

การศึกษาปัจจัยในกระบวนการผลิตที่มีผลต่อคุณภาพทางกายภาพของเต้าหู้หลอดไขไก่

โดย

นายบุญอนันต์ ศักดิ์บุญญารัตน์

# มหาวิทยาลัยศิลปากร สงวนลิขสิทธิ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

สาขาวิชาเทคโนโลยีอาหาร

ภาควิชาเทคโนโลยีอาหาร

บัณฑิตวิทยาลัย มหาวิทยาลัยศิลปากร

ปีการศึกษา 2547

ISBN 974-464-517-2

ลิขสิทธิ์ของบัณฑิตวิทยาลัย มหาวิทยาลัยศิลปากร

THE STUDY OF PROCESSING FACTORS AFFECTING PHYSICAL  
PROPERTIES OF PACKED EGG TOFU

By

Boonanan Sakboonyarat

มหาวิทยาลัยศิลปากร สงวนลิขสิทธิ์

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

Department of Food Technology

Graduate School

SILPAKORN UNIVERSITY

2004

ISBN 974-464-517-2

The graduate school, Silpakorn University accepted thesis entitled “THE STUDY OF PROCESSING FACTORS AFFECTING PHYSICAL PROPERTIES OF PACKED EGG TOFU” by Boonanan Sakboonyarat in partial fulfillment of the requirements for the degree of master science, program of food technology.

.....  
(Associate Professor Chirawan Kongklai, Ph.D.)

Dean of Graduate School

...../...../.....

Thesis Advisors

1. Bhundit Innawong, Ph.D.
2. Arunsri Leejeerajumnean, Ph.D.
3. Thouchpong Choosri

Thesis committee

มหาวิทยาลัยศิลปากร สงวนลิขสิทธิ์

.....Chairman

(Pramote Khuwijtjaru, Ph.D.)

...../...../.....

.....Member

(Bhundit Innawong, Ph.D.)

...../...../.....

.....Member

(Pramuk Parakulsuksatid, Ph.D.)

...../...../.....

.....Member

(Arunsri Leejeerajumnean, Ph.D.)

...../...../.....

.....Member

(Thouchpong Choosri)

...../...../.....

K 45403203 : สาขาวิชาเทคโนโลยีอาหาร

คำสำคัญ : เต้าหู้หลอดไข่ไก่ / การจืด / แมกนีเซียมซัลเฟต / แคลเซียมซัลเฟต

บุญอนันต์ ศักดิ์บุญญารัตน์ : การศึกษาปัจจัยในกระบวนการผลิตที่มีผลต่อคุณภาพทางกายภาพของเต้าหู้หลอดไข่ไก่ (THE STUDY OF PROCESSING FACTORS AFFECTING PHYSICAL PROPERTIES OF PACKED EGG TOFU) อาจารย์ผู้ควบคุมวิทยานิพนธ์ : อ.ดร.บัณฑิต อินดวงศ์, อ.ดร.อรุณศรี ลิขิตจำเนียร และ อ.รัชพงศ์ ชูศรี. 88 หน้า. ISBN 974-464-517-2

การศึกษาผลของสารโคแอกกูแลนต์ 5 ชนิด ประกอบด้วย กลูโคโนเดลตาแลคโตน (GDL), แมกนีเซียมซัลเฟต ( $MgSO_4$ ), แคลเซียมซัลเฟต ( $CaSO_4$ ), แมกนีเซียมคลอไรด์ ( $MgCl_2$ ), และแคลเซียมคลอไรด์ ( $CaCl_2$ ) ซึ่งเติมในเต้าหู้หลอดไข่ไก่ (น้ำในสูตรร้อยละ 50) ต่อคุณสมบัติทางกายภาพ พบว่า เต้าหู้หลอดไข่ไก่ที่ใช้  $CaSO_4$  และ  $MgSO_4$  ให้เนื้อสัมผัสที่ดีกว่าการใช้สารชนิดอื่น โดยมีค่าร้อยละการแยกตัวของน้ำ (%syneresis) เท่ากับ 5.37 และ 4.92 ตามลำดับ ซึ่งน้อยกว่าเต้าหู้หลอดไข่ไก่ทางการค้าที่มียอดจำหน่ายสูง (ตัวอย่างควบคุม) ถึงร้อยละ 47 จากนั้นจึงศึกษาผลของอัตราส่วนน้ำที่ 4 ระดับความแตกต่าง คือ ร้อยละ 50, 55, 60, และ 65 ตามลำดับ และเติม 0.3%  $MgSO_4$  ในสูตรการผลิต พบว่า เต้าหู้หลอดไข่ไก่ที่มีน้ำร้อยละ 55 จะให้ค่าความเค้นเฉือน (shear stress) และความแน่นเนื้อ (firmness) ใกล้เคียงกับตัวอย่างควบคุมมากที่สุด ( $p>0.05$ ) โดยมีความแตกต่างเพียงร้อยละ 2.3 และ 1.2 ตามลำดับ

เมื่อศึกษาผลของปริมาณการเติม  $MgSO_4$  ที่ระดับต่างๆ คือ ร้อยละ 0.1, 0.2, และ 0.3 โดยน้ำหนัก และอัตราส่วนน้ำ 2 ระดับ ที่ร้อยละ 55 และ 60 พบว่า เต้าหู้หลอดไข่ไก่ที่เติม 0.1%  $MgSO_4$  และอัตราส่วนน้ำที่ร้อยละ 55 จะให้เนื้อสัมผัสใกล้เคียงกับตัวอย่างควบคุมมากที่สุด ( $p>0.05$ ) โดยมีค่าความเค้นเฉือน 0.85 N/g.mm<sup>2</sup> และค่าความแน่นเนื้อ 2.26 N.mm จากการวิเคราะห์ทางสถิติ อิทธิพลร่วมระหว่างปริมาณการเติม  $MgSO_4$  และอัตราส่วนน้ำที่เติมในสูตรการผลิตนั้นไม่แตกต่างอย่างมีระดับนัยสำคัญ ( $p>0.05$ ) ถ้าเติม  $MgSO_4$  ในปริมาณที่สูงขึ้น เต้าหู้หลอดไข่ไก่จะมีสีเหลืองคล้ำ ( $p<0.05$ ) จากการวิเคราะห์หาอุณหภูมิและระยะเวลาในการต้มเต้าหู้หลอดไข่ไก่ที่เหมาะสมโดยใช้วิธีพื้นผิวตอบสนอง เต้าหู้หลอดไข่ไก่ที่ต้ม ณ อุณหภูมิในช่วง 87 ถึง 90 องศาเซลเซียส และใช้ระยะเวลาการต้มประมาณ 31 ถึง 40 นาที จะให้คุณสมบัติทางกายภาพที่ดีกว่าตัวอย่างควบคุม

จากการศึกษาผลการเติมการจืด (ร้อยละ 0.1 และ 0.2) ร่วมกับการใช้สารโคแอกกูแลนต์ ( $CaSO_4$  และ  $MgSO_4$ ) ในสูตรการผลิตเต้าหู้หลอดไข่ไก่ พบว่า ความเค้นเฉือนของเต้าหู้หลอดไข่ไก่ที่เติมการจืดและการใช้สารโคแอกกูแลนต์มีค่ามากกว่าเต้าหู้หลอดไข่ไก่ที่เติมสารโคแอกกูแลนต์เพียงอย่างเดียว การทดลองนี้ยังคงสนับสนุนว่า ความเค้นเฉือนของเต้าหู้หลอดไข่ไก่ที่เติม  $CaSO_4$  มีค่าสูงกว่าเต้าหู้หลอดไข่ไก่ที่เติม  $MgSO_4$  ส่วนค่าความแน่นเนื้อกลับพบว่าไม่มีความแตกต่าง ( $p>0.05$ ) การเติมการจืดในปริมาณสูงขึ้นไปยังสามารถช่วยลดการแยกตัวของน้ำในเต้าหู้หลอดไข่ไก่ และไม่ส่งผลต่อการเปลี่ยนแปลงสีของเต้าหู้หลอดไข่ไก่ ( $p>0.05$ ) จากการวิเคราะห์ทางสถิติไม่พบอิทธิพลร่วมระหว่างชนิดสารโคแอกกูแลนต์และปริมาณการจืดที่ใช้

ภาควิชาเทคโนโลยีอาหาร

บัณฑิตวิทยาลัย มหาวิทยาลัยศิลปากร

ปีการศึกษา 2547

ลายมือชื่อนักศึกษา.....

ลายมือชื่ออาจารย์ผู้ควบคุมวิทยานิพนธ์ 1.....2.....3.....

K45403203 : MAJOR : FOOD TECHNOLOGY

KEY WORD : PACKED EGG TOFU, CARRAGEENAN, CALCIUM SULPHATE, MAGNESIUM SULFATE.

BOONANAN SAKBOONYARAT : THE STUDY OF PROCESSING FACTORS AFFECTING PHYSICAL PROPERTIES OF PACKED EGG TOFU. THESIS ADVISORS : BHUNDIT INNAWONG, Ph.D., ARUNSRI EEJEERAJUMNEAN , Ph.D., AND THOUCHPONG CHOOSRI. 88 pp. ISBN 974-464-517-2.

The changes in physical properties of packed egg tofu containing 50% added water and 0.3% of 5 potential coagulants (including GDL,  $MgSO_4$ ,  $CaSO_4$ ,  $MgCl_2$ , and  $CaCl_2$ ) were intensively investigated. The packed egg tofu with  $CaSO_4$  and one with  $MgSO_4$  exhibited the best physical properties (such as shear stress, firmness, and syneresis). The synereses of both egg tofu consisting of  $CaSO_4$  and  $MgSO_4$  were 5.37% and 4.92%, respectively. As comparison with the best-seller commercial products (used as the control), the addition of both  $CaSO_4$  and  $MgSO_4$  substantially decreased the syneresis approximately 47%. The following experiment was conducted to explore the textural attributes of packed egg tofu with various %added water (50, 55, 60 and 65%). The packed egg tofu with 0.3%  $MgSO_4$  and 55% added water represented the best formulation with respect to its considerable reduction in syneresis and insignificantly differences in shear stress and firmness by 2.3% and 1.2%, respectively as compared with the control. For further studies, the most suitable formulation of packed egg tofu was consequently carried out and developed by using  $MgSO_4$  at 0.1, 0.2, and 0.3% (w/w) and adding the different water amounts (55 and 60%). As expected, the packed egg tofu with 0.1%  $MgSO_4$  and 55% of added was the most suitable and compatible with similarly textural control characteristics. No interaction effect was found ( $p>0.05$ ) between  $MgSO_4$  concentrations and water usage.

Regarding to response surface methodology, the process parameters including the cooking temperature and time were evaluated. The packed egg tofu cooked in the range of 87-90°C at 31-40 min could obtained more superior physical properties than the control. Finally, the textural improvement of packed egg tofu via the addition of both carrageenan (0.1, and 0.2%) and 0.1% coagulants ( $MgSO_4$  and  $CaSO_4$ ) was evaluated. The packed egg tofu added carrageenan remarkable incremented shear stress and firmness. The more carageenan usage, the higher shear stress and firmness obtained. In contrast, the syneresis of packed egg tofu tended to decrease with higher carrageenan concentration. The lightness ( $L^*$ ), redness ( $a^*$ ), and yellowness ( $b^*$ ) of all test samples were not significantly ( $p>0.05$ ) differentiate. The  $MgSO_4$ -egg tofu contributed greater shear stress than the  $CaSO_4$ -egg tofu, but firmnesses of both egg tofu were not distinguished. In contrast, the  $CaSO_4$ -egg tofu showed higher water content than the  $MgSO_4$ -egg tofu ( $p<0.05$ ). No interaction effect between coagulants and carrageenan concentration was indicated.

---

Department of Food Technology Graduate School, Silpakorn University Academic Year 2004

Student's signature.....

Thesis Advisors' signature 1.....2.....3.....

## ACKNOWLEDGEMENTS

I wish to express my sincere gratitude and respect to my advisor Dr. Bhundit Innawong for his kindness, ideas, patience, and knowledgeable advice throughout my thesis. I am thankful for his willingness to take the time to make suggestions and help me achieve my goals. His support, guidance, and encouragement were most appreciated.

I thank Dr. Arunsri Leejeerajumnean, Dr. Pramote Khuwijitjaru, Dr. Pramuk Parakulsuksatid and Mr. Thouchpong Choosri, who expressed to the members of my thesis. I would like to thank all of the graduate students that assisted in this research. I sincerely thank all staff in department of food technology.

Many thanks are extended to all my friends. Finally, I would especially like to thank my parents for their love, financial support, and encouragement that they are given me throughout my academic career.

มหาวิทยาลัยศิลปากร สงวนลิขสิทธิ์

## TABLE OF CONTENTS

	<b>Page</b>
THAI ABSTRACT .....	IV
ENGLISH ABSTRACT.....	V
ACKNOWLEDGEMENTS .....	VI
LIST OF TABLES .....	IX
LIST OF FIGURES .....	X
<b>CHAPTER</b>	
1 INTRODUCTION .....	1
REFERENCES.....	2
2 LITERATURE REVIEW .....	3
EGG INDUSTRY .....	3
TOFU .....	7
TYPE OF COAGULANTS.....	7
MECHANISM OF COAGULATION OF EGG.....	9
HEAT PROCESSING OF SOYMILK AND TOFU GELATION MECHANISM .....	12
FACTORS AFFECTING COAGULATION OF EGG .....	13
PROTEIN-POLYSACCHARIDE INTERACTIONS.....	17
REFERENCES.....	18
3 EFFECT OF DIFFERENT COAGULANTS ON THE PHYSICAL PROPERTIES OF PACKED EGG TOFU .....	21
ABSTRACT .....	21
INTRODUCTION.....	22
MATERIAL AND METHODS .....	24
RESULT AND DISCUSSION .....	26
CONCLUSIONS.....	28
REFERENCES.....	29

	<b>Page</b>
<b>CHAPTER</b>	
4	
OPTIMIZATION OF PROCESSING PROCEDURE FOR THE PHYSICAL PROPERTIES OF PACKED EGG TOFU USING RESPONSE SURFACE METHODOLOGY .....	37
ABSTRACT .....	37
INTRODUCTION.....	37
MATERIAL AND METHODS .....	39
RESULTS AND DISCUSSION .....	41
CONCLUSIONS.....	44
REFERENCES.....	45
5	
EFFECT OF CARRAGEENAN ON THE PHYSICAL PROPERTIES OF PACKED EGG TOFU.....	63
ABSTRACT .....	63
INTRODUCTION.....	63
MATERIAL AND METHODS .....	65
RESULTS AND DISCUSSION .....	67
CONCLUSIONS.....	70
REFERENCES.....	71
6	
SUMMARY .....	79
APPENDIX	
APPENDIX 1 .....	81
APPENDIX 2.....	82
VITA.....	88



## LIST OF TABLES

Tables	Page
3.1 Shear stress, and firmness of packed egg tofu used different coagulants (50% added water) .....	30
3.2 Shear stress, and firmness of packed egg tofu employed different added water ratios (%) at 0.3% MgSO <sub>4</sub> .....	31
3.3 Shear stress, and firmness of packed egg tofu at different water ratios and MgSO <sub>4</sub> concentration.....	32
3.4 Color for packed egg tofu employed different water ratios and coagulants concentration.....	33
3.5 Syneresis of packed egg tofu employed different water ratios and coagulants concentration.....	34
4.1 Experimental range levels of two independent variables in terms of actual factors. ....	46
4.2 3-Level factorial circumscribed design arrangement and responses.....	47
4.3 Aanalysis of variance (ANOVA) and model fitting for response variables .....	48
4.4 Predicted and observed values for the responses at optimum conditions .....	49

## LIST OF FIGURES

<b>Figure</b>		<b>Page</b>
2.1	Egg consumption between 1985 to 1999 .....	4
2.2	Regional Egg Consumption .....	4
2.3	World layers and egg production .....	6
2.4	Regional egg production .....	6
2.5	Gelation mechanism of soybean proteins in the presence of GDL or CaSO <sub>4</sub> ...	13
3.1	Effect of different coagulants (0.3%) at 50% added water on the syneresis of packed egg tofu .....	35
3.2	Effect of different water ratios at 0.3% MgSO <sub>4</sub> on the syneresis of packed egg tofu .....	36
4.1	Response surface plots (contour plot) of shear stress showing effects of cooking temperature and cooking time.....	50
4.2	Response surface plots (contour plot) of firmness showing effects of cooking temperature and cooking time.....	51
4.3	Response surface plots (contour plot) of syneresis showing effects of cooking temperature and cooking time.....	52
4.4	Response surface plots (contour plot) of color L* showing effects of cooking temperature and cooking time.....	53
4.5	Response surface plots (contour plot) of color a* showing effects of cooking temperature and cooking time.....	54
4.6	Response surface plots (contour plot) of color b* showing effects of cooking temperature and cooking time.....	55
4.7	Response surface plots (three-dimension surface plot) of shear stress showing effects of cooking temperature and cooking time.....	56

<b>Figure</b>	<b>Page</b>
4.8 Response surface plots (three-dimension surface plot) of firmness showing effects of cooking temperature and cooking time.....	57
4.9 Response surface plots (three-dimension surface plot) of syneresis showing effects of cooking temperature and cooking time.....	58
4.10 Response surface plots (three-dimension surface plot) of color L* showing effects of cooking temperature and cooking time.....	59
4.11 Response surface plots (three-dimension surface plot) of color a* showing effects of cooking temperature and cooking time.....	60
4.12 Response surface plots (three-dimension surface plot) of color b* showing effects of cooking temperature and cooking time.....	61
4.13 Optimum production procedure as a function of the independent variables after superimposition of the contour plots.....	62
5.1 Effect of coagulants and carrageenan on shear stress of packed egg tofu .....	72
5.2 Effect of coagulants and carrageenan on firmness of packed egg tofu.....	73
5.3 Effect of coagulants and carrageenan on syneresis of packed egg tofu .....	74
5.4 Effect of coagulants and carrageenan on water content of packed egg tofu.....	75
5.5 Effect of coagulants and carrageenan on lightness (L*) of packed egg tofu ....	76
5.6 Effect of coagulants and carrageenan on green-red chromaticity (a*) of packed egg tofu .....	77
5.7 Effect of coagulants and carrageenan on blue-yellow chromaticity (b*) of packed egg tofu .....	78

## CHAPTER 1

### INTRODUCTION

Hen's egg is valuable agricultural product with regard to contain a great variety of nutritious substances and be recommended as an inexpensive source of food protein (Jeamarnukulkij, 2002). In general, the egg proteins normally comprised 54% of ovalbumin, 12% of ovotransferrin, 11% of ovomucoid, and others and remarkably demonstrated good functional properties in some specific food commodities (Jaroonroj, 1980). According to the report of Agricultural Ministry of United States of America, there existed 795,711 millions eggs available in the world market (Exporters, 2002). In fact, over 95% eggs sold in Thailand have been used for the domestic consumptions and occasionally exported when only oversupplying (Exporters, 2002).

Regarding to the fluctuation of Thai domestic demand, this always promotes an excessive deviation of egg price. Several researchers were indicated that the crisis of egg price's fluctuation directly relies on various uncontrollable conditions including the available chicken meat in the market, the season of the year, and the requirement from the international trading (Jeamarnukulkij, 2002; Ouisoongneun, 1999; Suwannee, 1999). The inclination of egg production in Thailand has been considerably impeded due to Thai's eating behavior. According to the statistical recorded data, each Thai citizen normally consumes egg annually about 135.20 while one in either USA or Europe regularly have about 300 eggs (Somakornpin, 1999).

Fresh egg is considered as a perishable raw material because of the limitations of shelf storage. During over demand in the market as eggs are the product which has short shelf life, the agriculturists have to exported them in the low price. But modified it to another product, like freezed eggs and eggs powder, is also another choice to extent shelf life and add value to the products (Exporter, 2002). From the point of view, bring to idea of packed egg tofu. Nowadays, people have more concern about health so healthy products like packed egg tofu will have more demand in the market.

In the studies of coagulation ability of egg products, several factors such as salts, sugar, acid, alkaline, and temperature have been listed as the major parameters influencing the heat induce gelling of egg (Johnson and Zabik, 1980). The first objective of this study is to determine the appropriate type and concentration of coagulants and water per liquid whole egg ratio on the physical properties of packed egg tofu.

The second objective is to establish the optimum cooking temperature and cooking time of packed egg tofu. Moreover there is the third objective for the study, to understand the interaction between polysaccharides and protein that occurs widely in food systems. The effect of polysaccharide and protein interactions on coagulation abilities should be evaluated in relation to performance in food systems. Another objective of the present study is to evaluate the possible interaction among the carrageenan and egg proteins on the physical properties of packed egg tofu.

## REFERENCES

- Ouisoongneun C. 2003. Hen's egg, 4<sup>th</sup>. Agricultural Publishing.  
Nontaburi. 70 p.
- Johnson TM, Zabik ME. 1981. Gelation properties of albumen proteins, singly and in combination. Poultry Sci. 60:2071-2083.
- Jeamarnukulkij P. 2002. Eggs and Exported Products. Magazine for exporter and management (Export). 16 vol. 365 Oct. 58 p.
- Jaroonroj P. 1980. About Eggs. Food Science. Bangkok. 50 p.
- Kasetsuwan S. 1976. Egg and Chicken Meat. Kasetsart University. Bangkok. 396 p.
- Somakornpin S. 1999. Hen's egg. Rajabhat Thonburi Institute Bangkok. 336 p.

## CHAPTER 2

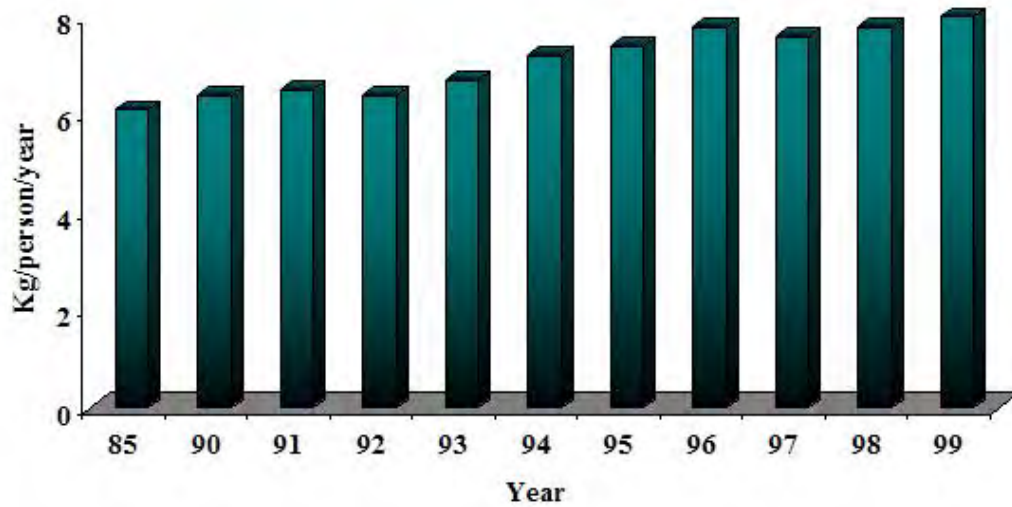
### LITERATURE REVIEW

#### EGG INDUSTRY

Birds' eggs have been used as a natural food by human beings since early antiquity. Compared with the hen's egg, no other single food of animal origin is eaten and relished by so many peoples the world over; none is served in such a variety of ways. Its popularity is justified because it is almost unsurpassed in nutritive excellence (Romanoff and Romanoff, 1988).

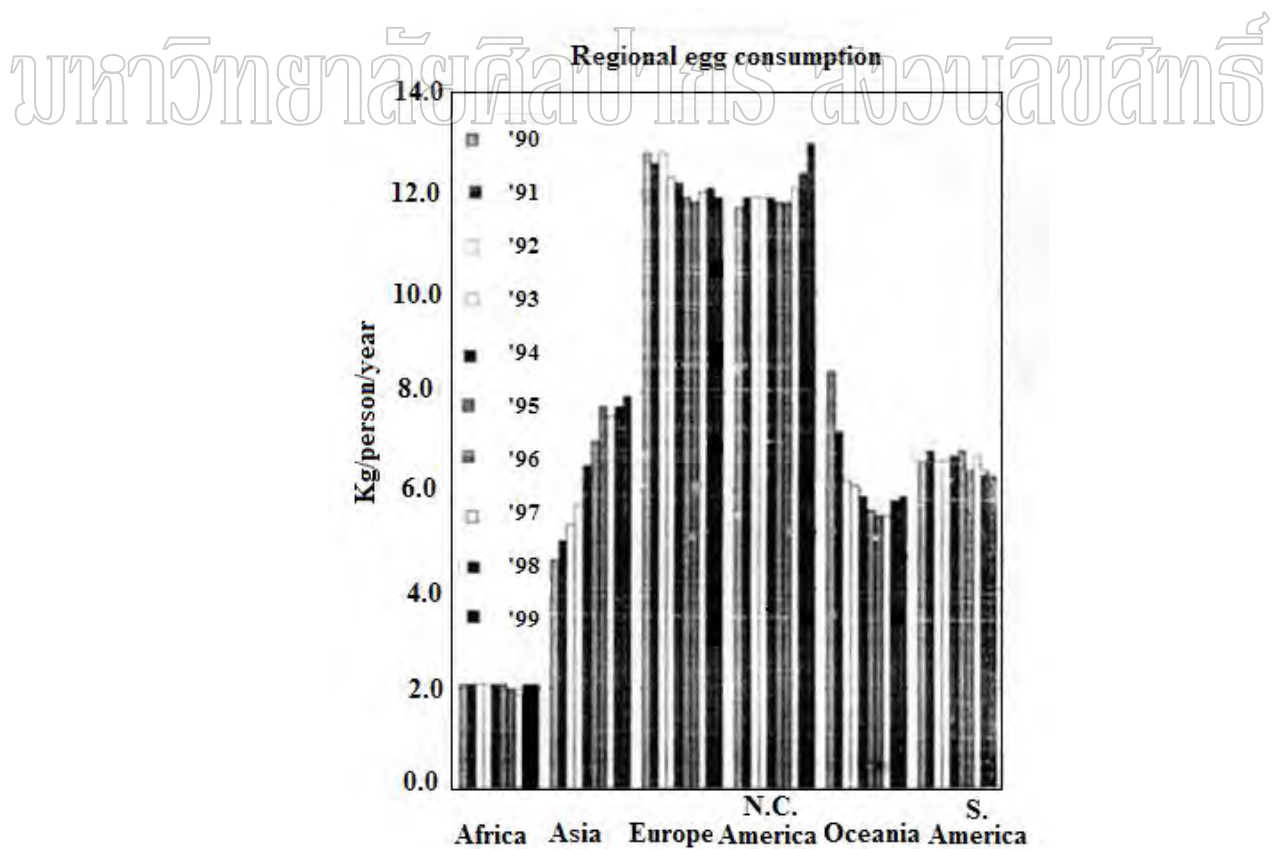
For many years, the trend has been toward an ever larger consumption of eggs throughout the world. The average number of eggs eaten annually by the individual varies greatly in different nations (Romanoff and Romanoff, 1988). Consumers are having increased interest in functional foods. Firms are now marketing egg enriched with certain nutrients such as vitamins, minerals and omega-3 fatty acids. There are also those consumers interested in natural or organic foods (Froning, 2002).

While the real income changes have little affected egg purchases and hence consumption in the developed economies, it is a key factor influencing the purchasing habits among those in the developing countries. Thus, the chart of average uptake/person has peaked in 1996 then suffered a sharp reversal. However, this setback has been overcome and consumption is back on an upward course reaching 8 kg/person at the end of the last decade (Fig. 2.1) (Egg industry, 2002). Over the period 1975 to 1981, the average number of meals eaten out/person/week showed an 8% increase since then far fewer meals are eaten out at the place of work or in the schools. In addition, the commercial fast-food and pub-food sectors have certainly continued to expand. Both these sectors are potentially excellent markets for egg and egg products (Well and Belyavin, 1980).



**Fig. 2.1** Egg consumption between 1985 to 1999.

source: Egg industry, 2002



**Fig. 2.2** Regional Egg Consumption.

source: Egg Industry, 2002

The regional chart revealed that this growth had been almost entirely because of an increase in egg eating in Asia (Fig. 2.2). Indeed, the average level of uptake in Europe, S. America and Oceania declined quite dramatically in the nineties. After a period of steady consumption, average uptake had risen in the USA, primarily due to the success of the egg promoters. This caused in changing people's attitudes towards eating egg and also, partly as a result of a steady rise in the consumption of egg products (Egg Industry, 2002). Between 1961 and 2001, the annual world egg production rose by more than 3.5 times to reach almost 57 million metric tons (all egg-not just hen egg); about 6% was hatching eggs. The Food and Agriculture Organization (FAO) has been predicted that it will increase by 24% by 2015 with a further increase of 28% being foreseen by 2030. This large, rather linear increase will result form a rapid expansion in output in the developing countries (Fig 2.3) (Gillin, 2002).

Regional showing (Fig 2.4) the fastest rate of growth was Asia where, for the first time the number of layers topped 300m (representing 59% of the total), which compared with less than 1900m (48%) back in 1993. While the numbers continued to rise on Africa, N. & C. America and S. America, growth in both Europe and Oceania has come to a halt the total flock showing no significant increase in the past five years from their respective levels of 700m and 19m. Indeed, since 1993, the number of layers in Europe has declined by more than 8% from 756m (Evan, 2002).



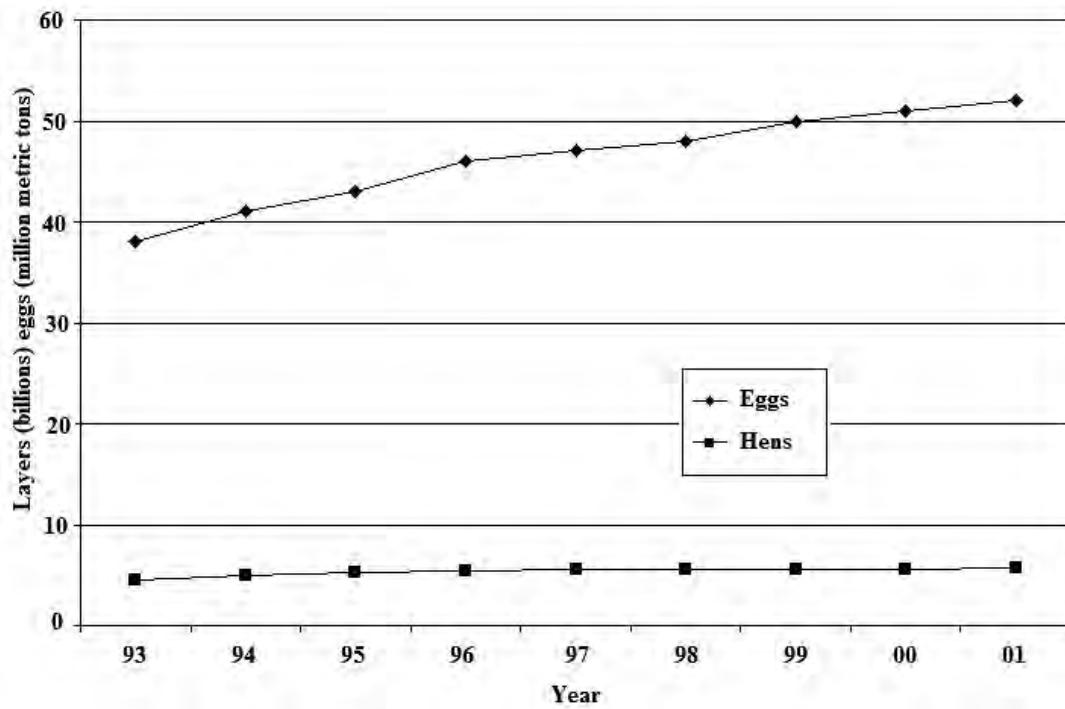


Fig. 2.3 World layers and egg production.

source: Evan, 2002

มหาวิทยาลัยศิลปากร สงวนลิขสิทธิ์

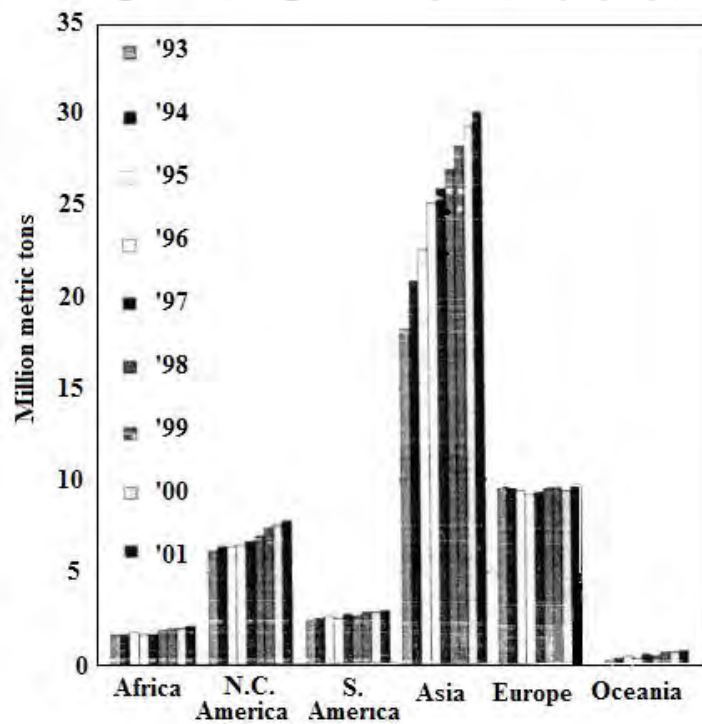


Fig. 2.4 Regional egg production.

source: Evan, 2002

## **TOFU**

Tofu is a traditional soybean-based food in Asia, and known as a protein gel like product (Cai et al., 1997; Shih, Hou, and Chang, 1997). It is a traditional and important source of protein in Asian countries. In the United States and Canada, tofu production has increased due to an increase in Asian immigrants and acceptance by the general population (Cai et al., 1997). Tofu is a curd that made directly from soybeans and resembles a soft white cheese or a very firm yogurt (Liu, 1997). Types of tofu include soft tofu, regular, and packed tofu (Shih, Hou, and Chang, 1997). Acceptable soft tofu requires a bland taste and fine texture with 88-89% moisture content. Tofu is inexpensive, nutritious, and versatile. On a wet basis, a typical pressed tofu with moisture content in the range of 85% contains about 7.8% protein, 4.2% lipid, and 2 mg/g calcium. Soft tofu has a soft cheeselike texture but is firm enough to retain shape after slicing (Liu, 1997; Tsai et al., 1981).

Because of its bland taste and porous texture, tofu can be prepared with virtually any other food. Most popular, it is served in soups or cooked with meat and/or vegetables. It can also be seasoned and served without further cooking. In the West, tofu-based ingredients have emerged. Together with soy-based dairy and meat analogs, they are referred to as the new generation of soyfoods (Liu, 1997).

## **TYPE OF COAGULANTS**

Coagulation is the most important step in tofu making. At least four variables involve in this step including the type of coagulants, the concentration of coagulants, the temperature of soymilk at which a coagulant is added, and the mode of adding and mixing the coagulant. Especially, the type and amount of coagulants is the first thing a tofu maker needs to decide since it ultimately affects tofu quality and yield (Cai, and Chang, 1998; Liu, 1997; Shih et al., 1997). The most commonly used coagulants are  $\text{CaSO}_4$ , nigari, and glucono-delta-lactone (GDL). The appropriate types of coagulant added in tofu normally depend on types of tofu made since each type of coagulant has a unique and optimum concentration (Shih et al, 1997).

### The salt coagulants

Calcium sulfate is the most widely used as a basic tofu coagulant. It is also the oldest. Over 2000 years ago, tofu makers in inland China first began to gather a translucent, crystalline white stone named gypsum, a dehydrate from of calcium sulfate, from mountains. It was baked and crushed before being used as a coagulant. The practice has been still popular in China even presently. In United States, calcium sulfate is currently produced from high purity gypsum with modern equipment. The white powder product is completely natural (Liu, 1997). Although calcium sulfate and glucono-delta-lactone are the coagulants of choice, other soluble salts of calcium, such as calcium acetate and calcium chloride have also been recommended for the coagulation of soy milk (Karim et al., 1999)

Nigari or chloride-type of salts includes natural nigari, refined nigari, and calcium chloride. Natural nigari is extracted from sea water by removing most or all of the table salt (NaCl) and water. A mixture of natural sea minerals, nigari consists primarily of magnesium chloride plus all of the other salts and trace minerals in sea water. Refined nigari is a relatively pure form of  $MgCl_2 \cdot 6H_2O$  crystal. In addition, calcium chloride is not a nigari type of coagulant because it is not found in sea water. However, it is a common coagulant used by tofu makers in North American. Nigari-type coagulants ( $MgCl_2$  99.60%) remarkably produce a delicious tofu with subtly sweet flavor and aroma. However, the texture of the tofu is not as soft and smooth as that made with a calcium sulfate. Therefore, nigari-type coagulants are not suitable for making silken tofu because they do not incorporate as much as others and they prone to give lower bulk yield. Futhermore, the optimum concentration level of nigari is so limited and the coagulation reaction takes place rapidly. Thus, its use requires more skill and attention from tofu makers. It must be added slowly, several times to the soymilk. Most tofu shops in Japan use a nigari-type coagulant in combination with calcium sulfate or GDL rather than nigari alone (Liu, 1997).

Numerous studies had been conducted to investigate the effect of coagulants on tofu quality and yield. Tsai et al. (1981) reported that significantly different qualities of tofu products were prepared by using various coagulants. Calcium sulfate and nigari were suitable coagulants for making the Chinese-style (firm) tofu, but not GDL and other types. Liu (1997) found among the four salts tested including  $\text{CaSO}_4$ ,  $\text{CaCl}_2$ ,  $\text{MgSO}_4$ , and  $\text{MgCl}_2$ ,  $\text{CaSO}_4$  remarkably provided a tofu with the greatest fresh weight. The chloride salts yielded tofu with much greater hardness and brittleness than with sulfate salts.

In general, each type of coagulants has its own advantages and disadvantages. For example, calcium sulfate is the coagulants of the first choice among most tofu makers. It can be used to make any type of tofu-silken, firm, or extra firm. However, tofu made from calcium sulfate has slightly inferior flavor as compared with that made from nigari. When mixed with water, calcium sulfate forms an unstable suspension because of its limited solubility. This contributes the difficulty when mixing well in soymilk, and results in the tofu having less consistency in texture. In addition, reactivity (or coagulation potency) of calcium sulfate decreases gradually during storage, particularly in the presence of moisture. Therefore, during tofu preparation, calcium sulfate should be mixed with water just before use and stirred into soymilk within 30 seconds of mix. If left longer in water, its potency will decrease.

## **MECHANISM OF COAGULATION OF EGG**

The mixture of proteins in egg albumin exhibits this property of heat gelation. Of the six egg albumin proteins tested for gel formation by Johnson and Zabik (1981), four egg proteins comprising lysozyme, the globulins, conalbumin, and ovalbumin were capable to produce gels after heat coagulation in pure aqueous solution. The heat mediated gelation of protein-water systems had been described as a two-stage process by Shimada and Matsushita (1980). The first step was the denaturation of native protein and followed by protein-protein interactions resulting in a three dimensional protein network which formed the gel. No evidence for the existence of

a denatured monomeric form of ovalbumin or conalbumin in heated solutions had been found (Woodward and Cotterill, 1987).

The specific conditions of temperature, pH, protein concentration, and ionic strength all had affected the natural formation of the protein aggregation. Substantial protein-water interaction in the system at the time of heating resulted in a highly hydrated, and created the viscoelastic gel. However a low degree of protein-water interaction in the system resulted in aggregation or precipitation due to an exclusion of water from the network (Shimada and Matsushita, 1980). Thus, the degree of protein-water interaction could be used as the index of the structure developing and setting protein gels during heating (Goldsmith and Toledo, 1985).

Coagulation involved some changes in the structure of egg-protein molecules and might be brought about by heat, mechanical means, salts, acids, alkalines, and other reagents such as urea. Hermansson (1979) described coagulation as the random aggregation of already denatured protein molecules. In this aggregation of molecules, polymer-polymer interactions were favored over polymer-solvent reactions (Schmidt, 1981). The coagulum was often turbid and the formation of the coagulum was usually thermally irreversible (Shimada and Matsushita, 1981). In general, the terms “coagulation” and “gelation” are used interchangeably as a “gel.” By definition, however, gelation is an orderly aggregation of proteins, which may or may not be denatured, forming a three dimensional network. Hermansson (1979) and Smith (1964) investigated the influence of urea on ovalbumin and found fold, and unfolding of the molecule followed by aggregation and possibly a secondary unfolding during or after aggregation. Kauzman (1959) suggested that formation of intermolecular hydrophobic bonds, hydrogen bonds, and disulfide bonds caused the protein to become insoluble. The extent of the unfolding of the molecule was related to the particular protein and various conditions of the system: the higher the concentration of unfolded molecules in the system and the faster the rate of unfolding, the finer the gel network (Woodward and Cotterill, 1987). A strong electrostatic force played a role in keeping proteins in the sol state. When the coulombic repulsion was lessened by the presence of a neutral salt, the polypeptide chains tended to associate and form a gel network. It was possible for gelation to occur before a large number of free chains

had formed. When this happened, a coarse network resulted. (Woodward and Cotterill, 1987).

Shimada and Matsushita (1980) reported that the first step in heat coagulation involved the formation of disulfide bonds and the exposure of hydrophobic groups. During further heating, egg albumen was polymerized by inter-molecular sulfhydryl-disulfide exchange and a protein network formed. Ma and Holme (1982) postulated that thermocoagulation required a balanced electrostatic attraction between protein molecules and presented evidence of hydrophobic interaction during gel formation. Marked oxidation of sulfhydryl groups during coagulation was not indicated.

Although formation of a protein network had been described as a two stage process (Woodward and Cotterill, 1987), the presence of soluble aggregates prior to network formation had been reported for a number of proteins (Ma and Holme, 1982; Mori, et al, 1986; Nakamura et al, 1984). Therefore, a three-step mechanism had been proposed involving protein denaturation followed by soluble aggregation and then interaction of the aggregates into a network. Further support for this mechanism had come from electron micrographs in which the strands of gel networks from whey protein concentrate and egg white were shown to contain spherical or bead-like structures.

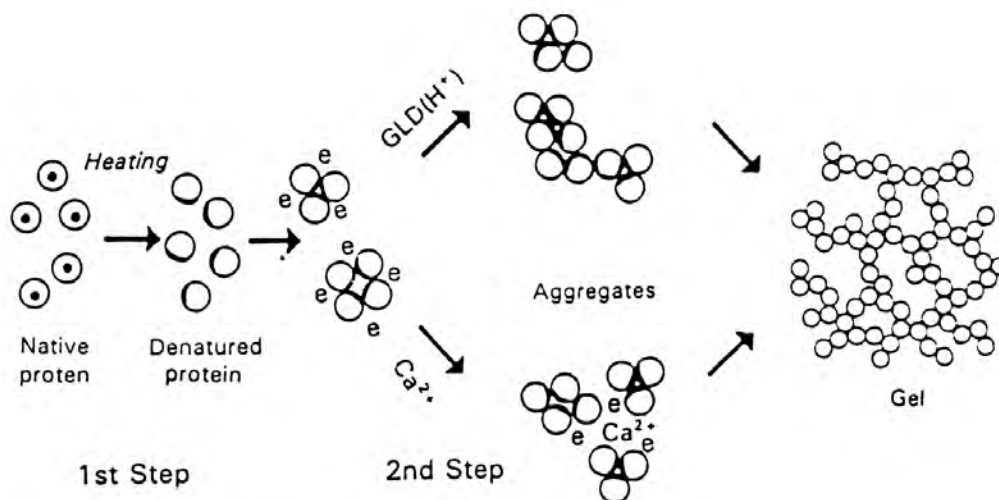
When Woodward and Cotterill introduced the two-stage mechanism, they speculated on the importance of the relationship between denaturation and association. If the attractive forces between chains are low, denaturation should proceed quickly relative to chain association. This would result in an accumulation of free denatured proteins as an intermediate. Under these conditions, a fine gel network should form. A coarse led to gelation prior to accumulation of many free chains. As attractive forces were further increased only a precipitate should form. For either the two-stage or three-stage mechanism, the relationship between denaturation and association influences the type of network formed.

## HEAT PROCESSING OF SOYMILK AND TOFU GELATION MECHANISM

Before adding coagulants, soymilk must be properly heated. This heat treatment is essential not only for improving nutritional quality and reducing beany flavor but also for denaturing proteins so that they can coagulate into curds in the presence of a coagulant.

Saio (1979) reported that heating soymilk led to aggregation and polymerization of soy protein, particularly of 11s fraction, in which the sulfhydryl-disulfide interchange reaction played an important role. The number of SH-groups increased immediately after heating, reached maximum, and then decreased. In limited heating, soybean protein did not dissociate into subunits but resulted in a pastelike gel, whereas in excessive heating, SH-groups were oxidized by air, leading to a decrease in gel cohesion. The optimum heating time-temperature combination corresponded approximately to the maximum range of SH-group.

Recently, after a series of studies on rheological characteristics of tofu made from 7s, 11s, their mixtures or soybean protein isolated in the presence of GDL or calcium sulfate. Liu (1997) proposed a gelation mechanism of tofu (Fig. 2.5). The gelation of tofu was a two-step process: protein denaturation by heat and hydrophobic coagulation promoted by protons from GDL or by calcium ion. In the raw soymilk, the hydrophobic regions of the protein molecules in the native state were located inside. Upon heating, these regions were exposed to the outside. Since the heat-denatured soy protein was negatively charged, either protons induced by GDL or calcium ions neutralized the net charge of the protein in second step. As a result, the hydrophobic interaction of the neutralized protein molecules became more predominant and led to aggregation. The gels were formed by random aggregation as imaged by scanning electron microscopy (SEM) observation and became turbid as they were generally developed near the isoelectric point due to a pH drop resulting from coagulant addition.



**Fig. 2.5** Gelation mechanism of soybean proteins in the presence of GDL or  $\text{CaSO}_4$ : Circle = protein molecules; dot = hydrophobic regions.

source: Liu (1997)

### FACTORS AFFECTING COAGULATION OF EGG

Under the usual conditions of food preparations, several things exert an influence simultaneously, and some of which may be in opposition to each other.

**Temperature.** Heat coagulation of albumen does not occur instantaneously at a given temperature, but is a time process in which heat is the accelerator. The average speed of coagulation albumen is increased, 191 times with a rise in temperature of  $1^\circ\text{C}$  ( $1.8^\circ\text{F}$ ) and approximately 635 times with a  $10^\circ\text{C}$  ( $18^\circ\text{F}$ ) increase in temperature. At high temperatures, coagulation occurs almost instantaneously (Lowe, 1955). Coagulation of albumen begins at about  $62^\circ\text{C}$ , and the coagulum ceases to flow when a temperature of about  $65^\circ\text{C}$  is reached. At  $70^\circ\text{C}$  the coagulum is fairly firm, but tender and it becomes very firm at higher temperatures (Beveridge et al., 1980; Romanoff and Romanoff, 1949). When heated by exposure to microwaves, albumen was observed to coagulate at  $57.2^\circ\text{C}$  (Baldwin et al., 1967). The white from duck egg coagulates at  $55^\circ\text{C}$  if held at this temperature for 10 min (Rhodes et al., 1960). Egg yolk begins to coagulate at  $65^\circ\text{C}$  and ceases to flow when it reaches a temperature of about  $70^\circ\text{C}$  (Romanoff and Romanoff, 1949).



Firmness of egg white gels increased with heating time from 7 to 60 min and temperatures ranging from 77°C to 90°C (Beveridge et al., 1980). Temperature was the most important factor in controlling gel rheology. Montejano et al. (1984) reported that rigidity of egg white was initiated at 71°C and increased through 83°C, while elasticity developed from 70 to 74°C. Woodward and Cotterill (1986) observed that as temperature and time of heating were increase from 75°C to 90°C and 50 min, the concentration of proteins in egg white gel serum decreased from about 10% to 0.5% while gel hardness increased. The denaturation of ovalbumin at temperatures ranging from 70 to 84°C may partially account for the increased hardness of gels heated above 80°C. Increasing temperature and time of heating also caused improved water-binding and a highly cross-linked gel structure.

Woodward and Cotterill (1987) found significant temperature and time interactions for hardness, cohesiveness, and springiness, indicating that the effects of time and temperature on the texture of beaker-cooked egg yolk (BCY) were not independent. At 10 and 30 min of heating, hardness of BCY increased significantly as the temperature increased from 75 to 90°C. When BCY was heated for 50 min, no significant differences in hardness occurred as the temperature increased. Both cohesiveness and springiness of BCY increased significantly as the temperature increased from 75° to 90°C at 10 min of heating and as time was extended from 10 to 30 min at 75°C. Nakamura et al. (1982) reported that a solution of low density lipoprotein isolated from yolk started to become rigid when heated at 65°C and increased in rigidity as the temperature increased to 85°C.

Shear force values for whole egg coagulum increased with both cooking time and temperature (Beveridge and Ko, 1984). In contrast to albumen, whole egg toughens extensively at 80°C, yielding shear values nearly equal to those obtained at 85° and 90°C after 30 min heating. Shear press values for whole-egg coagula were generally larger than those for egg white, but the increase did not reflect the difference in solids content between the two with whole egg being approximately twice as concentrated as egg white. Yang (1993) showed that toughness of egg tofu (a cooked flavored whole egg gel) increased with increasing egg protein concentration, heating temperature and heating time, while changes in cohesiveness

remained irregular. The optimum conditions for processing egg tofu were 4.5% whole egg protein with heating at 90°C (hot water) for 30 min.

**Dilution.** The temperature required for coagulation of egg is elevated by dilution, and the firmness of the coagulum decreases with increased dilution (Beveridge et al., 1980). The amount of liquid combined with egg for scrambling determines whether a small, firm mass or large, soft one is obtained. If excessive liquid is added to egg the cooked product is likely to appear curdled. If too little liquid is added and the egg are cooked to long, a rubbery mass results (Griswold, 1962). According to Andross (1940), 10 to 25 mL of milk per egg is suitable for scrambled egg of optimum consistency. With this amount of milk, there is a sufficient margin of safety between the coagulation and curdling temperatures to allow ease in handling the product. Dilution is also a factor in coagulation of custards. Custards containing a high proportion of eggs thicken at a lower temperature than those with a lesser egg content.

An investigation (Baldwin et al., 1967) of microwave cooking revealed that firmness of the coagulum decreased less with increasing dilution with water when coagulation was brought about by exposure to microwaves, rather than to heat in a conventional oven at 163°C. Also, the final temperature of the coagulum exposed to microwaves was lower when the amount of dilution increased. Conversely, as dilution increased in conventionally heated albumen, higher final temperatures were attained. Within each method of cookery, the amount of evaporation from the product remained constant, but there was less evaporation with microwave cookery than with conventional heating.

**Salts.** It has been reported that salts rupture ionic attraction on proteins, affect hydrogen bonds, and indirectly enhance hydrophobic interaction (Nandi and Robinson, 1972). Salts inhibit interactions between water molecules and hydrophilic groups in proteins, so that protein molecules become difficult to disperse. Bull and Breese (1970) found that several salts decreased the amount of water bound to egg albumen. Egg albumen contains many hydrophobic amino acids and easily formed a turbid coagulum even at an alkaline pH when salts were added.

The practice of adding NaCl to the water in which egg are poached improves the appearance of the finished product due to its tendency to promote coagulation (Andross, 1940; St. John and Flor, 1931). Beveridge et al. (1980) noted that  $\text{AlCl}_3$  and  $\text{CuSO}_4$  decreased firmness of coagula. The gel strength was a function of the balance of cation and anion activity of the salt. Curdling was caused by high salt concentration. However, when  $\text{FeCl}_3$  was used, the curds were dispersed by heating. Decreasing amounts of salts were required to promote coagulation as the valence of the cation increased (Lowe, 1955).

There appears to be an optimum salt concentration for network formation. It has been suggested that at very low concentrations, salts aid in protein solubilization prior to heating and provide a cross-link in the network (Mulvihill and Kinsella, 1988), while further addition of salts simply promotes aggregation than univalent ones, while the influence of polyvalent salts tends to be moderate (Nakamura et al., 1978). Optimum network characteristics were obtained with 300 mM NaCl or 5mM  $\text{CaCl}_2$  for ovalbumin. At higher NaCl concentrations the storage ( $G'$ ) and loss ( $G''$ ) moduli decreased while  $\tan \delta$  ( $G''/G'$ ) increased, indicating disruption of the network structure (Arntfield et al, 1990). Shimada and Matsushita (1981) observed that the critical pH for coagulum formation shifted to a more alkaline pH as salt concentrations increased. The effect of ions on egg albumen coagulation indicated an order of  $\text{SO}_4^{2-} > \text{Cl}^- > \text{Br}^- > \text{I}^- > \text{SCN}^-$  for anions and  $\text{Ca}^{+2} > \text{Li}^+ > \text{Na}^+ > \text{Cs}^+$  for cations.

The texture and appearance of ovalbumin gel are affected by NaCl concentration and heating method. Kitabatake et al. (1989A,B) observed that the aggregation of ovalbumin molecules heated without NaCl resulted in soluble linear aggregate. As NaCl concentration increased, the heated ovalbumin solutions became a sol, transparent gel, turbid gel, and suspension, successively. In the case of two-step heating, the soluble linear aggregates gelled immediately even at room temperature. Similar results were found with egg white. The gel network formed upon heating with NaCl was probably due to the hydrophobic interaction among the soluble linear aggregates without decomposition of the structure of the aggregate. Therefore, a gel could remain transparent after heating with NaCl.

## PROTEIN-POLYSACCHARIDE INTERACTIONS

Many processed and formulated foods are multicomponent systems which contain protein-polysaccharide-fat mixtures (Samant et al., 1993; Xu et al., 1992; Bernal et al., 1987). In order to achieve desirable functional properties in such foods, the use of various additives has been widely practiced. Of particular interest in this regard, because of their ability to bind water and form stable emulsions, suspensions and foams, are the group of complex polysaccharides known as hydrocolloids or, industrially, as gums (Xu et al., 1993). The protein-polysaccharide interaction has generated considerable research interest (Bernal et al., 1993; DeFreitas et al., 1997; Hansen, 1968; Hood and Allen, 1977; Karim et al., 1998; Schmidt and Smith, 1992; Xu et al., 1992). Understanding the mechanisms involved in the interactions between proteins and polysaccharides and the way in which these interactions are affected during processing is important when these components are added into foods to improve their functional properties (Bernal et al., 1987). Although the evidence seems to indicate that the major forces responsible for these interactions are electrostatic in nature, other interactions such as hydrogen, hydrophobic or covalent bonds may also be significant in the stabilization of these complexes. Before the potential of polysaccharides as ingredients in protein foods can be fully realized their impact on functional properties must be understood (Bernal et al., 1987; Samant et al., 1993).

When foods containing protein or polysaccharides are heated, gelation often takes place. This process involves the orderly interaction of macromolecules to form a three-dimensional matrix structure. In polysaccharide gels such an ordered network will be formed through specific and regularly interrupted chain-to-chain interactions between two or more aligned chain segments. Because these interactions involve relatively weak force (e.g., hydrogen and ionic bonds), cooperative association of a large number of interactions will be necessary to obtain a firm polysaccharide gel structure (Bernal et al., 1987).

## REFERENCES

- Arntfield SD, Murray ED, Ismond MAH. 1900. Influence of salts on the microstructural and rheological properties of heat-induced protein network from ovalbumin and vicilin. *J Agric Food Chem.* 38:1335-1343.
- Baldwin RE, Upchurch R, Cotterill OJ. 1968. Effect of additives on meringues cooked by microwaves and by baking. *Food Technol.* 22:1573-1576.
- Bernal VM, Smajda CH, Smith JL, Stanley DW. 1987. Interactions in Protein/Polysaccharide/Calcium Gels. *J Food Sci.* 52(2):1121-1136.
- Beveridge T, Ko S. 1984. Firmness of heat-induced whole egg coagulum. *Pult Sci.* 63:1372-1377.
- Beveridge T, Arntfield S, Ko S, Chung JKL. 1980. Firmness of heat induced albumin coagulum. *Pult Sci.* 59:1229-1236.
- Bull HB, Breese K. 1970. Water and solute binding by proteins. *Arch. Biochem. Biophys.* 137:299-303.
- Cai TD, Chang KC, Shih MC, Hou HJ, Ji M. 1997. Comparison of bench and production scale methods for making soymilk and tofu from 13 soybean varieties. *Food Research International.* 30(9):659-668.
- DeFreitas Z, Sebranek JG, Olson DG, Carr JM. 1997. Carragenan effects on salt-soluble meat proteins in model systems. *62(3):539-543.*
- Evans T. 2002. Asia shows fastest egg growth. *Egg Industry.* 107(8):10-18.
- Froning GW. 2002. Composition and marketing of specialty eggs. *Egg Industry.* 107(3):22.
- Gillin E. 2002. Production to reach 90mt by 2030. *Egg Industry.* 107(12):12-14.
- Goldsmith SM, Toledo RT. 1985. Studies on Egg Albumin Gelation Using Nuclear Magnetic Resonance. *J Food Sci.* 50:59-62.
- Hansen PMT. 1968. Stabilization of  $\kappa$ -casein by carrageenan. *J Dairy Sci.* 51(2):192-195.
- Hermansson AM. 1982. Gel Characteristics-Structure as Related to Texture and Waterbinding of Blood Plasma Gels. *J Food Sci.* 47:1965-1972.
- Hood LF, Allen. JE. 1997. Ultrastructure of carrageenan-milk sols and gels. *J Food Sci* 42:1062-1065.

- Johnson TM, Zabik ME. 1981A. Egg albumin proteins interactions in an angel food cake system. *J Food Sci.* 46:1231-1236.
- Johnson TM, Zabik ME. 1981B. Gelation properties of albumen proteins, singly and in combination. *Pult Sci.* 60:2071-2083.
- Karim A, Sulebele GA Azhar, ME, Ping CY. 1999. Effect of carrageenan on yield and properties of tofu. *Food Chem.* 66:159-165.
- Kauzman W. 1959. Some factors in the interpretation of protein denaturation. *Adv Protein Chem.* 14:1-63.
- Liu K. (1997). *Soybean*. New York: Chapman&Hall. 532p.
- Ma CY, HOLEME J. 1982. effect of chemical modifications on some physiochemical proteins and heat coagulation of egg albumen. *J Food Sci.* 47:1454-1459.
- Montejano JG, Hamann DD, Lanier TC. 1984. Thermally induced gelation of selected comminuted muscle systems-Rheological changes during processing, final strengths and microstructure. *J Food Sci.* 49:1496-1501.
- Mori T, Nakamura Y, Utsumi S. 1986. Behavior of intermolecular bond formation in the late stage of heat-induced gelation of glycinin. *J Agri Food Chem.* 34:33-37.
- Nakamura R, Fukano T, Taniguchi M. 1982. Heat-induced gelation of hen's egg yolk low density lipoprotein (LDL) dispersion. *J Food Sci.* 47:1449-1453.
- Nakamura T, Utsumi S, Mori T. 1984. Network structure formation in thermally induce gelation of glycinin. *J Agri Food Chem.* 32:349-352.
- Rhodes MB, Adams JL, Bennett N, Feency RE. 1960. Properties and food users of duck egg. *Poult Sci.* 39:1473-1478.
- Romanoff AL, Romanoff AJ. 1949. *The Avian Egg*. John Wiley and Sons Co., New York. 159-257p.
- Saio K. 1979. Tofu-relationships between texture and fine structure. *Cereal Foods World.* 24(8):343.
- Samant SK, Singhal RS, Kulkarni PR, Rege DV. 1993. Protein-polysaccharide interactions: a new approach in food formulations. *Int J Food Sci Technol.* 28: 547-562.
- Schmidt KA, Smith DE. 1992. Rheological properties of gum and milk protein. *J Dairy Sci.* 75:36-42.

- Schmidt RH. 1981. Gelation and coagulation. In Protein Functionality in Foods, Cherry, J.P. (Editor), ACS Symposium Series 147, Amer. Chem. Soc. 131-147.
- Shih MC, Hou HJ, Chang KC. 1997. Process optimization for soft tofu. *J Food Sci.* 62(4):833-836.
- Shimada K, Matsushita S. 1980. Relationship between thermo coagulation of proteins and amino acid compositions. *J Agri Food Chem.* 29:15-20.
- Smith MB. 1964. Studies on ovalbumin. I. Denaturation by heat, and the heterogeneity of ovalbumin. *Aust. J. Biol. Sci.* 17:261-270.
- Tsai SJ, Lan CY, Kao CS, Chen SC. 1981. Studies on the yield and quality characteristics of tofu. *J Food Sci.* 46:1734.
- Well RG, Belyavin CG. 1943. Egg quality-current problems and recent advances. Poultry science symposium number twenty. 1-191.
- Woodward SA, Cotterill OJ. 1986. Texture and microstructure of heat-formed egg white gels. *J Food Sci.* 51:333-339.
- Woodward SA, Cotterill OJ. 1987. Texture and microstructure of cooked whole egg yolks and heat-formed gels of stirred egg yolk. *J Food Sci.* 52:63-67.
- World consumption growing again. 2002. *Egg Industry.* 107(7):16-18.
- Xu SY, Stanley, DW, Goff HD, Davidson VJ, LE Maguer M. 1992. Hydrocolloid/Milk Gel Formation and Properties. *J Food Sci.* 57(1):96-102.
- Yang SC, Cotterill OJ. 1989. Physical and functional properties of 10% salted egg yolk in mayonnaise. *J Food Sci.* 54:210-213.

## CHAPTER 3

### EFFECT OF DIFFERENT COAGULANTS ON THE PHYSICAL PROPERTIES OF PACKED EGG TOFU

#### ABSTRACT

The utilization of individual 5 different coagulants (including GDL,  $\text{MgSO}_4$ ,  $\text{CaSO}_4$ ,  $\text{MgCl}_2$ , and  $\text{CaCl}_2$ ) operated with 55% added water on the physical properties of packed egg tofu were investigated and explored that the egg tofu with  $\text{CaSO}_4$  and  $\text{MgSO}_4$  contributed the highest firmness and shear stress, in texture and the syneresis retained at 5.37 and 4.92%, respectively. As comparison to the control, the syneresis of the developed product was lower up to 47%. The following experiment was conducted to measure the textural changes with respect to use 4 added water ratio (50, 55, 60 and 65%) and also contained 0.3%  $\text{MgSO}_4$ . The packed egg tofu with 55% added water was the best formulation due to its highest ability to decrease the syneresis and also exhibited identical nearly the shear stress and firmness as compared to the commercial control (different from the control about 2.3 and 1.2%, respectively). The effects of  $\text{MgSO}_4$  (0.1, 0.2, and 0.3% w/w) and 2 different added water ratio (55 and 60%) was further investigated. The studied found, the packed egg tofu with 0.1%  $\text{MgSO}_4$  and utilized the water:liquid whole egg at 55:45 exhibited the most similar textural characteristics to the control. As a result of statistical analysis no interaction effect was found between  $\text{MgSO}_4$  concentration and added water ratio.

**Keywords:** packed egg tofu, coagulation, syneresis



## INTRODUCTION

Tofu, a protein gel product, is one of the most popular food products in east and south eastern Asian countries. A variety of tofu products including soft tofu, regular, and packed tofu were commercially available in allover food market. In the US, the consumption of tofu products has being increased at a rate of 15% per year due to its excellent nutrition and health benefits for human (Cai and Chang, 1998). Acceptable packed tofu requires a bland taste and fine texture containing about 88 to 89% of moisture content. The texture of soft tofu is soft like cheese but still firm enough to retain shape after slicing (Shih et al., 1997).

Coagulation made the structural changes of egg-protein molecules and was also induced via a great variety of factors such as heat, mechanical means, salts, acids, alkalies, and other reagents such as urea. For example, salts ruptured ionic attractions on proteins, subsequently affected hydrogen bonds, and indirectly enhance hydrophobic interactions. Salts inhibit interactions between water molecules and hydrophilic group in proteins, so that protein molecules become difficult to disperse. Several salts decreased the amount of water bound to egg albumen. Egg albumen contained many hydrophobic amino acids and easily formed a turbid coagulum even at an alkaline pH when salts were added. Removal of naturally occurring salts from egg white impaired its ability to coagulate. Similarly, when the salts concentration of a custard mix was lowered by substitution of water for milk, gelation did not occur. Coagulation was accomplished in this custard by adding the salts to it, but curdling rather than smooth coagulation resulted if the salts were added after heating had completed. Lactates, chlorides, sulfates, phosphates, and combinations of  $MgCl_2$  and  $NaSCN$ , and  $NaCl$ ,  $Na_2SO_4$ , and  $CaCl_2$  also promoted coagulation (Stadelman and Cotterill, 1995).

Various coagulants have been used in the preparation of tofu to promote the textural characteristics varying from soft to firm with moisture content ranging from 70 to 90 % (Karim et al., 1998). Basically, three types of substances have the ability to coagulate proteins: salts, acids, and enzymes. Coagulants widely used for tofu

making are calcium sulfate, nigari or chloride-type, glucono- $\delta$ -lactone (GDL, or simply known as lactone), and a combination of two.

Numerous studies have been conducted to investigate the effect of coagulants on tofu quality and yield. Tsai et al. (1981) reported that significantly different qualities of tofu products were prepared using various coagulants. Although calcium sulphate and glucono-delta-lactone are the coagulants of choice, other soluble salts of calcium, such as calcium chloride and salts of magnesium, such as magnesium sulphate, magnesium chloride have also been recommended for the coagulation of tofu (Karim et al., 1998).

The quality of tofu products was significantly influenced by type of coagulant (Tsai et al., 1981). Calcium sulfate and bittern (nigari, in Japan) were suitable coagulants for making Chinese-style tofu regular tofu, but glucono-delta-lactone (GDL) was not. Bean curd made with  $\text{CaCl}_2$  and  $\text{MgCl}_2$  was coarse, granular, and hard, whereas calcium sulfate and GDL provided a smooth, soft, and uniform curd (Hou et al., 1997).

Dilution was also a major factor in coagulation affecting the firmness of tofu. The temperature required for coagulation of egg was elevated via dilution (Liu, 1997). The firmness of the coagulum reduced as higher dilution applied. Tofus containing high proportion of egg usually thickened at a lower temperature than those with a lesser egg content (Stadelman and Cotterill, 1995).

The objectives of this research was to investigate the effect of difference coagulants (including GDL,  $\text{MgSO}_4$ ,  $\text{MgCl}_2$ ,  $\text{CaSO}_4$ , and  $\text{CaCl}_2$ ) and water dilution on textural properties and syneresis of packed egg tofu.

## MATERIAL AND METHODS

### Preparation of Egg Tofu

1-Day old eggs were obtained from local market and then individually cleaned throughout the shell with warm water before breaking process. Liquid whole egg was blended in kitchen aid at low speed for 5 min, subsequently filtered using the wet cheese cloth for removing chalazas and then measure total soluble solid by hand refractometer. The preliminary experiment was conducted to investigate the effect of 5 different coagulants (consisting of GDL,  $MgSO_4$ ,  $MgCl_2$ ,  $CaSO_4$ , and  $CaCl_2$ ) on physical properties of packed egg tofu each 0.3% concentration (w/w) and 50% added water. After that, the combination 2 factors between 2 different amount of added water (55, and 60%), 3 different concentrations of  $MgSO_4$  (0.1, 0.2, and 0.3%) were studied. The packed egg tofu was also formulated via the addition of 0.8% sodium chloride, 0.5% soy sauce, 0.05% monosodium glutamate, and 0.3% sodium hexametaphosphate. The mixture was subsequently homogenized using APV homogenizer (model Rannie LAB2000, APV, Denmark) with 700 bars of 1<sup>st</sup> stage and 70 bars of 2<sup>nd</sup> stage, then packaged into cylindrical polyethylene bags (140 g) and heat sealed. The mixture of egg tofu was cooked at 85°C for 30 min, and then cooled to 10°C. The packed egg tofu was stored at 4°C for 24 hr before further analyses.

### Final Product Analyses

#### Texture measurement

Textural properties of egg tofu samples were determined using Texture Analyser (model TA.XT2, Stable Micro Systems, Goldaming, UK). All egg tofu samples were kept at 6°C after remove from cool room and then formed to the same cylindrical shape (25 mm of diameter, 20 mm of height). The compression test with a HDP/BSW blade set with warner bratzler probe was performed. The pre-test, test and post-test speeds were set to 5, 5 and 10 mm s<sup>-1</sup>, respectively. Each test was performed triplicate replications to measure maximum shear force (N). Shear stress (N/g.mm<sup>2</sup>)

was calculated as the maximum shear force multiplied by (2 × width × thickness of sample). Firmness (N.mm) was calculated as the area under graph from start to maximum force.

### **Color**

The color of the packed egg tofu was measured by Hunter Lab Colorimeter (model 9000, Gardner, USA). Calibrated to a white plate (CIE L\* = 98.76, a\* = -0.19, b\* = -0.06). The L\*, a\* and b\* values were used to calculate the derived color parameters. The test was replicated three times.

### **Syneresis**

A modified method Karim et al (1998) was employed in this test. Three pieces of tofu samples (3.5 cm of diameter) were weighed and placed on a sieve (3.5 cm x 3.5 cm) putting on the top of the PVC tube (3.0 cm of diameter, 3.0 cm of height) over 250 ml beaker. The beaker was wrapped with plastic wraps in a hanging position for 24 hr at 4 °C. Percentage syneresis was calculated as the weight of water released from the tofu in 24 hr divided by the original weight of sample and multiplied by 100.

### **Statistical analysis**

Statistic analyses were performed using complete randomize design (CRD) to test the effect of different coagulants and using augmented factorial design experiment to test the effects of concentration of coagulants and added water on physical properties of packed egg tofu compared to the commercial benchmark. All Data were evaluated using the Statistical Analysis System program (Ver. 8.1, SAS Inst., Cary, NC, USA). Analysis of variance (ANOVA) was conducted, and the differences between group means were analyzed using the Least Significant Difference (LSD). Statistical significance was established at  $p \leq 0.05$ .

## RESULT AND DISCUSSION

### Textural Properties

Textural characteristic of packed egg tofu was tremendously discriminated among the different use of coagulant (including GDL,  $\text{MgSO}_4$ ,  $\text{CaSO}_4$ ,  $\text{MgCl}_2$ , and  $\text{CaCl}_2$ ) at 55% added water. Regarding to this experiment,  $\text{MgSO}_4$ -egg tofu exhibited the highest shear stress ( $10.20 \times 10^{-2} \text{ N/g.mm}^2$ ), but GDL-egg tofu inversely provide the lowest shear stress ( $5.43 \times 10^{-2} \text{ N/g.mm}^2$ ) and firmness (1.89 N.mm) as shown in Table 3.1. However,  $\text{CaSO}_4$ -egg tofu had the highest firmness as compared to the control. It has been reported that the different coagulant and type of anion directly affected shear stress and firmness of packed egg tofu since the egg protein probably interacted with calcium and other constituents, such as anions (sulfate group) in liquid whole egg to form the stable microstructure (Liu,1997). In comparison with control,  $\text{CaSO}_4$ -egg tofu and  $\text{MgSO}_4$ -egg was appropriate to further studies. However, tofu made from  $\text{CaSO}_4$  has slightly inferior flavor as compared with that made from nigari. When mixed with water,  $\text{CaSO}_4$  forms an instable suspension because of its limited solubility. This makes it difficult to mix well in liquid whole egg, resulting in packed egg tofu with less consistency in texture. In addition, reactivity (or coagulation potency) of  $\text{CaSO}_4$  decreases gradually during storage, particularly in the presence of moisture. Therefore, during egg tofu preparation,  $\text{CaSO}_4$  should be mixed with water just before use and stirred in to liquid whole egg within 30 seconds of mixing. If longer in water, its potency will decrease (Liu,1997).

The effect of 4 added water ratio (50, 55, 60 and 65%) at 0.3%  $\text{MgSO}_4$  on the physical properties of packed egg tofu. Texture analysis revealed that packed egg tofu with 50% added water obtained the highest shear stress ( $12.61 \times 10^{-2} \text{ N/g.mm}^2$ ) and firmness (3.28 N.mm) when compared to the benchmark (Table 3.2). But the packed egg tofu at 55% added water was the best formulation since it is capable to increase the shear stress and firmness nearly equal also as compared to the commercial (benchmark) (different from the benchmark at 2.3% and 1.2%, respectively). At 65% added water exhibited the lowest shear stress ( $5.38 \times 10^{-2} \text{ N/g.mm}^2$ ) and firmness (1.28 N.mm). The increase in the textural characteristic

directly related to the amount of water held by the curd. The same result as Stadelman, and Cotterill, 1995 found that dilution was also a factor in coagulation of custard. The temperature required for coagulation of egg was elevated by dilution, and firmness of the coagulum decrease with an increase in dilution.

The effect of  $\text{MgSO}_4$  (0.1, 0.2, and 0.3% w/w) and 2 different added water ratio (55 and 60%) were studied. The effect of different added water treatments exhibited an decrease shear stress and firmness when more amount of water usage and found that the shear stress and firmness had significant ( $p < 0.05$ ) different (Table 3.3). Effect of  $\text{MgSO}_4$  concentration on shear stress and firmness was not significantly ( $p > 0.05$ ) different. From the experimental, the result reported that shear stress and firmness were not significantly different. This may be that the  $\text{MgSO}_4$  concentration was not significant (narrow range). The studied found that the packed egg tofu with 0.1%  $\text{MgSO}_4$  and utilized the water:liquid whole egg at 55:45 exhibited the most similar textural characteristics to the control. No interaction effect between  $\text{MgSO}_4$  concentration and added water ratio was found.

### **Color**

Egg tofu in good quality was generally bright and light-yellow in color. All the tofu sample prepared in this study had a light yellow color. The effects of added water and  $\text{MgSO}_4$  concentration on the color of packed egg tofu were shown in Table 3.4. There were no significant differences ( $p > 0.05$ ) for lightness ( $L^*$ ), but a slightly higher on  $L^*$  (81.17 to 82.8). In contrast there existed, significantly ( $p < 0.05$ ) different on redness ( $a^*$ ) and yellowness ( $b^*$ ). Packed egg tofu was significantly ( $p < 0.05$ ) darker when added  $\text{MgSO}_4$  at higher concentration as confirmed by decreasing in  $L^*$ ,  $a^*$ , and  $b^*$  values. From the statistical analysis, there were no interactions between added water and  $\text{MgSO}_4$  concentration on color.

## Syneresis

The syneresis of packed egg tofu with 0.3%  $\text{CaSO}_4$  and with 0.3%  $\text{MgSO}_4$  at 55% added water decreased to 49.3% and 44.7%, respectively as compared to the control.  $\text{MgSO}_4$  and  $\text{CaSO}_4$  were suitable coagulants for making the packed egg tofu (Fig 3.1). Packed egg tofu made from 50% added water gave the lowest syneresis (6.0%), but 65% added water had the highest syneresis (17.3%) (Fig. 3.2). As the present work indicated, 55% added water was appropriate for making packed egg tofu because at 55% added water could reduce the syneresis nearly the commercial. The effect of  $\text{MgSO}_4$  (0.1, 0.2, and 0.3% w/w) and 2 different added water ratios (55 and 60%) were studied. The result found that the syneresis of packed egg tofu was higher with an increase in added water (Table 3.5). In addition, the syneresis of packed egg tofu was higher with increased  $\text{MgSO}_4$  concentration. Increase of syneresis from the curd could be due to increased bonding occurring during storage, making the protein matrix more dense or compacted.

มหาวิทยาลัยศิลปากร สงวนลิขสิทธิ์

## CONCLUSIONS

Coagulation involved changes in the structure of egg-protein molecules and may be brought by salt and dilution effects. The present study confirmed the appropriate usage of chemical substances to coagulate whole egg in the manufacture of packed egg tofu. In general, each coagulant has its advantages and disadvantages. For this study  $\text{MgSO}_4$  showed the best potential one to produce packed egg tofu because of its high solubility and consistency in texture. Furthermore, the study had been examined the influence between the added water ratio and  $\text{MgSO}_4$  concentration and found that packed egg tofu made using  $\text{CaSO}_4$  and  $\text{MgSO}_4$  contained lower syneresis but higher textural characteristic than the others. Added water ratio less than 65% was the most appropriate to make packed egg tofu and avoid breaking curd. The combination between 55% added water and 0.1% magnesium sulfate provided the best products and selected to use for the next study.

## REFERENCES

- Cai TD, Chang KC. 1998. Characteristics of production-scale tofu as affected by soymilk coagulation method: propeller blade size, mixing time and coagulant concentration. *Food Res Int.* 31(4):289-295.
- Hou HJ, Chang KC, Shih MC. 1997. Yield and textural properties of soft tofu as affected by coagulation methods. *J Food Sci.* 62(4):824-827.
- Karim A, Sulebele GA, Azhar ME, Ping CY. 1999. Effect of carrageenan on yield and properties of tofu. *Food Chem.* 66:159-165.
- Ruey-Ling L, His-Shan C. 1992. Texture and microstructure of frozen/thawed egg tofu. *Taiwan sugar.* 39(4):26-19.
- Liu K. 1997. Soybean. New York: Chapman&Hall. 532p.
- Moizuddin S, Johnson LD, Wilson LA. 1999. Rapid method for determining optimum coagulant concentration in tofu manufacture. *J Food Sci.* 64(4): 684-687.
- Shih MC, Hou HJ, Chang KC. 1997. Process optimization for soft tofu. *J Food Sci.* 62(4):833-837.
- Stadelman WJ, Cotterill OJ. 1995. *Egg science and technology.* 4<sup>th</sup> ed. 591p.
- Tsai SJ, Lan CY, Kao CS, Chen SC. 1981. Studies on the yield and quality characteristics of tofu. *J Food Sci.* 46:1734-1737.



**Table 3.1** Shear stress, and firmness of packed egg tofu used different coagulants (50% added water)

Coagulants	Shear stress (N/g.mm <sup>2</sup> )x10 <sup>-2</sup>	Firmness (N.mm)
Benchmark	0.73 ± 0.01 <sup>c</sup>	2.00 ± 0.3 <sup>de</sup>
MgSO <sub>4</sub>	1.02 ± 0.06 <sup>a</sup>	2.40 ± 0.3 <sup>ab</sup>
CaSO <sub>4</sub>	0.85 ± 0.08 <sup>b</sup>	2.56 ± 0.1 <sup>a</sup>
MgCl <sub>2</sub>	0.90 ± 0.07 <sup>b</sup>	2.29 ± 0.1 <sup>abc</sup>
CaCl <sub>2</sub>	0.72 ± 0.02 <sup>c</sup>	2.22 ± 0.0 <sup>bcd</sup>
GDL	0.54 ± 0.05 <sup>d</sup>	1.89 ± 0.2 <sup>e</sup>

n = 9

<sup>abcde</sup> means within a column with unlike superscript letters are significantly different (P<0.05).

มหาวิทยาลัยศิลปากร สงวนลิขสิทธิ์

**Table 3.2** Shear stress, and firmness of packed egg tofu employed different added water (%) at 0.3% MgSO<sub>4</sub>

Added water (%)	Shear stress (N/g.mm <sup>2</sup> ) x 10 <sup>-2</sup>	Firmness (N.mm)
Benchmark	0.85 ± 0.05 <sup>b</sup>	2.45 ± 0.1 <sup>b</sup>
50	1.26 ± 0.01 <sup>a</sup>	3.28 ± 0.2 <sup>a</sup>
55	0.84 ± 0.02 <sup>b</sup>	2.48 ± 0.1 <sup>b</sup>
60	0.74 ± 0.01 <sup>c</sup>	2.08 ± 0.3 <sup>c</sup>
65	0.54 ± 0.03 <sup>d</sup>	1.28 ± 0.2 <sup>d</sup>

n = 9

<sup>abc</sup> means within a column with unlike superscript letters are significantly different (P<0.05).

มหาวิทยาลัยศิลปากร สงวนลิขสิทธิ์

**Table 3.3** Shear stress, and firmness of packed egg tofu at different water ratios and MgSO<sub>4</sub> concentration

Added Water (%)	MgSO <sub>4</sub> (%)	Shear stress (N/g.mm <sup>2</sup> )x10 <sup>-2</sup>	Firmness (Ns)
Benchmark	-	0.86 ± 0.03 <sup>a</sup>	2.45 ± 0.2 <sup>a</sup>
55	0.1	0.85 ± 0.04 <sup>ab</sup>	2.26 ± 0.4 <sup>ab</sup>
55	0.2	0.85 ± 0.02 <sup>ab</sup>	2.09 ± 0.4 <sup>b</sup>
55	0.3	0.85 ± 0.01 <sup>ab</sup>	2.34 ± 0.3 <sup>ab</sup>
60	0.1	0.83 ± 0.07 <sup>b</sup>	1.55 ± 0.3 <sup>c</sup>
60	0.2	0.83 ± 0.07 <sup>b</sup>	1.54 ± 0.2 <sup>c</sup>
60	0.3	0.83 ± 0.07 <sup>b</sup>	1.49 ± 0.2 <sup>c</sup>

n = 9

<sup>abc</sup> means within a column with unlike superscript letters are significantly different (p<0.05).

มหาวิทยาลัยศิลปากร สงวนลิขสิทธิ์

**Table 3.4** Color for packed egg tofu employed different water ratios and coagulants concentration

Added water (%)	MgSO <sub>4</sub> (%)	L*	a*	b*
Benchmark	-	83.4 ± 0.3 <sup>a</sup>	6.1 ± 0.2 <sup>a</sup>	19.8 ± 0.4 <sup>d</sup>
55	0.1	81.7 ± 0.5 <sup>d</sup>	5.9 ± 0.1 <sup>b</sup>	20.8 ± 0.3 <sup>a</sup>
55	0.2	82.8 ± 0.2 <sup>b</sup>	5.8 ± 0.1 <sup>bc</sup>	20.4 ± 0.4 <sup>b</sup>
55	0.3	83.3 ± 0.4 <sup>a</sup>	5.7 ± 0.0 <sup>cd</sup>	20.3 ± 0.1 <sup>b</sup>
60	0.1	82.5 ± 0.6 <sup>bc</sup>	5.6 ± 0.0 <sup>de</sup>	20.0 ± 0.1 <sup>cd</sup>
60	0.2	82.4 ± 0.2 <sup>c</sup>	5.5 ± 0.0 <sup>ef</sup>	20.1 ± 0.2 <sup>bc</sup>
60	0.3	82.8 ± 0.1 <sup>bc</sup>	5.4 ± 0.1 <sup>f</sup>	19.9 ± 0.2 <sup>cd</sup>

n = 9

<sup>abcdef</sup> means within a column with unlike superscript letters are significantly different (p<0.05).

มหาวิทยาลัยศิลปากร สงวนลิขสิทธิ์

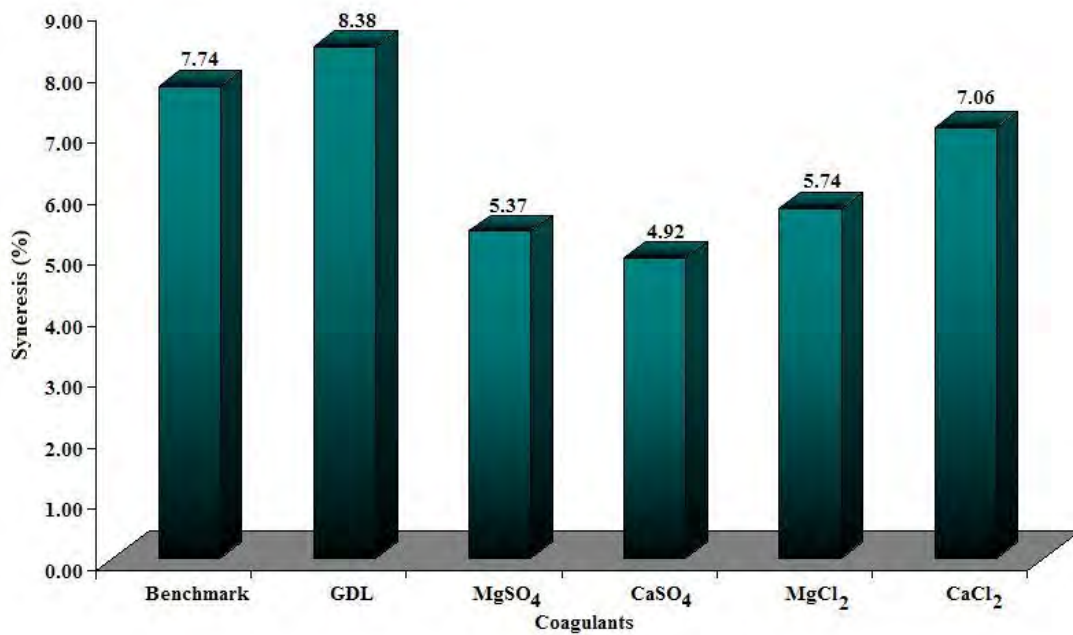
**Table 3.5** Syneresis of packed egg tofu employed different water ratios and coagulants concentration

Added water (%)	MgSO <sub>4</sub> (%)	Syneresis (%)
Benchmark	-	9.2 ± 0.4 <sup>d</sup>
55	0.1	8.4 ± 0.3 <sup>e</sup>
55	0.2	8.6 ± 0.2 <sup>e</sup>
55	0.3	9.1 ± 0.4 <sup>d</sup>
60	0.1	11.8 ± 0.6 <sup>c</sup>
60	0.2	12.4 ± 0.2 <sup>b</sup>
60	0.3	13.1 ± 0.8 <sup>a</sup>

n = 9

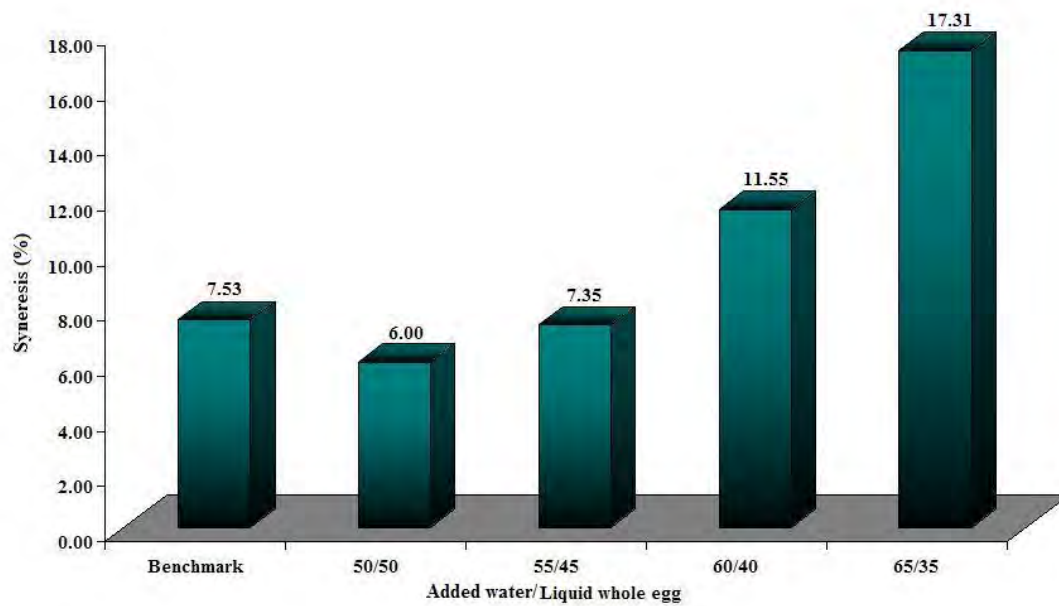
<sup>abcde</sup> means within a column with unlike superscript letters are significantly different (p<0.05).

มหาวิทยาลัยศิลปากร สงวนลิขสิทธิ์



**Fig.3.1** Effect of different coagulants (0.3%) at 50% added water on the syneresis of packed egg tofu

มหาวิทยาลัยศิลปากร สงวนลิขสิทธิ์



**Fig .3.2** Effect of different water ratios at 0.3% MgSO<sub>4</sub> on the syneresis of packed egg tofu

มหาวิทยาลัยศิลปากร สงวนลิขสิทธิ์

## CHAPTER 4

### OPTIMIZATION OF PROCESSING PROCEDURE FOR THE PHYSICAL PROPERTIES OF PACKED EGG TOFU USING RESPONSE SURFACE METHODOLOGY

#### ABSTRACT

Process optimization plays an important tool to control and monitor each individual processing unit operation, especially in heating units. Not only to achieve the desirable characteristics of finished product but also meet the economic goal due to remarkably decrease cost and improve energy consumption, the optimization of heating process must be investigated. Response surface methodology (RSM) was applied to optimize the heating procedure and determine the changes in physical attributes of in packed egg tofu. The optimized parameters were obtained by ridge analysis technique. In this experiment, the optimum ranges of each heating parameter consisted of 20-40 min of cooking time, 80-90°C of temperature. Optimum conditions were cooking temperature 87 to 90°C and cooking time 31 to 40 min.

**Keywords:** optimization; response surface methodology; packed egg tofu; water-binding.

#### INTRODUCTION

Gelation, as defined by Liang Hsieh and Regenstein (1992), involves a pre-gel stage, a gel point, and a post-gel stage. One important functional property of egg white, egg yolk, and whole egg is the ability to form gels upon heating. The physical attributes of the heat-formed gel, such as texture and water-binding ability, are highly dependent on the gel microstructure (Woodward and Cotterill, 1985).



Protein potentially forming gels after heating are referred to as thermo tropic proteins, e.g., egg albumen and ovalbumin. On heating, the heat-induced conformation of the protein molecules leads to their aggregation into filaments or densely branched clusters, which interact as ‘sticky’ reactive molecules rather than as unfolded random coils (Liang Hsieh and Regenstein, 1992). In addition, when heating, thermo tropic proteins, e.g., whey proteins and egg proteins form three-dimensional gels then stabilized by inter-molecular linkages such as disulfide cross-links, hydrogen bonds, and hydrophobic interaction (Liang Hsieh et al., 1993)

Speed of coagulation influences by temperature. Egg white begins to coagulate at 62°C mean while egg yolk starts to coagulate at 65°C. Over coagulation with extended time of heating, high temperature may reduce the difference between temperature at which optimum and over coagulation occurred. Firmness of egg white gels increases with longer heating time from 7 to 60 min and higher temperature from 77°C to 90°C. At 10 and 30 min of heating, hardness of beaker-cooked-egg yolk increases as temperature increases from 75°C to 90°C. Low-density lipoprotein from egg yolk starts to become rigid when heating at 65°C and more increases in rigidity as temperature increases up to 85°C. Toughness of egg tofu augments with an increasing egg protein concentration, heating temperature, and time (Stadelman and Cotterill, 1995).

Successful egg cookery is dependent upon the relation between time and temperature. Too much heat results in overcoagulation, regardless of whether the excess heat is the result of too high a temperature or exposure to heat for long er period of a time. It is possible to achieve excellent results with high temperatures applied, if the time is shortened appropriately. Heating beyond that required to cause optimum firmness results in undesirable syneresis (Stadelman and Cotterill, 1995).

The objective of this study was to examine the effect of heating temperature and time on the physical properties of packed egg tofu.

## MATERIAL AND METHODS

### Preparation of Egg Tofu

1-Day old eggs were obtained from local market and then individually cleaned throughout the shell with warm water before breaking process. Liquid whole egg was blended at low speed for 5 min. Liquid whole egg was filtered using the wet cheese cloth for removing chalazas and then measure total soluble solid. The packed egg tofu was also formulated via the addition of 0.8% sodium chloride, 0.5% soy sauce, 0.05% monosodium glutamate, and 0.3% sodium hexametaphosphate. The mixture was subsequently homogenized using APV homogenizer (model Rannie LAB2000, Denmark) with 700 bars of 1<sup>st</sup> stage and 70 bars of 2<sup>nd</sup> stage, then packaged into cylindrical bags (140g) and heat sealed. The packed egg tofu was cooked at 3 different temperatures (80, 85 and 90°C), three different times of cooked (20, 30 and 40 min) shown in table 4.2, and then finally cooled to 10°C. The packed egg tofu was stored at 4°C for 24 hr before further analyses.

### Final Product Analyses

#### Texture Analyses

Textural properties of egg tofu samples were determined using Texture Analyser (model TA.XT2, Stable Micro Systems, Goldaming, UK). All egg tofu samples were kept at 6°C after remove from cool room and then formed to the same cylindrical shape (25 mm of diameter, 20 mm of height). The compression test with a HDP/BSW blade set with warner bratzler probe was performed. The pre-test, test and post-test speeds were set to 5, 5 and 10 mm s<sup>-1</sup>, respectively. Each test was performed triplicate replications to measure maximum shear force (N). Shear stress (N/g.mm<sup>2</sup>) was calculated as the maximum shear force multiplied by (2 × width × thickness of sample). Firmness (N.mm) was calculated as the area under graph from start to maximum force.

## Syneresis

A modified method Karim et al (1998) was employed in this test. Three pieces of tofu samples (3.5 cm of diameter) were weighed and placed on a sieve (3.5 cm x 3.5 cm) putting on the top of the PVC tube (3.0 cm of diameter, 3.0 cm of height) over 250 ml beaker. The beaker was wrapped with plastic wraps in a hanging position for 24 hr at 4 °C. Percentage syneresis was calculated as the weight of water released from the tofu in 24 hr divided by the original weight of sample and multiplied by 100.

## Color

The color of the packed egg tofu was measured by Hunter Lab Colorimeter Labscan XE (model 9000, Gardner, USA). Calibrated to a white plate (CIE  $L^* = 98.76$ ,  $a^* = -0.19$ ,  $b^* = -0.06$ ). The  $L^*$ ,  $a^*$  and  $b^*$  values were used to calculate the derived color parameters. The test was replicated three times.

## Experimental Design

RSM was employed for optimizing production procedure in the physical properties from packed egg tofu samples. The production procedure was assumed to be affected by two independent variables: cooking time and cooking temperature. A fractional 3-levels-2-factors experimental design with three replicates at the centerpoint was adopted. The two factors (time and temperature), levels and experimental design in terms of coded and uncoded were given in Table 4.1.

## Data Analysis

The Response Surface Regression (RSREG) Procedure of Design-Expert Version 6.0.5 (Stat-Ease, Inc.) was used to fit the experimental data to the linear (1), quadratic (2) or cubic (3) polynomial equation to obtain coefficients of the equations

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 \quad (1)$$

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_3 X_1 X_2 \quad (2)$$

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_3 X_1 X_2 + \beta_{111} X_1^3 + \beta_{222} X_2^3 + \beta_{112} X_1^2 X_2 + \beta_{122} X_1 X_2^2 \quad (3)$$

where  $y$  is response;  $X_1$  = cooking temperature;  $X_2$  = cooking time;  $\beta_0$  = constant;  $\beta_1$  and  $\beta_2$  = linear coefficient.

## RESULTS AND DISCUSSION

### Model fitting from RSM

Table 4.2 showed the optimization experiments on process procedure of packed egg tofu. The experiments were performed at random order and the Y values were responses i.e., shear stress, firmness, syneresis and color to the independent variables,  $X_1$  and  $X_2$  which were cooking temperature and cooking time. The independent variables were coded -1, 0 and +1 for three levels that were equidistant from each other (Table 4.1). All data as shown in Table 4.2 were input into Design-Expert program and executed, resulting in various ANOVA tables exhibiting the significant independent variables as well as interactions of these variables.

Analysis of Variance (ANOVA) for response variables (Table 4.3) indicated that the statistical models were effective ( $p < 0.05$ ) for shear stress ( $y_1$ ), firmness ( $y_2$ ), syneresis ( $y_3$ ), and color parameter of packed egg tofu ( $y_4, y_5, y_6$ ). According to a model fitting technique, there was no significant linear relationship shear stress, cubic on firmness and quadratic on syneresis and color, whereas the linear relationship affected most of responses with high significance ( $p < 0.01$ ), except in color  $L^*$  and  $b^*$ .

All models showed no lack of fit. The coefficients of determination ( $R^2$ ) of the regression for responses with significant effects of the model were 0.892 to 0.994. These result indicated that the equations developed for response variables ( $y_1, y_2, y_3, y_4, y_5, \text{ and } y_6$ ) were adequate as following:

Simplified equation

$$y_1 = -0.030838 + 4.53333 \times 10^{-4} X_1 + 4.48148 \times 10^{-5} X_2 \quad (4)$$

$$y_2 = +2.51 + 0.84 X_1 - 0.044 X_2 - 0.042 X_1^2 - 0.14 X_2^2 + 0.067 X_1 X_2 + 0.68 X_1^2 X_2 + 0.41 X_1 X_2^2 \quad (5)$$

$$y_3 = +321.90173 - 6.84331 X_1 - 0.026385 X_2 + 0.037811 X_1^2 + 2.49732 \times 10^{-3} X_2^2 - 1.97222 \times 10^{-3} X_1 X_2 \quad (6)$$

$$y_4 = -131.80986 + 4.76974 X_1 + 0.57719 X_2 - 0.026396 X_1^2 - 8.65709 \times 10^{-4} X_2^2 - 5.99444 \times 10^{-3} X_1 X_2 \quad (7)$$

$$y_5 = +44.14329 - 0.84732 X_1 - 0.10675 X_2 + 4.51724 \times 10^{-3} X_1^2 + 6.01533 \times 10^{-4} X_2^2 + 6.27778 \times 10^{-4} X_1 X_2 \quad (8)$$

$$y_6 = +127.77073 - 2.52300 X_1 - 0.13707 X_2 + 0.014449 X_1^2 + 3.67816 \times 10^{-4} X_2^2 + 1.24444 \times 10^{-3} X_1 X_2 \quad (9)$$

### Response surface plotting

Contour and surface plots were generated using each significant parameter for individual response. The contour plots of the responses as a function of the independent variables presented the lines of constant value. Figs 4.1 to 4.12 showed examples of contour and three-dimensional surface plots (Fig. 4.1 to 4.3 and Fig. 4.7 to 4.9) for shear stress, firmness, and syneresis respectively, as a function of cooking temperature and cooking time of packed egg tofu. We also analyzed shear stress of packed egg tofu as affected by cooking temperature and cooking time. Results

showed that higher cooking temperature produced tofu with higher shear stress (Fig. 4.1 and 4.7).

The tofu firmness considerably increased via an increment of both cooking temperature and time (Fig. 4.2 and 4.8). However, the process procedure was to limited by temperature. For example, when temperature over 90°C, the interior water will be boiled and then directly affected the textural characteristic of packed egg tofu. It was suggested that the temperature might be kept at or below 90°C.

The effect of cooking temperature and cooking time on syneresis of packed egg tofu (Fig. 4.3 and 4.9) found that syneresis was reduced by an increase in cooking temperature and time. The regression coefficient of determination ( $R^2$ ), which was a measure of how well a equation model could can be fitted to the raw data, was approximately 0.930 for linear model, 0.994 for cubic model, and 0.991 for quadratic model, respectively. These ensured a satisfactory adjustment of the linear, cubic, and quadratic models to the experimental model to the experimental data and also indicated that approximately 94.2, 99.4, and 99.1% respectively of the variability in the dependent variable (response) could be explained by the model.

Fig. 4.4 to 4.6 showed examples of contour and three-dimension surface plots (Fig. 4.10 to 4.12) for  $L^*$  value,  $a^*$  value, and  $b^*$  value. The plots indicated that  $L^*$  values increased with longer cooking temperature and cooking time but decrease when after 90°C 30 minute (Fig. 4.4 and 4.10). Fig. 4.5 and 4.11 represented the redness ( $a^*$  value) of packed egg tofu and found that the redness decreased with an increase in cooking temperature and time. Whereas the yellowness (Fig. 4.6 and 4.12) gave the contrast result.

The optimum process conditions could then be determined by superimposing the contour plots of relevant and statistically significant responses. An optimum area was generated and calculated the centroid of the area forming the basis of the optimum process conditions. Fig. 4.13 showed a feasible representation of the optimum process conditions for the process procedure of packed egg tofu. As shown, the optimum process conditions could derive at point A. The conditions at point A could be described: the cooking temperature was about 90°C and cooking time was 40 minute to obtain the most desirable physical characteristics of packed egg tofu.

Validation tests were performed to determine the adequacy of the standard operating procedure (SOP) models. A model seemed adequate if the predicted values (of the model) were close to the experimental values observed during the validation tests. Table 4.4 showed the predicted and observed values for the responses at optimum conditions. The experimental values were averages of three replicates and were very close to the predicted values indicating that the SOP models generated were acceptable.

## CONCLUSIONS

Cooking temperature was the most pronounced parameter affecting the physical properties of packed egg tofu. Cooking time dominately lessen effect. The regression equation obtained in this study could be use to find optimum conditions for the desired physical properties of packed egg tofu within the range of conditions applied in this research. RSM methodology could be use to optimize production procedure of packed egg tofu parameters. Optimizing the processing operations should be performed in order to recommend best conditions for processing resulting in superior capacity and reducing processing costs. RSM was found to be useful approach and it should be recommended that this methodology be adapted to all optimization studies

**REFERENCES**

- Hou HJ, Chang KC, Shih MC. 1997. Yield and textural properties of soft tofu as affected by coagulation methods. *J Food Sci.* 62(4):824-827.
- Liang HY, Regenstein JM. 1992. Modeling gelation of egg albumen and ovalbumin. *J Food Sci.* 57(4):856-861.
- Liang HY, Regenstein JM, Anandha RM. 1993. Gel point of whey and egg proteins using dynamic rheological data. *J Food Sci.* 58(1):116-119.
- Stadelman WJ, Cotterill OJ. 1995. *Egg science and technology*. 4<sup>th</sup> ed. New York: The Haworth Press, Inc. 591p.
- Woodward SA, Cotterill OJ. 1985. Preparation of cooked egg white, egg yolk and whole egg gels for scanning electron microscopy. *J Food Sci.* 50:1624-1628.

มหาวิทยาลัยศิลปากร สงวนลิขสิทธิ์



**Table 4.1** Experimental range levels of two independent variables in terms of actual factors.

Variables	Symbols	Coded-variable level		
		-1	0	+1
Temperature (°C)	X <sub>1</sub>	20	30	40
Time (min)	X <sub>2</sub>	80	85	90

มหาวิทยาลัยศิลปากร สงวนลิขสิทธิ์

**Table 4.2** 3-Level factorial circumscribed design arrangement and responses

Run Order	Temperature (X <sub>1</sub> )	Time (X <sub>2</sub> )	Shear stress (×10 <sup>-2</sup> N/g.mm <sup>2</sup> )	Firmness (N.mm)	Syneresis (%)	Color L*	Color a*	Color b*
1	90	20	1.08	2.84	9.34	84.10	3.69	17.36
2	85	40	1.02	2.39	9.62	84.17	3.60	17.06
3	85	30	0.94	2.56	9.63	84.07	3.74	17.15
4	80	20	0.68	0.47	13.91	82.28	4.37	17.88
5	85	30	0.94	2.49	9.86	84.29	3.66	16.97
6	85	20	0.81	2.48	10.21	84.03	3.95	17.18
7	80	30	0.65	1.70	12.96	83.21	4.13	17.49
8	85	30	0.84	2.35	10.06	84.16	3.62	17.08
9	85	30	0.91	2.49	9.99	84.18	3.72	17.11
10	90	30	1.15	3.37	8.39	83.84	3.54	17.41
11	90	40	1.12	4.26	8.42	83.91	3.41	17.26
12	80	40	0.66	1.62	13.08	83.28	3.96	17.54
13	85	30	0.95	2.54	9.94	84.02	3.71	17.24

Refer to Table 4.1 for coded symbols and levels of independent variables (X<sub>1</sub> and X<sub>2</sub>)

**Table 4.3** Analysis of variance (ANOVA) and model fitting for response variables

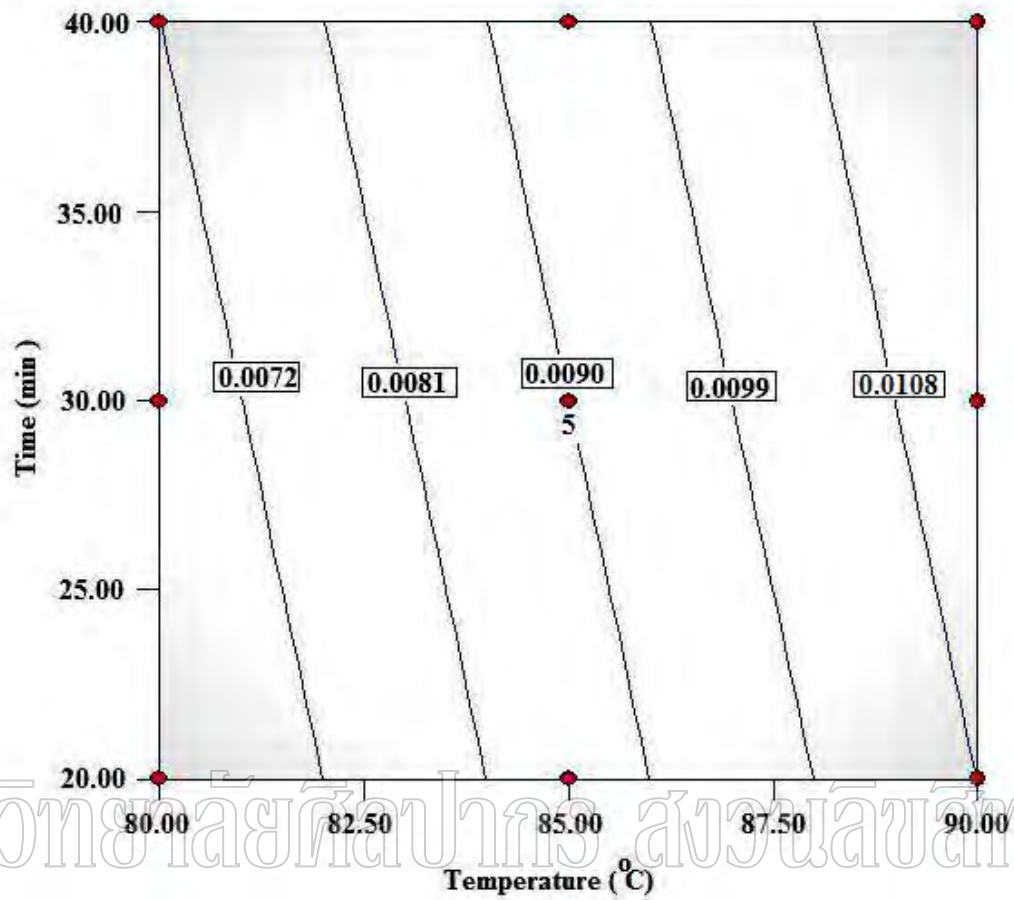
Source	df	Sum of square					
		$Y_1$	$Y_2$	$Y_3$	$Y_4$	$Y_5$	$Y_6$
Model	5	1.60x10 <sup>-5</sup> ***	1.35***	7.73***	0.73***	0.16***	0.13**
Linear	2	1.60x10 <sup>-5</sup> ***	4.24***	17.47***	0.86	0.36***	0.09
Quadratic	2	1.04x10 <sup>-7</sup>	0.04	1.83***	0.79***	0.04**	0.23**
Cubic	2	5.80x10 <sup>-7</sup>	0.43***	0.03	0.07*	8.45x10 <sup>-5</sup>	0.02
Crossproduct	1	2.02x10 <sup>-7</sup>	0.02	0.04	0.36	3.94x10 <sup>-3</sup>	0.02
Residual	7	2.40x10 <sup>-7</sup>	0.01	0.05	0.03	1.91x10 <sup>-3</sup>	0.01
Lack of fit	3	2.62x10 <sup>-7</sup>	0.02	0.08	0.05	9.42x10 <sup>-4</sup>	0.01
Pure error	4	2.10x10 <sup>-7</sup>	6.69x10 <sup>-3</sup>	0.03	0.01	2.63x10 <sup>-3</sup>	9.99x10 <sup>-3</sup>
Coefficient of determination (R <sup>2</sup> , %)		93.0	99.4	99.1	95.1	98.4	89.2

$Y_1$ =Shear stress,  $Y_2$ =Firmness,  $Y_3$ =Syneresis,  $Y_4$ =Color L\*,  $Y_5$ =Color a\*,  $Y_6$ =Color b\* Significance level: \*\*\*p<0.001, \*\*p<0.01, \*p<0.05.

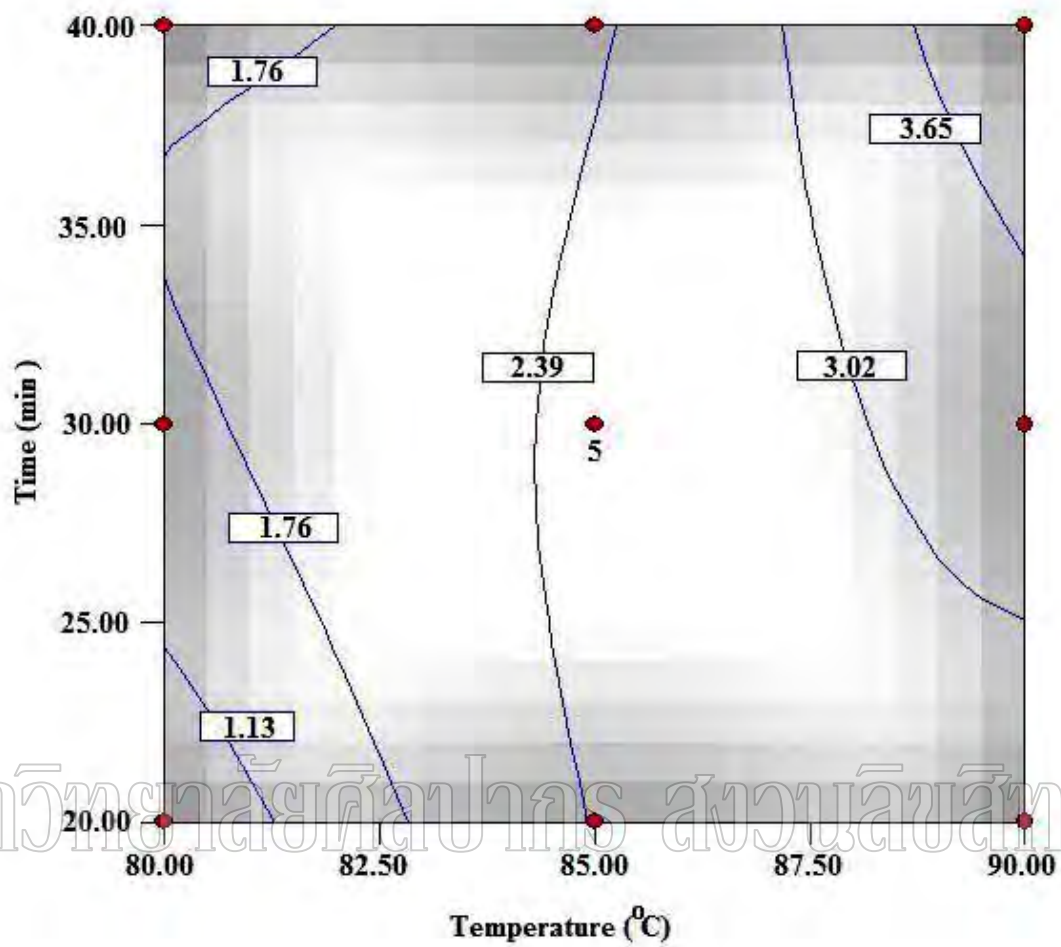
**Table 4.4** Predicted and observed values for the responses at optimum conditions

Response variable	Predicted value	Actual value
Shear stress ( $\times 10^{-2}$ N/g.mm <sup>2</sup> )	1.17	1.13 $\pm$ 0.02
Firmness (N.mm)	4.24	4.21 $\pm$ 0.08
Syneresis (%)	8.12	8.10 $\pm$ 0.05
L* value	83.79	83.94 $\pm$ 0.9
a* value	3.42	3.47 $\pm$ 0.9
b* value	17.31	17.32 $\pm$ 0.3

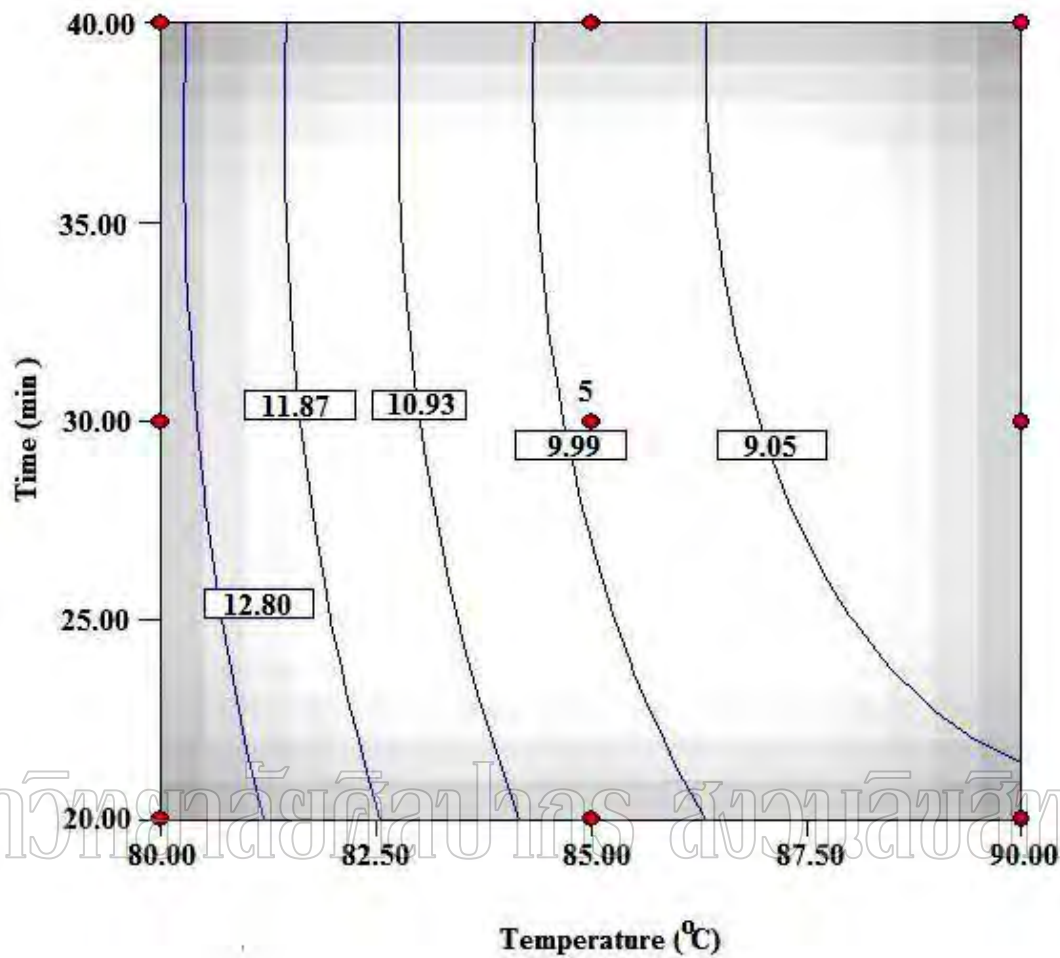
มหาวิทยาลัยศิลปากร สงวนลิขสิทธิ์



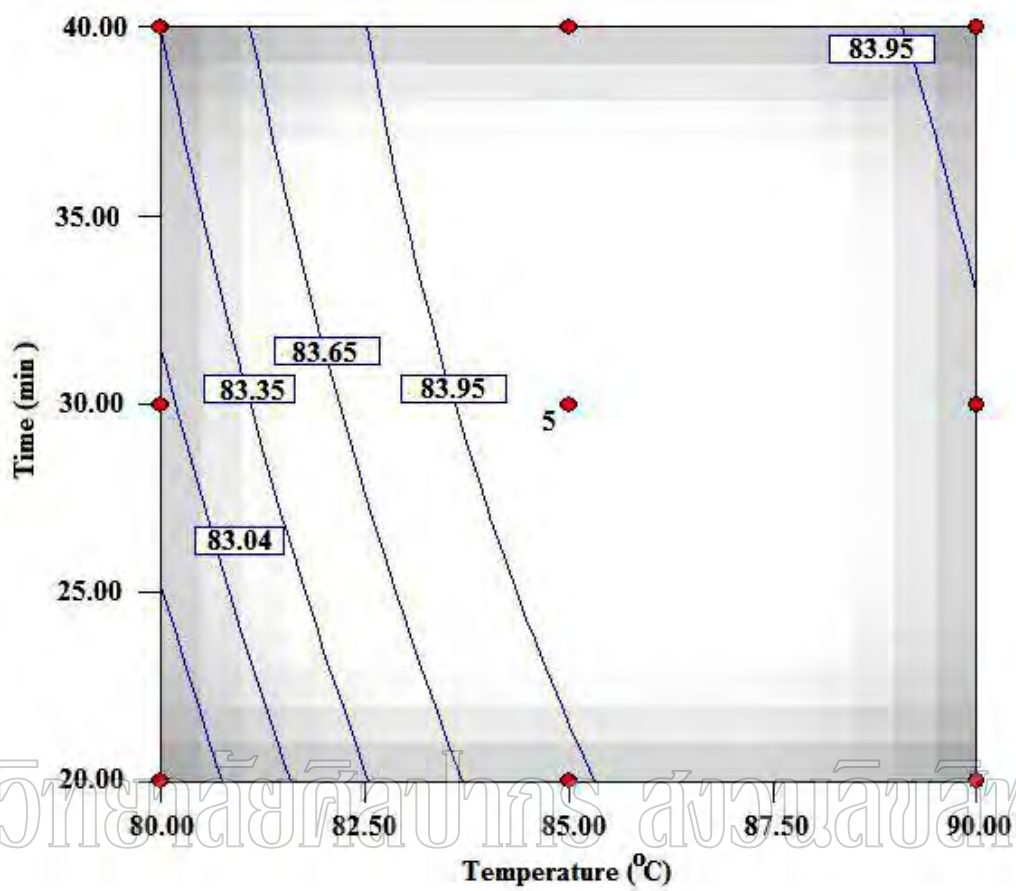
**Fig. 4.1** Response surface plots (contour plot) of shear stress showing effects of cooking temperature and cooking time. The number inside plots represent shear stress (N/g.mm<sup>2</sup>).



**Fig. 4.2** Response surface plots (contour plot) of firmness showing effects of cooking temperature and cooking time. The number inside plots represent firmness (N.mm).

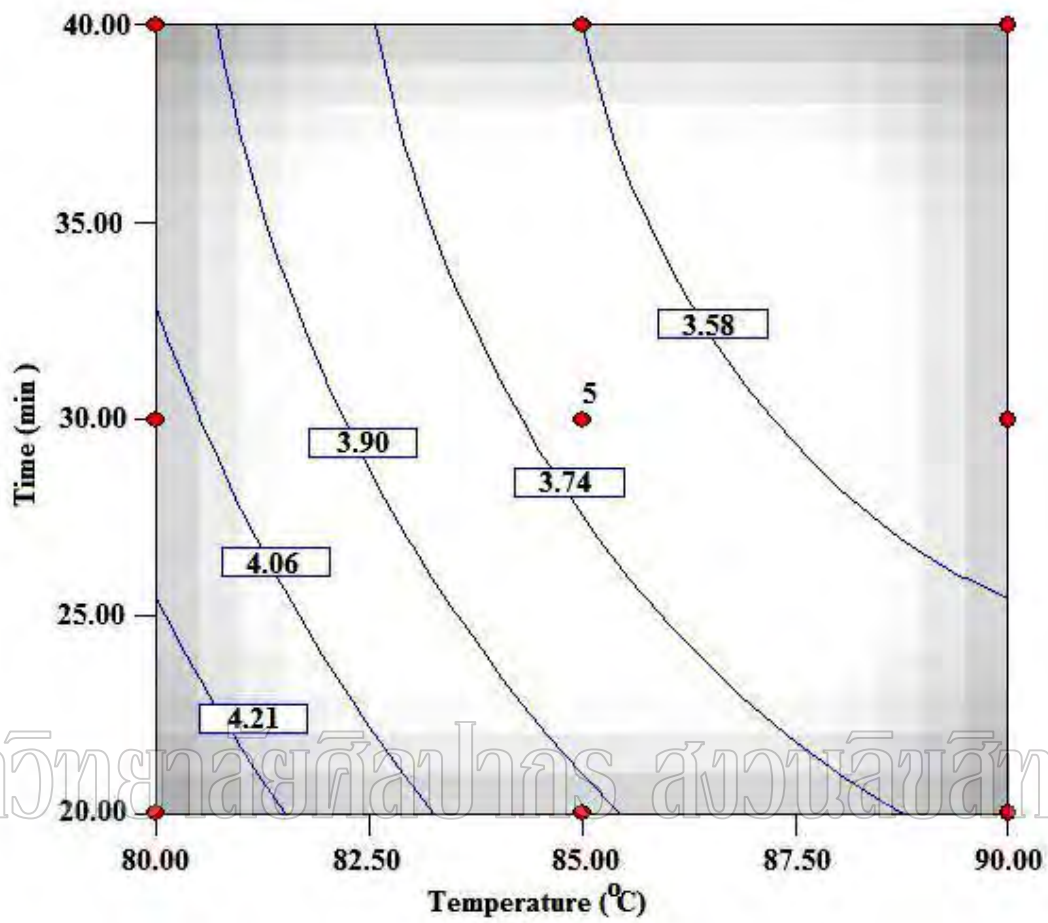


**Fig. 4.3** Response surface plots (contour plot) of syneresis showing effects of cooking temperature and cooking time. The number inside plots represent syneresis (%).

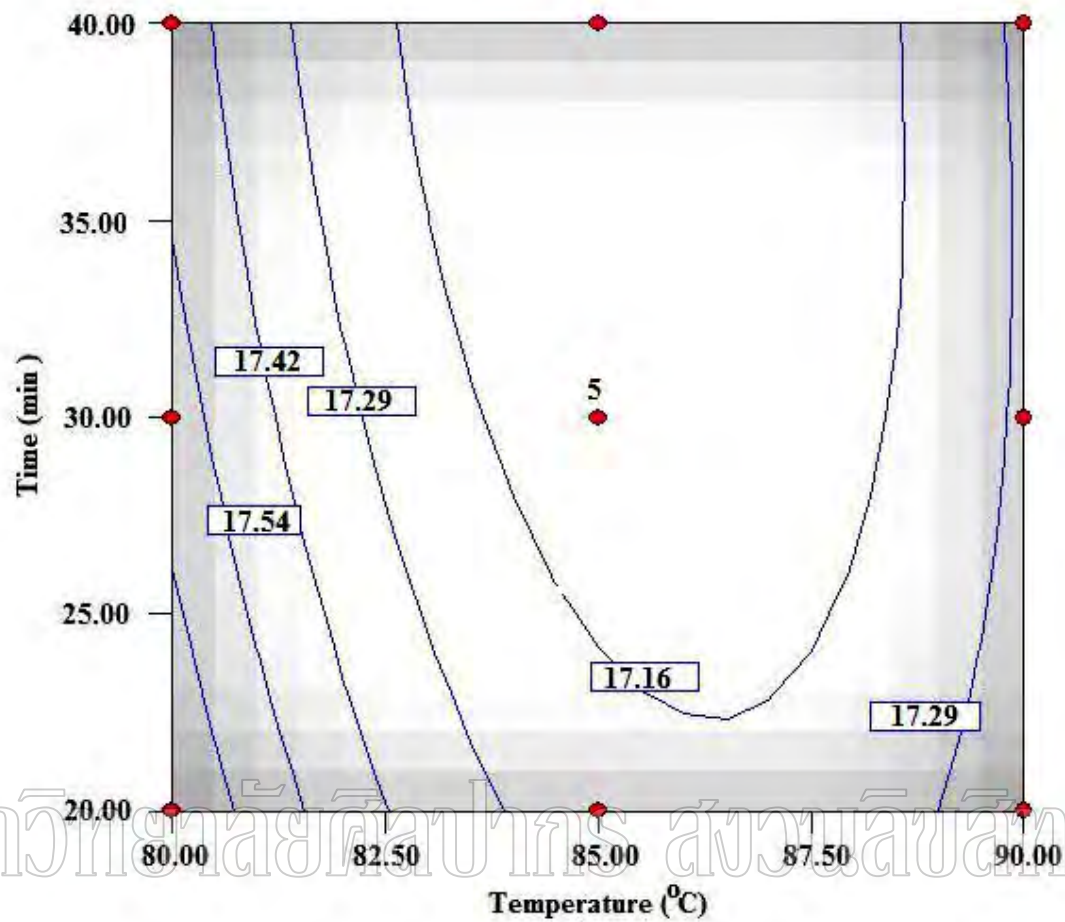


**Fig. 4.4** Response surface plots (contour plot) of color L\* showing effects of cooking temperature and cooking time. The number inside plots represent L\* value.

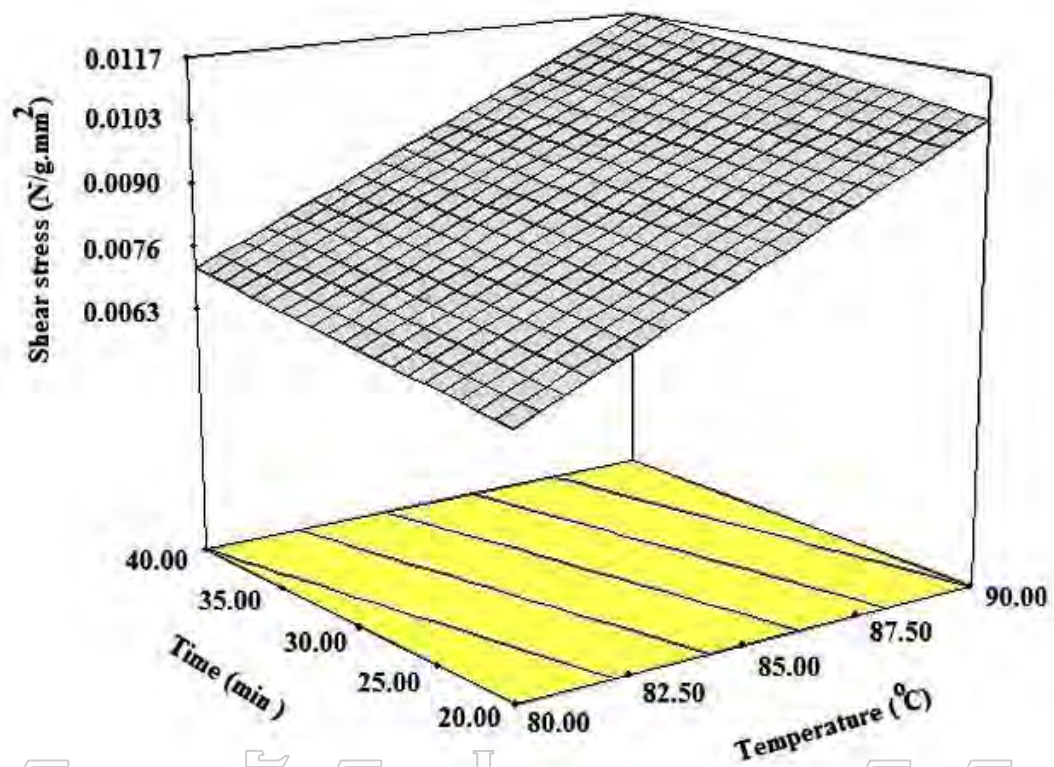




**Fig. 4.5** Response surface plots (contour plot) of color  $a^*$  showing effects of cooking temperature and cooking time. The number inside plots represent  $a^*$  value.

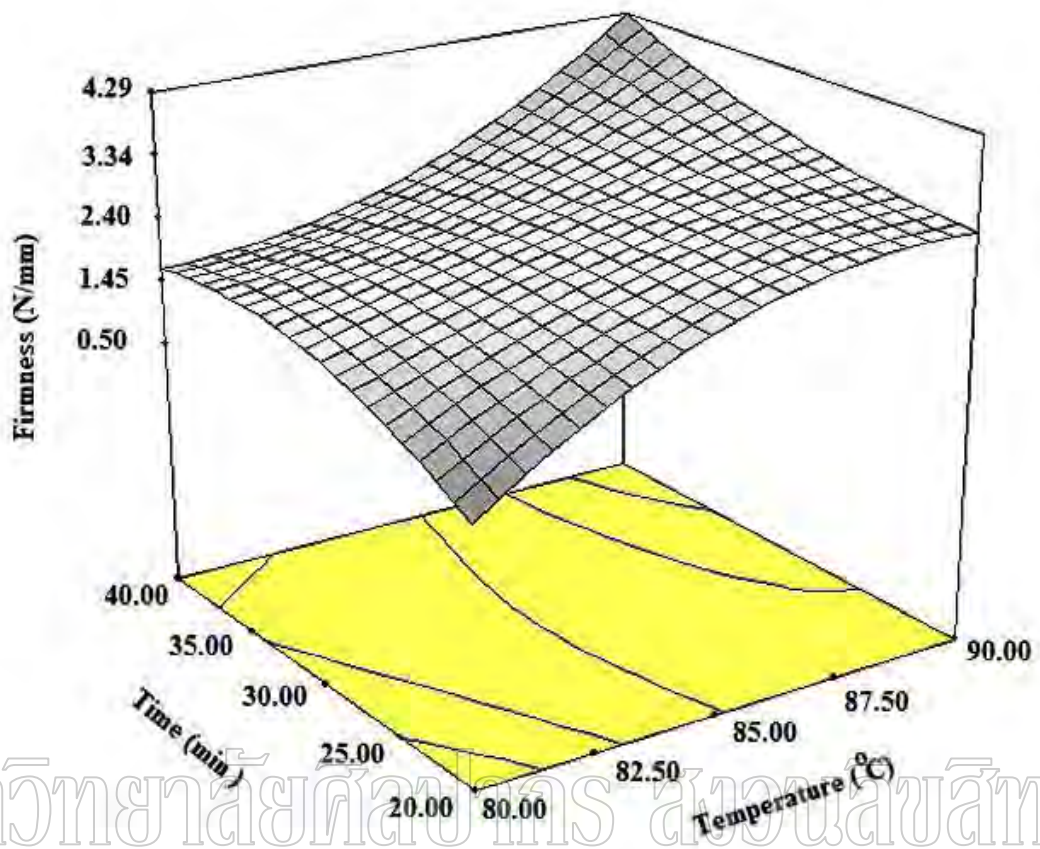


**Fig. 4.6** Response surface plots (contour plot) of color b\* showing effects of cooking temperature and cooking time. The number inside plots represent b\* value.

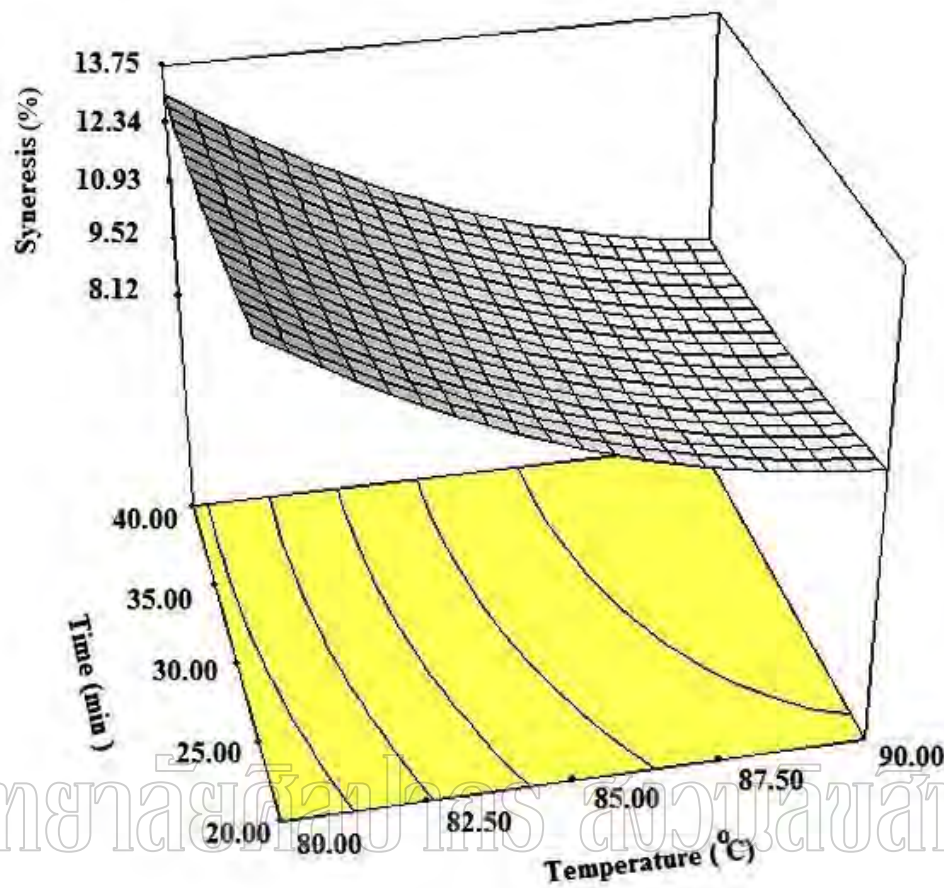


มหาวิทยาลัยศิลปากร สงวนลิขสิทธิ์

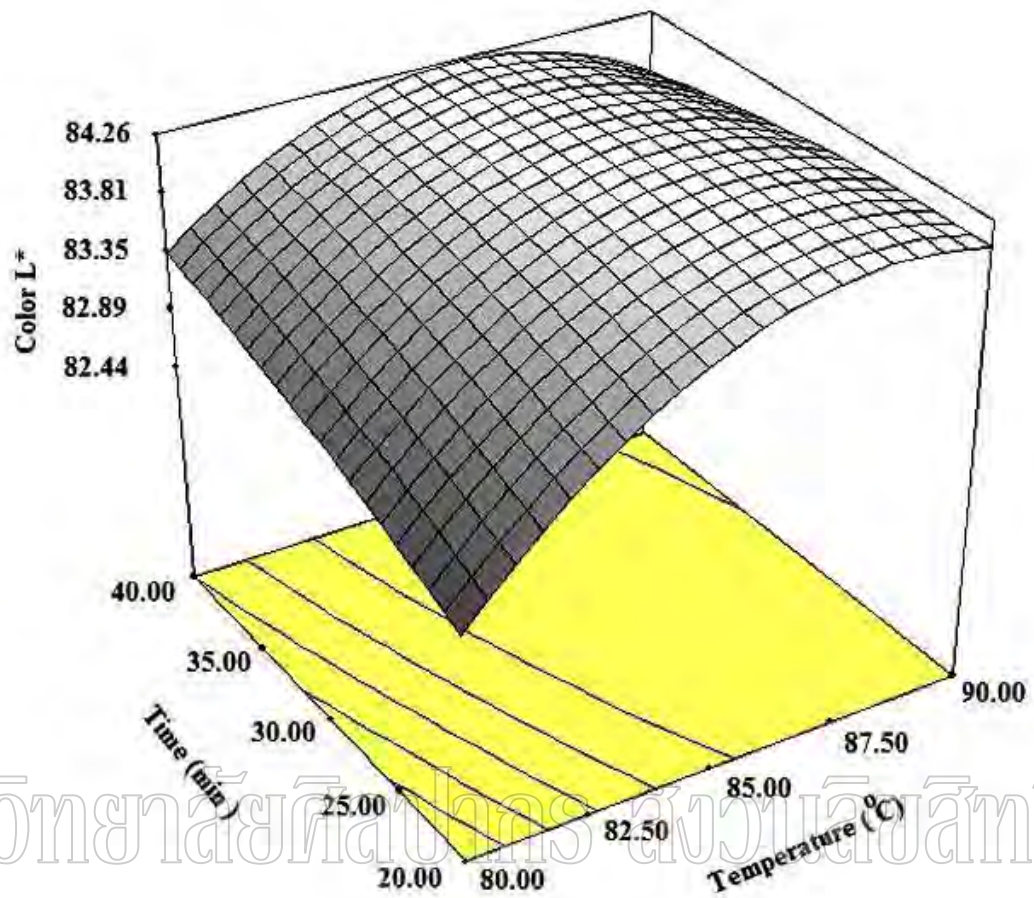
**Fig. 4.7** Response surface plots (three-dimension surface plot) of shear stress showing effects of cooking temperature and cooking time. The number inside plots represent shear stress ( $\text{N/g.mm}^2$ ).



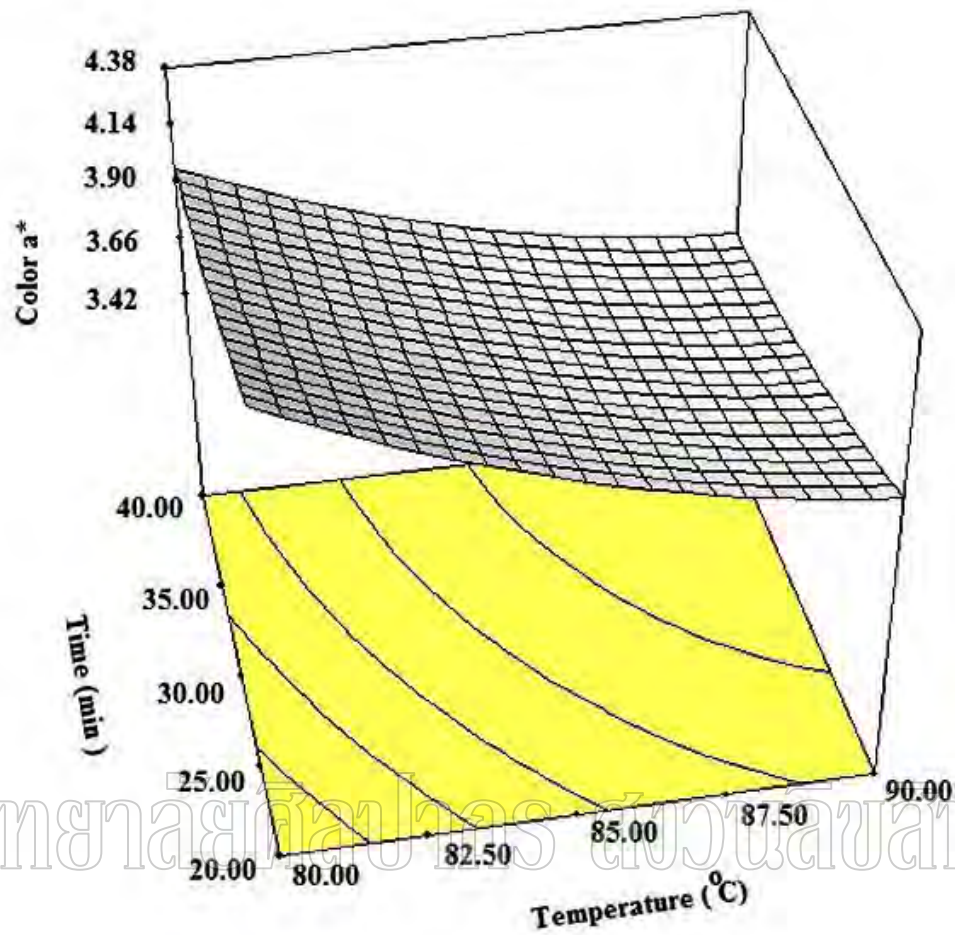
**Fig. 4.8** Response surface plots (three-dimension surface plot) of firmness showing effects of cooking temperature and cooking time. The number inside plots represent firmness (N.mm).



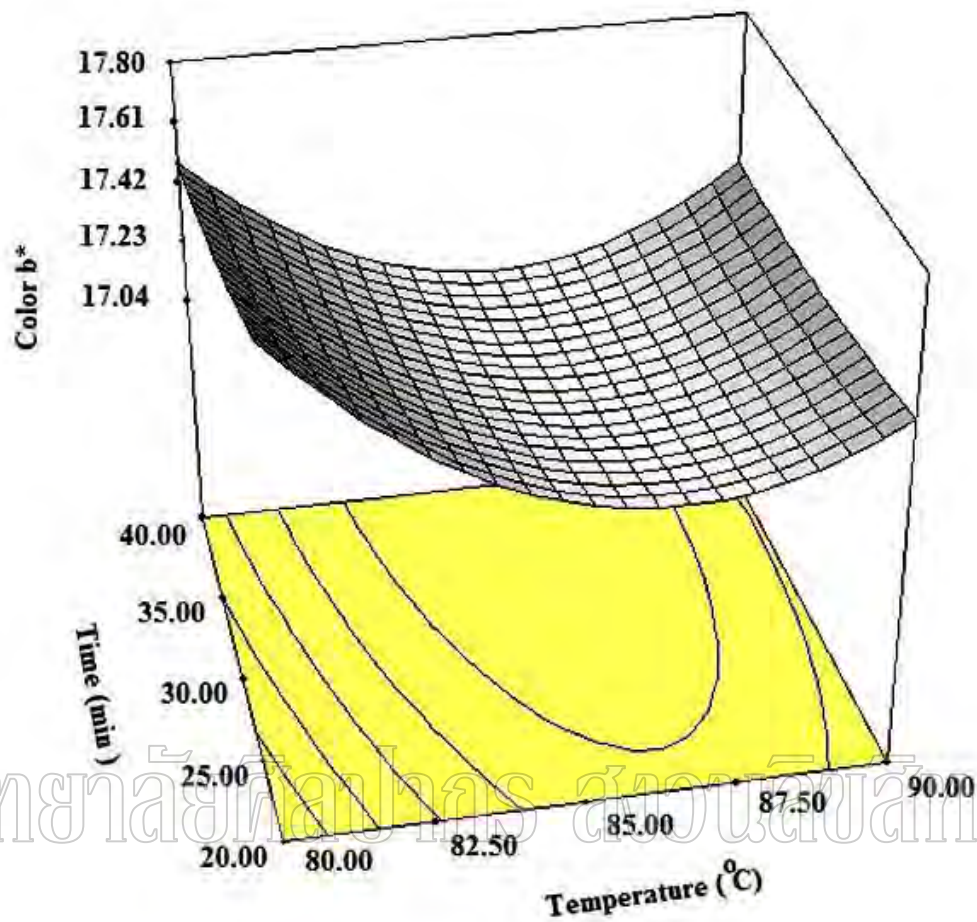
**Fig. 4.9** Response surface plots (three-dimension surface plot) of syneresis showing effects of cooking temperature and cooking time. The number inside plots represent syneresis (%).



**Fig. 4.10** Response surface plots (three-dimension surface plot) of color L\* showing effects of cooking temperature and cooking time. The number inside plots represent L\* value.

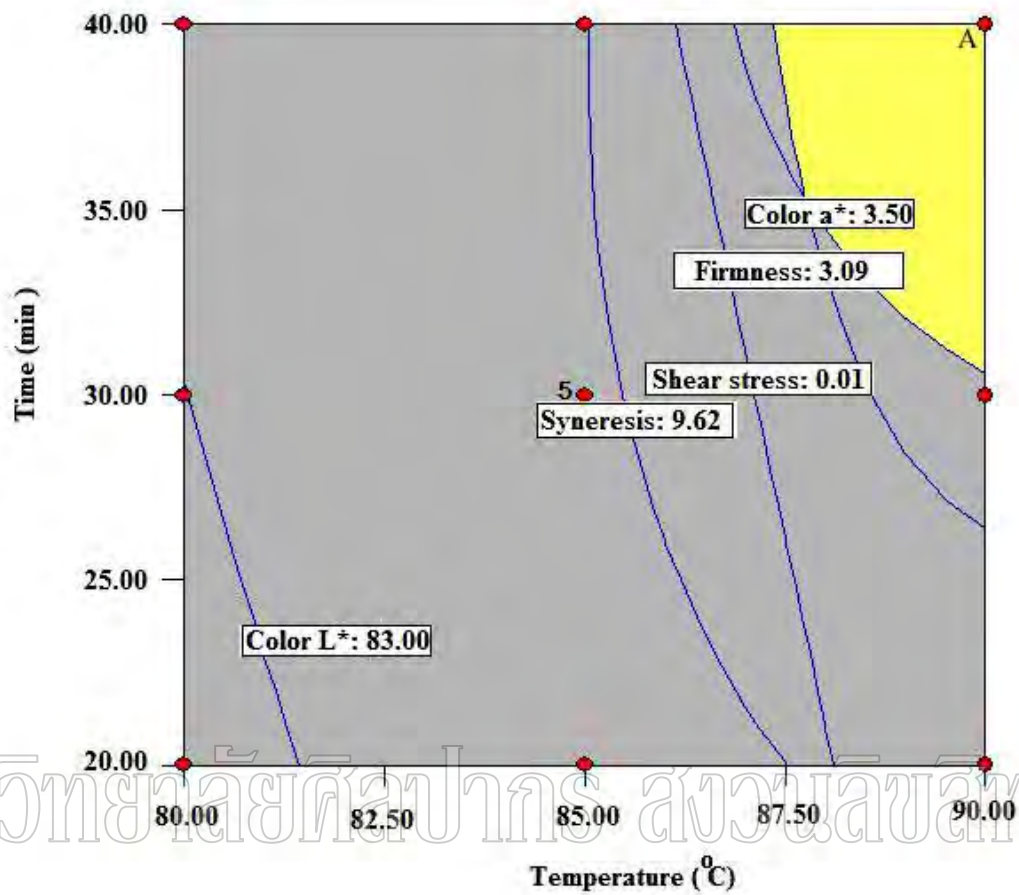


**Fig. 4.11** Response surface plots (three-dimension surface plot) of color  $a^*$  showing effects of cooking temperature and cooking time. The number inside plots represent  $a^*$  value.



**Fig. 4.12** Response surface plots (three-dimension surface plot) of color b\* showing effects of cooking temperature and cooking time. The number inside plots represent b\* value.





**Fig. 4.13** Optimum production procedure as a function of the independent variables after superimposition of the contour plots. Shade region is optimum area.

## CHAPTER 5

### EFFECT OF CARRAGEENAN ON THE PHYSICAL PROPERTIES OF PACKED EGG TOFU

#### ABSTRACT

The effects of the polysaccharide carrageenan and two types of coagulants including magnesium sulfate and calcium sulfate on the physical properties of packed egg tofu were investigated. Shear stresses of MgSO<sub>4</sub>-egg tofu were higher than that coagulated with CaSO<sub>4</sub>, but not significant difference in firmness of packed egg tofu. Addition of carrageenan in CaSO<sub>4</sub>-egg tofu substantially decreased the syneresis more than MgSO<sub>4</sub>-egg tofu. Carrageenan remarkably provided the benefit to significantly decrease syneresis ( $p < 0.05$ ) of packed egg tofu which was more pronounced at higher gum concentration applied. Significant differences ( $p < 0.05$ ) in color ( $L^*$ ,  $a^*$ , and  $b^*$ ) was found in packed egg tofu with increasing carrageenan concentration. Addition of carrageenan in packed egg tofu significantly ( $p < 0.05$ ) improved the physical properties of packed egg tofu.

**Keywords:** packed egg tofu, carrageenan, syneresis, calcium sulfate, magnesium sulfate

#### INTRODUCTION

A number of coagulants had been used in preparation of tofu (soybean curd). Each coagulant resulted in tofu with special textural characteristics which varied from softness to firmness with moisture ranging from 70 to 90% (Shen et al., 1991). Tofu manufactures preferred soybean varieties that produced tofu with high yield and good textural properties. The texture of tofu must be coherent, smooth and firm but not hard and rubbery. Due to its bland nature, the textural properties of tofu plays an important role in overall quality and consumer acceptability (Lim et al., 1990).

Tofu making involved the factors including intrinsic characteristics, such as soybean total protein content (Rajni et al., 2003), time and temperature of heating, type and concentration of coagulant (Lim et al., 1990; Shen et al., 1991; Shih, et al, 1997; Sun and Breene, 1991).

The coagulant of choice among most tofu makers was calcium sulfate; Tsai et al. (1981) found it superior to other calcium salts. Although calcium sulfate and glucono-delta-lactone were the coagulants of choice, other soluble salts of calcium, such as calcium acetate and calcium chloride had also been recommended for the coagulation of soymilk (Lim et al., 1990; Shen et al., 1991; Sun and Breene, 1991; Tsai et al., 1981)

Food was a complex and heterogeneous system containing many different chemical types and species (Samant et al., 1993). The rheological and other physiochemical properties of many processing and convenience foods were determined to a large extent by the behavior of protein and polysaccharide components (Karim et al., 1999). The protein-polysaccharide interactions had generated considerable research interest (Bernal et al., 1987; Foegeding and Ramsey, 1987; Hansen, 1968; Xu et al., 1992;). Interaction between polysaccharides (also termed as hydrocolloids and polyanions) and protein was first received attention when discovered that many sulfated polysaccharides stimulated the appearance in vivo of a lipemia clearing factor in blood serum, and that lipoprotein lipase was activated by heparin in vitro (Samant et al., 1993). Understanding the mechanisms involved in the interactions between these components was important to exploit their potential to meet new technological requirements (Karim et al., 1999).

Polysaccharides played a key that roled in modifying the textural properties of protein food systems (Karim et al., 1999). Addition of polysaccharide gum such as carrageenan to meat products was of interest to processors for production of lean economical structured beef products (Shand, et al, 1994). Carrageenans comprised a family of sulfated linear polysaccharides which extracted from red marine algae (*Rhodophyceae*) (Hill, et al, 1998; Karim et al., 1999). Carrageenans, a linear polysaccharides of D-galactose, and 3, 6 anhydro-D-galactose were used extensively in the food industry for their thickening, stabilizing and gelling properties (Karim et al., 1999). The kappa and iota fractions formed thermoreversible gels. After heating

to solubilize, their gel cooled and remelted on reheating. The lambda fraction did not gel. In general, in aqueous solutions, the remelt temperature was about 10-20°C that greater than the setting temperature (Shand, Sofos, and Schmidt, 1994). Almost all carrageenans and related polysaccharides were structurally heterogeneous with structural variations occurring both within and between polysaccharides. Choice of seaweed and preparative technique would favor particular structures and improve the structural regularity of the sample (Hill, Ledward, and Mitchell, 1998).

The objectives of this study at the outset was to study the feasibility to improve the physical properties of packed egg tofu via exploiting the carrageenan-protein interaction, and to determine how the physical properties of packed egg tofu were influenced by carrageenan addition to whole egg prior to coagulation by calcium sulfate and magnesium sulfate.

## MATERIAL AND METHODS

### Preparation of Egg Tofu

1-Day old eggs were obtained from local market and then individually cleaned throughout the shell with warm water before breaking process. Liquid whole egg was blended at low speed for 5 min. Liquid whole egg was filtered using the wet cheese cloth for removing chalazas and then measure total soluble solid. The ingredients including 0.8% sodium chloride, 0.5% soy sauce, 0.05% monosodium glutamate, and 0.3% sodium hexametaphosphate were added into liquid whole egg. Calcium sulfate and magnesium sulfate were added at level of 0.1% in the liquid whole egg. The require quantity of carrageenan (1 or 2  $\text{gl}^{-1}$ ) was added to the liquid whole egg after addition of coagulants. The mixture was subsequently homogenized using APV homogenizer (model Rannie LAB2000, APV, Denmark) with 700 bars of 1<sup>st</sup> stage and 70 bars of 2<sup>nd</sup> stage, then packaged into cylindrical bags (140g) and heat sealed. The mixture of egg tofu was cooked at 90°C for 40 min, and then cooled to 10°C. The packed egg tofu was stored at 4°C for 24 hr before further analyses.

## Final Product Analyses

### Texture Analyses

Textural properties of egg tofu samples were determined using Texture Analyser (model TA.XT2, Stable Micro Systems, Goldaming, UK). All egg tofu samples were kept at 6°C after remove from cool room and then formed to the same cylindrical shape (25 mm of diameter, 20 mm of height). The compression test with a HDP/BSW blade set with warner bratzler probe was performed. The pre-test, test and post-test speeds were set to 5, 5 and 10 mm s<sup>-1</sup>, respectively. Each test was performed triplicate replications to measure maximum shear force (N). Shear stress (N/g.mm<sup>2</sup>) was calculated as the maximum shear force multiplied by (2 ×width ×thickness of sample). Firmness (N.mm) was calculated as the area under graph from start to maximum force.

## มหาวิทยาลัยศิลปากร ส่วนวนลิขสิทธิ์

A modified method Amstrong et al (1994) was employed in this test. Three pieces of tofu samples (3.5 cm of diameter) were weighed and placed on a sieve (3.5 cm x 3.5 cm) putting on the top of the PVC tube (3.0 cm of diameter, 3.0 cm of height) over 250 ml beaker. The beaker was wrapped with plastic wraps in a hanging position for 24 hr at 4 °C. Percentage syneresis was calculated as the weight of water released from the tofu in 24 hr divided by the original weight of sample and multiplied by 100.

### Color

The color of the packed egg tofu was measured by Hunter Lab Colorimeter (model 9000, Gardner, USA). Calibrated to a white plate (CIE L\* = 98.76, a\* = -0.19, b\* = -0.06). The L\*, a\* and b\* values were used to calculate the derived color parameters. The test was replicated three times.

### **Water content**

Tofu samples were cut into pieces of weight 5-8 g. Each sample was placed in a crucible, vacuum dried at 105°C for 4 h. Then quickly take the samples out of the dryer and put them into desiccators for 30 min. The difference between the initial weight and final dry weight was use to calculate the water content (AOAC, 1984).

### **Statistical design and analysis**

Statistic analyses were performed using 2 x 3 factorial design experiment to test the effects of different coagulants and different carrageenan concentration on the physical properties of packed egg tofu. All data were evaluated using the Statistical Analysis System program (SAS Institute, Inc., 1985) Analysis of variance (ANOVA) was conducted, and the differences between group means were analyzed using the Least Significant Difference (LSD). Statistical significance was established at  $p \leq 0.05$ .

## **RESULTS AND DISCUSSION**

### **Textural properties**

The shear stress and firmness of packed egg tofu prepared with the different coagulant and carrageenan concentrations shown in Fig. 5.1 and Fig 5.2. It found that MgSO<sub>4</sub>-egg tofu and CaSO<sub>4</sub>-egg tofu did not significantly ( $p > 0.05$ ) different in shear stress and firmness. Shear stress and firmness of packed egg tofu could also relate to the addition of carrageenan for example, (Fig. 5.1 and 5.2), shear stress and firmness of packed egg tofu increased with an increasing concentration of carrageenan. In most cases, MgSO<sub>4</sub>-egg tofu (without carrageenan) contained the lowest shear stress ( $1.74 \times 10^2$  N/g.mm<sup>2</sup>) and firmness (4.07 N.mm), but with addition of carrageenan,

especially at  $2 \text{ g l}^{-1}$ ,  $\text{MgSO}_4$ -egg tofu exhibited the highest shear stress ( $1.88 \times 10^2 \text{ N/g.mm}^2$ ). According to statistic results, no interaction effect was found between type of coagulants and carrageenan concentration. Being a sulphated polysaccharide, carrageenan could exist as a negatively charged polymer over a wide range of pH. Above the isoelectric point, polyvalent metal ions such as  $\text{Ca}^{2+}$  could form bridges between the negatively charged carboxyl groups of the protein and the ester sulphate groups of the polysaccharide. In such protein-polysaccharide-calcium systems, both the protein and the polysaccharide could interact independently with the calcium ions (Karim et al., 1999).

Gelation of food protein involved heat denaturation followed by aggregation. If aggregation was relatively slower than denaturation an ordered structure would be promoted by allowing the denatured molecules to orient themselves in a systematic fashion prior to aggregation (Hermansson, 1978). Conditions that retard intermolecular interaction would result in a more homogeneous and regular network and consequently a stronger gel (Bernal et al., 1987). The observed effect of  $\text{MgSO}_4$  and  $\text{CaSO}_4$  with carrageenan in the present study the results showed that  $\text{MgSO}_4$  could be interacts with carrageenan greater than  $\text{CaSO}_4$ .

In protein/polysaccharide/calcium systems the components could interact in more than one way. Both the protein and the polysaccharide could interact on their own, with calcium ions, or with each other, with or without calcium involvement. Calcium bridges would maximize interactions between negative charged molecules and might improve gel firmness and stability (Bernal et al., 1987).

### Syneresis

After gels had allowed to stand protected against evaporation for a number of hours there was a tendency for the gel to separate into two phases. Syneresis of packed egg tofu with different coagulants type resulted in significantly ( $p < 0.05$ ) different in physical properties of packed egg tofu. Similar results were obtained from carrageenan concentration. As shown in Fig. 5.3, syneresis was found to be lower in packed egg tofu made form  $\text{MgSO}_4$ -egg tofu (15.79%) than in that made form  $\text{CaSO}_4$ -egg tofu (13.21%) as an increase in carrageenan concentration. No

interaction effect between type of coagulants and carrageenan concentration was noticeable. It generally accepted that syneresis in the protein gel during storage caused by an increased cross-linking among protein molecules through various interactions that made the protein gel matrix denser. At higher protein and polysaccharide concentrations, one might expected an increased number of cross-linking in the gel network. This may induce an increased in syneresis.

### **Water content**

The water content of packed egg tofu directly depended on the methods of preparation (Fig.5.4). CaSO<sub>4</sub>-egg tofu at 0% carrageenan concentration contained the largest amount of water (89.12%). The water content of packed egg tofu with 2 gl<sup>-1</sup> of carrageenan concentration decreased to 88.53% and 88.72% in MgSO<sub>4</sub>-egg tofu and CaSO<sub>4</sub>-egg tofu, respectively. No interaction effect between type of coagulants and carrageenan concentration was reported. Water content of packed egg tofu samples seemed to influence their gel strengths. However, the lower water content packed egg tofu exhibited the highest gel strength. This suggested that water content also substantially affected the gel properties of tofu.

### **Color**

Effect of coagulants and carrageenan on color of packed egg tofu was reported in Fig. 5.5, Fig. 5.6, and Fig. 5.7. All packed egg tofu samples preparing in this study retained a light yellow color. Lightness (L\*) decreased from 83.44 to 82.92 in MgSO<sub>4</sub>-egg tofu and 84.12 to 83.78 in CaSO<sub>4</sub>-egg tofu with an increase in carrageenan concentration (Fig. 5.5), while the redness (a\*) augmented from 3.88 to 4.22 in MgSO<sub>4</sub>-egg tofu and 3.18 to 3.47 in CaSO<sub>4</sub>-egg tofu with higher carrageenan concentration applied (Fig. 5.6). The yellowness (b\*) increased from 17.15 to 17.89 in MgSO<sub>4</sub>-egg tofu and 15.10 to 17.60 in CaSO<sub>4</sub>-egg tofu with an increase in carrageenan concentration (Fig. 5.7). There were no significant differences ( $p>0.05$ ) for blue-yellow chromaticity (b\*) of packed egg tofu as a function of coagulant types.



Conclusively, no interaction effect between type of coagulants and carrageenan concentration was found.

Whereas, there were significant differences ( $p < 0.05$ ) for lightness ( $L^*$ ), and green-red chromaticity ( $a^*$ ) of packed egg tofu as a function of carrageenan concentration. The color changes in packed egg tofu due to a combination of high temperature and low moisture content initiated non-enzymatic browning reaction such as Maillard reaction and the caramelization of sugar.

Maillard reactions included those involving reducing sugars, aldehydes, and ketones with amines, amino acids, peptides, and proteins. In tofu, the reactants could have been reducing sugars and amino acids. Caramels, melanoidin pigments, were probably generated by the heat treatment of carbohydrates such as sucrose, glucose, or inverted sugar in the tofu (Baik and Mittal, 2002).

## CONCLUSIONS

The present study had compared the effectiveness of  $\text{CaSO}_4$  and  $\text{MgSO}_4$  with carrageenan interaction for the improvement in syneresis and textural properties of packed egg tofu. Carrageenan at relatively low concentration ( $2 \text{ g/l}^{-1}$ ) achieved to enhance the water holding capacity of the egg protein gel that significantly affected the textural properties of both  $\text{MgSO}_4$ -egg tofu and  $\text{CaSO}_4$ -egg tofu. Results also suggested that the functionality of carrageenan under various conditions in packed egg tofu products directly relate to the type of coagulants and carrageenan concentration. An increment in physical properties of packed egg tofu upon the addition of carrageenan was probably due to more physical entrapment of protein and water by carrageenan. Carrageenan had also been shown to have no interaction effect with the coagulant. Optimization conditions on gelation for egg protein and the carrageenan-induced modifications of the gel network that may be useful in modifying the egg tofu processing technology for tangible commercial advantages.

## REFERENCES

- Abd Karim A, Sulebele GA, Azhar ME, Ping CY. 1999. Effect of carageenan on yield and properties of tofu. *J. Food Sci.* 66:159-165.
- Bernal VM, Smajda CH, Smith JL, Stanley, D.W. 1987. Interactions in Protein/Polysaccharide/Calcium Gels. *J. Food Sci.* 52(2):1121-1136.
- Hansen PMT. 1968. Stabilization of  $\kappa$ -Casein by Carageenan. *J. Dairy Sci.* 51(2):192-195.
- Hill SE, Ledward DA, Mitchell JR. 1998. Functional Properties of Food Macromolecules. 2<sup>nd</sup> ed. New York: Chapman&Hall. p 72-277.
- Lim BT, DeMan JM, DeMan L, Buzzell RI. 1990. Yield and quality of tofu as affected by soybean and soymilk characteristics. calcium sulfate coagulant. *J. Food Sci.* 55(4):1088-1092.
- Mujoo R, Trinh DT, Ng PKW. 2003. Characterization of storage proteins in different soybean varieties and their relationship to tofu yield and texture. *Food Chem.* 82: 265-273.
- Samant SK, Singhal RS, Kulkarni PR, Rege DV. 1993. Protein-Polysaccharide interactions: a new approach in food formulation. *Int. J. Food Sci. and Tech.* 28:547-562.
- Shand PJ, Sofos JN, Schmidt GR. 1994. Differential Scanning Calorimetry of Beef/Kappa-Carageenan Mixtures. *J. Food Sci.* 59(4):711-715.
- Shen CF, De Man L, Buzzell RI, De Man JM. 1991. Yield and quality of tofu as affected by soybean and soymilk characteristics: glucono-delta-lactone coagulant. *J. Food Sci.* 56(1):109-112.
- Sun N, Breene WM. 1991. Calcium sulfate concentration influence on yield and quality of tofu from five soybean varieties. *J. Food Sci.* 56(6):1604-1607.
- Tsai SJ, Lan CY, Kao CS, Chen SC. 1981. Studies on the Yield and Quality Characteristics of Tofu. *J. Food Sci.* 46: 1734-1737.
- Xu SY, Stanley DW, Goff HD, Davidson VJ, Le Maguer M. 1992. Hydrocolloid/Milk Gel Formation and Properties. *J. Food Sci.* 57(1):96-102.

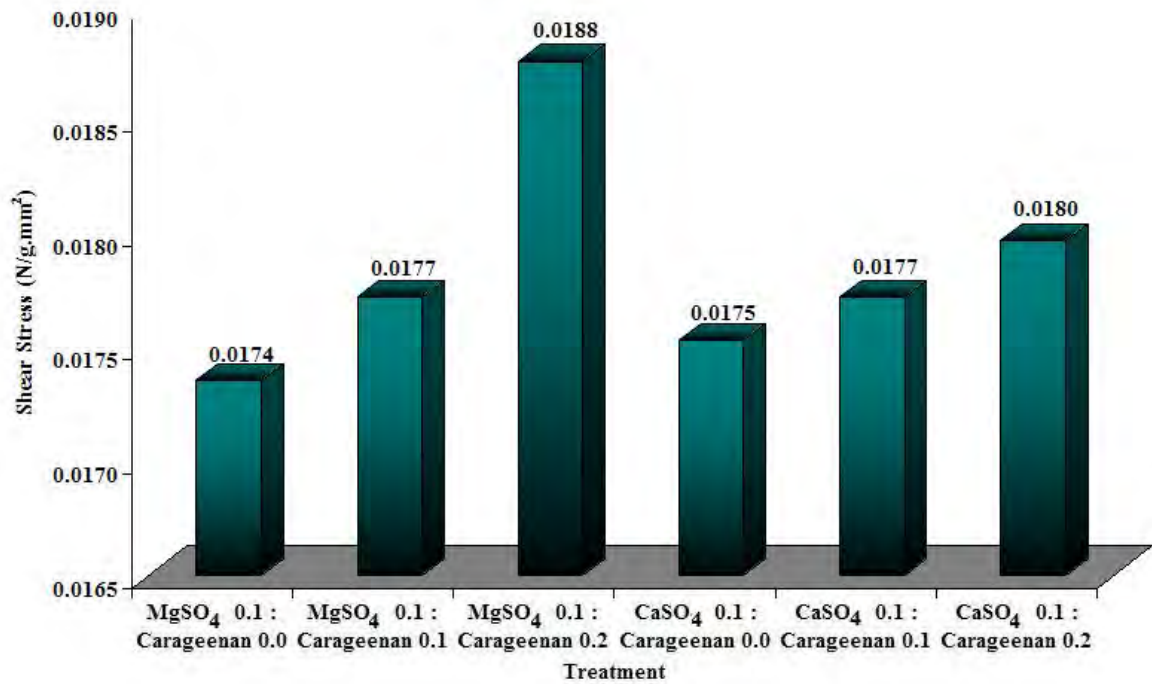
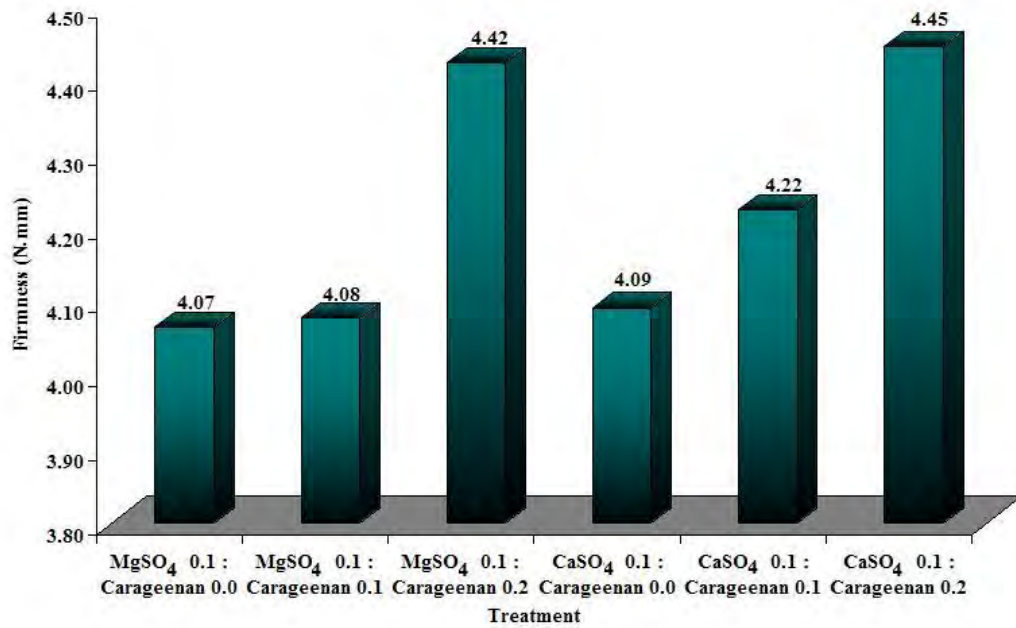
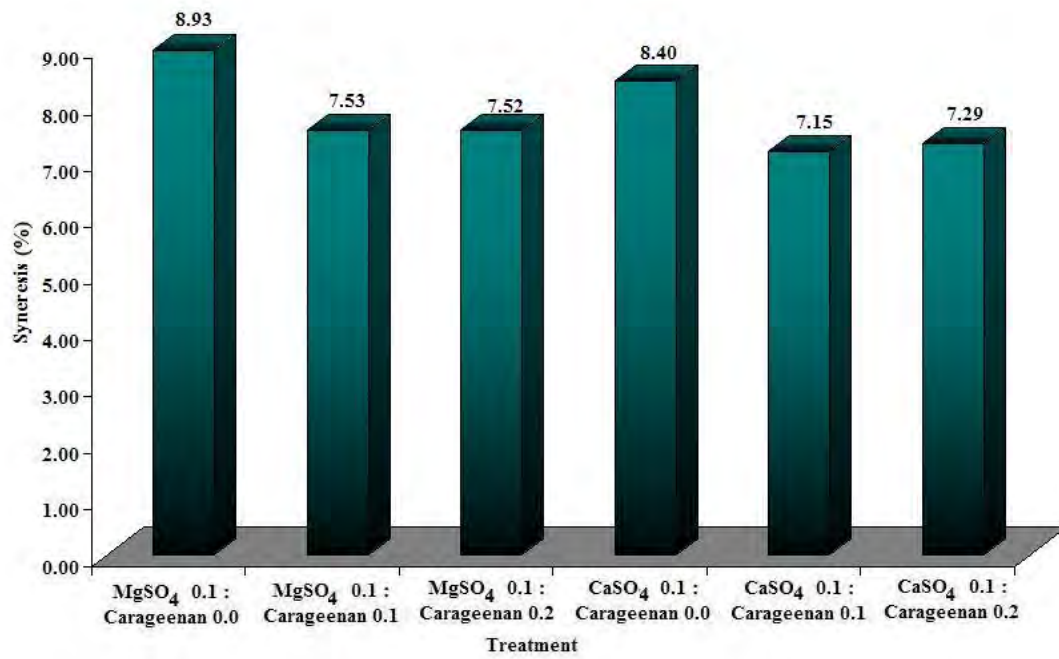


Fig.5.1 Effect of coagulants and carrageenan on shear stress of packed egg tofu



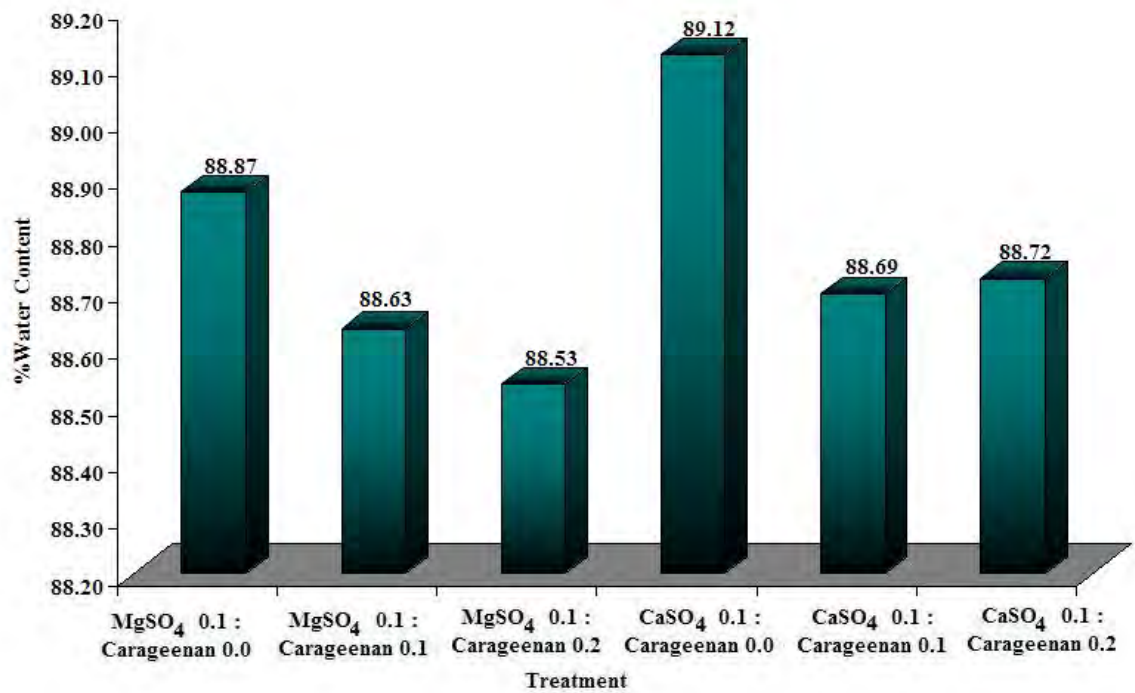
**Fig.5.2** Effect of coagulants and carrageenan on firmness of packed egg tofu

มหาวิทยาลัยศิลปากร สงวนลิขสิทธิ์



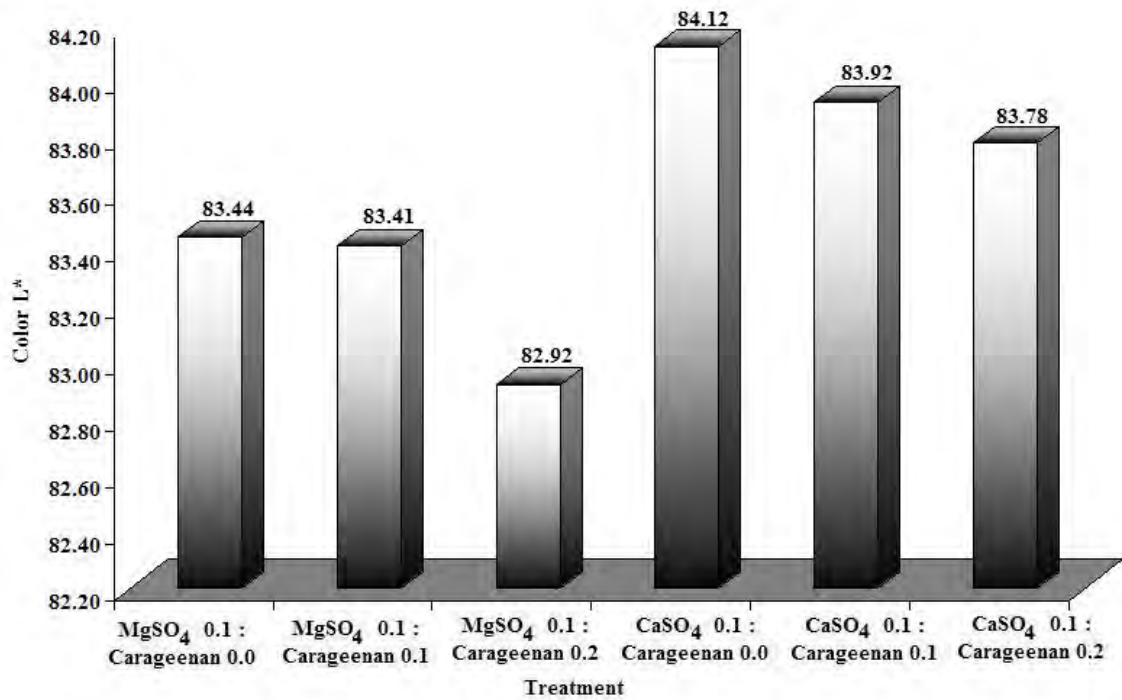
**Fig.5.3** Effect of coagulants and carrageenan on syneresis of packed egg tofu

มหาวิทยาลัยศิลปากร สงวนลิขสิทธิ์



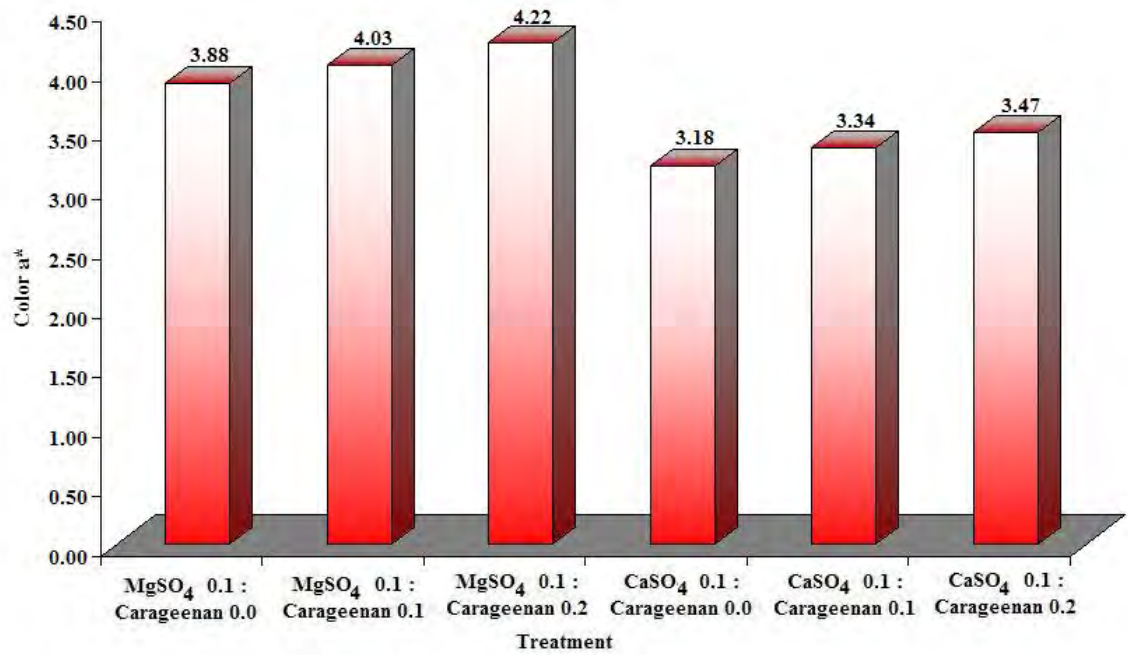
**Fig.5.4** Effect of coagulants and carrageenan on water content of packed egg tofu

มหาวิทยาลัยศิลปากร สงวนลิขสิทธิ์



**Fig.5.5** Effect of coagulants and carrageenan on lightness (L\*) of packed egg tofu

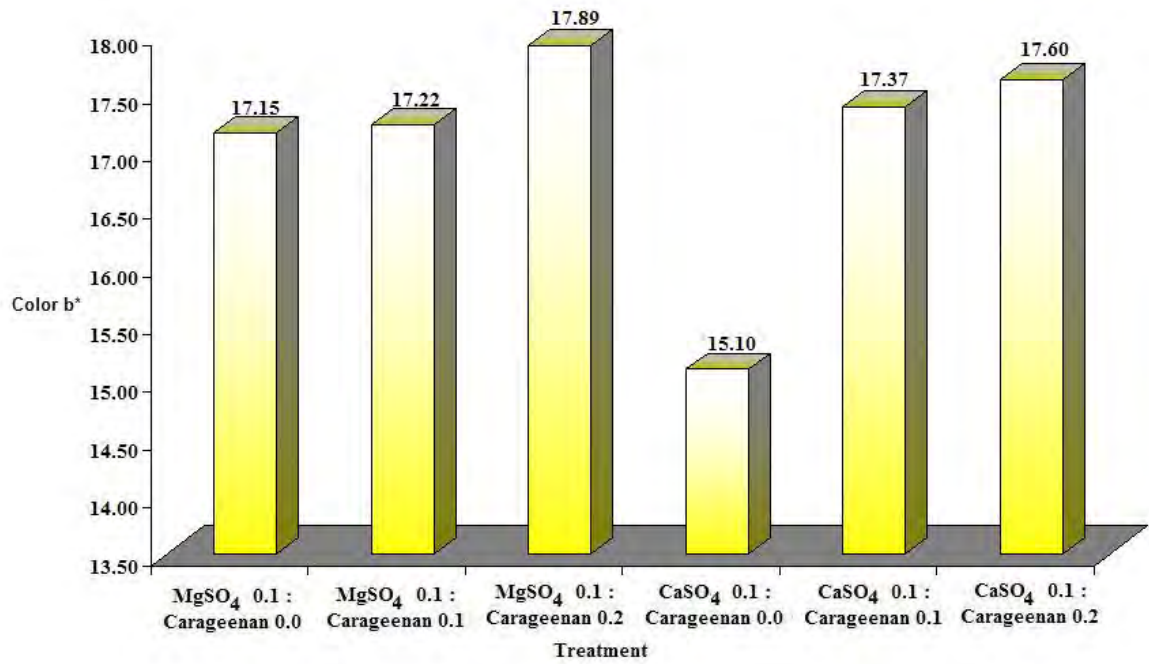
มหาวิทยาลัยศิลปากร สงวนลิขสิทธิ์



**Fig.5.6** Effect of coagulants and carrageenan on green-red chromaticity (a\*) of packed egg tofu

มหาวิทยาลัยศิลปากร สงวนลิขสิทธิ์





**Fig.5.7** Effect of coagulants and carrageenan on blue-yellow chromaticity (b\*) of

packed egg tofu

มหาวิทยาลัยศิลปากร สงวนลิขสิทธิ์

## CHAPTER 6

### SUMMARY

The objectives of this study were to determine the effect of ingredients comprising 5 different coagulants, the concentration usage, and the added water level on the physical and chemical properties of the packed egg tofu. The following experiment was conducted to figure out the optimum processing parameters during the thermal process such as temperature and time applied to receive the best textural characteristic of packed egg tofu. Finally, a potential food additive, for instance, carrageenan, was applied in the formulation to improve the syneresis and textural characteristics of the products.

The packed tofu at 55% added water and either 0.3% MgSO<sub>4</sub> or 0.3% CaSO<sub>4</sub> was selected for the best formulation since it is capable to decrease the syneresis and also increment the shear stress and firmness as compared to the commercial control. Regarding to the further investigation, the packed egg tofu with 0.1% MgSO<sub>4</sub> and utilized the water:liquid whole egg at 55:45 exhibited the most similar textural characteristics to the control.

According to response surface analysis, the process parameters including the cooking time and temperature were evaluated. The packed egg tofu cooked in the conditions of 87-90°C at 31-40 min absolutely provided the test results as greatly same as the control (benchmark). The selected condition at optimum point (90°C and 40 min) using in the thermal process of the packed egg tofu achieved higher shear stress and firmness (1.20 N/g.mm<sup>2</sup>, 4.15 N.mm, respectively), but lower syneresis (8.42%). However, the L\*, a\*, and b\* color of the test samples at this condition were pretty the same as the control (approximately 83.91, 3.41, and 17.26, respectively).

The additional 0.1% and 0.2% carrageenan in the packed egg tofu formulation attempted to sufficiently improve all the physical attributes ( $p < 0.05$ ). The more amount of carrageenan usage, the greater shear stress and firmness were obtained. In addition, the syneresis of tofu with carrageenan tended to decrease. The color of packed egg tofu was darker, redder and more yellow while as higher concentration of carrageenan employed.

มหาวิทยาลัยศิลปากร สงวนลิขสิทธิ์

## APPENDIX 1

### DEFINITION AND CALCULATION OF TEXTURAL PARAMETER

#### Shear stress

The force per unit area acting in the direction parallel to the surface of the plane in unit of N/g.mm.<sup>2</sup>, This experiment, area acting in the direction parallel to Warner Bratzler's blade was the two surfaces (width x length) of sample

$$\begin{aligned} \text{Shear stress} &= \text{Shear force}/(2 \times \text{width} \times \text{thickness of sample}) \\ &= F/A ; A = 2w \times l \end{aligned}$$

#### Firmness

The work while cutting under specified conditions to attain a given deformation in unit of N.mm

$$\text{Firmness} = \text{Area under graph from start to maximum force}$$

มหาวิทยาลัยศิลปากร สงวนลิขสิทธิ์

## APPENDIX 2

## ANALYSIS OF VARIANCE TABLE (PARTIAL SUM OF SQUARES)

**Appendix table 1.** Analysis of variance (ANOVA) for response surface linear model (Shear stress)

Source	Sum of squares	DF	Mean square	F Value	Prob>F	
Model	$13.203 \times 10^{-5}$	2	$1.602 \times 10^{-5}$	66.47	<0.0001	significant
A	$3.083 \times 10^{-5}$	1	$3.083 \times 10^{-5}$	127.94	<0.0001	
B	$1.205 \times 10^{-6}$	1	$1.205 \times 10^{-6}$	5.00	0.0493	
Residual	$12.409 \times 10^{-6}$	10	$2.409 \times 10^{-7}$			
Lack of fit	$1.572 \times 10^{-6}$	6	$2.619 \times 10^{-7}$	1.25	0.4331	not significant
Pure Error	$8.379 \times 10^{-7}$	4	$2.095 \times 10^{-7}$			
Total	$3.444 \times 10^{-5}$	12				

A = Temperature (°C)

B = Time (min)

**Appendix table 2.** Analysis of variance (ANOVA) for response surface linear model (Firmness)

Source	Sum of squares	DF	Mean square	F Value	Prob>F	
Model	19.43	7	1.35	131.76	<0.0001	significant
A	1.41	1	1.41	137.88	<0.0001	
B	$3.880 \times 10^{-3}$	1	$3.880 \times 10^{-3}$	0.38	0.5648	
A <sup>2</sup>	$4.844 \times 10^{-3}$	1	$4.844 \times 10^{-3}$	0.47	0.5218	
B <sup>2</sup>	0.055	1	0.055	5.35	0.686	
AB	0.018	1	0.018	1.76	0.2415	
A <sup>3</sup>	0.000	0				
B <sup>3</sup>	0.000	0				
A <sup>2</sup> B	0.63	1	0.63	61.19	0.0005	
AB <sup>2</sup>	0.23	1	0.23	22.20	0.0053	
Residual	0.051	5	0.010			
Lack of fit	0.024	1	0.024	3.64	0.1292	not significant
Pure Error	0.027	4	$6.690 \times 10^{-3}$			
Total	9.48	12				

A = Temperature (°C)

B = Time (min)

**Appendix table 3.** Analysis of variance (ANOVA) for response surface linear model (Syneresis)

Source	Sum of squares	DF	Mean square	F Value	Prob>F	
Model	38.65	5	7.73	160.06	<0.0001	significant
A	33.78	1	33.78	699.25	<0.0001	
B	1.17	1	1.17	24.25	0.0017	
A <sup>2</sup>	2.47	1	2.47	51.09	0.0002	
B <sup>2</sup>	0.17	1	0.17	3.57	0.1009	
AB	0.039	1	0.039	0.81	0.3993	
Residual	0.34	7	0.048			
Lack of fit	0.23	3	0.076	2.71	0.1798	not significant
Pure Error	0.11	4	0.028			
Total	38.99	12				

A = Temperature (°C)

B = Time (min)

**Appendix table 4.** Analysis of variance (ANOVA) for response surface linear model (Color L\*)

Source	Sum of squares	DF	Mean square	F Value	Prob>F	
Model	3.66	5	0.73	26.88	0.0002	significant
A	1.58	1	1.58	57.97	0.0001	
B	0.15	1	0.15	5.45	0.0523	
A <sup>2</sup>	1.20	1	1.20	44.20	0.0003	
B <sup>2</sup>	0.021	1	0.021	0.76	0.4121	
AB	0.36	1	0.36	13.20	0.0084	
Residual	0.19	7	0.027			
Lack of fit	0.15	3	0.049	4.69	0.0084	not significant
Pure Error	0.042	4	0.011			
Total	3.85	12				

A = Temperature (°C)

B = Time (min)



**Appendix table 5.** Analysis of variance (ANOVA) for response surface linear model (Color a\*)

Source	Sum of squares	DF	Mean square	F Value	Prob>F	
Model	0.80	5	0.16	84.24	<0.0001	significant
A	0.55	1	0.55	288.47	<0.0001	
B	0.18	1	0.18	94.14	<0.0001	
A <sup>2</sup>	0.035	1	0.035	18.47	0.0036	
B <sup>2</sup>	9.994 x 10 <sup>-3</sup>	1	9.994 x 10 <sup>-3</sup>	5.24	0.0559	
AB	3.941 x 10 <sup>-3</sup>	1	3.941 x 10 <sup>-3</sup>	2.07	0.1937	
Residual	0.013	7	1.907 x 10 <sup>-3</sup>			
Lack of fit	2.825 x 10 <sup>-3</sup>	3	9.416 x 10 <sup>-3</sup>	0.36	0.7875	not significant
Pure Error	0.011	4	2.631 x 10 <sup>-3</sup>			
Total	0.82	12				

A = Temperature (°C)

B = Time (min)

**Appendix table 6.** Analysis of variance (ANOVA) for response surface linear model (Color b\*)

Source	Sum of squares	DF	Mean square	F Value	Prob>F	
Model	0.65	5	0.13	11.56	0.0028	significant
A	0.13	1	0.13	11.40	0.0118	
B	0.051	1	0.051	4.51	0.0714	
A <sup>2</sup>	0.36	1	0.36	31.83	0.0008	
B <sup>2</sup>	3.737 x 10 <sup>-3</sup>	1	3.737 x 10 <sup>-3</sup>	0.33	0.5836	
AB	0.015	1	0.015	1.37	0.2805	
Residual	0.079	7	0.011			
Lack of fit	0.039	3	0.013	1.31	0.3870	not significant
Pure Error	0.040	4	9.992 x 10 <sup>-3</sup>			
Corrected Total	0.73	12				

A = Temperature (°C)

B = Time (min)

## VITA

**Name**                                 **Boonanan Sakboonyarat**

**Home address**                   **39 Ratchadumri Rd. Praphathomjadee District. Muang  
Nakhon Pathom 73000**

**Education**                         **Studying in Master Degree**  
   **Institute: Silpakorn University**  
   **Majority: Master of Science (MSc.)**  
   **Branch: Food Technology**

**Highest Education Level: Bachelor Degree**  
   **Institute: Silpakorn University**  
   **Majority: Bachelor of Science (BSc.)**  
   **Branch: Food Technology**

**High School**  
   **Institute: Phraphathom Wittayalai, Nakhon Pathom**

### **Rewards and Honors**

- Thesis Fund from Graduate School of  
Silpakorn University (2004)
- Good oral presentation award on the 4<sup>th</sup> National  
Symposium on Graduate Research,  
Chiang Mai University (2004)
- Poster presentation on The 6<sup>th</sup> Agro-Industrial  
Conference (THAIFEX & HALFEX 2004 )