# Impact of Salting on Chemical Compositions, Physicochemical and Functional Properties of Duck Egg

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**ชื่อวิทยานิพนธ์** ผลของการดองเกลือต่อองค์ประกอบทางเคมี สมบัติทางเคมีกายภาพ และสมบัติเชิงหน้าที่ของไข่เป็ด
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## บทคัดย่อ

จากการศึกษาการเปลี่ยนแปลงองค์ประกอบทางเคมี สมบัติทางกายภาพ และโครงสร้างทางจุลภาคของไข่เป็ดทางการค้าระหว่างการดองเกลือด้วยวิธีการพอกดินเค็มเป็นเวลา 2 สัปดาห์พบว่า การดองเกลือมีผลต่อการเพิ่มสัดส่วนไข่ขาวในขณะที่สัดส่วนไข่แดงลดลง นอกจากนี้ปริมาณความชื้นของไข่ขาวและไข่แดงลดลงอย่างต่อเนื่องในขณะที่ปริมาณเถ้าเพิ่มขึ้น ปริมาณโปรตีนและไขมันของไข่แดงทั้งส่วนนอกและส่วนในเพิ่มขึ้นเพียงเล็กน้อย ปริมาณไขมันที่ไหลเยิ้มเพิ่มขึ้นอย่างเห็นได้ชัดเมื่อระยะเวลาการดองเพิ่มขึ้นโดยเฉพาะในไข่แดงส่วนนอก ไตรกลีเซอไรด์และฟอสโฟไลปิดซึ่งเป็นไขมันหลักที่พบในไข่แดงมีการเปลี่ยนแปลงเล็กน้อยแต่ไม่พบการเปลี่ยนแปลงของรูปแบบโปรตีนของไข่แดงและไข่ขาวระหว่างการดองเกลือ เมื่อตรวจสอบโครงสร้างทางจุลภาคด้วยกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด พบว่าไข่แดงมีลักษณะโครงสร้างเป็นเม็ดที่มีเหลี่ยมขนาดเล็กจัดเรียงตัวกันและพบการจัดเรียงตัวชิดมากขึ้นเมื่อผ่านการดองเกลือ เมื่อตรวจสอบด้วยกล้องจุลทรรศน์อิเล็กตรอนแบบส่องผ่านพบว่าไข่แดงที่ผ่านการดองเกลือมีการกระจายตัวอย่างของโปรตีนทรงกลมอย่างสม่ำเสมอและพบหยดไขมันขนาดเล็กภายในโครงสร้างของเม็ดไข่แดงดังกล่าว การตรวจสอบด้วยกล้องจุลทรรศน์แบบส่องกราดด้วยแสงเลเซอร์ชี้ให้เห็นว่าไข่แดงที่ผ่านการดองเกลือมีการสูญเสียน้ำและปลดปล่อยไขมันออกมาจากโครงสร้างเพิ่มขึ้น

เมื่อเปรียบเทียบวิธีการดองเกลือสองวิธีคือ วิธีการพอกดินเค็มและการแช่น้ำเกลือ พบว่าวิธีการดองทั้งสองมีผลต่อการลดลงของปริมาณความชื้นและการเพิ่มขึ้นของปริมาณเกลือของทั้งไข่ขาวและไข่แดง วิธีการดองแบบพอกดินเค็มมีแนวโน้มให้ปริมาณไขมันที่ไหลเยิ้มของไข่แดงมากกว่าการดองด้วยวิธีการแช่น้ำเกลือ อย่างไรก็ตามไม่พบความแตกต่างของสัดส่วนไข่แดงที่แข็งตัวจากทั้งสองวิธี ไข่แดงเค็มที่ผลิตโดยวิธีการพอกดินเค็มมีค่าความแข็งและค่าการเกาะติดสูงกว่าไข่แดงเค็มจากการดองด้วยวิธีแช่น้ำเกลือ ในขณะที่ค่าความเปราะ ค่าความยืดหยุ่นและค่า

พลังงานที่ใช้ในการบดเคี้ยวของไข่แดงนั้นมีค่าสูงกว่าไข่แดงที่ดองด้วยวิธีแช่น้ำเกลือ อย่างไรก็ตาม การดองทั้งสองวิธีให้ค่าการยึดเกาะที่ใกล้เคียงกันโดยมีการสูญเสียน้ำและการปลดปล่อยไขมันที่เพิ่มขึ้นเมื่อเวลาในการดองเพิ่มขึ้นแต่พบได้มากกว่าในไข่แดงที่ดองด้วยวิธีการพอกดินเค็ม ดังนั้น กระบวนการดองเกลือจึงมีผลต่อสมบัติของไข่ในระดับหนึ่ง

สำหรับการลดเวลาในการดองเกลือให้สั้นลง ไข่เป็ดที่แช่ในสารละลายกรดอะซิติกความเข้มข้นร้อยละ 5 ก่อนแช่ในสารละลายโปรตีนเอสทางการค้า 4 ชนิด คือ ฟลาโวไซม์ โปรตาเม็กส์ อัลคาเลส และนิวเทรส ก่อนการดองเกลือ พบว่าการแช่ไข่เป็ดในสารละลายกรดอะซิติกร้อยละ 5 นาน 30 นาที ตามด้วยการแช่ด้วยสายละลายฟลาโวไซม์และนิว เทรสก่อนทำการดองด้วยวิธีแช่น้ำเกลือให้ค่าสัดส่วนของไข่แดงที่แข็งตัวสูงสุดสอดคล้องกับการเพิ่มขึ้นของปริมาณเกลือในไข่ขาวและการลดลงของปริมาณความชื้นในไข่แดง เมื่อเปรียบเทียบกับชุดการทดลองอื่นๆ ($p< 0.05$) การแช่ไข่เป็ดในกรดอะซิติกร้อยละ 5 และสารละลายนิวเทรสที่ระดับความเข้มข้นร้อยละ 0.25 เป็นเวลา 90 นาทีสามารถการลดระยะเวลาในการดองเกลือได้มากสุด 1 สัปดาห์ อีกทั้งเพิ่มปริมาณไขมันที่ไหลเยิ้มของไข่แดงได้

เมื่อทำการตรวจสอบองค์ประกอบทางเคมี สมบัติทางเนื้อสัมผัส และโครงสร้างทางจุลภาคของไข่เป็ดต้มสุก พบว่าเมื่อระยะเวลาการดองเกลือเพิ่มขึ้นทั้งไข่ขาวและไข่แดงต้มสุกมีปริมาณความชื้นลดลงในขณะที่ปริมาณเกลือเพิ่มขึ้น ($p< 0.05$) ปริมาณไขมันที่ไหลเยิ้มของไข่แดงต้มสุกและปริมาณน้ำจากการบีบอัดของไข่ขาวต้มสุกเพิ่มขึ้น เมื่อเวลาการดองเกลือเพิ่มขึ้น ($p< 0.05$) ภายหลังการต้ม ไข่แดงมีการปลดปล่อยไขมันอิสระร่วมกับการละลายสารให้สีออกมาโดยเฉพาะบริเวณไข่แดงส่วนนอก เมื่อระยะเวลาการดองเพิ่มขึ้นพบว่าไข่ขาวต้มสุกมีค่าความแข็ง ค่าความยืดหยุ่น ค่าพลังงานที่ใช้ในการบดเคี้ยว และค่าการคืนตัวลดลง ในขณะที่ค่าการเกาะติดและค่าการยึดเกาะเพิ่มขึ้นซึ่งสามารถพบได้จากการดองทั้งสองวิธี ในทางตรงข้ามกัน ไข่แดงต้มมีค่าความแข็งสูงขึ้นเมื่อระยะเวลาการดองเกลือเพิ่มขึ้น ไข่แดงที่ผ่านการดองเกลือและต้มสุกมีขนาดอนุภาคเม็ดไข่แดงที่เล็กกว่าเมื่อเปรียบเทียบกับอนุภาคเม็ดไข่แดงจากไข่แดงต้มสุกที่ปราศจากการดองเกลือ และพบว่าเจลไข่ขาวของไข่เค็มต้มสุกมีลักษณะเป็นเจลโปรตีนเกาะกลุ่ม (coagulum type gel)

การติดตามผลของความเข้มข้นของโซเดียมคลอไรค์ต่อการจับรวมตัวของไข่ขาวจากไข่เป็ดในระหว่างการให้ความร้อนที่ช่วงอุณหภูมิระหว่าง 20 ถึง 90 °ซ พบว่าสารละลายไข่ขาว (1 มก. โปรตีน/มล.) มีค่าความขุ่นและปริมาณไฮโดรโฟบิกซิตีบริเวณพื้นผิวเพิ่มขึ้น ในขณะที่ปริมาณหมู่ซัลฟ์ไฮดริลลดลงเมื่อให้ความร้อนในช่วงอุณหภูมิ 70-90 องศาเซลเซียส ($p< 0.05$) ซึ่ง

บ่งชี้ถึงการจับรวมตัวของโปรตีนที่เพิ่มขึ้น ณ อุณหภูมิสูง การจับรวมตัวของโปรตีนไข่ขาวเพิ่มขึ้นเมื่อเพิ่มความเข้มข้นของโซเดียมคลอไรด์ ภายหลังการให้ความร้อนจนถึง 90 °ซ พบว่าเมื่อความเข้มข้นของโซเดียมคลอไรด์เพิ่มขึ้น ความเป็นประจุลบของโปรตีนไข่ขาวมีค่าลดลง ในขณะเดียวกันโปรตีนมีการจับตัวกันเป็นกลุ่มอนุภาคที่มีขนาดใหญ่ขึ้นจากการตรวจสอบด้วยกล้องจุลทรรศน์แบบส่งกราดด้วยแสงเลเซอร์

จากการศึกษาผลของโซเดียมคลอไรด์และการกำจัดน้ำต่อพฤติกรรมทางวิสโคอิลาสติกของไข่แดงพบว่า การเพิ่มความเข้มข้นของโซเดียมคลอไรด์ร้อยละ 0 จนถึงร้อยละ 3 มีผลต่อพฤติกรรมทางวิสโคอิลาสติกของไข่แดงคือ ทำให้เกิดการเปลี่ยนแปลงจากของไหลไปเป็นเจล โดยเฉพาะอย่างยิ่งที่ระดับการเติมโซเดียมคลอไรด์เข้มข้นร้อยละ 1.5 จากการศึกษาผลของการกำจัดน้ำของไข่แดงต่อพฤติกรรมทางวิสโคอิลาสติกพบว่า การกำจัดน้ำมีอิทธิพลต่อการจับตัวและการเกิดโครงข่ายของเจลมากกว่าการเติมโซเดียมคลอไรด์เพียงอย่างเดียว อย่างไรก็ตามเมื่อมีการกำจัดน้ำที่ระดับต่ำการเติมโซเดียมคลอไรด์สามารถเปลี่ยนพฤติกรรมทางวิสโคอิลาสติก ของไข่แดงได้ คือ มีผลต่อการพัฒนาโครงข่ายของเจลที่ดีขึ้น การเติมโซเดียมคลอไรด์ในไข่แดงทำให้เกิดความคงตัวของโมเลกุลโปรตีนซึ่งสังเกตจากเพิ่มขึ้นของอุณหภูมิการสูญเสียสภาพธรรมชาติเช่นเดียวกับการชะลอการเกิดโครงข่ายของเจล ในทางตรงข้ามกันการกำจัดน้ำออกจากไข่แดงมีผลกระทบต่อการเกิดโครงข่ายของเจลเพียงเล็กน้อยในระหว่างการให้ความร้อน จากการศึกษาสมบัติทางความร้อนด้วย Differential Scanning Colorimetry ชี้ให้เห็นว่าการเพิ่มปริมาณโซเดียมคลอไรด์และการเพิ่มระดับการกำจัดน้ำของไข่แดงมีผลต่อการเพิ่มอุณหภูมิสูญเสียสภาพธรรมชาติและลดเอนธาลปี จากการตรวจสอบโครงสร้างทางจุลภาคพบว่าเจลไข่แดงมีโครงข่ายแน่นขึ้นและมีช่องว่างภายในโครงข่ายลดลงเมื่อความเข้มข้นของโซเดียมคลอไรด์และระดับการกำจัดน้ำเพิ่มขึ้น

จากการศึกษาการเพิ่มการใช้ประโยชน์จากไข่ขาวเค็มโดยการผลิตไข่ขาวเค็มไฮโดรไลเสทที่ระดับการย่อยสลาย 3 ระดับคือร้อยละ 3 6 และ 9 เพื่อใช้สำหรับการปรับปรุงคุณภาพของกุ้งขาว พบว่ากุ้งขาวที่ผ่านการแช่ในสารละลายโซเดียมคลอไรด์ร้อยละ 4 ที่ประกอบด้วยโปรตีนไข่ขาวเค็มไฮโดรไลเสทร้อยละ 7 (ที่ระดับการย่อยสลายร้อยละ 3) และฟอสเฟตผสม (โซเดียมแอซิดไพโรฟอสเฟตร้อยละ 0.625 และเตตระโซเดียมไพโรฟอสเฟตร้อยละ 1.875) ร้อยละ 2.5 มีผลผลิตหลังการให้ความร้อนสูงสุดและมีการสูญเสียภายหลังการให้ความร้อนต่ำสุด อีกทั้งมีคะแนนความชอบทางประสาทสัมผัสทั้งลักษณะปรากฏ เนื้อสัมผัส และ ความชอบโดยรวมที่มากกว่าแต่มีค่าแรงเฉือนที่น้อยกว่าเมื่อเทียบเท่ากับกุ้งที่แช่ด้วยโซเดียมคลอไรด์ร้อยละ 4 ที่มีฟอสเฟตผสมร้อยละ 3.5 การศึกษาลักษณะโครงสร้างทางจุลภาคแสดงให้เห็นว่าเส้นใย

กล้ามเนื้อกุ้งขาวจากทั้งสองชุดการทดลองมีลักษณะการพองตัวและพบช่องว่างระหว่างเส้นใยกล้ามเนื้อ ในขณะที่ชุดควบคุมมีโครงสร้างที่แน่น ดังนั้นการใช้โปรตีนไฮโดรไลเสทจากไข่ขาวเค็มสามารถลดปริมาณการใช้ฟอสเฟตโดยปราศจากผลกระทบต่อสมบัติทางประสาทสัมผัสของกุ้งขาว

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## ABSTRACT

Freshly prepared commercial salted duck eggs with paste coating method were monitored for chemical composition, physical properties and microstructure during salting up to 2 weeks. Salting resulted in an increase in weight proportion of egg white, but a decrease in yolk proportion. Moisture contents of both egg white and yolk decreased gradually with concomitant increases in salt and ash contents as the salting time increased. Protein and lipid contents slightly increased in both interior and exterior egg yolk with increasing salting time. Salting had an impact on oil exudation content in yolk, particularly in exterior yolk. Triacylglycerols and phospholipid found as the major lipids in egg yolk underwent slight changes, but no differences in protein patterns of both egg white and egg yolk were observed during salting. Scanning electron microscopic study revealed that yolk granule was polyhedral in shape and aligned closely when the salting proceeded. Protein spheres distributed uniformly together with oil droplets in salted yolk as visualized by transmission electron microscopy. Confocal laser scanning microscope (CLSM) micrographs indicated that greater dehydration and release of lipids took place in egg yolk during salting.

When two salting methods, paste coating and brine immersing, were compared, the decreases in moisture content with coincidental increases in salt content in both egg white and yolk were observed during salting, regardless of salting processes. Paste coating method tended to yield the greater oil exudation of egg yolk than immersing method. Similar hardening ratio of yolk was observed between both processes. The higher hardness and adhesiveness were found in yolk with paste

coating method, whereas the greater fractureability, springiness, gumminess and chewiness were observed with immersing method. Nevertheless, both processes rendered the yolk with similar cohesiveness. Dehydration and release of lipids in egg yolk increased with increasing salting time and were more pronounced with paste coating method. Thus salting processes affected the egg properties of egg to some degree.

To shorter salting time, duck egg pretreated with 5% acetic acid and different commercial proteases (Flavourzyme, Protamex, Alcalase and Neutrase) was subjected to salting. Duck eggs soaked in 5% acetic acid for 30 min, followed by socking in 5% (w/v) Flavourzyme and Neutrase prior to salting had the highest hardening ratio with the coincidental increase in salt content in egg white and decrease in moisture content of yolk, compared with those with other treatments ($p< 0.05$). Eggs pretreated with 0.25% (w/v) Neutrase for 90 min had the shorter salting time and enhanced oil exudation of yolk upon salting.

Chemical composition, textural properties, and microstructure of cooked egg were analyzed. Cooked egg had the decreases in moisture content with coincidental increases in salt content for both egg white and yolk obtained with increasing salting time ($p< 0.05$). Oil exudation of cooked yolk and expressible water content of cooked egg white obtained from both salting methods increased as salting proceeded ($P< 0.05$). After cooking, the release of free lipid accompanied by the solubilized pigments, especially at the outer layer of yolk, was obtained. As salting times increased, the lower hardness, springiness, gumminess, chewiness and resilience with higher adhesiveness and cohesiveness were found in cooked salted egg white ($p< 0.05$), irrespective of salting methods and times. Conversely, the higher hardness of cooked yolk was found with increasing salting time ($p< 0.05$). The smaller yolk granules with more release of free lipid were observed in salted egg after heating, compared with the fresh counterpart. After heating, the coagulum type gel of salted egg white was obtained.

Thermal aggregation of duck egg white solution were also monitored in the presence of different NaCl concentration (0-6%, w/w) at the temperature range

of 20-90°C. Egg white solution exhibited the higher turbidity with coincidental increases in surface hydrophobicity and the decreases in sulfhydryl group content as the heating temperatures increased from 70 to 90°C ($p < 0.05$), suggesting the increased formation of aggregate at high temperatures. The aggregation of egg white protein became more pronounced with increasing NaCl concentrations. After heating to 90°C, as NaCl concentration increased, the negative charge decreased with coincidental increases in particle size of aggregate and the larger cluster of protein were observe under visualization by confocal laser scanning microscopy.

The effects of NaCl and dehydration on the linear viscoelastic behaviour of egg yolk were evaluated. An increase in NaCl concentrations from 0 to 3% (w/w) resulted in a remarkable change in the linear viscoelastic behaviour by inducing a sol-gel transition. The transition was more pronounced when 1.5% (w/w) NaCl was incorporated. The influence of dehydration on viscoelastic behaviour of egg yolk at various NaCl concentrations was also examined. It was found that the effect of dehydration on aggregation and network formation was predominant than that of the NaCl addition. Nevertheless, at a lower degree of dehydration, the addition of NaCl could modulate the viscoelastic behaviour of egg yolk, resulting in a well-developed gel network. Addition of NaCl into egg yolk could stabilize the protein molecules as evidenced by an increase in denaturation temperature as well as a delay in gel network formation. Conversely, dehydration had a little impact on the gel formation of egg yolk during heating. DSC study suggested a progressive increase in denaturation temperature, but a lower enthalpy when NaCl concentration and degree of dehydration increased. As visualized by a scanning electron microscope, the denser network with smaller voids was observed in egg yolk gel with increasing NaCl concentration and degree of dehydration.

To enhance the use of salted egg white, protein hydrolysate from salted egg white (PHSEW) with different degrees of hydrolysis (DH) (3, 6 and 9%) was produced using pepsin and used for quality improvement of Pacific white shrimp (*Litopenaeus vannamei*). Shrimp soaked in 4% NaCl containing 7% PHSEW (DH= 3%) and 2.5% mixed phosphates (0.625% sodium acid pyrophosphate (SAPP) and

1.875% tetrasodium pyrophosphate (TSPP)) had the highest cooking yield with the lowest cooking loss ($p < 0.05$) Cooked shrimp treated with 4% NaCl containing 7% PHSEW and 2.5% mixed phosphate or those treated with 4% NaCl containing 3.5% had the higher score of appearance, texture and overall likeness but less shear force ($p < 0.05$). Microstructure study revealed that muscle fibers of cooked shrimp from both treatments had the swollen fibrils and gaps, while the control had the swollen compact structure. Therefore, use of PHSEW could reduce phosphate residue in shrimps without an adverse effect on sensory properties.

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# CONTENTS

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## CONTENTS (Continued)

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Thammarat Kaewmanee

## LIST OF TABLES

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## LIST OF FIGURES

## LIST OF FIGURES (Continued)

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# CHAPTER 1

# INTRODUCTION AND REVIEW OF LITERATURE

## 1.1 Introduction

Salted egg is pickled by salt and the salted egg yolk is a traditional preserved egg product, which is very popular in Asia (Chi and Tseng, 1998). Salted eggs have been produced in Thailand and consumed nationwide. Chaiya, Suratthani Province, is known as the largest producer of salted egg in Thailand. It is commonly consumed, especially with rice gruel for breakfast. Salted egg yolk is also widely used in bakery products, such as moon cake or chinese cake. Currently, there are two methods for salted egg production: by immersing the eggs in brine and by covering the eggs with salted soil paste. Salting process normally takes 3-4 weeks. The salty taste and appearance of salted egg vary with salting time as well as salting method. Traditionally, salted eggs are made from duck eggs because they attain more desirable characteristics than do hen eggs (Li and Hsieh, 2004). To shorten the salting time, new technology had been recently developed using the pressurizing technology. Even though the process could reduce the salting time to 2-3 days, egg yolk became watery and did not attain desirable characteristics (Chiang and Chung, 1986). Presently, the salted egg yolks production has been successfully developed by pickling the separated yolk in salt solution at high pressure condition. This process takes only 1 day to obtain the salted yolk (Thipayarut and Nopharatana, 2005). Salted egg white (containing 10% protein and 4-7% sodium chloride) is discarded as waste which produces environmental pollution because of the coagulated egg yolk are used for secondary processing in bakery products (Huang *et al*., 1999). In general, egg whites have varying functional properties such as foaming ability, emulsifying activity and gelation and have been used widely in food processing (Nakamura and Sat, 1964; Yang and Lin, 1990). These functional properties can be changed by sodium chloride added (Kakalis and Regenstein, 1986; Arntfield *et al*., 1990; Elizalde *et al*., 1991;

Vani and Zayas, 1995; Mine *et al.*, 1991). To maximize the utilization of salted egg white, the extraction of lysozyme (Chang and Liu, 1994; Liu *et al.*, 1994), and the use as ingredient frankfurters (Lin *et al.*, 1996) were carried out. Gel forming ability and emulsion stability of dried salted duck egg white powder were also investigated (Huang *et al.*, 1996). However, dried salted duck egg white has 30% sodium chloride and hygroscopic properties, which makes it less suitable for food application. Therefore, salted duck egg white is generally recognized as a useless by-product. In order to make it more utilizable in food manufacturing, alternative uses should be further studied.

Though salted egg has been consumed widely and new technologies have been developing, the formation of salted egg as appeared by the changes in characteristics of both egg white and egg yolk has not been clearly understood. Shorter time of commercial salting process should be developed. Also, the utilization of salted egg white by hydrolysis process has not been studied. Therefore, the studies of chemical, physico-chemical and microstructure properties of duck egg during salting as well as the mechanism of salted egg formation should be elucidated in order to manipulate the process to obtain the desired quality of salted eggs. Also, the new method, which could shorten the salting times, but still yields the salted egg with the acceptability, should be developed. The information gained would be of benefit for egg farmer and processor to understand and come across the appropriate processes for both salted egg production and the maximized use of salted egg residue.

## 1.2 Review of Literature

### 1.2.1 Structures and compositions of the egg

Egg is composed of three main parts; shell, albumen (egg white), and yolk. The yolk is surrounded by an albumen layer, and this structure is covered by a hard eggshell (Yamamoto *et al*., 1996). The weight of an egg and the weight distribution of the three parts differ considerably, depending on the kind of egg and their age (Okubo *et al.*, 1997). The egg is surrounded by a 0.2– 0.4 mm thick

calcareous and porous shell (Figure 1). Shells of chicken eggs are white-yellow to brown; duck's shells are greenish to white, and those of most wild birds are characteristically spotted. The inside of the shell is lined with two closely adhering membranes (inner and outer). The two membranes separate at the large end of the egg to form an air space, the so-called air cell. The air cell is approx. 5 mm in diameter in fresh eggs and increases in size during storage, hence it can be used to determine the age of eggs. The egg white (albumen) is aqueous, consisting of three fractions that differ in viscosity. The inner portion of the egg, the yolk, is surrounded by albumen. A thin but very firm layer of albumen (chalaziferous layer) closely surrounds the yolk and it branches on opposite sides of the yolk into two chalazae that extend into the thick albumen. The chalazae resemble two twisted rope-like cords, twisted clockwise at the large end of the egg and counterclockwise at the small end. They serve as anchors to keep the yolk in the center. In an opened egg, the chalazae remain with the yolk. The germinal disc (blastoderm) is located at the top of a clubshaped latebra on one side of the yolk. The yolk consists of alternate layers of dark- and light-colored material arranged concentrically (Belitz *et al*., 2009).

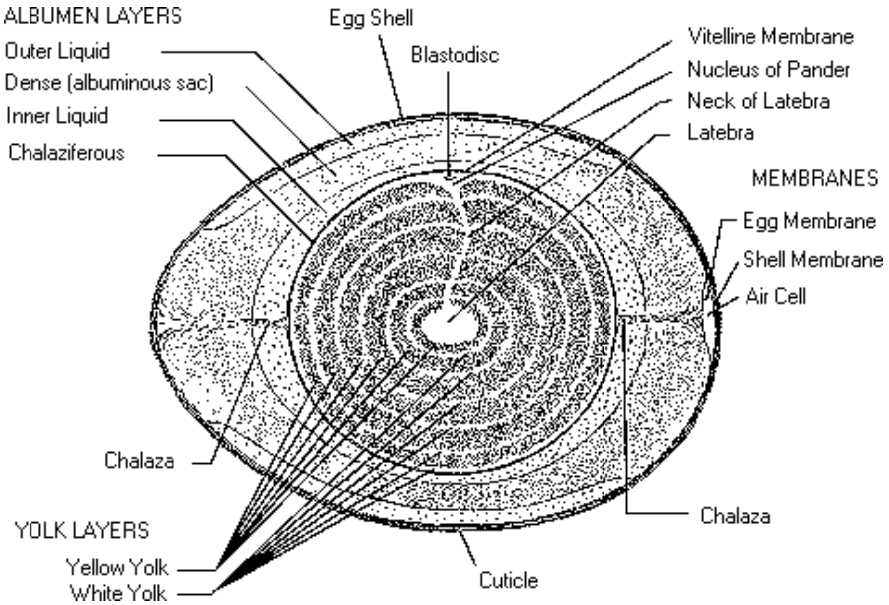



**Figure 1.** Structure of the egg.

**Source:** Yamamoto *et al.* (1996)

#### 1.2.1.1 Egg shell

The shell consists of calcite crystals embedded in an organic matrix or framework of interwoven protein fibers and spherical masses (proteinmucopolysaccharide complex) in a proportion of 50:1 (Figure 2). There are also small amounts of magnesium carbonate and phosphates (Belitz *et al*., 2009). The shell structure is divided into four parts: the cuticle or bloom, the spongy layer, the mammillary layer and the pores membrane (Okubo *et al.*, 1997). The outermost shell coating is an extremely thin (10 μm), transparent, mucilaginous protein layer called the cuticle, or bloom (Figure 3). The spongy (calcareous layer), a matrix comprising two-thirds of the shell thickness, is below the thin cuticle. The mammillary layer (cone layer) consists of a small layer of compressed, knob-like particles, with one side firmly cemented to the spongy layer and the other side adhering closely to the outer surface of the shell membrane (Okubo *et al.*, 1997).

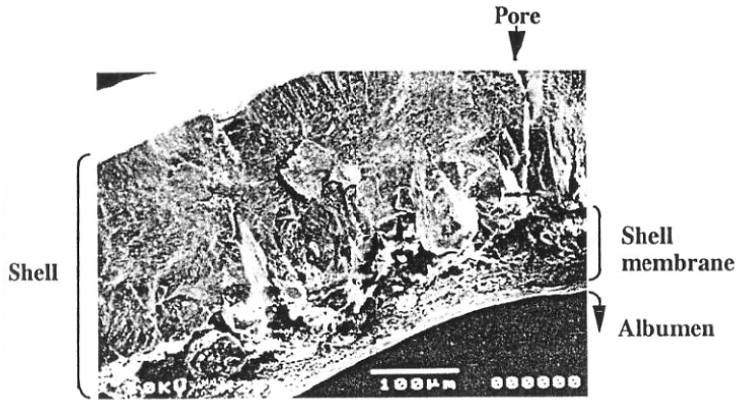



**Figure 2.** Scaning electron microscopic photograph of the shell of hen egg
**Source:** Okubo *et al.* (1997)

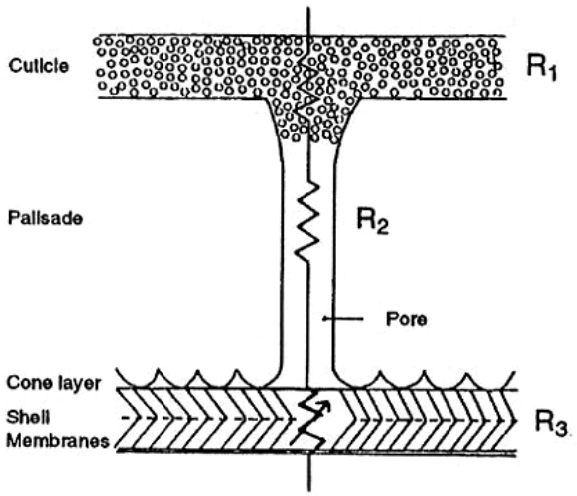



**Figure 3.** Schematic drawing of radial section of hen's egg showing the major components of the resistance network. R1 is the cuticle, R2 is the pore canal, and R3 is the shell membranes

**Source:** Board and Tranter (1997)

**a) Pore**

There are funnel-shaped small holes called pore canals on the surface of the shell for gas exchange (Figure 2 and 3). The pore canals are scatteringly located between the palisade layers of the shell, directed to the exterior. The diameter of the pore canal ranges from 10 to 30 μm. An egg has about 10,000 pore canals on the shell surface. The pore canal allows air and moisture to pass through, but does not allow liquid or water (Board and Tranter, 1997).

**b) Cuticle**

The cuticle, the most external layer of eggs, is about 10 μm thick and covers the pore canals. It protects the egg from moisture and invasion of microorganisms (Board and Hall, 1973). The cuticle permits the exchange of gas in the egg. The cuticle is removed from the shell easily by soaking eggs in either weak acid solutions or metal chelator containing solutions or by washing with water (Belyavin and Boorman, 1980). Therefore, washing of eggs often facilitates bacterial

invasion of the egg. The cuticle consists of protein small amounts of carbohydrates and lipids (Baker and Balch, 1962).

**c) Shell**

The egg shell components are 95% inorganic substance, 3.3% protein, and 1.6% moisture (Okubo *et al.*, 1997). Calcium carbonate is the major component of the inorganic substances. The egg shell (palisade layer) is very dense and hard. Its crystalline structure is formed by calcification of calcium carbonate and contains a small amount of magnesium, which constructs a spongy matrix together with collagen (Okubo *et al.*, 1997). The palisade layer is called the spongy layer.

**d) Shell membrane**

The shell membrane is made of two layers each an interwoven network of protein polysaccharide fibers. The outer layer adheres closely to the mammillary layer. The egg shell membrane is composed of inner and outer membranes (Nakano *et al*., 2003). The thickness of outer and inner membrane is about 50 μm and 15 μm, respectively. Their structure looks like entangled threads or randomly knitted nets (Figure 4). This structure is important in obstructing and invading microorganisms by catching them in the meshwork (Okubo *et al.*, 1997). They consist of 70% organic substance, 10% inorganic substance, and 20% moisture. The main organic constituent is protein with a small amount of lipids and carbohydrates (Ternes, 2001). Yi *et al*. (2004) reported the preparation and characterization of soluble egg shell membrane (SEP). The dissolution process, which is the key step of the preparation of SEP, has been followed by scanning electron microscopy (SEM) (Figure 4) to observe the changes of the surfaces of the eggshell membrane (ESM). SEP has a higher content of sulfur. In the presence of mercaptoethanol, SEM was dissolved to a higher extent as the time increased, suggesting the presence of disulfide bond in SEP.

**1.2.1.2 Whole egg**

The principal parts of the egg are the yolk and the white or albumen. The relative proportions of each of these constituents can vary considerably (Table 1). Linden and Lorient (2000) reported the average composition of hen egg: 61.5% white, 29.0% yolk and 9.5% shell. A whole egg contains approximately 66% water, 11%

mineral substances and 23% organic substances (12% proteins; 11% lipids) (Linden and Lorient, 2000).

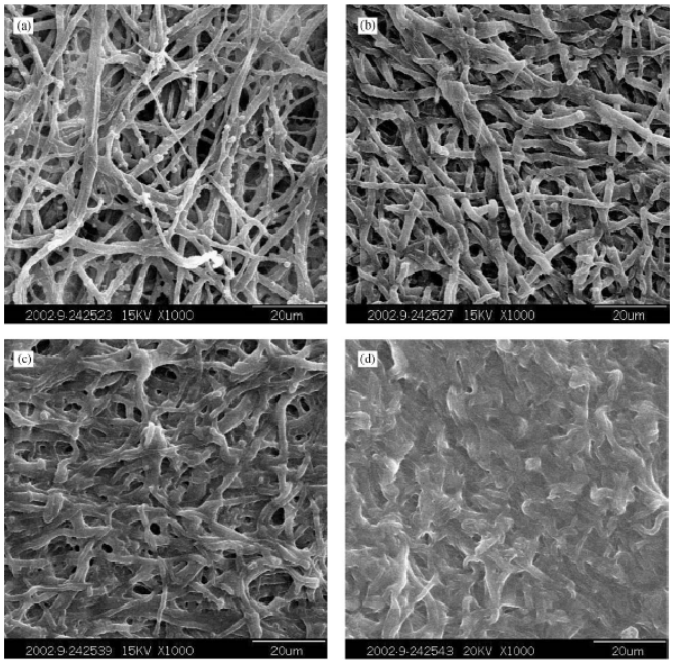



**Figure 4.** Scanning electron micrographs of outside surface of egg shell membrane treated by mercaptoethanol for (a) 0 h, (b) 1 h, (c) 3 h and (d) 5 h.

**Source:** Yi *et al*. (2004)

**Table 1.** Average composition of chicken eggs

| Fraction | Total weight (%) | Dry matter (%) | Protein (%) | Fat (%) | Carbohydrates (%) | Minerals (%) |
|---|---|---|---|---|---|---|
| Shell | 10.3 | 98.4 | 3.3[a] | | | 95.1 |
| Egg white | 56.9 | 12.1 | 10.6 | 0.03 | 0.9 | 0.6 |
| Egg yolk | 32.8 | 51.3 | 16.6 | 32.6 | 1.0 | 1.1 |

[a] protein mucopolysaccharide complex

**Source:** Belitz *et al*. (2009)

#### 1.2.1.3 Albumen (Egg White)

Albumen or Egg white is made up of outer thin white, thick white, inner thin white and chalaziferous layer (inner thick white). The proportion of the layer varies, dependent on the breed, environmental condition, size of the egg and rate production (Romanoff and Romanoff, 1949). The proximate analysis of albumen is present in Table 1. Water is the major constituent of albumen. The moisture content decreases from the outer to inner albumen layers (Powrie, 1977) (Table 2). Carbohydrates in the albumen are in the free form and in combination with protein. The amount of free carbohydrate, usually present as glucose, is 0.4% of the albumen and 0.5% is present as glycoprotein, which contains mannose and galactose units (Romanoff and Romanoff, 1949).

**Table 2.** Albumen and moisture content of different albumen layers.

| Layers | Albumen (%) | | Moisture (%) |
|---|---|---|---|
| | Mean | Range | |
| Outer thin white | 23.2 | 10-60 | 88.8 |
| Thick white | 57.3 | 30-80 | 87.6 |
| Inner thin white | 16.8 | 1-40 | 86.4 |
| Chalaziferous | 2.7 | | 84.3 |

**Source:** Powrie (1977)

##### 1.2.1.3.1 Egg white protein

Protein is the only major constituent which consists of more than 40 different kinds of proteins. Many of them are still uncharacterized because of their low concentration (Sugino *et al*., 1997). Table 3 lists the most important albumen proteins in order of their abundance in egg white. Several albumen proteins have biological activity (Table 3), such as enzymes (e. g., lysozyme), enzyme inhibitors (e. g., ovomucoid, ovoinhibitor) and complex-forming agents for some coenzymes (e. g., flavoprotein, avidin). The biological activities may be related to protection of the egg

from microbial spoilage. Egg white protein separation is relatively easy: the albumen is treated with an equal volume of saturated ammonium sulfate; the globulin fraction precipitates together with lysozyme, ovomucin and other globulins; while the major portion of the egg white remains in solution. This albumen fraction consists of ovalbumin, conalbumin and ovomucoid. Further separation of these fractions is achieved by ion-exchange chromatography (Belitz *et al*., 2009).

**Table 3.** Protein of egg white

| Protein | Percent of the total protein [a] | Denaturation temperature (°C) | Molecular weight (kDa) | Isoelectric point (pH) | Characteristic |
|---|---|---|---|---|---|
| Ovalbumin | 54 | 84.5 | 44.5 | 4.5 | |
| Conalbumin (Ovotransferrin) | 12 | 61.5 | 76 | 6.1 | bind metal ions, bacteria inhibitor |
| Ovomucoid | 11 | 70.0 | 28 | 4.1 | proteinase inhibitor |
| Ovomucin | 3.5 | | 5.5-8.3 × $10^6$ | 4.5-5.0 | inhibits viral hemagglutination, viscosity factor |
| Lysozyme (Ovoglobulin $G_2$) | 3.4 | 75.0 | 14.3 | 10.7 | N-acetylmuramidase |
| Ovoglobulin $G_2$ | 4 | 92.5 | 30-45 | 5.5 | good foam builders |
| Ovoglobulin $G_3$ | 4 | | | 5.8 | good foam builders |
| Flvoprotein | 0.8 | | 32 | 4.0 | Binds vitamin B2 |
| Ovoglycoprotein | 1.0 | | 24 | 3.9 | |
| Ovomacroglobulin | 0.5 | | 760-900 | 4.5 | inhibits serine and cysteine proteinases |
| Ovoinhibitor | 1.5 | | 49 | 5.1 | proteinase inhibitor, Inhibits serin proteases |
| Avidin | 0.05 | | 68 [b] | 9.5 | binds biotin |
| Cystain (ficin inhibitor) | 0.05 | | 12.7 | 5.1 | inhibits cysteine peptidases |

[a] Average values are presented.
[b] Four times 15.6 kDa + approx. 10% carbohydrate.

**Source:** Belitz *et al*. (2009)

**a) Ovobumin**

This is the main albumen protein. It is a glycophospho-protein with 3.2% carbohydrates (Table 4) and 0–2 moles of serine-bound phosphoric acid per mole of protein (ovalbumin components A3, A2 and A1, approx. 3, 12 and 85%, respectively) (John *et al*., 1972). Ovalbumin consists of a peptide chain with 385 amino acid residues. It has a molecular weight (Mr) of 42,699 Da and contains four thiol and one disulfide group. The phosphoric acid groups are at Ser-68 and Ser-344 (Belitz *et al*., 2009). During the storage of eggs, the more heat-stable S-ovalbumin (coagulation temperature 92.5°C) is formed from the native protein (coagulation temperature 84.5°C) probably by a thiol-disulfide exchange. The content of S-ovalbumin increases from 5% in fresh eggs to 81% in eggs cold stored for 6 months (Sugino *et al*., 1996). Ovalbumin is relatively readily denatured, for example, by shaking or whipping its aqueous solution. This is an interphase denaturation which occurs through unfolding and aggregation of protein molecules (Belitz *et al*., 2009).

**Table 4** Carbohydrate composition of some chicken egg white glycoproteins

| Protein | Carbohydrate (%) | Components (moles/mole protein) | | | | |
|---|---|---|---|---|---|---|
| | | Gal | Man | GlcN | GalN | Sialic acid |
| Ovobumin | 3.2 | | 5 | 3 | | |
| Ovomucoid | 23 | 2 | 7 | 23 | | |
| α-Ovomucin[a] | 13 | 21 | 46 | 63 | 6 | 7 |
| Ovoglycoprotein | 31 | 6 | 12 | 19 | | 2 |
| Ovoinhibitor (A) | 9.2 | | 10[b] | 14 | | 0.2 |
| Avidin[c] | 10 | | 4(5) | 3 | | |

[a] In addition to carbohydrate, it contains 15 moles of esterified sulfuric acid per mole protein
[b] Sum of Gal + Man
[c] Data per subunit (16 kDa)

**Source:** Spiro (1973)

**b) Conalbumin (Ovotransferrin)**

This protein, unlike ovalbumin, is not denatured at the interphase but coagulates at lower temperatures. Conalbumin consists of one peptide chain and contains one oligosaccharide unit made of four mannose and eight N-acetylglucosamine residues. Binding of metal ions (2 moles $Mn^{3+,}$ $Fe^{3+,}$ $Cu^{2+}$ or $Zn^{2+}$ per mole of protein) at pH6 or above is a characteristic property of conalbumin (Belitz *et al*., 2009). These metal complexes are more thermostable than protein in the native state (Linden and Lorient, 2000). The occasional red discoloration of egg products during processing originates from a conalbumin-iron complex (Belitz *et al*., 2009). The complex is fully dissociated at a pH less than 4. Tyrosine and histidine residues are involved in metal binding. Conalbumin has the ability to retard growth of microorganisms (Belitz *et al*., 2009).

**d) Ovomucoid**

It is a glycoprotein which consists of three sub-units. It is heat resistant, except in an alkaline medium, and has an anti-trypsin activity (Linden and Lorient, 2000). Ion-exchange chromatography or electrophoresis reveals 2 or 3 forms of this protein, which apparently differ in their sialic acid contents. The carbohydrate moiety (Table 4) consists of three oligosaccharide units bound to protein through asparagine residues. The carbohydrate moiety consists of three oligosaccharide units bound to protein through asparagine residues (Spiro, 1973). The protein has 9 disulfide bonds and, thereby providing the stability against heat coagulation. Hence, it can be isolated from the supernatants of heat coagulated albumen solutions, and then precipitated by ethanol or acetone (Belitz *et al*., 2009). Ovomucoid inhibits bovine trypsin but not human trypsin (Feeney *et al*., 1967; Travis, 1979).

**e) Lysozyme (Ovoglobulin G1)**

Lysozyme is widely distributed and is found not only in egg white but in many animal tissues and secretions, in latex exudates of some plants and in some fungi (Belitz *et al*., 2009). This protein, with three known components, is an N-acetylmuramidase enzyme that hydrolyzes the cell walls of Gram-positive bacteria. Lysozyme consists of a peptide chain with 129 amino acid residues and four disulfide bonds (McKenzie and White, 1991).

**f) Ovomucin**

This protein, of which three components are known, can apparently form fibrillar structures and contribute to a rise in viscosity of albumen, particularly of the thick, gel-like egg white, where it occurs in a four-fold higher concentration than in fractions of thin albumen (Donovan *et al*., 1970). Ovomucin has been separated into a low carbohydrate (carbohydrate content ca. 15%) α-fraction and a high-carbohydrate (carbohydrate content ca. 50%) β-fraction. It appears to be associated with polysaccharides (Belitz *et al*., 2009). The compositions of its carbohydrate moieties are given in Table 4. Ovomucin is heat stable. It forms a water insoluble complex with lysozyme. The dissociation of the complex is pH dependent. Presumably it is of importance in connection with the thinning of egg white during storage of eggs (Rocculi *et al*., 2009).

**g) Flavoprotein**

This protein binds firmly with riboflavin and probably functions to facilitate the transfer of this coenzyme from blood serum to egg (Belitz *et al*., 2009).

**h) Ovoinhibitor**

This protein is, like ovomucoid, a proteinase inhibitor. It inhibits the activities of trypsin, chymotrypsin and some proteinases of microbial origin. Its carbohydrate composition is given in Table 4 (Belitz *et al*., 2009).

**j) Avidin**

Avidin is a basic glycoprotein (Table 4) (Spiro, 1973). Avidin is a tetramer consisting of four identical subunits (Laitinen *et al*., 2002), each of which binds one mole of biotin. Avidin, in its form in egg white, is practically free of biotin, and presumably fulfills an antibacterial role.

**k) Cystatin (Ficin Inhibitor)**

Chicken egg cystatin C consists of one peptide chain with a ca. 120 amino acid residues (Mr= 12,700). The two isomers known differ in their isoelectric point (pI 5.6 and pI 6.5) and their immunological properties (Belitz *et al*., 2009). This inhibitor inhibits cysteine endopeptidases such as ficin and papain. In fact, cathepsins B, H, and L and dipeptidyl peptidase I are also inhibited by cystatin (Sen and Whitaker, 1973).

#### 1.2.1.3.2 Egg white lipid

Fresh egg white contain only a trace amount of lipid (about 0.02%) (Sato *et al*., 1973). However, in eggs stored for a long time when the yolk membrane becomes weak, triglycerides and cholesterol esters are considered to move into the albumen, causing a change in the foaming nature of egg white (Sato *et al*., 1973).

#### 1.2.1.3.3 Egg white carbohydrate

Carbohydrates (approx. 1%) are partly bound to protein (approx. 0.5%) and partly free (0.4–0.5%). Free carbohydrates include glucose (98%) and mannose, galactose, arabi-nose, xylose, ribose and deoxyribose, totally 0.2–2.0mg/100g egg white. There are no free oligosaccharides or polysaccharides. Bound carbohydrates were covered previously with proteins (Table 4). Mannose, galactose and glucosamine are predominant, and sialic acid and galactosamine are also present. (Tunmann and Silberzahn, 1961; Belitz *et al*., 2009).

#### 1.2.1.3.4 Egg white minerals and vitamin

The mineral content of egg white is 0.6%. Its composition is listed in Table 5 (Romanoff and Romanoff, 1949; Belitz *et al*., 2009). Data on vitamins found in egg white are summarized in Table 6 (Romanoff and Romanoff, 1949; Belitz *et al*., 2009).

**Table 5.** Mineral composition of egg

| | Egg white (%) | Egg yolk (%) |
|---|---|---|
| Sulfur | 0.195 | 0.016 |
| Phosphorus | 0.015–0.03 | 0.543–0.980 |
| Sodium | 0.161–0.169 | 0.026–0.086 |
| Potassium | 0.145–0.167 | 0.112–0.360 |
| Magnesium | 0.009 | 0.016 |
| Calcium | 0.008–0.02 | 0.121–0.262 |
| Iron | 0.0001–0.0002 | 0.0053–0.011 |

**Source:** Belitz *et al*. (2009)

**Table 6.** Vitamin content of whole egg, egg white and yolk (mg/100g edible portion)

| Vitamin | Whole egg | Egg white | Egg yolk |
|---|---|---|---|
| Retinol (A) | 0.22 | 0 | 1.12 |
| Thiamine | 0.11 | 0.022 | 0.29 |
| Riboflavin | 0.30 | 0.27 | 0.44 |
| Niacin | 0.1 | 0.1 | 0.065 |
| Pyridoxine (B6) | 0.08 | 0.012 | 0.3 |
| Pantothenic acid | 1.59 | 0.14 | 3.72 |
| Biotin | 0.025 | 0.007 | 0.053 |
| Folic acid | 0.051 | 0.009 | 0.15 |
| Tocopherols | 2.3 | 0 | 6.5 |
| α -Tocopherol | 1.9 | | 5.4 |
| Vitamin D | 0.003 | | 0.0056 |
| Vitamin K | 0.009 | | |

**Source:** Belitz *et al*. (2009)

#### 1.2.1.4 Egg Yolk

Yolk is a fat-in-water emulsion with about 50% dry weight. It consists of 65% lipids, 31% proteins and 4% carbohydrates, vitamins and minerals. The main components of egg yolk are LDL (68%), HDL (16%), livetins (10%) and phosvitins (4%). The yolk is a dispersion of particles (granules) in a continuous aqueous phase (plasma). The composition of granules and plasma was reported by Anton and Gandemer (1997) (Table 7). Granules contain mainly 70% high-density lipoprotein (HDL), 16% phosvitin and 12% low-density lipoproteins (LDL). Plasma is composed of 85% LDL and 15% livitin (McCully *et al*., 1962). The yolk is actually a source of lipids, which are easily dispersed in water, thus permitting emulsification of other substances. These properties are due to their high content in phospholipids and to the fact that all the lipids (including the triglycerides) are associated with at least two proteins, vitellin and vitellenin (Table 8) (Linden and Lorient, 2000).

**Table 7.** Composition of yolk, granules and plasma (g/100g dried matter)

| | Yolk | Granules | Plasma |
|---|---|---|---|
| Protein | 33.2 | 63.8 | 24.6 |
| Lipid | 63.4 | 30.7 | 72.6 |
| Triglycerides (TG) | 43.2 | 19.6 | 51.7 |
| Phospholipids (PL) | 17.7 | 11.1 | 18.2 |
| Phosphatidylcholine (PC) | 15.7 | 10.0 | 16.0 |
| Phosphatidylethanolamine (PE) | 2.0 | 1.1 | 2.1 |
| Cholesterol | 2.6 | 1.3 | 2.7 |
| Lipids/Protein | 1.9 | 0.5 | 3.0 |

**Source:** Modified from Anton and Gandemer (1997)

#### 1.2.1.4.1 Egg yolk protein

The first steps in the analysis of the proteins present in egg yolk are orientated towards the method used to classify lipoproteins. First, the granules are separated by the centrifugation of the diluted yolk (Figure 5).

**Table 8.** Composition of chicken egg yolk protein

| | Dry | Proportion of yolk proteins (%) | Molecular matter | Lipid content | Phosphate content of proteins (%) | Location |
|---|---|---|---|---|---|---|
| Phosvitin | 4 | 10 | 36000 | 0 | 0 | Granules |
| HDL lipovitellin | 16 | 36 | 400000 | 20 | $\alpha = 0.5$<br>$\beta = 0.25$ | Granules |
| LDL lipovitellenin | 68 | 24 | $3 - 10 \times 10^6$ | 88 | 0.1 | |
| Livetins | 10 | 30 | $\alpha = 80000$<br>$\beta = 45000$<br>$\gamma = 150000$ | 0 | - | Continuous phase |
| Yolk Riboflavin Binding Protein | 1.5 | 0.4 | 36000 | 0 | 0.2 | Continuous phase |

**Source:** Linden and Lorient (2000)

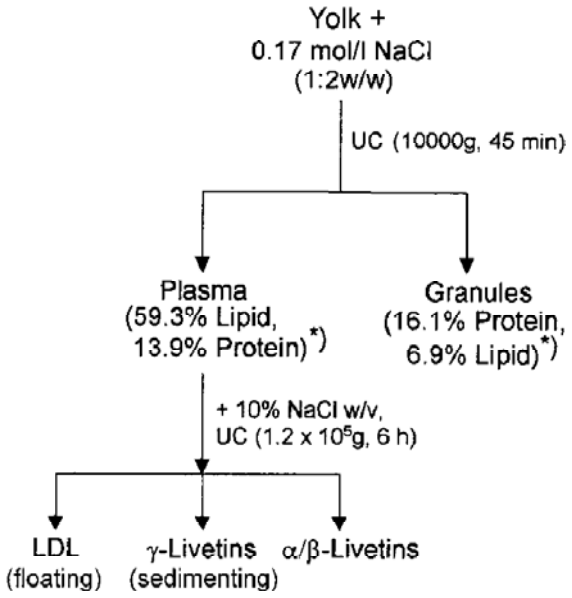



**Figure 5.** A schematic representation of the fractionation of egg yolk.

UC: ultracentrifuge

*Numbers: proportions of the yolk dry weight

**Source:** Belitz *et al*. (2009)

**a) Proteins of granules**

- Lipovitellins

The lipovitellin fraction represents high density lipoproteins (HDL). Its lipid moiety is 22% of dry matter and consists of 35% triglycerides, approx. 60% phospholipids and close to 5% cholesterol and cholesterol esters (Belitz *et al*., 2009). The lipovitellins can be separated by electrophoretic and chromatographic methods into their α- and β-components, which differ in their protein bound phosphorus content (0.39 and 0.19% P, respectively) (Eernardi and Cook, 2006). α-Lipovitellin consists of two polypeptide chains (MW 111,000 and 85,000 Da), but β-lipovitellin has only one chain (MW 110,000 Da) (Belitz *et al*., 2009). The vitellins are covalently bound to oligosaccharides made up of mannose, galactose, glucosamine and sialic acid. The stronger acidic character of α-lipovitellin is based not only on the higher phosphoric acid content, but also on the higher content of sialic acid (Belitz *et al*., 2009). The two lipovitellins form a quaternary structure (MW 420,000 Da), which

decomposes into subunits above pH 9. In the yolk, lipovitellins are present as a complex with phosvitin, with about two phosvitin molecules (MW 32,000 Da) for each lipovitellin molecule (MW 420,000 Da). The lipovitellins are heat stable. However, they lose this property if the lipids are separated (Belitz *et al*., 2009).

- Phosvitin

Phosvitin is a glycophosphoprotein with an exceptionally high amount of phosphoric acid bound to serine residues (Sugino *et al*., 1997). For this reason, it behaves like a polyelectrolyte (polyanion) in aqueous solution. On electrophoresis, two components are obtained, α- and β-phosvitin, which are protein aggregates with molecular weights of 160,000 and 190,000 Da (Belitz *et al*., 2009). In the presence of sodium dodecyl sulfate, α-phosvitin dissociates into three different subunits (Mw = 37,500, 42,500 and 45,000 Da) and β-phosvitin into only one subunit (Mw = 45,000 Da) (Guilmineau *et al*., 2005). The partial specific volume (0.545 mL/g) is very low, probably due to the large repulsive charges of the molecule. The frictional ratio suggests the presence of a long, mostly stretched molecular form. The sequences of 6–8 phosphoserine residues, interrupted by basic and other amino acid residues, are typical for this protein. Phosvitin is relatively heat stable. In fact, no changes can be electrophoretically detected after 10 minutes at 110°C. Phosphate is eliminated at 140°C. Coagulating egg yolk is frequently enclosed in coagulates of other proteins. Phosvitin very strongly binds multivalent cations, depending on the type of metal and the pH (Belitz *et al*., 2009). The iron in eggs, present as $Fe^{3+}$, is bound to an extent of 95% to phosvitin and its availability for nutrition is greatly limited. The $Fe^{3+}$ complex is monomeric and phosvitin is saturated with iron at a molar ratio of Fe/P = 0.5. Since phosvitin traps iron and other heavy metal ions, it can synergistically support antioxidants.

**b) Plasma proteins**

- Lipovitellenin

Lipovitellenin is obtained as a floating, low density lipoprotein (LDL) by ultracentrifugation of diluted yolk. Several components with varying densities can be separated by fractional centrifugation (Evans *et al*., 1973; Belitz *et al*., 2009). The lipid moiety represents 84– 90% of the dry matter and consists of 74% triglycerides

and 26% phospholipids. The latter contain predominantly phosphatidyl choline (approx. 75%), phosphatidyl ethanolamine (approx. 18%) as well as sphingomyelin and lysophospholipids (approx. 8%) (Belitz *et al*., 2009). Eleven bands appear on electrophoresis of the apoproteins .

- Livitin

Livitin is a water-soluble globular protein which accounts for 30% of the plasma proteins. Its fraction can be separated electrophoretically into α-, β- and γ-livetins. These have been proven to correspond to chicken blood serum proteins, i. e. serum albumin, $\alpha_2$-glycoprotein and γ-globulin (Williams, 1962.; Belitz *et al*., 2009)

**1.2.1.4.2 Egg yolk lipid**

Egg yolk contains 32.6% of lipid, whose composition is given in Table 9. The lipid includes triglycerides, phospholipids, cholesterol, cerebroside, and some other minor lipids (Romanoff and Romanoff, 1949). These lipids occur as the lipoproteins and are closely associated with the proteins occurring in yolk. The fatty acid composition of the lipids depends on that of the feed. However, the extent to which individual fatty acids are incorporated varies greatly. Highly unsaturated ω-3-fatty acids (20:5, 22:6) from fish oils do appear in egg lipids, but not in proportion to their content in the feed (Romanoff and Romanoff, 1949). Furthermore, it has been observed that the fatty acid pattern of the feed is reflected more clearly in the triglyceride fraction of egg lipids than in the polar lipids. About 4% of the egg lipids consist of sterols. The main component is cholesterol (96%), ca. 15% of which is esterified with fatty acids. The cholesterol content is 2.5%, based on the egg yolk solids. The quality of egg products is endangered by autoxidation of cholesterol (Belitz *et al*., 2009).

**Table 9.** Egg yolk lipids

| Lipid fraction | a | b |
|---|---|---|
| Triacylglycerols | 66 | |
| Phospholipid | 28 | |
| Phosphatidyl choline | | 73 |
| Phosphatidyl ethanolamine | | 15.5 |
| Lysophosphatidyl choline | | 5.8 |
| Sphingomyelin | | 2.5 |
| Lysophosphatidyl ethanolamine | | 2.1 |
| Plasmalogen | | 0.9 |
| Phosphatidyl inositol | | 0.6 |
| Cholesterol, cholesterol esters and other compounds | 6 | |

[a] As percent of total lipids.
[b] As percent of phospholipid fraction.

**Source:** Belitz *et al*. (2009)

**1.2.1.4.3 Egg yolk carbohydrate**

Egg yolk carbohydrates are about 1% of the dry matter, with 0.2% bound to proteins. The free carbohydrates present in addition to glucose are the same monosaccharides identified in egg white (Sugino *et al*., 1997; Belitz *et al*., 2009).

**1.2.1.4.4 Egg yolk minerals and vitamins**

Egg yolk contains 1% minerals and phorphorus is the most abundant mineral component. More than 61% of the total phosphorus of egg yolk is contained in phospholipids (Sugino *et al*., 1997). The minerals and vitamins in egg yolk are listed in Table 5 and 6, respectively (Sugino *et al*., 1997; Belitz *et al*., 2009).

**1.2.1.4.5 Egg yolk aroma Substances**

The typical aroma substances of egg yolk are still unknown. The "fishy" aroma defect that can occur in eggs is caused by trimethylamine TMA, which has an odor threshold that depends on the pH (25 μg/kg, pH7.9) because only the undissociated form is odor active. TMA is formed by the microbial degradation of

choline, e. g., on feeding fish meal or soy meal. Normally, TMA does not interfere because it is enzymatically oxidized to odorless TMA oxide. However, in feed, e. g., soy meal, substances exist which could inhibit this reaction (Maga, 1982).

**1.2.1.4.6 Egg yolk colorants**

The color of the yolk, which is produced by carotenoids in the feed, is considered to be a quality characteristic. The yellow-orange color of yolk is attributed to the presence of fat-soluble cartenoids in the lipid portion of lipoproteins (Shenstone, 1968). The majority of carotenoids in yolk are hydroxyl compounds called xanthophylls with minor amounts of carotenes. The types and amounts of carotenoids in yolk are diet dependent (Brown, 1938). The major xanthophylls in the yolk of commercial eggs are lutein, zeaxanthin, and cryptoxanthin, all of which are derived from commonly used pigment feed ingredients such as yellow corn, alfalfa meal, etc. (Smith and Perdue, 1966). Lutein is the principal xanthophyll in plant materials and has also been found to be the dominant carotenoid (about 62% of the total carotenoid) in the yolk from eggs of the hens fed yellow-corn and alfalfa-meal diets (Sugino *et al*., 1996). Zeaxanthin was the second highest with a level of 12.9% of the total carotenoid. The factors that influence the degree of yolk pigmentation include the chemical structure of the xanthophylls, the presence of antioxidants in feed, and the fat content of feed (Scott *et al*., 1968). Matsuno *et al.* (1986) reported a total carotenoids of 2.5 mg/100g yolk. The major carotenoids included lutein (40%), zeaxanthin (19.8%), cantaxanthin (17.9%) and β-cryptoxanthin (17.3%).

### 1.2.2 Salted eggs

Salted eggs are preserved food product made by soaking duck eggs in brine. Traditionally, salted eggs are produced by the coating method. The egg is usually coated with a 1-2 cm thick layer of salted charcoal paste (Yang, 1994). The salted duck eggs have a briny aroma, a very liquid egg white and a yolk that is bright orange-red in color, round, and firm in texture (Chi and Tseng, 1998). Salted duck eggs are normally boiled or steamed before being peeled and eaten as a condiment

with other foods. Normally, after 3-9 days, scramble salted egg can be made, and after 3-5 days it can be used for fried salted egg. Egg salted for 12-25 days is generally suitable for boiling (Yang, 1994). The egg white has a salty taste. The orange red yolk is rich, fatty, and less salty. The yolk is prized and is used in Chinese moon cakes to symbolize the moon. Salted duck eggs can also be made from chicken eggs though the taste and texture will be somewhat different, and the egg yolk will be less rich. Desirable characteristics of salted egg yolk include orange color, oil exudation and gritty texture (Chi and Tseng, 1998). The moisture contents of raw and cook salted duck eggs were 30.22 and 27.05% respectively, whereas the lipid contents of raw and cooked salt duck egg were 43.52 and 46.06%, respectively (Yang and Chen, 2001). The TBA value of cooked salted duck egg was higher than that of raw salted duck egg. This difference was due to the accelerated lipid oxidation during cooking.

#### 1.2.2.1 Developed methods for salted egg production

Varies methods have been developed to produce salted egg. Yuwawutto (1995) developed the process for soil-salted egg. The amount of salt had significant effect on salted egg attribute. The optimum formulation was composed of 60% clays and 40% solar salts. Water at a level of 32.4 mL/100 g of processed soil should be added to make the suitable soil for coating. The salted eggs were obtained after coating for 25 days. During salting, egg albumen changed from white to translucent white with tender texture and the yolk changed its color to deeper orange color; its texture became firmer and its appearance became oily. Moreover, Yimto *et al*., (2000) studied the development of low sodium salted eggs coated with rice straw pulp. Rice straw pulp was prepared by boiling in alkaline solution, washing and drying. The rice straw pulp was blended with water and then was mixed with NaCl 60% (solar salt) and KCl 40%. The mixture was spread out into thin layer and coated around the egg. After the egg was salt-coated for 25 days, egg white contained 3.98 % NaCl and 82.07% moisture. For yolk, it comprised 1.71% NaCl, 34.78% moisture and 40.04% fat. The consumer test indicated that they liked the product moderately and 78 % of the consumer accepted the product.

Since the egg shell most likely obstructs the penetration of NaC1 into the yolk, the reduction of shell thickness could be a means to accelerate the salt

penetration into the egg. Acid treatment of the egg shell resulted in a decrease in thickness and an increase in salt penetration (Williams and Dillard, 1973; Heath and Wallace, 1978). To induce salt penetration into egg, duck eggs were treated with 0.1 N HCl and an increased penetration rate of 2-10 folds was obtained. As a result, the formation of gelatinous yolk was accelerated (Lai *et al*., 1997). Shell thickness of less than 0.2 mm was observed when soaking the egg in 0.1 N HCl for 30 min (*Lai et al*., 1997). However, inorganic acid might not be acceptable for consumer and organic acid may be a promising alternative with the safety concern. In order to maintain the albumen functionalities, several research groups (Chiang and Chung, 1986; Chen *et al*., 1991; Wang, 1991) have tried to manufacture the salted yolks separated from eggs. Most of these salted processes were unsuccessful in obtaining salted yolks. Wang (1991) indicated that the formation of salted yolk is probably related to the diffusion speed and final concentration of NaCl.

#### 1.2.2.2 Effect of salting on compositions of egg

Moisture reduction during pickling is due to the difference in osmotic pressure between egg white and egg yolk and pickling medium. Generally, water migrates from egg yolk to egg white, then to the environment through the egg shell. NaCl gradually diffuses into the egg white and yolk through the pores and membrane of the shell during brining in saturated saline. The shell eggs become dehydrated during this period because the decrease in moisture is greater than the increase in NaCl. More reduction in moisture content of egg yolk occurred for duck yolk than for chicken yolk (Chi and Tseng, 1998) which might be due to the difference in pore sizes and structure of the shell (Feeney *et al*., 1956). In general, the moisture in the yolk increased in the late stage of brining. This phenomenon of yolk hydration is due to the weaker yolk membrane in the later stage. The moisture content in duck and chicken egg white decreased at similar rate (Chi and Tseng, 1998).

The change in the total lipid content of the egg during salting is related to NaCl absorption and moisture gain and loss. (Lai *et al*., 1997). The total lipid content, on a wet basis of yolk, increased from 34 to 46% in the first 6 weeks and then reduced to 31%, whereas the total lipid content, on a dry basis, decreased from 64 to 53%. The total lipid, based on salt-free solids, was unchanged. This illustrates that

total lipid content was not changed during brining (Lai *et al*., 1999). The free lipid content increased in the first 6 weeks and decreased afterward (Lai *et al*., 1999). Most lipids in the egg yolk exist in low-density lipoproteins (LDL) (Gilbert, 1971). Schultz *et al*. (1968) pointed out that the removal of water from egg yolk increased due to the structural change of low density lipoprotein. Thus, the dehydration during pickling probably enhanced oil exudation (Schultz, 1968).

#### 1.2.2.3 Effect of salting on color of egg

The change in color might be due to the dehydration of egg yolk during pickling. Yellow color of yolk comes from xanthophyll and zeaxanthin (Hinton *et al*., 1974) and is influenced by concentration of pigments. Tai *et al*. (1985) found that synthetic oxycarotenoid such as citranaxanthin and canthaxanthin can produce a golden yellow color in the duck egg yolk. The egg yolk stored at low moisture for a long time became darker yellow, due to removal of moisture (Lin, 1983). This suggested that the orange color of salted egg yolk may be due to the increase in concentration of pigments. The color of chicken egg yolk also became darkened during pickling but it did not result in the desirable orange color (Chi and Tseng, 1998). The color of cooked yolk is yellow before brining. From the outer region to the center, the cooked yolk gradually changes into a yellowish brown, dark brown, reddish orange and light yellow during 24 weeks of brining. The change in the yolk color of cooked eggs during brining is related to moisture gain or loss and amount of free lipid in the yolk (Lai *et al*., 1999).

#### 1.2.2.4 Effect of salting on hardening of egg yolk

Fresh duck egg was brined in 26% NaCl for up to 24 weeks. NaCl content for albumen and yolk increased to 14.8 and 8.9%, respectively (Lai et al., 1999). During pickling, the yolk gradually becomes solidified and hardened (Chi and Tseng, 1998). The salted egg yolk turns to be elastic gel. In egg yolk, several types of particles like spheres, granules or low-density lipoproteins, are suspended in a protein solution or plasma. Both fractions of liquid eggs have the capacity to form gels (Woodward and Cotterill, 1987). Chi and Tseng (1998) studied the permeability of salt through egg shell. Salt migrated from coating- paste into egg white, then to egg yolk. Salt content in egg yolk also increased with pickling time and dehydration of

egg yolk and diffusion of salt into egg white and egg yolk occurred concurrently during pickling. Both effects could cause the hardening of egg yolk. Lai *et al.* (1999) explained the relationship between salted egg yolk formation and NaCl penetration degree. Granulation and gelation of yolk appeared in the different stages of the brining process. Therefore, the formation of salted egg yolk was governed by the degree of NaCl penetration (Lai *et al.*, 1999).

Adding salt to the egg yolk produces disruption of granules, providing additional protein to improve the functional properties of yolk-containing food systems (Kiosseoglou and Sherman, 1983; Foegeding *et al*., 2001). The ability of electrolytes to influence the conformation of globular proteins has been reported to depend on the concentration of salt and/or the ionic strength of the salt (Shenstone, 1968). At low ionic strength, the influence that salt exerts on protein structure is governed by electrostatic interactions. At higher ionic strength, the ability of salts to stabilize protein structure is related to the preferential hydration of the protein molecule as a result of salt -induced alteration of water in the vicinity of the protein (Harrison and Cunningham, 1986). Protein-protein interactions are favored over protein-solvent interactions at high salt content due to lack of water molecules, which may lead to aggregation of protein molecules as reported by Puppo and Anon (1999a).

**1.2.2.5 Utilization of salted egg white**

Salted egg white (containing 10% protein and 4-7% sodium chloride) is discarded as waste, which produces environmental pollution. This is because the coagulated egg yolks are used for the secondary processing in bakery products. In general, egg white has varying functional properties such as foaming ability, emulsifying activity and gelation (Nakamura and Sat, 1964; Kato *et al.*, 1981, 1986, 1989; Yang and Lin, 1990). These functional properties can be changed by adding sodium chloride (Kakalis and Regenstein, 1986; Arntfield *et al.*, 1990; Kitabatake *et al.*, 1988; Elizalde *et al.*, 1991; Vani and Zayas, 1995; Mine *et al.*, 1991). Separation of duck egg white lysozyme from salted and fresh egg by anionic polysaccharides (Na-alginate, Na-carboxyl-methylcellulose, high methoxy pectin and κ-carrageenan) was studied by Yang *et al*. (1998). Only κ-carrageenan interacted and formed precipitates with lysozyme. Recovery of lysozyme from fivefold diluted salted duck

egg whites was 60–65% and 78–81% from fresh duck and hen egg whites using 0.7 % κ-carrageenan. The recovered lysozyme from the salted duck egg whites was stable during storage at 4°C for 35 days.

Functional properties of salted egg were studied by Huang *et al*. (1999). The effects of different salting time and electrodialysis desalination on foaming, emulsifying and gelation properties of salted duck egg white (SDEW) were investigated. The decreases in pH and zeta potential with the increase in surface hydrophobicity were observed after salting for 8 weeks. After electrodialysis desalination treatment, the pH of salted egg white was slightly increased and zeta potential was decreased; however, the NaCl content was reduced by 95%. They found that the electrodialysis desalination treatment did not affect emulsifying properties. The foam ability and foam stability of salted egg white decreased with increasing salting time, but increased with electrodialysis desalination treatment. The gel strength of salted egg white decreased with salting time, but decreased sharply after electrodialysis desalination treatment (Huang *et al*., 1999).

### 1.2.3 Gelation and viscoelastic properties of egg proteins

Protein gelation is important to obtain desirable sensory and textural structures in foods (Totosaus *et al*., 2002). Gelation requires a driving force to unfold the native protein structure, followed by an aggregation retaining a certain degree of order in the matrix formed by association between protein strands (Clark *et al*., 2001). Protein gelation has been traditionally achieved by heating, but some physical and chemical processes form protein gels in an analogous way to heat-induction. A physical means, besides heat, is high pressure. Chemical means are acidification, enzymatic cross-linking, and use of salts and urea, causing modifications in protein–protein and protein–medium interactions (Totosaus *et al*., 2002). The characteristics of each gel are different and dependent upon factors like protein concentration, degree of denaturation caused by pH, temperature, ionic strength and/or pressure (Hermansson, 1979; Kinsella *et al*., 1994).

#### 1.2.3.1 Gelation of egg white protein

Heat-induced gel egg white yields the more stable gel because it contains no lipids (Aguilera *et al*., 2004). Gel formation of egg white is similar to whey proteins in that a two-step process of denaturation followed by aggregation of denatured proteins takes place as temperature increases. At temperatures above 61°C, egg white begins to lose fluidity initiated by the denaturation of ovotransferrin (conalbumin), which is the least stable protein fraction at native pH and followed by denaturation of lysozyme and ovalbumin. Denaturation of ovalbumin, being the predominant protein fraction in egg white, determines the optimum temperature for gel formation and contributes to the increase of gel strength at temperatures above 80°C. Exposure to higher temperatures leads to higher rates of gelation, resulting in stronger gels (Aguilera *et al*., 2004). However, temperatures above 90°C or excessive heating times may lead to over-processing, resulting in a decrease in gel strength, shrinkage of the gel and syneresis. Depending on the environmental conditions, the structure of egg-white gels can range from fine-stranded to particle gels and from transparent to opaque structures. Similar to gels of most other globular proteins, the functionality of egg white gels is strongly influenced by pH and ionic conditions. These variables determine the relation between rates of denaturation and aggregation reactions. This is especially important since the pH of egg white increases during storage from approx. 7.6 to 9.4. In addition, the influence of pH has to be considered if egg white is to be used in complex food systems. Gels of good quality concerning firmness and water-holding capacity are obtained at pH 9 due to the high degree of cross-linking, minimal pore size and uniformity of network structure (Woodward and Cotterill, 1987). Hatta *et al*. (1997) reported that egg white gels with the addition of various concentrations of NaCl (pH 7.0) and heated at 90°C for 30 min had the varying properties. The strong gel was obtained at 0.5% NaCl. The higher the concentration of NaCl, the less gel strength was observed. On the other hand, the release of water was not affected by the concentration of NaCl. Since proteins are ampholyte substances, their electrostatic charges are influenced by the addition of electrolytes. The presence of salts such as NaCl suppresses the net charge of the protein molecules, resulting in a decrease in the electrostatic repulsive force working

among the protein molecules. Therefore, the hydrophobic interaction generated among heat denatured protein molecules is relatively strengthened by the presence of salt (Hatta *et al*., 1997). Therefore, the addition of salts in an appropriate amounts increases the gel formation capacity of egg albumin. However, the addition of excessive amounts of salt produces the inverse effect (Hatta *et al*., 1997). The gelation of ovalbumin by heating was examined as a function of ionic strength. At pH 7.5, in the presence of 20-50 mM and 60-80 mM NaCl, the ovalbumin solution remained as a transparent solution. In the presence of 20-50 mM and 60-80 mM NaCl, a transparent and a turbid gel were obtained, respectively (Hatta *et al*., 1986).

#### 1.2.3.2 Gelation of egg yolk protein

Yolk proteins have been known to possess gel forming ability. Gel network formation of yolk is attributed to the denaturation of its proteins, leading to molecular interactions and development of a hard and rubbery structure (Kiosseoglou, 2003). Yolk is not a pure protein solution, but rather a dispersion of particle (LDL micelles and HDL granules), where the neutral triglycerides are buried in the particle interior, while the protein dominate the particle surface (Paraskevopoulou and Kiosseoglou, 1997). The gelation of yolk, therefore, can be envisaged as a process of yolk particle destabilization brought about by denaturation. Upon heating of particle-stabilizing yolk protein molecules, an interparticle network formation takes place (Kiosseoglou *et al.*, 2005). Apolipoprotein of LDL micelles appear to dominate the process of yolk gelation. Anton *et al*. (2003) reported that yolk gelation process is dominated by LDL apolipoprotein molecular interactions. Similar gelation patterns were followed by yolk plasma and liquid yolk while the granules were less effective in gel network development. This difference in behavior was attributed to lower sensitivity of granular protein due to their globular structure, compared to that of LDL apolipoprotein, which denature at a relatively lower temperature (Le Denmet *et al*., 1999). Heat treatment of yolk may lead to gels exhibiting elasticity (Woodward and Cotterill, 1987). According to Woodward and Cotterill (1987), raw yolk is made up of "spheres" in adjoining polyhedrons (grains) ranging in size from 40 to 100 μm and capable of gelling. Gentle stirring disrupts 90 to 95% of these grains, releasing protein into the solution. During heating, the formation of a three-dimentional protein

network with a hard, cohesive and rubbery texture was developed. Raikos *et al*. (2007) reported that the gelation of whole egg, white and yolk protein was induced by heat. When heated, both yolk and white proteins unfolded and interacted to form high-molecular weight aggregates. Whole egg gels exhibited higher levels of firmness as compared to yolk and white (Raikos *et al*., 2007). A linear relationship between whole egg and yolk gel strength and pH was observed. Significant differences of gel hardness were detected for egg yolk and white, which were attributed to the addition of sugar and/or salt. Guerrero *et al*. (2004) studied the thermally-induced transitions of egg yolk and protein and found that gelation was affected by the pH, ionic strength and salt type. Kiosseoglou and Paraskevopoulou (2005) studied that molecular interaction in gel of egg yolk and its plasma and granules fraction, upon heating at 90 $^{0}$C for 30 min in the presence of D, L-dithiothreitol (DTT), N-ethylmaleimide (NEM) or Tween 40 to establish the role of disulfide covalent bonds and hydrophobic interactions between yolk protein constituents in the formation of gel network structure. It was found that yolk and its plasma fraction exhibit a similar behaviour. The involvement of disulfide bonds between their protein constituents in gel structure development was elucidated. Granule fraction gels, on the other hand, were not markedly affected by disulfide bond-splitting or sulfhydryl group-blocking reagent. (Kiosseoglou and Paraskevopoulou, 2005).

The effect of sodium salt on aggregation of egg yolk phosvitin, a phosphorylated protein in hen egg yolk protein was studied by Castellani *et al*., (2005). NaCl concentration had a great influence on the flocculation process of phosvitin molecules. The flocculation of phosvitin at 0.15 M of ionic strength was higher than that observed at 0.05 M. The presence of phosphocalcic bridges between phosphate groups of the three proteins was reported (Causeret *et al*., 1991). The modification of one of these could lead to an irreversible destruction of the granules. The existence of ionic bridges between the cations and the phosphate group of the phosphoseryl residues of phosphoprotein was hypothesized. Nevertheless, Chang *et al*. (1977) observed the total dissociation of yolk granules when adding 1.71 M NaCl to the yolk.

Several factors including pH, ionic strength, solid concentration or temperature may give rise to relevant modifications in the viscoelastic properties of egg yolk (Guerrero *et al*., 2004). Effect of pH and added electrolyte on the rheological properties of egg yolk was studied by Guerrero *et al*. (2004). Reduction in the pH value using citric acid produced a dramatic evolution in the microstructure and the rheological properties from fluid like to gel behavior of egg yolk dispersions (45% wt solid). An increase in electrostatic interactions that may give rise to protein denaturation and subsequently to formation and growth of aggregates which may extend to form three-dimensional structures. Moreover, the addition of sodium or calcium chloride suggests with ionic strength of 0.15 M is required to produce significant modifications, leading to the aggregation of yolk protein molecules.

Laca *et al*. (2009) studied on the rheological characteristics of egg yolk fractions. Egg yolk granule showed the higher linear vicoelastic properties than that of yolk plasma, indicating that the structure of granule was more resistant than that plasma of fraction. The different stress resistance are due both to the high protein content in granule fraction (since proteins are responsible of structure support) and also to the high lipid content in yolk plasma fraction (since lipids favor the flow). Fresh and freeze-drying of both fractions were also studied. Fresh granule fraction was more stable than plasma fraction as correlated with the higher three-dimensioned structure and the strength of the interaction.

Influence of high processing on the linear viscoelastic properties of egg yolk dispersions was studied by Aguilar *et al*. (2007). An increase in the pressure level produces a dramatic change in the linear viscoelastic behaviour, in the way which a sol–gel transition occurs. The impact of high pressure on aggregation and network formation can be modulated by pH. High-pressure processing may produce some relevant modifications in the linear viscoelastic properties of egg yolk dispersions, particularly at pH close to the isoelectric point. Both protein denaturation and subsequent hydrophobically driven random aggregation were enhanced with increasing pressure level. However, high-pressure processing of egg yolk systems is highly affected by protein concentration particularly, compared with heat processing.

Mayonnaise-like emulsions containing reduced cholesterol yolk were prepared and their structure was elucidated by applying dynamic oscillatory rheometry (Paraskevopoulou *et al*., 2000). The mayonnaise samples could be characterized as 'weak' gels. The yolk extracted with an ethanol: water mixture, containing 1.5% polysorbate 80, resulted in emulsions which exhibited higher viscoelastic moduli values compared to those prepared with supercritical $CO_2$-extracted yolk. Mayonnaise prepared with the control spray dried yolk exhibited the lowest viscoelasticity values but the highest stability when aged for a certain period of time.

Rheological properties of low-cholesterol mayonnaises prepared using egg yolk granules as emulsifying agent were studied by Laca *et al*. (2010). Rheological data showed that mayonnaise containing low-cholesterol has the closest behaviour to that of the commercial sample and/or to that of the mayonnaise prepared with raw egg yolk. No significant rheological differences were found between samples made with the same quantity of granules, either in fresh state or freeze-dried. Santipanichwong and Suphantharika (2008) elucidated the effects of β-glucans on the rheological properties of egg yolk stabilized oil-in-water emulsions. Small amplitude oscillatory shear measurement was used to determine viscoelastic properties of emulsions. The results indicated the dominant effect of β-glucans on the viscoelastic properties of emulsions which classified rheologically as a typical weak gel structure. The addition of β-glucans therefore enhanced the viscoelastic behavior of emulsions in the following order: curdlan > yeast > barley ≈ oat β-glucans.

### 1.2.4 Proteases and applications

Proteases are one of the most important industrial enzymes, accounting for nearly 60% of total worldwide enzyme sales (Sangeetha and Abraham, 2006). Protease is the generic name given to those enzymes hydrolyzing the peptide bond in proteins and some synthetic substrate and coded as the EC 3.4.11-99. Proteases, including peptidases and proteinases, are polyfunctional enzymes catalyzing the hydrolytic degradation of protein (Garcia-Carreno and Hernandez-Cortes, 2000).

Proteases can be classified based on their similarities to well characterized proteases, as trypsin-like, chymotrypsin-like, etc., their pH activity profiles as acid, neutral or alkaline proteases, substrate specificity and mechanism of catalysis (Haard and Simpson, 1994). Proteases have been exploited in the food industries in many ways. The basic function of proteases is to hydrolyze proteins; and this property has been exploited for the preparation of protein hydrolysates of high nutritional value.

**1.2.4.1 Classification of proteases**

Proteases are classified according to their source (animal, plant, microbial), their catalytic action (endopeptidase or exopeptidase) and the nature of the catalytic site. In EC system for enzyme nomenclatures, peptide hydrolases belong to subclass 3.4, which is further divided into 3.4.11-19, the exopeptidases and 3.4.21-24, the endopeptidases or proteinases (Nissen, 1993). Endopeptidases cleave the polypeptide chain at particularly susceptible peptide bonds distributed along the chain, whereas exopeptidases hydrolyze one amino acid from N terminus (aminopeptidases) or from C terminus (carboxypeptidases) (Figure 6).

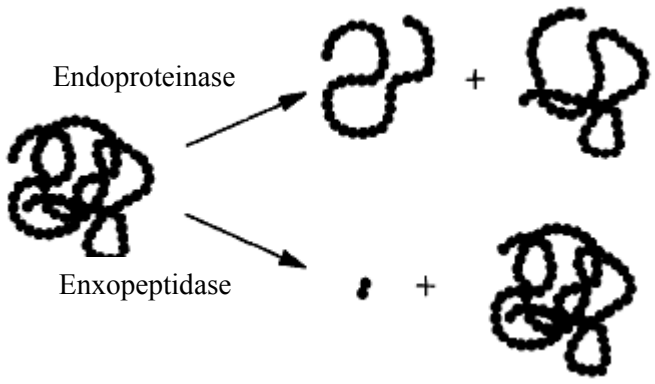



**Figure 6.** Effects of endopeptidases and exopeptidases on protein structure.
**Source:** An *et al*. (1996)

**a) Endopeptidases**

The four major classes of endopeptidases are serine proteinases (EC 3.4.21), cysteine proteinases (EC 3.3.22), aspartic proteinases (EC 3.4.23) and metalloproteinase (EC 3.4.24) (Nissen, 1993). Serine, cysteine and aspartic proteinases have serine, cysteine and aspartic acid side chains, respectively, as a part of the catalytic site. Modification or blocking of this side chain usually leads to complete inactivation of the enzyme and is a standard way of determining the nature of an unknown proteinase (Nissen, 1993). The serine proteinases have maximal activity at alkaline pH, while the closely related cysteine proteinases usually show maximal activity at more neutral pH values. The aspartic proteinases generally have maximal catalytic activity at acidic pH. Among the digestive enzymes, pepsin, an aspartic proteinase, is secreted in the stomach and the serine proteinases, trypsin and chymotrypsin, are excreted in the duodemum, in accordance with pH values of the digestive tract (acid in the stomach and alkaline in the gut) (Nissen, 1993). The metalloproteinases contain an essential metal atom, usually Zn and have optimal activity near neutral pH. $Ca^{2+}$ generally stabilizes these enzymes and the strong chelating agent, such as EDTA inhibits the activity (Nissen, 1993).

**b) Exopeptidaases**

The aminopeptidases (EC 3.4.11) are ubiquitous, but less readily available as commercial products, since many of them are intracellular or membrane bound. Carboxypeptidases are subdivided into serine carboxypeptidases (EC 3.4.16), metallocarboxypeptidases (EC 3.4.17) and cysteine carboxypeptidases (EC 3.4.18) according to the nature of the catalytic site (Nissen, 1993).

**1.2.4.2 Pepsin**

Pepsin is assigned the number EC. 3.4.23.1. It has preferential specificity for the aromatic amino acids, phenylalanine, tyrosine and tryptophan. Pepsin is the major digestive enzyme in stomach of animals, which is secreted as pepsinogen from chief cells of oxyntic glands located in the stomach wall epithelium (Kageyama, 2002). In acidic environment, pepsinogen rapidly converts to pepsin (Kageyama, 2002). During this activation reaction, both prosegment (activation segment) and the active enzyme undergo the conformational changes, and the

proteolytic cleavage of the prosegment can occur in one or more steps by either an intra or intermolecular reaction (Richter *et al*., 1998). Yoshino *et al*. (2004) assessed peptic digestibility of raw and heat-coagulated hen's egg white proteins at acidic pH range (1.5–4.0). Ovalbumin in raw egg white was slightly digested by pepsin at pH 1.5 and pH 2.0, and was almost resistant to the enzyme at pH 2.5 and above. For heat-coagulated egg white at the pH range from 1.5 to 2.5, the protein was well digested by pepsin. Peptic digestibility of ovomucoid in raw egg white was preferable at the pH range from 1.5 to 2.5, but was negligible of pH 3.0 and over, where the improvement of the digestibility of the protein was not found even in heat-coagulated egg white. Hydrolysis of hen egg white ovalbumin by porcine pepsin was examined at various pHs (Kitabatake *et al*., 1988). A strictly limited hydrolysis was observed at pH 4. Only single peptide bond in the original ovalbumin (MW 45000 Da) was cleft, and a peptide with a MW of about 3000 Da was released. Both the released peptide and the residual protein (MW 42000 Da) were resistant to further hydrolysis by prolonged incubation or addition of more pepsin. Miguel and Aleixandre (2006) isolated bioactive peptides from pepsin hydrolysis of ovalbumin. Davalos *et al*. (2004) reports the antioxidant activity of peptides produced by enzymatic hydrolysis of crude egg white with pepsin. Four peptides in ovalbumin hydrolysate possessed higher radical scavenging activity than that of Trolox. The hydrolysate of egg white prepared by pepsin for 3 h was found to exhibit a strong angiotensin I–converting enzyme (ACE) inhibitory activity *in vitro.* The peptide Tyr-Ala-Glu-Glu-Arg-Tyr-Pro-Ile-Leu, which was a strong ACE inhibitor (50% inhibitory concentration, 4.7 μM) also exhibited a high radical scavenging activity (oxygen radical absorbance capacity-fluorescein value, 3.8 μmol of Trolox equivalent per μmol of peptide).

#### 1.2.4.3 Microbial proteases

Commercial proteases, microbial proteases, are among the most important hydrolytic enzymes and have been studied extensively since the advent of enzymology. A large number of proteolytic enzymes, including commercially available exopeptidases and endopeptidases, can be used to make hydrolysates. Some have obtained a food-grade status and are complied with standards imposed by international regulatory agents (Wiseman, 1993). Proteases (EC 3.4.21-24) represent

one of the largest groups of industrial enzymes with increasing market demands due to their applications in industrial, biotechnological, medical, and basic research fields (Wiseman, 1993). Nowadays, commercially available crude proteases are used extensively in the food industry to prepare protein hydrolysates with improved nutritional or functional properties (Godfrey, 1996). Manipulation of hydrolysis reaction conditions can be used, to some extent, to define the characteristics of the final hydrolysates. However, the specificities of the enzymes determine the type of peptides produced and, therefore, the properties of specific food protein hydrolysates (Smyth and FitzGerald, 1998).

**a) Neutrase**

Neutrase (EC 3.4.24.28) is a bacterial endoprotease. As its name implies, Neutrase is a neutral protease with an optimum activity around pH 5.5-7.5 and 45-55 °C. It is a metallo- endoproteinase, requiring Zn ions for its activity. Consequently, it is stabilized by the presence of $Ca^{2+}$ ions and inhibited by EDTA. Neutrase is used commercially to 'upgrade' proteins of animal and vegetable origin. It attracts considerable interest due to a wide variety of possible applications, for example, in the production of functional food proteins by hydrolysis of mung bean (Li *et al*., 2005), corn gluten (Apar and Ozbek, 2007), cheese whey protein (Shin *et al*., 2008), tilapia (fish) (Raghavan *et al*., 2008), and soybean (Bao *et al*., 2008), in the improvement of the texture and sensory properties of dairy products (Kumar *et al*., 2000), and in peptide synthesis in organic media (Clape *et al*., 1997).

**b) Flavourzyme**

Flavourzyme (EC 3.4.11.1) is a fungal protease also known as leucyl aminopeptidase. It contains both endo- and exopeptidase activity with an optimum activity at pH 5–7 and 45–50°C. The hydrolysis results in the release of an N-terminal amino acid of the sequential order Xaa+ Yaa-, in which Xaa represents the first hydrolyzed amino acid and is preferably Leu, but may be other amino acids including Pro although not Arg or Lys, and Yaa represents the secondly hydrolyzed amino acid and may be Pro. Sunflower (Villanueva *et al*., 1999), heated whey (Kim *et al*., 2007) and Canola (Cumby *et al*., 2007) protein hydrolysates have been prepared by Flavourzyme.

**c) Alcalase**

Alcalase (EC 3.4.21.62) is a serine-endopeptidase produced by *Bacillus licheniformis* with an optimum activity at pH 8.0–8.5 and 55–60°C. Alcalase has a broad specificity for peptide bonds, and a preference for a large uncharged residue in the P1 position of the P1–P10 peptide bond, which interacts with the catalytic system of the serine protease according to the nomenclature of Schechter and Berger (1967), where the amino residues on the N-terminal side of the scissile bond are numbered P3, P2, P1 and those on the C-terminal side are numbered P10, P20, P30 , etc. The applied experimental conditions were pH 8.0 and 50°C. Alcalase has been proven repeatedly by many researchers to be one of the best enzymes used to prepare functional fish protein hydrolysates and other protein hydrolysates (Shahidi *et al*., 1995; Benjakul and Morrissey, 1997; Guerard *et al*., 2002). Alcalase is endopeptidase which is able to hydrolyze protein with broad specificity for peptide bond and prefers a large uncharged residue. The optimal working conditions for Alcalase are reported to be between pH 6.5 and 8.5 and at temperature of around 55-70$^{o}$C (Shahidi *et al.*, 1995). The optimum condition for the activity of Alcalase for the production of the protein hydrolysate from threadfin bream (*Nemipterus japonicus*) occurred at pH 8.5, 60$^{o}$C using a reaction time of 120 min and an enzyme substrate ratio at 2% (Normah *et al*., 2005). Kong *et al*. (2007) studied the enzymatic hydrolysis of wheat gluten by proteases and found that Alcalase served best for the preparation of wheat gluten hydrolysates with the maximum degree of hydrolysis of 15.8%. Aspmoa *et al*. (2005) reported that Alcalase and papain gave the highest yields of solubilized dry matter when compared with seven different commercial proteases (Alcalase 2.4L, Neutrase 0.8L, Protamex, Papain, Bromelain, Actinidin and a plant protease mix), approaching 95% for hydrolysis with high concentration of Alcalase. Holanda and Netto (2006) studied the enzymatic hydrolysis of the industrial waste from *Xiphopenaeus kroyeri* shrimp using Alcalase, resulting in 65% protein recovery.

**d) Protamax**

Protamax (EC 3.4.24.28) is protease from *Bacillus subtilis* with optimum pH of 7.0, and temperature of 50 °C. Protamex was the most efficient enzyme for preparing antioxidant peptides from Alaska pollack skin (Jia *et al*., 2010)

and sardine heads (Dumay *et al*., 2009). Loach (*Misgurnus anguillicaudatus*) proteins were hydrolyzed by papain and Protamex. The hydrolysates prepared by Protamex showed the strong antioxidant activity and the increase in peptides with MW below 500 Da was observed as the DH increased (You *et al*., 2009). Protein hydrolysate from herring (*Clupea harengus*) using Protamex was prepared, followed by membrane filtration (Beaulieu *et al*., 2009). The recovery of fish dry matter in the liquid hydrolysate was 67.8%. Most protein enriched fractions demonstrate a well-balanced amino acid composition, notably the most essential amino acids. These protein fractions contained peptides with relatively low MW (45 kDa and less). Studies on the nitrogen recovery in enzymic hydrolysis of Atlantic salmon (*Salmo salar*, L.) frames by Protamex protease was investigated by (Liaset *et al*., 2001). E: S ratio (90 AU/kg crude protein), temperature (50°C), starting pH (7.7) and the lowest level of frames–water ratio (0.71) resulted in the highest nitrogen recoveries (76%).

## 1.3 Objectives of study

1. To characterize commercially available duck egg and salted duck egg
2. To study the effect of salting processes and times on chemical composition, textural properties and microstructure of raw and cooked duck egg
3. To investigate the effect of pretreatment using acetic acid and commercial proteases on salting process and the characteristic of resulting salted egg.
4. To study the effect of NaCl on thermal aggregation of duck egg white.
5. To evaluate the influence of NaCl and dehydration on viscoelastic properties, thermal properties and the microstructure of duck egg yolk.
6. To produce protein hydrolysate from salted duck egg white and to study the impact of salted egg white hydrolysate on the properties of fresh and cooked Pacific white shrimp.

# CHAPTER 2

# CHANGES IN CHEMICAL COMPOSITION, PHYSICAL PROPERTIES AND MICROSTRUCTURE OF DUCK EGG AS INFLUENCED BY SALTING

## 2.1 Abstract

Changes in chemical composition, physical properties and microstructure of duck egg during salting up to 14 days were determined. Duck egg consisted of 10.87% shell, 54.73% egg white, and 33.94% yolk. Salting resulted in an increase in weight proportion of egg white, but a decrease in yolk proportion. Moisture contents of both egg white and yolk decreased gradually with concomitant increases in salt and ash contents as the salting time increased. Protein and lipid contents slightly increased in both interior (viscous liquid) and exterior (hardening) egg yolk with increasing salting time. Oil exudation was observed in yolk, particularly in exterior yolk. Triacylglycerols and phospholipid found as the major lipids in egg yolk underwent slight changes, but no differences in protein patterns of both egg white and egg yolk were observed during salting. Hardening ratio and hardness of egg yolk increased with increasing salting time. Adhesiveness and gumminess also increased, while springiness, cohesiveness and gumminess slightly decreased when the salting time increased. Scanning electron microscopic study revealed that yolk granule was polyhedral in shape and aligned closely when the salting proceeded. Protein spheres distributed uniformly together with oil droplets in salted yolk as visualized by transmission electron microscopy. Confocal laser scanning microscope (CLSM) micrographs indicated that the greater dehydration and release of lipids took place in egg yolk during salting.

## 2.2 Introduction

Eggs have been an important part of the human diet throughout the world. They have traditionally been used for breakfast, home meal preparation, baking and as the ingredient for many foods. The hen egg and duck egg are the most commonly eaten eggs, and are highly nutritious. They supply a large amount of complete, high-quality protein (which contains all essential amino acid for humans), and provide significant amounts of several vitamins and minerals (Gutierrez *et al*., 1996). They are also one of the least expensive single-food sources of complete protein (Watkins, 1994). Salted egg is one of the most traditional and popular preserved egg products. Generally, salted egg can be made by brining eggs in saturated saline or by coating the egg with soil paste mixed with salt for about 15-30 days (Chi and Tseng, 1998; Lai et al., 1999). Conventionally, salted eggs are made from duck eggs because they attain more desirable characteristics than do hen eggs (Li and Hsieh, 2004). The customer expect in egg yolk more than egg white. The desirable characteristics of salted egg yolk include orange color, oil exudation and gritty texture. During pickling, the yolk gradually becomes solidified and hardened. The egg white loses viscosity and become watery (Chi and Tseng, 1998). Chi and Tseng (1998) reported that the pickling appeared to cause moisture removal from egg yolk and the diffusion of salt into egg white and egg yolk. All changes occurring during the salting most likely determine the preferential characteristics of salted egg. However, a little information regarding the composition change of egg during salting process as well as microstructure of salted egg, particularly egg yolk, has been reported. Therefore, the objectives of this study were to investigate the changes in chemical composition, physical properties and microstructure of duck egg salted at different times.

## 2.3 Material and Methods

### 2.3.1 Egg samples

Fresh eggs of duck (*Anas platyrhucus*) with less than 3 days after laying, having the average weight of 65-75 g were obtained from a local producer in Chaiya, Suratthani Province, Thailand. Duck eggs were salted by coating with the salting paste (mud: salt, 4: 1 w/w). The thickness of coating was approximately 2-3 mm. Thereafter, the eggs were coated with rich hull ash. The prepared eggs were stored at room temperature for 7 and 14 days.

### 2.3.2 Chemicals

Petroleum ether, chloroform, methanol, diethyl ether, formic acid and nitric acid were purchased from Lab-Scan (Bangkok, Thailand). Glutaraldehyde, ethanol, n-hexane and silver nitrate were obtained from Merck (Darmstadt, Germany). Heptadecanoic acid, C17, was obtained from Sigma (St. Louis, MO, USA). Osmium tetroxide and potassium thiocyanate were purchased from Fulka (Buchs, Switzerland) and Bio-Rad (Richmond, CA, USA), respectively.

### 2.3.3 Proximate analysis and determination of salt content

Whole egg, egg yolk and egg white were analyzed for moisture, ash, lipid, and protein contents (AOAC, 2000). Salt content in egg samples was measured by the method of AOAC (2000). Sample (1 g) was added with 20 ml of 0.1 N $AgNO_3$ and 10 ml of $HNO_3$. The mixture was boiled gently on a hot plate until all solids except AgCl was dissolved (usually 10 min). The mixture was cooled using running water. Five ml of 5% ferric alum indicator ($FeNH_4 (SO_4)_2 \cdot 12 H_2O$) were added. The mixture was titrated with the standardized 0.1 N KSCN until solution became permanently light brown. The percentage of salt was then calculated as follows:

$$\text{Salt (\%)} = 5.8 \times [(V1 \times N1) - (V2 \times N2)]/W$$

where V 1 = volume of AgNO3 (mL); N1 = concentration of AgNO3 (N);
V 2 = volume of KSCN (mL); N2 = concentration of KSCN (N); and W = weight of sample (g).

### 2.3.4 SDS- polyacrylamide gel electrophoresis (SDS-PAGE)

Protein patterns of eggs white and yolk were determined according to the method of Laemmli (1970) using 4% stacking gel and 12% separating gel. Egg samples (3 g) were homogenized with 27 ml of 5% SDS using a homogenizer (Polytron, PT 2100, Kinematica AG, Luzern, Switzerland) at a speed of 12,000 rpm for 1 min. The homogenate was heated at 85°C for 1 h, followed by centrifugation at 7,500*x*g for 10 min at room temperature using a centrifuge (Sorvall, Model RC-B Plus, Newtown, CT, USA). The protein concentration of supernatant was determined by the Biuret method (Robinson and Hodgen, 1940) using bovine serum albumin (BSA) as standard. The prepared sample (20 µg protein) was loaded onto the gel. Electrophoresis was performed with the loads of 20 µg of protein using a vertical gel electrophoresis unit (Mini-protein II; Bio-Rad Laboratories, Richmond, CA., USA) at the constant voltage of 200 V/plate. The gels were stained with Coomassie Brilliant Blue R-125 (0.125%) in 25% methanol and 10% acetic acid. Destaining was performed using 40% methanol and 10% acetic acid.

### 2.3.5 Extraction of lipid from egg yolk

Lipid was extracted from egg yolks (whole yolks) using the Bligh and Dyer method (1959). Samples (25 g) were homogenized with 200 ml of the mixture of chloroform: methanol: distilled water (50: 100: 50, V/V/V) at 11,000 rpm using an IKA homogenizer (Model T25, Selangor, Malaysia) for 2 min. The homogenate was added with 50 mL of chloroform and homogenized for 1 min. Twenty five ml of distilled water was added and the mixture was homogenized for 30 sec at the same

speed. The mixture was centrifuged at 3,000*xg* for 10 min and transferred into a separating funnel. The chloroform phase (bottom phase) was drained off into the Erlenmeyer flask. Sodium sulfate anhydrous (1-2 g) was added and the mixture was shaken thoroughly to remove the residual water. Lipid in chloroform was decanted into a rounded bottom flask through a filter paper (Whatman No.4). The chloroform was evaporated at 25°C using a rotary evaporator (Rotavapor, model R-14, Buchi, Japan) and the residual solvent was removed by flushing nitrogen. The lipid was kept in an amber vial under nitrogen at -20°C until further analysis.

### 2.3.6 Determination of lipid composition and fatty acid profile of egg yolk

Egg yolk lipid composition was determined using thin layer chromatography/flame ionization detection analyzer (TLC-FID). Scanned quartz rods (Sillca gel powder coated chromarod S III) were dipped in 3% boric acid solution for 5 min, dried and rescanned with the TLC-FID analyzer. The sample solution (1 µl) was spotted on the rod and the separation was performed in the mixtures of benzene: chloroform: acetic acid (70: 20: 0.7, V/V/V) for approximately 30 min. Then the rods were dried in an oven (105°C) for 10 min before analyzing with flame ionization detector. The analytical condition was $H_2$ flow rate of 160 ml/min, air flow rate of 2000 ml/min and scanning speed of 30 s/scan. Retention time of lipid composition standards was used to identify chromatographic peaks. Peak area was quantitated and expressed as per cent of total lipid.

The fatty acid profile of egg yolk lipid was determined as fatty acid methyl ester (FAME) using a gas chromatography HP5890 series II equipped with flame ionization detection (FID). Silica capillary column carbowax-20 M (30 m, 0.25 mm ID) was used for separation. Helium was used as the carrier gas at a flow rate of 30 cm/s. The initial temperature of the column was set at 185 °C for 2 min, then increased at a rate of 5°C $min^{-1}$ to a temperature of 230°C, and maintained at 230°C for 24 min. The detector temperature at the injection port was maintained at 260°C. The total mass of FAME derived from each lipid class was calculated by reference to a Heptadecanoic acid (C17) standard which was added before methylation. The mass

of each lipid class in the extract was calculated from the derived mass of FAME, their mean molecular weight, and the fatty acyl contribution to the molecular weight of the lipid class (Speake *et al*., 2002). Fatty acid content was expressed as g/100 g lipid.

### 2.3.7 Determination of oil exudation of egg yolk

Oil exudation was measured according to the method of Lai *et al*. (1999) with a slight modification. Yolk (3 g) was homogenized with 35 ml of n-hexane/2-propanol (3:2 v/v) at 5,000 rpm for 10 min using a homogenizer (IKA, Labortechnik, Selangor, Malaysia). The filtrate obtained through Whatman No. 1 filter paper was evaporated in water bath and then dried at 105°C to constant weight. The residue was weighed and taken as total lipid content. To determine the oil exudation, yolk (5 g) was mixed with 25 ml of distilled water and homogenate at 5,000 rpm for 30 s. The homogenate was centrifuged at 9,500 xg for 30 min at 25°C and 25 ml of n-hexane/2-propanol (3:2 v/v) were added to the supernatant to dissolve the float. The solvent-lipid layer obtained was separated using a separation funnel. The solvent in the solvent-lipid layer was evaporated in a water bath and heated at 105°C until a constant weight was obtained The residue was weighed and taken as free lipid. Oil exudation was defined as the proportion of free to total lipid content.

$$\text{Oil exudation (\%)} = \frac{\text{Free lipid content}}{\text{Total lipid content}} \times 100$$

### 2.3.8 Texture profile analysis (TPA) of egg yolk

TPA was performed as described by Bourne (1978) with a TA-XT2i texture analyser (Stable Micro Systems, Surrey, England). Prior to analysis, salted egg yolks were rolled on a filter paper (Whatman No. 1) to remove egg white. The samples (whole egg yolks) were compressed twice to 50% of their original height with a compression cylindrical aluminum probe (50 mm diameter). Textural analyses were performed at room temperature. Force-distance deformation curves was

recorded at cross head speed of 5 mm/s and the recording speed was 5 mm/s. Hardness (N), fracturability (N) adhesiveness (N.s), springiness (mm), cohesiveness, chewiness (N.mm) and gumminess (N) were evaluated. These parameters were obtained using the MicroStable software (Surrey, England).

### 2.3.9 Hardening ratio of egg yolk

Hardening ratio of the salted yolk was determined following the method of Chi and Tseng (1998). The egg yolk was rolled on a filter paper (Whatman No. 1) to remove egg white. The weight of egg yolk was measured ($W_o$). The egg yolk was cut with a knife and the removable interior yolk (soft or liquid) was scraped out using a teaspoon. The weight of exterior yolk ($W_{ex}$) was measured. The hardening ratio of the egg yolk was calculated as follows:

$$\text{Hardening ratio} = (W_{ex}/ W_o) \times 100$$

### 2.3.10 Determination of microstructure of egg yolk using scanning electron microscopy

Microstructures of egg yolks were analyzed using a scanning electron microscopy (JEOL JSM-5800LV, Tokyo, Japan). Egg yolks were frozen, cut into a piece of 0.5×0.5 cm and fixed at room temperature in 2.5% glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 2 h. Fixed samples were rinsed with 0.2 M phosphate buffer (pH 7.2) for 15 min, followed by fixing in 0.1% osmium solution for 2 h at room temperature. The samples were dehydrated in graded series of ethanol (50, 70, 80, 90 and 100%) and then were mounted on SEM stubs using a double backed cellophane tape. The stub and samples were coated with gold and examined using a scanning electron microscope (JEOL JSM-5800LV, Tokyo, Japan).

### 2.3.11 Determination of microstructure of egg yolk using transmission electron microscopy

Microstructures of yolks were analyzed using a transmission electron microscopy (JEOL JEM 2010, Tokyo, Japan) at 160 kV. Egg yolk samples were fixed at room temperature in 2.5 % glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 2 h and were rinsed in 0.1 M sodium parahydroxybenzoate buffer (pH 7.4) for 1 h. The sample was then fixed in 1% osmium solution and 2% uranyl acetate for 1 h. The sample was dehydrated in graded series of ethanol (70, 80, 90 and 100%). Ethanol was removed with two successive baths in propylene oxide. The sample was embedded in Epone resin and polymerized for 24 h at 70°C. Thin sections were cut with a diamond knife in a LKB Ultramicrotome. The sections are 80 nm thick, and were deposited on copper grids, stained with 1% uranyl acetate and photographed.

### 2.3.12 Determination of microstructure of egg yolk using confocal laser scanning microscopy

The microstructures of egg yolks were examined with a confocal laser scanning microscopy (CLSM) (Olympus, FV300, Tokyo, Japan). Egg yolk samples were dissolved in Nile blue A solution (1:10) and manually stirred until the uniformity was obtained. Fifty μl of sample solutions were smeared on the microscopy slide. The CLSM was operated in the fluorescence mode at the excitation wavelength of 533 nm and the emisstion wavelength of 630 nm using a Helium Neon Red laser (HeNe-R) for lipid analysis and at the excitation wavelength of 488 nm and the emission wavelength of 540 nm using a Helium Neon Green laser (HeNe-G) for protein analysis.

### 2.3.13 Determination of microstructure of egg shell and shell membrane

Microstructure of shell was observed by scanning electron microscope (SEM). Membrane was manually removed after cleaning. Shell membrane was dried

with a series of ethanol (50-100%) and sputter-coated with gold prior to analysis. (Yi *et al*., 2004).

### 2.3.14 Statistical analysis

Completely random design was used throughout the study. The experiments were run in triplicate. Data were presented as mean values with standard deviations. One-way analysis of variance (ANOVA) was carried out and means comparisons were run by Duncan's multiple range tests (Steel and Torrie, 1980). Statistical analyses were performed with the statistical program (SPSS for windows, SPSS Inc, Chicago, IL, USA).

## 2.4 Results and Discussion

### 2.4.1 Changes in weight proportions and chemical composition of fresh duck egg and salted duck egg

Changes in weight proportions of different components of duck eggs during salting up to 14 days are shown in Table 10. Fresh duck egg consisted of 10.87% shell, 54.73% egg white, and 33.94% yolk. Generally, the proportion egg components are dependent on the strain and age of ducks (Powrie and Nakai, 1985; Sugino *et al*., 1996). Fresh duck egg had higher yolk proportion, when compared with hen egg which contained 28-29% of yolk (Sugino *et al*., 1996). During the salting, the increase in weight of egg was observed ($p< 0.05$). This might be due to the penetration of salt into the egg. The proportion of egg white increased with increasing salting time, whereas the decrease in egg yolk proportion was observed during salting. The proximate compositions of whole egg, egg white and egg yolk during salting are present in Table 11. Fresh duck eggs contained water as the major constituent. It had protein and lipid with the contents of 11.76% and 13.52%, respectively. Minerals expressed as the ash were also found in whole egg. Protein is the major constituent of egg white solids. The amount of lipid in egg white was

negligible. Egg yolk was rich in protein and contained a high content of lipids. Egg yolk contained a higher content of ash, compared with egg white. As the salting was proceeded, moisture content of egg white decreased gradually, most likely due to the loss of water from egg white to the outside caused by osmosis process. A slight decrease in protein content was observed during salting up to 14 days. It was noted that the marked increase in salted content was found in egg white as the salting time increased. This was in agreement with the increase in ash content. After 7 days of salting, the yolk became harden specially at the surface, named "exterior egg yolk"**,** while liquid yolk was found inside, termed "interior egg yolk". The reduction of moisture of egg yolk during salting was mostly associated with the hardening. High salt content in egg white might induce the water migration from egg yolk. During salting, water could be migrated from egg yolk to egg white, then to the environment through the egg shell as determined by pore sizes and structure of the shell (Chi and Tseng, 1998). Protein and lipid contents slightly increased in both interior and exterior egg yolk as salting time increased, mainly associated with dehydration of egg yolk. The dehydration resulted in the formation of exterior layer and egg yolk at the center became more concentrated. That was associated with the lower moisture content. Ash content in salted egg yolk also increased during the extended salting, indicating the migration of salts, mainly NaCl into the yolk. Potassium and sodium are the major mineral in albumen and the major element in fresh yolk are calcium, potassium and phosphorus (Powrie and Nakai, 1985). Most of the minerals are in conjugated form and only a small portion is present as inorganic compound or ions (Sugino *et al*., 1996). Additionally, salted eggs were produced by coating with the soil, which was the important source of minerals. NaCl content in the interior and exterior egg yolk increased after salting at a lower extent, compared with that found in egg white. The results confirmed the previous studies by Chi and Tseng, (1998) and Lai *et al*. (1999). After yolks become solidified, the migration of NaCl could be lowered. Furthermore, high lipid content in yolk might impede the migration of NaCl into the yolk. Salt content in salted egg can be varied with salting process. Salt content affects the acceptance of consumers. Generally, eggs salted for 7 days are recommended for pan

frying owing to the less salty taste. Eggs salted for 14 days or more are ready for boiling and commonly consumed, especially with rice gruel for breakfast.

**Table 10.** Weight proportion of different components of fresh shell eggs and shell salted for different times

| Samples | Fresh egg | Salted egg | |
|---|---|---|---|
| | | Day 7 | Day 14 |
| Whole shell egg (g) | 65.73±2.37[*, a†] | 68.22±3.04[b] | 68.89±1.44[b] |
| Egg white (%) | 54.73±1.68[a] | 59.88±1.81[b] | 62.20±1.74[c] |
| Egg yolk (%) | 33.94±1.65[c] | 28.85±2.13[b] | 25.88±1.59[a] |
| Shell and shell membrane (%) | 10.87±0.72[a] | 11.10±0.69[a] | 10.87±0.63[a] |

* Means ± SD from twenty determinations.

† Different superscripts in the same row indicate significant differences ($p < 0.05$)

### 2.4.2 Changes in oil exudation of egg yolk during salting

Oil exudation of egg yolk during salting is depicted in Figure 7. Oil exudation increased with increasing salting time ($p< 0.05$). The greater oil exudation was observed in exterior egg yolk, compared with interior counterpart ($p< 0.05$). Oil exudation is generally one of desirable characteristics of salted egg. Schultz *et al*., (1968) pointed out that removal of water from egg yolk increased the extracted lipid. Thus, the dehydration during salting probably enhanced oil exudation. Free lipid might be released from low density lipoprotein micelles, due to the structural changes of low density lipoprotein induced by dehydration and increased salt content. The dehydration of proteins might be caused by yolk protein denaturation associated with the loss in their emulsifying properties upon salting. Weak gel structure of yolk protein developed upon dehydration was attributed to hydrophobic and hydrogen interaction (Paraskeyopoulou *et al*., (2000).

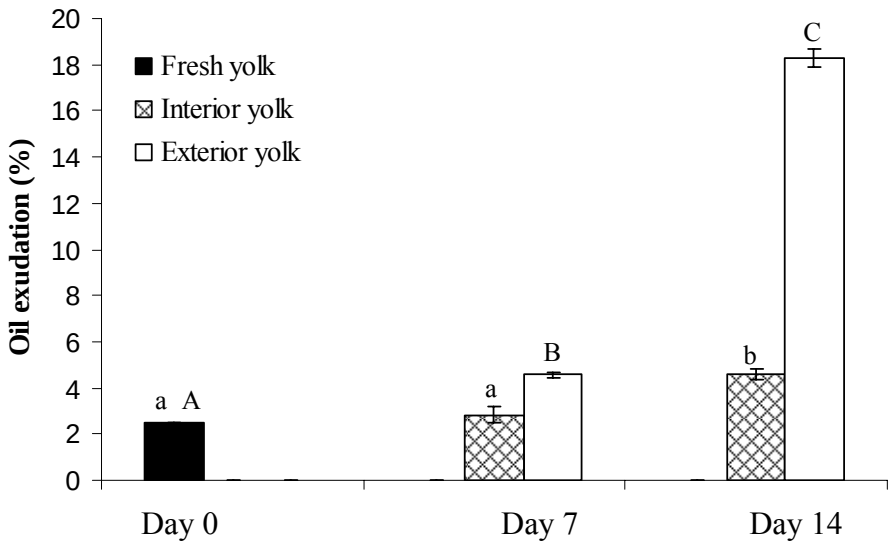



**Figure 7.** Oil exudation of fresh duck egg yolk and egg yolk salted for different times. Different letters on the bar indicates significant differences ($p < 0.05$).

**Table 11.** Proximate composition and salt content of fresh egg and egg salted for different times

| Samples | Composition (% wet wt. basis)* | | | | |
|---|---|---|---|---|---|
| | Moisture | Protein | Lipid | Ash | Salt |
| **Fresh egg** | | | | | |
| Whole egg | 71.77±0.78[*] | 11.76±1.15 | 13.52±0.14 | 1.17±0.02 | 0.33±0.00 |
| Egg white | 87.72±0.62[z†] | 10.52±0.14[z] | 0.03±0.012[x] | 0.74±0.01[x] | 0.39±0.07[x] |
| Egg yolk | 43.51±0.52 [c,C] | 16.04±0.48[a, A] | 37.25±0.16[a, A] | 1.59±0.11[a, A] | 0.45±0.04[a, A] |
| **Salted egg (Day 7)** | | | | | |
| Egg white | 85.19±0.59[y] | 10.05±0.05[y] | 0.03±0.02[x] | 3.02±0.10[y] | 3.96±0.10[y] |
| Interior egg yolk | 39.85±0.28[b] | 16.67±0.35[ab] | 38.39±1.19[a] | 1.91±0.11[b] | 0.54±0.20[a] |
| Exterior egg yolk | 26.57±1.51[B] | 19.51±0.90[B] | 47.59±2.74[B] | 2.28±0.01[B] | 0.67±0.07[B] |
| **Salted egg (Day 14)** | | | | | |
| Egg white | 83.59±0.68[x] | 9.55±0.12[x] | 0.05±0.014[x] | 4.04±0.18[z] | 6.90±0.20[z] |
| Interior egg yolk | 36.21±2.44[a] | 17.56±0.56[b] | 44.32±1.40[b] | 2.20±0.01[c] | 0.84±0.15[b] |
| Exterior egg yolk | 20.05±0.29[A] | 21.33±0.65[C] | 53.71±0.39[C] | 2.45±0.09[B] | 0.87±0.10[C] |

* Means ± SD from triplicate determinations.

† Different superscripts in the same column indicate significant difference ($p < 0.05$)

x, y and z in the same column indicate the significant difference between the egg white obtained from different salting times ($p < 0.05$).

a, b and c in the same column indicate the significant difference between interior salted yolk obtained from difference salting time and from fresh yolk ($p < 0.05$)

A, B and C in the same column indicate the significant difference between exterior salted yolk obtained from difference salting time and from fresh yolk ($p < 0.05$)

### 2.4.3 Changes in lipid composition and fatty acid profile of egg yolk during salting

Lipid from fresh and salted egg yolks contained triacylglycerols as the dominant lipid, followed by phospholipid. Diacylglycerol was found at low content (0.83-0.95 g/100g lipid). Free fatty acid was not found in egg yolks, indicating that salting had no impact on lipid lipolysis. Fatty acid compositions of lipids from fresh and salted duck eggs are shown in Table 12. The major fatty acids in fresh duck egg were oleic acid (C18:1n-9) and palmitic acid (16:0) which constituted 47.54% and 27.24% (w/w) of total fatty acids (Table 13). Linoleic acid (18:2n-6) was found at 8.08%. Arachidonic acid (C20:4n-6) and docosahexaenoic acid (C22:6n-3) were also found in egg yolk lipid. Fatty acid profiles of yolk lipid from different species of duck were similar (Speake *et al*., 2002). Fatty acid content of yolk lipid was influenced by the types of fatty acid in the feed (Powrie and Nakai, 1985). Duck egg yolk lipid has unique fatty acids including arachidonic acid and docosahexaenoic acid, which are not found in soy and other plant origins (Juneja and Kim, 1996). Arachidonic acid and docosahexaenoic acid are attached to phospholipids in egg yolk lipid (Juneja and Kim, 1996). ω-3 fatty acids are now regarded as essential in the diet for brain function and visual acuity in humans (Juneja and Kim, 1996). Fatty acid profile of salted egg lipid was similar with that of fresh yolk lipid. No marked changes in fatty acid profile were observed between egg salted for 7 and 14 days. However, stearic acid (C18:0) decreased slightly after 7 and 14 days of salting. Linoleic acid content decreased after salting for 14 days. Thus, salting generally had no impact on fatty acid composition of yolk lipids.

**Table 12.** Lipid composition of yolk from fresh egg and egg salted for different times

| Lipid composition (%(w/w) of total lipids) | Fresh egg | Salted egg | |
|---|---|---|---|
| | | Day 7 | Day 14 |
| Triacylglycerols (%) | 59.13±1.75[*, b†] | 58.14±2.62[ab] | 56.13±2.23[a] |
| 1, 3- Diacylglycerols (%) | 0.95±0.17[a] | 0.83±0.11[a] | 0.95±0.16[a] |
| Phospholipids (%) | 39.92±1.79[a] | 41.03±2.69[ab] | 42.98±2.41[b] |

[*] Means ± SD from five determinations

[†] Different superscripts in the same row indicate significant differences ($p < 0.05$).

**Table 13.** Fatty acid profile of yolk from fresh egg and egg salted for different times

| Fatty acid (% of total fatty acid) | Fresh egg | Salted egg | |
|---|---|---|---|
| | | Day 7 | Day 14 |
| C14:0 | 0.50 | 0.43 | 0.50 |
| C16:0 | 27.24 | 26.77 | 27.53 |
| C16:1 n-7 | 2.25 | 2.24 | 2.66 |
| C18:0 | 6.19 | 5.50 | 5.63 |
| C18:1 n-9 | 47.54 | 48.00 | 48.38 |
| C18:2 n-6 | 8.08 | 8.73 | 6.88 |
| C18:3 n-3 | 0.33 | 0.31 | 0.30 |
| C20:2 n-6 | 0.17 | 0.27 | 0.34 |
| C20:4 n-6 (ARA) | 2.62 | 2.57 | 2.79 |
| C22:4 n-6 | 0.18 | 0.15 | 0.18 |
| C22:5 n-3 | 0.37 | 0.41 | 0.23 |
| C22:5 n-6 | 0.35 | 0.22 | 0.31 |
| C22:6 n-3 (DHA) | 1.66 | 1.95 | 1.61 |
| Saturated fatty acid | 33.93 | 32.70 | 33.6 |
| Unsaturated fatty acid | 63.55 | 64.85 | 63.68 |

### 2.4.4 SDS-polyacrylamide gel electrophoresis (SDS-PAGE) patterns of fresh and salted duck egg

Whole egg contained 4 major protein bands with the apparent MW of 117, 105, 85, and 47 kDa. For egg white, ovalbumin was found as the major protein with MW of 47 kDa. Ovalbumin is the most abundant protein in egg white (54%) (Li-Chan *et al*., 1995; Linden and Lorient, 2000). Another protein band was found at MW of 76 kDa, most likely conalbumin (Linden and Lorient, 2000; Ternes, 2001). Electrophoretic study of yolk proteins (Figure 8) revealed several protein bands with MW range of 30-220 kDa. Among all proteins, that with MW of 100 kDa was dominant and most likely classified as LDL apoprotein, whose theoretical molecular weight value is estimated to 100-180 kDa (Ternes, 2001). Proteins with MW of 46 and 56 kDa likely corresponded to HDL apoprotein (Raikos *et al*., 2006). Egg yolk is a complex system of protein and lipid. All lipids of egg yolk are associated with proteins to form lipoproteins, commonly classified as low-density lipoproteins (LDL) and high-density lipoproteins (HDL) (Anton, 2003). For other protein bands, they might represent LDL protein of the plasma fraction of yolk. No change in protein patterns in egg white between fresh egg and egg salted for 7 and 14 days. Additionally, similar protein patterns were observed between yolk of fresh egg and salted egg, regardless of salting time and egg yolk portions, interior or exterior.

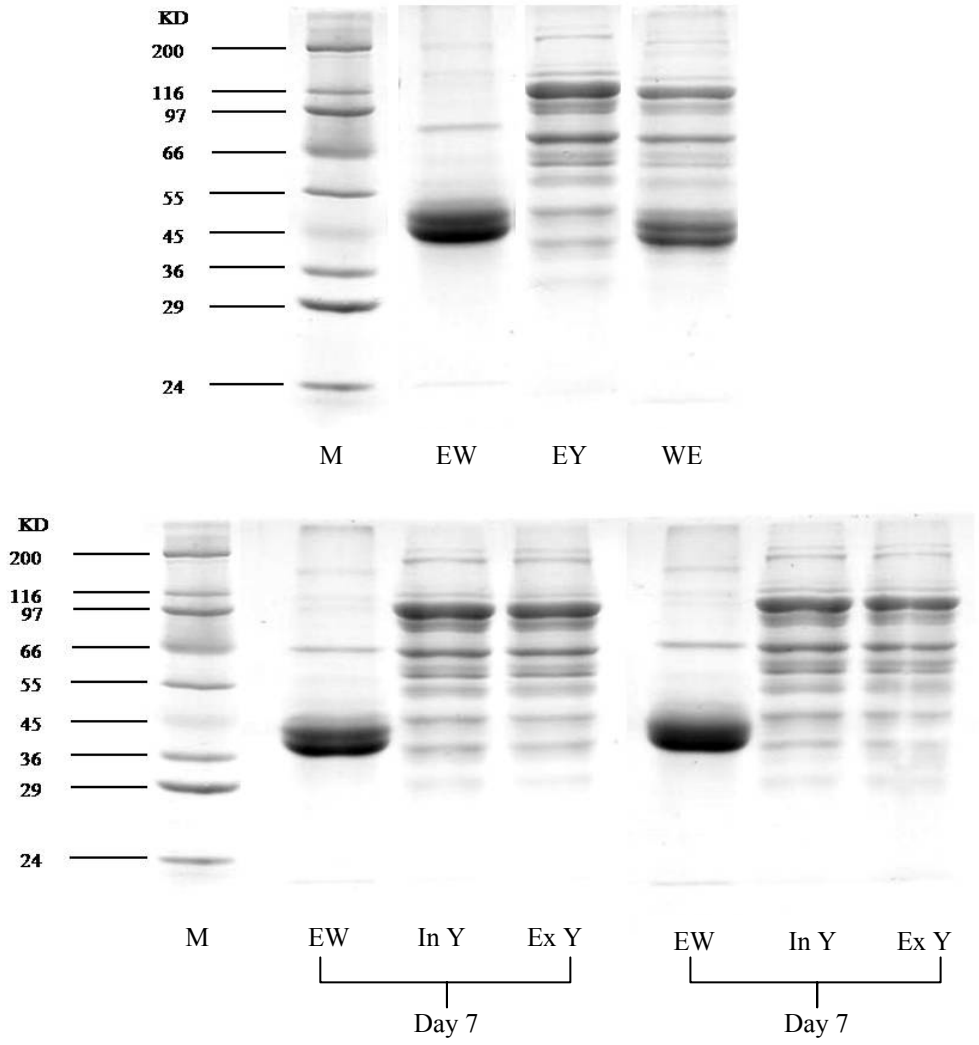



**Figure 8.** SDS-PAGE patterns of different portions of fresh duck egg and duck egg salted for different times. M: molecular weight standard; EW: egg white; EY: egg yolk; WE: whole egg; InY: interior yolk and ExY: exterior yolk

### 2.4.5 Changes in hardening ratio and texture profile of egg yolk during salting

Hardening ratio of yolk was defined as the weight percent of hard exterior yolk and used as an index for the completeness of salting. Hardening ratio of egg salted for 7 days was lower than that of egg salted for 14 days (Table 14). During salting, the solidification of egg yolk initiated near the vitelline membrane and proceeded toward the center, the exterior formation. The interior yolk was still in liquid form, but became more viscous with further dehydration of exterior salted yolk, hard and slightly rubbery structure was developed. When yolk protein became more concentrated, the interaction between protein molecules including lipoproteins could be formed. This resulted in the formation of gel-like network. The presence of native yolk lipids or emulsified oil droplet appears to influence the yolk gel rheological properties (Kiosseoglou, 2003). Egg yolk having relatively high lipid content could produce gel networks at low protein concentrations, suggesting that the yolk lipid molecules are somehow involved in gel structure formation (Kiosseoglou, 2003).

Texture profiles of egg salted for 7 and 14 days are shown in Table 14. The hardness of salted egg yolk increased with increasing salting time. This was in accordance with the increases in hardening ratio. The result suggested that the structure of salted egg yolk became more solidified. Fracturability of salted egg markedly increased from 0.11 to 3.76 N after salting for 7 and 14 days, respectively. Salted egg yolk was more dehydrated and could form the gritty texture. Grittiness is the major factor affecting consumer acceptance of salted egg product (Chi and Tseng, 1998). Adhesiveness and gumminess increased, while springiness, cohesiveness and gumminess slightly decreased when the salting time increased upto 2 weeks of salting.

**Table 14.** Hardening ratio and texture profile analysis (TPA) of yolk from egg salted for different times

| Hardening ratio/TPA Terms | Day 7 | Day 14 |
|---|---|---|
| Hardening ratio (%) | 66.61±3.82* | 88.22±4.50 |
| Hardness (N) | 3.45±0.79 | 9.25±3.28 |
| Fracturability (N) | 0.11±0.06 | 3.76±3.26 |
| Adhesiveness (N.s) | 1.01±0.71 | 1.68±0.78 |
| Springiness (mm) | 0.58±0.26 | 0.31±0.09 |
| Cohesiveness | 0.43±0.12 | 0.25±0.05 |
| Gumminess (N) | 1.56±0.76 | 2.27±0.90 |
| Chewiness (N.mm) | 1.05±0.96 | 0.71±0.31 |

* Means ± SD from ten determinations for hardening ratio and from twenty determinations for TPA analysis

### 2.4.6 Microstructures of shell of fresh and salted egg

Microstructures of shell and shell membrane of fresh and salted eggs are depicted in Fig. 3. Egg shells are composed of calcium carbonate layer, and two shell membranes (Okubo *et al*., 1996)**.** Egg shells contain funnel-shaped small holes called pore canals on the surface of the shell for gas exchange. The diameter of the pore canal ranges from 10 to 30 μm. An egg has about 7000-17,000 pore canals on the shell surface per egg (Powrie and Nakai, 1985; Okubo *et al*., 1996), allowing salt and water to pass through during salting process. No differences in shell structures were observed between fresh egg (Figure 9A and 9B) and salted egg (Figure 9E and 9F). The egg shell membrane consisted of inner and outer membranes. The structure was entangled thread or randomly knitted net in shape. The structure of shell

membrane of fresh egg (Figure 9C and 9D) was similar to that of salted egg (Figure 9G and 9H).

### 2.4.7 Microstructures of egg yolk of fresh and salted egg

Microstructures of yolk from fresh and salted egg obtained after 14 days of salting visualized by SEM are shown in Figure 10A and 10B. Egg yolk and salted egg yolk had polyhedral granules with the size range of 50-100 μm. Yang and Hsu (1989) reported polyhedral granules with the diameter ranging from of 23 to 127 μm in salted duck egg yolk. Chi and Tsung (1998) found the granules with the size range of 90-100 μm in salted egg yolk. The microstructure of salted egg yolk by SEM indicated that polyhedral granule was localized closely than fresh egg yolk (Figure 10B), mainly due to dehydration during salting. The presence of such granules probably provided the gritty texture sensation. Therefore, the stacking of granule was necessary to produce salted egg yolk with a gritty texture. The polyhedral granules were formed by yolk spheres (Mineki and Kobayashi, 1997). During salting, the dehydration was more pronounced and the granules were located closer, leading to the denser structure.

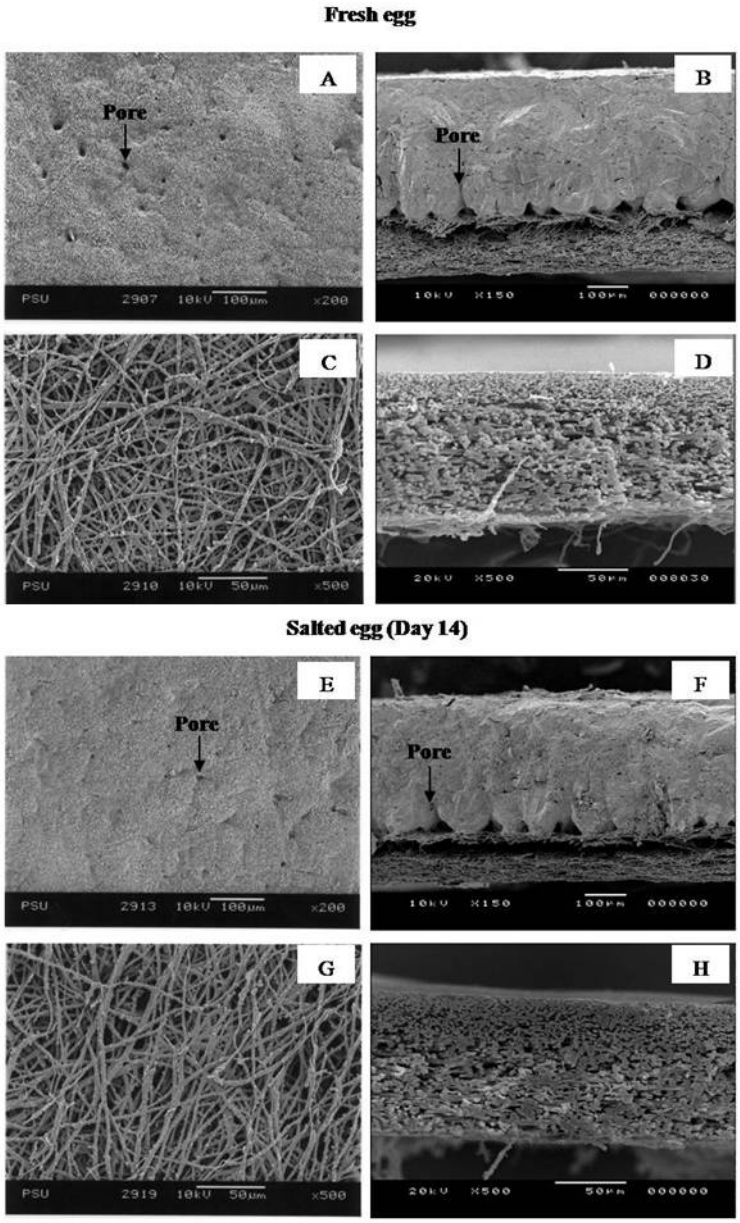



**Figure 9.** Scanning electron microscopic photograph of shell and shell membrane of fresh duck egg and salted duck egg after 14 days of salting. A, E: Top surface of shell; B, F: cross section of shell; C, G: Top surface of shell membrane and D, H: cross section of shell membrane

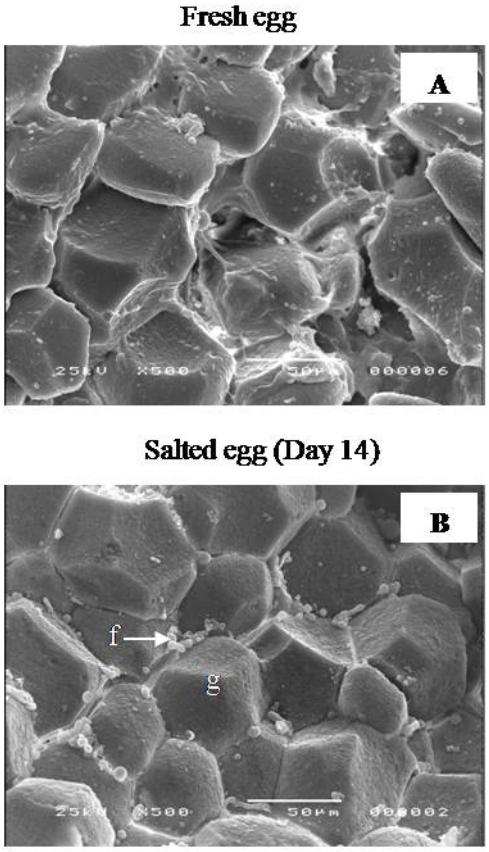



**Figure 10.** Scanning electron microscopic photograph of yolk granule from fresh duck egg (A) and after 14 days of salting (B) (from exterior counterpart) Magnification: 500X f: free fat released from the granule; g: granule

Transmission electron micrographs of fresh and salted egg yolk are shown in Figure 11. Larger protein spheres were observed in fresh egg (Figure 11FA), compared with those found in salted egg (Figure 11SA). Protein spheres with electron density and the diameter of 0.6-4 μm (Figure 11FB) and 0.2-2 μm (Figure 11SB) were found in fresh yolk and salted egg yolk, respectively. For salted yolk, oil spheres were observed at the interface of the yolk spheres (Figure 11SC), suggesting that oil droplets were released from lipid-protein structure. However, no oil droplets were found at the interface of fresh yolk (Figure 11FC). Egg yolk contains 22% of granule fraction and 72% of plasma (Sugino *et al*., 1996). Granules are composed of 17% phosvitin, 70% high density lipoprotein (HDL) and 12% low density lipoprotein,

whereas plasma consists of 85% LDL and 15% livitin (Sugino *et al*., 1996; Powrie and Nakai 1985; McBee and Cotterill, 1979). A low-density lipoprotein (LDL) fraction (lipovitellenin) contains 90% lipids. After salting, the structure of lipoprotein was destroyed and some parts of lipid in egg yolk became free (Lai *et al*., 1999).

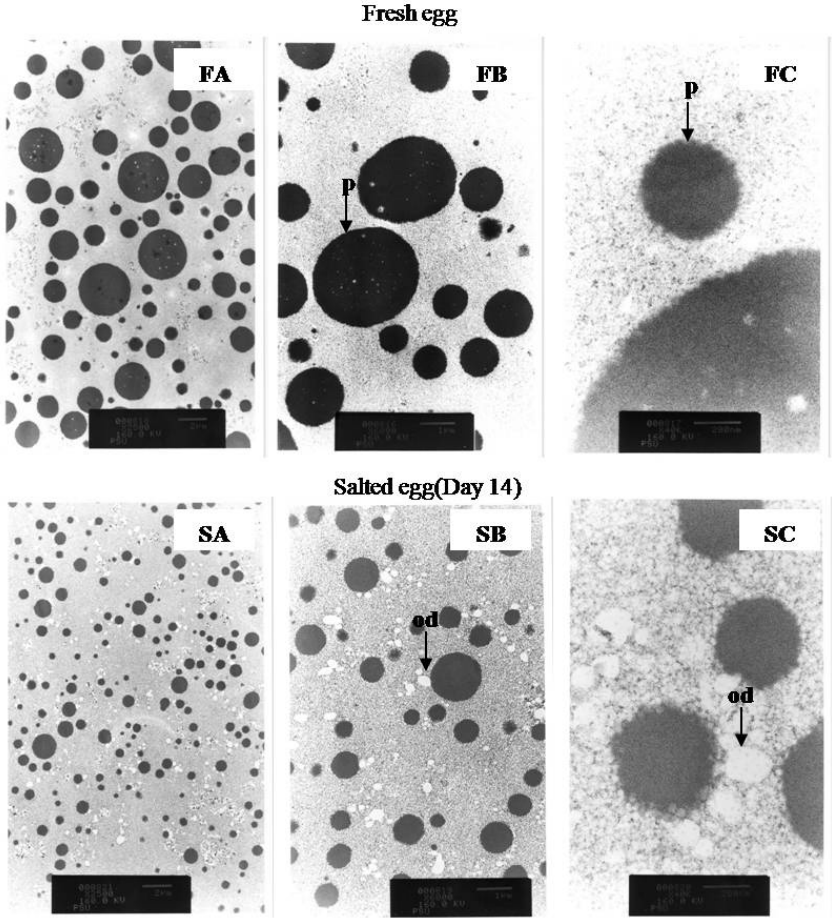



**Figure 11.** Transmission electron microscopic photograph of internal structure of yolk glanule from fresh egg yolk (F) and after 14 days of salting (S) (from exterior counterpart). Magnification; 2500X (A); 6000X (B); and 40, 000X (C) od: oil droplet; p: protein spheres

The confocal laser scanning microscope (CLSM) micrographs of fresh egg yolk and salted egg yolk are shown in Figure 12. Lipid and protein distributed uniformly in fresh egg yolk are shown in Figure 12FA and 12FB, respectively. On the other hand, lipid and shape of protein turned to be irregular with discontinuous

distribution after being solubilized in dye solution. CLSM micrographs indicated that egg yolk is actually a source of lipids and proteins, which are easily dispersed in water, thus permitting emulsification of other substances. The majority of proteins in yolk are organized into micellar and granular structures together with polar and non-polar lipid molecules (Kiosseoglou, 2003). All constituents of yolk (LDL, HDL, phosvitin and livitin) have a strong capacity to absorb at the oil-water interface (Anton *et al*., 2003). Among yolk components, proteins (including apoprotein of lipoproteins) are the main molecules that take part in adsorption at the water interface. Furthermore, they also control the colloidal interaction between covered oil droplets. As salting proceeded, the greater dehydration together with the release of lipids in egg yolk might reduce the emulsion capacity of protein portion. Irregular shape of both lipid and protein was found in both interior (Figure 12InA and 6InB) and exterior (Figure 12ExA and 12ExB) yolk after salting for 14 days. Increased viscosity in interior salted egg yolk and hardening of exterior salted egg yolk also made the yolk more difficult to be dispersed in the dye solution.

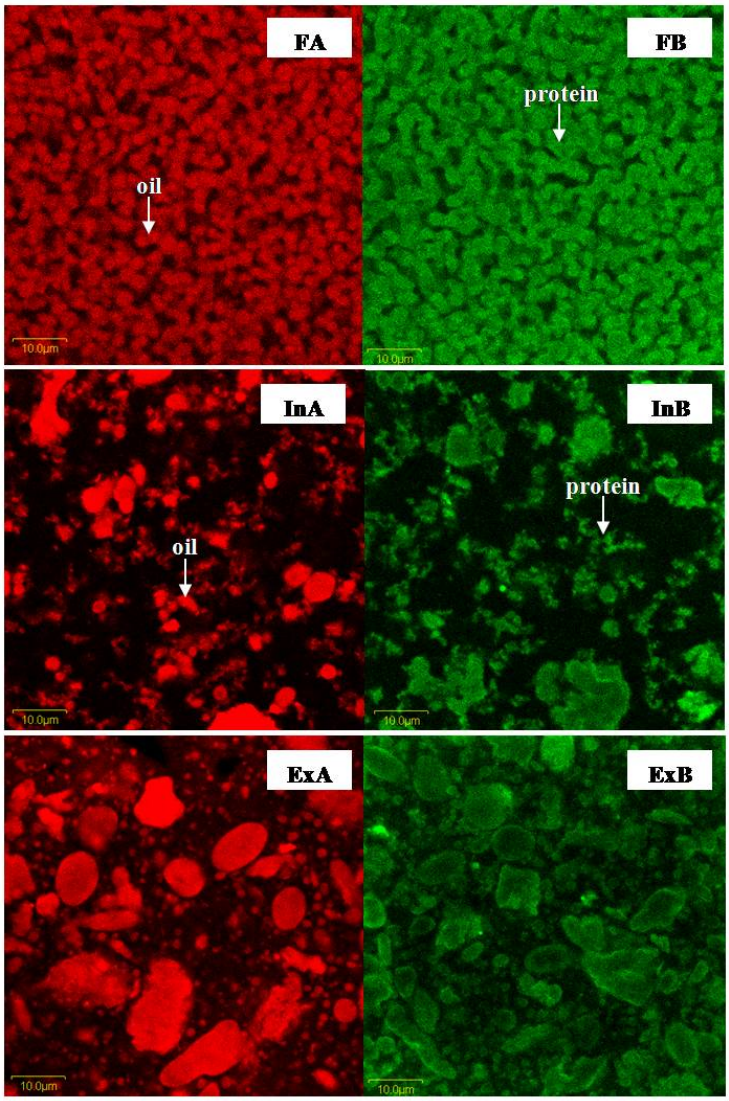



**Figure 12.** Confocal laser scanning microscope (CLSM) micrographs of fresh egg yolk (F), interior yolk (In) and exterior yolk (Ex) of egg after 14 day of salting Magnification: 600X (zoom X3.5) oil distribution (A) and protein distribution (B) Scale bar = 10 μm

## 2.5 Conclusions

Salted duck eggs were rich in protein and fat. Only slight changes in lipid composition and fatty acid profile were found in egg during salting, compare with fresh egg. Salting induced solidification of yolk accompanied with oil exudation and the development of gritty texture. Additionally, moisture removal and the association of egg yolk granules most likely contributed to the development of solidified yolk. After salting, polyhedral granules of egg yolk were localiaed closely, contributing to gritty texture. Moreover, the lager protein spheres were observed in fresh egg yolk, compared with those found in salted egg yolk. Oil spheres were observed at the interface of yolk spheres after salting.

# CHAPTER 3

# EFFECT OF SALTING PROCESSES ON CHEMICAL COMPOSITION, TEXTURAL PROPERTIES AND MICROSTRUCTURE OF DUCK EGG

## 3.1 Abstract

Changes in chemical composition, physical properties and microstructure of duck egg salted by two processes, paste coating and brine immersing, during salting up to 7 weeks were compared. Decreases in moisture content with coincidental increases in salt content in both egg white and yolk were observed during salting, regardless of salting processes. However, no differences in salt content was noticeable in yolks ($p>0.05$). Paste coating method tended to yield the greater oil exudation of egg yolk than immersing method. Thiobarbituric acid-reactive substance (TBARS) value in yolk slightly increases with increasing salting time. Similar hardening ratio of yolk was observed between both processes. Maximum transition temperature ($T_{max}$) of egg proteins increased when the salting time increased, regardless of salting processes. The higher hardness and adhesiveness were found in yolk with paste coating method, whereas the greater fractureability, springiness, gumminess and chewiness were observed with immersing method. Nevertheless, both processes rendered the yolk with similar cohesiveness. Yolk granule was polyhedral and aligned closely when the salting proceeded, irrespective of salting processes. Dehydration and release of lipids in egg yolk increased with increasing salting time and were more pronounced with paste coating method. Therefore salting processes effected for salted egg properties.

## 3.2 Introduction

Salted egg is one of the most popular traditional preserved egg products in oriental countries. Generally, salted egg can be made by brining eggs in saturated saline or by coating the egg with soil paste mixed with salt for about 15-30 days (Chi and Tseng, 1998; Lai *et al*., 1999). The immersing method is a faster and more convenient for production of salted egg (Yang and Chen, 2001). In addition to being consumed as the whole egg, salted egg yolks are widely used as a filling in some Chinese sweet such as moon cakes and other disserts. Conventionally, salted eggs are made from duck eggs because they attain more desirable characteristics than do hen eggs (Li and Hsieh, 2004). The customers generally expect in egg yolk rather than egg white. The desirable characteristics of salted egg yolk include orange color, oil exudation and gritty texture. During salting, the yolk gradually becomes solidified and hardened. Egg white loses viscosity and becomes watery (Chi and Tseng, 1998). Chi and Tseng (1998) reported that salting caused moisture removal from egg yolk and the diffusion of salt into egg white and egg yolk. All changes occurring during the salting most likely determine the preferential characteristics of salted egg.

Rate of salt penetration into egg white and yolk governed by salting methods may have the impact on the changes in composition as well as characteristic of egg, especially yolk. Additionally, salting time also plays a role in the formation of salted egg with desirable characteristics. However, no information regarding the changes in characteristics and properties of egg salted using different processes during salting has been reported. Therefore, the objective of this study was to determine chemical composition, textural properties and microstructure of duck egg obtained from coating method and immersing method at different salting times.

## 3.3 Materials and Methods

### 3.3.1 Chemicals

Chloroform, methanol, 2-propanol and nitric acid were purchased from Lab-Scan (Bangkok, Thailand). Glutaraldehyde, ethanol, n-hexane, isooctane and silver nitrate were obtained from Merck (Darmstadt, Germany). Nile blue A was obtained from Sigma (St. Louis, MO, USA). Osmium tetroxide and 2- thiobarbituric acid were purchased from Fluka (Buchs, Switzerland) and potassium thiocyanate was obtained from Bio-Rad (Richmond, CA, USA).

### 3.3.2 Duck egg collection

Duck eggs *(Anas plotyrhyncus)* with the weight range of 65-75 g were obtained within 1 day of laying from Satingphar, Songkhla Province, Thailand. The eggs were used within 3 days after laying.

### 3.3.3 Preparation of salted egg

Fresh duck shell eggs were separated into 2 groups. The first group was coated with coating paste (18.35±0.27% solar salt/sample), a mixture of clay (4 kg), solar salt (1 kg) and water (1.8 L). Duck eggs were covered with coating paste by dipping them for three times in the mixture to obtain a thickness of approximately 2 mm. Thereafter, coated eggs were covered with rice chaff ash. The second group was immersed in the brine solution (25%, w/v) using 1 egg/100 mL brine. Duck eggs from both groups were stored at room temperature (28-30ºC) and taken for analyses every 7 day up to 49 days. Before analyses, the coating paste was removed manually and the eggs were washed with water. Egg white and egg yolk were manually separated. Egg yolk was cut with a knife and the removable interior yolk was scraped out using a teaspoon. Interior yolk, exterior yolk and egg white were subjected to chemical analysis.

### 3.3.4 Determination of moisture and salt contents

Egg white, interior yolk and exterior yolk were determined for moisture content according to AOAC (2000). Salt content in egg samples was measured by the method of AOAC (2000). Samples (1 g for egg yolk or 0.5 g for egg white) were added with 20 mL of 0.1 N $AgNO_3$ and 10 mL of $HNO_3$. The mixture was boiled gently on a hot plate until all solids except $AgCl_2$ were dissolved (usually 10 min). The mixture was cooled using running water. Five mL of 5% ferric alum indicator ($FeNH_4$ $(SO_4)_2 \cdot 12$ $H_2O$) were added. The mixture was titrated with the standardized 0.1 N KSCN until the solution became permanently light brown. The percentage of salt was then calculated as follows:

$$\text{Salt (\%)} = 5.8 \times [(V1 \times N1) - (V2 \times N2)]/W$$

where V 1 = volume of $AgNO_3$ (mL); N1 = concentration of $AgNO_3$ (N);
V 2 = volume of KSCN (mL); N2 = concentration of KSCN (N); and W = weight of sample (g).

### 3.3.5 Determination of oil exudation of egg yolk

Oil exudation was measured according to the method of Lai *et al*. (1999) with a slight modification. Yolk (3 g) was homogenized with 35 ml of n-hexane/2-propanol (3:2, v/v) at 5,000 rpm for 10 min using a homogenizer (Polytron, PT 2100, Kinematica AG, Luzern, Switzerland). The filtrate obtained through Whatman No. 1 filter paper was evaporated in a temperature controlled water bath (Memmert, Schwabach, Germany) at 55ºC and then dried at 105°C in an oven (Memmert, Schwabach, Germany) until the constant weight was obtained. The residue was weighed and taken as total lipid content. To determine the oil exudation, yolk (5 g) was mixed with 25 mL of distilled water and homogenized at 5,000 rpm for 30 sec. The homogenate was centrifuged at 9,500 g for 30 min at 25°C and 25 mL of n-hexane/2-propanol (3:2, v/v) were added to the supernatant to dissolve the float.

The solvent-lipid layer obtained was separated using a separating funnel. The solvent in the solvent-lipid layer was evaporated in a water bath (55ºC) and the residue was heated at 105°C until a constant weight was obtained The residue was weighed and taken as free lipid. Oil exudation was defined as the proportion of free to total lipid content.

$$\text{Oil exudation (\%)} = \frac{\text{Free lipid content}}{\text{Total lipid content}} \times 100$$

### 3.3.6 Extraction of lipid from egg yolk

Lipid was extracted from whole egg yolks using the Bligh and Dyer method (1959). Samples (25 g) were homogenized with 200 mL of the mixture of chloroform: methanol: distilled water (50: 100: 50, V/V/V) at 11,000 rpm using an homogenizer (IKA, Labortechnik, Selangor, Malaysia) for 2 min. The homogenate was added with 50 mL of chloroform and homogenized for 1 min at the same speed. Twenty five mL of distilled water was added and the mixture was homogenized for 30 sec at the same speed. The mixture was centrifuged at 3,000*xg* for 10 min and transferred into a separating funnel. The chloroform phase (bottom phase) was drained off into the Erlenmeyer flask. Sodium sulfate anhydrous (1-2 g) was added and the mixture was shaken thoroughly to remove the residual water. Lipid in chloroform was decanted into a rounded bottom flask through a filter paper (Whatman No.4). The chloroform was evaporated at 25°C using a rotary evaporator (Rotavapor, model R-14, Buchi, Japan) and the residual solvent was removed by flushing nitrogen. The lipid was kept in an amber vial under nitrogen at -20°C until analysis.

### 3.3.7 Determination of Lipid oxidation

TBARS was determined according to the method of Buege and Aust (1978). Egg yolk oil sample (0.3 g) was mixed with 4 mL of TBARS solution (0.375% TBA, 15% TCA, and 0.25 N HCl). The mixture was heated for 10 min in boiling water (95-100°C) to develop a pink color. Then the mixture was cooled with

running water and centrifuged at 5,500×g for 25 min. The absorbance of the supernatant was measured at 532 nm using a spectrophotometer (UV-1601, Shimadzu, Japan). TBARS value was calculated from the standard curve of malonaldehyde and expressed as mg MDA/g sample.

Conjugated dienoic acid (CDA) of egg yolk oil samples were measured according to AOCS (1980). Sample (100 mg) was dissolved in 25 mL of isooctane and the mixture was allowed to stand for 10 min. Mixture was then 10-fold diluted with isooctane and the absorbance at 233 nm was determined by a spectrophotometer (UV-1601, Shimadza, Kyoto, Japan). CDA content was calculated as follows:

$$CDA\ (\%) = (0.84 \times A)/ (bc - K_0)$$

where A = absorbance at 233 nm; b = path length (cm); c = concentration (g/l)
$K_0$ = absorptivity by acid groups (0.03)

### 3.3.8 Measurement of hardening ratio of egg yolk

Hardening ratio of the salted yolk was determined following the method of Chi and Tseng (1998). The egg yolk was rolled on a filter paper (Whatman No. 1) to remove egg white. The weight of egg yolk was measured ($W_o$). The egg yolk was cut with a knife and the removable interior yolk (soft or liquid) was scraped out using a teaspoon. The weight of exterior yolk ($W_{ex}$) was measured. The hardening ratio of the egg yolk was calculated as follows:

$$Hardening\ ratio = (W_{ex}/ W_o) \times 100$$

### 3.3.9 Texture profile analysis (TPA) of egg yolk

TPA was performed as described by Bourne (1978) with a TA-XT2i texture analyzer (Stable Micro Systems, Surrey, England). Prior to analysis, salted egg yolks were rolled on a filter paper (Whatman No. 1) to remove egg white. The whole egg yolks were compressed twice to 50% of their original height with a

compression cylindrical aluminum probe (50 mm diameter). Textural analyses were performed at room temperature. Force-distance deformation curves were recorded at cross head speed of 5 mm/s and the recording speed was 5 mm/s. Hardness (g), adhesiveness (g.s), springiness (mm), cohesiveness and chewiness (g.s) were evaluated. These parameters were obtained using the MicroStable software (Stable Micro Systems, Surrey, England).

### 3.3.10 Differential Scanning Calorimetry

Thermal transition of egg proteins was determined using the differential scanning calorimetry (DSC) (Perkin-Elmer, Model DSCM, Norwalk, CT, USA). Fresh egg samples (10-15 mg wet weight) were placed in the DSC hermetic pans. For salted egg, moisture content was adjusted to the same level found in fresh egg (87% for egg white and 44% for egg yolk). An empty hermetic pan was used as a reference. The samples were scanned at 5ºC/min over the range of 20-110ºC. $T_{max}$ was measured and the denaturation enthalpies ($\Delta H$) were estimated by measuring the area under the DSC transition curve.

### 3.3.11 Determination of microstructure of egg yolk using scanning electron microscopy

Microstructures of egg yolks were analyzed using a scanning electron microscopy (JEOL JSM-5800LV, Tokyo, Japan). Egg yolks were frozen with liquid nitrogen, cracked into a small piece (0.5×0.5 cm) and fixed at room temperature in 2.5% glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 2 h. Fixed samples were rinsed with 0.2 M phosphate buffer (pH 7.2) for 15 min, followed by fixing in 0.1% osmium solution for 2 h at room temperature and washing with distilled water for 15 min. The samples were dehydrated in graded series of ethanol (50, 70, 80, 90 and 100%) and then were mounted on SEM stubs using a double backed cellophane tape. The stub and samples were coated with gold and examined using a scanning electron microscope (JEOL JSM-5800LV, Tokyo, Japan).

### 3.3.12 Determination of microstructure of egg yolk using confocal laser scanning microscopy

Microstructures of egg yolks were examined with a confocal laser scanning microscopy (CLSM) (Olympus, FV300, Tokyo, Japan) following the modified method of Mineki and Kobayashi (1997). Egg yolk samples were dissolved in 0.01% Nile blue A solution at a ratio of 1:10 (w/v) and stirred until the uniformity was obtained. Fifty μl of sample solutions and 0.01% acridine orange were smeared on the microscopy slide. The CLSM was operated in the fluorescence mode at the excitation wavelength of 533 nm and the emission wavelength of 630 nm using a Helium Neon Red laser (HeNe-R) for lipid analysis and at the excitation wavelength of 488 nm and the emission wavelength of 540 nm using a Helium Neon Green laser (HeNe-G) for protein analysis.

### 3.3.13 Statistical analysis

Completely random design was used throughout the study. The experiments were run in triplicate. Data were presented as mean values with standard deviations. One-way analysis of variance (ANOVA) was carried out and means comparisons were run by Duncan's multiple range tests (Steel and Torrie 1980). For pair comparison, T-test was used. Statistical analyses were performed with the statistical program (SPSS for windows, SPSS Inc, Chicago, IL, USA).

## 3.4 Results and Discussion

### 3.4.1 Changes in chemical composition of duck egg during salting

Changes in moisture and salt contents of duck egg obtained from different salting processes were monitored during salting up to 7 weeks. Decreases in moisture content with coincidental increases in salt content in both egg white and yolk were observed during salting, regardless of salting processes (Figure 13A). As the

salting time increased, moisture content of egg white decreased gradually ($p < 0.05$). The moisture content of egg white from immersing method was slightly lower than that of paste coating method, most likely due to the migration of water from egg white to the saturated brine mediated by osmosis process. At the first week of salting, the yolk became harden, especially at the surface, named "exterior egg yolk"**,** while viscous liquid yolk was found inside, termed "interior egg yolk". The solidification of egg yolk proceeded toward the center of egg yolk. The greater dehydration was observed in exterior egg yolk, compared with interior counterpart as evidenced by the lower moisture content in the former. No differences in moisture content of exterior yolk were obtained between paste coating and immersing methods ($p > 0.05$). Nevertheless, interior yolk from immersing method showed the lower moisture content than that of coating method after 4 weeks of salting ($p < 0.05$).

Salt contents of egg white from both salting methods increased with increasing salting time ($p < 0.05$) (Figure 13B). After 2 weeks of salting, the higher salt content was observed in egg white obtained from immersing method ($p < 0.05$). At week 7, egg white from immersing method and paste coating method had the salt content of 9.98 and 6.90%, respectively. A slight increase in salt content was noticeable in yolks from both methods; however no differences in salt content were obtained between interior and exterior portions of yolk ($p > 0.05$) (Figure 13B). The increase in salt content in egg white was faster and greater than that found in egg yolk. This result indicated that the dehydration of egg yolk mediated by osmotic process was accompanied with an increase in salt content in egg white. The reduction of moisture content of egg yolk during salting was mostly associated with its hardening. High salt content in egg white might induce the water migration from egg yolk. During salting, water could be migrated from egg yolk to egg white, and subsequently to the environment through the egg shell (Chi and Tseng, 1998). High diffusion of salt into egg white obtained from immersing method was mainly due to the higher salt concentration in brine (25%) when compared with the coating paste (18.35% salt). The higher difference in salt concentration, the higher the osmotic pressure was obtained. As a result, the greater migration of salt into the egg took place.

Oil exudation of egg yolk from paste coating and immersing methods during salting is shown in Figure. 14. Oil exudation of salted yolk from both methods increased with increasing salting time ($p< 0.05$). In general, exterior yolk showed the higher oil exudation than interior counterpart. This phenomenon was obvious in yolk obtained from egg salted by paste coating. However, no differences in oil exudation were noticeable between interior and exterior portions of yolk obtained from immersing method, particularly after 4 weeks of salting ($p> 0.05$). The dehydration during salting and the presence of salt in yolk probably enhanced oil exudation. Schultz *et al*. (1968) pointed out that the removal of water from egg yolk increased the extracted lipid. Free lipid might be released from low density lipoprotein micelles, due to the structural changes of low density lipoprotein induced by dehydration and increased salt content. Lower rate of salting might induce oil exudation more effectively. This gradual denaturation of yolk proteins as affected by salt might result in the greater loss in their emulsifying properties upon salting. As a consequence, the higher free fat was released.

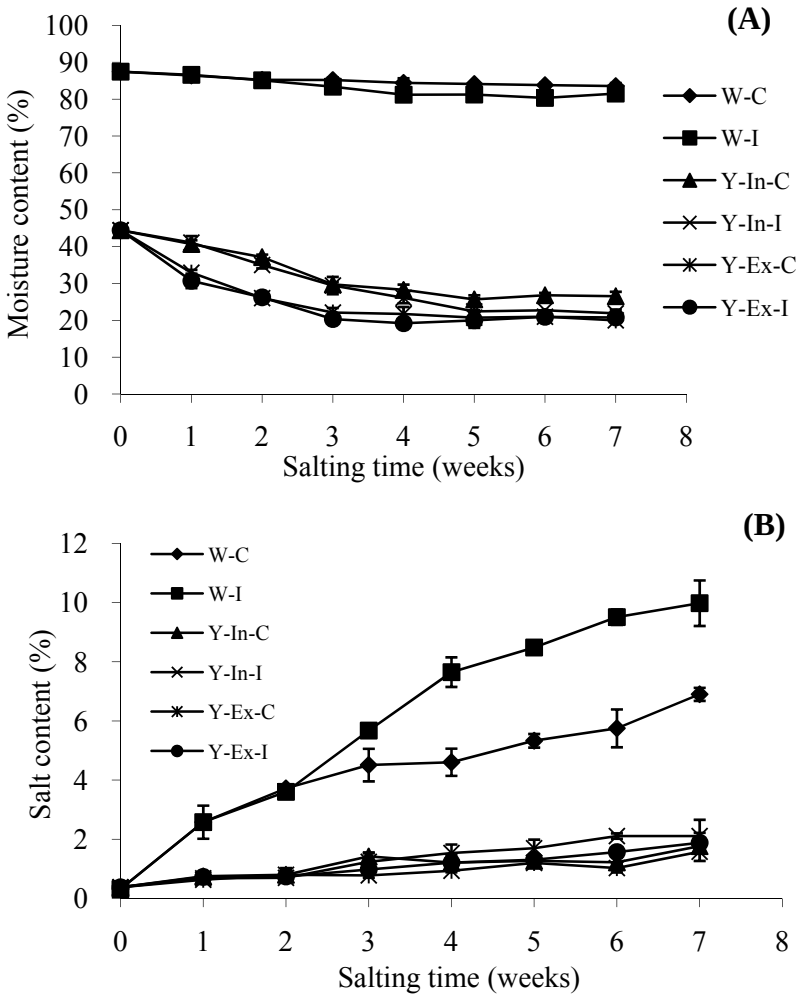



**Figure 13.** Moisture (A) and salt (B) contents of egg white and yolk from duck egg salted with different methods during 7 weeks of salting: W-C; egg white-Paste coating, W-I; egg white-Immersing, Y-In-C; Interior yolk-Paste coating, Y-In-I; Interior yolk-Immersing, Y-Ex-C; Exterior yolk-Paste coating, Y-Ex-I; Exterior yolk-Immersing**.** Bars represent standard deviation from triplicate determinations.

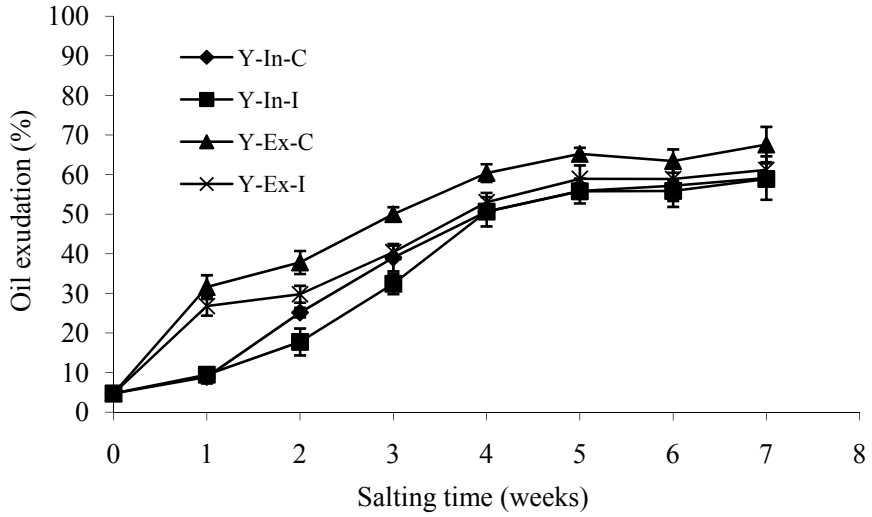



**Figure 14.** Oil exudation of yolk from duck egg salted with different methods during 7 weeks of salting: Y-In-C; Interior yolk-Paste coating, Y-In-I; Interior yolk-Immersing, Y-Ex-C; Exterior yolk-Paste coating, Y-Ex-I; Exterior yolk-Immersing**.** Bars represent standard deviation from triplicate determinations.

Oxidation of lipid extracted from salted egg yolk obtained from paste coating and immersing methods as monitored by TBARS and CDA value is depicted in Figure 15. TBARS value determines lipid oxidation products including malonaldehyde, which may be formed from polyunsaturated fatty acids with at least three double bonds (Gordon, 2001). During week 3 and 5, higher TBARS value was found in yolk obtained from coating method in comparison with immersing method (Figure 15A). This was coincidental with the more release of free lipid in that period of time. After week 5, yolk obtained from immersing method had the higher TBARS value than yolk from paste coating method ($p< 0.05$). Generally, higher CDA value of the lipid extracted from salted yolk was observed for immersing method after 2 weeks of salting (Figure 15B). The highest CDA value was observed at week 5 of salting. Thereafter, the decrease in CDA content was noticeable up to 7 weeks of salting. CDA has been used as indicator for the formation of hydroperoxides from polyunsaturated fatty acid (PUFA) (Gordon, 2001). In general, the decrease in CDA

content was coincidental with the increase TBARS values, especially after 2 weeks of salting. This indicated the decomposition of intermediate products as expressed as CDA content to the final oxidation products as monitored by TBARS values. Yang and Chen (2001) reported that the formation of cholesterol oxidation products in salted egg yolk depended upon the processing condition such as cooking, exposure to heat and oxygen, storage time and γ-irradiation.

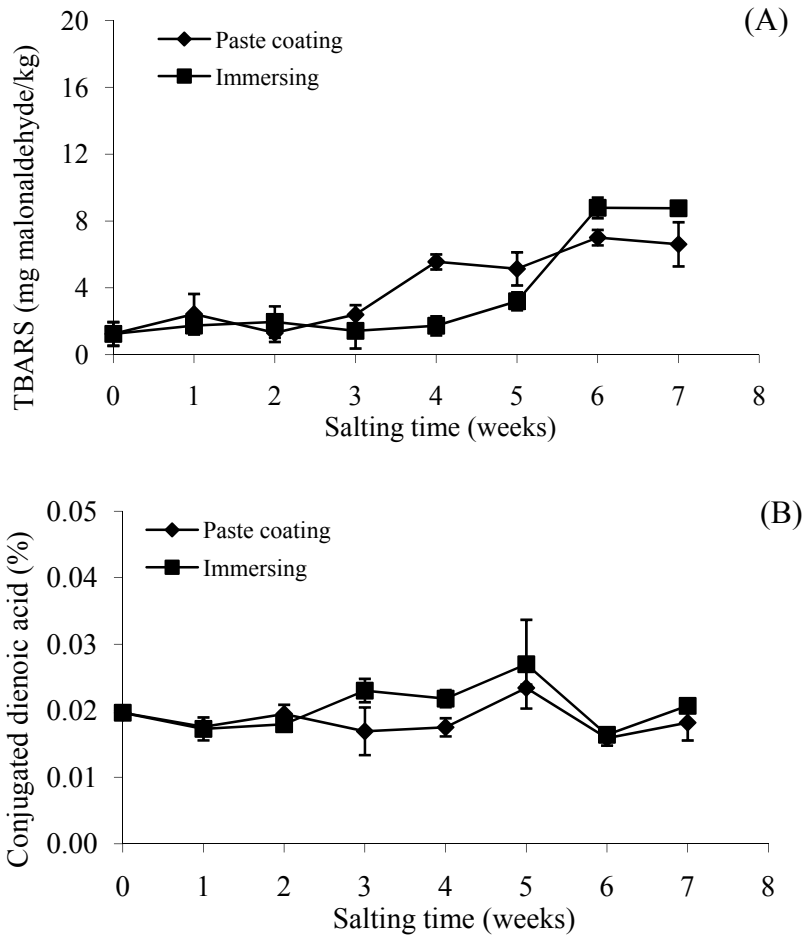



**Figure 15.** TBARS value (A) and conjugated dienoic acid content (B) of yolk from duck egg salted with different methods during 7 weeks of salting**.** Bars represent standard deviation from triplicate determinations.

### 3.4.2 Changes in textural properties of duck egg during salting

Hardening ratio of yolk was defined as the weight percent of hard exterior yolk and used as an index for the completeness of salting. Similar hardening ratio of yolk was observed between both salting processes throughout 7 weeks of salting ($p > 0.05$) (Figure 16). A remarkable increase in hardening ratio was observed within the first 2 weeks of salting. Thereafter, hardening ratio increased gradually up to 4 weeks. During 5-7 weeks, no changes in hardening ratio were found ($p > 0.05$). The solidification of egg yolk initiated near the vitelline membrane and proceeded toward the center. The interior yolk was further dehydrated and became harden when the salting proceeded. When yolk protein became more concentrated, the interaction between protein molecules including lipoproteins could be enhanced. Protein denaturation induced by the presence of salt and water loss that cause both effects, disruption of lipoprotein complexes that leads to the increased in oil exudation and formation of a hydrophobically driver gel-like network. It has been reported that the addition of salt to egg suspensions affects hydrogen bonds and inhibits interaction between water molecules and the hydrophilic groups in the protein backbone, promoting protein aggregation with may result in network formation, as a result of increased hydrophobicity (Yang and Baldwin, 1995). This resulted in the formation of gel-like network. The presence of native yolk lipids or emulsified oil droplet appears to influence the yolk gel rheological properties (Kiosseoglou, 2003). Yolk protein concentrates having relatively high lipid content underwent the formation of gel network at low protein concentrations, suggesting that the yolk lipid molecules are somehow involved in gel formation (Kiosseoglou, 2003).

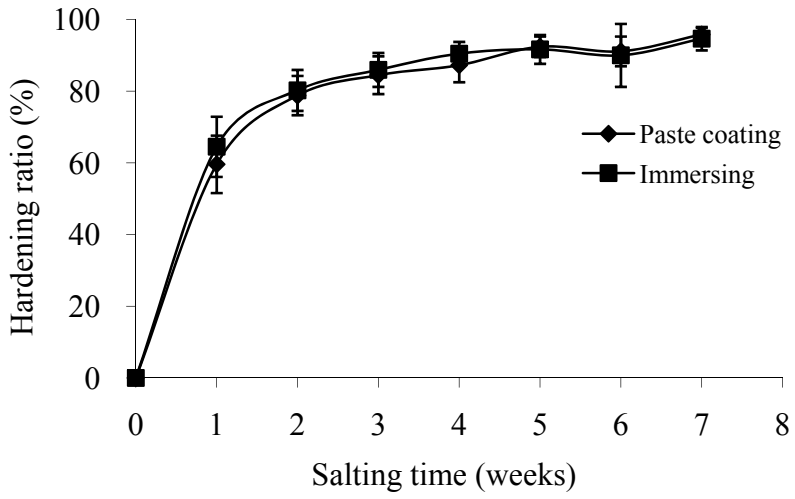



**Figure 16.** Hardening ratio of yolk from duck egg salted with different methods during 7 weeks of salting**.** Bars represent standard deviation from ten determinations

Texture profile analysis revealed that hardness and adhesiveness increased continuously and reached the maximum at week 5 ($p< 0.05$) (Figure 17A and 17C). At weeks 5, yolk obtained from paste coating method exhibited the adhesiveness than that from immersing method ($p< 0.05$). Free lipid released from the lipoprotein could make the yolk more adhesive. Additionally, increased in adhesiveness might be also related to gel-like network enhancement as the yolk is progressively hardening. The hardness value is the peak force of the first compression of salted egg yolk. The result suggested that the structure of salted egg yolk obtained from coating method had the similar resistant to compression in comparison with that from immersing method. Higher oil exudation of yolk from coating method might be associated with the greater interaction of proteins, resulting in the increased rigidity. Greater hardness of salted egg yolk from both salting methods was in accordance with the increases in hardening ratio. After 5 weeks of salting, the non-significant decrease in hardness of salted eggs from paste coating method was found. After 3 weeks of salting, higher adhesiveness was obtained in yolk from paste coating method, however no differences were observed during 6-7 weeks ($p> 0.05$) (Figure 17C). For

fracturability (originally called brittleness) was defined as the force of the significant break in the first bite, it increased from week 2 to week 5 of salting. Subsequently, the sharp decrease was noticeable and the samples almost completely lost in fracturability during 6-7 weeks of salting. The marked decrease in fracturability was coincidental with the increase in TBARS value (Figure 15). Oxidation product, such as aldehydes might act as proteins cross-linking resulting in more rigid structure. During week 4 and 5, yolk from immersing method had the greater fracturability than that from paste coating method ($p< 0.05$). Increased fractureability during week 2-5 probably related with dehydration of egg yolk, in which the lower moisture content of yolk was observed for immersing method. Salted egg yolk was more dehydrated and could form the gritty texture. Salted egg yolk from both methods could form the gritty texture after 2 weeks of salting. Grittiness is the factor affecting consumer acceptance of salted egg product (Chi and Tseng, 1998).

For cohesiveness, slightly lower values were found after 2 weeks of salting and no further changes were noticeable during salting up to 6 weeks. Nevertheless, both salting processes rendered the yolk with similar cohesiveness. Yolks obtained from both salting processes had the increases in gumminess and chewiness as the salting time increase ($p< 0.05$). After 5 weeks of salting, yolk from immersing method showed the higher gumminess and chewiness than that from paste coating method ($p< 0.05$). Greater dehydration of yolk obtained from immersing method more likely contributed to the higher gumminess and chewiness, in which the protein or lipoproteins became more concentrated or interacted each other to a greater extent. The commercial salted duck eggs in Thailand are generally produced by coating method. Egg salted for 2 weeks are recommended for pan frying, while those salted for 3-4 weeks are generally suitable for boiling. The present study indicated that no differences in textural properties of salted egg yolk from both salting methods at week 2 of salting. From the standpoint of costumers, gel-like texture and granular texture are desirable for fried and boiled salted eggs, respectively. The granular texture and oil exudation of yolk were obtained after brining for 4 weeks (Lai *et al*. 1999)

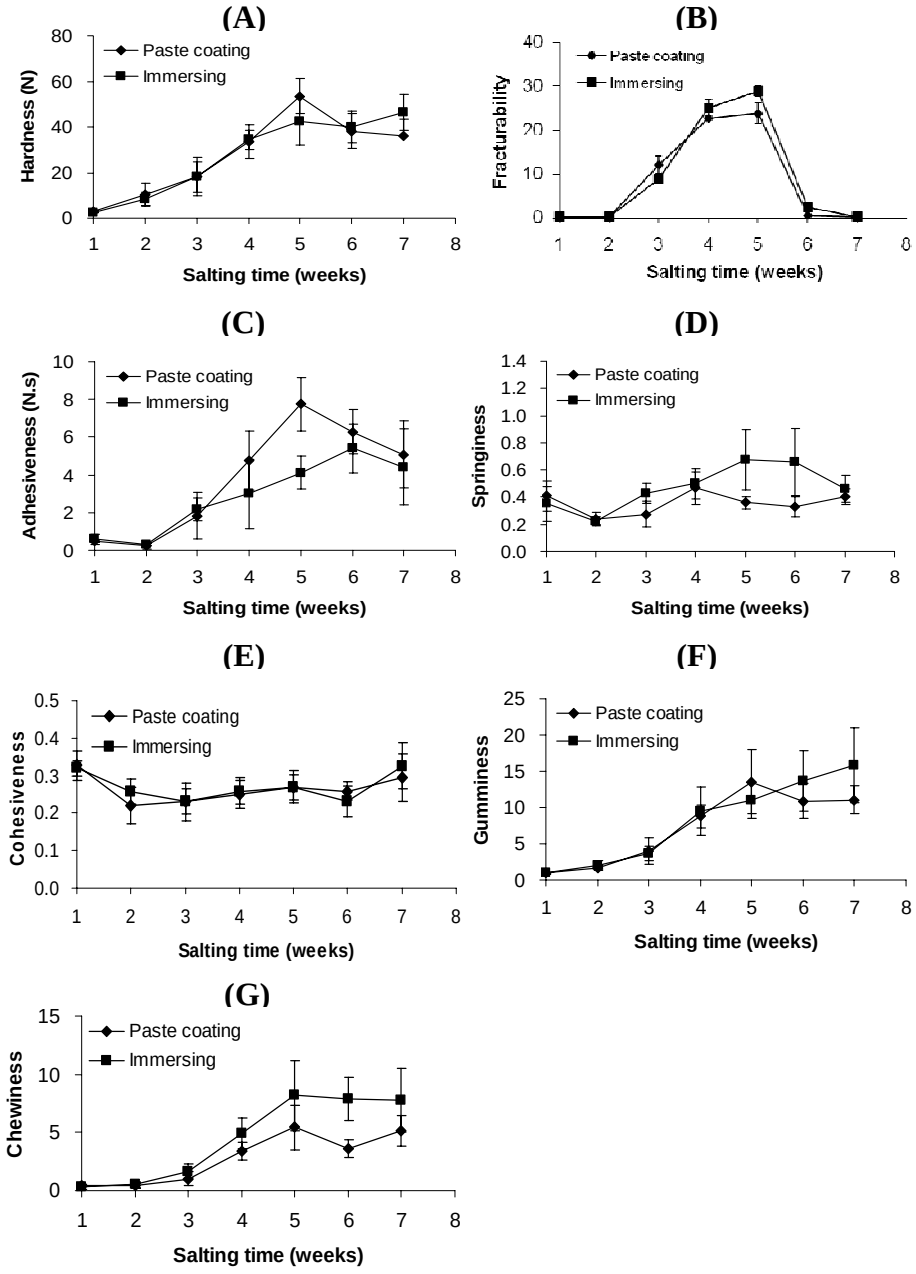



**Figure 17.** Texture profile analysis of yolk from duck egg salted with different methods during 7 weeks of salting**.** Bars represent standard deviation from ten determinations.

### 3.4.3 Changes in transition temperature of duck egg protein during salting

Transition temperature and enthalpy of egg white and egg yolk proteins before and after salting for different times as monitored by DSC are shown in Table 15. Fresh egg white proteins exhibited a major endothermic peak with $T_{max}$ values of 80.08 ºC, most likely due to the thermal denaturation of ovalbumin. However, a small peak with $T_{max}$ of 72.85ºC was noticeable. This peak might represent the interaction between as conalbumin and lysozyme (Donovan *et al.*, 1975). In generally, temperature of denaturisation of conalbumin and lysozyme were 61 and 75ºC respectively (Linder and Lorient, 2000). Moreover, the thermal stability of these proteins typically depends on pH and ionic strength to a high extent (Ichikava and Shimomura, 2007). Nevertheless, only one peak was found in egg white of egg salted for 1, 3 and 7 weeks. Thermal transitions of egg proteins are governed by the behavior of ovalbumin, which is the major protein of egg albumin. $T_{max}$ of ovalbumin increased when salting time increased and the highest $T_{max}$ was observed for both salting processes at 7 weeks of salting ($p< 0.05$). However, it was noted that the decreased $T_{max}$ was observed in egg white from egg salted with paste coating process at week 3. The increase in $T_{max}$ of egg white protein might be due to the increase in salt concentration in egg white. High salt concentrations have been shown to stabilize some proteins (Damodaran, 1996). The shift in $T_{max}$ of ovalbumin at high salt concentrations varied with the forms of ovalbumin, which are affected by the extents of phosphorylation (McKenzie and Frier, 2003). Salt-stabilized ovalbumin (higher $T_{max}$) most likely belonged to S-ovalbumin, which was thermally stable. For enthalpy, the increase in enthalpy was found in egg white from both salting processes after 1 week of salting. Nevertheless, the decrease in enthalpy was noticeable at week 3. Thereafter, the slight increase in enthalpy was observed at week 7. The changes in enthalpy indicated the alteration of protein conformation as affected by salting processes. Generally salting processes had no effect on enthalpy of egg white during salting, except for the salting time of 7 weeks, when the higher enthalpy was found in egg salted by coating process ($p< 0.05$).

Endothermic peak was observed for egg yolk with $T_{max}$ values of 79.64 ºC for fresh egg yolk and 79-85ºC for salted egg yolk. Granule protein (HDL and phosphitin) in egg yolk was more resistant to heat denaturation than plasma protein, in which heat sensitive LDL protein constituted as major component (Anton *et al*., 2001) $T_{max}$ of salted egg yolk increased when the salting time increased ($p< 0.05$). Higher $T_{max}$ of salted yolk tended to be found in immersing method especially, when the salting time increased. This might be associated with the higher salt content, which could have impact on the stability of yolk protein toward heat. According to Nishinari *et al*. (2000) salt tends to stabilize protein molecules as indicated by the shift of peak with increasing ionic strength. The addition of salt to egg yolk resulted in an increase in the denaturation temperature as well as a delay in gel network formation (Guerrero *et al*., 2004) For enthalpy, it varied with salting process and time. Interior yolk from coating process tended to have the decrease in enthalpy with increasing salting time, while the varying enthalpy was noticeable for immersing method. The similar trend was found in exterior yolk.

**Table 15.** $T_{max}$ and enthalpy of proteins from duck egg salted with different methods during 7 weeks of salting.

| Egg portion | Salting time (week) | $T_{max}$ (ºC) | | H (J/g) | |
|---|---|---|---|---|---|
| | | Paste coating | Immersing | Paste coating | Immersing |
| Interior yolk | 0 | $79.64\pm0.21^{*,A\dagger}$ | $79.64\pm0.21^{A}$ | $1.25\pm0.09^{D}$ | $1.25\pm0.09^{A}$ |
| | 1 | $79.63\pm0.18^{Aa\dagger\dagger}$ | $80.50\pm0.00^{Bb}$ | $1.55\pm0.77^{Ca}$ | $1.53\pm0.10^{Ba}$ |
| | 3 | $80.96\pm0.41^{Ba}$ | $81.13\pm0.13^{Ca}$ | $0.97\pm0.07^{Ba}$ | $1.05\pm0.13^{Aa}$ |
| | 7 | $80.62\pm0.06^{Ba}$ | $85.58\pm0.00^{Db}$ | $0.68\pm0.09^{Aa}$ | $1.81\pm0.06^{Cb}$ |
| Exterior yolk | 0 | $79.64\pm0.21^{A}$ | $79.64\pm0.21^{A}$ | $1.25\pm0.09^{B}$ | $1.25\pm0.09^{A}$ |
| | 1 | $79.83\pm0.35^{Aa}$ | $81.17\pm0.00^{Bb}$ | $0.76\pm0.05^{Aa}$ | $1.28\pm0.21^{Ab}$ |
| | 3 | $79.63\pm0.24^{Aa}$ | $80.88\pm0.88^{Ba}$ | $1.31\pm0.24^{Bb}$ | $0.80\pm0.21^{Aa}$ |
| | 7 | $83.96\pm1.12^{Ba}$ | $84.54\pm0.18^{Ca}$ | $0.83\pm0.01^{Aa}$ | $1.05\pm0.52^{Aa}$ |
| Egg white | 0 | $80.08\pm0.30^{B}$ | $80.08\pm0.30^{A}$ | $0.40\pm0.13^{A}$ | $0.40\pm0.13^{A}$ |
| | 1 | $81.00\pm0.00^{Ca}$ | $80.58\pm1.53^{Aa}$ | $1.82\pm0.05^{Ca}$ | $1.75\pm0.21^{Ba}$ |
| | 3 | $78.54\pm0.29^{Aa}$ | $82.79\pm0.18^{Bb}$ | $0.28\pm0.14^{Aa}$ | $0.19\pm0.18^{Aa}$ |
| | 7 | $84.25\pm0.94^{Da}$ | $84.46\pm0.49^{Ba}$ | $0.94\pm0.05^{Bb}$ | $0.41\pm0.05^{Aa}$ |

* Mean±SD from duplicate determinations.

† Different capital superscripts (A-D) within the same portion of egg in the same column indicate the significant differences ($p<0.05$)

†† Different superscripts (a-b) within the same parameter in the same row indicate the significant differences ($p<0.05$)

### 3.4.4 Changes in microstructure of duck egg yolk during salting

Scanning electron microscopic study indicated that salted egg yolk form both salting methods had polyhedral granules with the size range of 50-100 μm (Figure 18). Yang and Hsu (1989) reported polyhedral granules with the diameter ranging from 23 to 127 μm in salted duck egg yolk. Chi and Tsung (1998) found the granules with the size range of 90-100 μm in salted egg yolk. These polyhedral

granules were formed by yolk spheres with similar sizes (Woodward and Cotterill, 1987). The microstructure of salted egg yolk visualized by SEM indicated that polyhedral granules were aligned closely when the salting proceeded, mainly due to dehydration during salting, regardless of salting processes. The more reduction of moisture content, the more tightly the granules were packed. The result suggested that both salting methods could induce the formation of gritty texture. In general, granular is obtained after brining for 4 weeks (Lai *et al*., 1999) and this made the salted egg suitable for boiling.

Confocal laser scanning microscope (CLSM) micrographs using a two channel technique, in which both protein and lipid were stained. Figure 7 shows the images of labeled yolk protein (Figure 19A) and labeled yolk lipid (Figure 19B) in two separated channels together with a combined image (Figure 19C). The protein and lipid distributed uniformly in fresh egg yolk. As the salting proceeded, the shapes of lipid and proteins turned to be irregular with discontinuous distribution in dye solution. It might be due to the increased dehydration together with the release of lipid in egg yolk. The majority of proteins in yolk are organized into micellar and granular structures together with polar and non-polar lipid molecules (Kiosseoglou, 2003). Salting resulted in the dehydration together with the release of lipids in egg yolk, leading to the loss in emulsion capacity of protein portion. Most lipids in the egg yolk exist in low density lipoproteins (LDL) (Gilbert, 1971). Irregular shapes of both lipid and protein were found in egg yolk from both immersing and paste coating methods. Increased hardening of salted yolk also made the yolk more difficult to be dispersed in the dye solution. From the result, the greater release of free lipid from lipoprotein structure of egg yolk were obtained with egg salted by coating method when compared with immersing method as shown in the combined images (red portion) (Figure 19C). CLSM micrographs of yolk from egg salted for 7 weeks had the similar protein distribution to fresh yolk but showed the greater free lipid released (red portion).

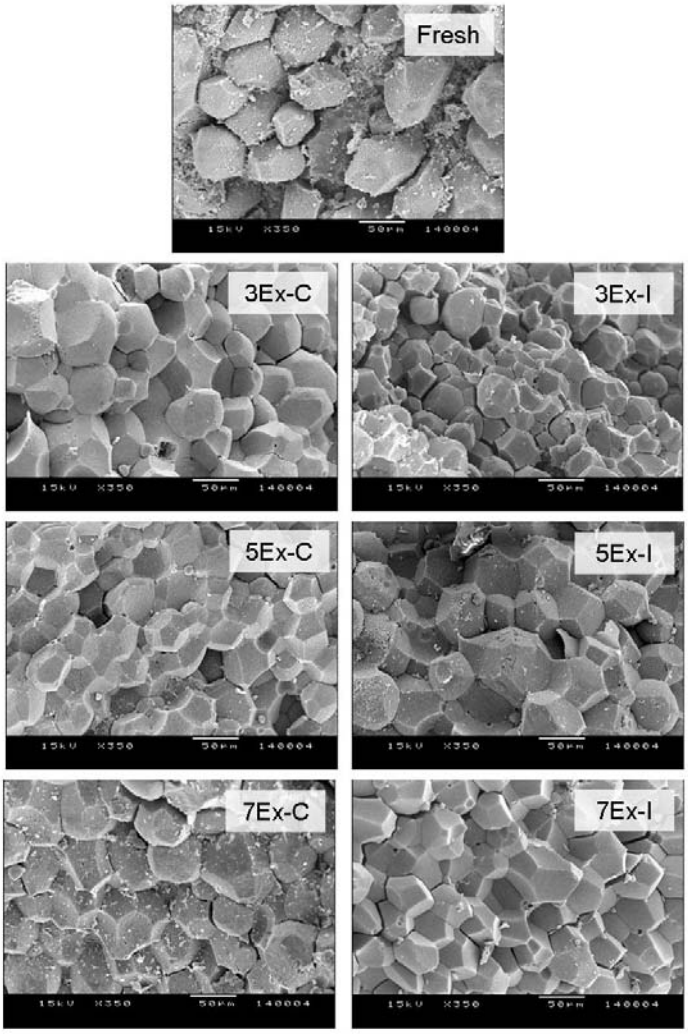



**Figure 18.** Scanning electron microscopic photograph of yolk granule from fresh duck egg and egg salted with different methods for different times. Fresh duck egg (Fresh), 3Ex-C; Exterior yolk-Paste coating (week 3), 3Ex-I; Exterior yolk-Immersing (week 3), 5Ex-C; Exterior yolk-Paste coating (week 5), 5Ex-I; Exterior yolk-Immersing (week 5), 7Ex-C; Exterior yolk-Paste coating (week 7), 7Ex-I; Exterior yolk-Immersing (week 7) Magnification: 350X

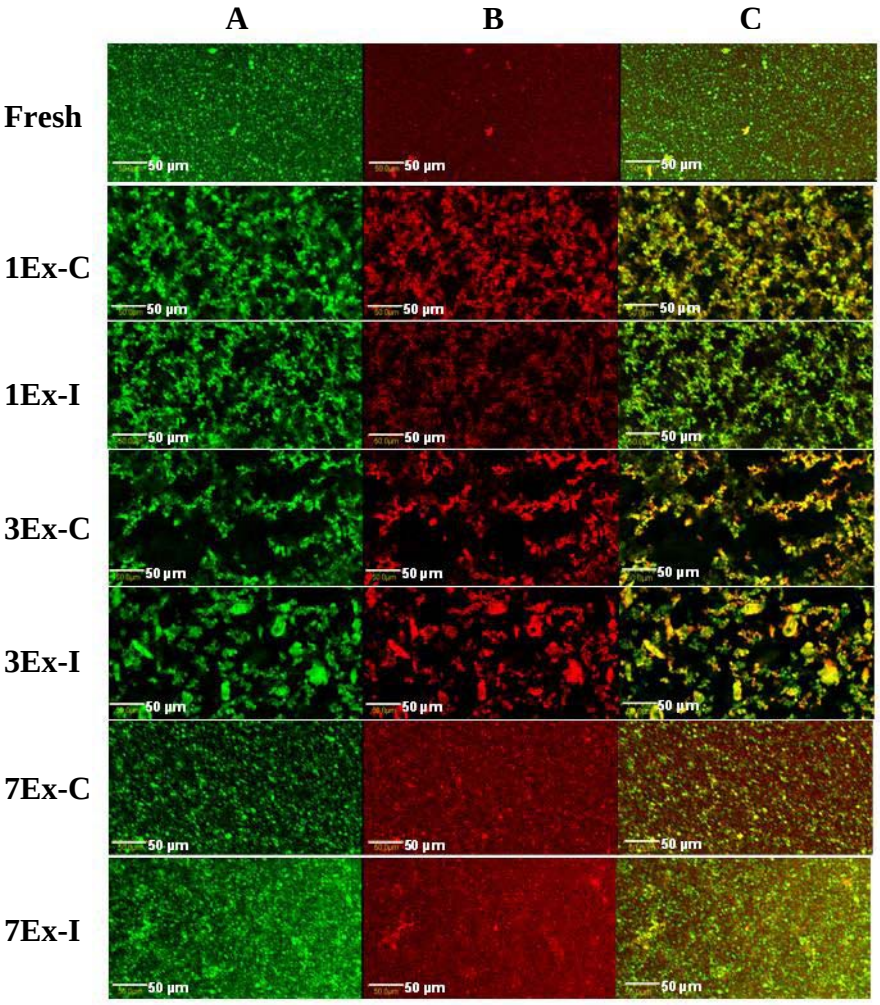



**Figure 19.** Confocal laser scanning microscope (CLSM) micrographs of yolk from fresh duck egg and egg salted with different methods for different times. 1Ex-C; Exterior yolk-Paste coating (week 1) 1Ex-I; Exterior yolk-Immersing (week 1) 3Ex-C; Exterior yolk-Paste coating (week 3) 3Ex-I; Exterior yolk-Immersing (week 3) 7Ex-C; Exterior yolk-Paste coating (week 7) 7Ex-I; Exterior yolk-Immersing (week 7) Magnification: 200X (zoom X2.5) protein distribution (A) and lipid distribution (B) and combined image of protein and lipid (C). Scale bar = 50 µm

## 3.5 Conclusions

Eggs white and yolk of duck egg salted with two salting methods, paste coating and brine immersing methods, had the slight differences in chemical composition and textural properties as salting proceeded. Both salting methods could induce solidification of yolk accompanied with oil exudation and the development of gritty texture. However, yolk obtained with different salting methods had different oil exudation as well as lipid oxidation. Salting methods also had the impact on textural properties of salted egg. Thus, salting methods somehow affected the characteristic of resulting egg white and egg yolk.

# CHAPTER 4

# EFFECT OF ACETIC ACID AND COMMERCIAL PROTEASE PRETREATMENT ON SALTING AND CHARACTERISTICS OF SALTED DUCK EGG

## 4.1 Abstract

Salting of duck egg pretreated with 5% acetic acid and different commercial proteases (Flavourzyme, Protamex, Alcalase and Neutrase) was studied. Duck eggs soaked in 5% acetic acid for 30 min, followed by socking in 5% (w/v) Flavourzyme and Neutrase had the highest hardening ratio with the coincidental increase in salt content in egg white and decrease in moisture content of yolk, compared with those with other treatments ($p< 0.05$). When eggs were pretreated with Neutrase at different concentrations, those pretreated with 0.25% (w/v) Neutrase for 90 min had the shorter salting time and enhanced oil exudation of yolk upon salting. No changes in viscosity were observed in egg white, regardless of Neutrase concentration and salting time. Microstructure study revealed that shell of salted egg pretreated with acetic acid had rough and porous surfaces when compared with control, whereas no changes in microstructure and FTIR spectra of shell membrane were found.

Salted eggs have been produced by taking 3-4 weeks of salting. To shorten the salting process, pretreatment of egg using acetic acid and Neutrase is recommended to increase the porosity of shell, allowing salting to penetrate into egg white and yolk. This developed process is applicable for the commercial scale.

## 4.2 Introduction

The salted duck egg is one of the most popular egg products in Thailand. It can be made by brining eggs in saturated saline or by coating the egg with a soil paste mixed with salt for 1-4 weeks (Peh *et al*. 1982; Lin *et al*. 1984; Chang and Lin, 1986). In addition to being eaten as the whole egg, salted egg yolk is widely used as fillings in foods such as moon cakes and glutinous rice dumplings (Chiang and Chung, 1986; Chi and Tseng, 1998). To preserve the functions of the albumen, several research groups separated the yolks from the albumen before brining, but these brining processes of egg yolk were not successful in which salted egg yolk became watery and did not attain desirable characteristic (Chang, 1995; Chen *et al*., 1991; Chiang and Chung, 1986; Tseng, 1994; Wang, 1991).

Generally, the dehydration and salt content are the major factors affecting the hardness of salted yolk (Kaewmanee *et al*., 2009). Granulation in salted egg yolk is related to salt and its interaction with low density lipovitellenin (Wang, 1991). Wang (1991; 1992) indicated that the formation of salted yolk might be related to the diffusion speed and final concentration of NaCl. From the standpoint of customers, cooked salted egg yolks with a granular texture are generally considered to be desirable (Chiang and Chung, 1986; Peh *et al*., 1982; Wang, 1992). To induce salt penetration into egg, duck eggs were treated with 0.1 N HCl and an increased penetration rate of 2-10 folds was obtained. As a result, the formation of gelatinous yolk was accelerated (Lai *et al*., 1997). However, inorganic acid might not be acceptable for consumer and organic acid may be a promising alternative with the safety concern. Moreover, the cleavage of egg proteinaceous membrane with the aid of protease might enhance the salt penetration into egg. Therefore, this study aimed to investigate the effect of pretreatment using acetic acid and commercial proteases on salting process and the characteristic of resulting salted egg.

## 4.3 Materials and Methods

### 4.3.1 Chemicals

Petroleum ether, chloroform, methanol, diethyl ether and nitric acid were purchased from Lab-Scan (Bangkok, Thailand). Silver nitrate was obtained from Merck (Darmstadt, Germany). Potassium thiocyanate was purchased from Fluka (Buchs, Switzerland). Nile blue A was obtained from Sigma (St. Louis, MO, USA).

### 4.3.2 Duck egg collection

Duck eggs *(Anas platyrhucus)* with the weight range of 65-75 g were obtained within 1 day of laying from Satingphar, Songkhla Province, Thailand. The eggs were used within 3 days after laying**.**

### 4.3.3 Commercial proteases

Alcalase 2.4 L (EC number: 3.4.21.62), Protamex (EC numbers: 3.4.21.62). Flavourzyme 1000 L (EC number: 3.4.11.1) and Neutrase 0.8 L (EC 3.4.24.28) were obtained from Novozymes (Novozymes, Bagsvaerd, Denmark).

### 4.3.4 Effect of pretreatment using different commercial proteases on salting and chemical composition of salted duck egg

Shell eggs were soaked in 5% acetic acid for 30 min at room temperature (28-30°C) and washed with running water to remove acid. The eggs were then air-dried. To study the effect of protease treatment on salting, the prepared eggs were immersed in distilled water containing different commercial proteases (Flavourzyme, Protamex, Alcalase and Neutrase) at the level of 0.5% (w/v) using 1 egg/100 mL protease solution for 60 min at room temperature (28-30°C). After draining, eggs were washed with water. The eggs were subsequently brined in 25%

NaCl solution. Eggs without any treatment were used as the control. During pickling, eggs were taken at week 1 and 2 for analyses including salt content of egg white, hardening ratio and moisture content of egg yolk. The enzyme rendering the high hardening ratio with the shorter salting time was selected for further study.

### 4.3.5 Effect of different Neutrase concentrations and soaking times on salting and chemical composition of salted duck egg

Shell eggs were soaked in 5% acetic acid for 30 min at room temperature and washed with running water. Prepared eggs were immersed in Neutrase solution at various concentrations (0.25, 0.5 and 0.75%, w/v) for different times (30, 60 and 90 min) (1 egg: 100 mL solution) at room temperature (25-28°C)**.** After draining, the eggs were washed and immersed in 25% NaCl. Eggs without any treatment were used as the control. Eggs were taken for analyses at week 1 and 2 as described above.

### 4.3.6 Changes in chemical composition and some characteristics of pretreated duck egg during salting.

Shell eggs were soaked in 5% acetic acid for 30 min at room temperature, followed by soaking in 0.25% Neutrase solution for 90 min. The samples were taken for analyses every week for up to 3 weeks. Hardening ratio, moisture and salt content of yolk were determined and salt content and viscosity of egg white were measured. FTIR spectra and microstructure of shell and shell membrane were also analyzed. Salted egg without soaking in acetic acid and Neutrase were also prepared and used as the control.

### 4.3.7 Measurement of hardening ratio of egg yolk

Hardening ratio of the salted yolk was determined following the method of Chi and Tseng (1998). The egg yolk was rolled on a filter paper (Whatman No. 1)

to remove egg white. The weight of egg yolk was measured ($W_o$). The egg yolk was cut with a knife and the removable interior yolk (soft or liquid) was scraped out using a tea spoon. The weight of exterior yolk ($W_{ex}$) was measured. The hardening ratio of the egg yolk was calculated as follows:

$$\text{Hardening ratio} = (W_{ex}/ W_o) \times 100$$

### 4.3.8 Determination of moisture and salt contents

Interior and exterior salted yolk was determined for moisture content according to AOAC (2000). Salt content in egg white was measured by the method of AOAC (2000). Samples (0.5 g) were added with 20 mL of 0.1 N $AgNO_3$ and 10 mL of $HNO_3$. The mixture was boiled gently on a hot plate until all solids except $AgCl_2$ were dissolved (usually 10 min). The mixture was cooled at room temperature. Five mL of 5% ferric alum indicator ($FeNH_4 (SO_4)_2 \cdot 12 H_2O$) were added. The mixture was titrated with the standardized 0.1 N KSCN until the solution became permanently light brown. The percentage of salt was then calculated as follows:

$$\text{Salt (\%)} = 5.8 \times [(V1 \times N1) - (V2 \times N2)]/W$$

where V 1 = volume of $AgNO_3$ (mL); N1 = concentration of $AgNO_3$ (N); V 2 = volume of KSCN (mL); N2 = concentration of KSCN (N) and W = weight of sample (g).

### 4.3.9 Viscosity of egg white

Salted egg white was separated from egg yolk manually. Viscosity of egg white (10 mL) was measured at room temperature using a Brookfield DV-II programmable viscometer (Brookfield, Middleblro, MA, USA) with a small sample adapter (SSA18/13RPY). The adapter consisted of a cylindrical sample holder and

SC4-18/13R spindle (No. 40). The rotational speed was controlled at 80 rpm. The viscosity was measured and expressed as centipoises (cP).

### 4.3.10 Preparation of egg shell membrane

Raw membrane-bound egg shell was collected and immediately stored in the iced water. The egg shell membrane was manually stripped from the shell. The stripped eggshell membrane was immersed in aqueous acetic acid (70%) for 2 days followed by rinsing with distilled water until the neutral pH of rinsed water was obtained. The shell membrane was finally dried in the oven (50°C) for 2 days (Tsai *et al*., 2006).

### 4.3.11 Fourier transform infrared spectroscopy (FTIR) analysis

Dried egg shell membranes were immersed in liquid nitrogen and further ground to obtain the fine powder. Dried powder was mixed with KBr (spectroscopic grade), followed by grinding and mixing in a mortar. Discs (12.7 mm ID and ≈1 mm thick) were prepared in a manual hydraulic press at about 10 tones for a pressing time of 30–60 s. A Bruker Model EQUINOX 55 FTIR spectrometer (Bruker, Ettlingen, Germany) equipped with a deuterated L-alanine triglycine sulfate (DLATGS) detector was used. The horizontal attenuated total reflectance (HATR) accessory was mounted into the sample compartment. The internal reflection crystal (Pike Technologies, Madison, WI, USA), made of zinc selenide, had a 45° angle of incidence to the IR beam. Spectra were acquired at a resolution of 4 $cm^{-1}$ and the measurement range was 4000–500 $cm^{-1}$ (mid-IR region) at room temperature. Automatic signals were collected in 32 scans at a resolution of 4 $cm^{-1}$ and were ratioed against a background spectrum recorded from the clean, empty cell at 25°C. Analysis of spectral data was carried out using the OPUS 3.0 data collection software programme (Bruker, Ettlingen, Germany).

### 4.3.12 Determination of microstructure of egg shell and shell membrane

Microstructure of shell and shell membrane was observed by scanning electron microscope (SEM). Membrane was manually removed after cleaning. Shell membranes were dried with a series of ethanol (50-100%). The samples were mounted on SEM stubs using a double backed cellophane tape. The stub and sample were coated with gold (Sputter coater SPI-module, West Chester, PA, USA) and examined using a scanning electron microscopy (JEOL JSM-5800LV, Tokyo, Japan).

### 4.3.13 Statistical Analysis

Experiments were run in triplicate with three different lots of eggs. Completely randomized design (CRD) was used throughout the study. Data were presented as mean values with standard deviations. One-way analysis of variance (ANOVA) was carried out and mean comparisons were done by Duncan's multiple range tests. For pair comparison, T-test was used. (Steel and Torrie, 1980). Statistical analyses were performed with the statistical program (SPSS for windows, SPSS Inc, Chicago, IL, USA).

## 4.4 Results and Discussion

### 4.4.1 Effect of pretreatment using different commercial proteases on chemical composition of duck egg during salting

Hardening ratio of yolk and salt content of egg white of duck egg without any pretreatment, egg pretreated with 0.5% acetic acid (Ac) and egg pretreated with acetic acid, followed by different proteases including Flavourzyme (Ac+F), Protamex (Ac+P), Alcalase (Ac+A) and Neutrase (Ac+N) after salting for 1 and 2 weeks are depicted in Figure 20. Hardening ratio of yolk is defined as the weight percents of hard exterior yolk and used as an index for the completeness of salting. Pretreatment with acetic acid did not have the impact on hardening ratio of

yolk and salt content of egg white, regardless of salting time ($p > 0.05$). On the other hand, pretreatment with acetic acid, followed by proteases resulted in the increases in hardening ratio of yolk and salt content of egg white, compared with eggs without any pretreatment and with only acetic acid pretreatment ($p < 0.05$).

The highest hardening ratio of yolk was obtained in salted egg, pretreated with acetic acid in combination with Flavourzyme and Neutrase ($p < 0.05$). At the first week of salting, the highest salt content of egg white was obtained with Ac+F and Ac+N samples, compared with other samples. Nevertheless, no differences in salt content in egg white were noticeable among all eggs pretreated with all proteases used ($p > 0.05$).

Changes in moisture content of yolk obtained from eggs with different pretreatments after salting for 1 and 2 weeks are shown in Figure 21. Decreases in moisture content of yolk were obtained from both interior and exterior counterparts with increasing salting time ($p<0.05$). The greater dehydration was observed in exterior egg yolk, compared with interior counterpart. At week 1 of salting, no difference in moisture content of interior yolk of egg with different treatments was noticeable ($p > 0.05$). Higher dehydration of interior yolk was observed in Ac+F and Ac+N samples after 2 weeks of salting, compared with other samples ($p < 0.05$). After 2 weeks of salting, the highest dehydration of exterior yolk was found in Ac+F, Ac+N and Ac+A samples ($p < 0.05$), compared with others. Generally, the dehydration caused the solidification of egg yolk initiated near the vitelline membrane, which proceeded toward the center. The interior yolk was dehydrated and became hardened when salting time increased. Dehydration of egg yolk induced by salt at higher levels in egg white resulted in hardening of egg yolk. Flavourzyme or Neutrase might cleave the protein in egg membrane or reduced viscosity of egg white to some degree. This led to the greater penetration of salt from egg white to the yolk, especially at the exterior portion. Kaewmanee *et al*. (2009) found that the increased salt content of egg white was associated with the dehydration of egg yolk mediated by osmotic process. Therefore, soaking eggs in 5% acetic acid, followed by immersing in Flavourzyme or Neutrase solutions was the appropriate process to induce the solidification of egg

yolk. As a result, salting time could be shortened. Neutrase was selected for further study due to the lower cost, in comparison with Flavourzyme.

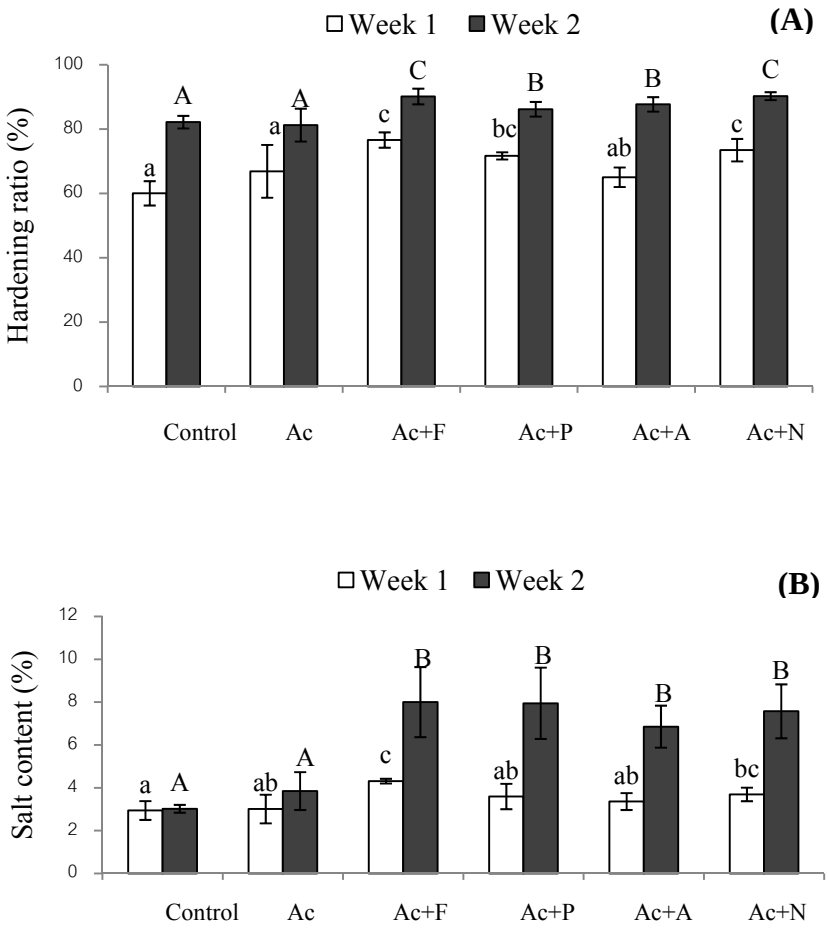



**Figure 20.** Hardening ratio of yolk (A) and salt content of salted egg white (B) of duck egg without pretreatment, egg pretreated with 5% acetic acid, and egg pretreated with 5% acetic acid, followed by immersing in different proteases after salting for 1 and 2 weeks. For salting, eggs were soaked in 25% NaCl at room temperature. Bars represent the standard deviation (n=3). Different letters or capital letters within the same salting time on the bar indicate the significant differences ($p<0.05$)

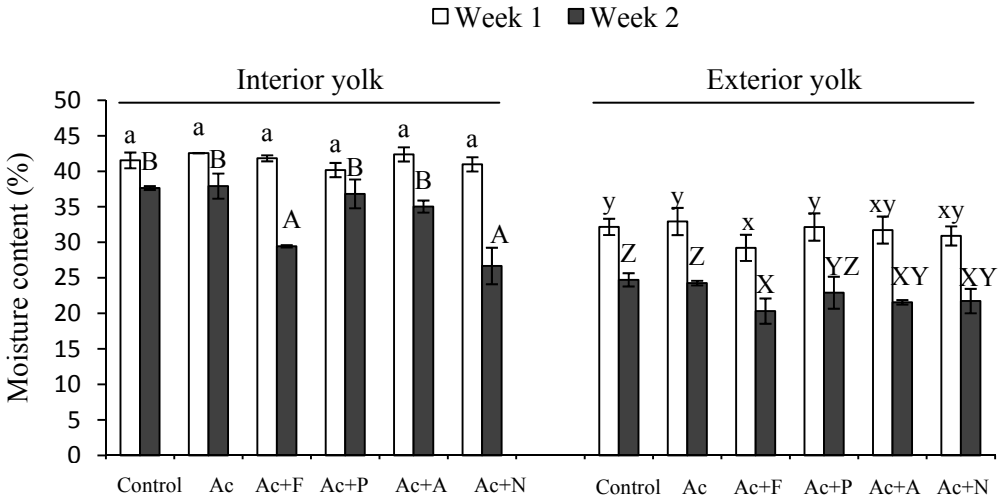



**Figure 21.** Moisture content of interior and exterior yolk of duck egg without pretreatment, egg pretreated with 5% acetic acid, and egg pretreated with 5% acetic acid, followed by immersing in different proteases after salting for 1 and 2 weeks. For salting, eggs were soaked in 25% NaCl at room temperature. Bars represent the standard deviation (n=3). Different letters or capital letters within the same salting time on the bar indicate the significant differences ($p<0.05$)

### 4.4.2 Effect of Neutrase concentrations and soaking times on chemical composition of duck egg during salting

Hardening ratio of yolk and salt content of egg white of duck egg pretreated with 5% acetic acid followed by soaking in Neutrase (Ac+N) at different concentrations and soaking time during salting are depicted in Figure 22A and 22B, respectively. At the first week of salting, the higher hardening ratio was obtained when Neutrase at levels of 0.5 and 0.75% were used compared with the control and that treated with only acetic acid ($p< 0.05$). Furthermore, pretreatment of prepared egg with 0.25% Neutrase for 90 min also resulted in the increased hardening ratio ($p< 0.05$). The higher hardening ratios were found after 2 weeks of salting, regardless of

treatment conditions ($p < 0.05$). However, after 2 weeks of salting, both Neutrase concentration and soaking times had no effect on hardening ratio ($p > 0.05$).

Similar impact of Neutrase pretreatment as affected by concentration and soaking time on salt content of egg white was noticeable. In general, the further increases in salt content were found as salting time increased up to 2 weeks ($p < 0.05$). Soaking prepared egg in Neutrase solutions for the longer time might induce salt penetration into egg white. After 2 weeks of salting, Neutrase concentration and time showed no impact on salt content in egg white.

Changes in moisture content of egg yolk, both interior and exterior portions, are shown in Figure 23. At week 1 of salting, no difference in moisture content was found in interior yolk among all samples ($p > 0.05$), except the lowest moisture content was noticeable in prepared egg soaked in 0.5% Neutrase for 30 min ($p < 0.05$). For the exterior portion, the lower moisture content was observed in egg pretreated with acetic acid, regardless of Neutrase concentration and soaking time ($p < 0.05$). After 2 weeks of salting, the decreased moisture content of yolk was found in egg pretreated with 0.75% Neutrase for 30-90 min ($p < 0.05$).

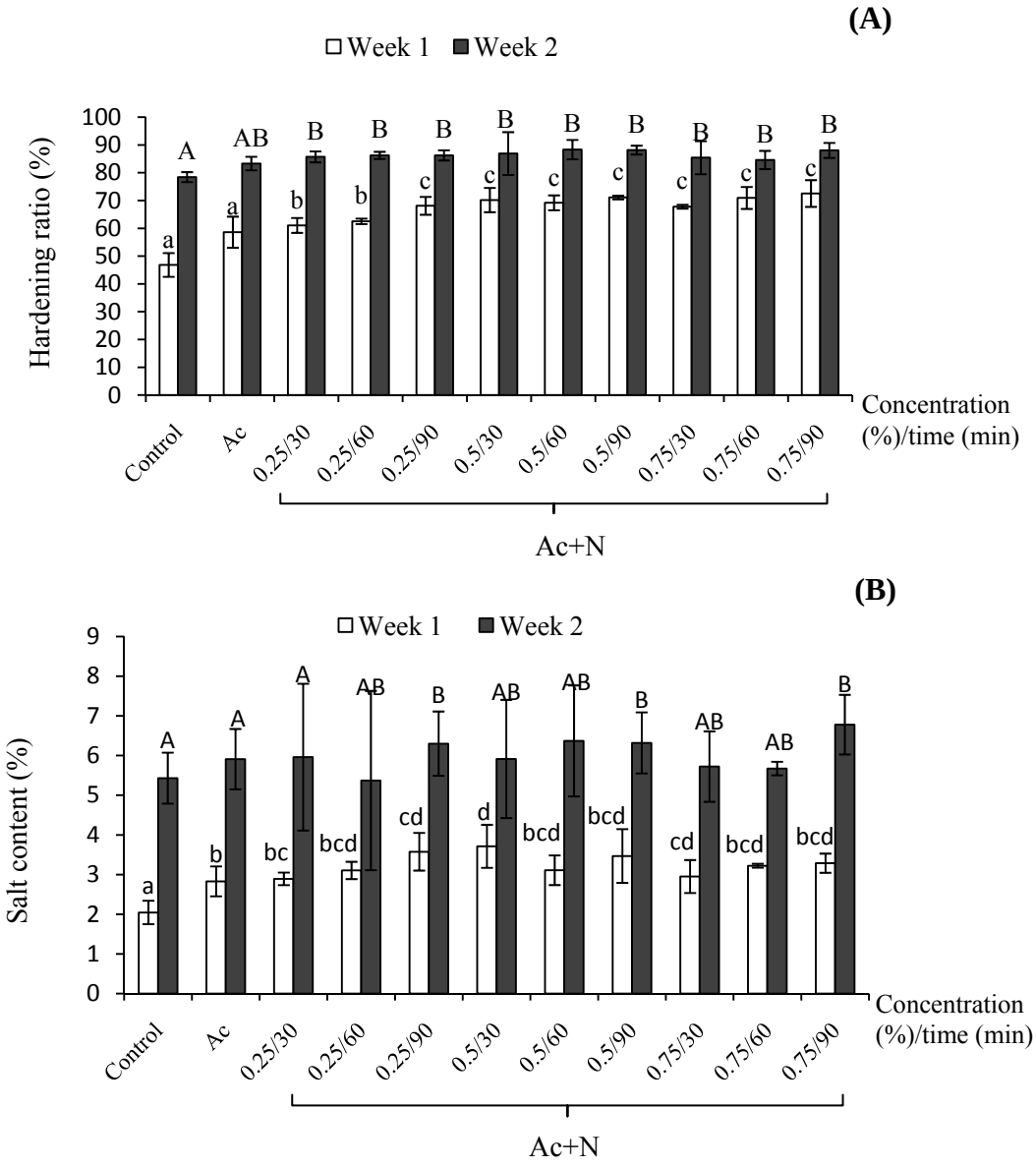



**Figure 22.** Hardening ratio of yolk (A) and salt content of egg white (B) of duck egg without pretreatment, egg pretreated with 5% acetic acid and egg pretreated with 5% acetic acid, followed by immersing in Neutrase solution with different concentrations and times after salting for 1 and 2 weeks. For salting, eggs were soaked in 25% NaCl at room temperature. Bars represent the standard deviation (n=3). Different letters or capital letters within the same salting time on the bar indicate the significant differences ($p<0.05$)

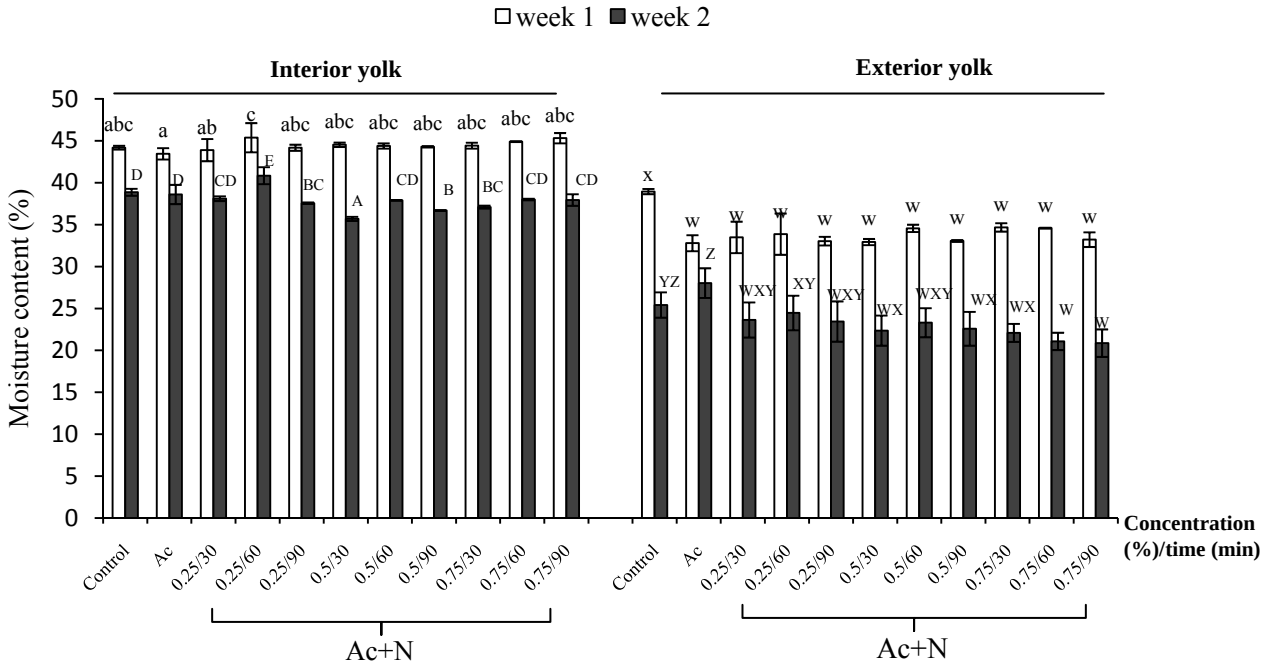



**Figure 23.** Moisture content of interior and exterior yolk of duck egg without pretreatment, egg pretreated with 5% acetic acid and egg pretreated with 5% acetic acid, followed by immersing in Neutrase solution with different concentrations and times after salting for 1 and 2 weeks. For salting, eggs were soaked in 25% NaCl at room temperature. Bars represent the standard deviation (n=3). Different letters or capital letters within the same salting time on the bar indicate the significant differences ($p < 0.05$)

### 4.4.3 Changes in chemical composition and some properties of duck egg pretreated with acetic acid and Neutrase during salting

Changes in hardening ratio, compositions and properties of egg without any pretreatment (control), egg pretreated with acetic acid (Ac) and egg pretreated with acetic acid, followed by socking in 0.25% Neutrase for 90 min (Ac+N) are shown in Table 16. Hardening ratio of egg yolk gradually increased when salting time increased and proceeded to the completeness of yolk gel formation. The highest hardening ratios of all samples were found at week 3 ($p< 0.05$). Ac+N sample had the highest hardening ratio of 95.32% after 3 weeks of salting. Moisture content of yolk, both interior and exterior portions, from all samples gradually decreased when salting time increased. At week 3, the lowest moisture content of interior yolk was found in Ac+N sample ($p< 0.05$), whereas no differences in moisture content were observed in exterior yolk among all samples ($p> 0.05$). Based on hardening ratio of yolk, pretreatment of prepared egg using Neutrase at a level of 0.25% for 30 min was chosen as the appropriate pretreatment condition. In general, the lowered moisture content of egg yolk was coincidental with the increase in salt content in egg white. No differences in salt content in egg yolk were obtained, regardless of pretreatment used.

Oil exudation of egg yolks with different treatments during salting are depicted in Figure 24A. Oil exudation of all samples increased with increasing salting time ($p< 0.05$). No differences in oil exudation were noticeable among all samples after week 1 of salting ($p> 0.05$). At week 2 and 3 of salting, the highest oil exudation was observed in Ac+N sample, followed by Ac sample and the control, respectively. Oil exudation is generally one of desirable characteristics of salted egg. The dehydration during salting and the presence of salt in yolk probably enhanced oil exudation. Schultz *et al*. (1968) pointed out that the removal of water from egg yolk increased the extracted lipid. Free lipid might be released from low lipoprotein micelles, due to the structural changes of low density lipoprotein induced by dehydration and increased salt content in egg white. Feeney *et al*. (1956) reported that the weakened yolk membrane causes the migration of water in albumin into the yolk

and diluted free lipid-protein (weakened lipoprotein) from surface of egg yolk, leading to oil exudation. With pretreatment using acetic acid and Neutrase, the thinner shell with increased porosity and weaker membrane, might favor the penetration of salt, with was associated with oil exudation.

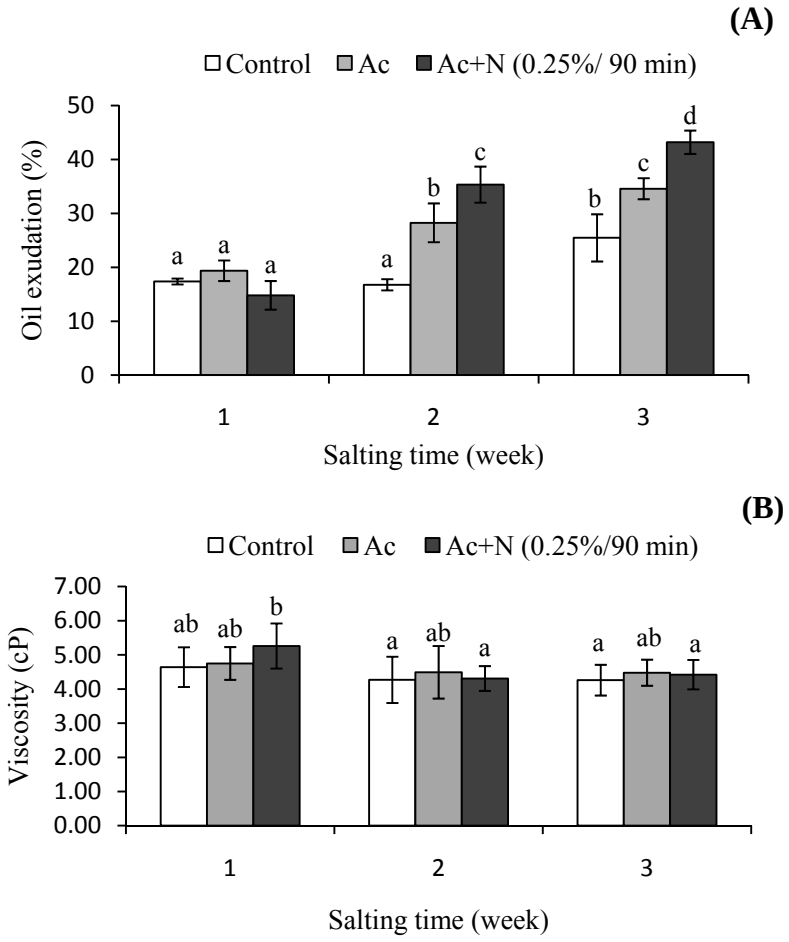



**Figure 24.** Oil exudation of yolk (A) and viscosity (B) of egg white of duck egg without pretreatment, egg pretreated with 5% acetic acid and egg pretreated with 5% acetic acid followed by immersing in 0.25% Neutrase solution for 90 min after salting for 1, 2 and 3 weeks. Bars represent the standard deviation (n=3). Different letters on the bars indicated the significant differences ($p<0.05$).

**Table 16.** Hardening ratio, salt content and moisture content of egg without and with different treatments during 3 weeks of salting

| Salting time | Treatment | Hardening ratio (%) | Salt content (%) | | Moisture content (%) | |
|---|---|---|---|---|---|---|
| | | | Egg white | Egg yolk | Interior yolk | Exterior yolk |
| Week 1 | Control[††] | 46.84±4.27[a*†] | 2.04±0.30[a] | 0.52±0.07[a] | 44.19±0.22[e] | 38.96±1.44[e] |
| | Ac | 58.61±5.61[b] | 2.83±0.38[ab] | 0.57±0.09[a] | 43.46±0.68[e] | 34.26±3.39[d] |
| | Ac+N (0.25%/90 min) | 68.11±3.21[c] | 3.58±0.47[b] | 0.64±0.08[a] | 44.18±0.37[e] | 32.39±2.08[d] |
| Week 2 | Control | 78.43±1.28[d] | 5.43±0.64[c] | 0.57±0.06[a] | 34.87±0.41[c] | 25.41±1.51[bc] |
| | Ac | 83.31±2.44[de] | 5.91±0.76[c] | 0.71±0.14[a] | 38.60±1.15[d] | 26.86±3.05[c] |
| | Ac+N (0.25%/90 min) | 86.24±1.29[ef] | 6.30±0.81[c] | 1.04±0.14[b] | 37.56±0.09[d] | 23.45±2.40[b] |
| Week 3 | Control | 88.92±3.49[ef] | 6.39±1.41[c] | 1.04±0.21[b] | 34.13±0.12[bc] | 20.33±1.74[a] |
| | Ac | 89.91±1.28[f] | 9.90±0.28[d] | 1.00±0.19[b] | 33.63±1.12[b] | 17.20±2.47[a] |
| | Ac+N (0.25%/90 min) | 95.32±2.56[g] | 9.10±1.75[d] | 1.07±0.06[b] | 28.45±0.76[a] | 17.58±1.39[a] |

*Means ± SD (n=3)

† Different subscripts in the same column indicate the significant differences ($p<0.05$).

††Control: No treatment; Ac: egg pretreated with 5% acetic for 30 min; Ac+N (0.25% Neutrase (w/v) / 90 min): egg pretreated with 5% acetic acid and immersed in 0.25% (w/v) Neutrase solution for 90 min.

Viscosity of egg white from egg subjected to different pretreatments during salting is shown in Figure 24B. Viscosity was determined to elucidate the effect of acetic acid or protease on the cleavages of egg white proteins. At the same periods of salting time, no differences in viscosity were found in all samples ($p > 0.05$). Therefore, proteases used more likely cleaved shell membrane rather than egg white protein, in which salt could be penetrated more efficiently.

Microstructures of egg shell and shell membrane of egg at week 2 of salting are shown in Figure 25. No difference in microstructure of shell membrane among all samples was observed (Figure 25A). The fibrous structure of shell membrane was not affected by acetic acid or acetic acid/Neutrase pretreatments. Fibrous material of shell membrane was initially identified as ovokeratin (Okubo *et al*., 1996). Similarly, the identification of desmosine and isodesmosine of shell membrane suggested the presence of elastin (Okubo *et al*., 1996). Neutrase might hydrolyze the interconnection region between membrane and shell, but did not the fibrous matrix of the membrane.

The rough surfaces of egg shell were observed in egg, which was pretreated with 5% acetic acid. Egg shells consists of mineral component, namely the trigonal phase of calcium carbonate ($CaCO_3$), known as calcite, which is the more stable polymorph at room temperature (Stadelman, 2000). Mineral can be removed from the egg shell by decalcification with EDTA or acetic acid (Nys *et al*., 2004). Egg treated with 5% acetic acid for 30 min with and without subsequent pretreatment using Neutrase had the lower thickness 0.37±0.03 and 0.36±0.02 mm, respectively, while the shell of control had the thickness of 0.43±0.02 mm (data not shown). Acetic acid can break apart the solid calcium carbonate crystals that make up the eggshell into their calcium and carbonate parts. The calcium ions float free, while the carbonate turn to be carbondioxide bubbles. This reaction causes the decrease in shell thickness (Nys *et al*., 2004). Shell thickness of less than 0.2 mm was observed when soaking the egg in 0.1 N HCl for 30 min (*Lai et al*., 1997). The pore sizes of egg shell become larger after pretreatment with acetic acid (Figure 25B). Generally, the outer surface of the eggshell is covered with a mucin protein

that acts as a soluble plug for the pores in the shell. The removal of cuticle and partial part of spongy layer opened the pores. As consequence, salt should be penetrated into the egg more effectively.

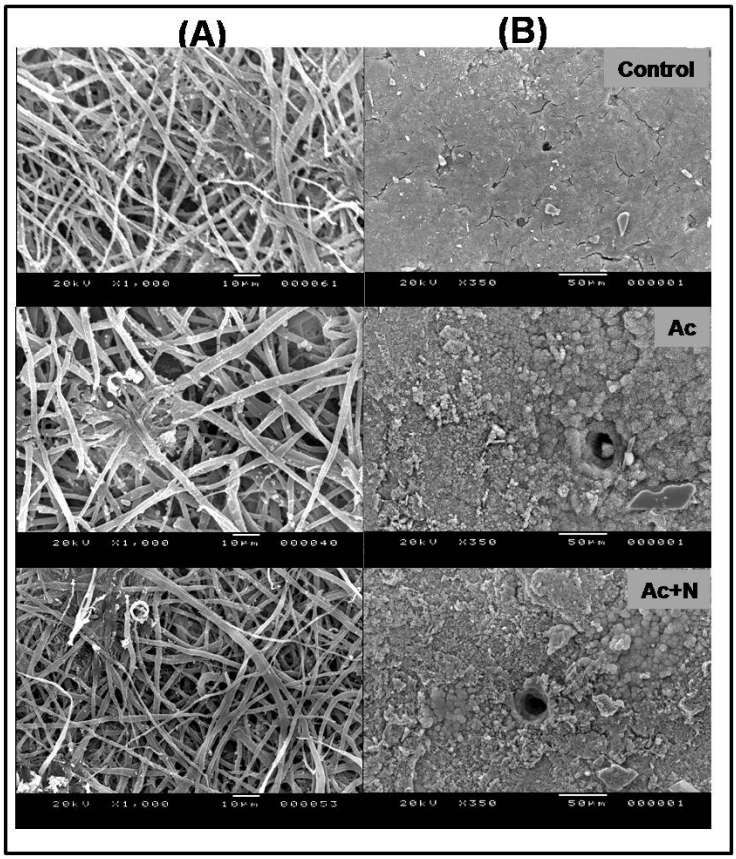



**Figure 25.** Scanning electron microscopic photograph of shell membrane (A) and shell (B) of duck egg without pretreatment, egg pretreated with 5% acetic acid and egg pretreated with 5% acetic acid followed by immersing in 0.25% Neutrase solution for 90 min.

FTIR spectra of egg shell membranes are shown in Figure 26. FTIR spectra shows the major absorption peaks at wavenumbers of 3297-3371, 1650-1653 and 1527-1534 $cm^{-1}$, strongly associated with the presence of hydroxyl group, amide I and amide II, respectively (Tsai *et al*., 2006). However, no differences in FTIR apectra of egg shell membrane among all samples. The result confirmed that Neutrase could not hydrolyze egg shell membrane, but more likely cleave the interconnection part between shell and shell membrane.

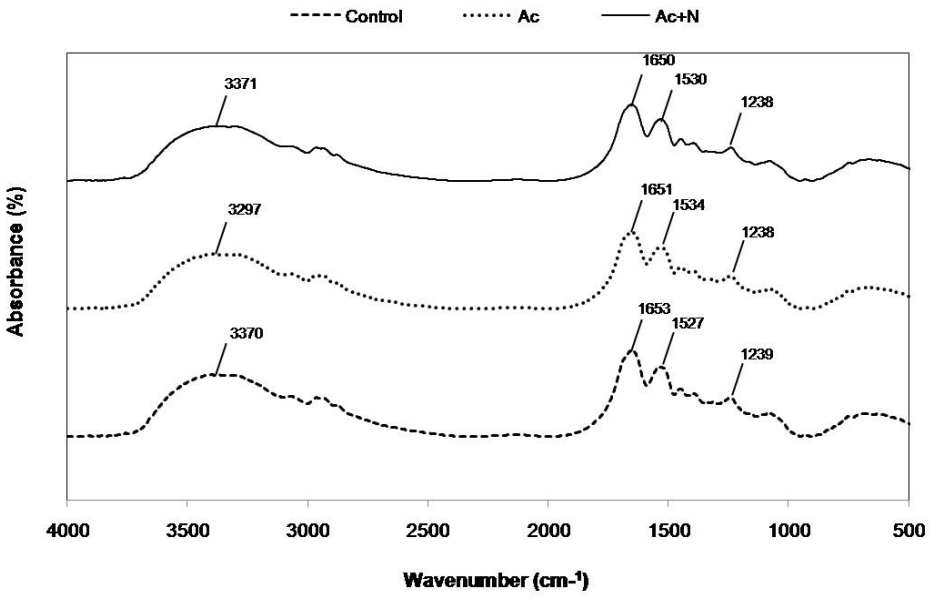



**Figure 26.** FTIR spectra of shell membrane of duck egg without pretreatment, egg pretreated with 5% acetic acid and egg pretreated with 5% acetic acid followed by immersing in 0.25% Neutrase solution for 90 min.

## 4.5 Conclusions

Salted egg could be produced with the aid of acetic acid in combination with commercial protease treatment. Soaking egg in 5% acetic acid, followed by immersing in 0.25% (w/v) Neutrase for 90 min could increase hardening ratio and oil exudation of yolk. Hardening ratio of 85% was obtained in salted egg with Neutrase treatment at week 2 of salting, whereas similar ratio was found in the control after 3 weeks of salting. Therefore, salting process could be shortened by 1 week with the aid of acetic acid and Neutrase prior to salting.

# CHAPTER 5

# CHEMICAL COMPOSITION, TEXTURAL PROPERTISE AND MICROSTRUCTURE OF COOKED DUCK EGG AS AFFECTED BY SALTING PROCESSES AND TIMES

## 5.1 Abstract

Chemical composition, textural properties, and microstructure of cooked duck egg salted by two methods (coating and immersing) were determined during four weeks of salting. As the salting time increased, both cooked salted egg white and yolk tended to have the decreases in moisture content with the increased salt content. Oil exudation of cooked yolk and expressible water content of cooked egg white obtained from both salting methods increased as salting proceeded ($P< 0.05$). After cooking, oil exudation accompanied by the solubilized pigments, especially at the outer layer of yolk, was obtained. Higher oil exudation was found in egg yolk from coating method, compared with that from immersing method, especially at week 3 of salting. As the salting times increased, the lower hardness, springiness, gumminess, chewiness and resilience with higher adhesiveness and cohesiveness were generally found in cooked salted egg white ($p< 0.05$), irrespective of salting methods and times. Conversely, the higher hardness of cooked yolk was found with increasing salting time ($p< 0.05$). Confocal laser scanning micrographs revealed the smaller yolk granules with more release of free lipid in salted egg after heating, compared with the fresh counterpart. As visualized by scanning electron microscope, gel of cooked salted egg white was coagulum type with larger voids. Therefore, salting processes had the impact on the characteristics of cooked salted egg to some degrees.

## 5.2 Introduction

Salted egg is one of the most popular preserved egg products in Thailand. Generally, salted egg can be made by brining eggs in saturated saline (immersing method) or by coating the egg with soil paste mixed with salt (coating method) for about 15-30 days (Chi and Tseng 1998; Lai *et al*., 1999). Salted egg is generally heated by boiling, pan frying, etc. before consumption. In addition to serving as a regular diet in the form of whole egg, the salted yolks are also used as stuffing material in some foods such as moon cake, other disserts and glutinous rice dumpling. The immersing method is a faster and more convenient for production of salted egg (Yang and Chen, 2001). During salting, the yolk gradually becomes solidified and hardened. On the other hand, egg white loses viscosity and becomes watery (Chi and Tseng, 1998). Chi and Tseng, (1998) reported that salting caused moisture removal from egg yolk and the diffusion of salt into egg white and egg yolk. All changes occurring during the salting most likely determine the preferential characteristics of salted egg, both raw and cooked forms.

In general, hard-cooked egg yolk has a crumbly and mealy texture (Angalet *et al*., 1976; Fischer *et al*., 1985). Nevertheless, salted egg with the granular texture and oil-off after cooking is preferable (Peh *et al*., 1982). Cooked egg white also has the impact on consumer's acceptability of salted egg. Egg white protein (mainly the abundant ovalbumin) underwent gelling after cooking. Huang *et al*. (1999) found that breaking force and gel strength of salted egg white gel decreased as salting time increased. Increasing salt content of egg white might induce aggregation of egg white protein, resulting in a coarser structure, in comparison with fresh egg. Rate of salt penetration into egg white and yolk governed by salting methods may have the influence on the changes in composition as well as characteristic of cooked salted egg white and yolk. Additionally, salting time more likely governs the changes in composition as well as the properties of eggs, especially after cooking. However, no information regarding the changes in characteristics and properties of cooked salted egg produced using different processes exists. Therefore, the objective of this study was to determine chemical compositions, textural properties and microstructure

of cooked salted duck egg, both egg white and yolk, obtained from coating method and immersing method at different salting times.

## 5.3 Materials and Methods

### 5.3.1 Chemicals

2-propanol and nitric acid were purchased from Lab-Scan (Bangkok, Thailand). Glutaraldehyde, ethanol, n-hexane and silver nitrate were obtained from Merck (Darmstadt, Germany). Nile blue A was procured from Sigma (St. Louis, MO, USA). Potassium thiocyanate was obtained from Bio-Rad (Richmond, CA, USA).

### 5.3.2 Duck egg collection

Duck eggs *(Anas platyrhucus)* with the weight range of 65-75 g were obtained within 1 day of laying from Satingphar, Songkhla province, Thailand. The eggs were used within 3 days after laying.

### 5.3.3 Preparation of salted duck egg

Salted duck eggs were prepared following the method of Kaewmanee *et al*. (2009b). Fresh duck eggs were separated into 2 groups. The first group was placing in coating paste, a mixture of clay (4 kg), salt (1 kg) and water (1.8 L) for three times to obtain a thickness of approximately 2 mm. Thereafter, coated eggs were covered with rice chaff ash. The second group was immersed in the brine solution (25%, w/v) using 1 egg/100 mL brine. Duck eggs of both groups were stored at room temperature (28-30ºC) and taken every week during salting up to 4 weeks. For coated sample, all coating materials were removed manually and washed with tap water until the shell was clean. Salted eggs prepared by brining method were also washed with tap water. Salted eggs were heated in boiling water for 20 min, following by cooling

in running water. Fresh duck egg were also cooked and used as the control. Cooked egg white and yolk were manually separated and subjected to analyses.

### 5.3.4 Determination of moisture and salt contents of cooked egg white and yolk

Moisture content was determined according to AOAC (2000). Salt content in cooked egg samples was measured by the method of AOAC (2000). Samples (1 g for egg yolk or 0.5 g for egg white) were added with 20 mL of 0.1 N $AgNO_3$ and 10 mL of $HNO_3$. The mixture was boiled gently on a hot plate until all solids except $AgCl_2$ were dissolved (usually 10 min). The mixture was cooled at room temperature. Five mL of 5% ferric alum indicator ($FeNH_4$ $(SO_4)_2 \cdot 12$ $H_2O$) were added. The mixture was titrated with the standardized 0.1 N KSCN until the solution became permanently light brown. The percentage of salt was then calculated as follows:

$$\text{Salt (\%)} = 5.8 \times [(V1 \times N1) - (V2 \times N2)]/W$$

where V1 = volume of $AgNO_3$ (mL); N1 = concentration of $AgNO_3$ (N);
V2 = volume of KSCN (mL); N2 = concentration of KSCN (N); and W = weight of sample (g).

### 5.3.5 Determination of oil exudation of cooked egg yolk

Oil exudation was measured according to the method of Lai *et al*. (1999) with a slight modification. Cooked egg yolk (3 g) was homogenized with 35 mL of n-hexane/2-propanol (3:2, v/v) at 5,000 rpm for 10 min using a homogenizer (Polytron, PT 2100, Kinematica AG, Luzern, Switzerland). The homogenate was filtered through Whatman No. 1 filter paper (Whatman International Ltd, Maidstone, UK) and the filtrate was evaporated in a temperature-controlled water bath (Memmert, Schwabach, Germany) at 55ºC and then dried at 105°C in an oven

(Memmert, Schwabach, Germany) until the constant weight was obtained. The residue was weighed and taken as total lipid content. To determine the oil exudation, yolk (5 g) was mixed with 25 mL of distilled water and homogenized at 5,000 rpm for 30 sec. The homogenate was centrifuged at 9,500×g for 30 min at 25°C and 25 mL of n-hexane/2-propanol (3:2, v/v) were added to the supernatant to dissolve the float. The solvent-lipid layer obtained was separated using a separating funnel. The solvent in the solvent-lipid layer was evaporated in a water bath (55ºC) and the residue was heated at 105°C until a constant weight was obtained The residue was weighed and taken as free lipid. Oil exudation was defined as the proportion of free to total lipid content.

$$\text{Oil exudation (\%)} = \frac{\text{Free lipid content}}{\text{Total lipid content}} \times 100$$

### 5.3.6 Texture profile analysis (TPA) of cooked egg white and yolk

TPA was performed as described by Bourne (1978) with a TA-XT2i texture analyzer (Stable Micro Systems, Surrey, England). Cooked egg white at narrow side with elliptical in shape was cut with a blade as indicated by a cutting line (Figure 27). The cooked egg white was subsequently cut in to a cube of 1×1×1 $cm^3$ (Figure 27B) and subjected to TPA analysis. Prepared samples (Figure 27C) were compressed twice to 50% of their original height with a compression cylindrical probe (25 mm diameter). Force-distance deformation curves were recorded at cross head speed of 3 mm/s and the recording speed was 5 mm/s. Hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness and resilience were evaluated. These parameters were obtained using the MicroStable software (Stable Micro Systems, Surrey, England).

Cooked egg yolk was subjected to hardness determination. The cooked whole yolks were compressed twice to 50% of their original height with a compression cylindrical aluminum probe (50 mm diameter). Textural analyses were performed at room temperature. Force-distance deformation curves were recorded at

cross head speed of 5 mm/s and the recording speed was 5 mm/s. Hardness of cooked yolk was evaluated.

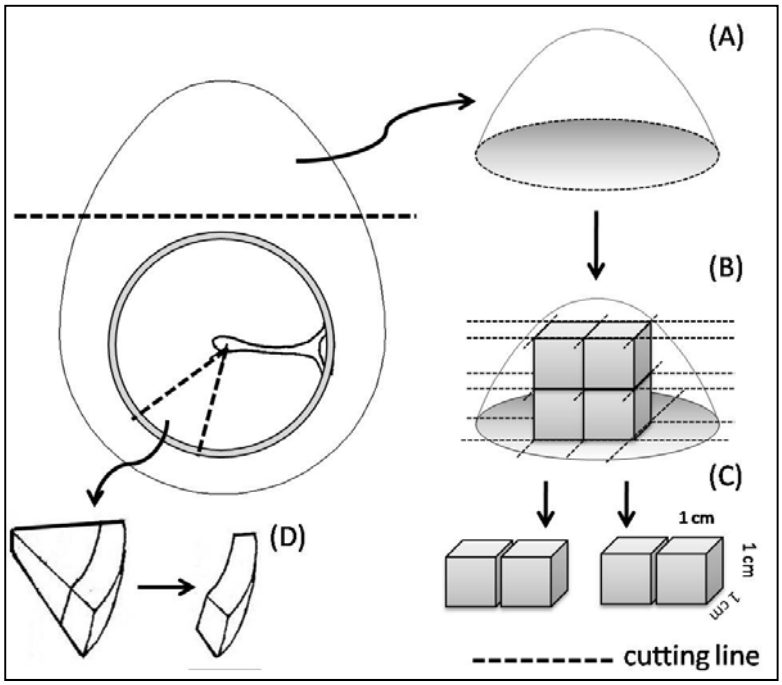



**Figure 27.** Cutting of cooked egg white and yolk A: sample for color measurement, B: cutting dimension, C: samples for texture profile analysis and expressible water content measurement and D: sample for microstructure measurement.

### 5.3.7 Determination of whiteness of cooked egg white

Cooked egg white was cut as demonstrated in Figure 27A. The color of cooked egg white was measured at cutting surface in the $L^*$ $a^*$ $b^*$ mode of CIE (angle 10°, illuminant D65) using HunterLab (ColorFlex, Hunter Associates Laboratory, VA, USA). $L^*$, $a^*$ and $b^*$ indicate lightness, redness/greenness and yellowness/blueness, respectively. Whiteness was calculated using the following equation (NFI, 1991).

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

### 5.3.8 Determination of expressible water of cooked egg white

The percentage of expressible water of cooked egg white was measured according to method of Visessanguan *et al*., (2004) with a slight modification. The expressible water was determined as the weight loss resulting from compression of sample. The samples were cut into cube (1×1×1 $cm^3$) (Figure 27C), placed between double layers of filter papers (3×3 $cm^2$) (Whatman No. 4) and subjected to compression using a texture analyzer with a cylindrical aluminum probe (50 mm diameter). The measurement was performed with crosshead speed of 3 mm/s to compress 70% of their original height for 30 s. The cooked egg white was subjected to moisture analysis by AOAC method (AOAC, 2000). The expressible water content was calculated as the ratio of the apparent expressible water to the total moisture content of cooked egg white according to the following equations:

$$\text{Expressible water (\%)} = \frac{100\times (\text{Apparent expressible water content})}{(\text{Total moisture content})}$$

where: $\text{Apparent expressible water content} = \frac{100\times (W_{before}-W_{after})}{W_{before}}$

$W_{before}$ = Weight before compression

$W_{after}$ = Weight after compression

### 5.3.9 Determination of microstructure of cooked egg white

Microstructures of cooked egg white were analyzed using a scanning electron microscopy. Cooked egg white was cut into a small piece (4×4×4 $mm^3$). Samples were fixed at room temperature in 2.5% glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 2 h. Fixed samples were rinsed with 0.2 M phosphate buffer (pH 7.2) for 15 min and with distilled water for 15 min. Prepared samples were dehydrated in graded series of ethanol (50, 70, 80, 90 and 100%) for 15 min each. All specimens were coated with 100% gold (sputter coater SPI-Module, West Chester, PA, USA).

The microstructure was visualized using a scanning electron microscope (JEOL JSM-5800LV, Tokyo, Japan).

### 5.3.10 Determination of microstructure of cooked egg yolk

Microstructures of cooked yolk were examined with a confocal laser scanning microscopy (CLSM) (Olympus, FV300, Tokyo, Japan) following the modified method of Mineki and Kobayashi (1997). Egg yolk samples (Figure 1D) were dissolved in 0.01% Nile blue A solution at a ratio of 1:10 (w/v) and stirred until the uniformity was obtained. Fifty μl of sample solutions and 0.01% acridine orange were smeared on the microscopy slide. The CLSM was operated in the fluorescence mode at the excitation wavelength of 533 nm and the emission wavelength of 630 nm using a Helium Neon Red laser (HeNe-R) for lipid analysis and at the excitation wavelength of 488 nm and the emission wavelength of 540 nm using a Helium Neon Green laser (HeNe-G) for protein analysis.

### 5.3.11 Statistical analysis

Completely random design was used throughout the study. The experiments were run in triplicate. Data were presented as mean values with standard deviations. One-way analysis of variance (ANOVA) was carried out and mean comparisons were performed by Duncan's multiple range tests (Steel and Torrie, 1980). For pair comparison, T-test was used. Statistical analyses were done with the statistical program (SPSS for windows, SPSS Inc, Chicago, IL, USA).

## 5.4 Results and Discussion

### 5.4.1 Chemical composition of cooked salted duck as influenced by salting processes and salting time

Moisture and salt contents of cooked duck egg obtained from coating and immersing methods during salting up to 4 weeks are shown in Figure 28. Decreases in moisture content (Figure 28A) with coincidental increases in salt content (Figure 28B) in both egg white and yolk were observed during salting ($p< 0.05$), regardless of salting methods. At the same salting times, no differences in moisture content were obtained between cooked salted egg white (CSEW) from both salting methods ($p> 0.05$). For raw salted egg white, the moisture content of egg white from immersing method was slightly lower than that from paste coating method, most likely due to the greater migration of water from egg white to brine mediated by osmosis process (Kaewmanee *et al*., 2009b). After cooking for 20 min, the water was removed from egg white and reached the similar equilibrium level. Moisture contents of cooked salted egg yolk (CSEY) from immersing method decreased as the salting time increased up to 3 weeks ($p<0.05$). No changes in moisture content were found between CSEY obtained at week 3 and 4 ($p> 0.05$). At the first week of salting, moisture contents of CSEY decreased from 45.28% to 40.31% and 40.57% for yolk obtained from coating and immersing method, respectively. No differences in moisture contents of CSEY obtained from paste coating method were obtained during 2-4 weeks of salting ($p> 0.05$). Nevertheless, the lowest moisture content was found in CSEY from immersing method at week 3 and 4 (25.26 and 24.22%, respectively). During salting, the solidification due to the dehydration of egg yolk proceeded with increasing salting time. Since egg yolk was localized inside, the migration of water as induced by heat during boiling was more likely retarded. Thus, the prior dehydration during salting determined the moisture content in cooked samples, rather than the dehydration taken place during cooking.

Salt content of CSEW from both salting methods increased up to 2 weeks of salting ($p< 0.05$) (Figure. 28B). No difference in salt content in CSEW at

week 2 and 3 was observed ($p > 0.05$). At week 4 of salting, no change in salt content was observed in CSEW obtained from coating method ($p > 0.05$), while the increase in salt content was obtained in CSEW from the immersing method ($p < 0.05$). For CSEY, the increase in salt content was ($p < 0.05$). During salting, the increase in salt content in CSEW was faster and greater than that found in CSEY. Solidified yolk obtained during salting might impede the migration of NaCl. Furthermore, high lipid content in yolk might lower the migration of NaCl into the yolk (Kaewmanee *et al*., 2009a). Kaewmanee *et al*., (2009b) found that high diffusion of salt into egg white obtained from immersing method was mainly due to the higher salt concentration in brine (25%) when compared with the coating paste (18% salt). Therefore, the difference in salt content was caused by the differences in osmotic pressure between both salting methods. During cooking, some water might be lost and salt became concentrated. As a result, salt content was slightly increased in both cooked egg white and yolk.

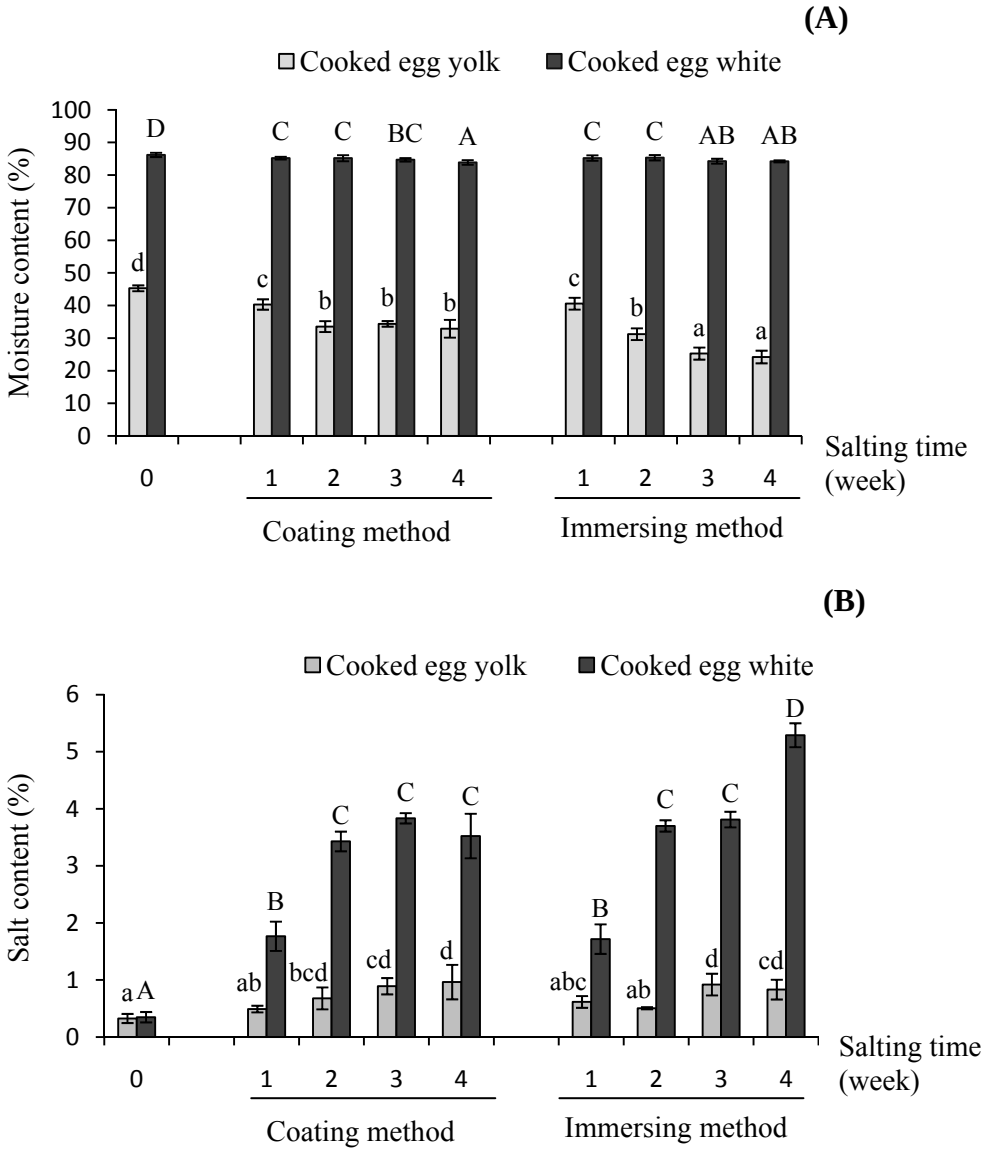



**Figure 28.** Moisture content (A) and salt content (B) of cooked egg white and cooked egg yolk from salted duck egg with different methods (coating and immersing) during 4 weeks of salting. Bars represent the standard deviation (n=3). Different letters on the bar indicates significant differences ($p< 0.05$).

Oil exudation of CSEY obtained from paste coating and immersing methods during salting up to 4 weeks is depicted in Figure 29A. Oil exudation of CSEY from both salting methods gradually increased with increasing salting time ($p< 0.05$). At the same salting time, no differences in oil exudation were found

between CSEY from coating and immersing methods ($p > 0.05$), except for CSEY obtained at week 3, where the higher oil exudation was noticeable in CSEY from immersing method ($p < 0.05$). The dehydration during salting probably enhances oil exudation (Schultz *et al*., 1968). Most lipids in egg yolk exist in low density lipoproteins (LDL) (Sugino *et al.,* 1997). During salting, free lipid might be released from low density lipoprotein micelles, due to the structural alteration of LDL induced by dehydration and high salt content. When the heat was applied, LDL might be more disrupted and other proteins stabilizing the oil in yolk might be more denatured. As a consequence, the free oil visualized as the exudates was increased.

Expressible water contents of CSEW are shown in Figure 29B. Cooked fresh duck egg (week 0) had the expressible water content of 18.13%. As the salting proceeded, the expressible water of CSEW increased ($p < 0.05$). However, no increase in expressible water content was found at week 3 and 4 of salting ($p > 0.05$). No differences in expressible water content of CSEW were obtained between paste coating and immersing methods during the first 2 weeks of salting ($p > 0.05$). On the other hand, at week 3 and 4 of salting, the higher expressible water content was obtained in CSEW from immersing method, compared with that from coating method ($p < 0.05$). Expressible water is water retained after cooking and could be used as an indicator of water imbibed in egg white gel network. Those with high expressible water showed the low water holding capacity in the gel network (Matsudomi *et al*., 2002). Egg white consists of 9.7 to 12% protein containing a numerous globular proteins in an aqueous solution (Mine, 1995; Li-Chan *et al*., 1995). The major proteins of egg white are regarded as ovalbumin, conalbumin (ovotransferrin), ovomucoid, lysozyme, globulin and ovomucin. More than 50% of egg white is ovalbumin (Li-Chan *et al*., 1995). Denaturation of ovalbumin occurred from 79 to 84 °C. The increase of temperature and the heating period improves the linkages between proteins and water molecules and increase protein cross-linking in the gel structure (Yang and Baldwin, 1995). The salt might destabilize protein molecules in egg white or induce the coagulation of egg white proteins, especially during heating. Disordered network in the presence of high salt could not imbibe water effectively. Generally, the retention ability of water decreased with increased randomness of aggregates,

resulting in a coarser network (coagulates) with larger pores, thereby lowering the water bound to protein (Hermanson, 1982). Therefore, the increased expressible water content of CSEW was mainly associated with the increasing salt content in egg white. The result was in agreement with Choi *et al*. (2000) who found that salt addition (2%) caused a slight reduction of water-retention ability of dried egg white gel.

### 5.4.2 Appearance of cooked salted yolk as influenced by salting processes and salting time

The color of cooked fresh yolk was yellowish orange or red. Cooked yolk obtained from both salting methods gradually became more reddish during 4 week of salting (Figure 30). The changes in the color of CSEY during salting might be related to moisture loss, increased salt content and increased amount of free lipids, especially at the outer layer of egg yolk. The lowered moisture was associated with the increased concentration of the pigments, and the free lipid was able to extract lipid soluble pigments, particularly carotenoid in yolk. The higher oil exudation was obtained in CSEY after 4 weeks of salting and the free lipid was abundantly localized at the outer layer of yolk. Simultaneously, soluble pigments were solubilized by those free oils. The pigments in egg yolk are carotenes and riboflavin (Sugino *et al*., 1997). Carotenes, which are the cause of the color of the yolk, cannot be synthesized by the duck or hen's metabolism. The feed is the major factor affecting carotenes content and the color of the yolk (Sugino *et al*., 1997). It was noted that similar appearance was observed between CSEY obtained from both methods. At week 4 of salting, cooked yolk from both salting processes showed the hard and dense core with pale color. The oil with the carotenoid in the core region might be migrated to the outer layer of yolk after heating and cooling. When heating was applied, the proteins located in that region with the lowered oil content underwent denaturation with ease. Therefore the dense network with pale color could be developed in that region.

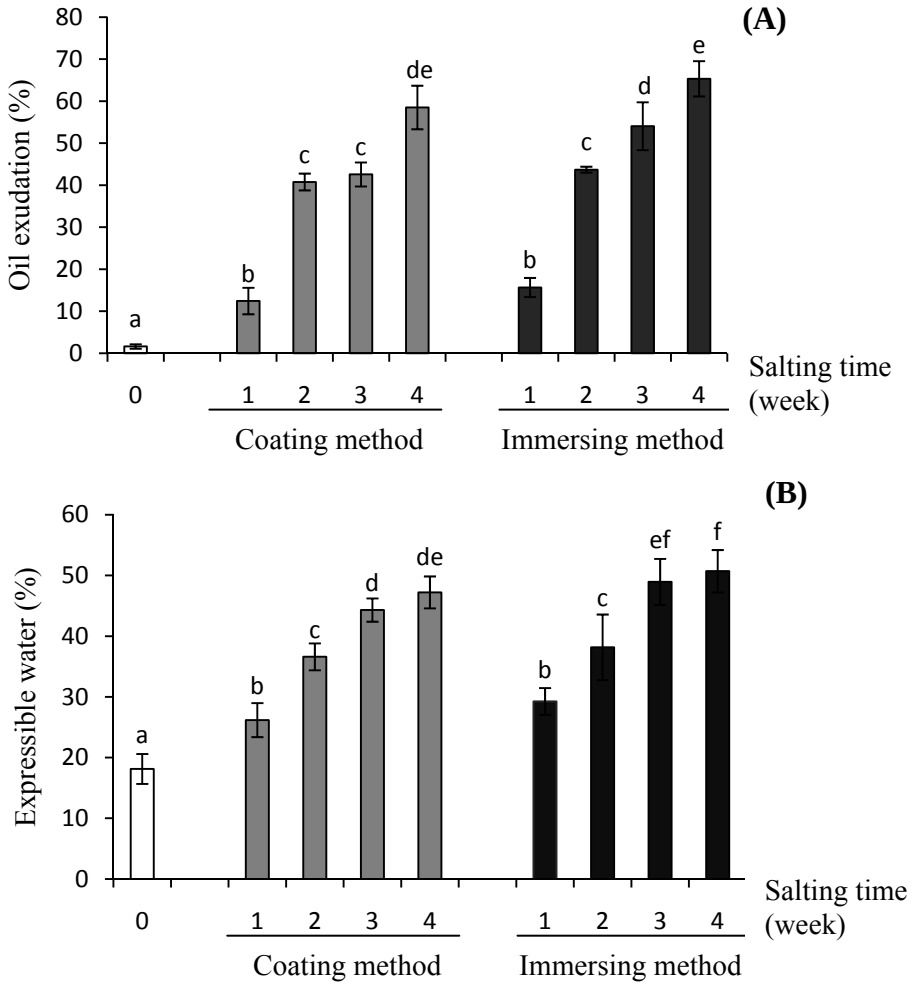



**Figure 29.** Oil exudations of yolk (A) and expressible water of egg white (B) from salted duck egg with different methods during 4 weeks of salting. Bars represent the standard deviation (n=3). Different letters on the bar indicates significant differences ($p < 0.05$).

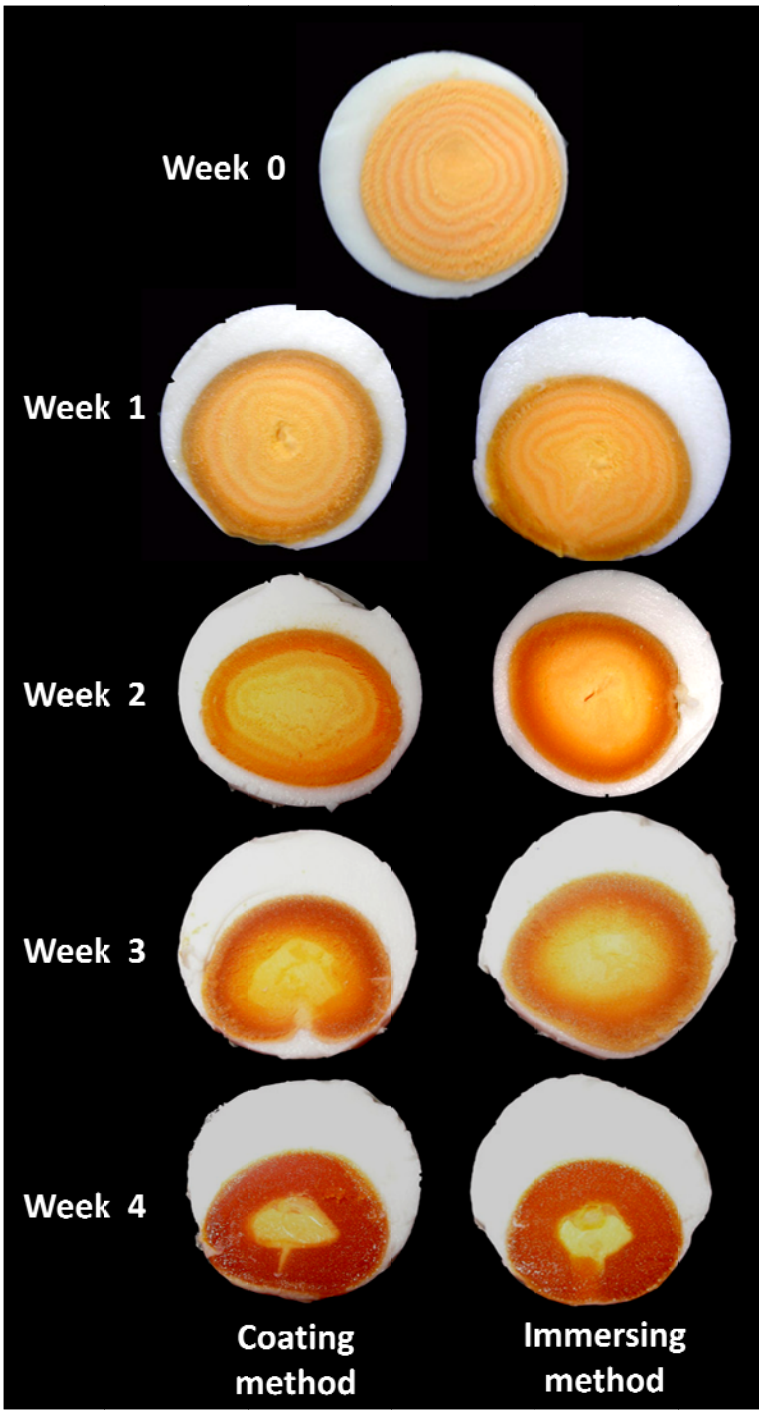



**Figure 30.** Cross sections of cooked fresh and salted duck egg with different methods during 4 weeks of salting.

### 5.4.3 Color of cooked salted egg white as influenced by salting processes and salting time

The colors of CSEW during salting of 4 weeks using two different methods are presented in Table 17. Lower lightness ($L^*$) values of cooked fresh egg (week 0) was obtained, compared with CSEW, regardless of salting method ($p< 0.05$). For CSEW from coating method, the differences in lightness were observed at different salting times, except at week 3, where the highest lightness was observed ($p< 0.05$). For CSEW from immersing method, the decrease in lightness was obtained at week 4 of salting ($p< 0.05$). In the presence of salt, the aggregation might be formed to a greater extent when the heat was applied. As a result, the cluster or coagulate was generated. This led to the higher light scattering of egg white gel formed. At week 4, the lower lightness was probably caused by the formation of large coagulate with less surface area, resulting in the decreased light scattering. At the same salting time, no differences in lightness were found, except for CSEW obtained from immersing method at week 1, which showed the higher lightness, than that from coating method. When a* and $b^*$ values, indicating redness/greenness and yellowness/blueness, respectively, were determined in CSEW, no changes in $a^*$ value were observed up to 4 week ($p> 0.05$), regardless of salting methods and times. During salting, slightly higher $b^*$ values were obtained for CSEW from coating method, compared with that of fresh egg ($p< 0.05$). However, salting time had no effect on b* value ($p> 0.05$). For CSEW from immersing method, the increase in b* value was observed at week 3 and 4, compared with that of fresh egg ($p< 0.05$). Generally, the higher a* and $b^*$ values were noticeable in CSEW than those of cooked fresh egg white. Salting process might soften yolk membrane, thereby facilitating the migration of pigment from yolk to egg white. This possibly contributed to the increase in redness/yellowness of CSEW with increasing salting time.

### 5.4.4 Textural properties of cooked salted egg white and yolk as influenced by salting processes and salting time

Textural properties of CSEW and CSEY during 4 weeks of salting are shown in Table 18. Salting markedly affected the hardness of cooked egg white ($p< 0.05$). Decreases in hardness of CSEW were obtained, compared with that of cooked fresh egg (week 0). At week 1, the higher hardness of CSEW was obtained from coating method ($p< 0.05$). No differences in hardness were found between both methods during 2 and 4 weeks of salting ($p> 0.05$). Hardness of CSEW decreased up to week 2 of salting by coating method ($p< 0.05$). Thereafter, no change in hardness was found ($p< 0.05$). No difference of hardness of CSEW from immersing method was observed during 1 and 4 weeks of salting ($p> 0.05$).

Egg albumin is a colloidal mixture of various proteins (Hatta *et al*., 1997). Only ovomucoid and ovomucin are not coagulable by heat (Johnson and Zabik, 1981) except when complexed with Fe or Al. Conalbumin is sensitive to heat. The denaturation temperatures of conalbumin, globulin, ovalbumin and lysozyme are 57.3, 72.0, 71.5, and 81.5°C, respectively (Yang and Baldwin, 1995). Ovalbumin behavior dominantly affects the gelation of egg white. In the presence of 0-10 mM NaCl, the ovalbumin solution (5%) remained as a transparent solution. In the presence of 20-50 mM and 60-80 mM NaCl, a transparent gel and a turbid gel was developed, respectively. Moreover, the gel of the highest gel strength was obtained in a transparent state, but opaque gel, where the higher NaCl content was present, had the lower hardness. Sato and Nakamura (1977) reported that egg albumin (15%) gel induced by heating at 90°C for 30 min was strongest in the presence of 0.5% NaCl. The higher the concentration of NaCl, the less gel strength was obtained. For the adhesiveness, the increases in adhesiveness were found in CSEW, compared with that of cooked fresh egg, regardless of salting methods and times. Adhesiveness increased as the salting time increased ($p< 0.05$). During the first 3 weeks of salting, no difference in adhesiveness was observed between both salting methods ($p> 0.05$). The highest adhesiveness was found at week 4, 44.76 and 38.81 g.s for coating method and immersing method, respectively. For springiness, presenting how well a product

physically springs back after it has been deformed during the first compression, the decrease in CSEW was obtained after 3 weeks of salting ($p< 0.05$). The lowest springiness was found in CSEW at week 4 of salting ($p< 0.05$). Cohesiveness slightly increased as salting time increased, regardless of salting methods. Decreases in cohesiveness were obtained for both methods after 4 weeks of salting ($p< 0.05$). Cohesiveness and springiness reflect the development of internal bonding in three-dimensional egg white gels network. In general, cohesiveness indicates how well the product withstands a second deformation and is relative to how it behaves under the first deformation. Cooked fresh egg white exhibited the highest gumminess and chewiness of 430 g and 590 g.mm, respectively. Decrease in gumminess was found in CSEW from coating method up to week 3-4 of salting ($p< 0.05$), whereas no differences in gumminess were noticeable in CSEW from immersing method during salting of 4 weeks ($p> 0.05$). For the chewiness of CSEW, the continuous decreases were found in immersing method during the salting of 4 weeks ($p< 0.05$). For CSEW from coating method, the continuous decrease in chewiness was also found during the salting, except that the chewiness at week 2 was higher than that found at week 1 ($p< 0.05$). Both gumminess and chewiness of CSEW were generally lower than those of cooked fresh egg white ($p< 0.05$). Resilience is defined as the energy accumulated that allows the sample to recover its original shape after deformation. Slight decreases in resilience were obtained in CSEW, compared with the cooked fresh egg white ($p< 0.05$). However, no difference in resilience was observed between cooked fresh egg white and CSEW from coating method at week 2 or CSEW from immersing method at week 3 ($p< 0.05$).

For CSEY, hardness of CSEY increased continuously and reached the maximum at week 2 and 2-3 for immersing and coating methods ($p< 0.05$), respectively. Thereafter, the decreases in hardness of CSEY were obtained with further salting. The results suggested that salted egg white and yolk had the varying texture properties, depending on salting process as well as salting time.

**Table 17.** Color of cooked egg white from salted duck egg with different methods during 4 weeks of salting

| Salting method | Salting time (week) | Color† | | | |
|---|---|---|---|---|---|
| | | $L^*$ | $a^*$ | $b^*$ | whiteness |
| No salting (Fresh egg) | 0 | 90.79±1.74$^{a††}$ | -2.75±0.35$^{a}$ | 2.33±0.54$^{a}$ | 90.09±1.70$^{a}$ |
| Coating method | 1 | 94.30±1.76$^{bc}$ | -1.64±0.21$^{b}$ | 2.99±0.38$^{bc}$ | 93.27±1.42$^{bc}$ |
| | 2 | 94.47±0.66$^{bc}$ | -1.82±0.17$^{b}$ | 3.38±0.29$^{c}$ | 93.25±0.54$^{bc}$ |
| | 3 | 95.44±0.37$^{cd}$ | -1.59±0.34$^{b}$ | 3.90±0.43$^{c}$ | 93.78±0.50$^{bc}$ |
| | 4 | 93.88±0.52$^{b}$ | -1.34±0.21$^{b}$ | 3.10±0.8$^{bc}$ | 93.14±0.38$^{bc}$ |
| Immersing method | 1 | 96.15±1.09$^{d}$ | -1.59±0.10$^{b}$ | 2.30±0.32$^{a}$ | 95.22±0.97$^{d}$ |
| | 2 | 94.84±0.53$^{bcd}$ | -1.15±0.87$^{b}$ | 2.14±0.32$^{a}$ | 94.15±0.58$^{cd}$ |
| | 3 | 95.32±0.83$^{cd}$ | -1.83±0.19$^{b}$ | 3.01±0.63$^{bc}$ | 94.13±0.95$^{cd}$ |
| | 4 | 93.61±0.40$^{b}$ | -1.43±0.59$^{b}$ | 2.89±0.31$^{bc}$ | 92.83±0.52$^{b}$ |

† Means ± SD (n=3)

†† Different superscripts in the same column indicate significant differences ($p < 0.05$)

**Table 18.** Texture profile analyses of cooked egg white and cooked egg yolk from salted duck egg with different methods during 4 weeks of salting.

| Salting method | Cooked egg white[†] | | | | | | | Cooked egg yolk[*] |
|---|---|---|---|---|---|---|---|---|
| | Salting time (week) | Hardness (g) | Adhesiveness (g.s) | Springiness (mm) | Cohesiveness | Gumminess (g) | Chewiness (g.mm) | Resilience | Hardness (g) |
| No salting | 0 | 822.67±26.79$^{d††}$ | 0.62±0.68$^{f}$ | 1.38±0.04$^{cd}$ | 0.50±0.04$^{c}$ | 426.68±25.08$^{d}$ | 587.28±45.98$^{e}$ | 0.35±0.02$^{c}$ | 968.79±244.32$^{a†}$ |
| Coating method | 1 | 560.47±33.07$^{c}$ | 4.17±1.06$^{e}$ | 1.43±0.12$^{d}$ | 0.54±0.04$^{d}$ | 243.18±39.42$^{c}$ | 308.84±27.72$^{c}$ | 0.28±0.02$^{a}$ | 1161.97±65.68$^{b}$ |
| | 2 | 488.65±25.52$^{b}$ | 6.74±0.83$^{d}$ | 1.34±1.12$^{c}$ | 0.57±0.01$^{d}$ | 238.48±20.15$^{c}$ | 354.08±32.48$^{d}$ | 0.32±0.04$^{bc}$ | 2160.06±190.05$^{ef}$ |
| | 3 | 491.37±41.45$^{b}$ | 9.21±1.07$^{c}$ | 0.93±0.06$^{b}$ | 0.55±0.02$^{d}$ | 188.20±38.60$^{ab}$ | 237.67±35.52$^{b}$ | 0.32±0.03$^{b}$ | 2215.76±84.60$^{f}$ |
| | 4 | 469.04±26.83$^{ab}$ | 44.76±4.01$^{a}$ | 0.57±0.09$^{a}$ | 0.42±0.04$^{a}$ | 159.41±23.65$^{a}$ | 127.90±34.97$^{a}$ | 0.31±0.03$^{b}$ | 1814.12±101.26$^{d}$ |
| Immersing method | 1 | 472.22±27.77$^{ab}$ | 3.96±0.86$^{e}$ | 1.32±0.12$^{c}$ | 0.53±0.01$^{d}$ | 228.16±9.03$^{c}$ | 305.58±27.47$^{c}$ | 0.31±0.03$^{b}$ | 1477.65±128.12$^{c}$ |
| | 2 | 467.54±25.87$^{ab}$ | 5.90±1.28$^{de}$ | 1.37±0.13$^{cd}$ | 0.54±0.02$^{d}$ | 205.46±22.11$^{bc}$ | 263.52±29.44$^{b}$ | 0.31±0.03$^{b}$ | 2542.72±156.96$^{g}$ |
| | 3 | 473.47±29.89$^{ab}$ | 9.52±1.02$^{c}$ | 0.93±0.02$^{b}$ | 0.54±0.05$^{d}$ | 231.35±17.02$^{c}$ | 251.76±36.41$^{b}$ | 0.33±0.04$^{bc}$ | 1996.81±99.86$^{e}$ |
| | 4 | 439.83±31.40$^{a}$ | 38.81±3.92$^{b}$ | 0.63±0.08$^{a}$ | 0.46±0.04$^{b}$ | 213.41±27.64$^{bc}$ | 159.69±33.80$^{a}$ | 0.31±0.06$^{b}$ | 1471.31±62.79$^{c}$ |

[†] Means ± SD (n=3)

[††] Different superscripts in the same column indicate significant differences ($p < 0.05$)

### 5.4.5 Microstructure of cooked egg white and yolk as influenced by salting process and salting time

Scanning electron micrographs of cooked salted egg white with two salting methods at week 2 and 4 of salting are shown in Figure 31. Cooked fresh egg white had the denser network with the smaller void, compared with CSEW. During heating, proteins underwent unfolding with subsequent aggregation gradually. As a result, the more compact network was developed. For CSEW gel, the larger voids (arrow head) were observed, compared with those of cooked fresh egg white. Less uniformity was found in CSEW. The lower hardness might be reflected by less ordered network. In the presence of NaCl, the coagulation was induced. During heating, that coagulum underwent interconnection, leading to the formation of coagulum type gel. Nevertheless, no marked difference in microstructure was obtained in CSEW after salting for 2 and 4 weeks.

Confocal laser scanning microscope (CLSM) micrographs using a two channel technique, in which both protein and lipid were stained, are illustrated in Figure 32. Images of labeled yolk protein (A) and labeled yolk lipid (B) in two separated channels together with a combined image (C) are also presented. The CLSM micrographs showed the polyhedral granules of cooked egg yolk with the diameter range of 50-100 μm. The smaller granules (25-50 μm) were observed in CSEY, compared with those found in the cooked fresh yolk, regardless of salting methods and times. Disruption of lipoprotein in yolk during salting might be associated with the reduction in granule size of yolk. Heating applied might facilitate the dissociation of granules. This was in agreement with the more distribution of oil in CSEY. The majority of proteins in yolk are organized into micellar and granular structures together with polar and non-polar lipid molecules (Kiosseoglou, 2003). The greater release of free lipid from lipoprotein structure of CSEY was obtained as shown in the combined images. CLSM obtained after 4 weeks of salting had the highest release of lipid from yolk granule, associated with the increasing in green portion (protein portion). The microstructure of yolk mainly determined the characteristic of cooked salted egg yolk, which was different from cooked fresh yolk.

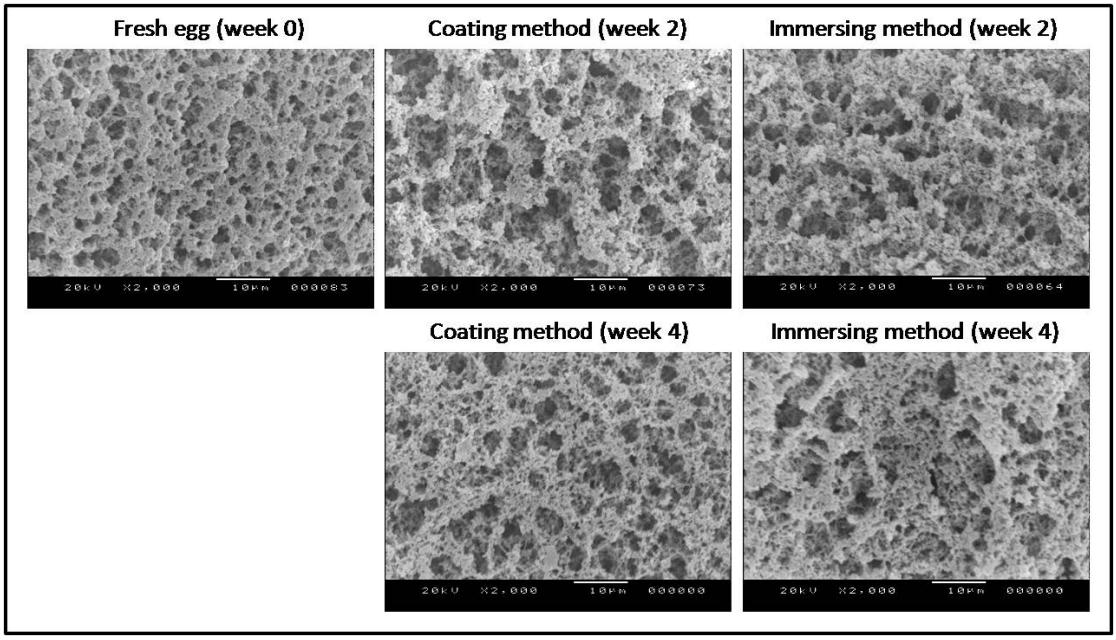



**Figure 31.** Scanning electron microscopic photograph of cooked egg white from salted duck egg with different methods at week 0, 2 and 4 of salting. Magnification: 2000X. Scale bar = 10 μm P= protein aggregate; arrow head= voids

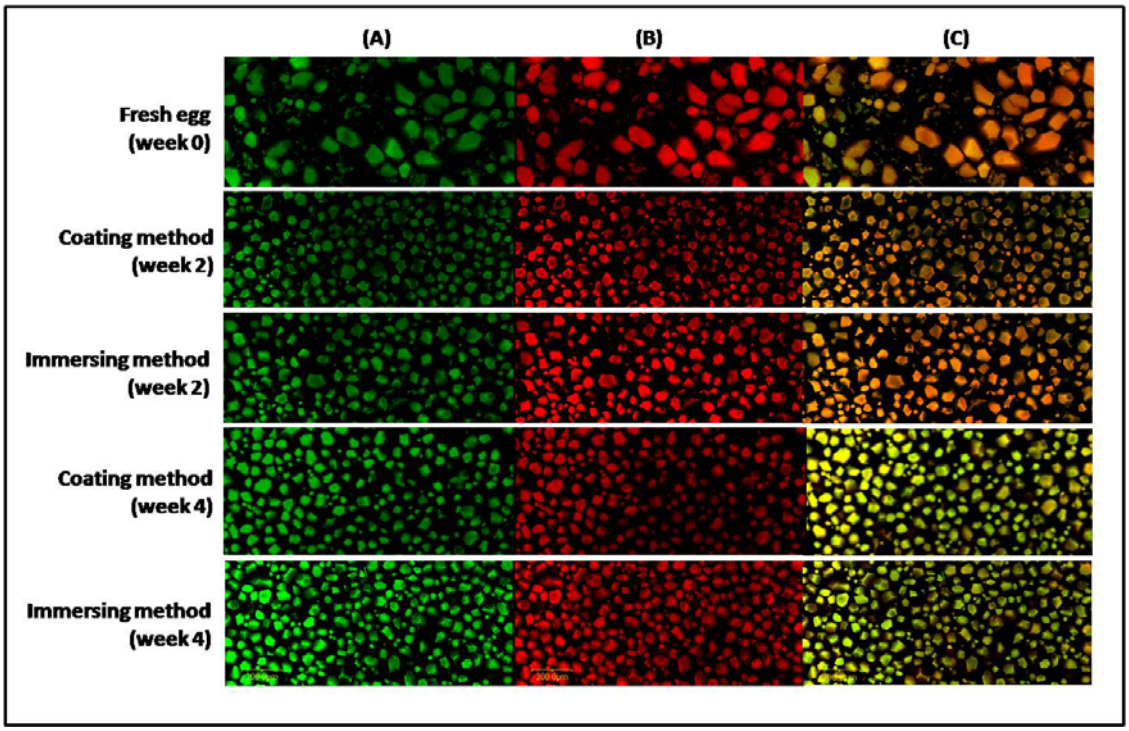



**Figure 32.** Confocal laser scanning microscopic (CLSM) photograph of cooked egg yolk from salted duck egg with different methods at week 0, 2 and 4 of salting. Protein distribution (A) and oil distribution (B) and combined image of protein and lipid (C). Magnification: 100X. Scale bar = 200 μ

## 5.5 Conclusions

Characteristics of salted egg white and yolk were affected by heating. Salting process caused the moisture removal and allowed the diffusion of salt into egg yolk and white. As a consequence, both cooked egg white and yolk contained the higher amount of salt. Salting affected the textural properties of cooked egg yolk and white to some degree. Immersing method had more impact on the softening of egg white gel than did coating method, particularly at the first week of salting. Moreover, oil exudation, a desirable characteristic of salted egg yolk, was more pronounced in egg yolk from coating method, especially at week 3 of salting. Marked decrease in hardness of cooked egg white with coincidental increase in hardness of egg yolk most likely determined the textural sensation during eating. After cooking, egg white gel turned to be coarser and lost water holding capacity, particularly as salting proceeded. Oil exudation with increased redness of cooked egg yolk was more pronounced with increasing salting time. Salting method therefore has the impact on the characteristic and properties of egg white and yolk after cooking.

# CHAPTER 6

# EFFECT OF NaCl ON THERMAL AGGREGATION OF EGG WHITE PROTEINS FROM DUCK EGG

## 6.1 Abstract

Thermal aggregation of duck egg white solution (1 mg protein/mL) were monitored in the presence of different NaCl concentration (0-6%, w/w) at the temperature range of 20-90°C. Egg white solution exhibited the higher turbidity with coincidental increases in surface hydrophobicity and the decreases in sulfhydryl group content as the heating temperatures increased from 70 to 90°C ($p< 0.05$), suggesting the increased formation of aggregate at high temperatures. The aggregation of egg white protein became more pronounced with increasing NaCl concentrations. As NaCl concentration increased, the negative charge decreased with coincidental increases in particle size of aggregate after heating to 90°C. As visualized by confocal laser scanning microscopy, the larger cluster of protein aggregate was observed with increasing NaCl concentrations. Major egg white protein with molecular weight of 45 kDa disappeared in the presence of 2-6% NaCl after heating at temperature higher than 80°C, regardless of concentrations. Therefore, NaCl, especially at high concentrations, could induce thermal aggregation of duck egg white protein, which could determine the characteristics of salted egg white after heating.

## 6.2 Introduction

Egg white is one of the best known food proteins, which is able to form a turbid gel upon heating. Egg white loses its fluidity around 60°C along with the subsequent formation of gel with the unique texture. Egg white shows 2 major endotherms, 60 to 65°C and 80 to 85°C, corresponding respectively to the denaturation temperatures of ovotransferrin and ovalbumin (Donovan *et al*., 1975).

The ovotransferrin is the most easily heat-denatured egg white protein, except when it forms a complex with iron, copper, or aluminum (Mine, 1995). Therefore, ovotransferrin plays a major role in the initiation of coagulation. The native protein is first converted to denatured proteins and then constructs insoluble aggregates of high molecular weight via intermolecular β-sheet interaction. Thermal coagulation of egg white is mediated by disulfide bonds, ionic interaction and hydrophobic interaction (Ma and Holme, 1982). Disulfide bonds and sulfhydryl (SH) group play an important role in the heat-induced aggregation of egg white proteins, while hydrophobic and electrostatic interactions initiate gel network formation after heat-induced partial denaturation of the protein (Plancken *et al*., 2005). Network of egg white gel also depends mainly on environmental conditions such as pH, ionic strength and temperature. Those factors affect availability of covalent and non covalent bonds for stabilization of tertiary structure and intermolecular aggregation of egg white protein (Sun and Hayakawa, 2002). The firmest gel of egg albumen was obtained when NaCl in the range of 0.05- 0.1 M was added (Holt *et al*., 1984). Salted egg white contained 4-7 % sodium chloride (Kaewmanee *et al*., 2009).

Salted egg is one of egg products commonly consumed in Asia and is considered as the preserved egg. Boiling is the popular cooking method for salted egg, in which the coarse texture and opaque gel is obtained, reflecting the characteristic of cooked salted egg white. The high salt level in salted egg might contribute to the thermal aggregation of egg white, in which the coagulum type gel could be formed. However, only gelation of egg white in the low salt concentration range has been reported (Xu *et al*., 1998; Matsudomi *et al*., 1985). However, no information regarding the thermal aggregation of egg white protein of duck egg in the presence of high salt content, representing salted egg, has been reported. Thus, the objective of this work was to study the effect of NaCl at various concentrations on thermal aggregation of egg white proteins during heating at different temperatures.

## 6.3 Materials and methods

### 6.3.1 Chemical reagents

8-anilino-1-naphthalenesulphonic acids (ANS), guanidine thiocyanate and β-mercaptoethanol (β-ME) were procured from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Sodium chloride was purchased from Merck (Darmstadt, Germany). 5,50-Dithiobis (2- nitrobenzoic acid) (DTNB) was obtained from Wako Pure Chemical Industries (Tokyo, Japan). Bovine serum albumin (BSA) was purchased from Fluka (Buchs, Switzerland).

### 6.3.2 Preparation of egg white solution

Duck eggs *(Anas platyrhucus)* with the weight range of 65-75 g were obtained within 1 day of laying from Satingphar, Songkhla province, Thailand. The eggs were used within 3 days after laying. Egg white was separated from the egg yolk and the chalazae were removed. The egg white was gently mixed and stored at 4$^{o}$C until used. Egg white (2 mg protein/mL) was mixed with 0.2 M Na-phosphate buffer (pH 7) containing different NaCl concentrations (0, 4, 8 and 12% w/v) at a ratio of 1:1 (v/v), in which the final NaCl concentrations were 0, 2, 4, and 6% (w/v), respectively.

### 6.3.3 Effect of NaCl concentrations on aggregation of egg white protein at different temperatures

Egg white solutions (1 mg protein/mL) with different NaCl concentrations were placed in test tube and placed in temperature-controlled water bath (Memmert, Schwabach, Germany). The solutions were heated from 20 to 90°C at heating rate of 1.62°C/min. A digital thermometer UN-305A Type K (Union, Kowloon, Hong Kong) was used to monitor the temperature of protein solution. The samples were collected when the temperature increased every 5°C and were cooled immediately with iced water (0-2°C). The samples were subjected to analyses.

### 6.3.4 Turbidity measurement

Egg white solutions were well mixed and placed in cuvette (light path length of 1 cm). The turbidity was determined by measuring the absorbance at 590 nm (Kitabatake and Kinekawa, 1995).

### 6.3.5 Determination of surface hydrophobicity

Protein surface hydrophobicity was measured according to the method of Benjakul *et al*. (2001) using 8-anilo-1 naphthalenesulfonic acid (ANS) as a probe. Egg white solutions were diluted to obtain the concentrations of 0.125, 0.25, 0.5 and 1 mg/mL using 0.2 M Na-phosphate buffer (pH 7) containing different NaCl concentrations (0, 2, 4 and 6% w/v). Two mL of prepared solutions were added with 10 μL of 10 mM ANS dissolved in 50 mM potassium phosphate buffer (pH 7.0). Fluorescence intensity was measured using a spectrofluorometer RF-1501 (Shimadzu, Kyoto, Japan) at the excitation and emission wavelength of 374 and 485 nm, respectively. Protein hydrophobicity was calculated from initial slope of plot of fluorescence intensity against protein concentration determined by the Biuret method (Robinson and Hodgen, 1940) using linear regression analysis. The initial slope was referred to as 'surface hydrophobicity, $S_0$ANS'.

### 6.3.6 Determination of total sulfhydryl group and disulfide bond contents

Total sulfhydryl group content was measured according to the method of Ellman (1959) as modified by Benjakul *et al*. (2001). To 2.5 mL of egg white solution, 3 mL of 0.2 M Tris- HCl buffer, pH 6.8 containing 8 M urea, 2% SDS and 10 mM EDTA were added. The mixture was then added with 0.25 mL of 10 mM DTNB in 0.2 M Tris-HCl buffer (pH 6.8) and incubated at 40°C for 40 min. The absorbance at 412 nm was measured using a spectrophotometer (UV-1601, Shimadzu, Kyoto, Japan). Reagent blank was prepared by replacing the sample with 0.2 M

phosphate buffer at pH (7.0) containing 2, 4 or 6% NaCl. Sample blank was prepared in the same manner, except that 0.2 M Tris-HCl (pH 6.8) was used instead of DTNB solution. Sulfhydryl group content was calculated using a molar extinction coefficient of 13,600 $M^{-1}cm^{-1}$. Disulfide bonds in proteins were also determined using 2-nitro-5-thiosulfobenzoate (NTSB) assay as described by Thannhauser *et al*. (1987).

### 6.3.7 Confocal laser scanning microscopy

Egg white solutions containing 0, 2, 4 and 6% NaCl heated to 90°C with heating rate of 1.62°C/min, followed by rapid cooling in ice water were subjected to microstructure determination using a confocal laser scanning microscopy (CLSM) (Olympus, FV300, Tokyo, Japan) following the modified method of Mineki and Kobayashi (1997). Fifty μL of heated egg white solutions added with 0.01% acridine orange were smeared on the microscopy slide. The CLSM was operated in the fluorescence mode at the excitation wavelength of 488 nm and the emission wavelength of 540 nm using a Helium Neon Green laser (HeNe-G). The images were taken using Fluoview program (Olympus Fluoview, FV300, Tokyo, Japan).

### 6.3.8 Measurement of zeta potential and particle size

Zeta potential and particle size of protein aggregates after heating to 90°C, followed by rapid cooling in iced water, were determined with ZetaPlus zeta potential analyzer (Brookhaven Instruments Corporation, Holtsville, NY, USA) at room temperature.

### 6.3.9 SDS- polyacrylamide gel electrophoresis (SDS-PAGE)

After heating to different temperatures (55-90°C), followed by rapid cooling, the suspensions were cooled rapidly in iced water and centrifuged at 10,000×g for 10 min using a refrigerated centrifuge (Beckman Coulter, Polo Alto, CA, USA). The supernatants were analyzed for protein pattern. Two hundred μL of supernatants were mixed with 100 μL of sample buffer (0.5 M Tris-HCl, pH 6.8 containing 4% SDS, 20% glycerol, 10% βME and 0.3% Bromophenol blue). Electrophoresis was run according to the method of Laemmli (1970) using 4% stacking gel and 10% separating gel. Samples (15 μL) were loaded and separated using a vertical gel electrophoresis unit (Mini-protein II; Bio-Rad Laboratories, Richmond, CA, USA) at the constant voltage of 15 mA/plate. The gels were stained with Coomassie Brilliant Blue R-125 (0.125%) in 25% methanol and 10% acetic acid. Destaining was performed using 40% methanol and 10% acetic acid. Protein standards were used to estimate the molecular weight of protein bands.

### 6.3.10 Protein determination

Protein content was determined by the Biuret method (Robinson and Hodgen, 1940) using bovine serum albumin as the standard.

### 6.3.11 Statistical analysis

A completely random design with three replications was used throughout the study. Data were presented as mean values with standard deviations. One-way analysis of variance (ANOVA) was carried out and means comparisons were run by Duncan's multiple range tests (Steel and Torrie, 1980). Statistical analysis was performed with the statistical program (SPSS for windows, SPSS Inc, Chicago, IL, USA).

## 6.4 Results and Discussions

### 6.4.1 Turbidity

Turbidity development of duck egg white solutions (1 mg protein/mL) heated from 20 to 90°C with the constant heating rate of 1.62°C/min (Figure 33) as monitored by the increase in $A_{590}$ is shown in Figure 33. No changes in $A_{590}$ were observed in all samples heated up to 65°C, regardless of NaCl concentrations ($p > 0.05$). At temperature higher than 70°C, the marked increases in $A_{590}$ were noticeable but the degree of increases varied with NaCl concentrations. The higher $A_{590}$ was found in the sample heated at higher temperatures ($p < 0.05$). At the same heating temperature, higher $A_{590}$ was observed with increasing NaCl concentrations ($p < 0.05$). Increase in $A_{590}$ or turbidity was due to the formation of large coagulums of heat-denatured egg white protein molecules and scattering effect of coagulums. The differences in turbidity were most probably associated with the varying size and rate of protein aggregation, which was governed by NaCl content. In general, egg white consists of water and proteins with a few minerals. Proteins constitute 90% of the dry matter of egg white (Linden and Lorient, 2000). It is a colloidal mixture of various proteins (Hatta *et al*., 1997). During heating, egg white proteins start to denature at a temperature greater than 70°C. Different proteins in egg white have the varying denaturation temperatures. The denaturation temperatures of ovalbumin, ovotransferrin, ovomucoid and lysozyme are 84, 61, 77, and 75°C, respectively (Belitz *et al*., 2009; Donovan *et al*., 1975). Ovalbumin, a major protein, constitutes 54% of egg white protein. During thermal denaturation and aggregation of egg white and ovalbumin, the stable intermolecular β-sheet structures were formed (Nakamura and Doi, 2000). The β-sheets are formed in extensive regions antiparallel between ovalbumin molecules. An interaction with the β-sheets of ovotransferrin and lysozyme is also observed (Nakamura and Doi, 2000). At the higher temperature used, the proteins underwent the aggregation to a higher extent as indicated by the higher $A_{590}$. The higher temperature could provide the sufficient enthalpy for

disruption of protein conformation, thereby inducing the formation of subsequent aggregation.

$A_{590}$ was higher in the sample with higher NaCl concentration. NaCl could induce the conformation changes of egg white protein mediated by the increasing ionic strength of a solution. $Na^+$ and $Cl^-$, especially, at higher concentrations, could have the impact on protein conformation by disruption ionic interaction of protein molecules. The ability of electrolytes to influence the conformation and stability of protein depends on the concentration and/or ionic strength of the salt (Von Hippel and Schleich, 1969). At low salt concentration (low ionic strength), the stabilizing effect has been attributed to an electrostatic response (Von Hippel and Schleich, 1969). At ionic strength > 0.5, the ability of salts to stabilize protein structure has been related to the preferential hydration of the protein molecule as a result of salt induced alteration of the water structure in the vicinity of the protein (Arakawa and Timasheff, 1982). Increasing solubility of protein molecules increases their susceptibility to thermal denaturtion (Boye *et al*., 1997). Also, at high NaCl content, 'salting out' effect became more pronounced. This led to the precipitation or coagulation of proteins. Thus, salt concentration played a crucial role in the protein aggregation of duck egg white proteins.

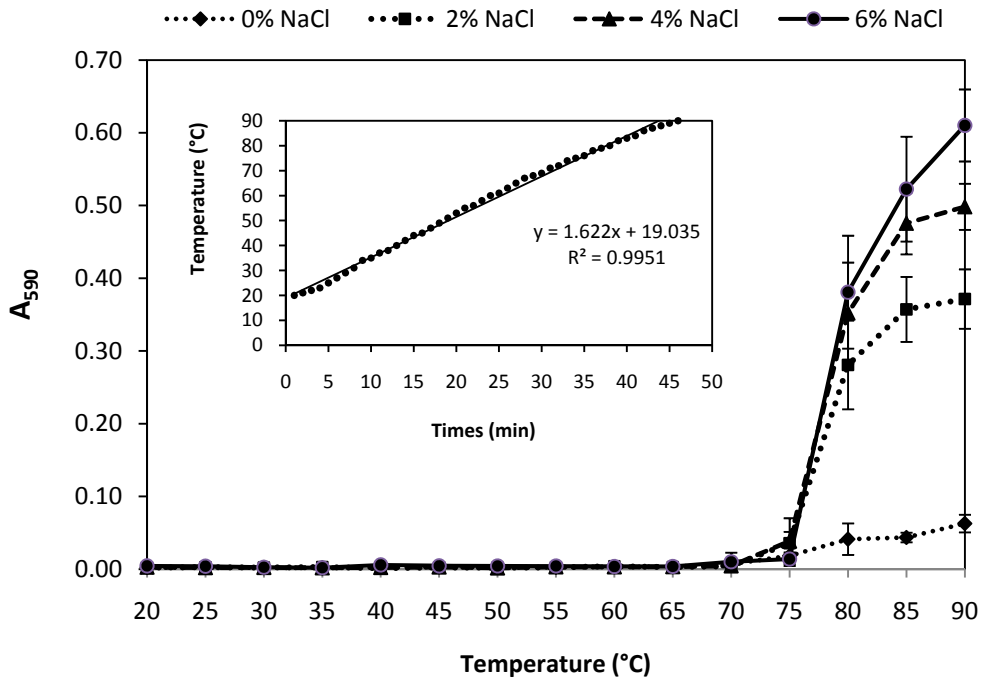



**Figure 33.** Turbidity of duck egg white solution (1 mg protein/mL) containing NaCl at different levels during heating from 20 to 90°C. Inset figure present a relation between temperature and time of egg white solution at different NaCl concentrations during heating. Bars represent standard deviation (n=3).

### 6.4.2 Surface hydrophobicity

Surface hydrophobicity of egg white solution in the presence of different NaCl concentrations (0, 2, 4 and 6% w/v) during heating from 20 to 90°C is depicted in Figure 34A. The increase in surface hydrophobicity was observed at temperature higher than 65°C, irrespective of NaCl concentrations ($p < 0.05$). After heating at 70°C, gradual increase in surface hydrophobicity content was observed ($p < 0.05$). At the same temperature, higher surface hydrophobicity was observed as NaCl concentrations increased ($p < 0.05$). Plancken *et al*., (2006) found that the increased surface hydrophobicity of egg white solution could be observed at temperatures above

55°C. This increase might occur in two steps: the exposure of hydrophobic protein of the unfolding of ovalbumin and lysozyme at higher temperature range and more heat sensitive proteins at the lower temperatures. For the samples containing NaCl at levels of 0-4%, the increase was found when being heated from 65 to 80°C ($p< 0.05$). When the samples were heated at 80-90°C, the constant or decreased surface hydrophobicity was noticeable. However, the continuous increase in surface hydrophobicity but at the lower increasing rate was found in the sample containing 6% NaCl. In general, the level of unfolding of protein can be measured by the value of the surface hydrophobicity (Plancken *et al*., 2006). The decreases in surface hydrophobicity of samples containing no salt or with 2% NaCl were possibly caused by the interaction of hydrophobic domains. As a result, those proteins were hidden inside the aggregate. Nevertheless, in the presence of high NaCl (6%), the unfolding still proceeded, though the some aggregates were formed simultaneously. With increasing temperatures, proteins became unfolded and hydrophobic domains were exposed. In the presence of NaCl (2-6%), egg white protein had the greater changes in suface hydrophobicity, which coincided with the higher protein turbidity (Figure 33). This indicates that NaCl induced the protein unfolding and the formation of insoluble turbid aggregates facilitated by increased hydrophobic interactions during heating.

### 6.4.3 Sulfhydryl group content

Changes in total sulfhydryl (SH) group content of egg white solution with different NaCl concentrations (0, 2, 4 and 6% w/v) during heating from 20 to 90°C are shown in Figure 34b. At the initial temperature (20°C), total amount of SH group of all treatments was 3.11 mole/$10^5$ g proteins. Sample containing 6% NaCl showed the slightly higher SH group content (3.26 mole/$10^5$ g protein). Salt at higher level might help in exposing SH group of proteins, which could interact with DTNB more effectively. Egg white protein has substantial cysteine content (2.17g/100g dry wt) (Watkins, 1995). Ovalbumin is the only fraction that contains the free SH groups (Mine, 1995), most of which exist in the interior of the molecule in the native state. Others egg white fractions, such as ovotransferrin, ovomucoid and lysozyme contains

S-S bridges (Onda *et al*., 1997). The constant increase of total SH group content was obtained during heating from 20 to 70°C ($p > 0.05$). It was assumed that most SH group was mainly buried in the protein core. After heating at 70°C, slight decrease in total SH group content was observed in samples containing 4 and 6% NaCl, whereas the higher decrease in total SH group content was obtained in sample containing no NaCl or 2% NaCl ($p < 0.05$). The lowest total SH group content was found after heating up to 90°C ($p < 0.05$). For SS content, the formation of disulfide bond was in agreement with the decreases in SH group content (data not shown). At higher temperature, proteins underwent the conformational changes to a greater extent. The exposed SH groups were more likely oxidized, in which disulfide bonds were formed. In the presence of high NaCl, the disulfide bond formation could be impeded. Ion-modified molecules might have the molecular constraints, in which the oxidation of SH group was impeded, whereas hydrophobic interaction or other bondings stabilizing the aggregates were still formed to some degree. The presence of $O_2$ is also reported to affect the disulfide bond formation of egg white proteins. Beveridge and Arntfield (1979) demonstrated that oxygen containing egg white solutions showed rapid losses of total SH groups, while solutions flushed with nitrogen showed no decrease in total SH content after 40 min of heating at 91°C. The result indicated that NaCl concentration had the impact on the disulfide bond of egg white protein during heating process.

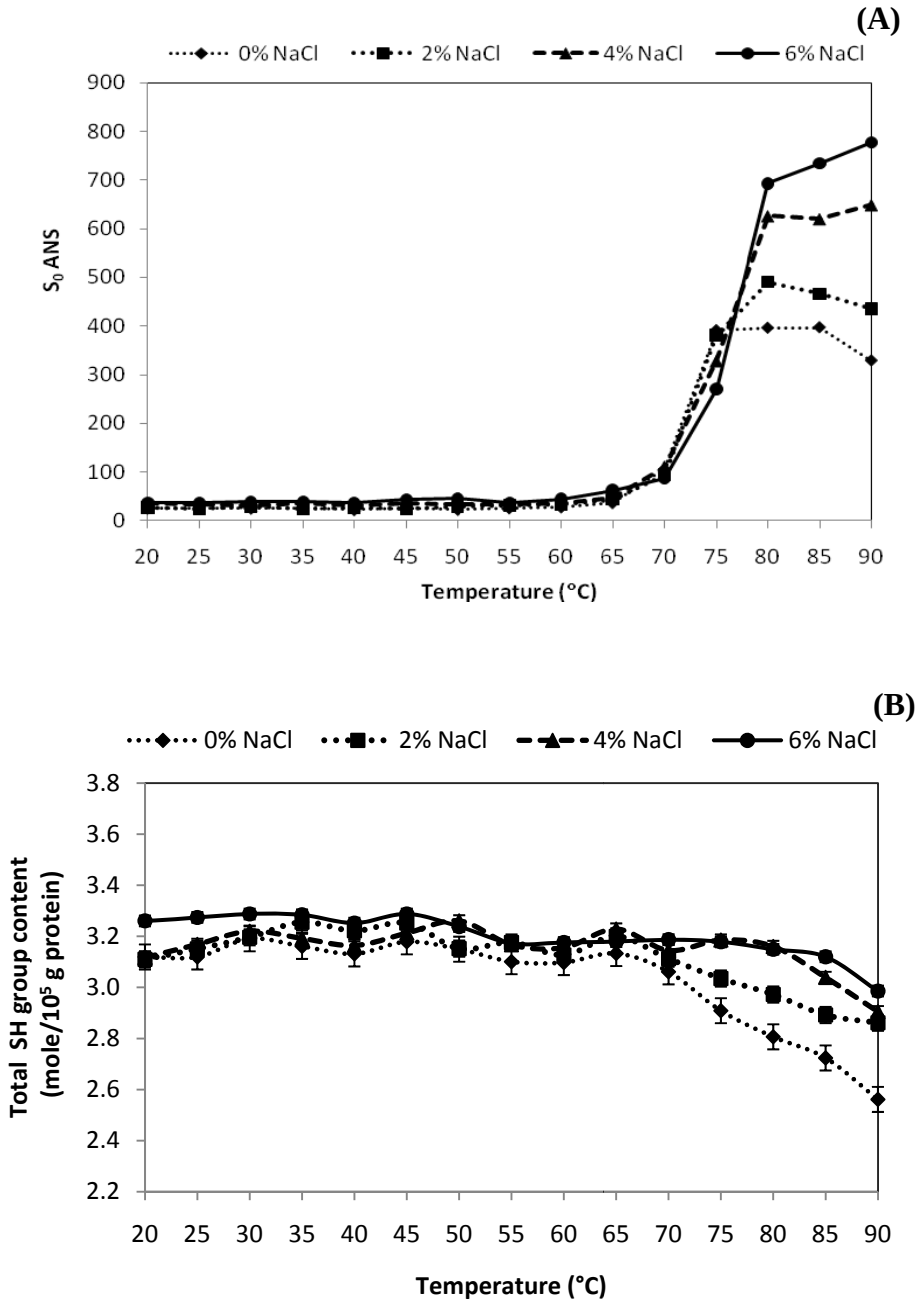



**Figure 34.** Surface hydrophobicity ($S_0$ANS) (A) and total sulfhydryl group content (B) of duck egg white solution (1 mg protein/mL) containing NaCl at different levels during heating from 20 to 90°C. Bars represent standard deviation (n=3).

### 6.4.4 Zeta potential

The zeta potential of duck egg white solution containing different levels of NaCl after heating to 90°C is shown in Table 19. The highest negative charge was found in solution with no NaCl ($p < 0.05$). Solution possessed less negative charge with increasing NaCl concentrations ($p < 0.05$). $Na^+$ ion might neutralize the negative charge of protein during heating as indicated by the lowered negative charge as NaCl concentration increased (Figure 35). When 6% NaCl was present in the solution, proteins had the positive charge after heating. Progressive decreases in electronegativity of the protein molecule with the lowered electrostatic repulsion between protein molecules could occur, resulting in the induced protein aggregation. Averages particle size of egg white protein after heating to 90°C is shown in Table 1. Particle size of egg white protein was obviously increased in the presence of NaCl, especially at high concentration ($p < 0.05$). The increase in particle sizes of heated egg white protein was in accordance with the increase in turbidity (Figure 33). The aggregation of heated protein molecules not only affected the size of particulates formed, but also had the impact on the charge of surface of aggregate. These results suggested that NaCl played a role in modification of protein charge. This might govern the physicochemical changes of protein molecules, both native and denatured forms. The charges were markedly altered after the sufficient energy was provided along with the involvement of $Na^+$ or $Cl^-$ in binding or interacting with protein molecules. As a consequence, the aggregation or coagulation was enhanced in the presence of NaCl at high level during heating at high temperature as evidenced by the increase in particle size of protein aggregate (Figure 35).

**Table 19.** Zeta potential and particle size of duck egg white solution (1 mg protein/mL) containing NaCl at different levels after heating up to 90°C.

| NaCl (%) | Zeta potential (mV) | Particle size (nm) |
|---|---|---|
| 0 | -18.30±2.55[*, a†] | 239.90±5.96[a] |
| 2 | -14.89±3.19[ab] | 3025.33±242.11[b] |
| 4 | -10.76±4.13[b] | 4365.65±266.23[c] |
| 6 | +12.61±6.46[c] | 4917.77±415.69[d] |

*Mean±SD (n=3)
† Different superscripts the same column indicate significant differences ($p < 0.05$).

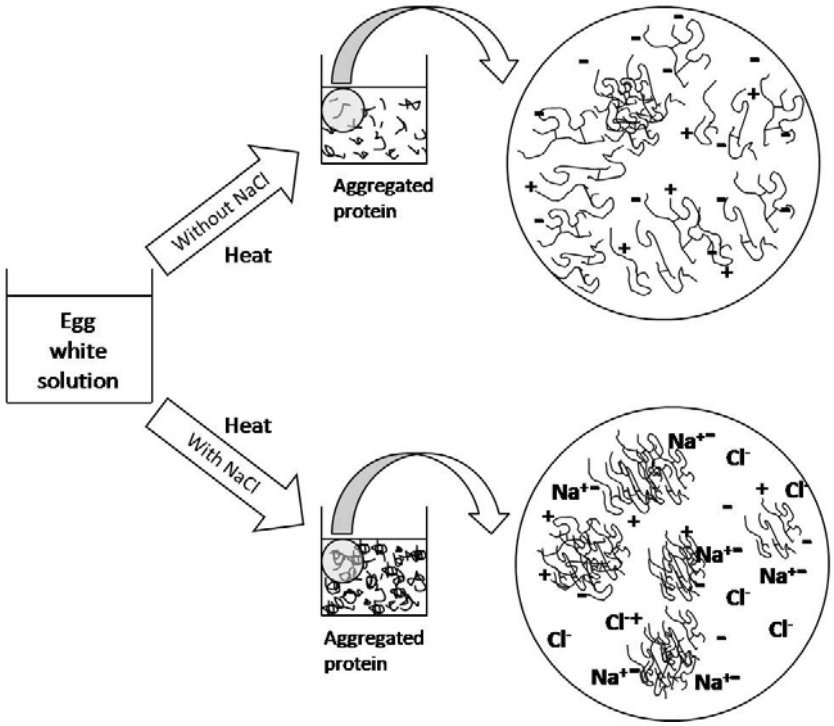



**Figure 35.** Proposed scheme for egg white protein aggregation without and with salt at high concentration.

### 6.4.5 Confocal laser scanning microscopic image

Confocal laser scanning microscope (CLSM) micrographs of duck egg white solution in the absence and presence of NaCl at different concentrations after heating to 90°C are shown in Figure 36. Without NaCl, egg white solution contained the small aggregate with the small number of cluster formed. In the presence of 2% NaCl, a large aggregate with increasing numbers of clusters was developed. When protein solution was heated in the presence of 6% NaCl, very large aggregates were formed and those aligned themselves as the large cluster (Figure 36). The increase in size of aggregate or cluster visualized by CLSM was in accordance with the increase in particle size determined by the particle size analyzer (Table 19). The large cluster of protein aggregated was reflected by the highest turbidity of solution heated to 90°C in the presence of 6% NaCl, which was in the range found in salted egg white (2-6%) (Kaewmanee *et al.*, 2009). When salted egg white was boiled or cooked, the coarse texture with opaque color is generally found as its characteristic, which is different from cooked fresh egg white (without NaCl). The latter has the smooth texture with the lower turbidity. This was explained by the large aggregate with the coagulum type of egg white protein in the former (salted egg) (Figure 35).

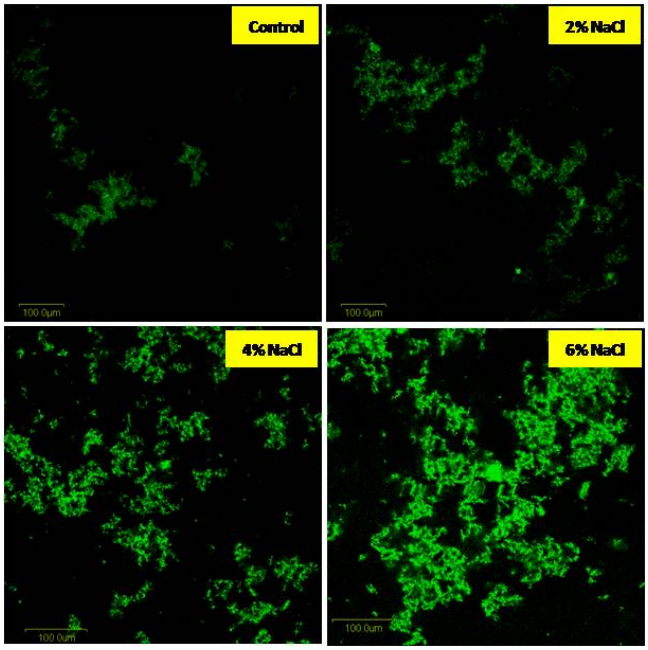



**Figure 36.** Confocal laser scanning microscopic (CLSM) photograph of duck egg white solution (1 mg protein/mL) containing NaCl at different levels after heating to 90°C. Magnification: 600×. Scale bar = 100 μm

### 6.4.6 Protein patterns

Protein in egg white solution lost the solubility at varying degrees after heating to different temperatures. The protein patterns of supernatant obtained after centrifugation (1000g, 10 min) of egg white solutions are shown in Figure 37. Egg white supernatant contained 5 major proteins, representing ovomucin (A), ovotransferrin (B), avidin (C), ovobumin (D) and ovomucoid (E), having the molecular weight of 110, 76, 68.3, 44.5 and 28 kDa, respectively (Belitz et al. 2009, Osaga and Feeney, 1974). When the solutions were heated to 55-65°C, egg white supernatant contained ovomucin, ovotransferrin and ovobumin bands, whereas smear bands of avidin and ovomucoid were observed. In the absence of NaCl, the band intensity of ovomucin, ovotransferrin and ovobumin decreased after heating at temperature above 80°C. In the presence of NaCl, the higher decreases in protein

band intensity were observed. Disappearance of most protein including ovabumin was noticeable in the supernatant of solutions containing NaCl heated to 85 and 90°C. The disappearance was more pronounced at 90°C, compared with 80°C. The result confirmed that NaCl could induce egg white protein aggregation when heating temperature was in the range of 85-90°C.

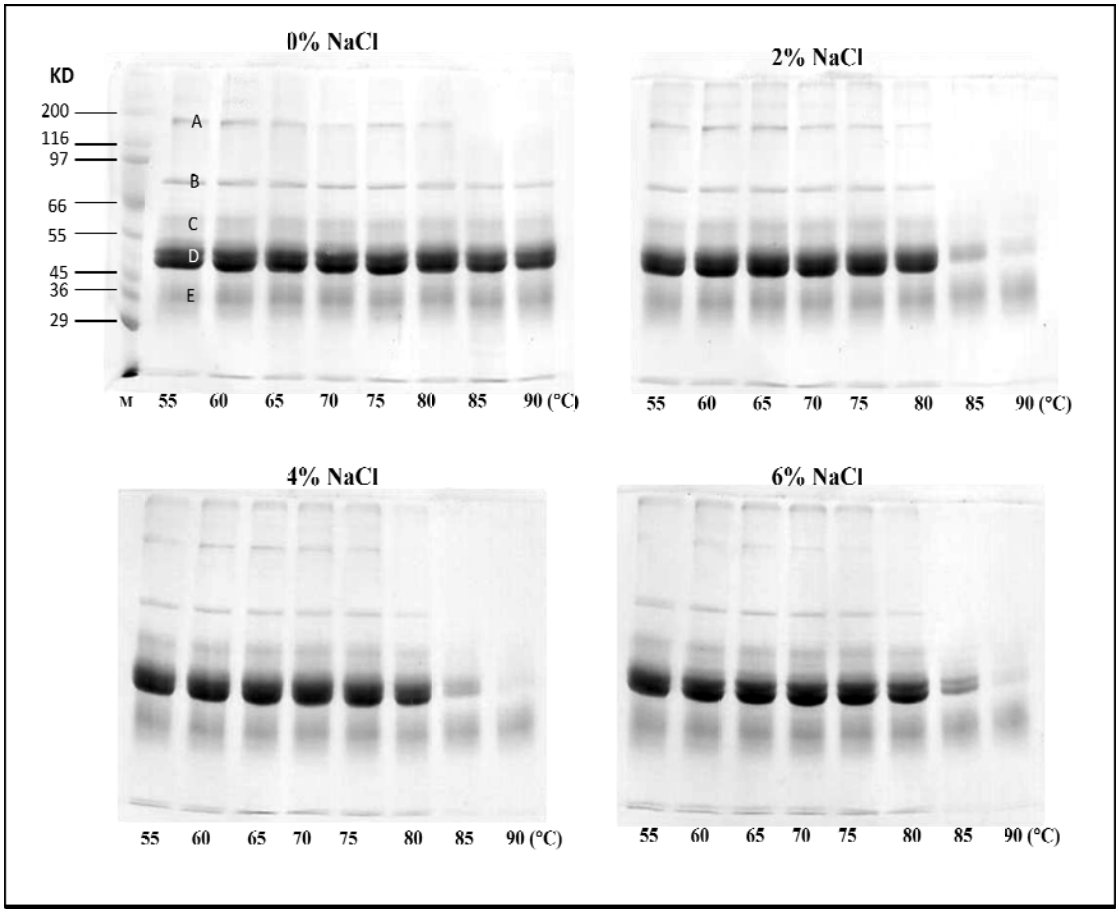



**Figure 37.** Protein patterns of the supernatant from duck egg white solutions containing NaCl at different levels after heating from 55 to 90°C. A: ovomucin; B: ovotransferrin; C: avidin; D: ovobumin; E: ovomucoid; M: molecular weight standard

## 6.5 Conclusions

Heat-induced aggregation of duck egg white solution was governed by heating temperature and NaCl concentrations. Higher NaCl concentration was associated with the induced thermal aggregation of egg white proteins mainly by induction of hydrophobic interaction or ionic interaction. NaCl plays a role in the formation of coagulum type gel of cooked salted egg white, which had the course texture with opaqueness.

# CHAPTER 7

# EFFECT OF SODIUM CHLORIDE AND DEHYDRATION ON VISCOELASTIC PROPERTIES AND THERMAL-INDUCED TRANSITIONS OF DUCK EGG YOLK

## 7.1 Abstract

The effects of NaCl and dehydration on the linear viscoelastic behavior of egg yolk were evaluated. An increase in NaCl concentrations from 0 to 3% (w/w) resulted in a remarkable change in the linear viscoelastic behaviour by inducing a sol-gel transition, specifically. The transition was more pronounced when 1.5%w/w NaCl was incorporated. The influence of dehydration on viscoelastic behavior of egg yolk at various NaCl concentrations was also examined. It was found that the effect of dehydration on aggregation and network formation was predominant than that of the NaCl addition. Nevertheless, at a lower degree of dehydration, the addition of NaCl could modulate the viscoelastic behaviour of egg yolk, resulting in a well-developed gel network. Addition of NaCl into egg yolk could stabilize the protein molecules as evidenced by an increase in denaturation temperature as well as a delay in gel network formation. Conversely, dehydration had a little impact on the gel formation of egg yolk during heating. DSC study suggested a progressive increase in denaturation temperature, but a lower enthalpy when NaCl concentration and degree of dehydration increased. As visualized by a scanning electron microscope, the denser network with smaller voids was observed in egg yolk gel with increasing NaCl concentration and degree of dehydration.

## 7.2 Introduction

Egg yolk is used world-wide as a key ingredient in many food products, such as mayonnaise, dressings, creams, custard, and a wide variety of confectionery and bakery products. Its popularity is due to the unique sensory characteristics that egg yolk provides to food products, as well as its highly nutritional and functional ingredients, including high-quality food proteins. Egg yolk is not a simple protein solution but must be considered together with lipids (Guerrero *et al*., 2004). All of lipids appear to be associated with at least two proteins, namely, vitellin and vitellenin (Shenstone, 1968). Egg yolk also constitutes a liquid dispersion that can be fractionated into plasma (75–81%) and granules (19–25%). The plasma is composed of 85% Low Density Lipoproteins (LDL) and 15% livetin. LDL are spherical micelles with a core of non-polar lipids (triglycerides, cholesterol, and cholesteryl esters) surrounded by a layer of apoproteins and phospholipids (Martin *et al*., 1964). The granules consist of 70% High Density Lipoproteins (HDL), 16% Phosvitin and 12% LDL (Burley and Cook, 1961). Consequently, the surfaces of both micelles and granules are dominated by proteins, which seem to be the yolk constituent that largely determines functional properties and stability to native egg yolk.

Salted duck eggs, also known as preserved eggs, are widely consumed in Asia. All changes of duck egg characteristics occurring during the salting most likely determine the preferential characteristics of salted egg. Gel-like texture of salted egg yolk with desirable characteristics includes orange color, oil exudation and gritty texture (Chi and Tseng 1998). During salting, the yolk gradually becomes solidified and hardened (Chi and Tseng 1998). The salted egg yolk turns to be an elastic gel as salting time increases. Several types of particles like spheres, granules or low-density lipoproteins, suspended in a protein solution or plasma have the ability to form gels (Woodward and Cotterill, 1897). During salting, salt migrates from coating-paste into egg white, then to egg yolk. Thus, the salt content in egg yolk increases with salting time and dehydration of egg yolk and diffusion of salt into egg white and egg yolk occurs concurrently during salting (Chi and Tseng, 1998). Wang

(1991; 1992) indicated that the formation of salted yolk could be related to the diffusion speed and final concentration of NaCl. The dehydration and salt content are key factors affecting the hardness of salted yolk (Kaewmanee *et al*., 2009). Moreover, the effect of ionic strength of NaCl on the solubility and emulsifying properties of egg yolk was reported by Anton and Gandemer (1997), Anton *et al*. (2003), Martinez *et al*. (2007) and Sousa *et al*. (2007).

Gelation of yolk can be envisaged as a process in which heat-induced denaturation of particle-stabilizing protein molecules leads to particle destabilization, that gives rise to interparticle network formation (Kiosseoglou, 2003). Thermal induced sol-gel transition of native egg yolk has been studied by using temperature-controlled small-amplitude oscillatory strain (SAOS) measurements and DSC (Cordobes *et al*., 2004). The changes in the protein system induced aggregation of partially denaturated protein molecules, and association of aggregates (Clark, 1998). However, the effect of NaCl and dehydration on thermal gelation of duck egg yolk, representing gelation of yolk at high salt and solid contents commonly found in salted egg, have not been elucidated. Therefore, the objective of this study was to evaluate the influence of NaCl and dehydration on viscoelastic and thermal properties, and microstructure of duck egg yolk subjected to thermal process.

## 7.3 Materials and methods

### 7.3.1 Duck egg collection

Duck eggs *(Anas plotyrhyncus)* with the weight range of 65-75 grams were obtained from Satingphar, Songkhla Province, Thailand. They were used within 3 days after laying**.**

### 7.3.2 Egg yolk preparation

Eggs were manually broken and the white carefully separated from the yolk. The vitellin membrane was then disrupted with a blade and yolk was collected

in a beaker. The collected liquid yolk was preserved by adding 0.02%w/w sodium azide. Total solid content (TSC) of yolk was determined using a hot oven method by heating samples at 105°C for 6 hours (Kiosseoglou & Paraskevopoulou, 2005).

### 7.3.3 Effect of NaCl concentrations on rheological behavior and thermal properties of egg yolk

Native yolk (TSC = 55.5 ± 0.4 %w/w) was added with finely ground NaCl at different concentrations (0, 0.5, 1, 1.5, 2, 2.5 and 3 %w/w). The mixtures were manually mixed thoroughly in a polyethylene bag without entrapping air until NaCl was completely dissolved. The prepared samples were then stored at room temperature (approximately 25-28°C) for 30 minutes prior to rheological experiments. Particularly, samples containing NaCl concentration of 0, 1, 1.5 and 3 %w/w were used in temperature sweep experiment Furthermore, samples containing NaCl at levels of 0, 1 and 3%w/w were examined through DSC measurement.

### 7.3.4 Effect of dehydration and NaCl coupling on rheological behavior and thermal-induced properties of egg yolk

Native yolk was filled into a 15 cm dialysis membrane tube *(*Seamless Cellulose Tubing, UC*20-32*-1000; Viskase Sales Corp., Tokyo, Japan) with a molecular weight cut-off of 12000-14000 daltons. Two ends of tubes were tied tightly and subjected to osmotic dehydration with polyethylene glycol powder (PEG 20,000 Fluka AG, Buchs, Switzerland) for 6, 12 and 24 h at room temperature (25-28°C). Subsequenly, tubes containing yolk were washed with isotonic solution (0.17 M NaCl) to remove the residual PEG. Prepared yolks with different degree of dehydration were manually mixed with NaCl to obtain different final concentrations (0, 1 and 3%wt) in polyethylene bag. These samples were then studied through rheological and differential scanning calorimetric measurements. Stress sweep and frequency sweep experiments were performed on all dehydrated egg yolk sample with three levels of salt concentration. In addition, temperature sweep and DSC

experiments were conducted on the dehydrated samples with 0 and 3%w/w salt additions.

### 7.3.5 Rheological experiments

All rheological experiments were performed using a stress-controlled Gemini $HR^{nano}$ rheometer (Malvern instruments, UK). The instrument was equipped with a parallel plate of 25 mm diameter and 1 mm gap height. The operating temperature was controlled by a peliter plate. All samples were maintained in the instrument for 20 minutes prior rheological tests. Firstly, shear stress sweep tests were performed on each sample at a constant frequency of 1 Hz in order to establish a region of linear viscoelasticity (LVE). Subsequently, dynamic viscoelasticity frequency sweep measurements were carried out at a constant stress within the LVE region (Aguilar *et al*., 2007).

Temperature ramp experiments were performed at 2°C/min heating rate and constant frequency of 1 Hz from 20 to 95°C. A low-viscosity paraffin oil (25–80 mPas) (Appli-Chem, Darmstadt, Germany) was used as a sealing fluid for all measurements in order to avoid coagulation of yolk protein at surface, and to prevent moisture loss. All rheological, measurements were performed using two replicates.

### 7.3.6 Differential scanning calorimetry experiments

A thermal-induced transition of egg yolk was determined using the differential scanning calorimetry (DSC) (Perkin-Elmer, Model DSCM, Norwalk, CT, USA). Samples were scanned at the rate of 2ºC/min over the temperature range of 20-120ºC. Denaturation temperature ($T_{max}$) was measured and the denaturation enthalpy ($\Delta H$) was estimated by measuring the area under the DSC transition curve.

### 7.3.7 Microstructural analysis

Microstructure of egg yolk gel obtained from temperature ramp experiments after being heated at 95°C were investigated using a scanning electron microscope (SEM). Gel samples were prepared by immersing in a 2.5% glutaraldehyde solution for 72 hours. They were then washed thoroughly with distilled before post-fixing in a 1% osmium tetroxide solution at 4°C. After rinsing for an hour with distilled water, they were dehydrated in an ethanol series (50-100%) and dried at the critical point. Each dried sample was then mounted on a bronze stub and coated with gold (Sputter coater SPI-module, West Chester, PA, USA) prior to microscopic examinations.

### 7.3.8 Statistical analysis

A completely random design with three replications was used throughout the study. Data were presented as mean values with standard deviations. One-way analysis of variance (ANOVA) was performed and means comparisons were analyzed by Duncan's multiple range tests (Steel and Torrie, 1980). Statistical analysis was performed using commercial statistical program SPSS version 16.0 (SPSS Inc, Chicago, IL, USA).

## 7.4 Results and Discussion

### 7.4.1 Effect of NaCl on rheological behavior of egg yolk

Stress sweep experiments were initially performed in order to determine the linear viscoelastic region. Such experiments can provide the information on critical stress ($\sigma_c$) that is the maximum stress up to which storage ($G'$) and loss ($G''$) moduli remain constant. $G'$ is proportional to the extent of the elastic component due to cross-linking, entanglement or aggregation in the system. $G''$ is

rational to the extent of the viscous component (from the liquid-like portion) of the system. The critical stress can be referred to as the dynamic yield stress which is the minimum stress required to cause the material to flow. A wider LVE region, thus a larger $\sigma_c$, indicates the system stability to better resist the external stress. Stress sweep experimental results of egg yolk as a function of NaCl concentrations are shown in Figure. 38(A). It is evident that an increase in the NaCl levels from 0-3%w/w resulted in a remarkable growth in the storage modulus. The values of critical stress and loss tangent, i.e. $\tan(\delta) = G''/G'$, obtained at different NaCl concentrations are shown in Figure. 38(B). It can be seen that the critical stress increased sharply with increasing salt level. In addition, the evolution of tan(δ) with salt concentration indicated that a sol-gel transition occurred at salt concentration approximately 1.5%w/w.

The evolution of mechanical spectrum as a function of NaCl concentration is shown in Figure 39A. It is observed that the frequency dependence on the storage and loss moduli became less pronounced with increasing NaCl levels. This implied that there was a change in network structure with increasing NaCl concentrations from 0-3%w/w. This phenomenon was confirmed in Figure. 39(B) by plotting tan(δ) at various frequencies as a function of NaCl concentration. It is apparent from this figure that two regions of different behaviour can be defined. Below 1.5%w/w NaCl concentration, egg yolk exhibited fluid-like behaviour, i.e. $\tan(\delta) > 1$. On the contrary, a solid-like behaviour of egg yolk was found beyond the concentration. Such findings are consistent with the observations from stress sweep experiments. The change in egg yolk behaviour could be attributed to the association of proteins which dehydrate by the addition of NaCl Harrison and Cunningham (1986) reported an increase in viscosity of egg yolk when 10%w/w NaCl was incorporated as a result of the increased association of proteins and dehydration caused by salt. The ability of electrolytes to influence the conformation of globular proteins has been reported to depend on the concentration of salt and/or ionic strength (Shenstone, 1968). At low ionic strength, the influence that salt exerts on protein structure is governed by electrostatic interactions. At high ionic strength, depending on the system, the ability of salts to stabilize protein structure is related to the

preferential hydration of the protein molecule as a result of salt induced alteration of water in the vicinity of the protein (Harrison and Cunningham, 1986). Protein-protein interactions are favored over protein-solvent interaction at high salt content due to the lack of water molecules, which may lead to aggregation of protein molecules as reported by Puppo and Anon (1999) for soybean protein dispersions. Generally, granules exist as insoluble particles in egg yolk, primarily as a result of strong interactions between lipoproteins and phosvitin molecules mediated by phosphocalcic bridges (Chang *et al*., 1977; Causeret *et al*., 1991).

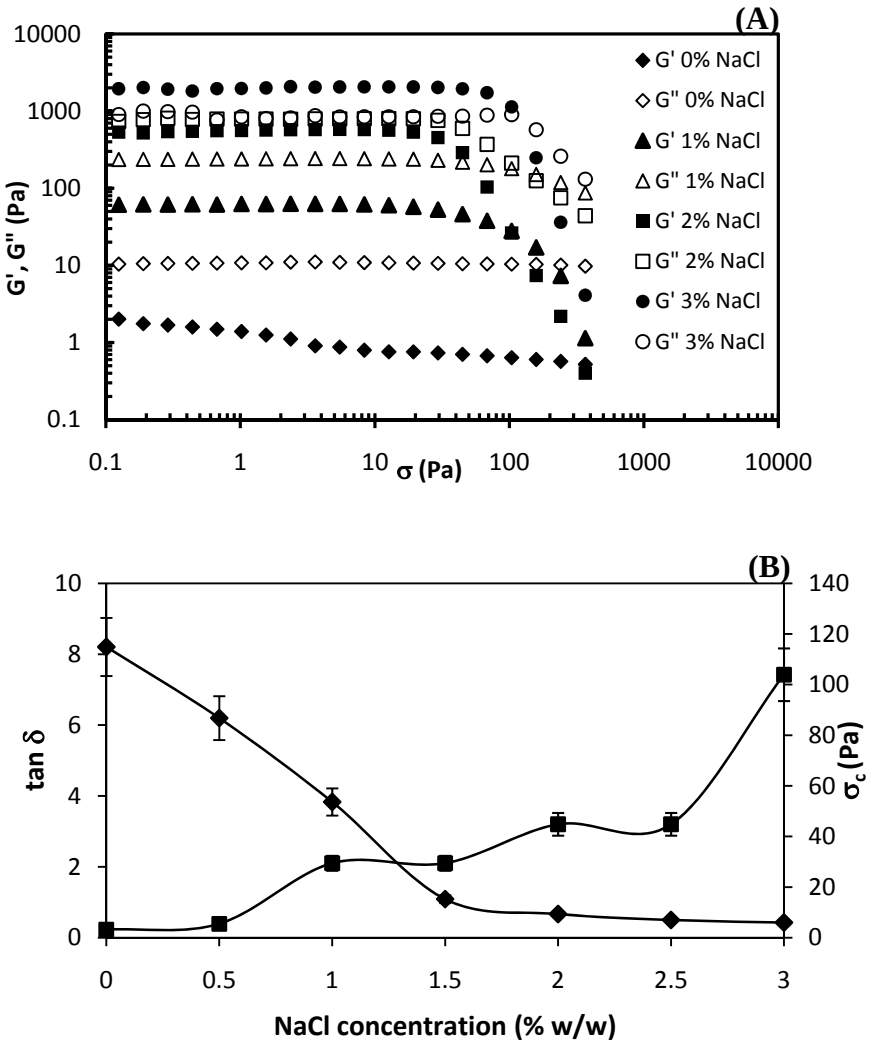



**Figure 38.** Stress sweep profiles of duck egg yolk at different NaCl concentrations showing evolutions of (A) storage and loss moduli and (B) critical stress (-■-) and loss tangent (-◆-).

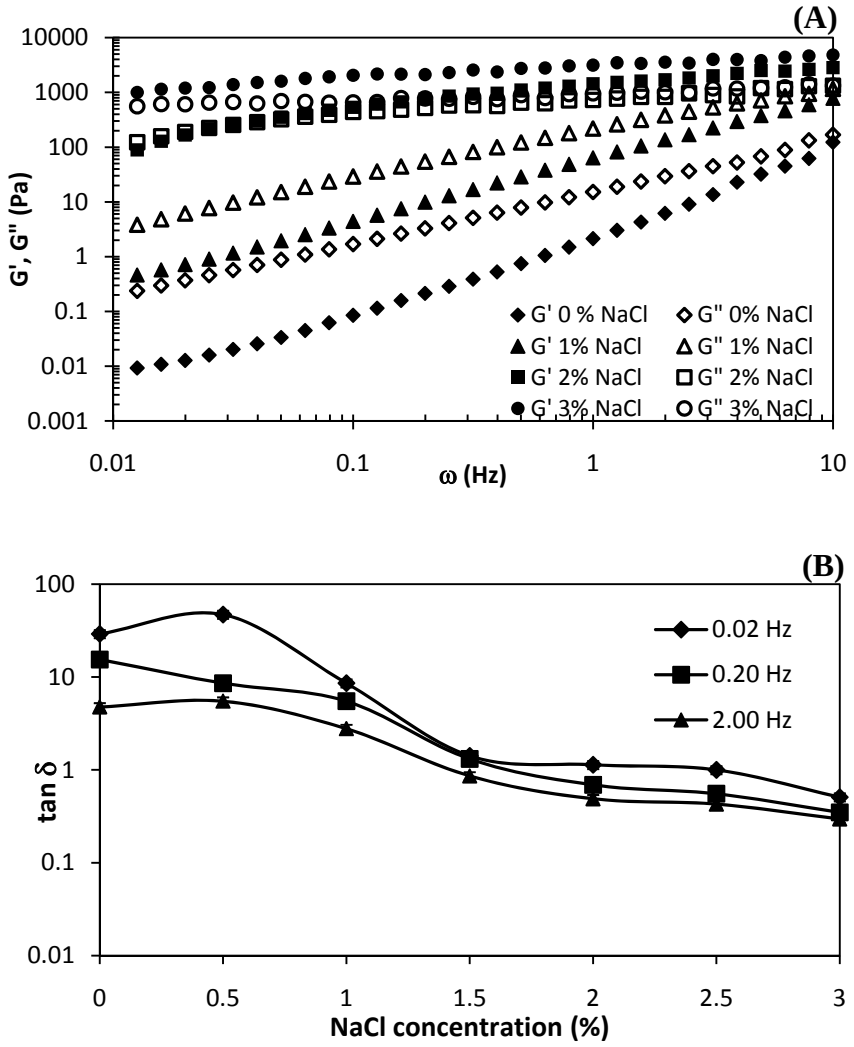



**Figure 39.** Viscoelastic properties of duck egg yolk at different NaCl concentrations showing evolutions of (A) storage and loss moduli and (B) loss tangent.

At 1.7M NaCl concentration, complete dissociation of the granule took place, leading to the release of lipoproteins and phosvitin into the salt solution (Chang *et al*., 1977; Ternes, 1989; Causeret *et al*., 1991). This disruption of granules is associated with liberation of low-density lipoproteins (Guerrero *et al*., 2004). Those released proteins may form aggregation with other molecules. Sodium chloride-induced dissociation of granule is believed to be as a result of substitution of monovalent sodium for bivalent cations, leading to the disruption of the phosphocalcic bridges and release of calcium (Causeret *et al*., 1991). Disruption of

granules associated with protein solubilization, provided additional protein to improve the functional properties of yolk-containing food systems (Kiosseoglou and Sherman 1983b; Foegeding *et al*., 2001). Therefore, the increased NaCl levels could lead to an increase in both viscoelastic moduli with the loss tangent depending on the NaCl level. Particularly, the viscous characteristic of yolk dispersions dominated at low NaCl concentration but the elastic dominant behaviour was observed at the presence of increasing NaCl contents.

### 7.4.2 Effect of dehydration on rheological behavior of egg yolk

Effect of dehydration on the rheological behavior of salted and unsalted egg yolk was investigated. The dehydration periods used for egg yolk samples were 6, 12 and 24 hours and their total solids contents were measured to be 63.9 ± 0.1, 66.9 ± 0.0 and 74.9 ± 0.1% w/w respectively. All dehydrated samples were added with NaCl at different concentrations (0, 1 and 3% w/w). Frequency sweep tests were performed at constant shear stress within the LVE range. Results are shown in Figreus 40A-C for NaCl concentrations of 0, 1 and 3% w/w, respectively. Without NaCl addition, the mechanical spectra of egg yolk sample dehydrated for 6 hours showed highly frequency dependent behavior (Figure 40A). In addition, the loss modulus was higher than the storage modulus at lower frequencies and the situation was reversed at higher frequencies. This suggests that the hydrated egg yolk had entanglement network structure and behaved as a concentrated solution. In addition, the crossover between the storage and loss moduli can be interpreted as follows; the macromolecular chains can disentangle during a long period of oscillation at a lower frequency and thus the system behaves as a solution while the entangled macromolecular chains do not disentangle during a short period at a higher frequency and then the entanglement point plays a role of a knot of three-dimensional networks, and the system shows a solid-like behaviour. Conversely, the mechanical spectra were weakly frequency-dependent and the storage modulus was higher than the loss modulus across the entire frequency range for egg yolk samples with longer

dehydration periods, e.g. 12 and 24 hours. Such results indicate that the egg yolk samples behaved like a strong elastic gel. By adding 1% w/w of NaCl, the previously observed frequency dependent behaviour at 6 hours dehydration period changed dramatically, thus, implying a change in network structure (Figure 40B). Particularly, both storage and loss moduli increased significantly at low frequencies. The mechanical spectra were weakly frequency dependent and the storage modulus was higher than the loss modulus at all frequencies. Such results could be caused by the absorption of water by NaCl, thus, decreasing the solubility of egg yolk. At longer dehydration period, the addition of 1% w/w NaCl did not have any substantial effect on the mechanical spectra. Increasing NaCl concentration to 3%w/w also showed insignificant effect on the viscoelastic properties of dehydrated egg yolks as can be observed in Figure 40C. These results suggest that the effect of dehydration is more predominant than that of the NaCl addition, especially at dehydration periods longer than 6 hours. This is also confirmed by the relationship between tan(δ) and NaCl concentration at varying degrees of dehydration as shown in Figure 41. It is apparent that an increase in NaCl concentration only affected the viscoelastic behaviour of egg yolk by inducing a sol-gel transition when the dehydration time was less than 6 hours. Exceeding this dehydration period, a similar gel network structure was observed at the same dehydration period regardless of the amount of NaCl concentration. Such findings could occur in a salting process of egg yolk. During salting, the solidification of egg yolk initialized near the vitelline membrane and proceeded towards the center. Consequently, the gel formation started to occur at the exterior yolk while the interior yolk was still in a liquid form. However, the interior yolk eventually became more viscous with further dehydration of the exterior yolk (Kaewmanee *et al*. 2009a). As egg yolk became solidified, the interaction between protein molecules including lipoprotein occurred (Kaewmanee *et al*. 2009a) resulting in a revolution of viscoelastic properties of the egg yolk. In addition, the reduction in moisture content concurrently with the increase in salt content of the egg yolk was observed during salting process (Kaewmanee *et al*. 2009b). Therefore, it can be said that both NaCl concentration and degree of dehydration more likely determined gel network formation of egg yolk during saltin

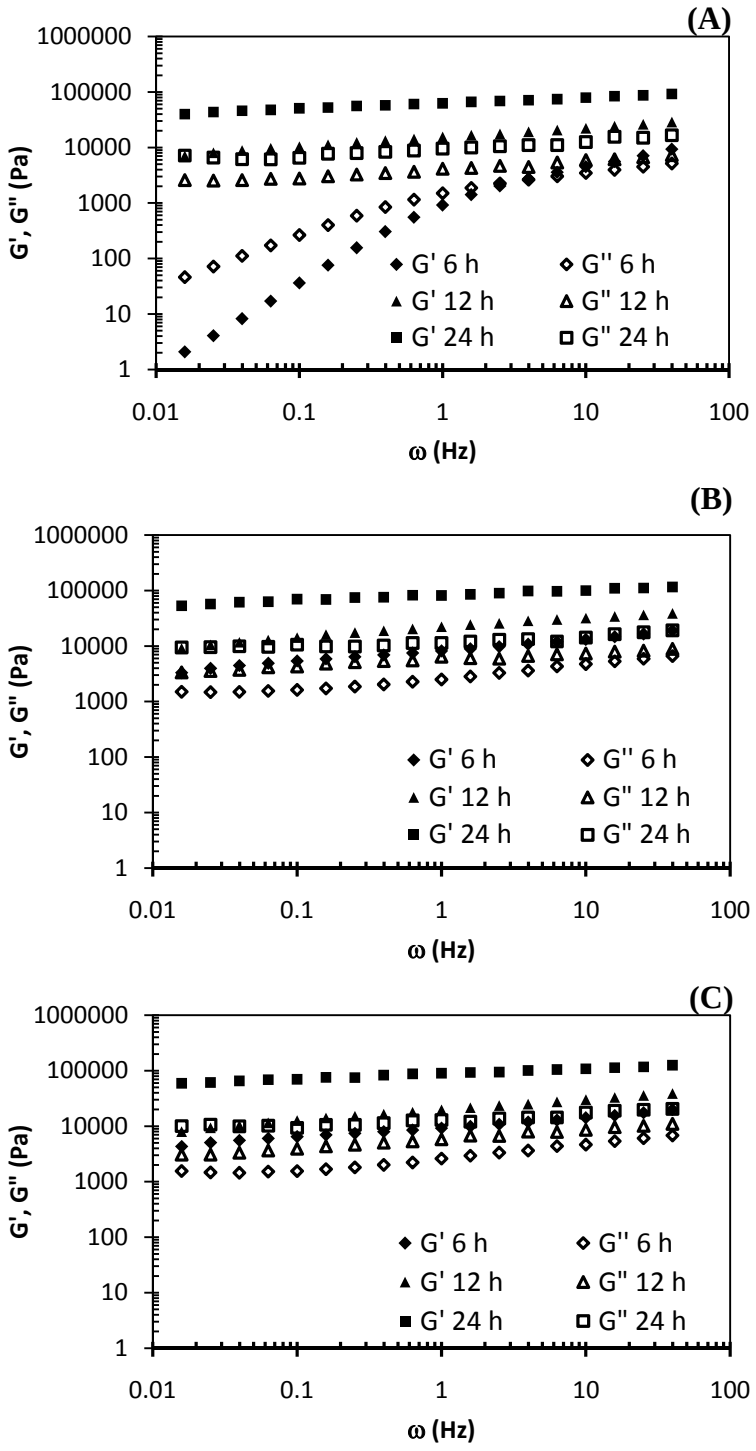



**Figure 40.** Viscoelastic properties of duck egg yolk as a function of degree of dehydration at (A) 0, (B) 1, and (C) 3%w/w NaCl concentrations.

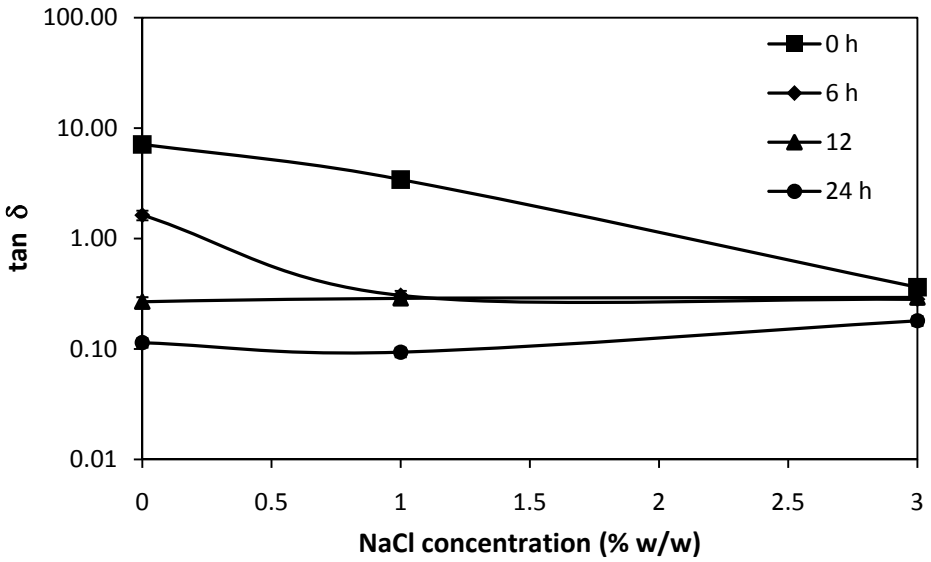



**Figure 41.** Relationship between loss tangent and NaCl concentrations at various degrees of dehydration at constant frequency of 1 Hz

### 7.4.3 Effect of NaCl concentration on thermal-induced transitions

Viscoelastic properties of egg yolk in the presence of NaCl at different concentrations, obtained from temperature ramp experiments at a constant frequency of 1 Hz and a heating rate of 2°C/min from 20 to 95°C, are shown in Figure 42. Depending on heating temperature, three different temperature regions can be obtained for egg yolk samples subjected to the test, regardless of NaCl concentrations. In the first region, at temperatures below 60°C, a fluid-like behavior was observed as tan(δ) was less than unity. Both storage and loss moduli were also observed to decrease with increasing temperature. A decrease in both moduli was more pronounced with increasing NaCl concentration. The second region, occurring at temperature between 60-85°C, showed a remarkable increase in both moduli and a slump in the loss tangent. A heat-induced gel point was located within this region at the crossover point between G′ and G″ (Cordobes *et al*., 2004). The crossover point corresponded to the sol-gel transition temperature ($T_c$) (Sanchez and Burgos 1997).

As can be observed from Figure 42(B), an increase in NaCl concentration led to an increase in $T_c$ as well as a reduction in the thermal-induced growth of both viscoelasticity functions. The final region appeared at temperature higher than 85$^{o}$C. Within this region, both G′ and G″ values were approximately constant and were independent on NaCl concentration. Furthermore, tan(δ) were approximately 0.1 for all studied NaCl levels, signifying a well-developed gel network with no further association of protein aggregates. The above-mentioned behavior may be explained in terms of the processes involved in protein gelation as describe by Clark (1998). Those processes include a) a change in the protein system from the native to the denatured state; b) aggregation of partially denatured proteins and c) association of protein aggregates. It should also be emphasized that most yolk proteins are organized into micellar and granular structures together with polar and non-polar lipid molecules, where the latter are embedded into the particle cores. As a result, an interparticle network gel is formed (Kiosseoglou 2003).

DSC thermograms of egg yolk samples with various NaCl concentrations are shown in Figure 43. An apparent endothermic peak, located at 79.4°C, was found the sample without NaCl addition. Cordobes *et al*. (2004) also reported a similar non-reversible endothermic behavior which was related to the denaturation temperature of the egg yolk. In the presence of NaCl, the denaturation temperatures were found at 84.8 and 91.1$^{o}$C for 1 and 3% w/w NaCl concentrations, respectively. The shift in the denaturation temperature was consistent with the increase in $T_c$ obtained in the temperature sweep experiments. These results confirm that the addition of salt tends to stabilize protein molecules in egg yolk. Nevertheless, a reduction in thermal denaturation enthalpy, which was calculated from the area under the transition peak, was observed when the NaCl concentration increased from 0-3% w/w.

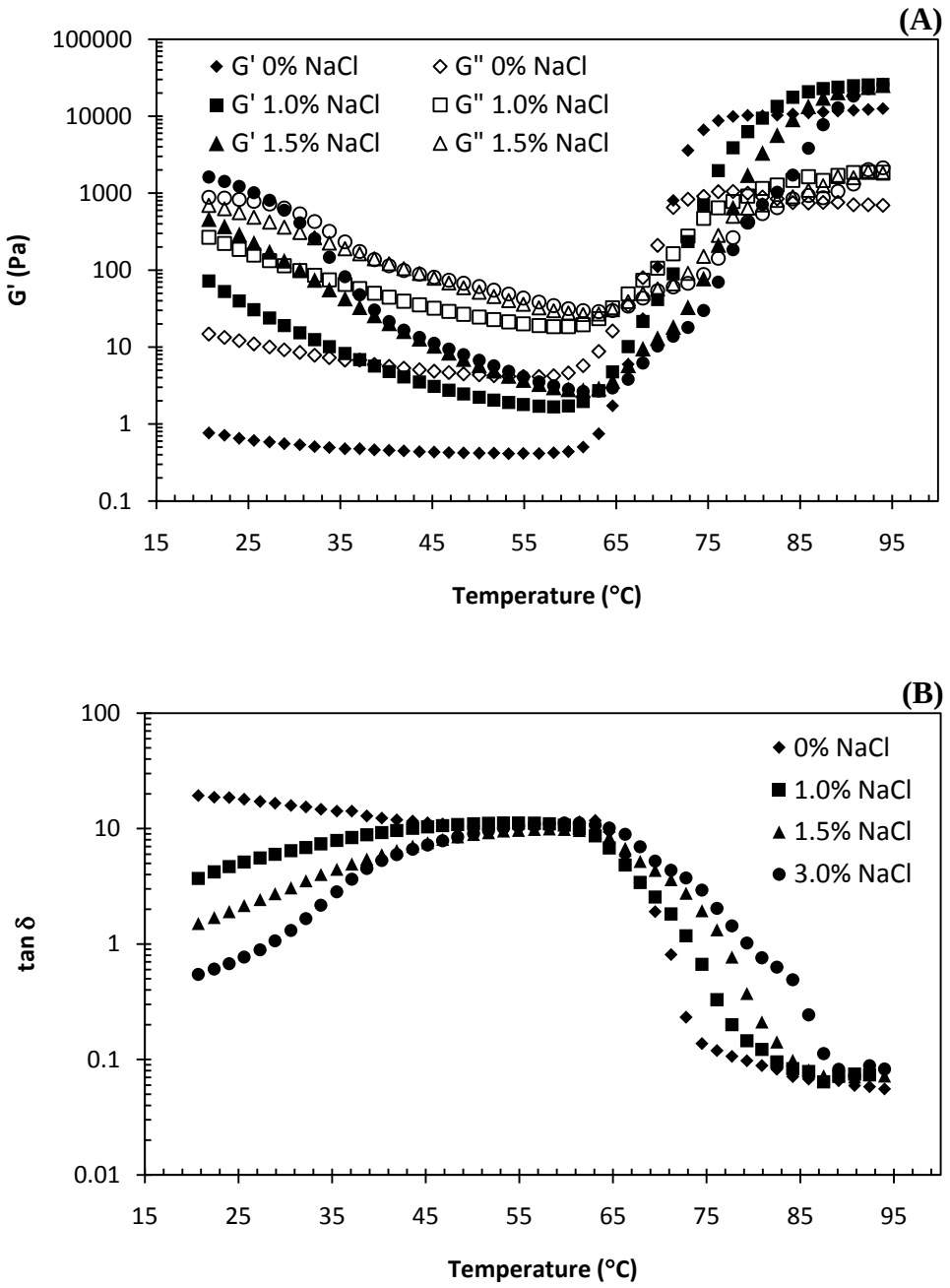



**Figure 42.** Thermal-induced properties of duck egg yolk at different NaCl concentrations showing evolutions of (A) storage and loss moduli and (B) loss tangent.

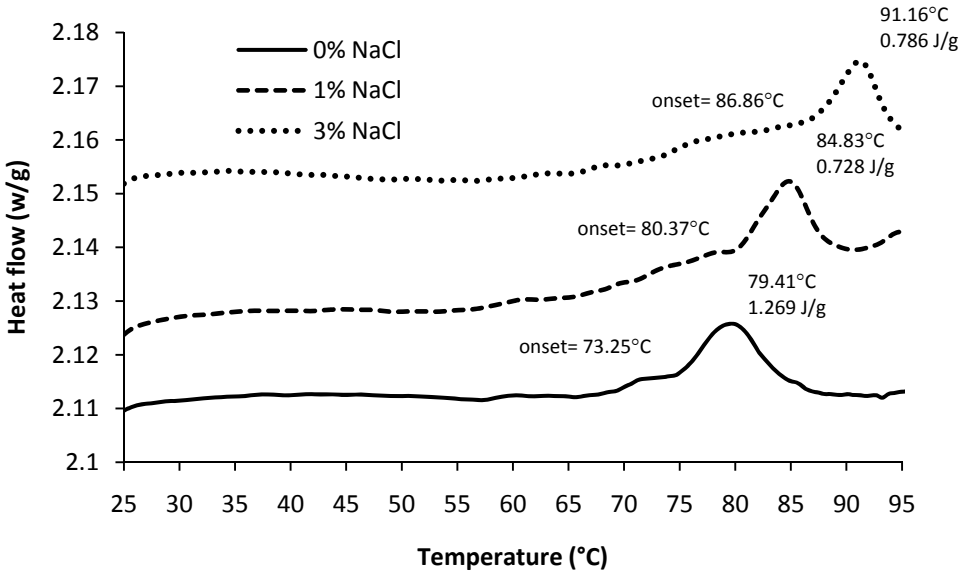



**Figure 43.** DSC thermograms of egg yolk at different NaCl concentration

In general, ions affect protein conformation stabilized by electrostatic interactions with the charged groups and the protein polar groups or by hydrophobic interactions between protein molecules (Damodaran and Kinsella 1982). In the latter case, the salt-induced change in water structure (destabilization of hydrogen bonds) caused by ion-dipole interaction may alter the degree of hydration as well as the orientation of water molecules around the non-polar residues of polypeptide chains (Damodaran and Kinsella 1982; Puppo and Anon 1998). Salt increases the denaturation temperature of egg proteins and delays the formation of aggregates. It also inhibits interactions between water molecules and hydrophilic groups in proteins, resulting in increased hydrophobicity that favors the native protein conformation (Danilenko *et al*., 1985). Moreover, the addition of salt has a ‘‘shielding’’ effect on the repulsive forces between protein molecules. As a result, proteins randomly aggregate to form gels, resulting from favorable protein–protein interactions over protein–water interactions (Choi *et al*., 2000). The increased gel point temperature in the presence of NaCl could arise from the non-ordered protein coagulation. Under

high ionic strength conditions, the granular structure is disintegrated and the liberated constituents may take part in network formation by interacting with other yolk constituents located in plasma during heating (Kiosseoglou and Paraskevopoulou, 2005). !

### 7.4.4 Effect of dehydration on thermal-induced transitions

Effect of dehydration on the thermal-induced transitions of egg yolk was investigated using temperature sweep and DSC experiments. Figures. 44A and B show the evolution of the mechanical spectra of dehydrated egg yolk samples at 0 and 3%w/w NaCl concentration, respectively. A decrease in $T_c$ from 70.4 to 60.5°C was observed when the sample was dehydrated for 6 hours (Figure 44A). However, such parameter could not be determined for the samples higher degree of dehydration (12 and 24 hours) as the gel-like network was already achieved at low temperature. With 3% w/w NaCl addition, all dehydrated egg yolk samples showed a well-developed gel network. In addition, the crossover temperature did not exist for all dehydrated samples. These results suggested that both dehydration and addition of NaCl could affect the gel formation of thermal-induced egg yolk. An increase in degree of dehydration results in a decrease in the onset of gelling temperature. The addition of NaCl also helped to achieve the gel formation in dehydrated egg yolk more effectively. A stronger gel network could be obtained with increasing degrees of dehydration and NaCl concentration.

Table 20 shows DSC results of dehydrated egg yolk samples at 0 and 3% w/w NaCl concentrations. It is evident that the denaturation temperature ($T_{max}$) increased with increasing dehydration period for both NaCl concentrations. At the same dehydration level, the higher denaturation temperature was found in egg yolk with higher NaCl concentration. Such results confirm that both dehydration and NaCl addition play important roles on the denaturature temperature of egg yolk. It is worth noting that peaks of the denaturation temperature tend to disappear with increasing dehydration time and NaCl addition.

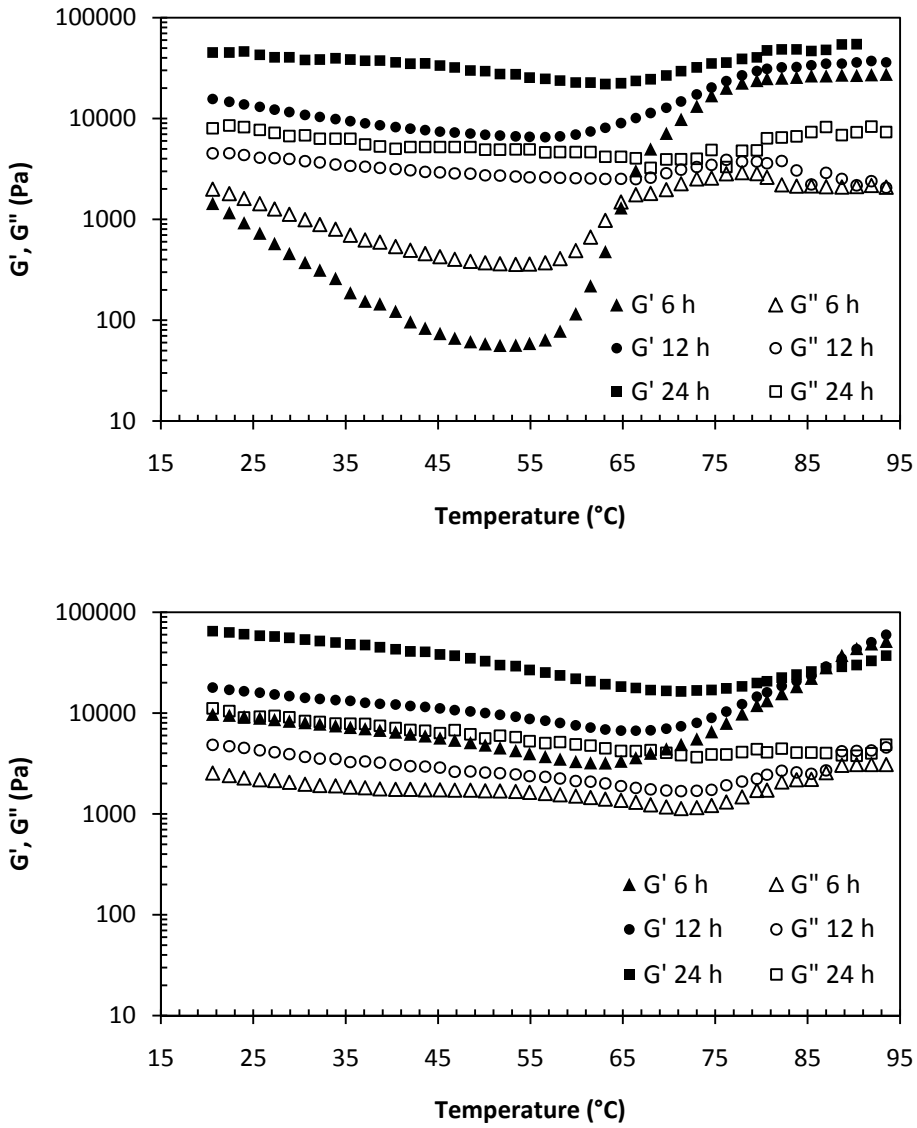



**Figure 44.** Thermal-induced properties of dehydrated duck egg yolk samples added with (A) 0 and (B) 3%w/w NaCl concentrations.

**Table 20**. DSC results of egg yolk as a function of dehydration time and NaCl concentration

| Treatment (NaCl concentration, dehydration time) | Temperature (°C) | | Enthalpy, ΔH (J/g) |
|---|---|---|---|
| | onset | $T_{max}$ | |
| 0%, 6 h | 77.78 | 79.53 | 0.478 |
| 0%, 12 h | 79.01 | 82.43 | 0.802 |
| 0%, 24 h | 83.69 | 89.56 | 0.259 |
| 3%, 6 h | 84.70 | 86.54 | 0.501 |
| 3%, 12 h | 87.77 | 90.57 | 0.545 |
| 3%, 24 h | 90.79 | 93.86 | 0.902 |

### 7.4.5 Microstructure of heat-induced egg yolk gels

Scanning electron microscopic images of egg yolk samples with different NaCl concentrations and degrees of dehydration obtained upon heating to 95$^{o}$C are shown in Figure 45. It can be seen from Figure 45A that egg yolk sample without NaCl addition has a number of round globules (1-5 μm in diameter) trapped in a fibrous network structure. These round globules (rich in high-density lipoproteins, HDL) represented egg yolk granules, which are suspended in the gel matrix (Hsu *et al*., 2009). The cooked egg yolk granule is constituted with embedded round globules and even holes resulting in a continuously lumpy appearance (Hsu *et al*., 2009). A distinctive homogenous structure, composed of the destroyed yolk globules and a pore like structure, was observed in the samples with NaCl addition. Smaller pore sizes and finer network strands could be observed in the samples with increasing NaCl contents. These networks have been described by globular proteins in terms of regions of high protein concentration separated by regions which form pores nearly devoid of protein (Stading *et al*., 1995). Figure 45B shows microstructure of thermally-induced dehydrated egg yolk samples with 0 and 3% w/w NaCl. For samples without NaCl addition, large amount of round globules could be observed.

Moreove, the gel structure became more compact with increasing dehydration period. The extent of these globules was decreased with NaCl addition, regardless of the degree of dehydration. The addition NaCl also produced a larger cluster of aggregation. During heating, hydrophobic interaction within LDL micelles plays a role in gel formation, which leads to random aggregation and rather heterogeneous particulate gel (Guerrero *et al*., 2004). Although HDL within granules may also participate in the gel network, they have been reported to be less sensitive to heat. Therefore, granules can be said to be less effective as gel network formation agents (Le Denmat *et al*., 1999; Anton *et al*., 2001). Disruption of yolk granules and disappearance of yolk globules can be induced by NaCl addition, whereas dehydration can lead to more compact gel network structure.

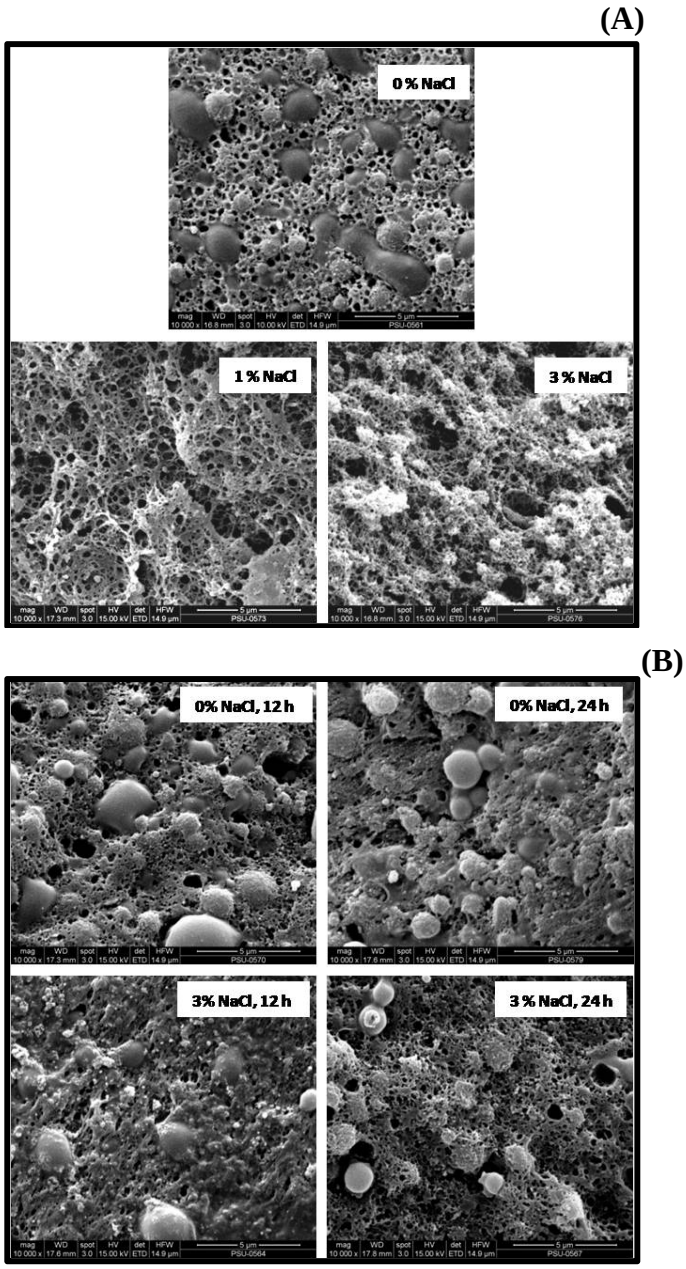



**Figure 45.** Scanning electron microscopic images obtained from heat-induced egg yolk gel as affected by (A) NaCl addition and (B) dehydration time.

## 7.5 Conclusions

Addition of NaCl and dehydration period played an important role on viscoelastic and thermal-induced properties of egg yolk. The former led to the aggregation of yolk protein molecules, especially at high NaCl concentration. The latter also resulted in a well-developed gel network. During heating process, the addition of NaCl to egg yolk tended to stabilize the protein molecules, leading to an increase in the denaturation temperature as well as a delay in gel network formation.

# CHAPTER 8

# PROTEIN HYDROLYSATE OF SALTED DUCK EGG WHITE AS A SUBSTITUTE OF PHOSPHATE AND ITS EFFECT ON QUALITY OF PACIFIC WHITE SHRIMP (*LITOPENAEUS VANNAMEI*)

## 8.1 Abstract

Protein hydrolysate from salted egg white (PHSEW) with different degrees of hydrolysis (DH) (3, 6 and 9%) was produced using pepsin. Disappearance of proteins with molecular weight (MW) of 108 and 85 kDa with the concomitant formation of proteins with MW of 23, 20, 13 and 5 kDa was observed in PHSEW. The use of PHSEW for quality improvement of Pacific white shrimp (*Litopenaeus vannamei*) was investigated. Shrimp soaked in 4% NaCl containing 7% PHSEW and 2.5% mixed phosphates (0.625% sodium acid pyrophosphate (SAPP) and 1.875% tetrasodium pyrophosphate (TSPP)) had the highest cooking yield with the lowest cooking loss ($p< 0.05$), compared with shrimps with other treatments. Nevertheless, no difference in weight gain was obtained in comparison with those treated with 4% NaCl containing 3.5% mixed phosphate ($p> 0.05$). Cooked shrimp treated with 4% NaCl containing 7% PHSEW and 2.5% mixed phosphate or those treated with 4% NaCl containing 3.5% mixed phosphate had the higher score of appearance, texture and overall likeness but less shear force, in comparison with the control (no treatment) ($p< 0.05$). Microstructure study revealed that muscle fibers of cooked shrimp from both treatments had the swollen fibrils and gaps, while the control had the swollen compact structure. Therefore, use of PHSEW could reduce phosphate residue in shrimps without an adverse effect on sensory properties.

## 8.2 Introduction

Salted egg is one of the most traditional and popular preserved egg products. Salted egg can be produced by coating with clay containing salt or immersing in saturated brine for 2-3 weeks (Kaewmanee *et al*., 2009). Salted egg yolks are more demanded in comparison with salted egg white. Generally, salted egg yolks have been used in many products such as moon cake or other bakery products. As a consequence, salted egg white containing 10% protein and 4-7% sodium chloride (Kaewmanee *et al*., 2009) is discarded as waste, causing the environmental pollution. To earn the benefit of salted egg white, it has been used for lysozyme extraction (Chang and Liu 1994; Liu *et al*., 1994), and used as ingredient in frankfurters (Lin *et al*., 1996). In general, egg white possesses various functional properties such as gelation, foaming ability and emulsifying activity and has been used widely in food processing (Kato *et al*., 1981; 1986; 1989). These functional properties can be altered by sodium chloride to some degrees (Arntfield *et al*., 1990; Elizalde *et al*., 1991; Kakalis and Regenstein 1986; Kitabatake *et al*., 1988; Mine *et al*., 1991; Vani and Zayas, 1995). However, Huang *et al*., (1996) reported gel forming ability and emulsion stability of dried salted duck egg white powder (Huang *et al*., 1996). Due to high salt content (30%) and hygroscopic property of dried salted duck egg white, it becomes less suitable for food application.

Protein hydrolysates have been paid increasing attention for improving food functional and technological properties or for medical and nutritional purposes (Pacheco *et al*., 2002). The hydrolysis effects of papain on color, solubility, foaming capacity, and gel strength of egg white solid were reported by Lee and Chen (2002). Enzymatic hydrolysis of egg white with pepsin resulted in the formation of peptides with free radical-scavenging capacity and lipid peroxidation inhibition ability (Davalos *et al*., 2004). Some peptides in egg white hydrolysate exhibited *in vitro* angiotensin converting enzyme (ACE) inhibitory activity (Migual *et al*., 2004; Miguel and Aleixandre, 2006). Due to the increased hydrophilicity and solubility of protein hydrolysate, it can be applied to improve water holding capacity of meat or fish products. Owing to the strict regulation for the use of phosphates in meat and fish

product, the alternative additives with the capability of holding water or reducing cooking loss should be taken into consideration.

Additionally, the treatment of shrimp with phosphates, especially at an excessive amount, causes a translucent and slimy texture (Rattanasatheirn *et al*., 2008). Therefore, the use of egg white hydrolysate, particularly from salted egg white, as phosphate substitute could minimize the translucence as well as improve the quality of shrimp. The objects of this investigation were to produce protein hydrolysate from salted duck egg white (PHSEW) and to study the effect of PHSEW on the properties of fresh and cooked Pacific white shrimp.

## 8.3 Materials and Methods

### 8.3.1 Chemicals

Tetrasodium pyrophosphate (TSPP) and sodium acid pyrophosphate (SAPP) were purchased from Fluka (Buchs, Switzerland). Pepsin from porcine stomach mucosa (EC 3.4.23.1; powdered; 624 $Umg^{-1}$ dry matter) and protein markers were purchased from Sigma Chemical Co. (St. Louis, MO, USA.).

### 8.3.2 Preparation of salted duck egg

Duck (*Anas plotyrhyncus*) eggs (60-70 g) were obtained from a farm in Satingphar, Songkhla Province, Thailand. Salted duck eggs were prepared following the method of Kaewmanee *et al*. (2009) with the salting time of 14 days. Salted egg white was manually separated from yolk and gently homogenized at 11,000 rpm, followed by filtration through a layer of cheesecloth to obtain the homogeneous mixture. Protein content of prepared salted egg white was determined using the biuret method (Robinson and Hodgen, 1940).

### 8.3.3 Hydrolysis of salted egg white using pepsin

Prepared salted egg white was adjusted to pH 2 using 4 M HCl and distilled water was added to obtain the final concentration of 10% protein content (3.2% salt). The solution was preincubated at 37ºC for 10 min prior to enzymatic hydrolysis using pepsin. The hydrolysis reaction was initiated by the addition of pepsin at levels of 1 and 3% (w/w, based on protein content). The reaction was conducted at 37ºC. Sample (10 mL) was taken at the designated times (0, 20, 40, 60, 90, 120, 150, 180, 240, 300 and 360 min) and adjusted pH to 7 with 4 M NaOH to inactivate pepsin.

Degree of hydrolysis (DH) of salted egg white was analyzed according to the method of Benjakul and Morrissey (1997). The samples (125 µl) were added with 2.0 mL of 0.2 M phosphate buffer, pH 8.2 and 1.0 mL of 0.01% TNBS solution. The solution was mixed thoroughly and placed in water bath at 50 ºC for 30 min in dark. To terminate the reaction, 2.0 mL of 0.1 M sodium sulfite were added. The mixtures were cooled for 15 min at room temperature (25-27ºC). The absorbance was measured at 420 nm and α-amino acid content was expressed in terms of L-leucine. DH was calculated as follows:

$$DH = [(L_t - L_0)/ (L_{max} - L_0)] \times 100$$

where $L_t$ is the amount of α-amino acid released at time t. $L_0$ is the amount of α-amino acid in the original salted egg white solution. $L_{max}$ is the total α-amino acid in original salted egg white solution obtained after acid hydrolysis with 6 N HCl at 100ºC for 24 h.

### 8.3.4 Preparation of protein hydrolysate from salted egg white with different DHs

Salted egg white solution (10% protein) adjusted to pH 2 and incubated at 37ºC for 10 min was added with pepsin at different concentrations (1, 3,

5, 7, 10 and 12%, w/w). The mixture was mixed thoroughly. After 300 min of hydrolysis, 10 mL of sample was taken and adjusted to pH 7 with 4 M NaOH. % DH of hydrolysate was determined as described by Benjakul and Morrissey (1997). $Log_{10}$ of pepsin amount was plotted against % DH to obtain the regressive equation. From the relationship, the amount of enzyme required for hydrolysis to obtain DHs of 3, 6 and 9% was calculated and used for preparation of PHSEW. The samples were cooled and the pH values of samples were then adjusted to 7.0 with 4 M HCl or 1 M HCl. The resulting hydrolysates were clarified by centrifuging at 4,500×g for 20 min at 4ºC to remove insoluble debris. The supernatant was collected and referred to as 'PHSEW'. PHSEWs with different DHs were subjected to analysis of protein pattern.

### 8.3.5 Determination of protein pattern of PHSEW

Protein patterns of PHSEW were determined using SDS-PAGE with 4.5% stacking gel and 12.5% separating gel according to the method of Laemmli (1970). PHSEWs were mixed with sample buffer (0.125 M Tris-HCl, pH 6.8 containing 4% SDS and 20% (v/v) glycerol) at a ratio of 1:1 (v/v). Proteins (15 μg) were loaded onto the gel. The electrophoresis was run at a constant current of 15 mA per gel by a Mini-Protein II Cell apparatus. The gels were fixed and stained with 0.05% (w/v) Coomassie Brilliant Blue R-250 in 15% methanol and 5% acetic acid and destained in 30% methanol and 10% acetic acid. Wide range molecular weight markers were used to estimate the molecular weight of proteins in PHSEW.

Quantitative analysis of protein band intensity was performed using a Model GS-700 Imaging Densitometer (Bio-Red Laboratories, Hercules, CA, USA) with Molecular Analyst Software version 1.4 (image analysis systems). The intensity of interested protein bands was expressed, relative to those of egg white without hydrolysis.

### 8.3.6 Study on the effect of DH and levels of PHSEW on the quality of Pacific white shrimp

Pacific white shrimps (*Litopenaeus vannamei*) with the size of 55-60 shrimps per kg were obtained from a farm in Songkhla province. Shrimps were kept in ice with a shrimp/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Thailand, within 1 h. Upon arrival, shrimps were washed with clean tap water, immediately deheaded and the shells were peeled off. The shrimps were placed in polyethylene bag and kept in wet ice during preparation and until use.

Shrimps were treated with 4% NaCl containing PHSEW with different DHs (3, 6 and 9%) at various concentrations (3, 5 and 7%) for 1 h at 4ºC. Shrimps soaked in 4% NaCl or 4% NaCl with 3.5% mixed phosphate (0.875% sodium acid pyrophosphate (SAPP) and 2.625% tetrasodium pyrophosphate (TSPP)) were also prepared. All shrimp samples were subjected to analyses. Solution rendering the lowest cooking loss with the maximized weight gain and cooking yield was chosen for further study.

### 8.3.7 Study on the effect of PHSEW in combination with mixed phosphates on the quality of Pacific white shrimp

Prepared shrimps were treated with 4% NaCl containing the selected PHSEW (DH= 3%) at a level of 7% in combination without and with mixed phosphates at different concentrations (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5%) for 1 h at 4ºC. All shrimp samples were subjected to analyses. Shrimps soaked in 4% NaCl or 4% NaCl containing 3.5% mixed phosphates were also prepared.

### 8.3.8 Determination of weight gain, cooking loss and cooking yield

Weight gain was determined by weighing the shrimps before and after soaking in solutions. After soaking, the samples were drained on plastic screen for 5 min at 4ºC. Weight gain was calculated as follows:

$$\text{Weight gain (\%)} = [(B-A)/A] \times 100$$

where: A= initial weight (before soaking); and B= weight after soaking, followed by draining

Cooking loss and cooking yield were measured by weighing the shrimps before and after steaming. Shrimps were cooked by steaming for 5 min, immediately cooled in iced water for 1 min and drained at 4ºC for 5 min. Cooking loss and cooking yield were calculated as follows:

$$\text{Cooking loss (\%)} = [(B-C)/B] \times 100$$
$$\text{Cooking yield (\%)} = (C/A) \times 100$$

where: A= initial weight (without soaking and steaming); B= weight after soaking, followed by draining; and C= weight after steaming, followed by cooling in iced water.

### 8.3.9 Determination of salt content

Salt content was determined by the method of AOAC (2000) with the analytical number of 935.43 Samples (1 g) were added with 10 mL of 0.1 N $AgNO_3$ and 10 mL of $HNO_3$. The mixture was boiled gently on a hot plate until all solids except $AgCl_2$ was dissolved (usually 10 min). The mixture was cooled using running water and the solids were removed through filter paper (Whatman No. 1) Five mL of 5% ferric alum indicator ($FeNH_4$ $(SO_4)_2 \cdot 12$ $H_2O$) were added. The mixture was

titrated with the standardized 0.1 N KSCN until solution became permanently light brown. The percentage of salt was then calculated as follows:

$$\text{Salt (\%)} = 5.8 \times [(V1 \times N1) - (V2 \times N2)]/W$$

where: V 1 = volume of $AgNO_3$ (mL); N1 = concentration of $AgNO_3$ (N); V 2 = volume of KSCN (mL); N2 = concentration of KSCN (N) and W = weight of sample (g)

### 8.3.10 Determination of microstructure of Pacific white shrimp meat

Microstructures of cooked Pacific white shrimps without and with the selected treatment were analyzed as described by Jones and Mandigo (1982). Samples were cut into a cube (4 × 4 × 4 mm) with a razor blade. The prepared samples were fixed in 2.5% glutaraldehyde in 0.2 M phosphate buffer, pH 7.2 at room temperature for 2 h. All specimens were washed three times with distilled water for 15 min each time. They were then dehydrated with a serial concentration of 50-100% ethanol for 15 min each. All specimens were coated with 100% gold (sputter coater SPI-Module, PA, USA). The microstructure was visualized using a scanning electron microscopy (JEOL, JSM-5800 LV, Tokyo, Japan).

### 8.3.11 Determination of opacity

Evaluation of opacity was carried out by 10 trained panelists according to the method of (Rattanasatheirn *et al.*, 2008). Trainings of 5 sessions (2 h each) were performed. Different references including 1) 7 days ice-stored shrimp soaked in 3.5% TSPP for 10 h at 4ºC; 2) fresh shrimp soaked in 3.5% TSPP for 2 h at 4ºC and 3) fresh shrimp were steamed for 5 min, followed by cooling in ice water for 1 min were used for training with the scale of 1, 3 and 5, respectively. Cooked white shrimps (fresh shrimps and shrimps soaked in 4% NaCl containing 3.5% mixed phosphates or 4% NaCl with 7% PHSEW and 2.5% mixed phosphates) were evaluated for opacity using

a point structured scale with a value of 1 for very translucent, 3 for moderately translucent and 5 for turbid or opaque. The opacity score was recorded. The higher values represent the higher opacity or less translucent. All samples were identified by a three digit code. Testing was held in a clean, well lighted and well ventilated room. Those three samples were also subjected to sensory evaluation.

### 8.3.12 Sensory evaluation

All cooked samples were evaluated for acceptance by 30 panelists. The panelists were graduate students in the Food Technology program, Faculty of Agro-Industry, Prince of Songkla University of age ranging from 22 to 35 years. Panelists had sensorial acquaintance with cooked shrimps. A nine-point hedonic scale, in which a score of 1= dislike extremely, 5= neither like nor dislike and 9= like extremely, was used for evaluation (Meilgaard *et al*., 1990). Shrimp samples were steamed for 5 min with boiling water and soaked in iced water for 1 min. The samples were allowed to stand at room temperature for approximately 30 min prior to evaluation. Samples were randomly selected and coded with three-digit random numbers and presented to the panelists at room temperature. Room temperature water was given to rinse the mouth between samples. The panelists were asked to evaluate each sample for color, appearance, texture, odour, taste and overall liking.

### 8.3.13 Determination of shear force

Shear force of shrimp meats, raw and cooked, without and with the selected treatment, was measured using the TA-XT2i texture analyzer equipped (Stable Micro Systems, Surrey, England) with a Warner-Bratzler shear apparatus (Bourne, 1978). The operating parameters consisted of a cross head speed of 2 mm/s and a 25 kg load cell. The shear force, perpendicular to the axis of muscle fibers, was measured in 3 replicates for each treatment. The peak of the shear force profile was regarded as the shear force value.

### 8.3.14 Statistical Analysis

Completely randomized design (CRD) was used throughout the study. The experiments were run in triplicate. Data were presented as mean values with standard deviations. One-way analysis of variance (ANOVA) was carried out and means comparisons were run by Duncan's multiple range tests (Steel and Torrie, 1980). Statistical analyses were performed with the statistical program (SPSS for windows, SPSS Inc, Chicago, IL, USA).

## 8.4 Results and Discussion

### 8.4.1 Hydrolysis of salted egg white using pepsin

Hydrolysis of salted egg white using pepsin at levels of 1 and 3% for different times was monitored in terms of degree of hydrolysis (DH) (Figure 46A). DH continuously increased during the first 1 h, regardless of pepsin concentrations added. Thereafter, a slightly lower rate of hydrolysis was obtained. The rapid hydrolysis in the initial phase indicated that a large number of peptide bonds were hydrolyzed. At the same hydrolysis time, higher DH was observed with samples hydrolyzed with 3% pepsin, compared to those treated with 1% pepsin. After 6 h of hydrolysis, DHs of 5.2% and 7.9% were obtained for hydrolysate prepared using 1 and 3% pepsin, respectively. DH indicates the percentage of peptide bonds cleaved (Adler-Nissen, 1979) and affects several properties of protein hydrolysates (Nielsen, 1997). The presence of indigenous protease inhibitors in egg albumen might influence pepsin activity to some degree. Egg white contains a substantial amount of ovomucoid, which is a strong inhibitor of pepsin. (Cigic and Zelenik-Blatnik, 2004). The ovomucin fraction contained various protease inhibitors including ovostatin, ovoinhibitor and ovomucoid. Ovostatin found in egg albumen has been known to inhibit metalloproteases (Nagase *et al*., 1983). Denaturation temperature and pH of albumen were optimized to denature or inactivate inhibitor irreversibly prior to hydrolysis. (Cigic and Zelenika-Blatnik, 2004). For the present study, no heat

treatment was applied since the large aggregate was formed upon heating in the presence of salt (5.65±0.40%). Such aggregates were more likely less susceptible to hydrolysis by pepsin added. No differences in DH were found between the hydrolysate with hydrolysis time of 5 and 6 h ($p > 0.05$). Therefore, the hydrolysis time of 5 h was used for preparation of hydrolysate from salted egg white. Linear regression was observed when DH and $\log_{10}$ pepsin concentration were plotted (Figure 46B). The amount of pepsin required for hydrolysis to obtain DHs of 3, 6 and 9% was calculated and used for preparation of protein hydrolysate from salted egg white (PHSEW).

### 8.4.2 Protein pattern of PHSEW with different DHs

Protein patterns of PHSEW with different DHs and salted egg white are show in Figure 47. Salted egg white contained 5 major protein bands with MW of 109 (protein A), 85 (protein B), 70 (protein C), 49 (protein D) and 30 (protein E) KDa, corresponding to ovomucin, ovotransferrin (conalbumin), avidin, ovalbumin and ovomucoid, respectively (Osuga and Feeney, 1974) (Figure 47). Ovalbumin is the most abundant protein in egg white (54%) (Li-Chan *et al*., 1995; Linden and Lorient 2000). When salted egg white was hydrolyzed to obtain different DHs, protein bands with MW greater than 70 kDa disappeared. As DHs increased, the intensity of bands of proteins C, D, and E gradually decreased with the coincidental formation of protein with MW of 23 kDa (F), 20 kDa (G), 13 kDa (H) and 5 kDa (I). Increase in band intensity of proteins G and H were noticeable when DHs increased. No marked difference in band intensity of protein I was observed in PHSEW with different DHs.

The results indicated that partial hydrolysis of salted egg white proteins, especially ovomucin, ovotransferrin and ovobumin, produced the smaller peptides or proteins. Increased formation of small peptides was in agreement with increasing DHs.

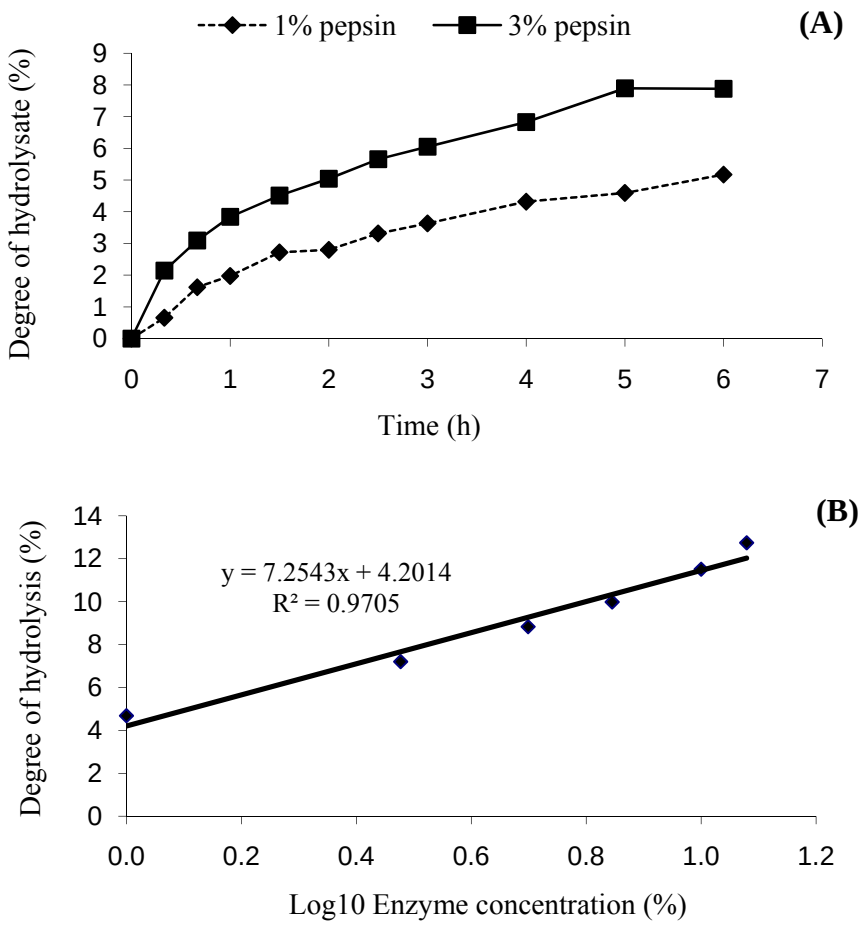
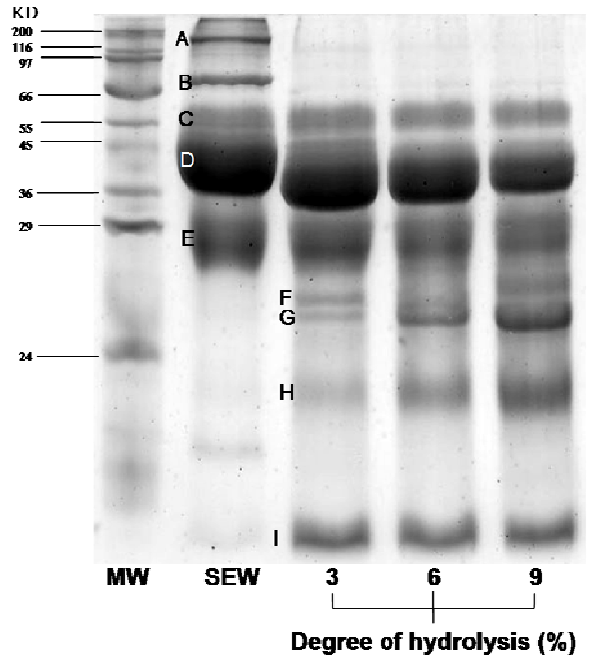



**Figure 46.** Change in degree of hydrolysis (DH) of salted egg white during hydrolysis with 1 or 3% pepsin (A) and relationship between DH of salted egg white and $\log_{10}$ of pepsin concentration (%) (B). The reaction was performed at pH 2.0 and 37ºC. The duration for obtaining data (B) was 5 h.




| Bands | Band density (%) | | | |
|---|---|---|---|---|
| | SEW | 3% DH | 6% DH | 9% DH |
| A | 7.28 | - | - | - |
| B | 6.42 | - | - | - |
| C | 9.82 | 10.35 | 10.79 | 8.18 |
| D | 48.63 | 40.15 | 38.71 | 28.85 |
| E | 27.86 | 22.55 | 20.55 | 18.25 |
| F | - | 4.54 | | |
| G | - | 3.81 | 7.36 | 16.14 |
| H | - | 6.56 | 9.23 | 16.68 |
| I | - | 12.04 | 13.37 | 11.91 |

**Figure 47.** SDS-PAGE patterns and band intensity of salted egg white and protein hydrolysate with different DHs. MW: molecular weight standard; SEW: Salted egg white.

### 8.4.3 Effect of PHSEW with different DHs at various concentrations on quality of Pacific white shrimp

Weight gain and salt content of shrimps soaked in 4% NaCl, 4% NaCl containing PHSEW with different DHs at various levels or 4% NaCl involving 3.5% mixed phosphates are shown in Figure 48A and 48B, respectively. Shrimp treated with 4% NaCl containing mixed phosphates had the highest weight gain (6.64%). When treated with 4% NaCl containing PHSEW having 6 and 9% DH, at levels of 3 and 5%, shrimps had the higher weight gain than those treated with 4% NaCl containing 3% DH PHSEW at the same concentration ($p< 0.05$). At a level of 7% PHSEW, shrimps treated with 3% DH PHSEW showed the higher weight gain, when compared with those treated with PHSEW having 6% and 9% DHs ($p< 0.05$). With increasing DHs of PHSEW (6 and 9%), shrimps treated with PHSEW at levels of 3 and 5% also had the highest weight gain ($p< 0.05$).

After soaking in 4% NaCl in the presence of PHSEW with different DHs at various levels, shrimps contained varying salt contents, depending on the treatments ($p< 0.05$) (Figure 48B). Highest salt content was found in shrimp treated with 4% NaCl containing 5% PHSEW (DH= 3%) or 7% PHSEW (DH= 6%) ($p< 0.05$). On the other hand, no differences in salt content were obtained in shrimps treated with 4% NaCl, shrimp treated with 4% NaCl containing mixed phosphates and shrimps with other treatments ($p> 0.05$). The lowest salt content was found in shrimp treated with 4% NaCl containing 5% PHSEW (DH= 6%) ($p< 005$). It was noted that the increase in salt content in all samples was not in a good accordance with the increase in weight gain. Increase in water binding and hydration in muscle fibers are generally attributed to enhanced electrostatic repulsions between myofibril filaments, causing the filamental lattices to expand for water entrapment (Xiong *et al*., 2000). The result indicated that NaCl showed the synergistic effect with PHSEW under the selected condition for the improvement of weight gain of shrimp. Salt at higher concentration resulted in muscle fiber expansion (swelling) mediated by electrostatic repulsions, which allows more water to be immobilized in the myofibril lattices (Offer and Knight, 1988). Swelling is strongly dependent on ionic strength of system (Offer

and Knight, 1988). With the loosed structure, peptides from PHSEW, particularly those with small MW, might penetrate into shrimp muscle effectively (Figure 47). Due to the hydrophilicity in nature, those peptides were effective in holding water. However, the use of PHSEW with higher DH at higher levels resulted in the lower weight gain, while the treatment with PHSEW (DH= 3%) at higher levels yielded the shrimps with higher weight gain ($p< 0.05$). This confirmed that size and level of PHSEW were the factors influencing weight gain of shrimps. Proteolytic modification of protein improved the moisture sorption and water binding of several proteins (Schwenke, 1997). Canola protein hydrolysates enhanced the water holding capacity of the meat, thus improving cooking yield. However, this effect was also concentration-dependent and influenced by the enzymes employed during the hydrolysis process (Cumby *et al*., 2008). Moisture uptake by proteins or peptides is related to the number of ionic groups present, which increases as a result of hydrolysis due to the liberation of amino and carboxyl groups (Schwenke, 1997).

Cooking loss of shrimp treated with 4% NaCl containing PHSEW with 3% or 9% DHs at a level of 7 % was lowest but was still higher than those treated with 4% NaCl containing mixed phosphates ($p< 0.05$; Figure 49). Shrimp treated with 4% NaCl containing mixed phosphate also showed the highest cooking yield, followed by those treated with 7% PHSEW having 3 and 9% DHs. It was noted that the decrease in cooking loss was concomitant with the increase in cooking yield. The lower cooking loss with higher cooking yield of shrimp treated with 4% NaCl containing 7% selected PHSEW indicated that the shrimp muscle had a higher water holding capacity even after cooking. Generally, water molecules might be bound with protein muscle or PHSEW via hydrogen bond. The water is probably lost due to heat induced denaturation of protein during cooking of the meat, which causes less water to be entrapped within the protein structures held by capillary forces (Aaslyng *et al*., 2003). PHSEW localized in shrimp muscles might be coagulated inside the muscle, leading to the entrapment of water in the muscle. Because PHSEW with 3% DH could be produced with the level of pepsin used and could improve the cooking yield and lower cooking loss effectively, it was chosen for further study.

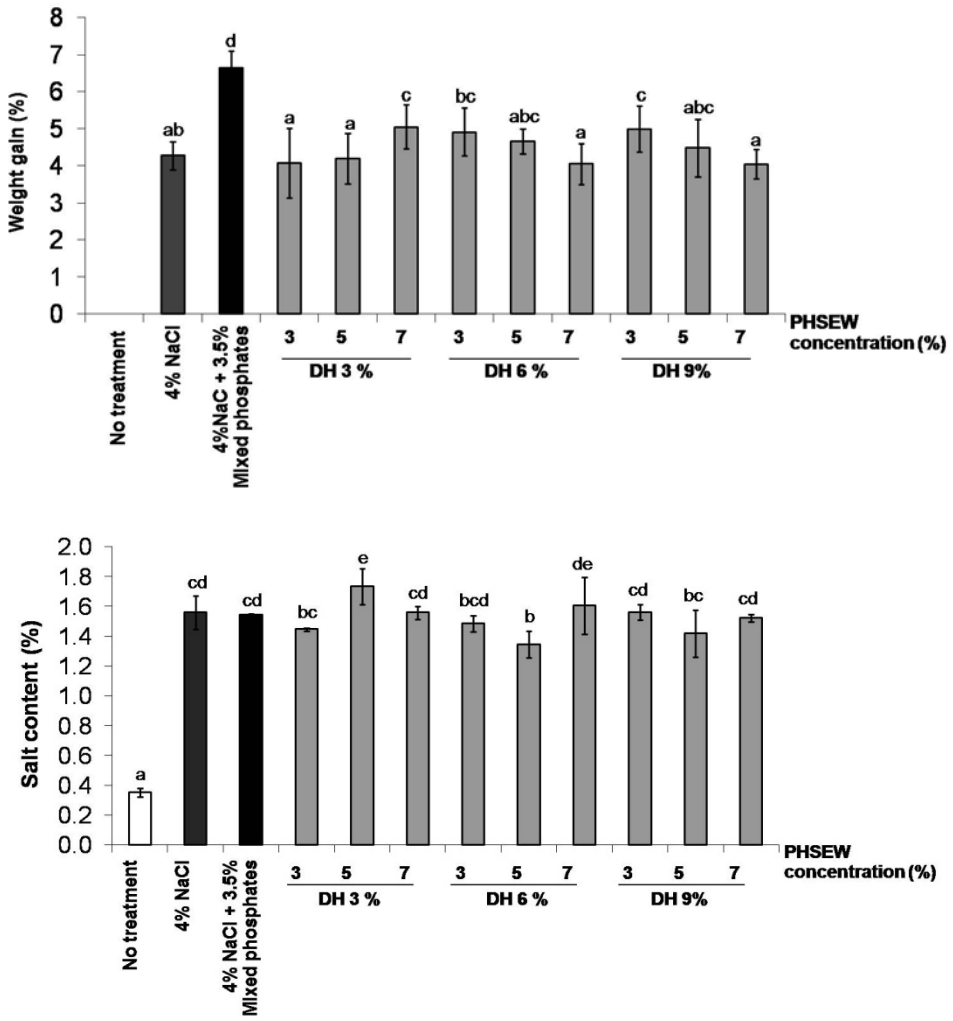



**Figure 48.** Weight gain (A) and salt content (B) of Pacific white shrimp soaked in 4% NaCl containing PHSEW with different DHs at various levels. Shrimps were also soaked in 4% NaCl without and with 3.5% mixed phosphates and used as the controls. Bars represent the standard deviation (n= 3). Different letters on the bar indicate the significant differences ($p<0.05$)

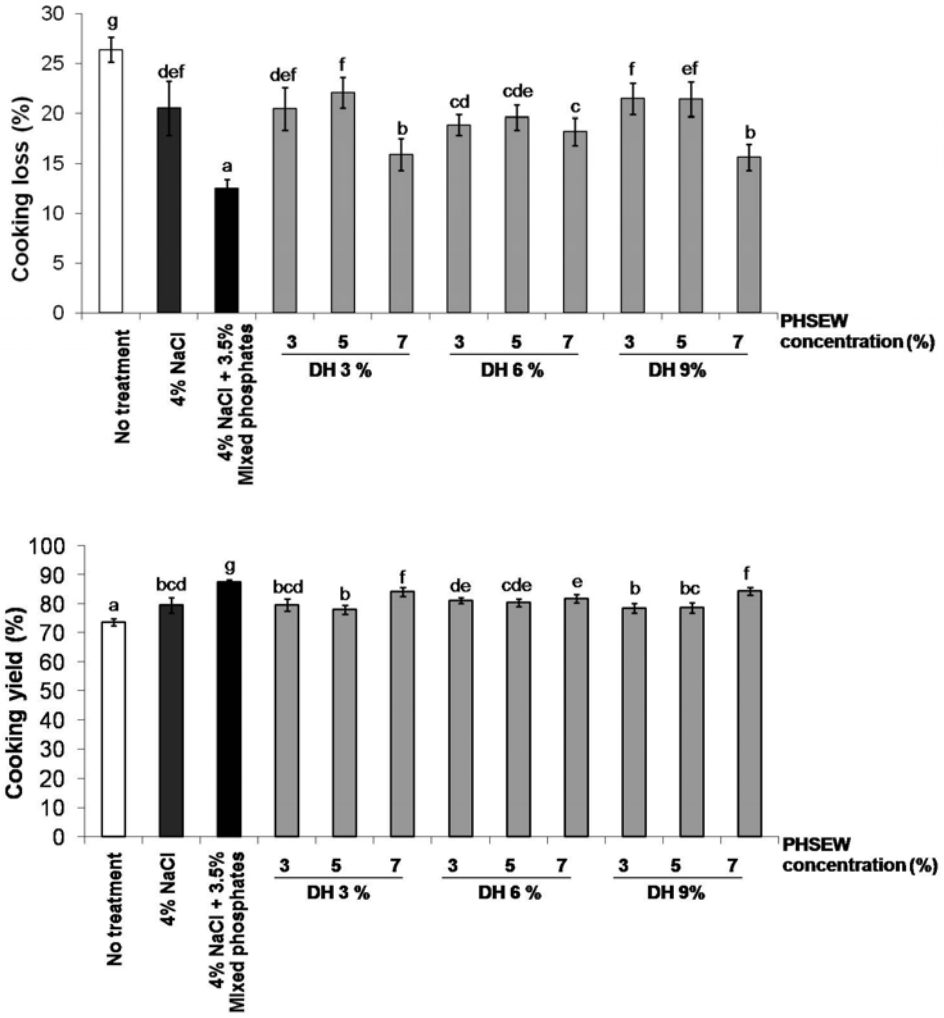



**Figure 49.** Cooking loss (A) and cooking yield (B) of Pacific white shrimp soaked in 4% NaCl containing PHSEW with different DHs at various levels. Shrimps were also soaked in 4% NalCl without and with 3.5% mixed phosphates and used as the controls. Bars represent the standard deviation (n=3). Different letters on the bar indicate the significant differences ($p < 0.05$)

### 8.4.4 Effect of PHSEW in combination with mixed phosphates on quality of Pacific white shrimp

Weight gain and salt content of shrimps soaked in 4% NaCl or 4% NaCl containing 7% PHSEW (DH= 3%) together with mixed phosphates at different levels are depicted in Figure 50A and 50B, respectively. Shrimp treated with mixed phosphates had the highest weight gain (6.16%) ($p< 0.05$). No differences in weight gain were found between shrimps soaked in 4% NaCl and those treated with 4% NaCl containing 7% PHSEW and mixed phosphates ranging from 0 to 1.5% ($p> 0.05$). Higher weight gain was observed in shrimp treated with 4% NaCl containing 7% PHSEW and mixed phosphates at levels greater than 1.5 % ($p< 0.05$). However, no further increases in weight gain were observed when mixed phosphates concentration increased up to 3.5% ($p> 0.05$).

Salt content of shrimps treated with 4% NaCl containing 3.5% mixed phosphates were similar to that of shrimps treated with 4% NaCl containing 7% PHSEW and mixed phosphates ranging from 1.0 to 3.5% ($p> 0.05$) (Figure 49B). A slight but significant increase in salt content was found in shrimp treated with 4% NaCl containing 7% PHSEW and 0.5% mixed phosphates compared with that treated with 4% NaCl ($p< 0.05$). Phosphate contents of shrimp soaked in 4% NaCl containing 7% PHSEW increased when the amount of mixed phosphates increased ($p< 0.05$) (Figure 50C). In the presence of 7% PHSEW, soaking solution containing 2 or 2.5% mixed phosphates yielded the shrimps with similar weight gain, compared to solution having only 3.5% mixed phosphate (without PHSEW) ($p>0.05$). Those solutions rendered shrimps with lower phosphate content, in comparison with 4% NaCl containing 3.5% mixed phosphates (without PHSEW). The highest phosphate content was found in shrimp treated with 4% NaCl containing 3.5% mixed phosphates ($p< 0.05$). However, phosphate contents in shrimp were less than the standard value (5000 ppm) (Official Journal of the European Communities, 1995). The result suggested that phosphates might penetrate into the shrimp muscle differently when 7% PHSEW was incorporated. Phosphate and salt have been known to increase swelling of the muscle

and retain more water (Offer and Knight, 1988). However, the amount of mixed phosphates could be reduced when PHSEW at a level of 7% was incorporated without lowering the efficacy in holding water as indicated by no difference in weight gain of treated shrimps.

Cooking loss and cooking yield of shrimps treated with 4% NaCl, 4% NaCl containing 3.5% mixed phosphate and 4% NaCl containing 7% PHSEW and mixed phosphate at different levels are shown at Figure 51A and 51B, respectively. Lower cooking loss with higher cooking yield was found in shrimp treated with 4% NaCl containing 7% PHSEW and mixed phosphates ranging from 2.5 to 3.5%, compared with those treated with 4% NaCl and 3.5% mixed phosphates (without PHSEW) ($p< 0.05$). However, when PHSEW at levels lower than 2.5% were incorporated in 4% NaCl containing 7% PHSEW, the treated shrimps had the higher cooking loss and lower cooking yield ($p< 0.05$). To obtain the increased weight gain and cooking yield with lowered cooking loss, soaking solution comprising 4% NaCl, 7% PHSEW and 2.5% mixed phosphates was recommended.

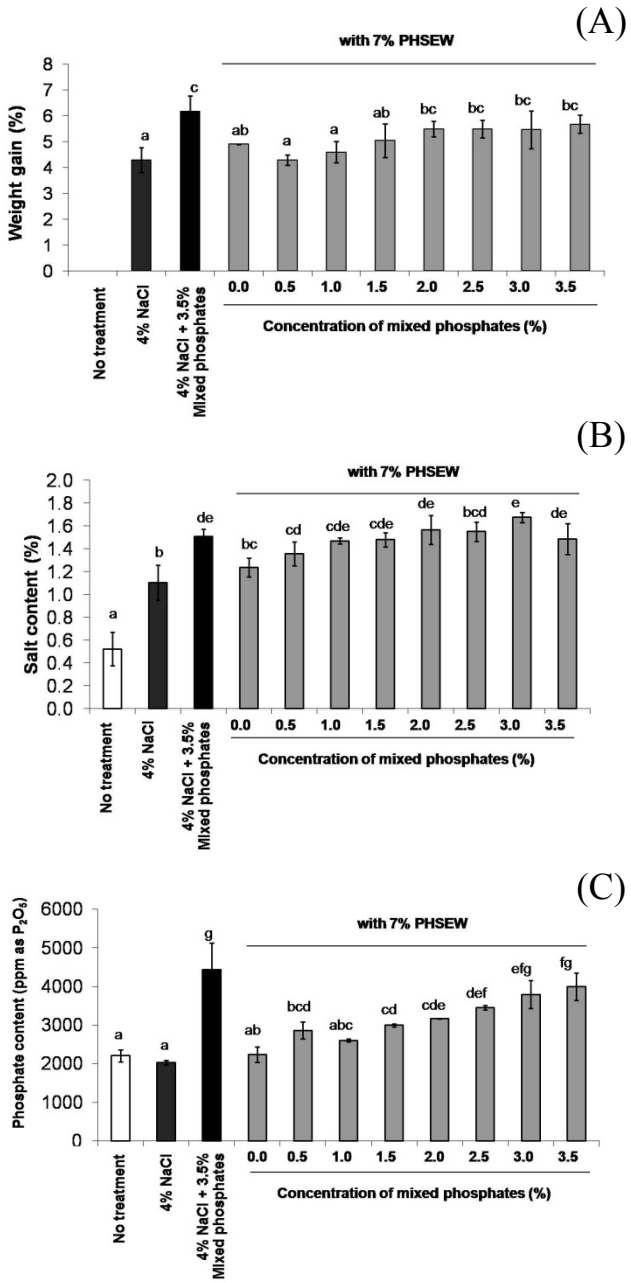



**Figure 50.** Weight gain (A), salt content (B) and phosphate content (C) of Pacific white shrimp soaked in 4% NaCl containing 7% PHSEW and mixed phosphates at various levels. Bars represent the standard deviation (n= 3). Shrimps were also soaked in 4% NaCl without and with 3.5% mixed phosphates and used as the controls. Different letters on the bar indicate the significant differences ($p < 0.05$)

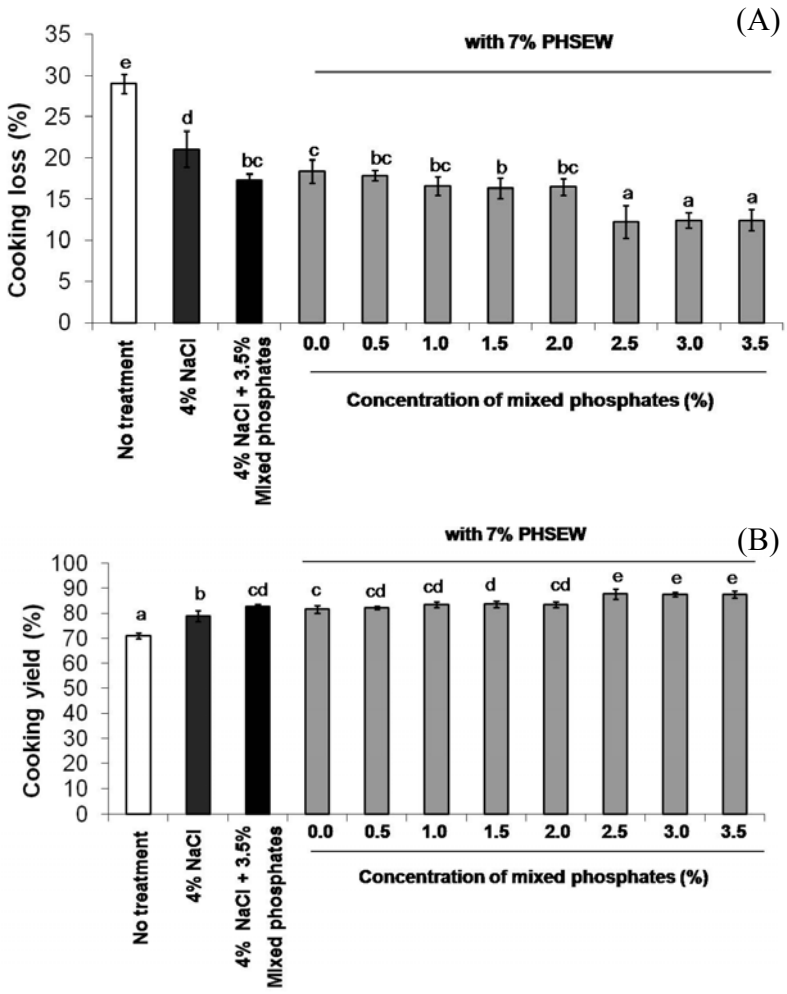



**Figure 51.** Cooking loss (A) and cooking yield (B) of Pacific white shrimp soaked in 4% NaCl containing 7% PHSEW and mixed phosphates at various levels. Bars represent the standard deviation (n= 3). Shrimps were also soaked in 4% NaCl without and with 3.5% mixed phosphate and used as the controls. Different letters on the bar indicate the significant differences ($p<0.05$).

### 8.4.5 Opacity score and sensory property

After being treated with 4% NaCl containing 3.5% mixed phosphates or 4% NaCl containing 7% PHSEW and 2.5% mixed phosphates, both samples become more translucent, compared with the control (no treatment) ($p< 0.05$) (Table 20). However, no differences were observed between both samples ($p> 0.05$). Translucence of shrimps increased with the treatment of phosphates as indicated by the decrease in opacity score. When the soaking solution contained PHSEW, the treated shrimps tended to have the non-significantly lower translucence.

The likeness scores of cooked white shrimp with different treatments are shown in Table 20. No differences in color, taste and odour likeness were observed among all samples ($p> 0.05$). Higher scores appearance, texture and overall likeness were found in shrimp treated with 4% NaCl containing 3.5% mixed phosphates or 4% NaCl containing 2.5% mixed phosphates and 7% PHSEW, compared with the control (no treatment). According to the criterion for acceptability limit, the score greater than 5 indicates acceptability (Meilgaard *et al*., 1990). Thus, shrimp treated with PHSEW in combination with mixed phosphates had the similar acceptability to those treated with only mixed phosphates. The higher likeness was most likely associated with the improved cooking yield with enhanced water holding capacity. This resulted in the increase juiciness of treated shrimps.

Shear force of fresh and cooked white shrimp with different treatments in comparison with the control is shown in Table 21. Without cooking, shrimp treated with 4% NaCl containing 7% PHSEW and 2.5% mixed phosphates had the lower shear force than the control ($p< 0.05$). No differences in shear force were found between shrimp treated with 4% NaCl and 3.5% mixed phosphates and those treated with 4% NaCl containing 7% PHSEW and 2.5% mixed phosphates ($p> 0.05$). For cooked shrimp, shrimps with both treatments had the lower shear force than the control ($p< 0.05$) and similar shear force was noticeable between both treatments ($p> 0.05$). Shrimp meat is enhanced in firmness or solidity by heat processing and gets too solid when its inner temperature is above 100ºC (Mizuta *et al*., 1999). From the result, shrimp treated with mixed phosphates had a lower shear force, regardless of PHSEW

incorporation, either before or after heating, when compared with the control. The differences in microstructure and the arrangement of muscle fiber after being treated with mixed phosphates were postulated. The swelling of muscle and retained water most likely had the impact on lower shear force of treated shrimp.

**Table 21.** Sensory scores of cooked Pacific white shrimp without and with different treatments.

| Parameters | NT†† | Mixed PP | Mixed PP+PHSEW |
|---|---|---|---|
| Opacity score | 4.5±0.4[b] | 3.4±0.4[a] | 3.6±0.6[a] |
| Likeness | | | |
| - Color | 7.2±1.1*, [a†] | 7.3±0.9 [a] | 7.5±0.9 [a] |
| - Appearance | 6.6±0.5 [a] | 7.6±0.7 [b] | 7.8±0.8 [b] |
| - Taste | 6.9±1.5 [a] | 7.4±1.1 [a] | 7.6±1.0 [a] |
| - Odor | 7.2±1.5 [a] | 7.0±1.2 [a] | 7.1±1.4 [a] |
| - Texture | 7.0±0.8 [a] | 7.9±1.0 [b] | 7.8±0.9 [b] |
| - Overall | 7.2±0.8 [a] | 7.7±0.7 [b] | 7.7±0.6 [b] |

* Means ± SD (n=3)

† Different subscripts in the same row indicate the significant differences ($p<0.05$).

††NT: No treatment; Mixed PP: 4% NaCl containing 3.5% mixed phosphates (0.875% SAPP, 2.625% TSPP); Mixed PP + PHSEW: 4% NaCl containing 7% PHSEW (DH= 3%) and 2.5% mixed phosphate. Opacity score = 1: very transparent; 3: moderately transparent and 5: Turbid, dull. Liking score 1: not like very much; 5: neither like and 9: like extremely.

**Table 22.** Shear forces of raw and cooked Pacific white shrimp without and with different treatments.

| Treatments | Shear force (N) | |
|---|---|---|
| | Raw Shrimp | Cook shrimp |
| NT[††] | 18.79±1.96[*, a†] | 20.79±2.01[a] |
| Mixed PP | 17.78±1.63[ab] | 16.83±2.01[b] |
| Mixed PP + PHSEW | 17.11±1.06[b] | 16.62±1.96[b] |

[*] Means ± SD (n=3)

[†] Different subscripts in the same row indicate the significant differences ($p<0.05$).

[††]NT: No treatment; Mixed PP: 4% NaCl containing 3.5% mixed phosphates (0.875% SAPP, 2.625% TSPP); Mixed PP + PHSEW: 4% NaCl containing 7% PHSEW (DH= 3%) and 2.5% mixed phosphate.

### 8.4.6 Microstructure of cooked Pacific white shrimp

Microstructures of cooked Pacific white shrimp muscle without and with two different treatments are illustrated in Figure 51. For the transverse section (Figure 52A-52C), cooked shrimps treated with mixed phosphate had loosened structure with the larger gap than those without phosphate treatment, irrespective of PHSEW incorporation. However, no difference in microstructure was noticeable between shrimps treated with 4% NaCl containing 3.5% mixed phosphates and 4% NaCl containing 2.5% mixed phosphates and 7% PHSEW. For longitudinal section (Figure 52D-52F), shrimp muscle obtained from both treatments had the larger myofibrils. However, myofibrils were less attached as indicated by gaping occurred. Heating process generally causes the shrinkage of muscle of shrimp. Heat processing enhanced the firmness and degree of shrinkage of *Penaeus japonicus* (Mizuta *et al.,* 1999). The release of water from protein molecules might facilitate the myofibrils to align closely, leading to the more compact structure. With phosphate treatment, water could be more retained and prevented the excessive shrinkage of muscle. For raw

shrimp, similar myofibril arrangement was observed between all samples (data not shown).

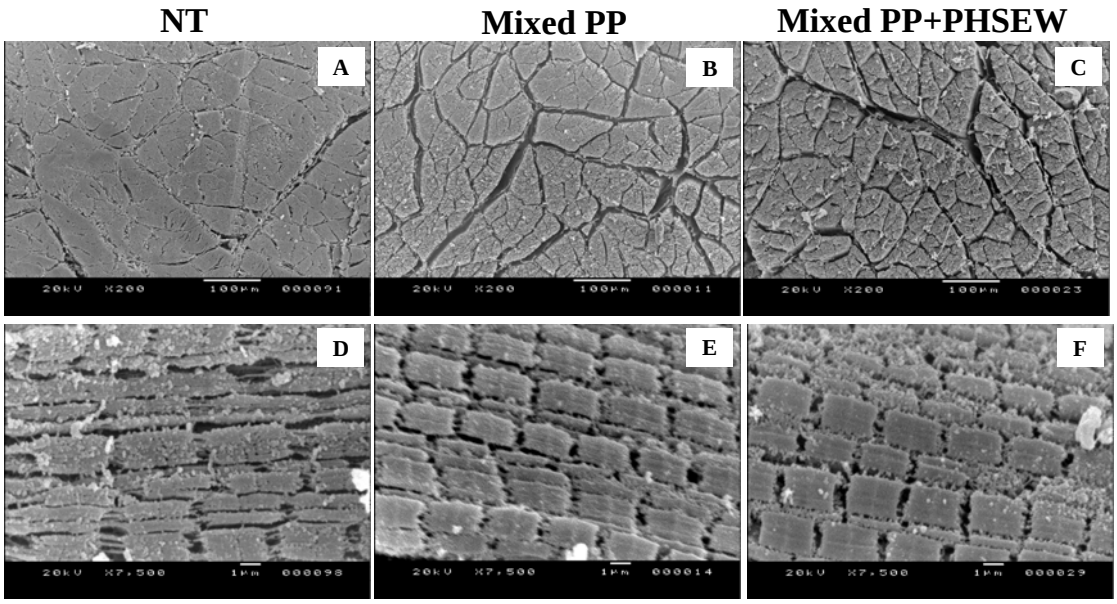



**Figure 52.** SEM micrographs of transverse (A-C) and longitudinal (D-F) sections of cooked Pacific white shrimp without and with different treatments. NT: No treatment; Mixed PP: 4% NaCl containing 3.5% mixed phosphates (0.875% SAPP, 2.625% TSPP); Mixed PP + PHSEW: 4% NaCl containing 7% HPSWE and 2.5% mixed phosphate.

## 8.5 Conclusions

Protein hydrolysate from salted egg white (PHSEW) could be produced with the aid of pepsin. Use of 7% PHSEW in combination with 4% NaCl and 2.5% mixed phosphates for treatment of Pacific white shrimp could lower the cooking loss and increase cooking yield and weight gain of resulting shrimp more effectively than the use of 4% NaCl containing 3.5% mixed phosphates. Thus, phosphate residue in treated shrimp could be reduced by incorporating with PHSEW at an appropriate level in the soaking solution without any significant changes in sensory properties.

# CHAPTER 9

# SUMMARY AND FUTURE WORKS

## 9.1 Summary

1. Salting process induced the solidification of yolk, accompanied with oil exudation and the development of gritty texture. Moisture removal and the association of egg yolk granules most likely contributed to the development of solidified yolk. Salting processes has no impact on the changes in lipid composition and fatty acid profile of duck egg during salting.

2. Salting processes affected the characteristic of resulting egg white and egg yolk. Both salting methods, coating and immersing, could induce solidification of yolk accompanied with oil exudation and the development of gritty texture. Salted duck egg produced by paste coating and brine immersing methods had the slight differences in chemical composition and textural properties as salting proceeded. Salted egg yolk obtained with different salting methods had different oil exudation as well as lipid oxidation during salting.

3. Salting process could be shortened with acetic acid pretreatment and the aid of Neutrase prior to salting. Soaking egg in 5% acetic acid, followed by immersing in 0.25% (w/v) Neutrase for 90 min could increase hardening ratio and oil exudation of yolk within the shorter time than tranditional method for about 1 week.

4. Characteristics of salted egg white and yolk were affected by heating. Salting process caused the moisture removal and allowed the diffusion of salt into egg yolk and white. As a consequence, both cooked egg white and yolk contained the higher amount of salt. Salting affected the textural properties of cooked egg yolk and white to some degree. Immersing method had more impact on the softening of egg white gel than did coating method, particularly at the first week of salting. Moreover, oil exudation, a desirable characteristic of salted egg yolk, was more pronounced in egg yolk from coating method, especially at week 3 of salting.

Marked decrease in hardness of cooked egg white with coincidental increase in hardness of egg yolk most likely determined the textural sensation during eating. After cooking, egg white gel turned to be coarser and lost water holding capacity, particularly as salting proceeded. Oil exudation with increased redness of cooked egg yolk was more pronounced with increasing salting time. Salting method therefore have the impact on the characteristic and properties of egg white and yolk after cooking.

5. Salting was associated with the induced thermal aggregation of egg white proteins mainly by induction of hydrophobic interaction or ionic interaction. NaCl plays a role in the formation of coagulum type gel of cooked salted egg white, which had the course texture with opaqueness.

6. Addition of NaCl and dehydration period played an important role on viscoelastic and thermal-induced properties of egg yolk. The former led to the aggregation of yolk protein molecules, especially at high NaCl concentration. The latter also resulted in a well-developed gel network. During heating process, the addition of NaCl to egg yolk tended to stabilize the protein molecules, leading to an increase in the denaturation temperature as well as a delay in gel network formation.

7. Phosphate residue in treated shrimp could be reduced by incorporating with protein hydrolysate from salted egg white (PHSEW) at an appropriate level in the soaking solution without any significant changes in sensory properties. Use of 7% PHSEW in combination with 4% NaCl and 2.5% mixed phosphates for treatment of Pacific white shrimp could lower the cooking loss and increase cooking yield and weight gain of resulting shrimp more effectively than the use of 4% NaCl containing 3.5% mixed phosphates.

## 9.2 Future works

1. The effect of storage on quality of cooked salted egg with the sensorial acceptability should be further study.

2. The utilization of salted egg white and should be further studied.

3. The bioactivity of peptides purified from protein hydrolysate from salted egg white should be investigated.

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**List of Publication and Proceedings**

**Publications**

1. Kaewmanee, T., Benjakul, S. and Visessanguan, W. 2009. Changes in chemical composition, physical properties and microstructure of duck egg as influenced by salting. Food Chem. 112: 560-569.
2. Kaewmanee, T., Benjakul, S. and Visessanguan, W. 2009. Effect of salting processes on chemical composition, textural properties and microstructure of duck egg. J. Sci. Food Agric. 89: 625-633.
3. Kaewmanee, T., Benjakul, S. and Visessanguan, W. 2010. Protein hydrolysate of salted duck egg white as a substitute of phosphate and its effect on quality of Pacific White Shrimp (*Litopenaeus vannamei*). J. Food Sci. 74: 351-361.
4. Kaewmanee, T., Benjakul, S. and Visessanguan, W. Effect of acetic acid and commercial protease pretreatment on salting and characteristics of salted duck egg. J. Food Biochem. (Submitted).
5. Kaewmanee, T., Benjakul, S. and Visessanguan, W. Chemical composition, textural properties and microstructure of cooked duck egg as affected by salting processes and times. J. Food Sci. (Submitted).

6. Kaewmanee, T., Benjakul, S. and Visessanguan, W. 2010. Effect of NaCl on thermal aggregation of egg white proteins from duck egg. J. Food Eng. (Submitted).

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1. Kaewmanee, T., Benjakul, S. and Visessanguan, W. 2008. Changes in chemical composition, physical properties and microstructure of duck egg as influenced by salting. IFT Annual Meeting. New Orleans, USA.
2. Kaewmanee, T., Benjakul, S. and Visessanguan, W. 2008. Effect of salting processes on chemical composition, textural properties and microstructure of duck egg. Food Innovation Asia 2008: The International Food Conference. BITEC Bangna, Bangkok.
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