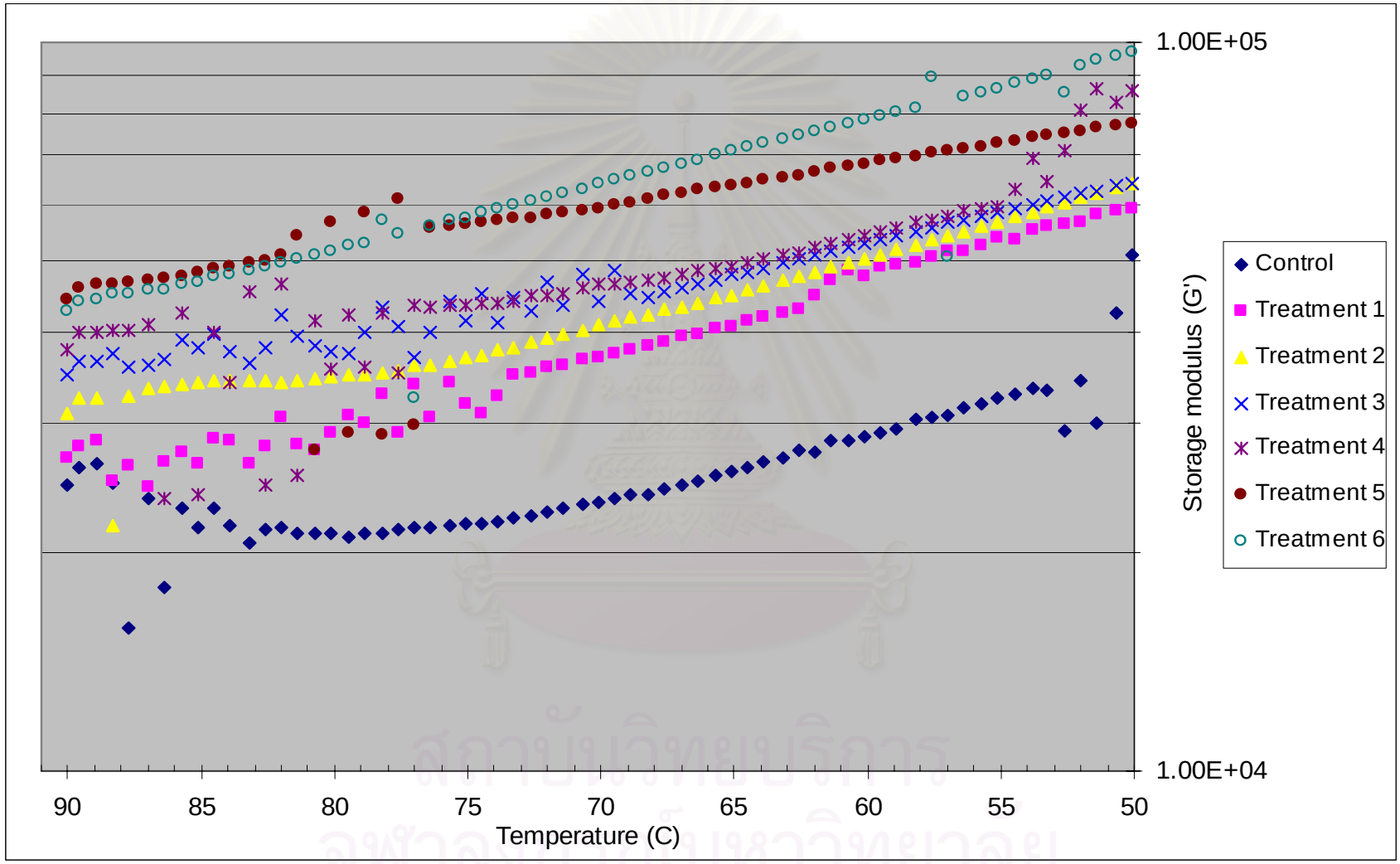


Figure 4.38 Elastic modulus or G´ (Pa) from oscillation temperature sweep test (cooling phase) of raw restructured chicken meat samples containing various concentrations of chitosan, chicken protein hydrolysates and their combinations.

168

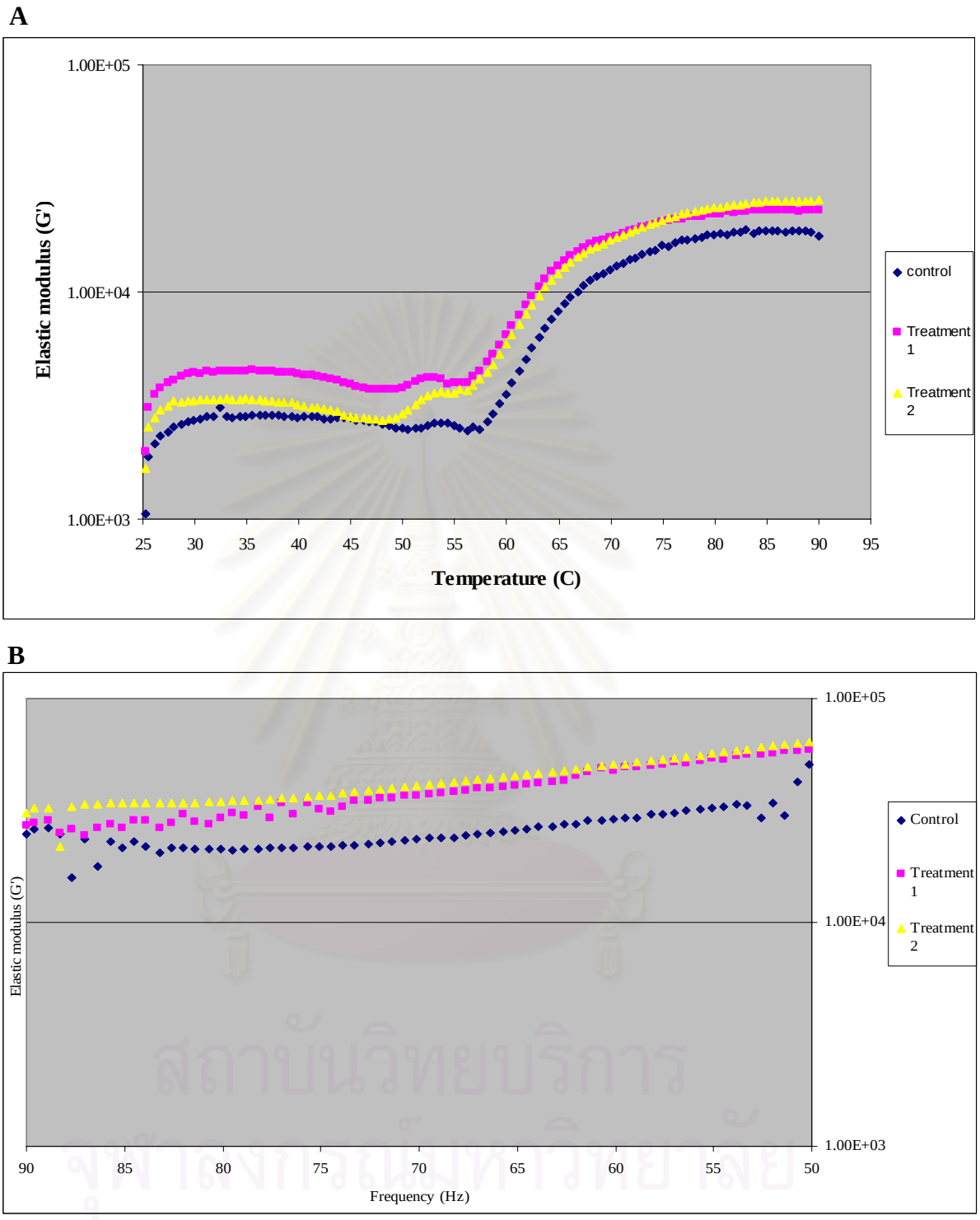


Figure 4.39 Elastic modulus or G′ (Pa) from oscillation temperature sweep test of raw restructured chicken meat samples containing various concentrations of chitosan. **A** represents the changes of G′ in heating phase and **B** represents the changes of G′ in cooling phase.

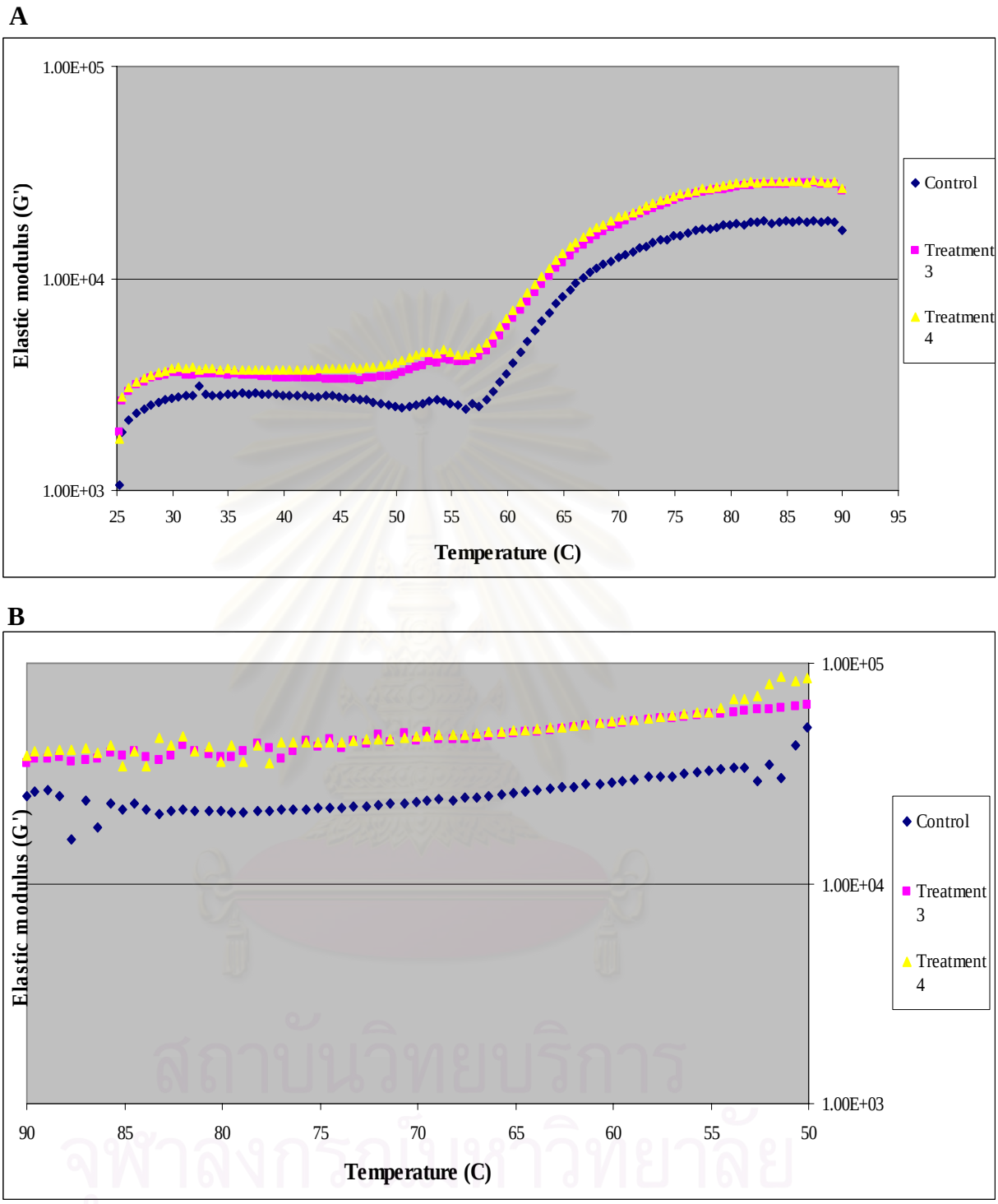


Figure 4.40 Elastic modulus or G′ (Pa) from oscillation temperature sweep test of raw restructured chicken meat samples containing various concentrations of chicken protein hydrolysates. **A** represents the changes of G′ in heating phase and **B** represents the changes of G′ in cooling phase.

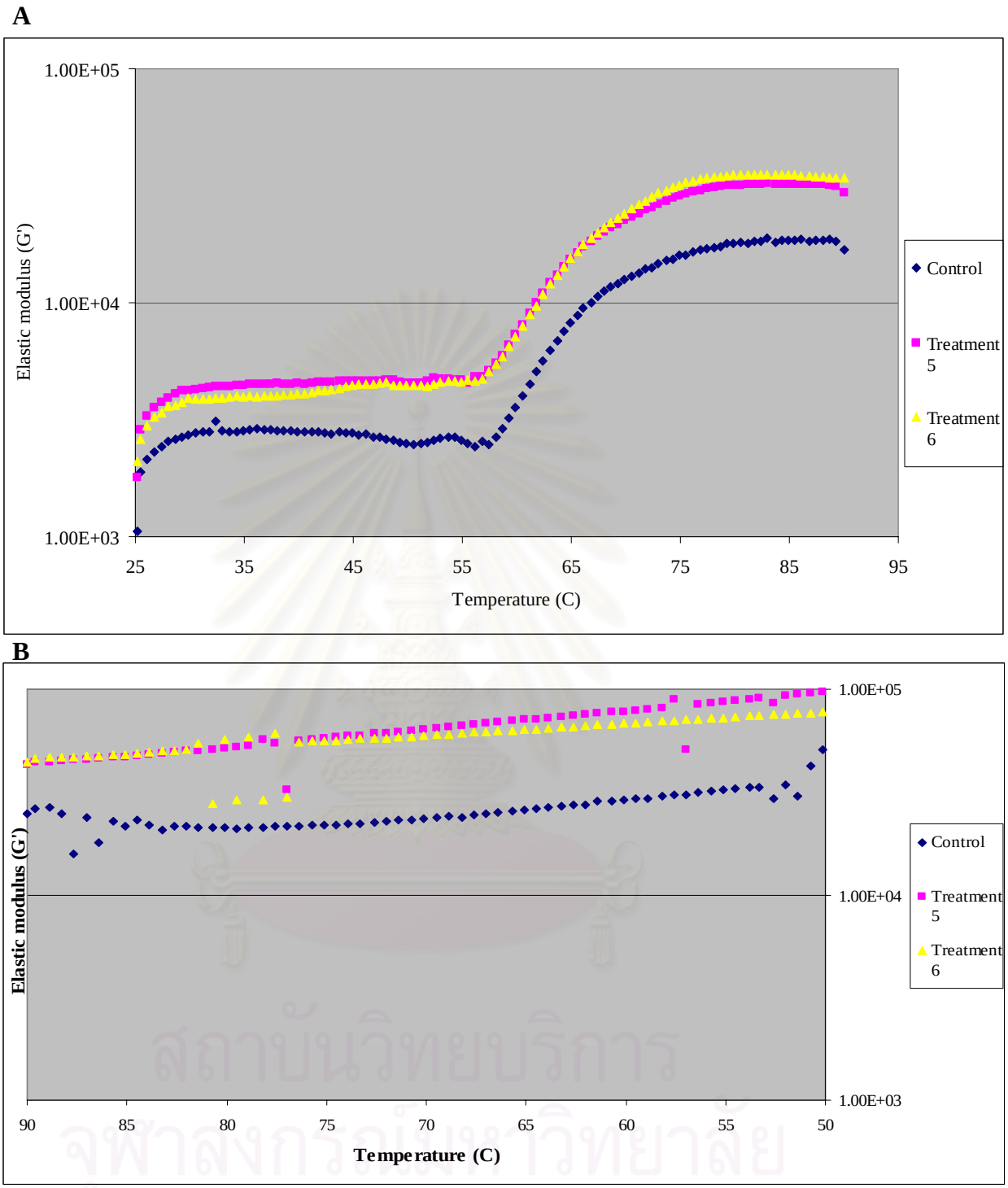


Figure 4.41 Elastic modulus or G′ (Pa) from oscillation temperature sweep test of raw restructured chicken meat samples containing the combinations of chitosan at chicken protein at various concentrations. **A** represents the changes of G′ in heating phase and **B** represents the changes of G′ in cooling phase.

4.5.4 Effect on Textural Characteristics

The texture of the obtained raw restructured chicken meat was determined using texture profile analysis (TPA) including hardness (N/cm^2), cohesiveness (ratio), adhesiveness (cm^3), gumminess (N/cm^2), springiness (cm) and chewiness (N/cm) as shown in table 4.13. TPA was selected to be used in this experiment because it provided the textural information which could be correlated with the sensory characteristics (Bourne, 1978). The obtained results completely explained the complicated texture of raw restructured meats which needed for the quality control. In SSP gels, only the measurement of maximum compression force or hardness was enough for elucidating effect of the added binders since this uncomplicated system contained only SSP and the binders.

In the previous studies of SSP gels, the improvement of textural parameter as expressed in term of maximum compression force was obtained when alkali-precipitated chitosan, chicken protein hydrolysates and their combinations were added. The highest improvement was found when combinations of the two cold-set binders were used. This phenomenon was also exhibited in raw restructured chicken meats. The hardness of such product was significantly increased ($p \leq 0.05$), especially in treatment with combination binders at high concentration. The addition of binders affected this parameter more than the others as shown by the increase of hardness from that of the control around 2-3 times in treatments with each binders and around 4-5 times in treatments with combination binders. These results might occur from the structural reinforcement of the added alkali-preciptiated chitosan and chicken protein hydrolysates. The report of Andrés, Zaritzky and Califano (2006) also revealed the textural

improvement detected by TPA in chicken sausages from the combination use of whey proteins and guar or xanthan gum.

The cohesiveness of the obtained raw restructured meats was significantly improved when cold-set binders were added ($p \leq 0.05$). Raw restructured chicken meats with chicken protein hydrolysates exhibited the higher cohesiveness than treatments with alkali-precipitated chitosan when using at 3% concentration. The increase of cohesiveness indicated the strength of the gel network which could be recovered from the first compression (Bourne, 1978). Chicken protein hydrolysates homogeneously fortified the raw restructured chicken meat structure with the more improvement than that of the alkali-precipitated chitosan. This result might occur from the higher water solubility and the smaller molecular size of chicken protein hydrolysates than that of the alkali-precipitated chitosan. When using the two binders concomitantly, the best improvement was obtained due to the complex formation as previously described. This result was agreed with the report of Chung *et al.* (2000). They also found the improvement of tensile strength and meat binding ability from the addition mackerel muscle protein hydrolysates as binder in restructured pork meats. Moreover, the report of Barbut in 2006 also revealed the enhancement of textural properties of chicken meat batters from the addition of whey protein hydrolysates.

The similar results were found in adhesiveness; however, the addition of alkali-precipitated chitosan had the major effect on this parameter. The use of this binder at 1.5% by weight improved the adhesiveness of the obtained products similar to that of the treatments with 2 and 3% chicken protein hydrolysates, and the combination of 1% alkali-precipitated chitosan with 2% chicken protein hydrolysates. This result

indicated that the addition of polysaccharide into the protein matrix increased the adhesive force from the thickening behavior of the high MW polysaccharides (Da Silva and Rao, 1992). The increase of system viscosity could be obtained as the increase of adhesiveness. This result was agreed with the report of López-Caballero *et al*. (2005). They also found the increase of adhesion from the addition of chitosan powder into fish sausage. The textural improvement from the addition of chitosan was also discovered by Popper *et al*. (2001). They also reported the use of chitosan as binder in gelled emulsion meat products with the improvement of gel firmness and adhesiveness.

The different result was observed in springiness. The addition of binders either alone or in combination significantly increased springiness of the obtained raw restructured meat from that of the control ($p \leq 0.05$); however, the significant effect of concentration and binder type was not observed ($p > 0.05$). Springiness of all treatments with cold-set binders was not significantly different from each others. This result indicated that these binders could not improve the elasticity of the gel. The harder gel with the more brittleness was obtained. This result was also reported in surimi products with the combination use of chitosan and transglutaminase (Benjakul *et al*., 2003; Gómez-Guillèn *et al*., 2005). According to the remaining textural parameters, gumminess and chewiness, these two parameters were calculated from the measured hardness and cohesiveness, and hardness, cohesiveness and springiness, respectively. Hence, their changes were totally depended on such measured parameters and the similar tendency to that of the gel hardness was observed.

Table 4.13 Texture profiles from TPA of raw restructured chicken meats samples containing various concentrations of chitosan, chicken protein hydrolysates and their combinations.

Treatment	Textural parameters					
	Hardness (N/cm^2)	**Cohesiveness** (ratio)	**Adhesiveness** (cm^3)	**Gumminess** (N/cm^2)	**Springiness** (cm)	**Chewiness** (N/cm)
Control	$35.09^{a} \pm 1.05$	$0.011^{a} \pm 0.005$	$20.52^{a} \pm 1.89$	$1.08^{a} \pm 0.19$	$0.15^{a} \pm 0.05$	$0.32^{a} \pm 0.09$
1	$87.03^{b} \pm 2.87$	$0.029^{b} \pm 0.012$	$47.76^{b} \pm 1.54$	$2.63^{b} \pm 0.22$	$0.34^{bc} \pm 0.10$	$1.01^{b} \pm 0.15$
2	$112.90^{bc} \pm 3.63$	$0.028^{b} \pm 0.011$	$72.38^{c} \pm 1.78$	$3.54^{bc} \pm 0.44$	$0.35^{bc} \pm 0.03$	$1.28^{b} \pm 0.52$
3	$106.93^{bc} \pm 5.67$	$0.023^{b} \pm 0.016$	$61.33^{bc} \pm 4.17$	$2.65^{b} \pm 0.34$	$0.32^{bc} \pm 0.07$	$0.57^{a} \pm 0.28$
4	$147.86^{c} \pm 7.99$	$0.031^{bc} \pm 0.023$	$78.93^{c} \pm 2.88$	$3.95^{c} \pm 0.55$	$0.35^{bc} \pm 0.08$	$1.51^{bc} \pm 0.74$
5	$142.12^{c} \pm 4.13$	$0.040^{c} \pm 0.029$	$84.82^{cd} \pm 9.57$	$5.53^{d} \pm 1.69$	$0.39^{c} \pm 0.11$	$2.03^{c} \pm 0.56$
6	$189.06^{d} \pm 7.85$	$0.050^{cd} \pm 0.024$	$98.21^{d} \pm 1.16$	$6.35^{d} \pm 1.12$	$0.42^{c} \pm 0.09$	$2.67^{d} \pm 0.49$

$^{a-d}$ Means followed by different letters within the same column are significantly different ($p \leq 0.05$).

4.5.5 Effect on Water Binding Properties

Water binding properties of the resulting raw restructured chicken meats were significantly improved by the addition of cold-set binders as shown by the reduction of cooking loss and the increase of WHC ($p \leq 0.05$).

The addition of alkali-precipitated chitosan alone significantly reduced cooking loss when comparing with that of the control ($p \leq 0.05$); however, a better result was obtained in the presence of chicken protein hydrolysates ($p \leq 0.05$). When comminuted muscle products were heated, denaturation of myofibrillar proteins was obtained followed by their coagulation, shrinkage of myofibrils and tightening of the microstructure, and resulted in expelling of free water which was easily to loose from the muscle structure and decreasing of immobilized water (Xiong, 1997). The addition of binders either proteins or polysaccharides which possessed high water binding properties provided the products with more juiciness. After mixing, these binders were dispersed to fill into the gel matrix of the products and promoted water binding during cooking by using hydroxyl, carbonyl and amino groups in their side chain (Zayas, 1997). When using alkali-precipitated chitosan alone, the free and reactive amino group inside its chains could bind with water in the products with electrostatic interactions and hydrogen bonding, hence the reduction of cooking loss was obtained. Meanwhile, the less cooking loss in treatments with chicken protein hydrolysates occurred due to hydrogen bondings between water and the short chain peptides which possessed high amount of hydroxyl, carboxyl and amino groups. Shahidi and Synowieki (1997) also reported the improvement of cooking loss and water binding properties from the addition of seal protein hydrolysates into restructured seal meats. Due to the complex formation in

treatments with combination of alkali-precipitated chitosan and chicken protein hydrolysates, it could be occurred because these two binders were homogeneously mixed before added into the raw restructured meats. The electrostatic complexes could be partially occurred (Tolstoguzov, 1986). When heat was applied to comminuted meat products with salt, the denaturation of the proteins occurred. The gels could hold water in the interstices of a myosin-muscle particle gels network. Most of the water was located in the large spaces occupied by the myofibrils and the myosin gel with the myofibrils (Offer and Knight, 1988). The improvement of cooking loss from the addition of binders, either polysaccharides or proteins, might occur from their filling effects which these binders might fill into the large space between muscle particles and the smaller voids with the higher water binding efficiency was obtained (Meste, Simatoes and Gervais, 1995; Xiong, 1997). Moreover, Tolstoguzov (1974) also reported that the inclusion of the electrostatic complexes could effectively fill into the matrix of meat proteins and trap water molecule inside. Hence, the highest reduction of cooking loss was resulted. Furthermore, the increasing of water binding sites from the addition of combination binders at high amount might be another reason responsible for the decrease of cooking loss.

The significant increase of WHC from that of the control was detected in all treatments with alkali-precipitated chitosan, chicken protein hydrolysates and their combinations (table 4.14) ($p \leq 0.05$). Raw restructured chicken meats with chicken protein hydrolysates exhibited higher WHC than treatments with alkali-precipitated chitosan, especially at high concentration (treatment 4) ($p \leq 0.05$). This result suggested that chicken protein hydrolysates functioned as water binding agent in the

obtained products. This result was agreed with the report of Satterlee, Zachariah and Levin (1973). They found the improvement of water binding capacity in sausages from the addition of protein hydrolysates from beef or pork skin. The enhancement of water binding properties which was mainly driven by chicken protein hydrolysates was from the increase of charged peptides after enzymatic hydrolysis with papain. These charged groups could interact and form hydrogen bonding with water molecules in the gel setting period at low temperature (Fik and Surówka, 1986; Fonkwe and Singh, 1996). The reports of Higuchi, Ojima and Nishita (2002), Hossain *et al*. (2003), and Khan *et al*. (2003) revealed that the added protein hydrolysates helped protecting myofibrillar proteins from denaturation at low temperature by interacting with water molecules around the myofibrillar proteins through their charged groups. Thus, the higher improvement of WHC in treatments with chicken protein hydrolystes was obtained. Furthermore, chicken protein hydrolystes was also functioned as good water binding agent even in the system with alkali-precipitated chitosan (treatment 5 and 6).

Table 4.14 Cooking loss (%) and water holding capacity (WHC) (%) of raw restructured chicken meats samples containing various concentrations of chitosan, chicken protein hydrolysates and their combinations.

Treatment	Cooking loss (%)	Water holding capacity (WHC) (%)
Control	$13.97^{d} \pm 1.19$	$90.65^{a} \pm 3.39$
1	$9.01^{c} \pm 1.58$	$95.58^{b} \pm 3.23$
2	$8.68^{c} \pm 1.44$	$97.13^{bc} \pm 3.58$
3	$7.67^{b} \pm 0.47$	$99.48^{c} \pm 0.73$
4	$7.15^{b} \pm 0.81$	$99.72^{c} \pm 0.47$
5	$7.84^{bc} \pm 1.05$	$99.62^{c} \pm 0.48$
6	$6.06^{a} \pm 0.86$	$99.96^{c} \pm 0.076$

[a-d] Means followed by different letters within the same column are significantly different ($p \leq 0.05$).

4.5.6 Effect on Structural Profiles

The structure profiles of the obtained raw restructured chicken meats with cold-set binders and control were studied by scanning electron microscope and the electron micrographs at 500, 1,000, 3,500 and 5,000 magnifications were collected. The results in figure 4.42-4.53 showed that the addition of alkali-precipitated chitosan, chicken protein hydrolysates, and their combinations drastically modified the resulting strtucture as comparing with that of the control. Moreover, their structure was significantly different from the structure of SSP gels. The network of fine protein strands as detected in SSP structure (figure 4.11, 4.12, 4.13, 4.22, 4.31, 4.32 and 4.33) could not be observed because the raw restructured chicken meats contained meat particles in the system. The continuous matrix of meat particles coated with the extracted SSP and cold-set binders were expected. From scanning electron micrographs at 500 magnifications, the structural modification effect of alkali-precipitated chitosan was observed (figure 4.42). The formation of dense gel zones were detected (figure 4.42 B and C). These gel zones occurred and dispersed uniformly in muscle protein gel matrix. Alkali-precipitated chitosan acted as binder in SSP gel which bonded with muscle proteins and induced macromolecules closer to form the dense muscle protein gel matrix. The increase of chitosan concentration resulted in the denser gel zones which dispersed homogeneously inside gel matrix. The results were confirmed when the scanning electron micrographs were magnified to higher magnifications at 1,000, 3,500 and 5,000 times (figure 4.43-4.45). At 1,000 magnifications, many voids were obvious inside the control gel matrix, while the denser and more uniform gel network was clearly elucidated in samples containing chitosan (figure 4.43). The electron micrographs at 3,500 magnifications

demonstrated that there was no pour inside structure of the products with alkali-precipitated chitosan and the dense gel zones were bonded with muscle gel matrixes (figure 4.44 B and C at the arrow pointer). Various interactions such as electrostatic interactions, salt bridges, hydrogen bonds and some of covalent bonds might occur to stabilize this gel structure. When increasing the magnifications to 5,000 times, the uniform arrangement of muscle proteins and alkali-precipitated chitosan became even clearer (figure 4.45 B and C). The junction between dense gel zones and muscle protein gel matrix was homogenously bound. These profiles accentuated the formation of the stronger gel matrix from the addition of alkali-precipitated chitosan.

The raw restructured chicken protein matrix became denser when chicken protein hydrolysates was added (figure 4.46-4.49). At 500 magnifications, the scanning electron micrographs of products with hydrolysates showed a compact structure (figure 4.46). The chicken protein hydrolysates which was composed of myosin and other myofibrillar proteins increased the amount of free and soluble myofibrillar proteins in the system, hence provided a glue-like substance for the muscle fibers. In samples with high amount chicken protein hydrolysates, the structures became denser and more homogeneous (figure 4.46 C), which indicated the structure reinforcement from these proteins. This result was confirmed by increasing the magnifications to 1,000, 3,500 and 5,000 times (figure 4.47-4.49). No porous was found even at low binder concentration. The area of chicken protein hydrolysates in raw restructured chicken meat structure was obviously observed (figure 4.47 C, 4.48 C and 4.49 C at the arrow pointer). This result confirmed the formation of filled gel which the muscle particles coated with SSP formed the continuous network with many voids (figure 4.47 A, 4.48 A and 4.49 A), and the

added chicken protein hydrolysates acted as gel filler which filled into such voids. This formation provided the stronger gel network. However, this phenomenon occurred only in treatment with 3% chicken protein hydrolysates. The electron micrographs of treatment with this binder at 2% could be observed only the tightly bound junction zone between the binder and the extracted SSP (figure 4.47 B, 4.48 B and 4.49 B), and this result might occur from the low binder concentration.

The combination use of alkali-precipitated chitosan and chicken protein hydrolysates in raw restructured chicken meat products resulted in drastic improvement of gel structure of the obtained products (figure 4.50-4.53). The most dense and uniform gel structure was observed while the structure of control was non-homogeneous and had many voids inside (figure 4.51-4.53). The formation of the dense gel zones similar to the structure of products with alkali-precipitated chitosan was detected, but more frequently (figure 4.50 B and C). This result indicated that alkali-precipitated chitosan could modify the resulting gel structure even though in the system with chicken protein hydrolysates. The increase of alkali-precipitated chitosan concentration to 1.5% incorporated with 3% chicken protein hydrolysates resulted in the formation of various tightly bound junctions between the dense gel zones and the muscle protein matrix (figure 4.52 C and 4.53 C). These profiles indicated the formation of the strong gel network resulted from structural modulation of the added cold-set binders. Alkali-precipitated chitosan could act as both cold-set gel binder and filler which modulated the muscle proteins and induced these macromolecules to form various interactions as previously described, while chicken protein hydrolysates could act as gel filler which filled and reinforced the structure of the resulting raw restructured chicken

meats. Samant *et al*. (1993) also explained that the proteins and polysaccharides in the system at suitable condition could interact and form the filled gel network. Furthermore, in this experiment, alkali-precipitated chitosan and chicken protein hydrolysates were mixed homogeneously before added into raw restructured chicken meats, hence there was an opportunity for some of these binders to interact and form the electrostatic complexes. These complexes and the remained portions of alkali-precipitated chitosan and chicken protein hydrolysates could disperse uniformly into the gel matrix of cold-set restructured chicken meat and might responsible for the structural changes and textural improvement of the resulting products. However, these complexes could not be observed from the scanning electron micrograph in this experiment due to the complexity of the system.

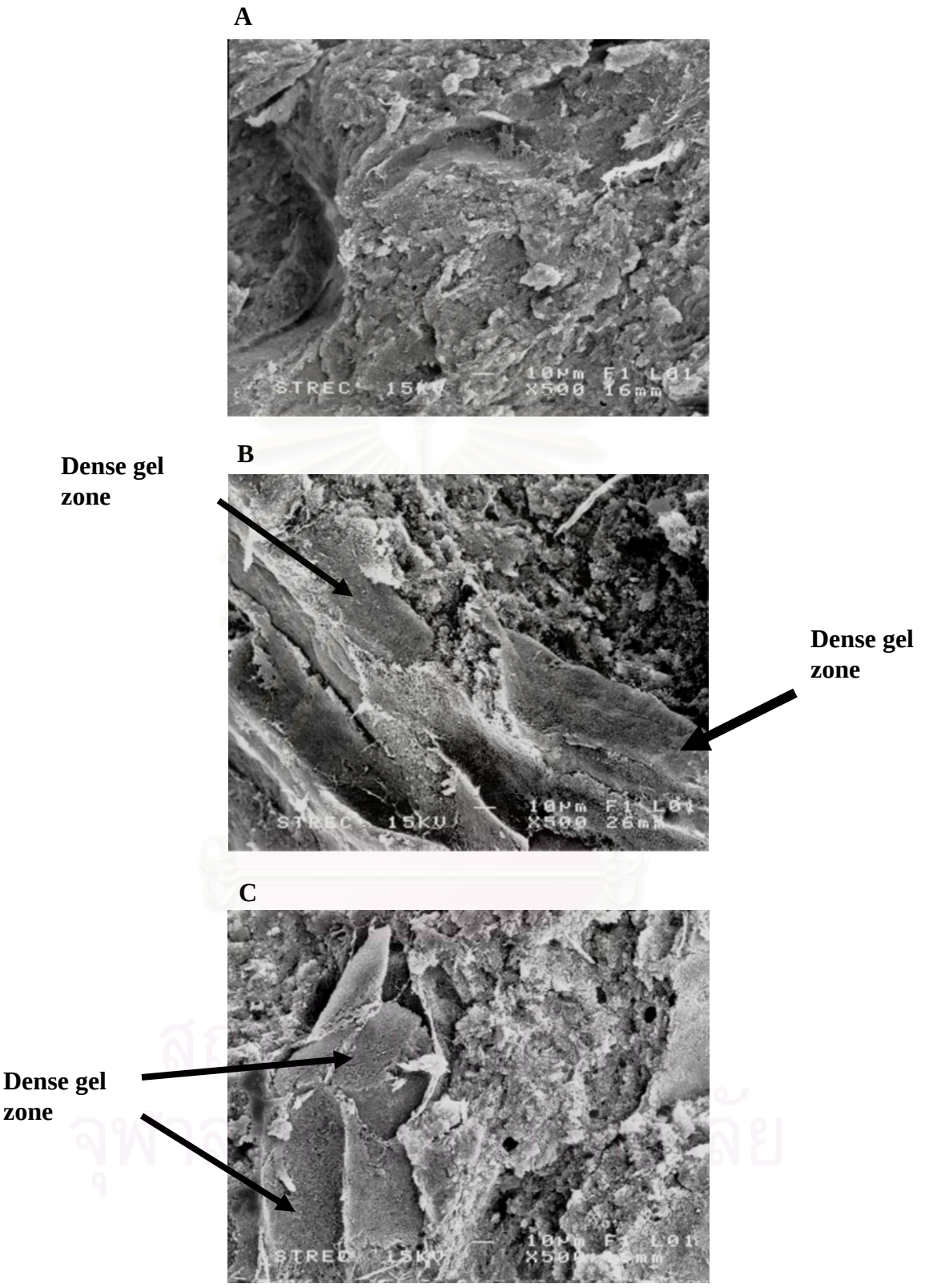


Figure 4.42 Scanning electron micrographs (500 x magnifications) of raw restructured chicken meats made from chicken broiler tenderloin meats without cold-set binder (A), and with chitosan at 1% by weight (B) and 1.5% by weight (C).

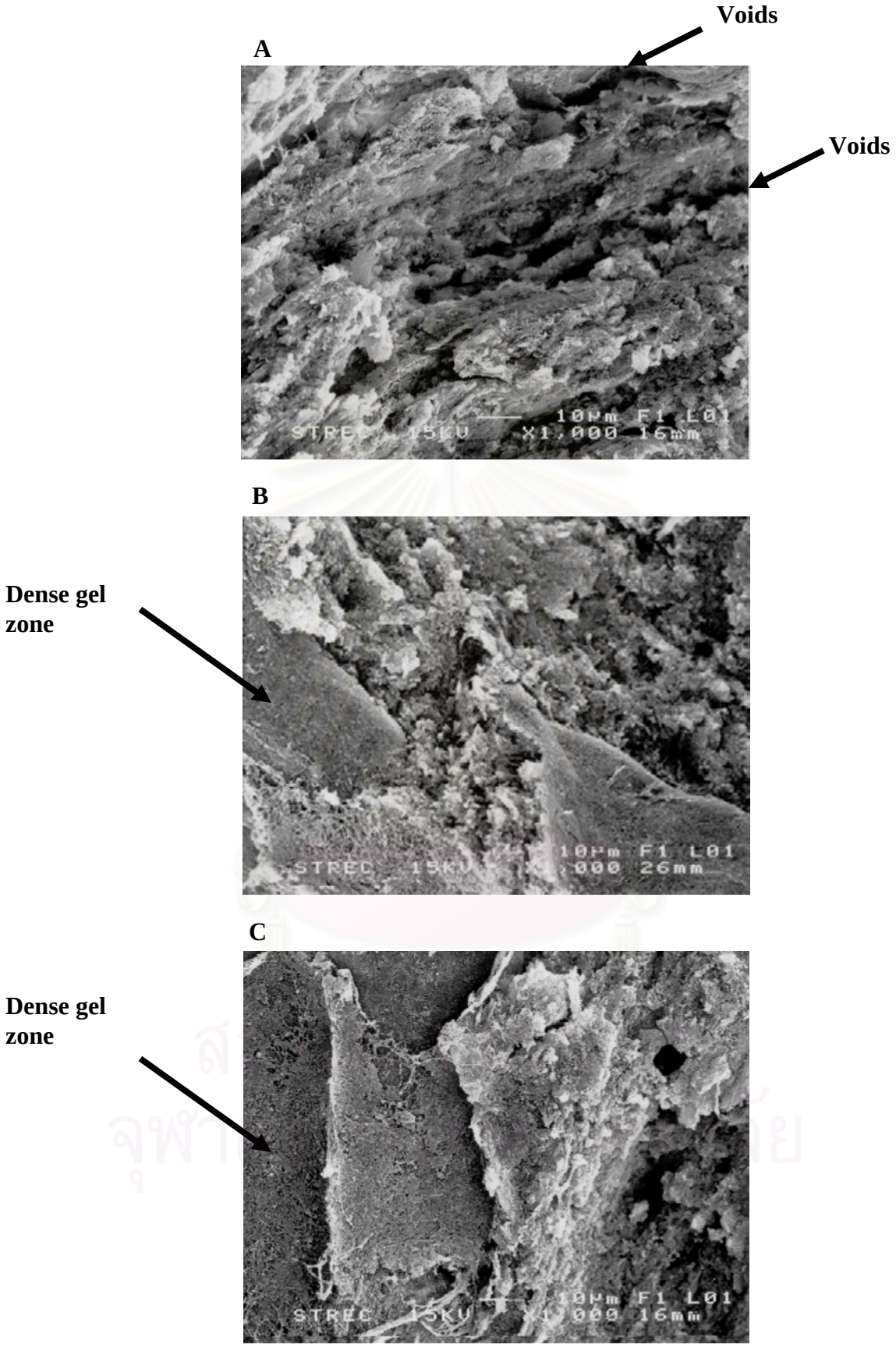


Figure 4.43 Scanning electron micrographs (1,000 x magnifications) of raw restructured chicken meats made from chicken broiler tenderloin meats without cold-set binder (A), and with chitosan at 1% by weight (B) and 1.5% by weight (C).

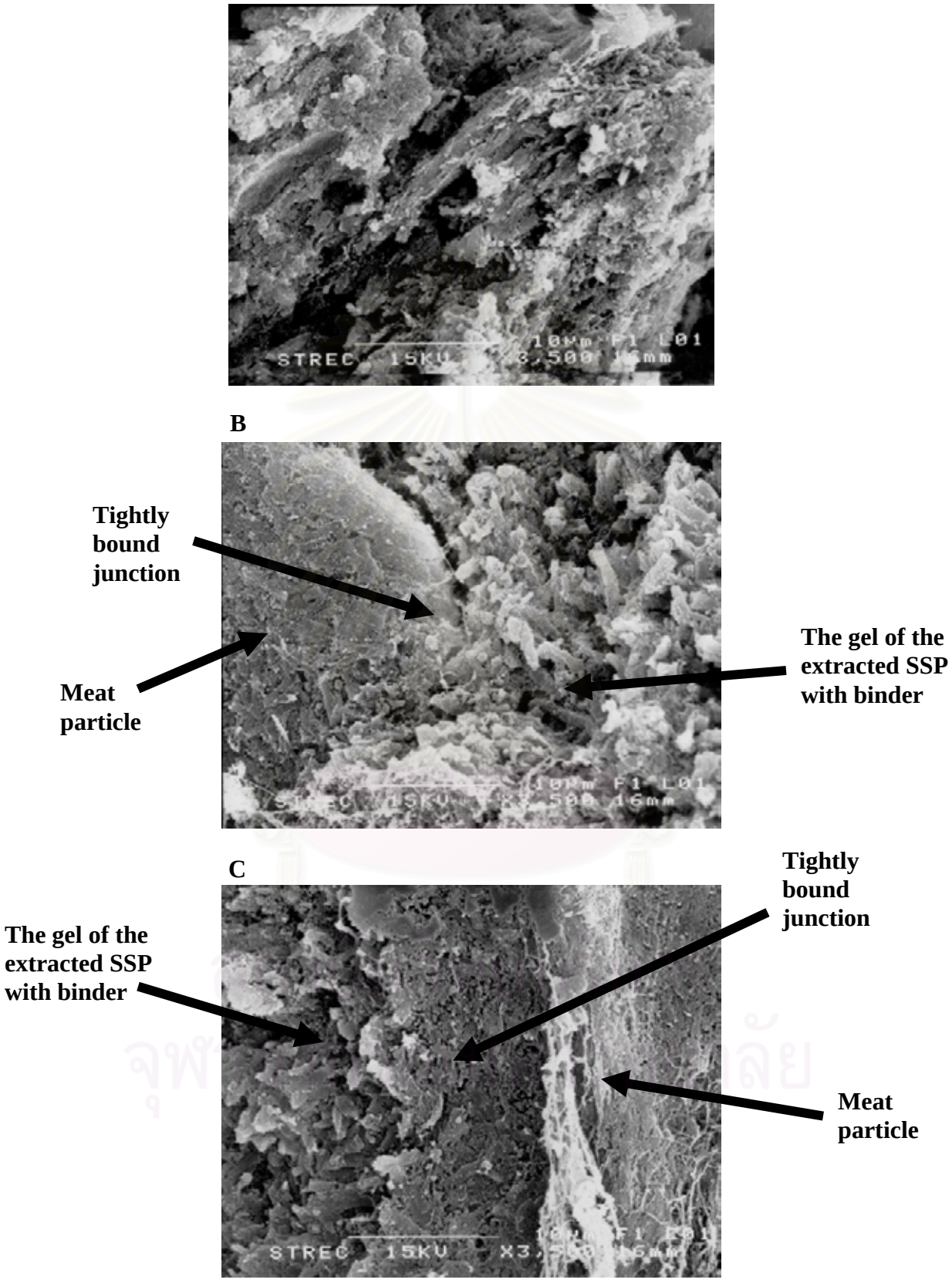


Figure 4.44 Scanning electron micrographs (3,500 x magnifications) of raw restructured chicken meats made from chicken broiler tenderloin meats without cold-set binder (A), and with chitosan at 1% by weight (B) and 1.5% by weight (C).

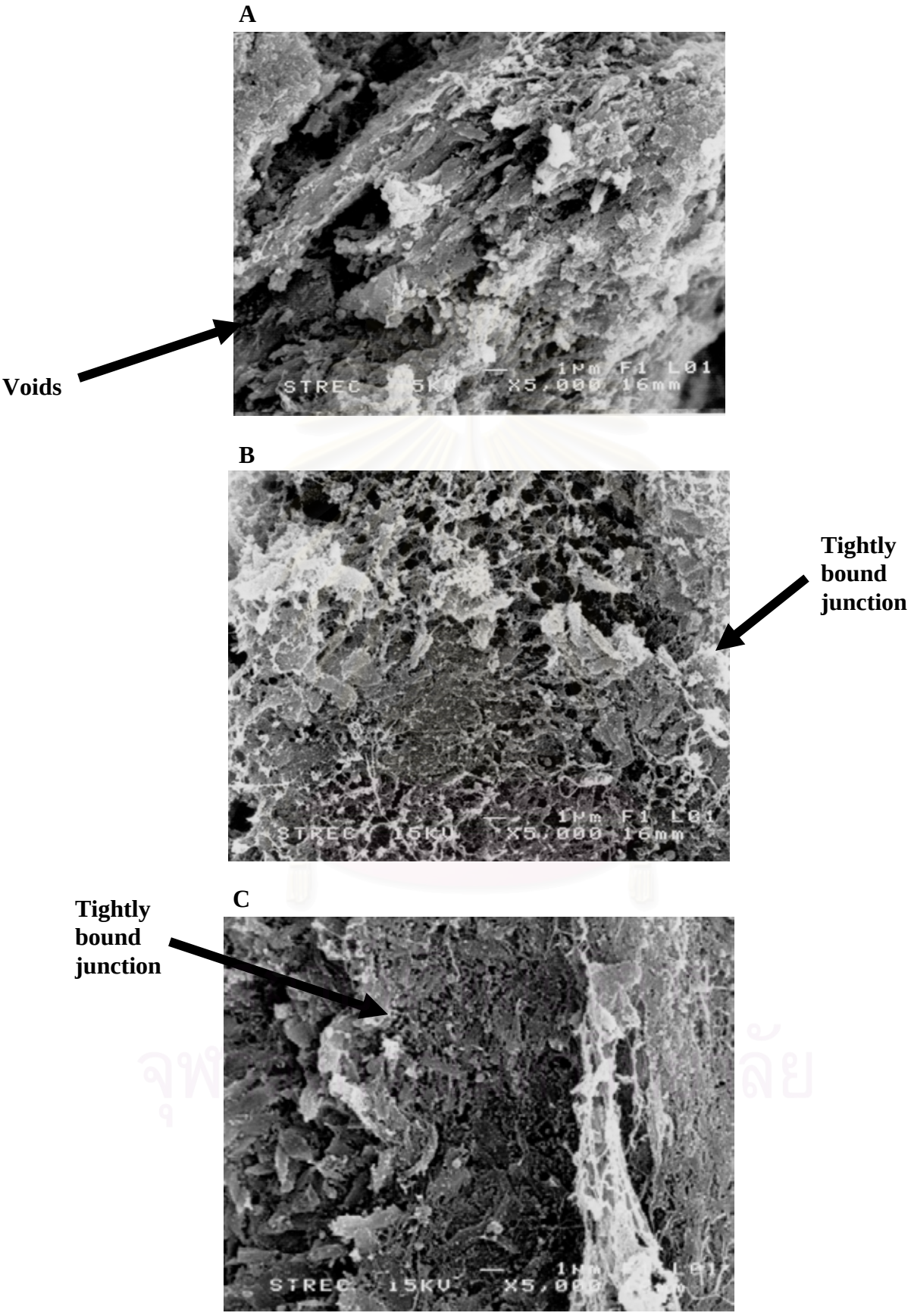


Figure 4.45 Scanning electron micrographs (5,000 x magnifications) of raw restructured chicken meats made from chicken broiler tenderloin meats without cold-set binder (A), and with chitosan at 1% by weight (B) and 1.5% by weight (C).

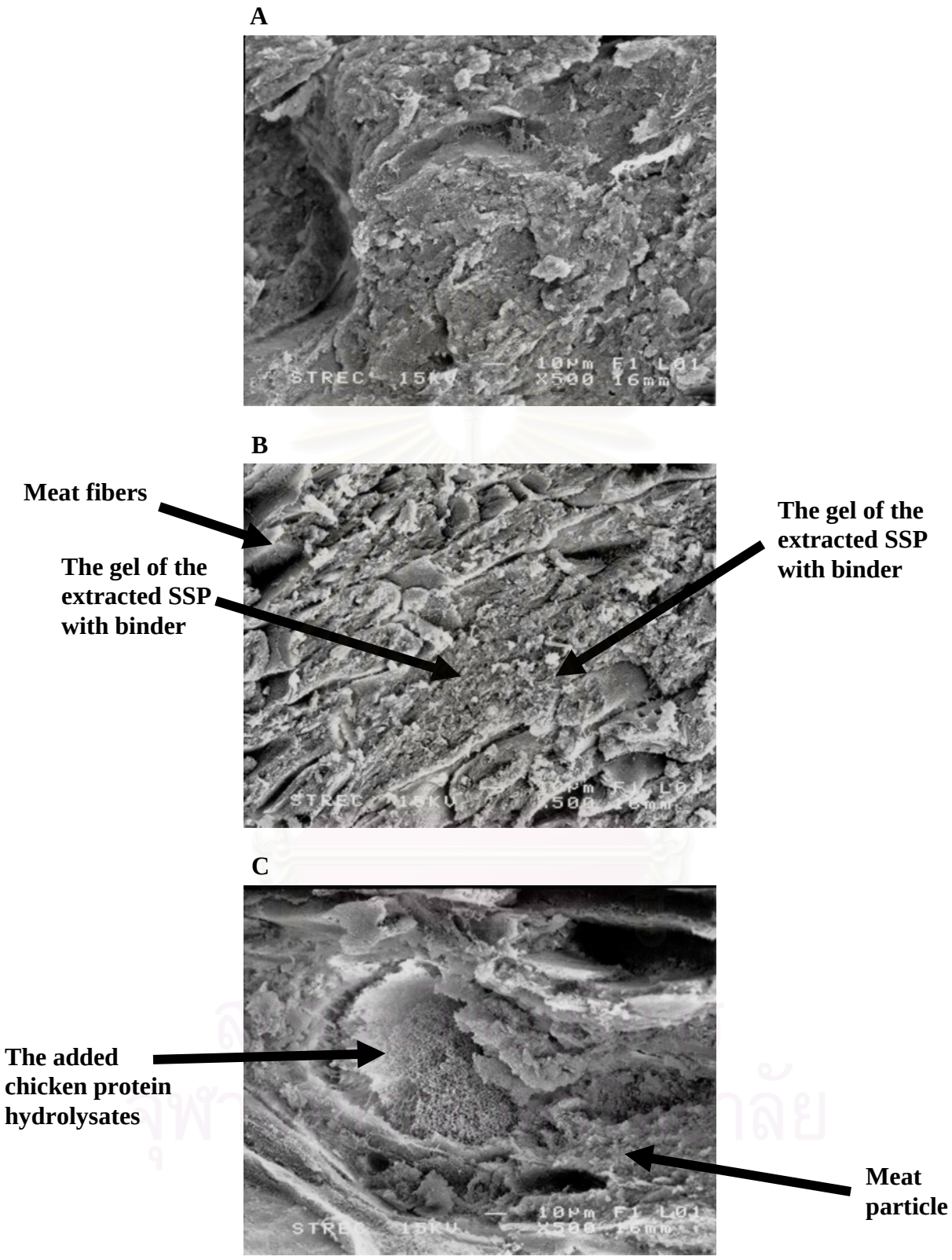


Figure 4.46 Scanning electron micrographs (500 x magnifications) of raw restructured chicken meats made from chicken broiler tenderloin meats without cold-set binder (A), and with chicken protein hydrolysates at 2% by weight (B), and 3% by weight (C).

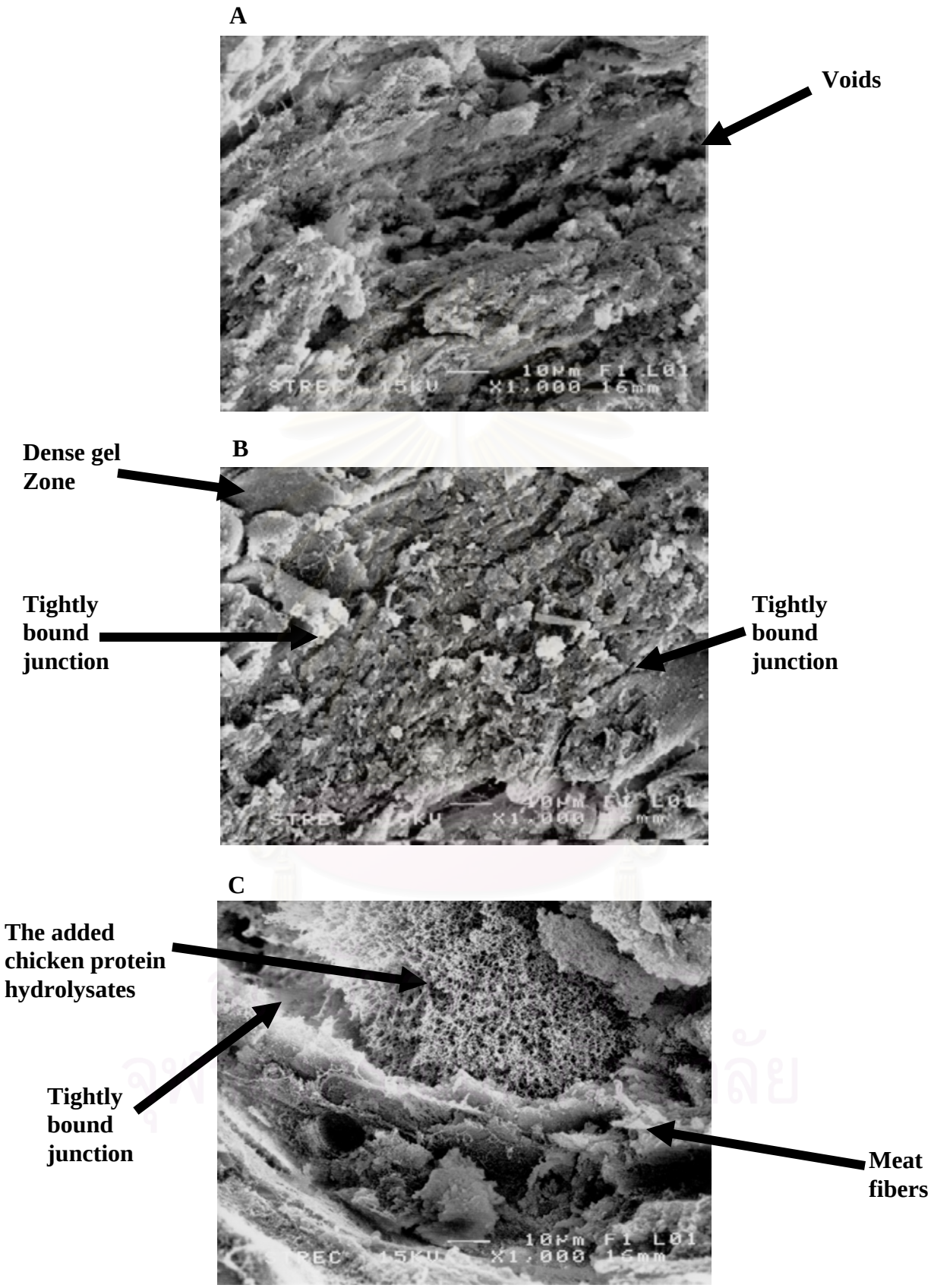


Figure 4.47 Scanning electron micrographs (1,000 x magnifications) of raw restructured chicken meats made from chicken broiler tenderloin meats without cold-set binder (A), and with chicken protein hydrolysates at 2% by weight (B), and 3% by weight (C).

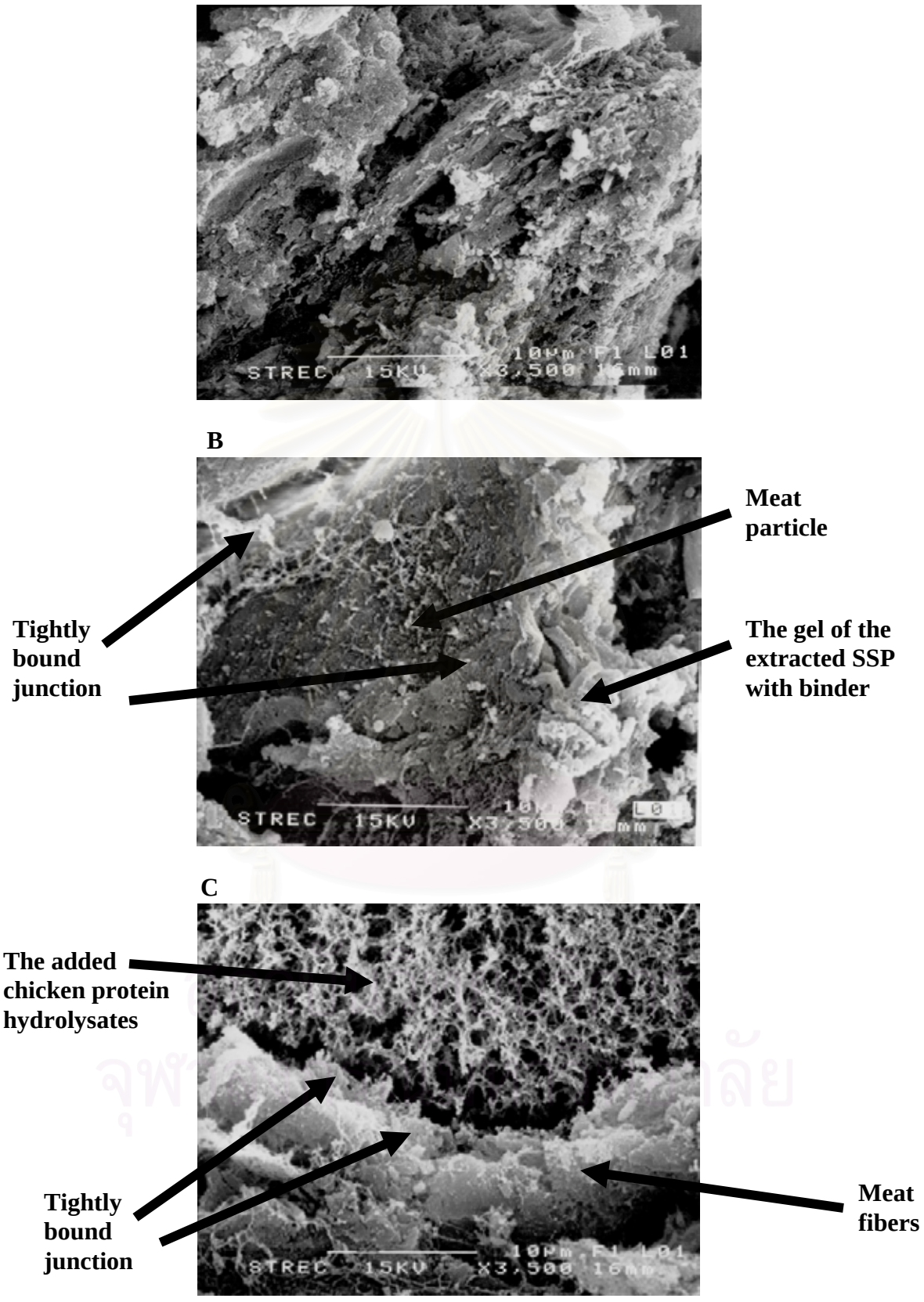


Figure 4.48 Scanning electron micrographs (3,500 x magnifications) of raw restructured chicken meats made from chicken broiler tenderloin meats without cold-set binder (A), and with chicken protein hydrolysates at 2% by weight (B), and 3% by weight (C).

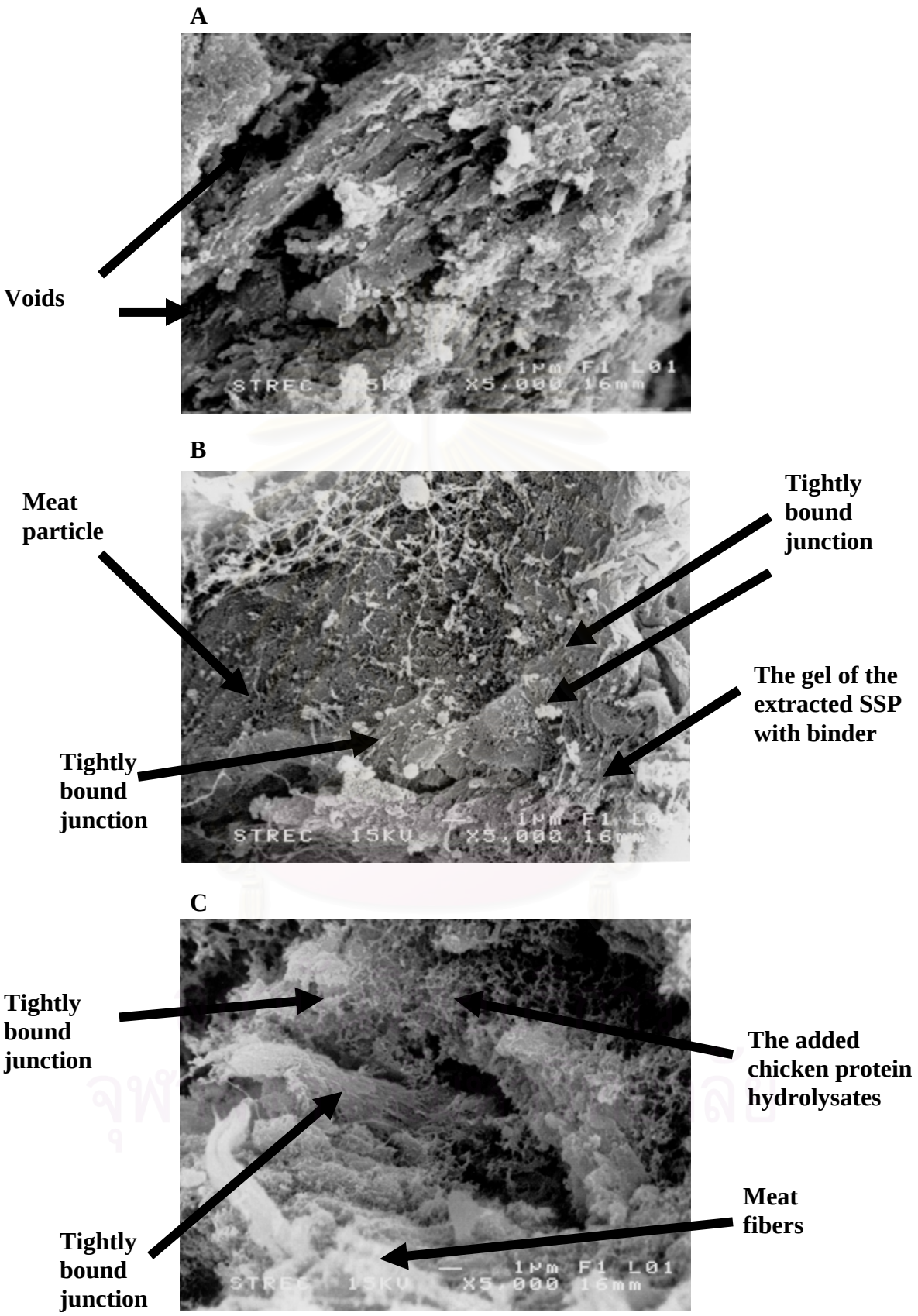


Figure 4.49 Scanning electron micrographs (5,000 x magnifications) of raw restructured chicken meats made from chicken broiler tenderloin meats without cold-set binder (A), and with chicken protein hydrolysates at 2% by weight (B), and 3% by weight (C).

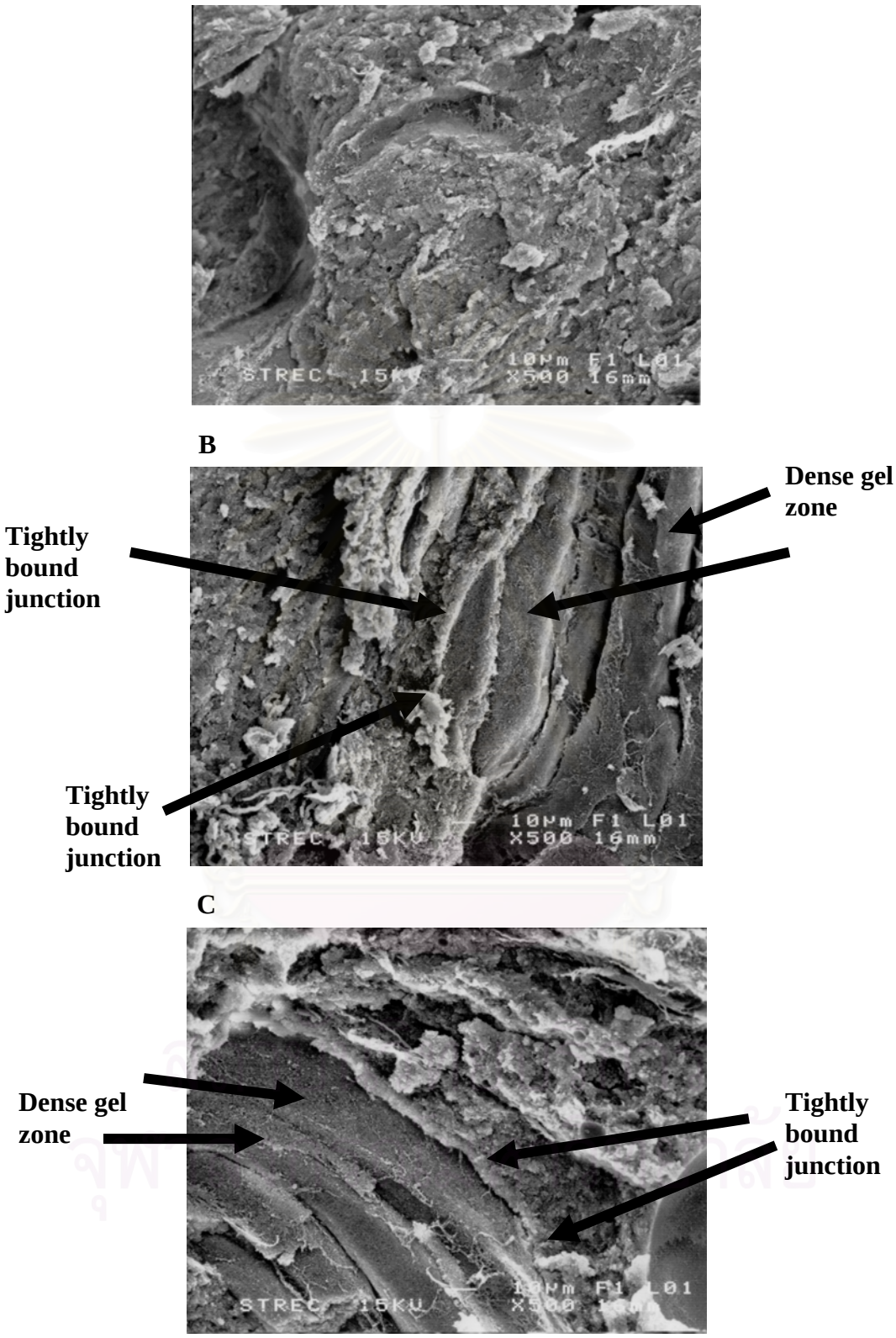


Figure 4.50 Scanning electron micrographs (500 x magnifications) of raw restructured chicken meats made from chicken broiler tenderloin meats without cold-set binder (A), with 1% by weight chitosan and 2% by weight chicken protein hydrolysates (B), and with 1.5% by weight chitosan and 3% by weight chicken protein hydrolysates (C).

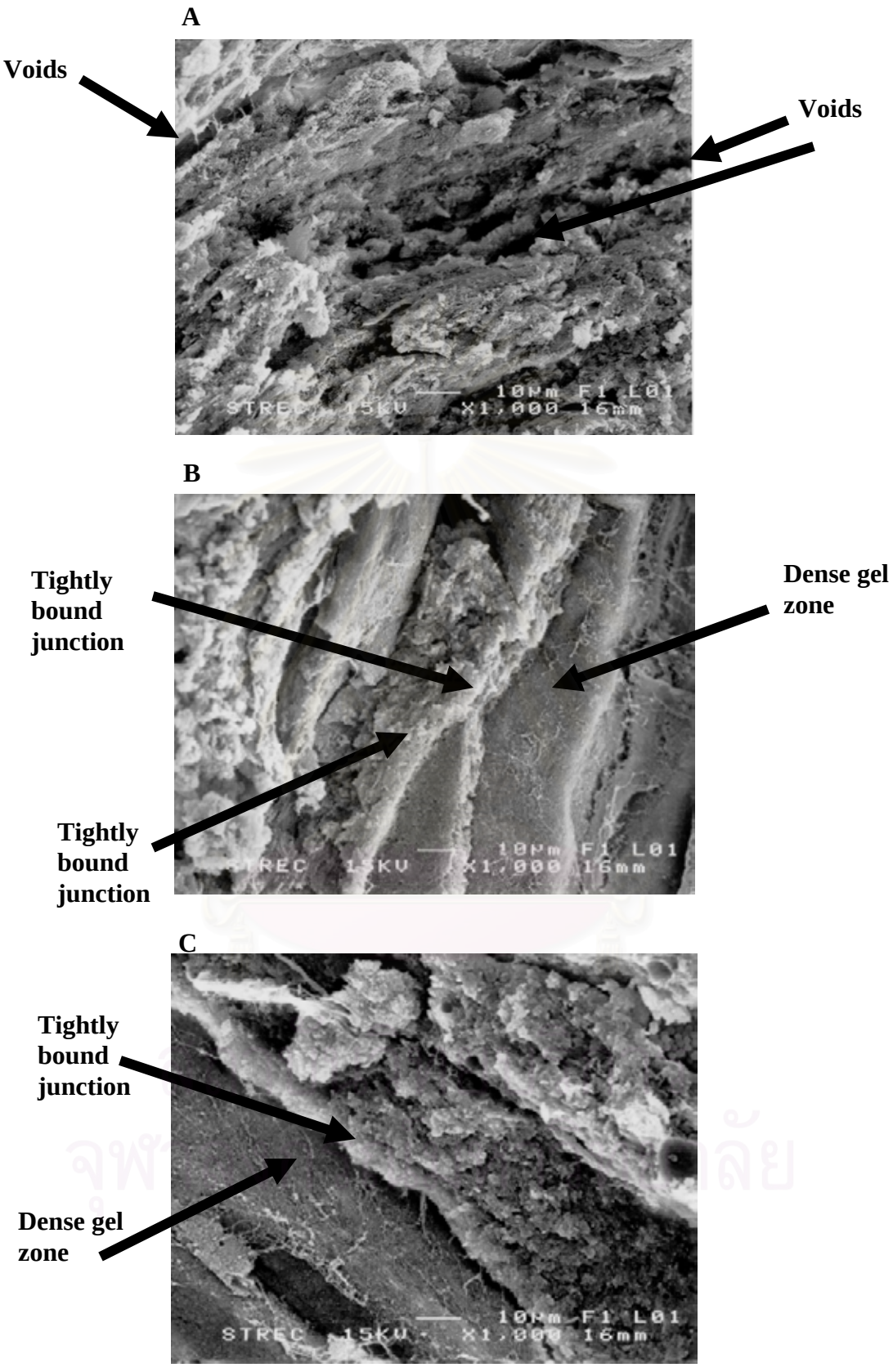


Figure 4.51 Scanning electron micrographs (1,000 x magnifications) of raw restructured chicken meats made from chicken broiler tenderloin meats without cold-set binder (A), with 1% by weight chitosan and 2% by weight chicken protein hydrolysates (B), and with 1.5% by weight chitosan and 3% by weight chicken protein hydrolysates (C).

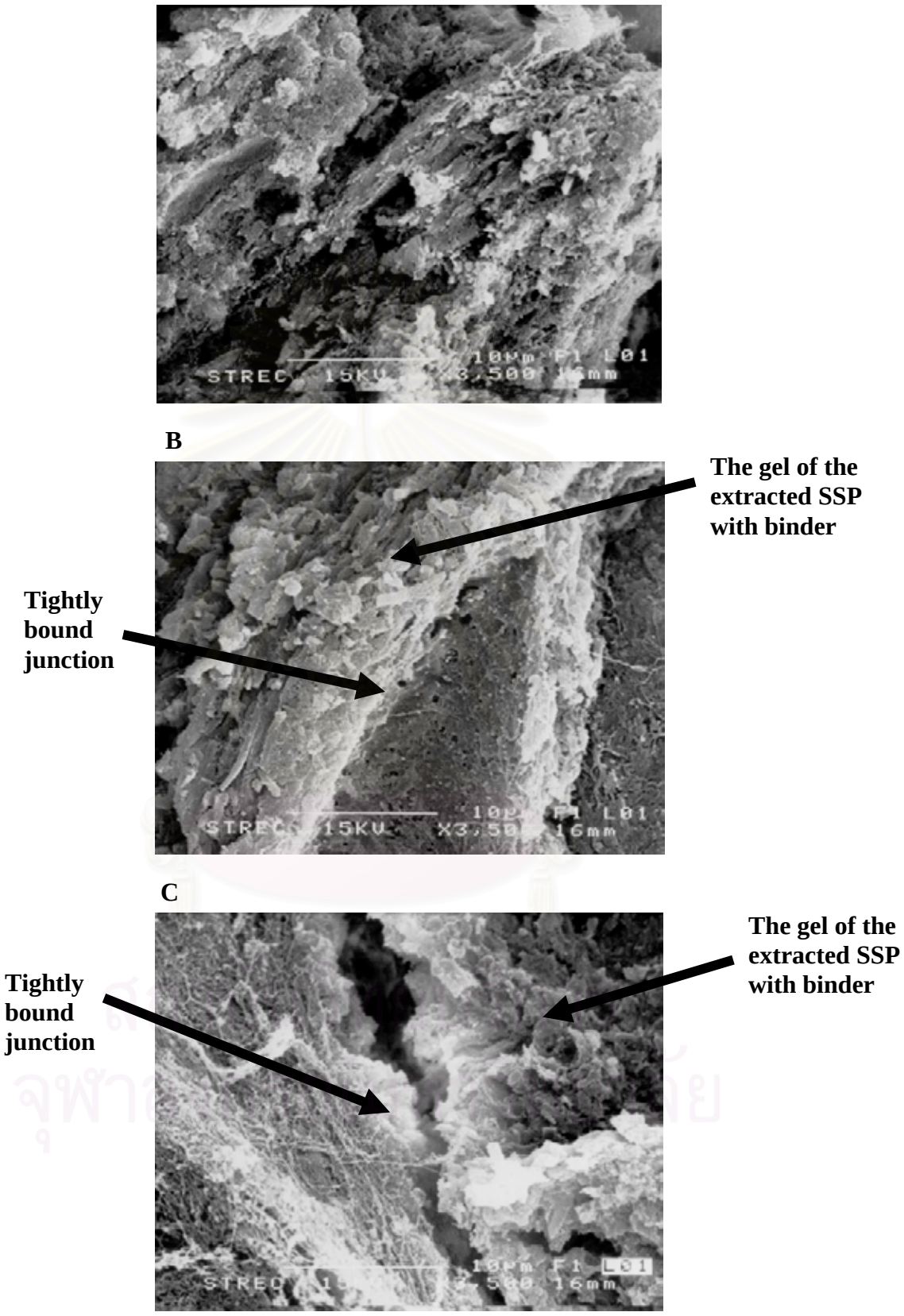


Figure 4.52 Scanning electron micrographs (3,500 x magnifications) of raw restructured chicken meats made from chicken broiler tenderloin meats without cold-set binder (A), with 1% by weight chitosan and 2% by weight chicken protein hydrolysates (B), and with 1.5% by weight chitosan and 3% by weight chicken protein hydrolysates (C).

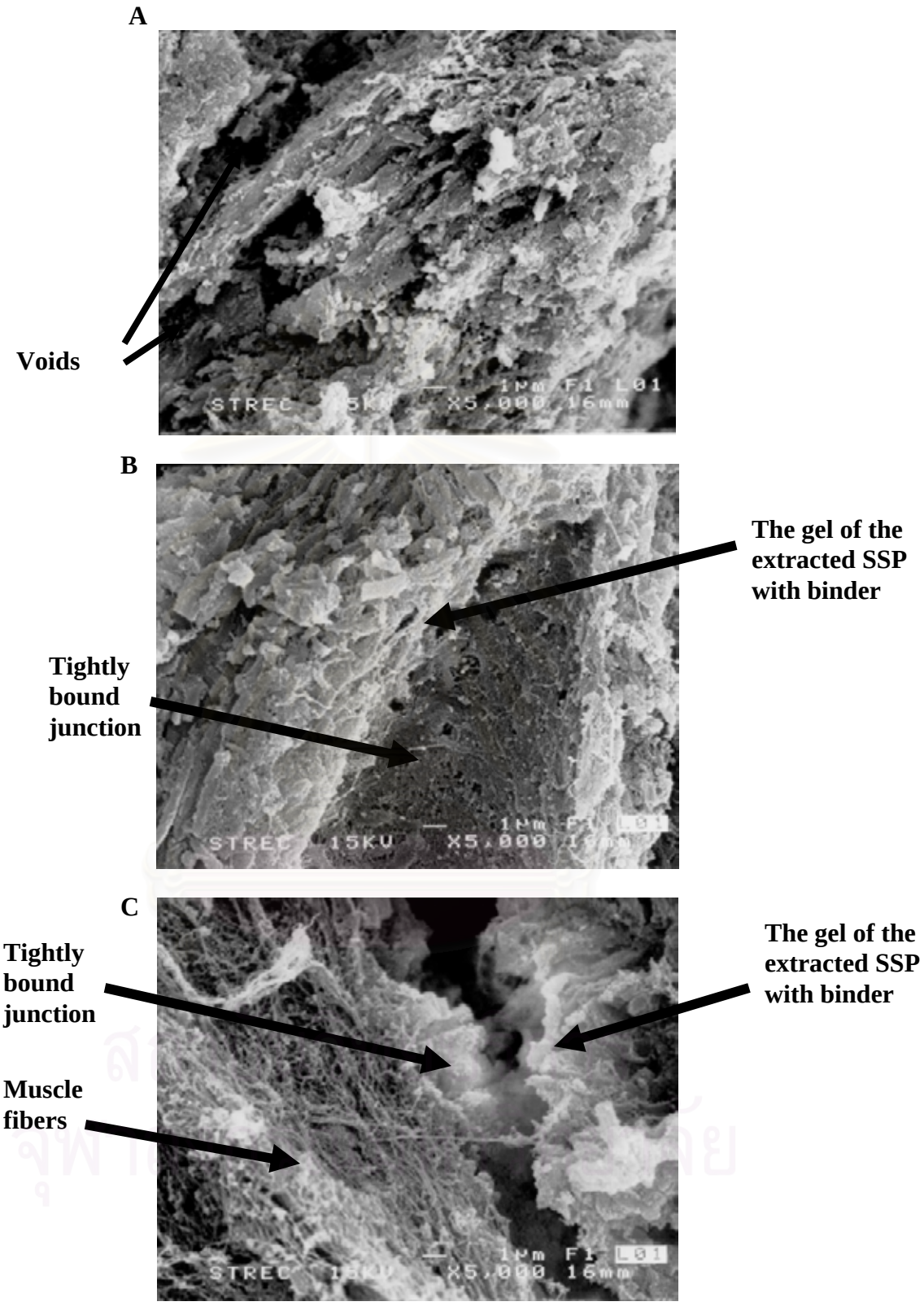


Figure 4.53 Scanning electron micrographs (5,000 x magnifications) of raw restructured chicken meats made from chicken broiler tenderloin meats without cold-set binder (A), with 1% by weight chitosan and 2% by weight chicken protein hydrolysates (B), and with 1.5% by weight chitosan and 3% by weight chicken protein hydrolysates (C).

4.5.7 Concluding Remarks

Alkali-precipitated chitosan and chicken protein hydrolysates functioned as good cold-set binders in raw restructured chicken meats as well as in SSP gels. The improvement of rheological characteristics, texture and structure was also observed when either of these cold-set binders was added. However, the combination use provided the highest improvement, especially at high concentration. The improvement of water binding properties of the obtained raw restructured chicken meats was also observed. The pH of the products containing the combined binders was not different from that of the control. This result was different from that shown in SSP gels. The highest quality improvement was resulted in treatment with combination of 1.5% alkali-precipitated chitosan and 3% chicken protein hydrolysates similar to that of the SSP gels.

CHAPTER V

CONCLUSIONS

5.1 Preparation and Characterization of Chitosan and Chicken Protein Hydrolysates

Clean and white chitosan flakes with desirable DD and MW were obtained in this study. The DD which were 78, 88 and 94%, depended on the deacetylation time and temperature. High MW chitosan (MW range from 1.02-1.10 x 10^6 Dalton) was naturally obtained after the preparation. Chitosanase from *Bacillus* sp. PP8 successfully hydrolyzed chitosan chain and gave low (MW range from 5.43-9.86 x 10^4 Dalton) and medium MW (MW range from 1.73-1.92 x 10^5 Dalton) chitosan samples with low polymer dispersibility.

Light yellow chicken protein hydrolysate samples with desirable DH from 0.03-60.12% were obtained by papain hydrolysis with the processing yield at 10-11%. The freeze-drying process used in this study did not affect color of the resulting hydrolysates. Increasing of DH resulted in lowering pH of the protein hydrolysates.

5.2 Effect of Chitosan at Different DD, MW and Concentration (C) on Cold-Set Gelation Abilities, Structures and Physico-chemical Properties of Chicken Salt-Soluble Protein (SSP) Gels

Alkali-precipitated chitosan improved rheological and textural characteristics, increased disulfide content, and improved the structure of SSP gels. Color of the developed cold-set gels was changed due to the increasing of L* and the decreasing of a*. The unchanged non-denaturing gel-electrophoretic patterns indicated the minor effect of strong disulfide bonds and the majority of the weak interactions such as van

der Waals force, electrostatic interactions, hydrophobic interactions, hydrogen bonds and salt bridges within the developed SSP gels. DD, MW and C of alkali-precipitated chitosan significantly influenced physico-chemical properties of the developed gels. The cold-set gel with 1.5% by weight alkali-precipitated chitosan at MW of $1.84x10^5$ Daltons and 94% DD exhibited the greatest quality improvement.

5.3 Effect of Chicken Protein Hydrolysates at Different DH and C on Cold-Set Gelation Abilities, Structures and Physico-chemical Properties of SSP Gels

The physico-chemical properties of the resulting cold-set gels, including rheological characteristics, texture, disulfide content and gel structure, significantly improved when using chicken protein hydrolysates as cold-set binder. The formation of high MW protein molecules as detected by the intense bands in non-denaturing gel-electrophoretic patterns suggested the formation of cold-set gels which mainly stabilized by disulfide bonds. The color difference value (ΔE^*_{ab}) of the developed SSP gels was increased in the presence of the hydrolysates. DH and C of chicken protein hydrolysates significantly influenced physico-chemical properties of the developed gels. The cold-set gel with 3% by weight chicken protein hydrolysates at 22.38% DH exhibited the greatest quality improvement.

5.4 Combination Effects of Chitosan and Chicken Protein Hydrolysates on Cold-Set Gelation Abilities, Structures and Physico-chemical Properties of SSP Gels

The highest improvement of the physico-chemical properties of the resulting cold-set gels, including rheological characteristics, texture, disulfide content and gel structure, were obtained when using the combination of alkali-precipitated chitosan at MW of $1.84x10^5$ Daltons and 94% DD and chicken protein hydrolysates at 22.38% as

cold-set binders. The formation of the accumulative high MW macromolecules on the top of non-denaturing gel-electrophoretic wells was observed in samples with these two cold-set binders suggesting the formation of electrostatic complexes and interactions between these binders and SSP chains. The significant decrease of pH and the significant increase of ΔE^*_{ab} was also observed in these gels. The developed cold-set gels with combination of 1.5% by weight alkali-precipitated chitosan and 3% by weight chicken protein hydrolysates exhibited the highest quality improvement.

5.5 Application of Chitosan, Chicken Protein Hydrolysates and Their Combinations in Raw Restructured Chicken Meat

In raw restructured chicken meats, the inclusion of alkali-precipitated chitosan at MW of 1.84×10^5 Daltons and 94% DD, chicken protein hydrolysates at 22.38% DH, and their combinations resulted in significant improvement of textural parameters, rheological characteristics and gel structure from that of the control. The highest improvement was occurred when using the combined binders, especially at high concentration. These results were in accord with the result in SSP gel system. The improvement of water binding properties was also obtained. The gel structure of the obtained raw restructured meat was different from the SSP gel in which the dense structure of the added binders-meat particles-SSP gel matrix was obtained instead of the structure of fine protein network. The L* and ΔE^*_{ab} of these products was increased, while the pH was not changed from that of the control. Raw restructured chicken meat with 1.5% by weight alkali-precipitated chitosan and 3% by weight chicken protein hydrolysates exhibited the highest quality improvement.

5.6 Suggestions and Future Work

Chitosan in alkali-precipitated form has potential as cold-set binder in raw restructured meat products. However, the effects of chitosan in other forms or some modifications of chitosan side groups on its cold-set gelation ability should also be investigated. The modification of chicken protein hydrolysate chains on their side groups is also suggested to perform in order to enhance their abilities as cold-set binders. The study of interactions between the added cold-set binder and other proteins in the system is suggested to be investigated using Raman spectroscopy or FT-IR techniques. With these techniques, the charged interactions such as dipole-dipole interactions and electrostatic interactions can clearly be elucidated, but not the covalent bonds such as disulfide bond.

The study of combination use of other proteins and polysaccharides, or alkali-precipitated chitosan with other proteins such as globular proteins, or chicken protein hydrolysates with other polysaccharides such as alginate, carrageenan and xanthan gum ect., as cold-set binders are also interesting, since the complexes of these macromolecules possess various unique characteristics and functionalities. Furthermore, the appropriate formulations for producing palatable raw restructured chicken meats from the developed cold-set binders are worth investigated.

REFERENCES

Adler-Nissen, J. 1986. Enzymatic hydrolysis of food proteins. London: Elsevier Applied Science Publishers.

Adriaan, B.M., Maria, K.M., and Johannes, V. 1999. Fat replacer, especially for foods and cosmetics. US Patent. 5,952,007.

Aida Penã-Ramos, E., and Xiong, Y.L. 2003. Whey and soy protein hydrolysates inhibit lipid oxidation in cooked pork patties. Meat Science. 64: 259-263.

Akhtar, M., and Dickinson, E. 2003. Emulsifying properties of whey protein-dextran conjugates at low pH and different salt concentration. Colloids and Surfaces B: Biointerfaces. 31: 125-132.

Aktaş, N. and Kiliç, B. 2005. Effect of microbial transgluaminase on thermal and electrophoretic properties of ground beef. LWT-Food Science and Technology. 38(8): 815-819.

Alting, A.C., Hamer, R.J., de Kruif, C.G., and Visschers, R.W. 2000. Formation of disulfide bonds in acid-induced gels of preheated whey protein isolate. Journal of Agricultural and Food Chemistry. 48: 5001-5007.

Alting, A.C., Hamer, R.J., de Kruif, C.G., Paques, M., and Visschers, R.W. 2003. Number of thiol groups rather than the size of the aggregates determines the hardness of cold-set whey protein gels. Food Hydrocolloids. 17: 469-479.

Andrès, S., Zaritzky, N., and Califano, A. 2006. The effect of whey protein concentrates and hydrocolloids on the texture and colour characteristics of chicken sausages. International Journal of Food Science and Technology. 41: 954-961.

Association of Official Analytical Chemist (AOAC). 1995. Official methods of analysis. 13th ed. Washington DC: Association of Official Analysis Chemists.

Barbanti, D., and Pasquini, M. 2005. Influence of cooking conditions on cooking loss and tenderness of raw and marinated chicken breast meat. LWT. 38: 895–901.

Barbut, S. 2006. Effects of casein, whey and milk powders on the texture and microstructure of emulsified chicken meat batters. LWT-Food Science and Technology. 39(6): 660-664.

Barbut, S., and Drake, D. 1997. Effect of reheating on sodium-induced cold gelation of whey proteins. Food Research International. 30: 153-157.

Benjakul, S., Visessanguan, W., Phatchrat, S., and Tanaka, M. 2003. Chitosan affects transglutaminase-induced surimi gelation. Journal of Food Biochemistry. 27: 53-66.

Berry, B.W. 1987. Texture in restructured meats. In A.M. Pearson and T.R. Dutson (eds.). Advance in meat research Volume 3: Restructured meat and poultry products, pp. 125-160. New York: AVI Publishing.

Beveridge, T., Toma, S.J., and Nakai, S. 1974. Determination of SH- and SS-groups in some food proteins using Ellman's reagent. Journal of Food Science. 39: 49-51.

Bishay, I.E., and Clark, E.R. 1996. Carbohydrate/protein cream substitutes. US Patent. 5,536,514.

Blaise, M. 1997. Protein-polysaccharide complex composition and method of use. US Patent. 5,645,880.

Boles, J.A., and Shand, P.J. 1998. Effect of comminution method and raw binder system restructured beef. Meat Science. 49(3): 297-307.

Boles, J.A., and Shand, P.J. 1999. Effects of raw binder system, meat cut and prior freezing on restructured beef. Meat Science. 53: 233-239.

Booren, A.M., and Mandigo, R.W. 1987. Fundamentals of production. In A.M. Pearson and T.R. Dutson (eds.). Advance in meat research Volume 3: Restructured meat and poultry products, pp. 351-382. New York: AVI Publishing.

Borderías, A.J., Sánchez-Alonso, I., and Pérez-Mateos., M. 2005. New applications of fibers in foods: Addition to fishery products. Trends in Food Science and Technology. 16(10): 458-465.

Bourne, M.C. 1978. Texture profile analysis. Food Technology. 7: 62-66.

Bryant, C.M., and McClements, D.J. 1998. Molecular basis of protein functionality with special consideration of cold-set gels derived from heat-denatured whey. Trends in Food Science and Technology. 9: 143-151.

Bryant, C.M., and McClements, D.J. 2000. Influence of NaCl and $CaCl_2$ on cold set gelation of heat denatured whey protein. Journal of Food Science. 65(5): 801-804.

Camou, J.P., Sebranek, J.G., and Olson, D.G. 1989. Effect of heating rate and protein concentration on gel strength and water loss of muscle protein gels. Journal of Food Science. 54(4): 850-854.

Carballo, J., Ayo, J., and Jiménez-Colmenero, F. 2006. Microbial transglutaminase and caseinate as cold set binders: Influence of meat species and chilling storage. LWT-Food Science and Technology. 39(6): 692-699.

Chang, K.L.B., Lin, Y.S., and Chen, R.H. 2003. The effect of chitosan on the gel properties of tofu (soybean curd). Journal of Food Engineering. 57: 315-319.

Chatterjee, S., Chatterjee, S., Chatterjee, B.P., and Guha, A.K. 2004. Clarification of fruit juice with chitosan. Process Biochemistry. 39(12): 2229-2232.

Chen, W.S., and Soucie, W.G. 1985. Edible fibrous serum milk proteins/xanthan gum complexes. US Patent. 4,559,233.

Chen, C.M., and Trout, G.R. 1991. Sensory, instrumental texture profile and cooking properties of restructured beef steaks made with various binders. Journal of Food Science. 56(6): 1457-1460.

Chen, Y.C., Wang, C.H., Lai, L.S., and Lin, K.W. 2003. Rheological properties of chitosan and its interaction with porcine myofibrillar proteins as influenced by chitosan's degree of deacetylation and concentration. Journal of Food Science. 68(3): 826-831.

Chiang, W.D., Shih, C.J., and Chu, Y.H. 1999. Functional properties of soy protein hydrolysate produced from a continuous membrane reactor system. Food Chemistry. 65: 189-194.

Chung, Y.C., Ho, M.L., Chyan, F.L., and Jiang, S.T. 2000. Utilization of freeze-dried mackerel (*Scomber australasicus*) muscle proteins as a binder in restructured meat. Fisheries Science. 66: 130-135.

Chung, Y.C., Tsai, C.F., and Li, C.F. 2006. Preparation and characterization of water-soluble chitosan produced by Maillard reaction. Fisheries Science. 72(5): 1096–1103.

Clemente, A., Vioque, J., Sánchez-Vioque, R., Pedroche, J., Bautista, J., and Millán, F. 1999. Protein quality of chickpea (*Cicer arietinum* L.) protein hydrolysates. Food Chemistry. 67: 269-274.

Cohen, M.R. 1990. Process for making a restructure meat product. US Patent. 4,975,294.

Copeland, R.A. 1994. Methods for protein analysis: A practical guide to laboratory protocols. London: Chapman and Hall.

Cornforth, D. 1994. Color-its basis and importance. In A.M. Pearson and T.R. Dutson (eds.). Advances in meat research-Volume 9 Quality attributes and their measurement in meat, poultry and fish products, pp. 34-78. Glasglow: Blackie Academic and Professional.

Correia, L.R. and Mittal, G.S. 1992. Viscoelastic properties of meat emulsions. In M.A. Rao, and J.F. Steffe (eds.). Viscoelastic properties in foods, pp. 185-206. London: Elsevier Science Publishing.

Cross, H.R., Moen, R., and Stanfield, M.S. 1978. Training and testing of judges for sensory analysis of meat quality. Food Technology. 7: 48-54.

Dalgleish, D.G., and Hunt, J.A. 1995. Protein-protein interactions in food materials. In A.G. Goankar (ed.) Ingredient interactions-Effects on food quality, pp. 199-268. New York: Marcel Dekker.

Darmadji, P., and Izumimoto, M. (1994). Effect of chitosan in meat preservation. Meat Science. 38: 243-254.

Da Silva, J.A.L., and Rao, M.A. 1992. Viscoelastic properties of food hydrocolloids dispersions. In M.A. Rao, and J.F. Steffe (eds.). Viscoelastic properties in foods, pp. 285-316. London: Elsevier Science Publishing.

DeFreitas, Z., Sebranek, D.G., Olson, D.G., and Carr, J.M. 1997. Carrageenan effects on salt-soluble protein in model systems. Journal of Food Science. 62(3): 539-543.

Devatkal, S., and Mendiratta, S.K. 2001. Use of calcium lactate with salt-phosphate and alginate-calcium gels in restructured pork rolls. Meat Science. 58(4): 371-379.

Devlieghere, F., Vermeulen, A., and Debevere, J. 2004a. Chitosan: antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables. Food Microbiology. 21: 703-714.

Devlieghere**,** F., Vermeiren, L., and Debevere, J. 2004b. New preservation technologies: Possibilities and limitations. International Dairy Journal. 14(4): 273-285.

Dickinson, E., and Izgi, E. 1996. Foam stabilization by protein-polysaccharide complexes. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 113: 191-201.

Diniz, F.M., and Martin, A.M. 1997. Effects of the extent of enzymatic hydrolysis on functional properties of shark protein hydrolysate. Lebensm.-Wiss.U.-Technol. 30: 266-272.

Dublier, J.L., Luannay, B., and, Cuvelier, G. 1992. Viscoelastic properties of food gels. In M.A. Rao, and J.F. Steffe (eds.). Viscoelastic properties in foods, pp. 371-434. London: Elsevier Science Publishing.

Doublier, J.L., Garnier, C., Renard, D., and Sanchez, C. 2000. Protein-polysaccharide interactions. Current Opinion in Colloid and Interface Science. 5:202-214.

Duarte, J., Vinderola, G., Ritz, B., Perdigón, G., and Matar, C. 2006. Immunomodulating capacity of commercial fish protein hydrolysate for diet supplementation. Immunobiology. 211: 341–350.

Edwards, S.F., Lillford, P.J., and Blanshard, J.M.V. 1987. Gels and networks in practice and theory. In J.M.V. Blanshard, and P. Lillford (eds.). Food structure and behavior, pp. 1-12. London: Academic Press.

Eric, K., and Christophe. 2004. Interface stabilization of a product with 2 or more phase with a protein-polysaccharide complex. WO Patent. 2,004,028,281.

Fadil, E., Babilker, E., Khan, M.A.S., Mutsudomi, N., and Kato, A. 1996. Polymerization of soy protein digests by microbial transgluminase for improvement of the functional properties. Food Research International. 29(7): 627-634.

Fernández-Martín, F., Cofrades, S., Carballo, J., and Jiménez-Colmenero, F. 2002. Salt and phosphate effects on the gelling process of pressure/heat treated pork batters. Meat Science. 61: 15-23.

Fik, M., and Surówka, K. 1986. Preparation and properties of protein concentrates from broiler chicken head. J. Sci. Food Agric. 37: 445-454.

Flaczyk, E., Rudińska, M., Wąsowicz, E., Korczak, J., and Amarowicz, R. 2006. Effect of cracklings hydrolysates on oxidative stability of pork meatballs fat. Food Research International. 39(8): 924-931.

Fletcher, D.L. 1999. Broiler breast meat color variation, pH and texture. Poultry Science. 78: 1323-1327.

Fonkwe, L.G., and Singh, R.K. 1996. Protein recovery from mechanically deboned turkey residue by enzymatic hydrolysis. Process Biochemistry. 31(6): 605-616.

Forrest, J.C., Abele, E.D., Hedrick, H.B., Judge, M.D., and Merkel, R.A. 1975. Principles of meat science. New York: W.H. Freemand and Company.

Fradin, M. 1991. Process for preparing restructured meat. US Patent. 5,017,393.

Freedman, P.G., and Blaise, M. 1997. Protein-polysaccharide complex composition, method of preparation and use. US patent. 5,591,473.

Gagliardi, Jr., and Eugene, D. 1988. Restructured meat products and methods of making same. US Patent. 4,728,524.

Gilchrist, A., and Nobbs, J. 1999. Colour by numbers. JSDC. 115(1): 4-7.

Gómez-Guillén, M.C., Montero, P., Solas, M.T., and Pérez-Mateos, M. 2005. Effect of chitosan and microbial transglutaminase on the gel forming ability of horse mackerel (*Trachurus* spp.) muscle under high pressure. Food Research International. 38: 103-110.

Gray, J.I., and Pearson, A.M. 1987. Rancidity and warmed-over flavor. In A.M. Pearson and T.R. Dutson (eds.). Advances in meat research Volume 3: Restructured meat and poultry products, pp. 221-270. New York: AVI Publishing.

Greaser, M.L., and Pearson, A.M. 1999. Flesh foods and their analogs. In A.J. Rosenthal (ed.). Food texture: Measurement and perception, pp. 228-258. Maryland: An Aspen Publication.

Hermansson, A.M. 1994. Microstructure of protein gels related to functionality. In R.Y. Yada, R.L. Jackman and J.L. Smith (eds.). Protein structure-function relationships in foods, pp. 22-42. Cambridge: Blackie Academic and Professional.

Higuchi, T., Ojima, T., and Nishita, K. 2002. Heat-induced structural changes and aggregation of walleye Pollack myosin in the light meromysin region. Fisheries Science. 68: 1145-1150.

Hongsprabhas, P., and Barbut, S. 1997. Effect of gelation temperature on Ca^{2+}-induced gelation of whey proteins. Lebensm.-Wiss.u.-Technol. 30: 45-49.

Hongsprabhas, P., and Barbut, S. 1999a. Effect of pre-heated whey protein level and salt on texture development of poultry meat batters. Food Research International. 32: 145-149.

Hongsprabhas, P., and Barbut, S. 1999b. Use of cold set whey protein gelation to improve poultry meat batters. Poultry Science. 78: 1074-1078.

Honikel, K.O. 1998. Reference methods for the assessment of physical characteristics of meat. Meat Science. 44(4): 447-457.

Honikel, K.O., and Hamm, R. 1994. Measurement of water-holding capacity and juiciness. In A.M. Pearson and T.R. Dutson (eds.). Advances in meat research-Volume 9 Quality attributes and their measurement in meat, poultry and fish products, pp. 125-161. Glasglow: Blackie Academic and Professional.

Hoppert, M. 2003. Microscopic techniques in biotechnology. Darmstadt: Wiley-VCH GmbH and Co.KGaA.

Hossain, Md. A., Abu Ali khan, Md., Ishihara, T., Hara, K., Osatomi, K., Osaka, K., and Nozaki, Y. 2004. Effect of proteolytic squid protein hydrolysate on the state of water and denaturation of lizardfish (*Saurida wanieso*) myofibrillar protein during freezing. Innovative Food Science and Emerging Technologies. 5: 73–79.

Hossain, Md. A., Ishihara, T., Hara, K., Osatomi, K., Abu Ali khan, Md., and Nozaki, Y. 2003. Effect of proteolytic squid protein hydrolysate on the state of water and dehydration-induced denaturation of lizard fish myofibrillar protein. Journal of Agricultural and Food Chemistry. 51: 4769-4774.

Huffman, D.L. 1981. Process for production of a restructured fresh meat product. US. Patent. 4,258,068.

Hung, T., and Smith, D.M. 1993. Dynamic rheological properties and microstructure of partially insolubilized whey protein concentrate and chicken breast salt-soluble protein gels. Journal of Agricultural and Food Chemistry. 41: 1372-1378.

Hunt, M.C., and Kropf, D.H. 1987. Color and appearance. In A.M. Pearson and T.R. Dutson (eds.). Advances in meat research Volume 3: Restructured meat and poultry products, pp. 125-160. New York: AVI Publishing.

Iwasaki, T., Noshiroya, K., Saitoh, N., Okano, K., and Yamamoto, K. 2006. Studies of the effect of hydrostatic pressure pretreatment on thermal gelation of chicken myofibrils and pork meat patty. Food chemistry. 95(3): 474-483.

Jeon, Y.J., Byun, H.G., and Kim, S.K. 1999. Improvement of functional properties of cod frame protein hydrolysates using ultrafiltration membranes. Process Biochemistry. 35: 471–478.

Jo, C., Lee, J.W., Lee, K.H., and Byun, W. 2001. Quality properties of pork sausage prepared with water-soluble chitosan oligomer. Meat Science. 59: 369-375.

Johannes, V.R., Cornelis, D.H., and Leendert, T. 1977. Globular protein-algal polysaccharide complex in food compositions. GB Patent. 1,440,181.

Jolley, P.D., and Purslow, P.P. 1988. Reformed meat products: Fundamental concepts and new developments. In J.M.V. Blanchard, and J.R. Mitchell (eds.), Food structure: Its creation and evaluation, pp. 231-264. London:Latimer Tread. Co.

Kamil, J.Y.V.A., Jeon, Y.J., and Shahidi, F. 2002. Antioxidative activity of chitosans of different viscosity in cooked comminuted flesh of herring (*Clupea harengus*). Food Chemistry. 79: 69-77.

Kanatt, S.R., Chander, R., and Sharma, A. 2004. Effect of irradiated chitosan on the rancidity of radiation-processed lamb meat. Journal of Food Science and Technolgy. 39(9): 997-1003.

Khan, M.A.A., Hossain, M.A., Hara, K., Osatomi, N., Ishihara, T., and Nozaki, Y. 2003. Effect of enzymatic fish-scrap protein hydrolysate on gel forming ability and denaturation of lizard fish *Saurida wanieso* surimi during frozen storage. Fisheries Science. 69: 1271-1280.

Kiliç, B., 2003. Efffect of microbial transglutaminase and sodium caseinate on quality of chicken doner kebab. Meat Science. 63:417-421.

King, N.L., and Macfarlane, J.J. 1987. Muscle proteins. In A.M. Pearson and T.R. Dutson (eds.). Advance in meat research Volume 3: Restructured meat and poultry products, pp. 21-72. New York: AVI Publishing.

Koide, S.S. (1998). Chitin-chitosan: properties, benefits and risks. Nutrition Research. 18(6): 1091-1101.

Kolle, D.S., and Savell, J.W. 2003. Using Activa^{TM} TG-RM to bind beef muscles after removal of excessive seam fat between the *m. longissimus thoracis* and *m. spinalis dorsi* and heavy connective tissue from within the *m. infraspinatus*. Meat Science. 64:27-33.

Kominz, D.R., Mitchell, E.R., Nihei, T., and Kay, C.M. 1965. The papain digestion of skeletal myosin A. Biochemistry. 4(11): 2373-2382.

Kranen, R.W., Van Kuppevelt, T.H., Goedhart, H.A., Veerkamp, C.H., Lambooy, E., and Veerkamp, J.H. 1999. Hemoglobin and myoglobin content in muscles of broiler chickens. Poultry Science. 78: 467-476.

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

Laneuville, S.I., Paquin, P., and Turgeon, S.L. 2000. Effect of prepatation conditions of the characteristics of whey protein-xanthan gum complexes. Food Hydrocolloids. 14:305-314.

Lahl W.J. and Braun, S.D. 1994. Enzymatic production of protein hydrolysate food use. Food Technology. 10: 68-71.

Larmond, E. 1976. The texture profile. In J.M. DeMan, P.W. Voisey, V.F. Rasper and D.W. Stanley (eds.). Rheology and texture in food quality, pp. 546-553. Connecticut: AVI Publishing.

LeBlanc, E.L., and LeBlanc, R.J. 1992. Determination of hydrophobicity and reactive groups in proteins of Cod (*Gadus morhua*) muscle during frozen storage. Food Chemistry. 43(1): 3-11.

Lertsuriwong, P., How, N. C., Chandkrachang, S., and Stevens, W.F. 2002. Effect of chemical treatment on the characteristics of shrimp chitosan. Journal of Metals, Materials and Minerals. 12(1): 11-18.

Lesiów, T., and Xiong, Y.L. 2001. Mechanism of rheological changes in poultry myofibrillar protein during gelation. Avian and Poultry Biology Reviews. 12(4): 137-149.

Lesiów, T., and Xiong, Y.L. 2003. Chicken muscle homogenate gelation properties: Effect of pH and muscle fiber type. Meat Science. 64: 399-403.

Li, Q., Dunn, E.T., Grandmaison, E.W., and Goosen, M.F.A. 1997. Applications and properties of chitosan. In M.F.A. Goosen (ed.) Applications of chitin and chitosan, pp. 3-29. Lancaster: Technomic publishing.

Lii, C.Y., Tomasik, P., Zaleska, H., Liaw, S.C., and Lai, V.M.F. 2002. Carboxymethyl cellulose-gelatin complexes. Carbohydrate Polymers. 50: 19-26.

López-Caballero, M.E., Gómez-Guillén, M.C., Pérez-Mateos, M., and Montero, P. 2005. A functional chitosan-enriched fish sausage treated by high pressure. Journal of Food Science. 70(3): M166-M171.

Ma, C.Y., and Harwalkar, V.R. 1991. Thermal analysis of food proteins. Adv. Food and Nutri.Res. 35:317-366.

Macdougall, D.B. 1994. Colour of meat. In A.M. Pearson and T.R. Dutson (eds.). Advances in meat research-Volume 9 Quality attributes and their measurement in meat, poultry and fish products, pp. 79-93. Glasglow: Blackie Academic and Professional.

Macfarlane, J.J., Schmidt, G.R., and Turner, R.H. 1977. Binding of meat pieces: A comparison of myosin, actomyosin and sarcoplasmic proteins as binding agents. Journal of Food Science. 42(6): 1603-1605.

Maehashi, K., Matsuzaki, M. Yamamoto, Y., and Udaka, S. 1999. Isolation of peptides from an enzymatic hydrolysate of food proteins and characterization of their taste properties. Biosci. Biotechnol.Biochem. 63(3): 555-559.

Mandigo, R.W. 1986. Restructuring of muscle food. Food Technology. 40(3): 85-89.

Manninen, A.H. 2004. Protein hydrolysates in sports and exercise; A brief review. Journal of Sports Science and Medicine. 3: 60-63.

Marangoni**,** A. G., Barbut, S., McGauley, S. E., Marcone, M., and Narine, S.S. 2000. On the structure of particulate gels - the case of salt-induced cold gelation of heat-denatured whey protein isolate. Food Hydrocolloids. 14(1): 61-74.

McWilliams, M. 1999. Food experimental perspective. 3rd ed. New Jersey: Merrill.

Means, W.J., and Schmidt, G.R. 1986. Algin/calcium as a raw and cooked binder in structured beef steaks. Journal of Food Science. 51(1): 60-65.

Means, W.J., and Schmidt, G.R. 1987. Restructuring fresh meat without the use of salt or phosephate. In A.M. Pearson and T.R. Dutson (eds.). Advance in meat research Volume 3: Restructured meat and poultry products, pp. 469-488. New York: AVI Publishing.

Means, W.J., Clarke, A.D., Sofos, A.D., and Schmidt, G.R. 1987. Binding, sensory, and storage properties of algin/calcium structured beef steaks. Journal of Food Science. 52(2):252-256.

Meste, M.L., Simatos, D., and Gervais, P. 1995. Interaction of water with food components. In A.G. Goankar (ed.). Ingredient interactions-Effects on food quality, pp. 85-123. New York: Marcel Dekker.

Mishra, S., Mann, B., and joshi, V.K. 2001. Functional improvement of whey protein concentrate on interaction with pectin. Food Hydrocolloids. 15: 9-15.

Moe, S.T., and Draget, K.I. 1995. Alginates. In M.S. Alistair (ed.). Food polysaccharides and their applications, pp. 245-286. New York: Marcel Dekker.

Morris, E.R. 1995. Polysaccharide rheology and in-mouth perception. In M.S. Alistair (ed.). Food polysaccharides and their applications, pp. 517-546. New York: Marcel Dekker.

Muzzarelli, R.A.A. 1996. Chitosan-based dietary foods. Carbohydrate Polymers. 29: 309-316.

Muzzarelli, R.A.A., and Rocchetti, R. 1997. Methods for the determination of the degree of acetylation of chitin and chitosan. In R.A.A. Muzzarelli, and M.G. Peter (eds.). Chitin Handbook, pp. 109-119. Italy: Atec Edizioni.

Nashef, A.S., Osuga, D.T., Lee, H.S., Ahmed, A.L., Whitaker, J.R., and Feeney, R.E. 1977. Effects of alkali on proteins, disulfides and their products. Journal of Agricultural and Food Chemistry. 25(2): 245-251.

Nielsen, G.R., Pedersen, B.R., and Møller, A.J. 1995. Impact of salt, phosphate and temperature on the effect of a transglutaminase (FXIIIa) on the texture of restructured meat. Meat Science. 41(3): 293-299.

Nielsen, P.M., Petersen, D., and Dambmann, C. 2001. Improved method for determining food protein degree of hydrolysis. Journal of Food Science. 66(5): 642-646.

No, H.K., Lee, S.H., Park, N.Y., and Meyers, S.P. 2003. Comparison of physicochemical, binding, and antibacterial properties of chitosan prepared without and with deproteinization process. Journal of Agricultural and Food Chemistry. 51: 7659-7663.

Oakenfull, D., Pearce, J., and Burley, R.W. 1997. Protein gelation. In S. Damodaran and A. Paraf (eds.). Food proteins and their applications, pp. 111-124. New York: Marcel Dekker.

Ockerman, H.W. 1977. Meat proteins. In H.D. Graham (ed.). Food colloids, pp. 240-291. Connecticut: AVI Publishing.

O'Connell, P. 1985. Structured meat product. US Patent. 4,544,560.

Offer, G., and Khight, P. 1988. The structural basis of water-holding in meat part 1: General principles and water uptake in meat processing. In R. Lawrie (ed.). Developments in meat science-Volume 4, pp. 63-171. London: Elsevier Applied Science.

Okada, M. 1985. Method for preparing fabricated meat products. US Patent. 4,559,236.

Panyam, D., and Kilara, A. 1996. Enhancing the functionality of food proteins by enzymatic modification. Trends in Food Science and Technology. 7: 120-125.

Pearson, A.M., and Tauber, F.W. 1984. Processed meats. 2nd ed. Westport:AVI Publishing Co.

Peng, Y., and Zhang, L. 2003. Characterization of a polysaccharide-protein complex from *Ganoderma tsugae* mycelium by size-exclusion chromatography combined with laser light scattering. J. Biochem. Biophys. Method. 56: 243-252.

Pietrasik, Z. 2003. Binding and textural properties of beef gels processed with κ-carrageenan, egg albumin and microbial transglutaminase. Meat Science. 63: 317-324.

Pietrasik, Z., and Jarmoluk, A. 2003. Effect of sodium caseinate and κ-carrageenan on binding and textural properties of pork muscle gels enhanced by microbial transglutaminase addition. Food Research international. 36: 285-294.

Pietrasik, Z., and Li-Chan., E.C.Y. 2002. Binding and textural properties of beef gels as affected by protein, κ-carrageenan and microbial transglutaminase addition. Food Research International. 35:91-98.

Pinto E Silva, M.E.M., Mazzilli, R.N., and Cusin, F. 1999. Composition of hydrolysates from meat. Journal of Food Composition and Analysis. 12: 219-225.

Pomeranz, Y. 1985. Functional properties of food component. Florida: Academic Press.

Popper, G., Ekstedt, S., Walkenstrom, P., and Hermansson, A.M. 2001. Gelled emulsion products containing chitosan. US Patent. 6,238,720.

Pouliot, Y., Wijers, M.C., Gauthier, S.F., and Nadeau L. 1999. Fractionation of whey protein hydrolysates using charged UF/NF membranes. Journal of Membrane Science. 158: 105-114.

Pyne, C.A. 2001. Meat restructuring using cold binding system ; In 2001 IFT Annual Meeting, Session 36: Industry needs new ingredient technologies for further processed meat products. [Online]. Available from: http://ift.confex.com/ift/2001/techprogram/paper6032.htm

Qiao, M., Fletcher, D.L., Northcutt, J.K., and Smith, D.P. 2002. The relationship between raw broiler breast meat color and composition. Poultry Science. 81: 422-427.

Qin, C., Du, Y., Xiao, L., Li, Z., and Gao, X. 2002. Enzymic preparation of water-soluble chitosan and their antitumor activity. International Journal of Biological Macromolecules. 31: 111-117.

Qin, C., Li, H., Xiao, Q., Liu, Y., Zhu, J., and Du, Y. 2006. Water-solubility of chitosan and its microbial activity. Carbohydrate Polymers. 63(3): 367-374.

Rabiskova, M., Song, J., Opawale, F. O., and Burgess, D. J. 1994. The influence of surface properties on uptake of oil into complex coacervates microcapsules. J. Pharm. Pharmacol. 46: 631–635.

Ramírez-Suárez, J.C., Addo, K., and Xiong, Y.L. 2005. Gelation of mixed myofibrillar/wheat gluten proteins treated with microbial transglutaminase. Food Research International. 38: 1143–1149.

Ramírez-Suárez, J.C., and Xiong, Y.L. 2003. Effect of transglutaminase-induced cross-linking on gelation of myofibrillar/soy protein mixtures. Meat Science. 65: 899-907.

Rao, M.A. 1992. Measurement of viscoelastic properties of fluid and semisolid foods. In M.A. Rao, and J.F. Steffe (eds.). Viscoelastic properties in foods, pp. 207-232. London: Elsevier Science Publishing.

Roller, S., and Covill, N. 1999. The antifungal properties of chitosan in laboratory media and apple juice. International Journal of Food Microbiology. 47: 67-77.

Roller, S., Sagoo, S., Board, R., Mahony, T.O., Caplice, E., Fitzgerald, G., Fogden, M., Owen, M., and Fletcher, H. 2002. Novel combinations of chitosan, carnocin and sulphite for the preservation of chilled pork sausages. Meat Science. 62: 165-177.

Ross-Murphy, S.B. 1995. Rheological of biopolymer solutions and gels. In E. Dickinson (ed.). New physico-chemical techniques for the characterization of complex food systems, pp. 139-156. Glasglow: Blackie Academic and Professional.

Rungsardthong, V., Wongvuttanakul, N., Kongpien, N., and Chotiwaranon, P. 2006. Application of fungal chitosan for clarification of apple juice. Process Biochemistry. 41(3): 589-593.

Ruttanapornvareesakul, Y., Ikeda, M., Hara, K., Osatomi, K., Osaka, K., Kongpun, O., and Nozaki, Y. 2006. Concentration dependent suppressive effect of shrimp head protein hydrolysate on dehydration-induced denaturation of lizardfish myofibrils. Bioresource Technology. 97: 762-769.

Sagoo, S., Board, R., and Roller, S. 2002a. Chitosan inhibits growth of spoilage micro-organisms in chilled pork products. Food microbiology. 19:175-182.

Sagoo, S., Board, R., and Roller, S. 2002b. Chitosan potentiates the antimicrobial action of sodium benzoate on spoilage yeasts. Letters in Applied Microbiology. 34: 168-172.

Sakanaka, S., and Tachibana, Y. 2006. Active oxygen scavenging activity of egg-yolk protein hydrolysates and their effects on lipid oxidation in beef and tuna homogenates. Food Chemistry. 95: 243-249.

Sakanaka, S., Tachibana, Y., Ishihara, N., and Juneja, L.R. 2004. Antioxidant activity of egg-yolk protein hydrolysates in a linoleic acid oxidation system. Food Chemistry. 86: 99-103.

Samant, S.K., Singhal, R.S., Kulkarni, P.R., and Rege, D.V. 1993. Protein-polysaccharide interactions: A new approach in food formulations. International Journal of Food Science and Technology. 28:547-562.

Sanchez, C., and Renard, D. 2002. Stability and structure of protein-polysaccharide coacervates in the presence of protein aggregates. International Journal of Pharmaceutics. 242: 319-324.

Satterlee, L.D., Zachariah, N.Y., and Levin, E. 1973. Utilization of beef and pork skin hydrolysates as a binder or extender in sausage emulsions. Journal of Food Science. 38 (2): 268-270.

Schmidt, G.R., and Means, W.J. 1986. Process for preparing algin/calcium gel structured meat products. U.S. Patent. 4,603,054.

Schmidt, G.R., and Means, W.J. 1991. Process for preparing algin/calcium gel structured meat products. U.S. Patent. 4,603,054.

Schmitt, C., Sanchez, C., Banon, S.D., and Hardy, J. 1998. Structure and technofunctional properties of protein-polysaccharide complexes: A review. Critical Review in Food Science and Nutrition. 38(8): 689-753.

Schmitt, C., Sanchez, C., Lamprecht, A., Renard, D., Lehr, C.M., de Kruif, C.G., and Hardy, J. 2001. Study of β-lactoglobulin/acacia gum complex coacervation by diffusing-wave spectroscopy and confocal scanning laser microscopy. Colloids and Surfaces B: Biointerfaces. 20: 267-280.

Secrist, J.L. 1987. Restructured meats-the past and present. In A.M. Pearson and T.R. Dutson (eds.). Advance in meat research Volume 3: Restructured meat and poultry products, pp. 1-20. New York: AVI Publishing.

Serrano, A., Cofrades, S., and Jiménez-Colmenero, F. 2003. Transglutaminase as binding agent in fresh restructured beef steak with added walnuts. Food Chemistry. 85(3): 423-429.

Shahidi, F., Han, X.Q., and Synowiecki, J. 1995. Production and characteristics of protein hydrolysates from capelin (*Mallotus villosus*). Food Chemistry. 53: 285-293.

Shahidi, F., and Synowieki, J. 1997. Protein hydrolysates from seal meat as phosphate alternatives in food processing applications. Food Chemistry. 60(1): 29-32.

Shahidi, F., Synowiecki, J., Venugopal, V., Pegg, R.B., and Botta, J.R. 1997. Characteristics of chicken-seal salami. Meat Science. 45(4): 551-559.

Shapiro, J.H., and Peck, B.J. 1983. Restructured meat product for roasting and method for preparing the same. US Patent. 4,377,597.

Shoemaker, C.F. 1992. Instrumentation for the measurement of viscoelasticity. In M.A. Rao, and J.F. Steffe (eds.). Viscoelastic properties in foods, pp. 233-246. London: Elsevier Science Publishing.

Sionkowska, A., Wisniewski, M., Skopinska, J., Kennedy, C.J., and Wess, T.J. 2004. Molecular interactions in collagen and chitosan blends. Biomaterials. 25: 795-801.

Simpson, B. K., Nayeri, G., Yaylayana, V., and Ashie, I. N. A. 1998. Enzymatic hydrolysis of shrimp meat. Food Chemistry. 61: 131-138.

Šližytė, R., Daukšasa, E., Falcha, E., Storrøa, I., and Rustadb, T. 2005. Characteristics of protein fractions generated from hydrolysed cod (*Gadus morhua*) by-products. Process Biochemistry. 40: 2021–2033.

Smewing, J. 1999. Hydrocolloids. In A.J. Rosenthal (ed.). Food texture: Measurement and perception, pp. 282-303. Maryland: An Aspen Publication.

Somjit, K., Ruttanapornvareesakul, Y., Hara, K., and Nozaki, Y. 2005. The cryoprotectant effect of shrimp chitin and shrimp chitin hydrolysate on

denaturation and unfrozen water of lizardfish surimi during frozen storage. Food Research International. 38(4): 345-355.

Soucie, W.G., and Chen, W.S. 1986. Edible xanthan gum-protein fibrous complexes. US Patent. 4,563,360.

Stanley, D.W. 1976. The texture of meat and its measurement. In J.M. DeMan, P.W. Voisey, V.F. Rasper and D.W. Stanley (eds.). Rheology and texture in food quality, pp. 405-426. Connecticut: AVI Publishing.

Suklim, K., Flick, G.J., Marcy, J.E., Eigel, W.N., Haugh, C.G., and Granata, L.A. 2004. Effect of cold-set binders: Alginates and microbial transglutaminase on the physical properties of restructured scallops. Journal of Texture Studies. 35: 634-642.

Synoweicki, J., and Al-Khateeb, N.A. 2003. Production, properties, and some new applications of chitin and its derivatives. Critical Reviews in Food Science and Nutrition. 43(2): 145-171.

Tarr, B.D., and Bixby, S.H. 1995. Fat substitute. US Patent. 5,393,550.

Terbojevich, M., and Cosani, A. 1997. Molecular weight determination of chitin and chitosan. In R.A.A. Muzzarelli, and M.G. Peter (eds.). Chitin Handbook, pp. 109-119. Italy: Atec Edizioni.

Tolaimate, A., Desbrieres, J., Rhazi, M., and Alagui, A. 2003. Contribution to the preparation of chitins and chitosans with controlled physico-chemical properties. Polymer. 44(26): 7939-7952.

Tolstoguzov, V. B., Izjumov, D. B., Grinberg, V. Y., Marusova, A. N., and Chekhovskaya, V. T. 1974. Method of making protein-containing foodstuffs resembling minced-meats. US Patent. 3,829,587.

Tolstoguzov, V.B. 1986. Functional properties of protein-polysaccharide mixtures. In J.R. Mitchell and D.A. Ledward (eds.). Functional properties of food macromolecules, pp. 385-416. Essex: Elsevier Applied Science Publishers.

Tolstoguzov, V.B. 1997. Protein-polysaccharide interactions. In S. Damodaran and A. Paraf (eds.). Food proteins and their applications, pp. 171-198. New York: Marcel Dekker.

Tornberg, E. 2005. Effects of heat on meat proteins-Implications on structure and quality of meat products. Meat Science. 70: 493-508.

Trautwein, E.A., Jürgensen, U., and Erbersdobler, H.F. 1997. Cholesterol-lowering of gallstone-preventing action of chitosans with different degree of deacetylation in hamster fed cholesterol-rich diets. Nutrition Research. 17(6): 1053-1065.

Trespalacios, P., and Pla, R. 2007. Simultaneous application of transglutaminase and high pressure to improve functional properties of chicken meat gels. Food Chemistry. 100(1): 264-272.

Trout, G.R., and Schmidt, G.R. 1987. Nonprotein additives. In A.M. Pearson and T.R. Dutson (eds.). Advances in meat research Volume 3: Restructured meat and poultry products, pp. 307-326. New York: AVI Publishing.

Tsai, G.J., Su, W.H., Chen, H.C., and Pan, C.L. 2002. Antimicrobial activity of shrimp chitin and chitosan from different treatments and applications of fish preservation. Fisheries Science. 68: 170-177.

Tsai, S.J., Unklesbay, N., Unklesbay, K., and Clarke, A. 1998. Water and absorptive properties of restructured beef products with five binders at four isothermal temperatures. Lebensm.-Wiss. U.-Technol. 31:78-83.

Tsumura, K., Saito, T., Tsuge, K., Ashida, H., Kugimiya, W., and Inouye, K. 2005. Functional properties of soy protein hydrolysates obtained by selective proteolysis. LWT. 38: 255–261.

Tung, M.A., and Paulson, A.T. 1995. Rheological concepts for probing ingredient interactions in food system. In A.G. Goankar (ed.). Ingredient interactions-Effects on food quality, pp. 45-83. New York: Marcel Dekker.

Turgeon, S.L., Beaulieu, M., Schmitt, C., and Sanchez, C. 2003. Protein-polysaccharide interactions: Phase-ordering kinetics, thermodynamic and structural aspects. Current Opinion in Colloid and Interface Science. 8:401-414.

USDA. 2003. Specification for dry whey protein concentrate (Effective April, 2003). [Online]. Available from: http://www.ams.usda.gov/dairy/dry_whey_prot_conc.pdf

Vårum, K.M., Holme, H.K., Izume, M., Stokke, B.T., and Smidsrød, O. 1996. Determination of enzymatic hydrolysis specificity of partially ***N***-acetylated chitosans Biochimica et Biophysica Acta (BBA) - General Subjects. 1291(1): 5-15.

Verbeken, D., Neirinck, N., Van Der Meeren, P. and Dewettinck, K. 2005. Influence of κ-carrageenan on the thermal gelation of salt-soluble meat proteins. Meat Science. 70(1): 161-166.

Vliet, T.V. 1999. Rheological classification of foods and instrumental techniques for their study. In A.J. Rosenthal (ed.). Food texture: Measurement and perception, pp. 65-98. Maryland: An Aspen Publication.

Voisey, P.W. 1976. Instrumental measurement of food texture. In J.M. DeMan, P.W. Voisey, V.F. Rasper and D.W. Stanley (eds.). Rheology and texture in food quality, pp. 79-141. Connecticut: AVI Publishing.

Wang, C.H., and Damodaran, S. 1990. Thermal gelation of globular proteins: Weight-average molecular weight dependence of gel strength. Journal of Agricultural and Food Chemistry. 38(5): 1157-1164.

Wang, L.L., and Xiong, Y.L. 2005. Inhibition of lipid oxidation in cooked beef patties by hydrolyzed potato protein is related to its reducing and radical scavenging ability. Journal of Agricultural and Food Chemistry. 53: 9186-9192.

Walsh, D.J., Cleary, D., McCarthy, E. Murphy, S., and FitzGerald, R.J. 2003. Modification of the nitrogen solubility properties of soy protein isolate following, proteolysis and tranglutaminase cross-linking. Food Research International. 36: 677-683.

Webster, J.D., Ledward, D.A., and Lawrie, R.A. 1982. Protein hydrolysates from meat industry by products. Meat Science. 7(2): 147-157.

Westphalen, A.D., Briggs, J.L., and Lonergan, S.M. 2005. Influence of pH on rheological properties of porcine myofibrillar protein during heat induced gelation. Meat Science. 70(2): 293-299.

Winterowd, J.G. and Sandford, P.A. 1995. Chitin and chitosan. In M.S. Alistair (ed.), Food polysaccharides and their applications, pp. 441-462. New York: Marcel Dekker.

Wong, D.W.S. 1989. Mechanism and theory in food chemistry. New York: AVI Publishing.

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

Xiong, Y.L. 2004. Muscle proteins. In R.Y. Yada (ed.). Protein in food processing, pp. 101-122. Baca Raton: CRC Press.

Xiong, Y.L. 2005. Role of myofibrillar proteins in water-binding in brine-enhanced meats. Food Research International. 38: 281-287.

Yamashita, Y., Zhang, N., and Nozaki, Y. 2003. Effect of chitin hydrolysate on the denaturation of lizard fish myofibrillar protein and the state of water during frozen storage. Food Hydrocolloids. 17: 569-576.

Zaleska, H., Ring, S., and Tomasik, P. 2001. Electrosynthesis of potato starch-whey protein isolate complexes. Carbohydrate Polymers. 45:89-94.

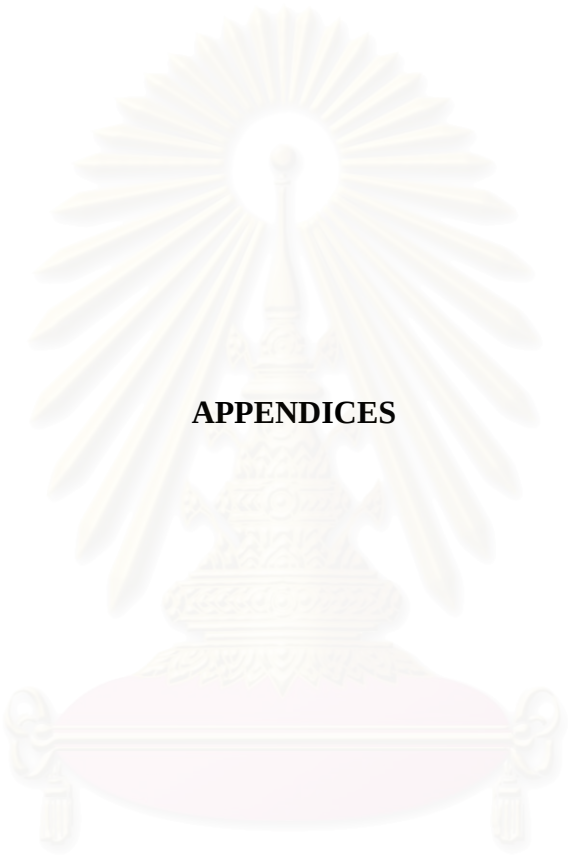
Zaleska, H., Tomasik, P., and Lii, C.Y. 2002. Formation of carboxymethyl cellulose-casein complexes by electrosynthesis. Food Hydrocolloids. 16:215-224.

Zayas, J.F. 1997. Functionality of proteins in food. New York: Springer-Verlag Berlin.

Zhang, M., Duan, Z.H., Huan, Y.J., and Tao, Q. 2003. Preparation technology for semi-fluid high-energy food. Journal of Food Engineering. 59: 327-330.

Zolper, J.T. 1995. Fat substitute based on carrageenan gels, processes for producing the same and food products containing fat substitutes. US Patent. 5,458,904.

APPENDICES



APPENDIX A

Additional Information of Chitosan

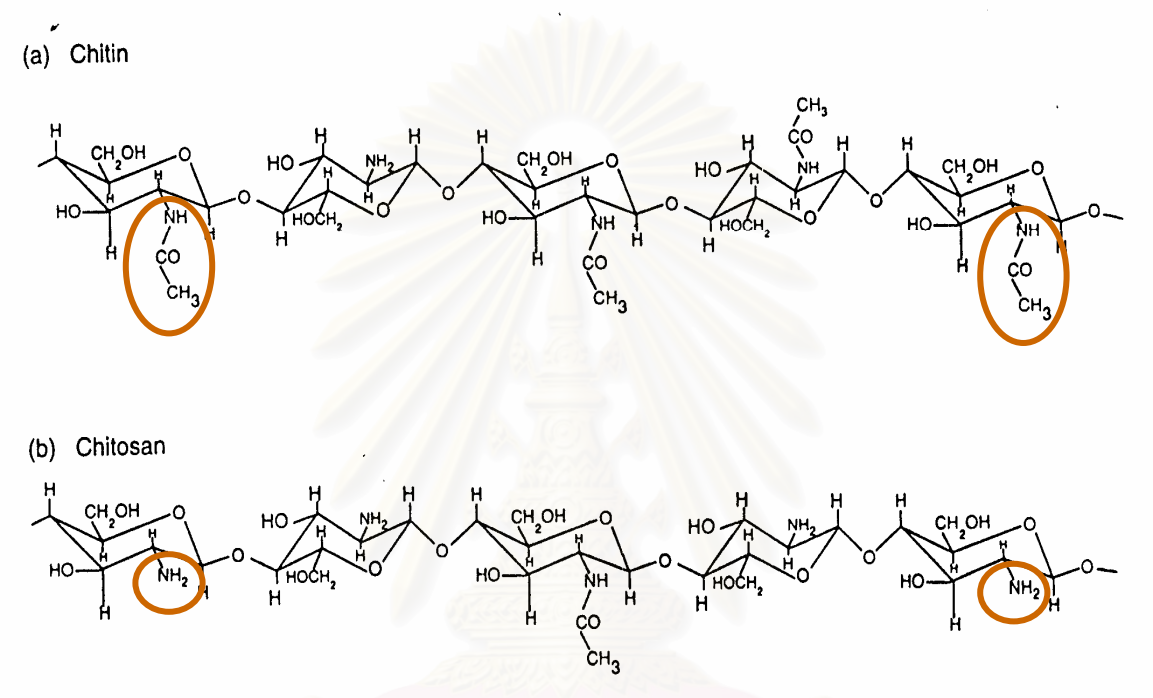


Figure 1: Molecular structure of chitin and chitosan

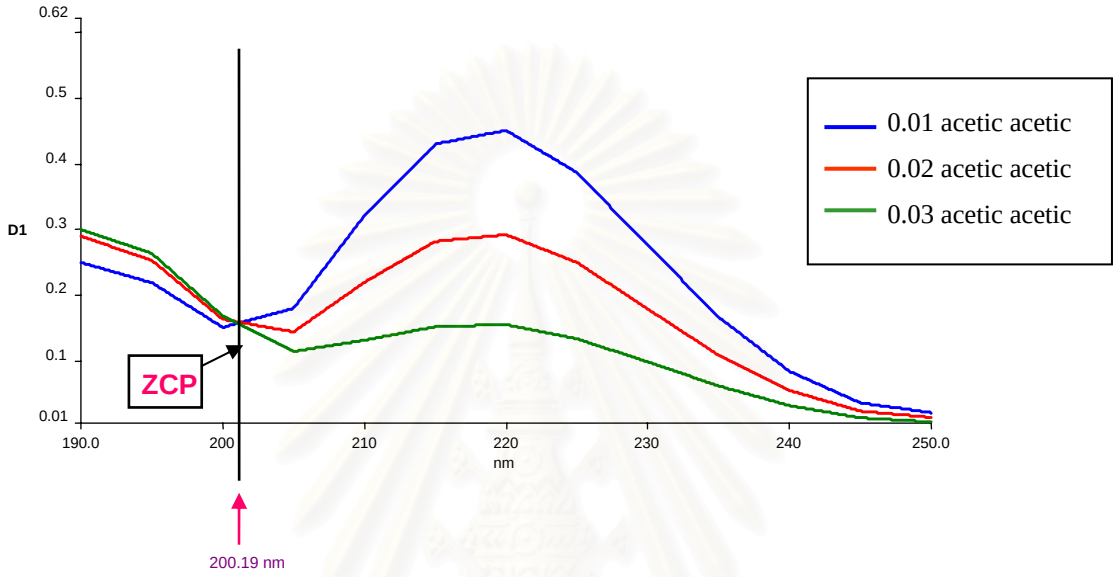


Figure 2: First derivatives of standard solution of 0.01, 0.02 and 0.03M acetic acid

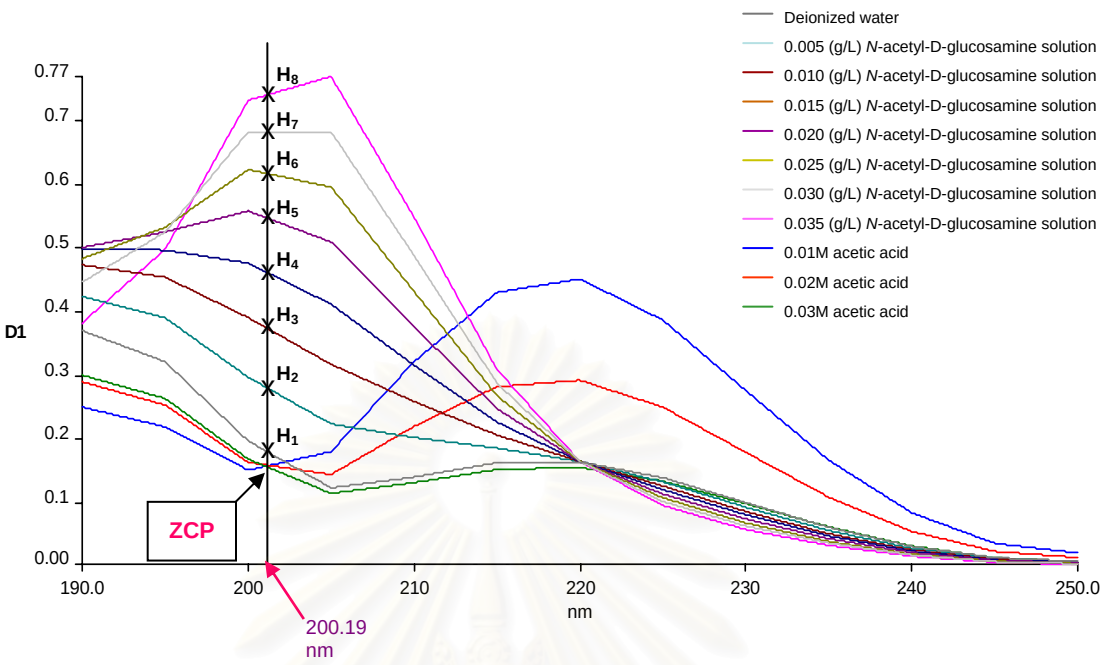


Figure 3: First derivatives of standard solution of *N*-acetyl-D-glucosamine

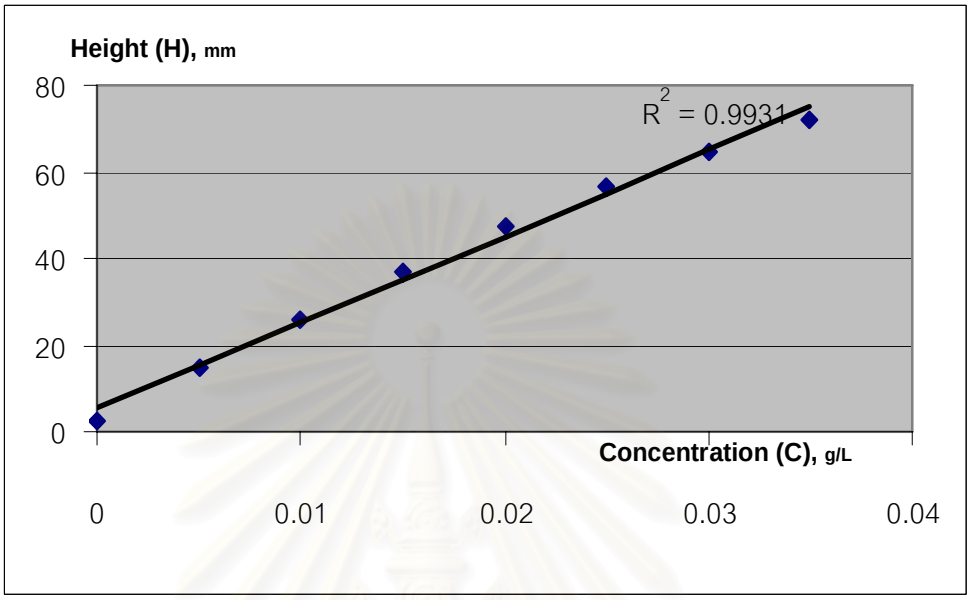


Figure 4: Standard curve of *N*-acetyl-D-glucosamine solution

APPENDIX B

Additional Information of Chicken Protein Hydrolysates

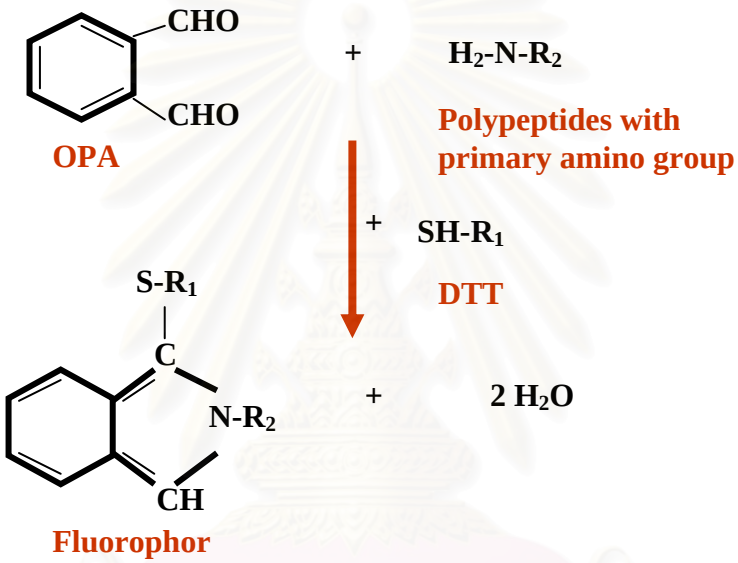


Figure 1: The OPA reaction; OPA reacts with primary amino groups and a SH-compound (dithiothreitol, DTT) to form a compound that will absorb light at 340 nm.

VITAE

Miss Tantawan Kachanechai was born on September 23, 1980 in Bangkok, Thailand. She obtained a B.Sc. degree (First Class Honors) in Food and Nutrition from Faculty of Public Health, Mahidol University in 2000 and continually studied in Food Technology Department, Faculty of Science, Chulalongkorn University. Her research areas are restructured meat via cold-set gelation mechanism, cold-set gelation of proteins, cold-set binders, chitosan and protein hydrolysates.