Production of Flavorant and Colorant from Pacific White Shrimp (*Litopenaeus vanamei*) Head and Their Food Applications

Sineenath Sukkwai

A Thesis Submitted in Fulfillment of the Requirements for the Degree of
Doctor of Philosophy in Food Science and Technology
Prince of Songkla University
2017

Production of Flavorant and Colorant from Pacific White Shrimp (*Litopenaeus vanamei*) Head and Their Food Applications

Sineenath Sukkwai

A Thesis Submitted in Fulfillment of the Requirements for the Degree of
Doctor of Philosophy in Food Science and Technology
Prince of Songkla University
2017

Thesis Title	Production of Flavorant and Colorant from Pacific White Shrimp (*Litopenaeus vanamei*) Head and Their Food Applications
Author	Miss Sineenath Sukkwai
Major Program	Food Science and Technology

Major Advisor

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

Co-advisor

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

…….…….....
(Prof. Dr. Witoon Prinyawiwatkul)

Examining Committee:

…..……...…....Chairperson
(Asst. Prof. Dr. Saowakon Wattanachant)

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

…….…….....Committee
(Asst. Prof. Dr. Withida Chantrapornchai)

…….…….....Committee
(Asst. Prof. Dr. Nopparat Cheetangdee)

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

…….…….....Committee
(Prof. Dr. Witoon Prinyawiwatkul)

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

…….....
(Assoc. Prof. Dr. Teerapol Srichana)
Dean of Graduate School

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

……..…Signature
(Asst. Prof. Dr. Kongkarn Kijroongrojana)
Major Advisor

…….……Signature
(Miss Sineenath Sukkwai)
Candidate

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

…….……Signature

(Miss Sineenath Sukkwai)

Candidate

ชื่อวิทยานิพนธ์ การผลิตสารให้กลิ่นรสและสารให้สีจากหัวกุ้งขาว (*Litopenaeus vanamei*) และการประยุกต์ใช้ในอาหาร

ผู้เขียน นางสาวสินีนาถ สุขไกว

สาขาวิชา วิทยาศาสตร์และเทคโนโลยีอาหาร

ปีการศึกษา 2559

บทคัดย่อ

การศึกษาการใช้ประโยชน์จากหัวกุ้งในการผลิตสารให้กลิ่นรสและสารให้สีโดยศึกษาการแปรรูปเบื้องต้นเพื่อหาสภาวะที่เหมาะสมในการป้องกันการเกิดเมลาโนซิสในหัวกุ้งขาวแวนนาไม (*Litopenaeus vanamei*) โดยการแช่หัวกุ้งในสารละลายต่าง ๆ ได้แก่ กรดซิตริกที่ความเข้มข้นร้อยละ 0.5, 1.0, 2.0 และสารละลายโซเดียมเมตาไบซัลไฟต์ที่ความเข้มข้นร้อยละ 1.0, 2.0, 4.0 ด้วยอัตราส่วน 1:5 (น้ำหนักหัวกุ้ง/ปริมาตรสารละลาย) เป็นเวลา 0, 15 และ 20 นาที พบว่าโซเดียมเมตาไบซัลไฟต์มีประสิทธิภาพมากกว่ากรดซิตริก ($p<0.05$) โดยตัวอย่างที่ผ่านการแช่สารละลายโซเดียมเมตาไบซัลไฟต์ ร้อยละ 2.0 เป็นเวลา 15 นาที มีค่ากิจกรรมของเอนไซม์โพลีฟีนอลออกซิเดสและคะแนนการเกิดเมลาโนซิสต่ำที่สุด ($p<0.05$) รวมถึงมีค่าต่ำกว่าการยับยั้งโดยการให้ความร้อนอีกด้วย (อุณหภูมิแกนกลาง 80 องศาเซลเซียส เป็นเวลา 30 นาที) ($p<0.05$)

การผลิตสารให้กลิ่นรสจากหัวกุ้งขาวแวนนาไมโดยย่อยด้วยเอนไซม์อัลคาเลส (พีเอช 8.0) และเอนไซม์ฟลาโวไซม์ (พีเอช 7.0) ร้อยละ 0.15 (น้ำหนัก/น้ำหนัก) ที่อุณหภูมิ 55 องศาเซลเซียส เป็นเวลา 5 ชั่วโมง พบว่าการสกัดเป็นเวลา 270 นาทีจะให้ปริมาณโปรตีนและฟอร์มาลดีไฮด์ไนโตรเจนสูงที่สุด ($p<0.05$) จากการทดสอบเชิงพรรณาพบว่าตัวอย่างสารให้กลิ่นรสที่สกัดด้วยฟลาโวไซม์มีความเข้มของกลิ่นปูม้าต้มมากกว่า แต่มีกลิ่นกุ้งแห้งคั่วและกุ้งแห้งต้มน้อยกว่าตัวอย่างที่สกัดด้วยอัลคาเลส ($p<0.05$) โดยตัวอย่างที่สกัดด้วยอัลคาเลสมีคะแนนความชอบด้านสีและความชอบโดยรวมสูงกว่า ($p<0.05$) ตัวอย่างสารให้กลิ่นรสที่ไม่ผ่านการแปรรูปเบื้องต้นมีปริมาณโปรตีนและฟอร์มาลดีไฮด์ไนโตรเจนที่สูงกว่า ($p<0.05$) ในขณะที่คะแนนความชอบโดยรวมของสารให้กลิ่นรสที่สกัดจากหัวกุ้งที่ผ่านการแปรรูปเบื้องต้นจะสูงกว่าตัวอย่างที่สกัดจากหัวกุ้งที่ไม่ผ่านการแปรรูปเบื้องต้น ($p<0.05$)

การศึกษาหาสภาวะที่เหมาะสมในการทำแห้งสารให้กลิ่นรสที่สกัดด้วยอัลคาเลสโดยใช้วิธีการต่าง ๆ ได้แก่ การทำแห้งแบบเยือกแข็ง (FD), การทำแห้งแบบอบแห้งในถาด (TD) และการทำแห้งแบบพ่นฝอย (SD) รวมทั้งการใช้มอลโตเดกซ์ตริน โดยระเหยสารสกัดจนมีปริมาณของแข็งร้อยละ 15 แล้วเติมมอลโตเดกซ์ตรินร้อยละ 5 (15+5M) เปรียบเทียบกับสารสกัดที่ระเหยจนมีปริมาณของแข็งร้อยละ 20 (20) จากการทดสอบการยอมรับแสดงให้เห็นว่าวิธีการทำแห้งไม่มีอิทธิพลต่อคะแนนความชอบด้านกลิ่น กลิ่นรส/รสชาติของลูกชิ้นปลาที่เติมสารให้กลิ่นรสจากหัวกุ้งในปริมาณร้อยละ 4 ($p \geq 0.05$) ในขณะที่ลูกชิ้นปลาที่เติมสารให้กลิ่นรส TD15+5M ได้รับคะแนนความชอบด้านสีต่ำที่สุดเมื่อเปรียบเทียบกับการทำแห้งด้วยวิธีอื่น ๆ ($p<0.05$) ลูกชิ้นปลาที่เติม SD20 SD15+5M และ TD15+5M ได้รับคะแนนความชอบโดยรวมสูงที่สุด ($p<0.05$) จากการทดสอบเชิงพรรณาพบว่าสารให้กลิ่นรสก่อนการทำแห้งมีความเข้มของกลิ่นกุ้งแห้งคั่ว กุ้งแห้งต้ม ปูม้าต้ม และกุ้งไม่สดต้มสูงที่สุด ($p<0.05$) โดยความเข้มของกลิ่นดังกล่าวจะลดลงหลังการทำแห้งในทุกสภาวะ ตัวอย่าง TD15+5M มีค่าความเข้มของกลิ่นมันกุ้งสูงที่สุด ($p<0.05$) โดยกลิ่นดังกล่าวเป็นผลมาจากสารประกอบไพราซีนที่เกิดขึ้นในระหว่างการทำแห้ง ในการเก็บรักษาตัวอย่างสารให้กลิ่นรสที่ทำแห้งด้วยวิธีอบแห้งในถาด (TD20) เป็นเวลา 4 เดือนที่อุณหภูมิห้อง (28±2 องศาเซลเซียส) พบว่าตัวอย่างที่เก็บภายใต้สภาวะสุญญากาศมีการเปลี่ยนแปลงของสัดส่วนสารระเหยน้อยกว่าการเก็บภายใต้สภาวะที่มีอากาศ มีสารประกอบไพราซีนเพิ่มขึ้น ขณะที่สารในกลุ่มแอลดีไฮด์มีปริมาณลดลง อย่างไรก็ตามการเปลี่ยนแปลงดังกล่าวไม่ส่งผลต่อความเข้มของคุณลักษณะด้านกลิ่นเมื่อทดสอบโดยผู้ทดสอบที่ผ่านการฝึกฝน ($p \geq 0.05$)

การสกัดสารให้สีธรรมชาติจากหัวกุ้งขาวแวนนาไมโดยศึกษาสัดส่วนที่เหมาะสมของไอโซโพรพานอลและเฮกเซนในการสกัดแคโรทีนอยด์จากหัวกุ้งโดยใช้ simplex lattice design พบว่า 40.731: 59.269 (โดยปริมาตร) เป็นสัดส่วนที่ให้ปริมาณผลผลิตสูงที่สุด ($p<0.05$) และพบว่าสารให้สีที่สกัดจากหัวกุ้งที่ผ่านการแปรรูปเบื้องต้นมีผลผลิตแคโรทีนอยด์ โปรตีนและเถ้าสูงกว่าตัวอย่างที่ไม่ผ่านการแปรรูปเบื้องต้น ($p<0.05$) อย่างไรก็ตามตัวอย่างทั้งสองมีปริมาณความชื้นและไขมันไม่แตกต่างกัน ($p \geq 0.05$)

การศึกษาการนำสารให้กลิ่นรสและสารให้สีที่สกัดจากหัวกุ้งขาวแวนนาไมในผลิตภัณฑ์อาหารโดยใช้สารให้กลิ่นรส (ร้อยละ 0-3 โดยน้ำหนัก) และสารให้สี (ร้อยละ 0-0.4 โดย

น้ำหนัก) ในผลิตภัณฑ์ขนมจีบกุ้งโดยใช้ Central composite design แสดงให้เห็นว่าการใช้ส่วนประกอบทั้งสองชนิดในปริมาณดังกล่าวไม่ส่งผลต่อคะแนนความชอบของขนมจีบกุ้ง ($p \geq 0.05$) และสังเกตได้ว่าการเติมสารให้กลิ่นรสส่งผลให้คะแนนความชอบทุกคุณลักษณะลดลง ($p<0.05$) ในขณะที่คะแนนความชอบด้านสีเพิ่มขึ้นเมื่อมีการเติมสารให้สี ($p<0.05$) และการเติมสารให้สีในร้อยละ 0.2 (7.63 ± 0.89) จะทำให้ตัวอย่างขนมจีบกุ้งได้รับคะแนนความชอบด้านสีสูงกว่าตัวอย่างที่ไม่เติมสี (6.73 ± 1.20) และเติมสีร้อยละ 0.4 (7.07 ± 0.60)($p<0.05$)

การศึกษาการใช้สารให้สีที่สกัดจากหัวกุ้งในผลิตภัณฑ์ซอสมายองเนสโดยใช้สารให้สี 3 ระดับความเข้มข้น (ไม่เติมสารให้สี (NC): ร้อยละ 0, สารให้สีระดับกลาง (MC): ร้อยละ 1.2 และ สารให้สีระดับสูง (HC): ร้อยละ 3.6, โดยน้ำหนัก) ร่วมกับการใช้เกลือที่แตกต่างกัน (เกลือที่ใช้โดยทั่วไป (RS): เกลือโซเดียมคลอไรด์, ลดโซเดียม (ReS): เกลือโพแทสเซียมคลอไรด์ และ ไม่เติมเกลือ (NS): ไม่เติมเกลือ) พบว่าการเติมสารให้สีทำให้ค่าคะแนนความชอบในรสเค็มลดลง ($p<0.05$) สีที่เข้มเกินไป (HC) ส่งผลให้คะแนนความชอบด้านสีลดลง ($p<0.05$) นอกจากนี้ยังพบว่าตัวอย่างที่ได้รับคะแนนความชอบด้านสีมากกว่าจะมีร้อยละของระดับความพอดีในรสเค็มมากกว่าเช่นกัน ความเข้มข้นของสารให้สีเดียวกัน RS เป็นตัวอย่างที่ได้รับคะแนนความชอบในรสเค็มมากที่สุดตามด้วย ReS และ NS ตามลำดับ ($p<0.05$) ความเข้มข้นของสารให้สีมีอิทธิพลอย่างยิ่งต่ออารมณ์ของผู้ทดสอบหลังการบริโภคซอสมายองเนส ทั้งนี้จากการวิจัยยังพบว่าการให้ข้อมูลเรื่อง ‘การใช้สารให้สีจากธรรมชาติ’ และ ‘ปริมาณโซเดียม’ มีผลเพียงเล็กน้อยต่ออารมณ์และความตั้งใจซื้อของผู้บริโภค

Thesis Title Production of Flavorant and Colorant from Pacific White Shrimp (*Litopenaeus vanamei*) Head and Their Food Applications

Author Miss Sineenath Sukkwai

Major Program Food Science and Technology

Academic Year 2016

ABSTRACT

Study of shrimp head utilization for flavorant and colorant productions, the pretreatment of Pacific white shrimp (*Litopenaeus vanamei*) head for melanosis prevention was conducted. Soaking shrimp head in 0.5, 1.0, 2.0 % citric acid solutions and 1.0, 2.0, 4.0 % sodium metabisulfite solutions in the ratio of 1:5 w/v for 10, 15 and 20 min were compared. The results showed that PPO activity and melanosis score of the samples were decreased with increasing in solution concentration and soaking time ($p<0.05$). The sample treated with 2.0% sodium metabisulfite solution for 15 min obtained the lowest PPO activity and melanosis score. Moreover, the values were lower than of heated sample (core temperature of 80^{o}C for 30 sec) ($p<0.05$).

To produce the shrimp flavorant from Pacific white shrimp head, the optimal condition of enzymatic flavorant extraction from shrimp head using 0.15% w/w Alcalase (pH 8.0) or Flavourzyme (pH 7.0) at 55^{o}C for 5 hr were studied. The results showed that extraction for 270 min yielded the highest protein and formaldehyde nitrogen contents for both enzymes ($p<0.05$). A descriptive analysis indicated that the flavorant using Flavourzyme gained higher intensity of boiled blue swimming crab odor, but lower intensities of boiled sundried shrimp and roasted sundried shrimp odors than that using Alcalase. The overall and color acceptance scores (n=30) of fish ball added with 4% (dry weight) flavorant extracted by Alcalase were higher than that by Flavourzyme ($p<0.05$). The flavorant solution from the non-pretreated sample had the higher protein and formaldehyde nitrogen contents than another and overall acceptance score of the pretreated sample was higher than that of non-pretreated sample ($p<0.05$).

The shrimp flavorant using Alcalase, dried with different drying methods (freeze drying (FD), tray drying (TD) and spray drying (SD)) and the 20% flavorant solutions (20) and the 15% flavorant solution with 5% maltodextrin added (15+5M) were compared. The acceptance test indicated that drying methods had no effect on odor and flavor/taste liking scores of fish ball added with 4% shrimp flavorant

($p \geq 0.05$). However, color acceptance scores of fish balls added with the TD flavorants were lower than those of other methods ($p<0.05$). The fish balls added with SD20, SD15+5M and TD15+5M had the highest overall acceptance score ($p<0.05$). Descriptive analysis revealed that the flavorant before drying had the highest intensity of roasted sundried shrimp, boiled sundried shrimp, boiled blue swimming crab and unfresh shrimp juice odors ($p<0.05$). The intensities of those odors decreased after drying the sample in all conditions. Mungoong (shrimp paste) odor was presented in tray dried sample and the highest intensity was found in tray dried sample with maltodextrin. The storage at room temperature ($28\pm2^{o}C$) for 2 and 4 months under vacuum condition had less effect on the volatile compounds in shrimp flavorant than that under air condition. However, the trained panelists could not detected the differences between the intensities of odor characteristics ($p \geq 0.05$).

To produce a natural colorant from Pacific white shrimp (*Litopenaeus vanamei*) head, the optimized mixture proportions of isopropanol and hexane for carotenoid extraction was studied by applying the simplex lattice design. The prediction model showed that the proportion gaining the highest carotenoid yield was 40.731: 59.269 (v/v). The colorant extracted from pretreated shrimp head had the higher carotenoid yield, protein and ash content than that from non-pretreated shrimp head ($p<0.05$). However, moisture and fat content of both samples were not different ($p \geq 0.05$).

The studies of applications of flavorant and colorant extracted from Pacific white shrimp head in foods were conducted. Central composite design was used to optimize the concentration of shrimp flavorant (0-3% w/w) and colorant (0-0.4% w/w) in shrimp shumai. It was noticeable that the shrimp flavorant decreased liking scores of color, flavor, texture and overall ($p<0.05$), while color liking score was increased with the shrimp colorant added ($p<0.05$). Shrimp shumai added with 0.2% colorant had the highest color liking score (7.63 ± 0.89) when compared with that without the colorant (6.73 ± 1.20) and added at 0.4% (7.07 ± 0.60) ($p<0.05$).

Another application, the shrimp colorant was applied to enhance color of mayonnaise-based dipping sauce. The colorant (NC: 0, MC: 1.2 and HC: 3.6%, w/w) and salts (RS: NaCl, ReS: KCl and NS: no salt added) were added to mayonnaise-based dipping sauce and the results showed that when colorant concentration increased the

saltiness liking score decreased ($p<0.05$). Too intense color (HC) affected the decreasing of color liking score ($p<0.05$). The samples with the higher color liking scores gained the higher percentage to be just-about-right samples for expected saltiness. At the given colorant concentration, the highest saltiness liking score was occurred in RS followed by ReS and NS, respectively ($p<0.05$). Scores of emotions elicited by dipping sauces were highly affected by colorant concentration. Statements of ‘colorant from a natural source’ and ‘sodium content’ had a few effects on elicited emotions and consumer’s purchase intent.

ACKNOWLEDGEMENTS

I would like to express my deepest appreciation and sincere gratitude to my advisor, Asst. Prof. Dr. Kongkarn Kijroongrojana of the Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, for her kindness, mentorship and assistance during my study. I also would like to thank for her care and always believe in me. In the Ph.D. student life, advisor is the most important factor that affects your happiness, successful as well as what you are going to be and I found the best for me.

I would like to express my sincere thanks to my co-advisor, Prof. Dr. Soottawat Benjakul of the Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University for his invaluable suggestion for my academic knowledge and support me on my research.

I would like to express my profound gratitude to my co-advisor, Prof. Dr. Witoon Prinyawiwatkul of School of Nutrition and Food Sciences, Louisiana State University, United State for his kindess, support and inspire me to learn the new things. As well as his lab members, I can say again that we are a good team. I thank Off, Paka, Dhara, Ryan, Valentina, Yupeng, Kennet, Dapeng and Ashley for their love, help and constant encouragement. Durind my stay in the US was wonderful and it could not be like that without you. Thank my Thai friends in Baton Rouge, Off, Som, Aew, Krit, Krit, Peun, Chompoo and Bas. All of you are a part of that wonderful time and I hope to see you again.

I also thank all my friends in Faculty of Agro-Industry, Prince of Songkla University for your support and encouragement, those mean so much to me. Especially, my trained panelists. You helped me a lot and never gave up on my training. Finally, we did it together.

I am grateful to my examining committee, Asst. Prof. Dr. Saowakon Wattanachant and, Asst. Prof. Dr. Nopparat Cheetangdee of the Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University and Asst. Prof. Dr. Withida Chantrapornchai of Department of Product Development, Faculty of Agro-

Industry, Kasetsart University for the valuable time devoted to my thesis and their kindness, comments and helpful suggestion.

This study could not be succeeded without the financial support from Thailand Research Fund under the Royal Golden Jubilee Ph.D. Program, the government budget and the Graduate School of Prince of Songkla University. I gratefully acknowledge this financial support.

Finally, I most gratefully acknowledge my family. They support me in every steps of my life. Thanks for always believed in me, your encouragement and your love. The most important person is my beloved grandmother, who is pleased with every moments of me. She is my idol. She never gives up on any obstacles and she had done all the good things not only for her family but for everyone. Even she could not be here to see but I know that from somewhere she is looking to me, smile and proud of me.

Sineenath Sukkwai

CONTENTS

CONTENTS (Continued)

CONTENTS (Continued)

CONTENTS (Continued)

LIST OF TABLES

LIST OF TABLES (Continued)

Table

LIST OF TABLES (Continued)

Table

LIST OF FIGURES

Appendixes

CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

1.1 Introduction

During shrimp processing, a large amount of shrimp waste including exoskeleton and cephalothorax are generated (Venugopol and Shahidi, 1995; Senphan and Benjakul, 2012). This waste can be represented 50% to 70% of weight of raw material (Holanda and Netto, 2006). The shrimp waste is a rich source of valuable components such as protein, carotenoid pigments, chitin and chitosan, as well as flavor compounds (Ramaswamy *et al*., 1991; Klomklao *et al*., 2009). However, this waste is processed for low value products as fertilizer, animal feed, and fish feed. The protein recovered in the form of hydrolysates can be used as flavorant and incorporated into fish-base foods (Teerasuntonwat and Raksakulthai, 1995; Holanda and Netto, 2006). The previous studies showed that peptides and free amino acids affected flavor in marine food (Konosu and Yamaguchi, 1982; Raksakulthai and Haard, 1992; Teerasuntonwat and Raksakulthai, 1995). Enzymatic hydrolysis, one of the effective approaches for protein recovery from shrimp waste has been widely studied (Simpson and Haad, 1985; Cano-Lopez *et al*., 1987; Synowiecki and Al-Khateeb, 2000; Gildberg and Stenberg, 2001; Mizani *et al*., 2005; Holanda and Netto, 2006). The studies were shown that enzymatic extraction conditions such as raw material, enzyme, and extraction time could be effected on flavorant yield and flavor (Teerasuntonwat and Raksakulthai, 1995; Simpson *et al*., 1998). Apart from the product mentioned above, carotenoid, a red-orange pigment, is one for the most interested component in shrimp waste. Astaxanthin, a major carotenoid found in shrimp, is a powerful biological antioxidant that occurs naturally in a wide variety of living organisms (Hussein *et al*., 2006). The extracted carotenoid can be used as natural colorant fortified foods in various industries such as beverage, ice cream, candy, confectionary, meat product, pet food and aquaculture food (Pu *et al*., 2010). In addition, the shrimp shells waste obtained after protein and astaxanthin recovery can be further used for chitin isolation. By this way, the liquid waste containing alkaline, protein, and protein degradation

products from tradition chitin isolation which cause environmental problems will be diminished (Chakrabarti, 2002).

Thus, the production of natural flavorant and colorant from shrimp waste can be a promising mean to gain the high market value products applied in seafood or related food industries.

1.2 Review of Literature

1.2.1 Shrimp waste

The shrimp wastes from shrimp processing represent 50% to 70% of weight of raw material. These waste include consists of 71.4% head and 28.6% shell (Mayers, 1986; Holanda and Netto, 2006). The major components of the shrimp waste consist of flavorant protein and carotenoid protein, chitin and minerals as shown in Table 1. Chemical compositions of shrimp by-product were varied depended on species, season, size, source etc. (Sriket *et al*., 2007; Liang *et al*., 2008; Rødde *et al*., 2008).

Total volatile base nitrogen (TVBN), trimethylamine (TMA), and pH were used to be the indicators to evaluate freshness and quality of shrimp as they were generated during postmortem handling (Bak *et al*., 1999). The study of Mu *et al*. (2012) on Pacific white shrimp (*Litopenaeus vannamei*) showed that during the first 2 days of storage the slightly increasing of TVBN was occurred and it sharply increased at day 3. Okpala (2014) studied the changes in TVBN and TMA of Pacific white shrimp during 12 days ice storage and found that after storage for 6 days, TVBN and TMA content of the shrimp were higher than the borderline of consumer's acceptability. The TVB-N scale of acceptability for raw shrimps was reported by Lannelongue *et al*. (1982) as <12 mg nitrogen/100 g for fresh, 12-20 mg nitrogen/100 g for edible but slightly decomposed, 20 to 25 mg nitrogen/100 g for borderline and 25 mg nitrogen/100 g for inedible and decomposed. The freshness borderline of TMA content was 5 mg nitrogen/100 g for shrimp as it still be acceptable for the consumers (Uchiyama and Kakuda, 1984; Mendess *et al*., 2002).

The previous studies showed the increasing in pH of shrimp during storage (Gonçalves *et al*., 2003; Lopez-Caballero *et al*., 2007; Mu *et al*., 2011; Nirmal 2011). Mu *et al*. (2011) reported the initial pH of fresh Pacific white shrimp as 7.04 and it was continuously increased until reach of 7.8 at day 6 during the storage at 4^{o}C. The similar results were also reported by Gonçalves *et al*. (2003) since pH of deepwater pink shrimp stored at 1.6 ± 0.4^{o}C was increased from 7.08 to 8.06 within 7 days. Huss (1995) revealed that microbial activity generated volatile amines those affect the increasing of pH. The enzymatic ammonia production was reported by Cobb (1979) and Finne (1982) as a cause of pH increasing in ice storage shrimp.

As by-product, shrimp head was not well treated and may hold at room temperature for a short time before freezing. The higher temperature could allowed microbial action as well as the enzymatic reaction and resulted in the accumulation of basis compounds accumulation those related to the increasing of pH (Lopez-Caballero, *et al*., 2007). However, Mehmet *et al.* (2009) reported that shrimp with a pH 7.7 and below still remained of good quality.

Table 1 Proximate composition of various shrimp wastes

Shrimp waste	Components (% dry basis)					References
	Moisture	**Protein**	**Fat**	**Ash**	**Chitin**	
Shrimp head						
Peneaus monodom Fabricius	78.5	60.93	14.42	23.26	-	Teerasuntonwat and Raksakulthai (1995)
Penaeus monodon (wild)	67.4	34.66	3.68	27.91	8.90	Babu *et al*. (2008)
Penaeus monodon (culture)	77.0	59.57	7.39	26.09	10.87	Babu *et al*. (2008)
Penaeus indicus	73.6	46.59	5.68	26.89	9.47	Babu *et al*. (2008)
Metapenaeus monocerous	75.8	46.28	6.20	43.39	7.02	Babu *et al*. (2008)
Farfantepenaeus paulensis	73.9	49.0	4.9	27.0	18.2	Sánchez-Camargo *et al.* (2011)
Hepatopancreas						
Litopenaeus vannamei	71.9	47.7	36.9	7.5	-	Senphan and Benjakul (2012)
Shrimp waste *						
Crangon crangon	71.12	40.6	9.95	27.5	17.8	Synowiecki and Al-Khateeb (2000)
Pandulas borealis	75.61	41.9	10.23	29.2	17.0	Shahidi and Synowiecki (1991)
Pandulas borealis	79.1	44.50	2.87	39.23	-	Heu *et al*. (2003)
Trachypena curvirostris	78.5	53.95	3.26	32.56	-	Heu *et al*. (2003)
Xiphopenaeus kroyeri	89.86	39.42	3.79	31.98	19.92	Holanda and Netto (2006)

* Shrimp waste including shrimp head and shell

1.2.2 Melanosis in shrimp

Main attributes used for the assessment of shrimp quality are usually odor and appearance (Bremner, 1998). Melanosis or blackspot appears very frequently during the postmortem storage of shrimp before the onset of spoilage. Melanosis is the formation of insoluble black pigment (melanin) in the internal shell surface due to enzymatic oxidation of phenolic precursor (Cobb, 1979). This is a natural mechanism caused by enzyme reactions that start as soon as the *Penaeidae* are taken from the water and come into contact with the oxygen of the atmosphere. After harvest and death, Polyphenol oxidase (PPO) systems are still active and can promote the development of black pigments around the shell and on the surface of the meat (Anonymous, 1992).

1.2.2.1 Polyphenol oxidase (PPO)

PPO catalyzes two basic reactions: hydroxylation to the *o*-position adjacent to an existing hydroxyl group of the phenolic substrate (monophenol oxidase activity), and oxidation of diphenol to *o*-benzoquinones (dephenol oxidase activity). Both reactions utilize molecular oxygen as a co-substrate. PPO is one of the metalloproteins with two copper atoms in the active site. A mechanism of the catalytic reactions of PPO is relatively well understood, in which PPO in met-type [Cu(II)CU(II)] interacts with molecular oxygen to form PPO in oxy-type [Cu(II)Cu(II)O_2] being capable of catalyzing the reactions of mono- and diphenols (Pérez-Gilabent and García-Carmona, 2000).

1.2.2.1.1 Monophenol oxidase

Monophenol oxidase catalyzes the hydroxylation of monophenols to *o*-diphenols (Figure 1). The enzyme was referred to as tyrosinase in animals, since L-tyrosine is the major monophenolic substrate (Whitaker, 1972). Monophenol oxidase (tyrosinase) has been given somewhat more attention in insect and crustacean systems, owing to its physiological significance in conjunction with diphenolase activity, in hardening of the cutical for sclerotization (Whitaker, 1972).

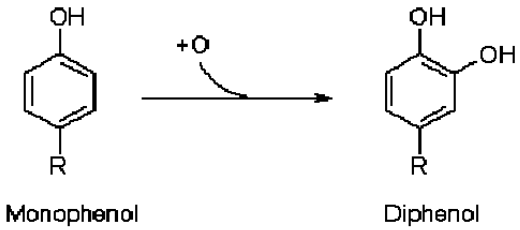


Figure 1 Monophenol oxidase pathway producing the diphenol
Source: Toussaint and Lerch (1987)

1.2.2.1.2 Diphenol oxidase

The oxidation of diphenolic substrates to quinones in the presence of oxygen is catalyzed by diphenol oxidase activity (Figure 2). Diphenol oxidases have received much attention owing to their high catalytic rate and their association with the formation of quinones, which lead to production of the browning pigment, melanin.

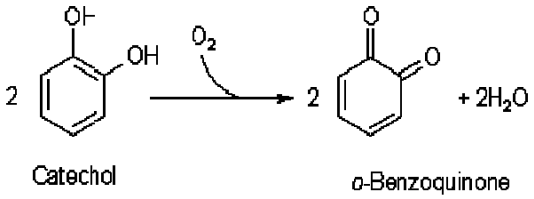


Figure 2 diphenol oxidase pathway producing the quinones
Source: Toussaint and Lerch (1987)

PPO is localized in the carapace of the cephalothorax, in the caudal zone and in the cutical of the abdomen, mainly where the cutical segments are joined and where the cutical is connected to the pleopods (Ogawa *et al*., 1984). In chilled shrimps, the melanosis reaction begins at the head and then spreads to the tail; the rate of spread of melanosis differs among the various species (Montero *et al*., 2001b).

The optimal temperature and pH of PPO has been reported to be varied, depending on species, habitat temperature or the physiological pH, in which the enzyme activity occurs in nature. PPO from carapace of shrimp (*Penaeus setiferus*) had the maximal activity at 45^{o}C, whereas the optimal temperature of PPO from carapace of shrimp (*Penaeus japonicus*) cultured in Spain was 55^{o}C (Simpson *et al*., 1988). The activity markedly decreased in very acidic and alkaline pH ranges. The unfolding of PPO molecules might occur at the extreme pHs owing to the increase in electrostatic repulsion, leading to the losses in its activity. Benjakul *et al*. (2005) reported that the optimal pH of PPO from the kuruma prawn cephalothorax was 6.5 at 35°C. PPO from the carapace of shrimp (*Penaeus setiferus*) showed the maximal activity at pH 7.5 (Simpson *et al*., 1987). PPO from the prawn (*Penaeus japonicus*) had two pronounced peaks at pHs 5.0 and 8.0 (Montero *et al*., 2001a), whereas the optimal pH of black tiger prawn PPO was 6.0 (Rolle *et al*., 1991). The optimal pH of PPO from the carapace of cephalothorax was 7.16, while it was 8.76 for PPO from abdominal cutical (Montero *et al*., 2001a). Nirmal and Benjakul (2012) reported that the optimal pH and temperature of PPO from Pacific white shrimp (*Litopenaeus vannamei*) were 6 and at 55°C, respectively.

1.2.3 Inhibition of melanosis

There are many studies reported the various techniques that have been applied to shrimp products of foodstuffs to prevent PPO activity throughout the post-harvest period. The techniques utilized aim to eliminate one or more of components essential for activity to occur (Williams, 2003). The essential components are oxygen, copper, the enzyme, or the substrate (Kim and Marshall, 2000).

1.2.3.1 Heat

Thermal processing is the most commonly used method for stabilization of foods due to its ability to destroy micro-organisms and deactivate enzymes (Kim and Marshall, 2000). Manheem *et al*. (2013) investigated the effect of pre-cooking at 80°C for various 0 to 120 s on enzyme activities and melanosis of Pacific white shrimp (*Litopenaeus vannamei*). They found that the residual activities of PPO and protease decreased with increasing pre-cooking times (0-120 s) ($p < 0.05$). Marked decreases in the relative PPO and protease activities were observed within the first 30 s of pre-cooking, and negligible activities were detected after 120 s. This result was related to the melanosis score which showed that shrimps pre-cooked with longer time showed a lower development of black spots as evidenced by a lower melanosis score throughout storage of 7 days at 4^{o}C. The time-temperature profile required to achieve an irreversible deactivation varies between species since thermal stability of the enzyme is dependent on the relative amounts of free and bound water, pH of the solution, and the presence of salts (Kim and Marshall, 2000). William (2003) reported the thermodurablity of the PPO in western rock lobster which required up to 90^{o}C for 36 min to ensure denaturation of the enzyme and thus prevention of melanosis.

1.2.3.2 Refrigeration or freezing

Refrigeration and freezing result in a reduction of the enzyme's catalytic rate during storage. However, lowering the temperature only delays the onset of melanosis, it does not prevent it (Ogawa, 1985). In freezing the increase in solute concentration combined with decreased water activity, and resultant changes in pH, appear to exert an inhibitory effect. Upon thawing disruption of the cells by ice crystals results in enhanced enzyme activity due to increased contact between the enzyme and substrates (Ogawa 1985). The increased activity results in higher levels of enzymatic browning after freezing and thawing when compared to unfrozen product (Ogawa,

1985; Kim and Marshall, 2000). Diaz-Tenorio *et al*. (2007) also reported that frozen storage can effectively retard physicochemical changes of shrimp, however, black spot formation (melanosis) could take place after thawing. During freezing and thawing, the inactive PPO stored in hemocytes and digestive gland are easily released and activated, and in the presence of suitable substrates and oxygen, melanosis develops more rapidly.

1.2.3.3 Modified atmosphere

An atmosphere modified with carbon dioxide (CO_2) has been used as an application to influence PPO activity. Chen *et al*. (1993) observed that purified Florida spiny lobster, brown shrimp and potato PPO exhibited a time-related decline in activity following treatment at 43ºC with high-pressure CO_2 at 58 atm. After two minutes at 43°C in the presence of high pressure (5.88 MPa) CO_2, Florida spiny lobster (*Panulirus argus*) PPO showed no activity. High pressure increased the susceptibility of PPO to the effects of heat and CO_2, which meant lower temperatures could be used during processing to deactivate the enzyme (Chen *et al*., 1992). Bak *et al*. (1999) found that shrimp packed in modified atmosphere (flushed with nitrogen before sealing) during frozen storage was better overall quality in relation to color fading of rancid flavor and toughening of the meat when compared to the control.

1.2.3.4 High hydrostatic pressure treatment

High hydrostatic pressure (HHP) promotes protein denaturation which is associated with conformational changes. It can increase or reduce enzymatic biological activity or change its substrate specificity, modifying the functionality of an enzyme. The effectiveness of treatment depends on the type of enzyme, pH, medium composition, temperature, time and pressure level applied (Hendrickx *et al*., 1998). For several PPOs, it has been reported that pressure-induced inactivation proceeds faster at lower pH. In addition to pH, pressure inactivation is influenced by the addition of salts, sugars or chemical antibrowning effectors (Rapeanu *et al*., 2005). Montero *et al*. (2001a) found that pressure at 300-400 MPa for 10 min at lower than 10ºC induced inactivation of PPO extracted from imperial tiger prawn (*Penaeus japonicus*). Total inhibition of the extract was achieved only with ascorbic acid and citric acid at pH 3.0.

1.2.3.5 Enzyme treatments

Modification of the enzyme or its substrate through the use of appropriate enzymes, such as oxygenases, *o*-methyl transferases and protesases, has been proposed as a method of prevention of enzymatic browning. However, the use of these methods is restricted due to the high cost of the enzymes and the impacts they may have on the food system (McEvily and Iyengar, 1992).

1.2.3.6 Complexing agents

Cyclodextrins inhibit enzymatic browning by the entrapment or formation of inclusion complexes with the substrates or products of PPO activity. A similar activity is exhibited by chitosan, a naturally occurring polymer. However, due to the large molecular size of these compounds their use is restricted to liquid systems (McEvily and Iyengar, 1992).

1.2.3.7 Polyphenol oxidase inhibitor

The use of melanosis or browning inhibitors in shellfish is restricted by consideration relevant to toxicity, wholesomeness and effect on taste, flavor, texture and cost. Melanosis inhibitors may be classified in accordance with their primary mode of action. Four categories of PPO inhibitors are applicable in the prevention of enzymatic browning. These include reducing agents, acidulants, chelating agents and enzyme inhibitors (McEvily *et al*., 1992).

1.2.3.7.1 Reducing agents

Reducing agents or antioxidants react with the *o*-quinones produced to form stable colorless compounds or to reduce them back to the less reactive colorless diphenols and so prevent pigment formation. However, reducing agents are only effective for the time period determined by their rate of consumption as they are irreversibly oxidized during the reaction with the pigment intermediaries. They are also non-specific in nature and react with other compounds that may result in production of undesirable flavors and colors (McEvily and Iyengar, 1992).

- Sulfiting agents

Sulfiting agents are compounds that release sulfur dioxide (SO_2) under the conditions of use. They exist as a mixture of ionic species in aqueous solution, bisulfite (HSO_3^-) and sulfite (SO_3^{2-}). The equilibrium between species is dependent on the pH of the solution. Bisulfite is the most effective species as it reduces the quinone

products of the enzyme and acts as a competitive inhibitor by binding to the Sulfhydryl group found at the enzyme's active site (Kim and Marshall, 2000).

Until recently, sulfites were the most widely used inhibitors of enzymatic browning. However, they also exhibit several undesirable characteristics. High levels of sulfites result in the bleaching of naturally occurring pigmentation, as well as having a negative impact on flavor (McEvily and Iyengar, 1992). Health risks also exist for sensitive individuals. As a result, there is an increasing level of regulatory control over the use of sulfites with maximum residue limits of 100 ppm being set for those products (Food Standards Australia New Zealand, 2004).

- Ascorbic acid and analogs

The main role of ascorbic acid and its isomer, erythorbic acid, in the prevention of enzymatic browning is to reduce the *o*-quinones to diphenols and to act as free radical or oxygen scavengers. There also appears to be some degree of interaction with the enzyme resulting in its deactivation (McEvily and Iyengar, 1992). Ascorbic acid is irreversibly oxidized to dehydroascorbic acid during reaction with the *o*-quinones. Once the ascorbic acid becomes fully oxidized, enzyme activity will recommence and browning will occur (Kim and Marshall, 2000).

While the mode of action of ascorbic and erythorbic acids are similar, ascorbic acid appears to be more efficient in most food systems. In particular, erythorbic acid is more susceptible to the copper-catalysed oxidation carried out by PPO and is therefore used up faster than an equivalent concentration of ascorbic acid

Ascorbyl phosphate esters have been utilized as more stable sources of ascorbic acid. They release ascorbic acid when hydrolysed by endogenous acid phosphatases. They are not as effective in acid systems possibly due to the low activity of the endogenous acid phosphatases. Therefore, the suitability of ascorbyl phosphate esters as browning inhibitors is dependent on the ability of the food system to absorb the compound, the pH of the system and the activity of the endogenous acid phosphatases (McEvily and Iyengar 1992).

Ascorbic acid and/or its derivatives are commonly used in conjunction with other anti-browning agents such as citric acid. This results in a synergistic effect allowing for the use of lower concentrations (Kim and Marshall, 2000).

- Sulfhydryl compounds

Sulfhydryl compounds react with *o*-quinones to produce stable colorless compounds or reduce the carbonyl groups and double bonds of the pigment so that a colorless compound results (Kim and Marshall, 2000). In addition, the amino acids containing thiol groups are more effective than others due to their high affinity for the Cu^{2+} at the enzyme's catalytic site (McEvily and Iyengar, 1992). Many Sulfhydryl containing compounds are not approved for food use by the regulatory authorities and others are too expensive to be practicable alternatives, for example glutathione. Practical alternatives are the sulfur containing amino acids such as cystine, methionone and cysteine. Of these, cysteine has been shown to be as effective as sodium bisulfite in preventing browning in some food systems (Kim and Marshall, 2000). Unfortunately the concentrations required also produce negative impacts on the taste and coloration of the treated foods (McEvily and Iyengar, 1992; Kim and Marshall, 2000). Benjakul *et al*. (2006) reported that cysteine and glutathione inhibited the oxidation of 3-(3,4-dihydroxylphenyl)-L-alanine (L-DOPA) catalyzed by kuruma prawn (*Penaeus japonicus*) PO. Those thiol compounds showed competitive inhibition toward PO. Additionally, both cysteine and glutathione prevented the color development by trapping the color intermediates or reducing *o*-quinone to colorless compounds.

1.2.3.7.2 Acidulates

Acidulants, such as citric, malic, or phosphoric acid, can inhibit PPO activity by reducing pH and/or chelating copper in a food product (Guerrero-Beltran *et al*., 2005). The pH of food affect ionization groups on the enzyme. These groups must be in the proper ionic form in order to maintain the conformation of active site, bind the substrates, or catalyze the reaction. However, most of these changes in ionization are reversible. Irreversible denaturation occurs usually at extreme pH value (Kim *et al*., 2000). The role of acidulants is to maintain the pH below what is needed for optimum catalytic activity of PPO. Acidulants such as citric, malic and phosphoric acids lower the pH of the system to below 3 where PPO is inactive (Richardson and Hyslop, 1985). Furthermore, citric acid act as chelating agent and acidulant (Laurila *et al*., 1998). The inhibitor reaction mechanism differs, depending on the reducing agent employed.

1.2.3.7.3 Chelating agents

Enzymes have metal irons at their active sites, so the removal of these ions by chelators would render the enzyme inactive. Chelating agents complex with prooxidative agents such as copper and iron ions through an unshared pair of electrons in their molecule structure which provides the complexing of chelating action (Kim *et al*., 2000). Since PPO is a metalloprotein with copper as the prosthetic group, it can inhibited by metal chelation agents such as citric acid, ethylenediamine tetraacetic acid (EDTA) (Whitaker, 1972).

- Kojic acid

Kojic acid is a metabolite of several species of *Aspergillus* and *Penicillium* and so is found as a natural constituent in many Japanese fermented foods. It is a natural antibacterial and anti-fungal agent, a reducing agent and an antioxidant. Kojic acid has been shown to prevent melanosis in shrimp when used as a 1% dipping solution and is thus comparative in activity to sulfiting agents. It is effective through the direct inhibition of the enzyme by chelation of the copper ions at the active site, and it is thought to reduce the pigment or its precursors to colorless compounds (Kim and Marshall, 2000). A blend of kojic acid and ascorbic acid has been patented as an anti-browning agent for foods in Japan (Chen *et al*., 1991). However, it has been shown that when kojic acid is added to tissues possessing peroxidase activity, and low levels of hydrogen peroxide, a yellow pigmentation results even though enzymatic browning is prevented (Kahn *et al*., 1995). When consideration is given to this, and kojic acid's potential as a mutagenic agent and concerns over toxicity, it is doubtful if kojic acid will be permitted as a food additive (Kim and Marshall, 2000).

- EDTA

EDTA is a chelating agent permitted for use in the food industry as a chemical preservative. Calcium disodium EDTA (21 CFR 172.120) and disodium EDTA (21 CFR 172.135) have been approved for us as food additives. In shrimp (cooked or canned) products, not exceed 250 ppm EDTA was available for by the United State Food and Drug Administration (Anon, 1992). Highly stable complexes are formed by the sequestering action of EDTA compounds on iron, copper and calcium. Maximum chelating efficiency occurs at the higher pH value where carboxyl groups

exist in a dissociated state (Dziezak, 1986). EDTA is generally used in combination with other chemical treatments for the prevention of enzymatic browning in foods.

1.2.3.7.3 Enzymatic inhibitors

- Substituted resorcinol

Extracts of figs were shown to have an inhibitory effect on PPO activity (Taoukis *et al*., 1990). Isolation of the inhibitory fractions showed that the inhibitors present were substituted resorcinols. These compounds are structurally related to the phenolic substrates of PPO in that they are *m*-diphenols (Kim and Marshall, 2000). Resorcinol can be substituted at the 1,3-,2-,4- and 5-positions. Those substituted at the 1,3- and 2- positions were the least effective as antibrowning agents (McEvily and Iyengar, 1992). 4- and 5-position substitutions showed the same high level of inhibition, however the 5-substituted resorcinols also posses toxic and irritant properties that make them unsuitable for use in the food industry (McEvily *et al*., 1991).

- Aromatic carboxylic acids

These act to inhibit PPO due to their structural similarities to the phenolic substrates (Kim and Marshall, 2000). Their effectiveness varies from system to system dependent on the substrate specificity of the PPO present and therefore the type of inhibition that occurs. Inhibition can be competitive, noncompetitive or mixed. Unfortunately these compounds may be converted to PPO substrates by other enzymes present in the system and therefore act to induce browning rather than inhibit it (Kim and Marshall, 2000).

- Anions

Inorganic halides act as inhibitors of PPO by reacting with the positively charged imidazole group at the active site of PPO. Their effectiveness is pH dependent and decreases as pH increases. Ideally the pH of the system should lie between 3.5 and 5. Other anions such as nitrite or sulphates are ineffective due to steric effects (McEvily and Iyengar, 1992).

1.2.4 Seafood flavorant

Seafood flavorant can be used as an additive in surimi based products such as crab analog and shrimp analog, and cereal based extrusion products. The flavorant in fish and shell fish can be classified according to nitrogen compounds including free amino acid, low molecular weight peptide, neucleotide, organic base and non-nitrogen compounds such as organic acid, sugar, inorganic constituents (Konosu and Yamaguchi, 1982). In addition, Manley *et al*. (2005) and Vandanjon *et al*. (2002) classified flavorant compounds in seafood extracts into 2 groups according to volatility as discuss below.

1.2.4.1 Volatile Compounds

The volatile aroma compounds can be classified as most of the top notes. These compounds consists of low molecular weight (MW) compounds (<400 g/mol), very volatile and belonging to various chemical classes such as aldehydes, ketones, alcohols, esters, N- and S-containing compounds, etc. These flavor compounds provide the pleasant aroma i.e. cucumber/green, almond/nutty, potato, etc. which characterize seafood products (Vandanjon *et al*., 2002).

1.2.4.1.1 N-containing compounds

Among this category, the most common chemical is trimethylamine, which results from a degradation of trimethylamine oxide (TMAO) by microbial enzymes. This degradation happens in freshly harvested fish; and when the fish is frozen, degradation of TMAO leads to the formation of formaldehyde and dimethylamine, these components contributing to the flavor or "off-flavor" of fish (Reineccius, 1994).

1.2.4.1.2 Alcohols, aldehydes, and ketones

These components can be resulted either from the oxidative degradation of polyunsaturated fatty acids, as is the case of tetradecatrien-2-one, which yields a cooked shrimp-like note, or from the enzymatic degradation (mainly catalyzed by a lipoxygenase) of the same polyunsaturated fatty acids to produce aldehydes, ketones, and alcohols. The precursors for these reactions are arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid (Gordon and Josephson, 2000).

1.2.4.1.3 Bromophenols

These compounds (Figure 3) are unique to food flavors, but they have been identified in a number of marine species, and are considered to be responsible for the iodine-like flavor of shrimp and fish (Boyle *et al.*, 1993).

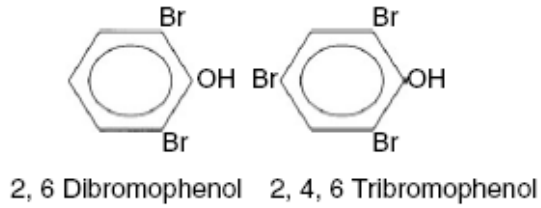


Figure 3 Bromophenols found in seafood

Source: Manley *et al*. (2005)

1.2.4.1.4 Sulfur-containing compounds

The typical of sulfur-containing compound in seafood is dimethyl sulfide, resulting from the degradation of sulfury amino acids, such as cysteine and methionine, by endogenous enzymes, through a Strecker degradation (Iida, 1998). Table 2 summarizes the sulfur compounds found in seafood and their characterized flavors (Manley *et al*., 2005)

1.2.4.2 Nonvolatile Compounds

Nonvolatile compounds can be classified as savory or seasonings as follows (Manley *et al*., 2005). The compounds consist of water soluble flavor compounds including low MW free amino acids, peptides, nucleotides, quaternary ammonium bases, organic acids (lactic acid), sugar and inorganic salts (Vandanjon *et al*., 2002).

1.2.4.2.1 Amino acids

These components are believed to either contribute to the umami taste, like glycine and glutamic acid, or giving body to the product, like arginine and alanine. They occur naturally in their free form in crab meat.

1.2.4.2.2 Organic acids

The two acids related to seafood tastes are lactic acid which is found in the muscle of the fish, and succinic acid which occurs in mollusks.

Table 2 Some important sulfur-containing compounds occurring in seafood

Compound	**Occurrence**
Dimethyl sulfide	Shrimp
Carbon disulfide	Cooked shrimp
Dimethyl disulfide	Cooked crayfish, oyster, cooked shrimp, spray-dried shrimp powder
Dimethyl trisulfide	Cooked crayfish, oyster, cooked shrimp
Methyl propyl trisulfide	Cooked shrimp
Dimethyl sulfoxide	Oyster, roasted shrimp
Methanethiol	Cooked shrimp
Methyl trithiomethane	Roasted clam
2-Methylthioethanol	Roasted shrimp
3-Methylthiopropanol	Roasted shrimp
Methional	Raw, fermented, and cooked shrimp
2-(Methylthio)-methyl-2-butenol	Spray-dried shrimp powder
3-Methylthiopropanol	Fermented and cooked shrimp
3-Methylthiobutanal	Cooked shrimp

Source: Manley *et al*. (2005)

1.2.4.2.3 Ribonucleotides

As previously mentioned, the 5'-ribonucleotides have a synergistic effect with glutamic acid to enhance the umami impact. The nucleotide 5'-inosinate monophosphate (IMP) occurs widely in fish and meats, whereas 5'-guanylate monophosphate (GMP) is found in mushrooms, but not in fish or shellfish.

1.2.4.2.4 Peptides

These compounds are chains of five or fewer amino acids, such as creatine, anserine (naturally occurring in salmon), cadaverine, and creatinine. They also have a certain type of umami-enhancing effect in surimi food.

1.2.4.2.5 Sugars

These compounds contribute sweet taste of the seafood products. Glucose and ribose are commonly found in cooking juice of sea products (Vandanjon *et al*., 2002).

1.2.4.2.6 Inorganic salt

These components include Na^+, K^+ and Cl^- (Vandanjon *et al*., 2002). Raksakulthai and Haard (1992) reported on the correlation between the concentration of peptides and amino acids and the flavor of fish sauce. They concluded that both free Glu and peptides containing Glu were important to the flavor of fish sauce. Konosu and Yamaguchi (1982) reported that the flavor of fish and shellfish were from water soluble, low molecular weight components, especially free amino acids. Glycine (Gly), proline (Pro), arginine (Arg), taurine (Tau) and alanine (Ala) were major amino acids in shrimp flavor.

Erickson *et al*. (2007) studied the sensory differentiation of raw and cooked shrimp attributes for both fresh and frozen commercially available shrimps including Gulf brown shrimp (*Penaeus aztecus*), Gulf white shrimp (*P. setiferus*), Gulf pink shrimp (*P. duorarum*), Georgia brown shrimp (*P. aztecus*), Georgia white shrimp (*P. setiferus*), Burma black tiger shrimp (*P. monodon*), Belise white shrimp (*P. vannamei* or *P. stylirostris*), Columbia white shrimp (*P. vannamei* or *P. stylirostris*), Honduras white shrimp (*P. vannamei* or *P. stylirostris*), and Mexico white shrimp (*P. vannamei* or *P. stylirostris*) using a trained descriptive analysis panels. The descriptors and standard references of both raw and cooked shrimp are shown in Tables 3 and 4, respectively. References for these descriptors were also defined and intensity ratings for these references were established by consensus of the panels. The results showed that significant differences in sensory attributes existed among the types of frozen shrimp evaluated. The flavor attributes were not unique to one type of frozen shrimp, hence no single attribute could be used for identifying of shrimp. The frozen shrimp had greater intensities of cooked shrimp flavor and aroma, but lower intensity of sweetness and juiciness when compared to fresh shrimp.

Table 3 Standard references and ratings used in descriptive analysis of 'raw shrimp' samples

Category	Attribute	Definition	Reference/source
Raw aroma	Ocean/seawater	Aromatic associated with the ocean or seawater, from slight to strong	Clam juice (Doxsee All Natural, Snow's Food Co., Portland, ME)
	Shrimp	Aromatic associated with raw shrimp, from slight to strong	Raw fresh shrimp was used as the qualitative descriptor while taste solutions (see Table 4) were used as a reference for intensity of this attribute
	Old shrimp	Aromatic associated with old fish, from slight to strong	Shrimp powder

Source: Modified from Erickson *et al*. (2007)

Table 4 Standard references and definition of attributes used in descriptive analysis of ‘cooked shrimp’ samples

Category	Attribute	Definition	Reference/source
Raw aroma	Ocean/seawater	Aromatic associated with the ocean or seawater, from slight to strong	Clam juice
	Cooked shrimp	Aromatic associated with fresh cooked shrimp, from slight to strong	Fresh cooked shrimp was used as the qualitative descriptor while taste solutions were used as a reference for intensity of this attribute
	Old shrimp	Aromatic associated with old fish, from slight to strong	Shrimp powder
Flavor	Cooked shrimp	The flavor associated with cooked shrimp, from slight to strong	Fresh cooked shrimp was used as the qualitative descriptor while taste solutions were used as a reference for intensity of this attribute

Table 4 (Continued)

Category	Attribute	Definition	Reference/source
Tastes	Bitter	The intensity of the taste associated with caffeine solutions	0.05/100 g caffeine 0.08/100 g caffeine 0.15/100 g caffeine
	Salty	The intensity of the taste associated with salt solutions	0.2/100 g NaCl 0.35/100 g NaCl 0.5/100 g NaCl
	Sour	The intensity of the taste associated with citric acid solutions	0.05% citric acid 0.08% citric acid 0.15% citric acid
	Sweet	The intensity of the taste associated with sugar solutions	2 g/100 g sugar 5 g/100 g sugar 10 g/100 g sugar 16 g/100 g sugar

Source: Modified from Erickson *et al*. (2007)

1.2.5 Enzymatic extraction of flavorant from shrimp waste

In the previous studies, many methods were used to extract flavorant from shrimp waste including enzymatic and non-enzymatic methods. Water, acid solution and brine were used as extraction solution with the various extraction temperatures and times for non-enzymatic methods (Teerasuntonwat and Raksakulthai, 1995; Holanda and Netto, 2006). For enzymatic methods, many enzymes were used such as bromelain, papain, neutrase, pancreatin, Alcalase and Flavourzyme (Teerasuntonwat and Raksakulthai, 1995; Holanda and Netto, 2006; Benjakul *et al*., 2009).

The taste and volatile components of shrimp heads were recovered by enzymatic digestion, followed by spray drying (Chakrabarti, 2002). Some previous studies on the protein extraction from shrimp waste indicated that enzymatic extraction was an effective in improving the recovery of protein (Cano-Lopez *et al*., 1987; Ya *et al*., 1991; Holanda and Netto, 2006; Klomklao *et al*., 2009). Generally, the enzyme absorbs rapidly onto the insoluble protein particles, and the polypeptide chains that are loosely bound to the surface are cleaved. The more compacted core proteins are hydrolyzed more slowly. The rate of enzymatic cleavage of peptide bond controls the overall rate of hydrolysis (Benjakul and Morrissey, 1997). However, available substrate for hydrolysis decreases as time of reaction increases (Benjakul and Morrissey, 1997).

Holanda and Netto (2006) compared the enzymatic and alkaline methods used for protein recovery/deproteinization from shrimp (*Xiphopenaeus kroyeri*) processing waste. For alkaline process, NaOH and KOH were used at concentrations of 1%, 3%, and 5% (w/v) with raw waste to alkaline proportion of 1:10 and heated at 3 different temperatures (50, 70, and 90^{o}C), with stirring for 1, 2 or 3 hr. For enzymatic hydrolysis, the hydrolysis reaction conditions were at pH 8.5, enzyme/substrate ratio (E/S) of 3%, at 60^{o}C for Alcalase and pH 8.5, E/S ratio of 1%, at 40^{o}C for pancreatin. The shrimp waste was hydrolyzed until DH of protein hydrolysate reached 6% and 12% measured by pH-state. The result indicates that alkaline hydrolysis was more efficient, giving a maximum yield of 88.39% as against 59.50% for enzymatic hydrolysis. However, it might be pointed out that alkaline hydrolysis was not the best method for protein recovery since some amino acids were lost, resulting in the decreased functionality and nutritional quality. Protein hydrolysis at extreme temperatures and pH generally yields products with reduced nutritional quality, poor functionality, and restricted use as flavor

enhancers (Loffler, 1986). In addition, several deleterious reactions occur in alkaline solutions during hydrolysis. These are initiated by hydrogen abstraction and include racemization of the *L*-amino acids, producing *D*-amino acids, which are not absorbed by humans. Moreover, disulfide bonds are split with a loss of cysteine, serine, and threonine via β-elimination reactions (Krause and Freimuth, 1985).

There are some factors affecting on yields and properties of enzyme extracted protein from shrimp waste as discussed below.

1.2.5.1 Enzyme type

The effect of different enzymes on yield and properties of protein extracted from shrimp waste have been reported from previous studies (Teerasuntonwat and Raksakulthai, 1995; Holanda and Netto, 2006; Klomklao *et al*., 2009). Holanda and Netto (2006) extracted protein from *Xiphopenaeus kroyeri* processing waste, containing cephalothorax, shell, and tail with DH of 6 and 12% using 3% Alcalase at 60^{o}C and 1% pancreatin at 40^{o}C, pH 8.5. The result showed that protein recovered (PR) with Alcalase was higher than obtained with pancreatin for both DH. Many authors compared proteolytic enzymes such as Alcalase, Neutrase and papain to recover protein form shrimp waste. Alcalase resulted in higher protein recovery, in addition to providing hydrolysates with good functional properties and a mild bitter taste (Quaglia and Orban, 1987; Rebeca *et al*., 1991; Baek and Cadwallader, 1995; Shahidi *et al*., 1995; Mizani *et al*., 2005).

Moreover, the hydrolysis curves for *X. kroyeri* waste using Alcalase and pancreatin showed that at the same hydrolysis time, DH of protein hydrolyzed by Alcalase was higher than pancreatin. This might be due to the different specificities of Alcalase and pancreatin leading to different yields of soluble product. There are relationship between protein recovery and DH (Baek and Cadwallader, 1995; Synowiecki and Al-Khateeb, 2000). However, Holanda and Netto (2002) showed that the protein recovery did not improve significantly at DH values higher than 12%.

Some studies have been reported on affecting of source of enzyme on protein recovery. Sila *et al*. (2012) showed that the recovery yield of protein attained from pink shrimp (*Parapenaeus longirostris*) waste with barbel (*Barbus callensis*) trypsin were higher ($p<0.05$) than those obtained with bovine trypsin. The two trypsins were noted to show more efficiency in the extraction of carotenoprotein compared to

the control (without addition of enzyme). The results are well agreement with Cano-Lopez *et al*. (1987), who showed that Atlantic cod trypsin was more efficient in the extraction of carotenoprotein from shrimp waste than bovine trypsin.

In addition, Klomklao *et al*. (2009) studied the comparison of bovine and bluefish trypsin as extraction aids for carotenoprotein recovery from black tiger (*Penaeus monodon*) shrimp shell by hydrolyzed minced shrimp shell with 1.2 units/g sample of bluefish (*Pomatomus saltatrix*) trypsin or bovine trypsin. The results showed that at the same level of enzyme added (1.2 units/g sample), trypsins from both sources were effective in aiding the extraction of carotenoprotein, compared to the control (without trypsin addition) ($p<0.05$). Bluefish trypsin showed similar recovery efficacy of protein or carotenoids, compared with bovine trypsin ($p\geq0.05$). In this regard, bluefish trypsin differed from Atlantic cod trypsin, which was shown to be a more effective extraction aid than bovine trypsin for recovering carotenoprotein from shrimp process waste at 4^{o}C (Cano-Lopez *et al*., 1987). Chakrabarti (2002) reported that trypsin showed higher recovery of carotenoprotein from brown shrimp-shell waste than did pepsin and papain. From the results, bluefish trypsin has a potential for use in facilitating the hydrolysis and recovery of carotenoproteins from shrimp shells.

Benjakul *et al*. (2009) used Flavourzyme as a processing aid for the production of Mungoong (a shrimp extract paste) from the cephalothorax of white shrimp (*Litopenaeus vanamei*). Flavourzyme containing both endopeptidase and exopeptidase has been used to produce protein hydrolysates with less bitterness (Suh *et al*., 2003). Nilsang *et al*. (2005) found that fish protein hydrolysates produced using Flavourzyme had a bitterness less than that of a 1 ppm caffeine solution. Therefore, Flavourzyme may have an advantage compared to other proteases for the production of Mungoong, which is consumed directly or used as the condiment. With more hydrolysis, smaller peptides could be produced and might exhibit more biological activities.

1.2.5.2 Enzyme concentration

Klomklao *et al*. (2009) showed that the recovered carotenoprotein content from black tiger shrimp (*Penaeus monodon*) shells increased ($p<0.05$) with the increasing in bluefish trypsin concentration up to 1.2 units/g sample at 25^{o}C for 1 hr. However, no significant increases in protein content was found with treatment of

bluefish trypsin at concentrations above 1.2 unit/g sample (1.8 to 3.6 units/g sample) ($p \geq 0.05$). This was due to the limited amount of protein substrate for hydrolysis reaction by proteinase. Similar to Sila *et al.* (2012) who observed that the recovery of carotenoproteins from deep-water pink shrimp (*Parapenaeus longirostris*) processing waste using barbel (*Barbus callensis*) trypsin and bovine trypsin at 25◦C for 1 hr was maximized by the hydrolysis using 1.0 trypsin unit/g of shrimp waste, beyond which the protein tended to stabilize. Additionally, the recovery yields of protein attained with barbel trypsin were higher than those obtained with bovine trypsin ($p<0.05$). The two trypsins were noted to show more efficiency in the extraction of carotenoprotein compared to the control (without addition of enzyme). However, Benjakul *et al*. (2009) showed that the Flavourzyme concentrations (0.15 % and 0.30 %) at 50^{o}C for 90 min had no effect on yield of Mungoong extracted form cephalothorax of white shrimp (*Litopenaeus vanamei*).

1.2.5.3 Raw material

1.2.5.3.1 Source of raw material

Liang *et al*. (2008) compared the flavor components in shrimp (*Litopenaeus vannamei*) cultured in sea water and low salinity water. The extracts were produced by incubation homogenized shrimp muscle with distilled water at 90^{o}C for 1 hr, then centrifugation to collect supernatant, precipitation the protein by ethanol, and vaporization the ethanol to collect the extract. The result showed that the shrimp muscle extracted from samples cultured in sea water was significantly lower moisture but higher protein content than samples cultured in low salinity water ($p<0.05$). However, crude lipid and ash values did not differ significantly between the two types of samples ($p \geq 0.05$). The flesh pH values (7.57) in sea water were lower than that in low salinity water specimens (7.01) ($p<0.05$).

In addition, free amino acid composition as shown in Table 5 revealed that glycine, glutamate contents and total free amino acid concentration were higher in samples cultured in sea water than those cultured in low salinity water ($p<0.05$). Nevertheless, the total combined amino acid concentration in samples from low salinity water was higher than that in samples cultured in sea water ($p<0.05$). Crustacean muscle contains high concentrations of free amino acids, particularly glycine, proline, arginine, glutamate and alanine (Simpson *et al*., 1959; Schoffeniels

and Gilles, 1970; Cobb *et al*., 1979; D'Aniello, 1980). In the study, higher concentrations of these amino acids were found in the muscle extracts of samples in sea water versus those cultured in low salinity water. Free amino acids have been shown to function as osmoregulators in crustaceans and they also are a major contributor to the flavor of seafoods. Therefore, changes in environmental salinity might possibly be used to produce freshwater shrimp with different flavor characteristics. In addition, the amino acid composition and concentration in the muscle of shrimp may affect muscle quality (Liang *et al*., 2008).

Table 5 Free amino acid composition and amino acid composition of peptides (including oligopeptides) in muscle extracts from *Litopenaeus vannamei* cultured in sea water and low salinity water (mg/100 g)

Amino acids	Free amino acid composition		amino acid composition of peptides	
	sea water	salinity water	sea water	salinity water
Aspartic acid	8.45 ± 1.05^{a}	9.91 ± 2.12^{a}	42.31 ± 1.32^{b}	66.80 ± 0.28^{a}
Threonine	14.84 ± 0.80^{b}	19.52 ± 1.05^{a}	50.11 ± 2.09^{b}	58.40 ± 1.86^{a}
Serine	21.64 ± 1.52^{a}	19.57 ± 2.36^{a}	62.54 ± 1.77^{b}	72.85 ± 3.65^{a}
Glutamate	34.46 ± 1.72^{a}	28.17 ± 1.80b	390.83 ± 2.78^{a}	356.00 ± 3.84^{b}
Glycine	333.67 ± 2.50^{a}	300.98 ± 1.52^{b}	939.09 ± 11.25^{a}	1002.58 ± 22.87^{a}
Alanine	97.82 ± 2.33^{b}	177.39 ± 3.06^{a}	313.05 ± 3.35^{b}	327.27 ± 2.91^{a}
Valine	32.01 ± 0.76^{b}	41.86 ± 0.65^{a}	252.97 ± 1.42^{a}	247.29 ± 1.54^{b}
Methionine	15.12 ± 3.07^{a}	11.77 ± 2.12^{b}	96.24 ± 4.12^{b}	88.37 ± 0.58^{a}
Isoleucine	16.43 ± 1.16^{b}	25.70 ± 2.01^{a}	88.81 ± 1.55^{a}	87.83 ± 1.32^{a}
Leucine	27.37 ± 0.74^{b}	45.43 ± 1.19^{a}	144.66 ± 2.55^{b}	152.86 ± 1.89^{a}
Tyrosine	16.80 ± 0.78^{b}	24.94 ± 1.03^{a}	84.56 ± 0.59^{a}	77.62 ± 0.78^{b}
Phenylalanine	20.47 ± 2.11^{a}	23.94 ± 1.12^{a}	70.65 ± 1.23^{a}	64.91 ± 1.08^{b}
Lysine	23.24 ± 1.87^{b}	32.27 ± 2.18^{a}	113.10 ± 3.42^{b}	137.95 ± 4.56^{a}
Histidine	16.45 ± 0.55^{a}	11.22 ± 0.87^{b}	39.14 ± 0.65^{a}	35.29 ± 0.53^{b}
Arginine	244.45 ± 8.23^{a}	136.58 ± 2.27^{b}	448.22 ± 8.08^{b}	514.46 ± 10.05^{a}
Proline	74.57 ± 1.15^{a}	69.35 ± 0.95^{b}	609.81 ± 3.54^{a}	519.02 ± 6.83^{b}
Taurine	22.41 ± 1.37^{b}	25.32 ± 2.06^{a}	-	-
Total amino acids	1020.20 ± 10.85^{a}	1003.92 ± 11.59^{b}	3746.13 ± 17.67^{b}	3809.48 ± 22.13^{a}

Values are mean ± SD of three analyses.

Means within a row having different superscripts are significantly different ($p<0.05$).

Source: modified from Liang *et al*. (2008)

1.2.5.3.2 State of raw material

Benjakul *et al*. (2009) studied the effect of pretreatment process of raw material on yield, chemical composition and properties of Mungoong extracted form cephalothorax of white shrimp (*Litopenaeus vanamei*) using Flavourzyme (0.15 % and 0.30 %) at 50^{o}C for 90 min. The yield of Mungoong from raw cepharothorax was higher than that produced from its cooked counterpart ($p<0.05$). It was suggested that the state of the substrate played an essential role in protein hydrolysis caused by Flavourzyme. During the cooking process, the denaturation of protein together with the removal of water from protein molecules most likely caused the aggregation of proteins. The larger aggregates formed were less susceptible to hydrolysis by Flavourzyme (Thiansilakul *et al*., 2007). As a consequence, fewer proteins were hydrolyzed by Flavourzyme, when cooked cephalothorax was used as a substrate. In addition, the ability of an enzyme to disperse in the aggregated protein could be lowered, leading to the decreased yield (Šlizyte *et al*., 2005).

1.2.6 Drying of flavorant from shrimp waste

Several drying methods for seafood flavorant have been reported. Teerasuntonwat and Raksakulthai (1995) compared dextrose, dextrin and sodium chloride at 10% (w/w) as binders for drying of shrimp flavor extract using hot air oven at 50 ± 5°C for 72 hr. After drying, the samples were ground and tested. The sample with dextrose was sticky brown powder color. It had sweet taste with shrimp-like and caramel odors. The sample using dextrin as a binder was light brown with very mild shrimp odor but strong potato starch odor. Although, it did not stick to the drying trays and required a shorter drying time, it was not suitable as a flavor binder. Samples using NaCl as binder required longer drying times and stuck slightly to the drying trays but had the strongest shrimp flavor. In general, shrimp flavor powder is applied in soups or snacks, therefore NaCl is the most appropriate binder for shrimp flavor powder.

Bueno-Solano *et al*. (2009) hydrolyzed protein in shrimp (*Penaeus* spp.) by-product (cephalothorax and exoskeleton) using lactic acid fermentation. In producing of the dry powder, the liquid hydrolysate, rich in protein, was dehydrated using a spray dryer. The liquid hydrolysate was heated to a constant 80^{o}C. The temperature of the air inlet was 180^{o}C and the air outlet was 140^{o}C. The speed of the

peristaltic pump was minimized to produce a slow flow of fluid input (1 l/hr); the flow of air in the chamber was 100%. Dry powder has 5.78% moisture content and higher content of minerals (8.25% wet weight), when compared to the liquid hydrolysate (2.03% wet weight). The dry powder protein hydrolysate has a brown color, showed low values for brightness (L*= 44.42), as in b* (17.86), however; b* values are greater than a* (11.93). The brown color of dry powder is possibly due to the components that were generated in the Maillard reaction during the drying period. They also concluded that, spray drying is a novel technique that can concentrate the protein content. Also, the powder has a lower moisture content which extends the storage life, reduces the microbial flora and also facilitates transport.

Kanpairo *et al*. (2012) studied the drying method for tuna precooking juice. The concentrated tuna precooking juice with 15% total soluble solid content (TSS) was heated to 70^{o}C and maltodextrin (DE = 9) was added to increase the TSS to 20, 22, 24 and 26%. The tuna precooking concentrate was dried by spray dryer, with an inlet air temperature at 180°C and outlet air temperature of 75°C. Based on compositions and properties, tuna flavor powders with 22% TSS was the most appropriable. Kurozawa *et al*. (2009) also studied chicken meat protein hydrolysate powder using spray dryer. Before the spray drying process, the carrier materials (maltodextrin (MD) or gum Arabic (GA)) were added directly (10, 20 and 30 g carrier agent/100 g feed solution, which correspond to 17.3, 26.0 and 34.6 g total solids/100 g feed solution, respectively). The laboratory spray dryer equipped with a spray nozzle two-fluid atomizer with an orifice of 0.7 mm in diameter was used. The inlet air temperature was 180^{o}C and the outlet air temperature varied from 91 to 102^{o}C. The feed flow rate and compressed air flow rate were 0.2 kg/h and 0.6 m^3/hr, respectively. The results indicated that an increasing carrier agent concentration decreased the powder moisture content and bulk density. Mean diameter particle increased with increasing maltodextrin or gum Arabic concentration, which is related to the feed viscosity. The addition of maltodextrin or gum Arabic in the feed solution also contributed significantly to powder stability since powder hygroscopicity decreased and glass transition temperature increased with increasing carrier agent concentration.

1.2.7 Utilization of shrimp flavorant in foods

The volatile aroma components are susceptible to losses during thermal treatments and, depending on the degree of volatility, can be severely affected during surimi seafood manufacturing. Flavorings can be coated with a protective material to allow them to withstand degradation by heat and oxidation. Several techniques create encapsulated flavor materials. Spray-drying is widely used by adding encapsulating materials, such as gums and other hydrocolloids, dextrins, waxes, oils, and other food additives, to the slurry prior to the drying operation. The resulting product is a powder with good shelf life and handling properties while retaining the original character of the flavor through many types of processing conditions. Moreover, the utilization of those carrier agent can stabilize and present a flavorant in a stable form allow the flavor to be released when eaten (Manley *et* al, 2005).

Flavor interactions with food constitute a very complex study. The oxidation, thermal effects, and reactions with proteins and other food materials can affect flavor and then the problems that develop when a flavor is put into a food matrix can be understood. Adding a flavoring ingredient into a complex matrix such as surimi can also lead to a significantly different aromatic perception due to the texture, processing, and composition of the matrix. This partly results from the interactions of the flavor with the chemical reactions in the matrix and the physical nature of the texture on the release of the flavor when the food is eaten (Manley *et al*., 2005).

Teerasuntonwat and Raksakulthai (1995) produced flavoring agent from shrimp (*Peneaus monodon* Fabricius) head by extraction of nitrogenous compounds with water, sodium chloride, hydrochloric acid, sodium hydroxide, bromelain, papain and neutrase. The extracted samples were dried using 10% (w/v) sodium chloride as a binder and dried in hot air oven at 50±5 ^{o}C for 72 hr. All samples were applied in shrimp flavored cracker and compared with the sample containing commercial flavoring agent (krill extract) without spices. The crackers were fried and evaluated in terms of color, flavor, odor and overall acceptability using a 9-point hedonic scale by 16 panelists. The results showed that shrimp flavored crackers containing commercial flavorant had the highest score. In addition, cracker with shrimp flavor extracted by bromelain had higher score than the control (crackers without flavor extract) but was not significantly different to samples with flavor extract prepared by papain, water, NaCl, neutrase or

HCl. However, Gildberg (1993) reported that hydrolysates prepared with bromelain had better organoleptic quality than those prepared with other proteinases.

1.2.8 Color of shrimp

In crustaceans the basic nature of body coloration relies on specific pigments present in the subepidermal chromatophores and/or in the principal layer of the animals exoskeleton. Among the different types of pigments found in crustaceans such as hemoglobins, hemocyanins, flavins, cytochromes, carotenoid, melanins, ommochromes, pterins, and rhodopsin, the carotenoids are by far the most significant ones in determining their pigmentation (Goodwin, 1954; Schiedt, 1987; Latscha, 1989).

Carotenoids are exogenously derived isoprenoid compounds representing not only one of the most wide-spread groups of natural pigments, but probably also have the most varied structures and functions (Latscha, 1989). Regarding the structures, formally, all carotenoids can be derived from the acyclic $C_{40}H_{56}$ polyenelycopene by reactions involving e.g. hydrogenation, dehydrogenation, cyclization, chain elongation and oxygen insertion. Their wide range of colors from almost colorless to yellow and dark red is due to the varying chromophore in different groups of carotenoids and consisting in different groups of a varying conjugated polyene system in the molecule (Latscha, 1989).

In crustaceans including the commercially most important penaeids, the most prevalent carotenoid found in the integuments is astaxanthin, 3,3'-dihydroxy-i,i-carotene-4,4'-dione representing about 65-98 % of the total carotenoids. In addition, three different forms of this particular pigment namely diesters, monoesters and the free form are recognized in crustaceans as reported in Table 6. The esters generally representing the bulk of astaxanthin in the integumental tissues. Pigmentations in crustaceans or shrimps respectively is further complicated by the wide occurrence of this pigment in carotenoid-protein complexes commonly termed carotenoproteins or chromoproteins which due to usually marked bathochromic shift in the light absorption maximum caused by characteristics of the pigment-protein bonding exhibit colors (e.g. green to purple) which largely differ from the color of the pigment itself (Chersman *et al*., 1967). It is obvious from these, that pigmentation in general, but particularly that of crustaceans is rather complex and largely influenced by a multiplicity of pigment-,

feed-, animal-, and disease related as well as environmental factors and giving rise to significant variations e.g. between wild-caught and farmed animals.

Table 6 Carotenoid content and composition in various wild species of *Penaeidae*

Carotenoids	*P. vannamei*	*P. monodon*	*P. japonicus*	*Metapenaeus monoceros*
Content µg/g tissue	56	52	38	44
Composition	%	%	%	%
β, β-Carotene	0.5	0.2	0.0	3.0
Yellow xanthophylls*	30.0	0.4	19.0	23.0
7,8-Didehydroastaxanthin	5.0	1.0	2.0	2.0
Astaxanthin total	65.0	98.0	75.0	72.0
Diesters	50.8	27.5	46.8	30.5
Monoesters	44.6	58.2	36.7	52.8
Free form	4.6	14.3	16.5	16.7

* Ester of fatty acids, no lutein, zeaxanthin, presumably three hydroxy groups

Source: Latscha (1989)

Astaxanthin, the main carotenoid found in crustacean and salmonids provides the desirable reddish-orange color in these organisms (Gentles and Haard, 1991; Higuera-Ciapara *et al*., 2006; Shahidi and Synowiecki, 1991). Astaxanthin possesses two identical asymmetric atoms at C-3 and C-3' making possible three optical isomers with all-trans configuration of the chain: 3S,3'S ; 3R,3'S ; 3R,3'R (Figure 4). The distribution of the isomers in natural astaxanthin differs from that of the synthetic product. The latter is a racemic mixture, with a typical ratio of 1:2:1 (3S,3'S : 3R,3'S : 3R,3'R), while astaxanthin from natural sources has a variable distribution of the isomers deriving from the different biological organism that synthesized it (Moretti *et al*., 2006). The composition of optical isomer of astaxanthin in various Penaeidae are shown in Table 7.

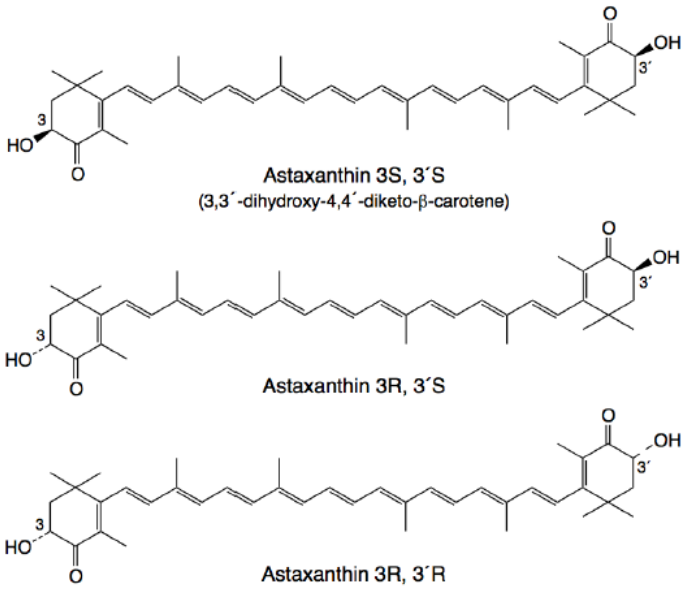


Figure 4 Sterioisomers of astaxanthin

Source: Fassett and Coombes (2012)

Table 7 Composition of optical isomers of astaxanthin in various wild species of *Penaeidae.*

Optical isomers	*P. vannamei*	*P. monodon*	*P. japonicus*	*Metapenaeus monoceros*
(3R, 3'R)	23*	19*	15*	20*
(3R,3'S; meso)	44	45	40	42
(3S, 3'S)	32	36	45	38

* % of total astaxanthin.

Source: Latscha (1989)

In addition to its pigmentation function, one of the most important properties of astaxanthin is its antioxidant activity. It has been reported that the antioxidant activity of astaxanthin is ten times higher than other carotenoids such as zeaxanthin, lutein, canthaxantin, and β-carotene (Miki, 1991). Numerous studies have identified astaxanthin antioxidant mechanisms that quench active oxygen species and free radicals in *vitro* and in *vivo* (Edge *et al.*, 1997; Palozza and Krinsky, 1992; Rengel *et al.*, 2000).

Because of its antioxidant properties, astaxanthin may have a role in the treatment of chronic diseases such as cardiovascular diseases, cataract development, macular degeneration, and some types of cancer (Mayne, 1996).

1.2.9 Shrimp colorant extraction

Colorants can even function as flavor enhancers, as the intensity of taste perceptions can be enhanced by a color which fits into our sensory experience (Koza *et al*., 2005; Luisa *et al*., 2006). From the previous studies, various methods were used to recover carotenoid and astaxanthin from shrimp by- product such as edible oil (Sachindra and Mahendrakar, 2005) and organic solvent process (Sachindra *et al*., 2006).

1.2.9.1 Organic Solvent

Several studies have been conducted to extract astaxanthin from crustacean by-products using organic solvents. Sachindra *et al*. (2006) assessed the extractability of shrimp waste carotenoids in polar and non-polar organic solvents as well as solvent mixtures. The polar solvents used were acetone, methanol, ethyl methyl ketone, isopropyl alcohol (IPA), ethyl acetate and ethanol; while the non-polar solvents were petroleum ether and hexane. The solvent mixtures were prepared by mixing equal quantities of a polar and non-polar solvent. The result showed that A 50:50 mixture of isopropanol and hexane gave the highest carotenoid extraction yield (43.9 µg/g waste) compared to acetone, methanol, ethanol, isopropanol, ethyl acetate, ethyl methyl ketone, petroleum ether, and hexane individually and to a mixture of acetone and hexane ($p<0.05$). The optimized extraction conditions for maximum yield of carotenoids were 60% hexane in solvent mixture, solvent mixture to waste ratio of 5:1 in each extraction of three times extractions. Similar to Takeungwongtrakul *et al*. (2013) who investigated the impacts of extraction conditions on lipid and carotenoid yields from hepatopancreas of Pacific white shrimp (*Litopenaeus vannamei*). Among single solvents (acetone, isopropanol and hexane) and their mixtures, a mixture of hexane and isopropanol (50:50, v/v) rendered lipids with the highest carotenoid yield (336.40 mg/kg hepatopancreas) with the extraction yield of 18.22% (w/w hepatopancreas) ($p<0.05$). The use of hepatopancreas to solvent ratio of 1.0:4.5 (w/v) and three repetitions showed the highest carotenoid yield (363.94 mg/kg

hepatopancreas) with the extraction yield of 18.08% (w/w hepatopancreas). Moreover, Suhnel *et al*. (2009) studied the effect of composition in organic solvents on the carotenoid extracting solutions of scallop (*Nodipecten nodosus*), two organic solvents were tested: acetone and hexane (Ac = O:Hex) at four ratios, 1:1, 1:3, 1:5, and 2:3, in four static extraction times: 0, 5, 10, and 15 minutes. The results indicated that the best single extraction (0.312 ± 0.016 μg carotenoids/mg) was attained with Ac = O: Hex 1:3, for 15 minutes. Through exhaustive extraction methodology (10x), a superior yield (0.41 ± 0.001 μg carotenoids/mg) was obtained from a gonad sample in comparison to the highest value found for a single extraction. Astaxanthin content was reduced by 8.6% in carotenoid extract preservation assay, i.e., –18°C, 26 days incubation, under N_2 atmosphere.

1.2.9.2 Edible Oils

In addition to organic solvents, edible oils are widely used as the media to extract astaxanthin from crustacean by-products due to lipid solubility of astaxanthin. Sachindra and Mahendrakar (2005) used a number of different vegetable oils such as sunflower oil, groundnut oil, gingelly oil, mustard oil, coconut oil, and rice bran oil to extract carotenoids from shrimp by-products and compared the carotenoids yield. The results showed that using of sunflower oil with oil to shrimp waste ratio of 2:1 and heating the mixture at 70^{o}C for 150 min obtained the highest yield ($p<0.05$). Handayani *et al*. (2008) found that both of mass transfer and reaction kinetic control the extraction of astaxanthin from giant tiger (*Panaeus monodon*) shrimp waste using palm oil. The thermodynamic parameters of extraction were also obtained in their study. It was found that both of mass transfer and reaction kinetic control the extraction of astaxanthin from shrimp waste using palm oil.

1.2.10 Stability of shrimp carotenoid and oil

Astaxanthin, a liposoluble carotenoid from xanthophylls family, is the most abundant pigment in aquatic animals such as salmon, trout, shrimp and lobster. To extract lipid and carotenoid, several solvents have been widely used (Shahidi and Brown 1998; Higuera-Ciapara *et al*., 2006). Takeungwongtrakul *et al*. (2013) reported that astaxanthin, astaxanthin diester and canthaxanthin were the major carotenoids of

carotenoid-containing lipids from hepatopancreas of Pacific white shrimp (*Litopenaeus vannamei*). These components constituted as the coloring pigment in the lipids. Another study of Takeungwongtrakul *et al*. (2012) showed that phospholipid was found as the dominant components of lipids extracted from cephalothorax of Pacific white shrimp (*Litopenaeus vannamei*) accounting 82.51% of total lipid. The lipid contained 29.51% saturated fatty acid, 25.91% monounsaturated fatty acid and 39.30% polyunsaturated fatty acid of which linoleic acid (C18:2(n-6)) was the most abundant fatty acid, followed by palmitic acid (C16:0), oleic acid (C18:1(n-9)) representing 19.69%, 18.9% and 18.66%, respectively. A number of studies reported that shrimp oil contained a large amount of long chain polyunsaturated fatty acids as eicopentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Heu *et al*., 2003; Sriket *et al*., 2007; Senphan and Benjakul, 2012; Takeungwongtrakul *et al*., 2012) those contribute the prevention of geriatric, cardiovascular disorders and certain forms of cancer related diseases (Yazawa and Kageyama, 1991). However, shrimp oil extracted from shrimp by-product may not be the good source of those important fatty acid due to the lipid oxidation during transportation and storage affected the decreasing of those compounds (Heu *et al*., 2003). The products with high content of polyunsaturated fatty acids are highly susceptible to oxidative spoilage and the rate of oxidation is significantly different from that of other oils (Boran *et al*., 2006). During the autoxidation, undesirable flavors and odors develop at very low peroxide values at an early stage of oxidation, even during the induction period. The hydroperoxides do not themselves contribute appreciably to the deteriorated flavors and odors of oxidised oils (Stansby, 1967). In most cases, the organoleptically detectable materials appear to have low molecular weights and are formed by the decomposition of peroxides and by further oxidation of the peroxides and their breakdown products. A large number of saturated and unsaturated aldehydes, ketones, acids, and other products have been isolated from oxidized oils, and have been shown to contribute to the undesirable flavors and odors.

1.3 Objectives

1.3.1 To study the chemical compositions and qualities of Pacific white shrimp head.

1.3.2 To study the effect of different pretreatment conditions on melanosis prevention in Pacific white shrimp head.

1.3.3 To extract and characterize flavorant from Pacific white shrimp head using different enzymes.

1.3.4 To study the effect of pretreatment on flavorant extracted from Pacific white shrimp head.

1.3.5 To study the effect of different drying methods on qualities of flavorant extracted from Pacific white shrimp head.

1.3.6 To study the effect of packaging method on flavorant powder from Pacific white shrimp head.

1.3.7 To extract and characterized colorant from Pacific white shrimp head using different solvents and their mixtures as well as the effect of pretreatment on the extracted colorant.

1.3.8 To study the physical, chemical, and sensory properties of food product added with flavorant and colorant from Pacific white shrimp head.

CHAPTER 2

ENZYMATIC EXTRACTION OF SHRIMP FLAVORANT AND EFFECT OF MELANOSIS PREVENTION PRETREATMENT ON SHRIMP FLAVORANT FROM PACIFIC WHITE SHRIMP HEAD

2.1 Abstract

Pacific white shrimp head contained 76.05% moisture, 12.93% protein, 3.17% fat and 3.81% ash. pH, TVBN, TMA, and salt content were 7.62, 6.35, 4.05 mg nitrogen/100 g and 3.75 % (wet basis), respectively. To prevent melanosis in Pacific white shrimp (*Litopenaeus vanamei*) head, soaking shrimp head in 0.5, 1.0, 2.0 % citric acid solution and 1.0, 2.0, 4.0 % sodium metabisulfite solution in the shrimp head to solution ratio of 1:5 w/v for 0, 15 and 20 min were conducted. The polyphenoloxidase (PPO) activity and melanosis score of the pretreated samples were compared with the control and the sample soaked in distilled water. The results showed that PPO activities were decreased with increasing solution concentration and soaking time ($p<0.05$). Citric acid treatments had no effect on melanosis scores when compared with the control after 7 days storage ($p\geq0.05$). The sample treated with 2.0% sodium metabisulfite solution for 15 min obtained the lowest score. Moreover, the value was lower than that of heated sample (core temperature of 80^{o}C for 30 sec) ($p<0.05$).

To produce the shrimp flavorant from Pacific white shrimp head, the optimal condition of enzymatic flavorant extraction from shrimp head using 0.15% w/w Alcalase (pH 8.0) or Flavourzyme (pH 7.0) at 55^{o}C for 5 hr was studied. The results showed that extraction for 270 min yielded the highest protein and formaldehyde nitrogen contents for both enzymes ($p<0.05$). However, the flavorant solution extracted by Alcalase showed the higher protein content than that of Flavourzyme ($p<0.05$). A descriptive analysis indicated that the flavorant using Flavourzyme gained higher intensity of boiled blue swimming crab odor, but lower intensity of boiled sundried shrimp and roasted sundried shrimp odor than that using Alcalase. The overall and color acceptance scores (n=30) of fish ball added with 4% (dry weight) flavorant extracted by Flavourzyme were higher than that by Alcalase ($p<0.05$). However, no differences

in odor, flavor/taste and texture scores between both samples were observed ($p \geq 0.05$). The comparison of flavorants extracted from non-pretreated and pretreated shrimp head by soaking in 2% sodium metabisulfite solution for 15 min for melanosis prevention showed that flavorant solution from the non-pretreated sample had the higher protein and formaldehyde nitrogen contents than another. However, overall acceptance score of the pretreated sample was significantly higher than that of non-pretreated sample ($p < 0.05$).

2.2 Introduction

During shrimp processing, a large amount of shrimp waste including exoskeleton and cephalothorax are generated. This waste can be represented 50% to 70% of weight of raw material and this waste consists of 71.4% head and 28.6% shell (Holanda and Netto, 2006). They are the rich source of valuable components such as protein, carotenoid pigments, chitin and chitosan (Ramaswamy *et al*., 1991; Klomklao *et al*., 2009). Number or studies have been reported on the extraction of carotenoprotein or carotenoid pigment used as a colorant or bioactive compound in functional food (Sachindra and Mahendrakar, 2005; Sachindra *et al*., 2006; Sachindra *et al*., 2007; Pu *et al*., 2010, Cahú *et al*., 2012, Sadighara *et al*., 2015), the isolation of chitin and chitosan applied in many areas as waste water treatment, food, agriculture, cosmetic and pharmaceutical industries (Cahú *et al*., 2012; Sadighara *et al*., 2015) as well as the utilization of protein recovered from shrimp waste (Benjakul and Morrissey, 1997; Cahú *et al*., 2012; Dey and Dora, 2014; Sowmya *et al*., 2014; Jeyakumari *et al*., 2016). However, when the shrimp heads were left under uncontrolled condition or kept for long time, they will turn to be black even under a frozen condition. This is a natural mechanism caused by enzymatic reactions that occurs as soon as the *Penaeidae* taken from the water and come into contact with the oxygen of the atmosphere. After harvest and death, PPO systems are still active and can promote the development of black pigments around the shell and on the surface of the meat (Anonymous, 1988). Therefore, the pretreatment for melanosis prevention are crucial for the qualities of shrimp and its by-products during storage.

The protein recovered in the form of hydrolysates can be used as flavorant and incorporated into fish-base foods (Teerasuntonwat and Raksakulthai, 1995; Holanda and Netto, 2006). The previous studies showed that peptides and free amino acids contributed to flavors in marine food (Konosu and Yamaguchi, 1982; Raksakulthai and Haard, 1992; Teerasuntonwat and Raksakulthai, 1995). Enzymatic hydrolysis, one of the effective approaches for protein recovery from shrimp waste has been widely studied (Simpson and Haad, 1985; Cano-Lopez *et al.*, 1987; Synowiecki and Al-Khateeb, 2000; Gildberg and Stenberg, 2001; Mizani *et al*., 2005; Holanda and Netto, 2006). Holanda and Netto (2006) extracted protein from *Xiphopenaeus kroyeri* processing waste, containing cephalothorax, shell, and tail with degree of hydrolysis (DH) of 6 and 12% using 3% Alcalase at 60^{o}C and 1% pancreatin at 40^{o}C, pH 8.5. The result showed that protein recovered with Alcalase was higher than obtained with pancreatin for both DH. Benjakul *et al*. (2009) used Flavourzyme as a processing aid for the production of Mungoong (a shrimp extract paste) from the cephalothorax of white shrimp (*Litopenaeus vanamei*). Flavourzyme containing both endopeptidase and exopeptidase has been used to produce protein hydrolysates with less bitterness (Suh *et al*., 2003). Nilsang *et al*. (2005) found that fish protein hydrolysates using Flavourzyme had less bitter than 1 ppm caffeine solution. With more hydrolysis, smaller peptides could be produced and might exhibit more biological activities. Teerasuntonwat and Raksakulthai (1995) produced flavoring agent from shrimp (*Peneaus monodon* Fabricius) head by extraction of nitrogenous compounds with water, sodium chloride, hydrochloric acid, sodium hydroxide, bromelain, papain and neutrase and drying with 10% (w/v) sodium chloride in hot air oven at 50±5 ^{o}C for 72 hr. The acceptability scores of cracker added with commercial flavorant and dried extract showed that the sample with commercial flavorant had the highest score. In addition, crackers with the extract using bromelain had higher score than the control (without flavor extract) but was not significantly different to those using papain, water, NaCl, neutrase or HCl. Moreover, Gildberg (1993) reported that hydrolysates prepared with bromelain had better organoleptic quality than those prepared with papain, ficin, bromelin, trypsin, pancreatin, Alcalase, and pronase. However, limited information regarding pretreatment for blackening prevention of shrimp head and sensory characteristic of the shrimp flavorant has been reported. Therefore, the aims of this study were to study the

enzymatic extraction conditions to produce flavorant from shrimp head and to investigate different pretreatments on melanosis prevention of Pacific whit shrimp head as well as its effect on the characteristics of the extracted flavorant.

2.3 Meterials and methods

2.3.1 Chemicals

Flavourzyme (protease from *Aspergillus oryza*) and Alcalase (protease from *bacillus* licheniformis) were procured from Sigma, Capricorn, Singapore. Formaldehyde was obtained from Merck (Darmstadt, Germanyl). Hydrochloric acid (37%), sodium hydroxide, sulfuric acid and petrolium ether were purchased from LAB-SCAN (Thailand). Potassium sulfate, copper sulfate and boric acid were procured from Ajax Finechem (New Zealand).

2.3.2 Shrimp head preparation

Frozen Pacific white shrimp heads obtained from Sea wealth Frozen Food Co., Ltd, Songkhla, Thailand were kept at -20^{o}C for not longer than 1 month. The samples were thawed by running water and minced for 1 min using blender (AY46, Moulinex, China). The shrimp head was mixed and subjected to analyses as well as study of enzymatic extraction.

2.3.2.1 Proximate analysis

Minced shrimp head was determined for moisture, protein, fat and ash content according to AOAC (2000).

2.3.2.2 Determination of pH

Minced shrimp head (2 g) was homogenized thoroughly with 10 volumes of distilled water (w/v) using homogenizer (T25 basic, IKA LABORTECHNIK, Selangor, Malaysia). The pH of homogenate was measured using pH meter (CG842, SCHOTT, Deutschland, Germany) (Benjakul *et al*., 1997).

2.3.2.3 Determination of salt content

Salt content of minced shrimp head was determined by the method of AOAC (2000). Sample (1 g) was mixed with 10 ml of 0.1N $AgNO_3$ and 10 ml of conc. NHO_3. The mixture was gently boiled on a hot plate until all solid except $AgCl_2$ was dissolved (usually 10 min). The sample was cooled with running water before added with 5 ml of 5% ferric alum indicator. The mixture was titrated with standardized 0.1 N KSCH until solution becomes permanent light brown and the percentage of salt was calculated as follows:

$$\text{Salt (\%)} = \frac{5.8 \times (\text{volume of } AgNO_3 \times N) - (\text{volumn of KSCN} \times N)}{\text{Weight of sample}}$$

2.3.2.4 Determination of total volatile basic (TVB) and trimethylamine (TMA) contents

Total volatile base (TVB) contents in shrimp head were determined using the Conway micro-diffusion method (Conway and Byrne, 1936). Sample (2 g) was extracted with 8 ml of 4% (w/v) trichloroacetic acid (TCA) solution. The mixture was homogenized at 8000 rpm for 1 min using a homogenizer (model T25 basic, ULTRA TURRAX®, IKA LABORTECNIK, Selangor, Malaysia). The homogenate was kept at 28±2°C for 30 min, then filtered through Whatman No. 41 filter paper (Schleicher & Schuell, Maidstone, England). The filtrate was collected and the final volume was adjusted to 10 mL using 4% TCA. The inner ring solution (1 ml) and filtrate (1 ml) were added to inner ring and outer ring of the Conway unit, respectively. One milliliter of saturated K_2CO_3 solution was then added into outer ring. To determine the TMA content, formaldehyde was added to the filtrate to fix ammonia present in the sample. The Conway unit was closed and the solution was mixed slowly. The mixture was incubated at 37°C for 60 min and the inner ring solution was titrated with 0.02 N HCl using a micro-burette until green color turned into pink. For the blank, TCA solution (4%) was used instead of sample extract. The amounts of TVB were calculated and the results were expressed as mg N/100 g shrimp head.

2.3.3 Effect of pretreatment conditions on melanosis prevention of Pacific white shrimp head

Frozen Pacific white shrimp head was thawed in running water. The shrimp heads were subjected to 2 different pretreatment methods.

2.3.3.1 Soaking in solution

Shrimp head was soaked in different soaking solutions as follows:

- Distilled water
- Citric acid with concentration of 0.5, 1.0, 2.0 %
- Sodium metabisulfite with concentration of 1.0, 2.0, 4.0 %

The shrimp head samples were immersed with shrimp head to solution ratio of 1:5 w/w for 10, 15 and 20 min at 28±2^{o}C. Then, the pretreated shrimp head was rinsed with water for 5 min, drained through strainer for 10 min and subjected to analyses as follows:

2.3.3.1.1 Determination of polyphenol oxidase activity

The pretreated shrimp head samples were powderized by grinding with liquid nitrogen in a warring blender (AY46, Moulinex, China). PPO was extracted from the prepared powder according to the method of Simpson *et al*. (1987) with a slight modification. The powder (50 g) was mixed with 150 mL of the extracting buffer (0.05 M sodium phosphate buffer, pH 7.2, containing 1.0 M NaCl and 0.2% Brij-35). The mixture was stirred continuously at 4^{o}C for 30 min, followed by centrifugation at 8000×*g* at 4^{o}C for 30 min using a refrigerated centrifuge (Beckman Coulter, Avanti J-E Centrifuge, Fullerton, CA, USA). Solid ammonium sulfate was added into the supernatant to obtain 40% saturation and the mixture was allowed to stand at 4^{o}C for 30 min. The precipitate was collected by centrifugation at 12,500×*g* at 4^{o}C for 30 min using the refrigerated centrifuge. The pellet obtained was dissolved in a minimum volume of 0.05 M sodium phosphate buffer, pH 7.2 and dialyzed against 15 volumes of the same buffer at 4^{o}C with three changes of dialysis buffer. The insoluble materials were removed by centrifugation at 3,000×*g* at 4^{o}C for 30 min and the supernatant was used as "crude PPO extract".

PPO activity was assayed using L-3,4-dihydroxyphenylalanine (L-DOPA) as a substrate according to the method of Simpson *et al*. (1987) with a slight modification. The assay system consisted of 100 µl of crude PPO extract, 600 µl of 15

mM L-DOPA in deionized water, 400 µl of 0.05 M phosphate buffer, pH 6.0 and 100 µl of deionized water. The reaction mixture was incubated for 3 min at 45^{o}C and the formation of dopachrome was monitored by measuring the absorbance at 475 nm (A475) using a UV-160 spectrophotometer (Shimadzu, Kyoto, Japan). One unit of PPO activity was defined as an increase in A475 by 0.001/min/ml. Enzyme and substrate blanks were prepared by excluding the substrate and enzyme, respectively, from the reaction mixture and the deionized water was used instead.

2.3.3.1.2 Determination of melanosis

Pretreated shrimp heads were stored at -20^{o}C in polyethylene bag and placed at 28±2^{o}C for an hour at day 0, 3, 5, and 7 for melanosis determination. Melanosis or blackening of shrimp head samples during storage was evaluated by twelve trained panelists as described by Otwell and Marshall (1986). A 10-points scale was used (Appendix 1), where 0 = absent; 2 = slight, noticeable on some shrimps; 4 = slight, noticeable on most shrimps; 6 = moderate, noticeable on most shrimps; 8 = heavy, noticeable on most shrimps; and 10 = heavy, totally unacceptable.

The soaking solution exhibited in the lowest polyphenol oxidase activity and melanosis score was selected to compare with heating treatment in part 2.3.3.2.

2.3.3.2 Heat treatment

Shrimp head was heated in water bath with the shrimp head to water ratio of 1:5 (w/v) at 85^{o}C until the core temperature reached 80^{o}C for 30 sec. The heated sample was immediately cooled in iced water for 5 min and drained for 10 min (Manheem *et al*., 2013). The drained heated and soaked shrimp head selected from Part 2.3.3.1 were subjected to analyses as mentioned in Part 2.3.3.1.

The selected pretreated method with the lowest PPO activity and melanosis score was chosen for the further study.

2.3.4 Enzymatic extraction of shrimp flavorant from Pacific white shrimp head

The non-pretreated shrimp head was hydrolyzed by Flavourzyme or Alcalase (0.15% w/w of shrimp head). Protease activity of enzyme was analyzed using the method of Banik and Prakash (2006). The hydrolysis method was described in Figure 5.

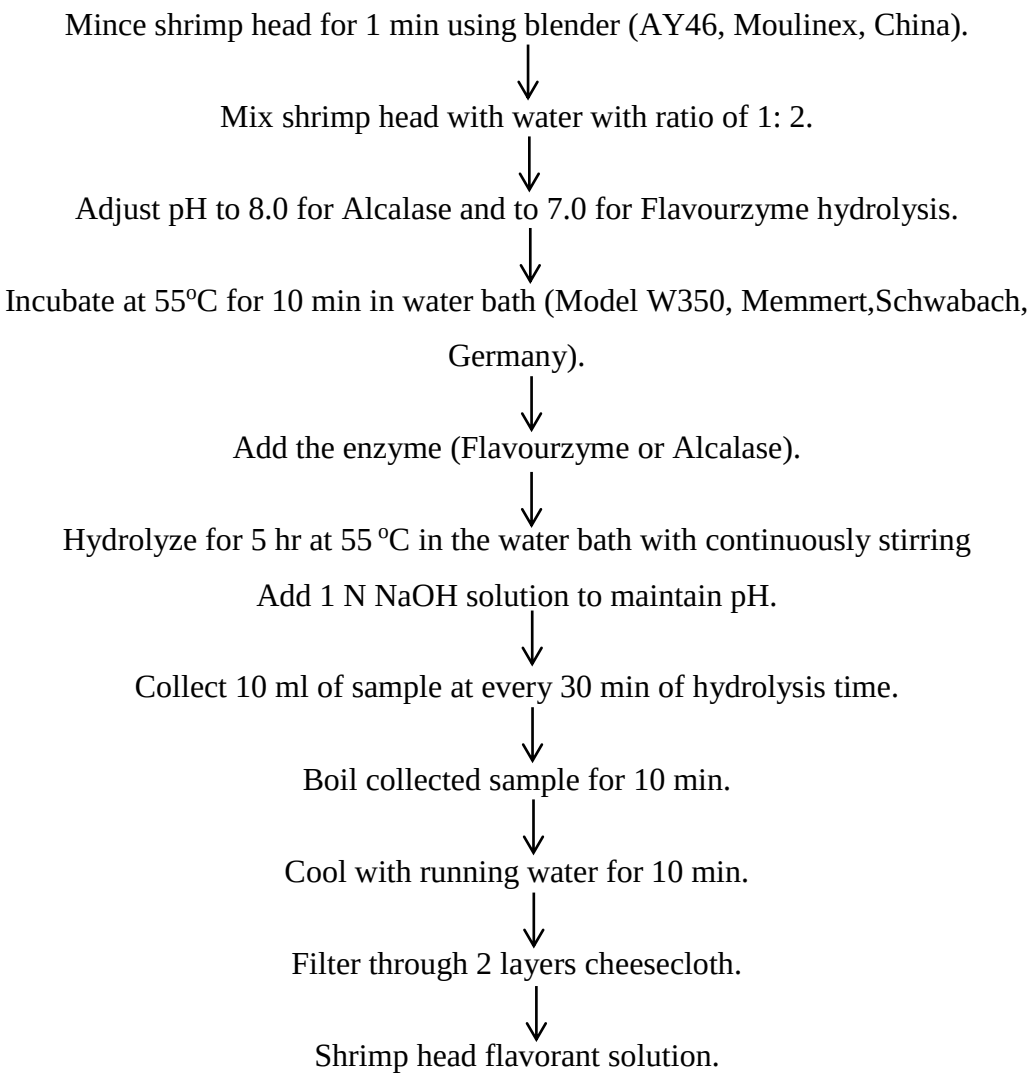


Figure 5 Schematic of shrimp head flavorant extraction using enzymatic hydrolysis

The shrimp flavorant solution was subjected to analyses.

2.3.4.1 Determination of protein content

Protein content was determined using Kjeldahl's method (AOAC, 2000). The sample (1 ml) was placed in Kjeldahl flask and hydrolyzed at 220°C by 20 ml H_2SO_4 with 5 g of Kjeldahl catalyst (K_2SO_4: $CuSO_4$ in a ratio of 9:1) of 2 hr. Distilled water (60 ml) and access 20% (w/v) NaOH were added into the mixture before distilling for 15 min to release volatile nitrogen into 50 ml of 4% boric acid containing methyl red-bromocresol green. The distillate was finally titrated with 0.5 M HCL until the end-point was obtained. Protein content was calculated as follows:

$$\text{Protein content} = \frac{(A - B) \times N \times 1.4007 \times F}{W}$$

When A was volume (ml) of 0.5 M HCl titrated with sample.
B was volume (ml) of 0.5 M HCl titrated with blank.
N was standardized concentration of HCl.
F was protein-nitrogen conversion factor (6.25).
W was volume of the shrimp flavorant solution.

2.3.4.2 Determination of formaldehyde nitrogen content

Formaldehyde nitrogen content was determined by the titration method as described by the Thai Industrial Standard (1983) with a slight modification. Shrimp flavorant solution (1 ml) was added to 10 ml of distilled water and the pH was adjusted to 7 using 0.1M NaOH. Subsequently, 10 ml of formaldehyde (38% v/v; pH 9) was added and mixed well. The mixture was titrated with 0.1 N NaOH to obtain a pH of 9.0. Formal nitrogen content was calculated and expressed as mg formal nitrogen per ml sample as shown below:

$$\text{Formaldehyde nitrogen content (mgN/g)} = \frac{\text{mL of NaOH (pH7} - \text{pH9)} \times 0.1 \times 14}{\text{Weight of sample (g)}}$$

The shrimp flavorant solutions with the highest extracted protein content and formaldehyde nitrogen content from each enzyme were subjected to sensory evaluation as follows:

2.3.4.3 Sensory evaluation

2.3.4.3.1 The samples preparation for sensory evaluation

- Shrimp flavorant solutions extracted by Alcalase or Falvourant were diluted by distilled water to gain 4% solid content. Each flavorant solution (4 ml) was kept in a 10 ml amber glass bottle and warmed in a 45^{o}C water bath before serving. The bottle was labelled with random three-digit codes.

- Fish ball added with the shrimp flavorant was prepared using the formula and method as described in Table 8 and Figure 6, respectively. The flavorant solution extracted using either Alcalase or Flavourzyme was evaporated by a rotary evaporator (Buchi rotavapor, Switzerland) at 50^{o}C to obtain 25% total solid content. The concentrated solution was frozen at -20^{o}C until used. The fish ball was contained 4% of flavorant (dry weight, approximately 15 g of frozen solution).

2.3.4.3.2 Generic Descriptive Analysis

The human subjective training method was based on International Standard ISO 8586-1 (1993). Members of the panel were recruited from post graduate students of Food Science and Technology Program at Prince of Songkla University, Songkhla, Thailand. Thirteen panelists were selected, based on interest, availability, verbal expression, and the liking for shrimp. The panelists were trained 20 times for 1 hr each. A brief background to sensory evaluation was discussed by the researcher and panelists. The discussion included term and definition of each key sensory attribute in shrimp flavorant. To generate the list of odor characteristics, the flavorant solutions from both enzymes in the amber glass bottles were given to the panelists for odor evaluation. Each panelist was asked to characterize odors perceived in the flavorants. The odor characteristics were generated and screened by the consensus of all selected panelists. The trainees were ready to evaluate the intensity of the flavor and odor in shrimp flavorants extracted by various conditions when they could identify the intensity of the attribute. The references and the attributes used in this study are shown in Table 9.

2.3.4.3.3 Acceptance test

The acceptance test was judged by 30 consumer-type panelists recruited from the Faculty of Agro-Industry, Prince of Songkhla University, HatYai, Thailand using a 9-point hedonic scale (Appendix 2).

The shrimp flavorant solution was evaluated for color, odor and overall likings, while fish ball samples were used for assessment of color, odor, flavor/taste, texture and overall likings.

Table 8 Ingredients of fish ball added with shrimp flavorant

Ingredients	%
Surimi from bigeye snapper	79.05
Ice	15.81
Tapioca starch	3.16
Salt	1.98

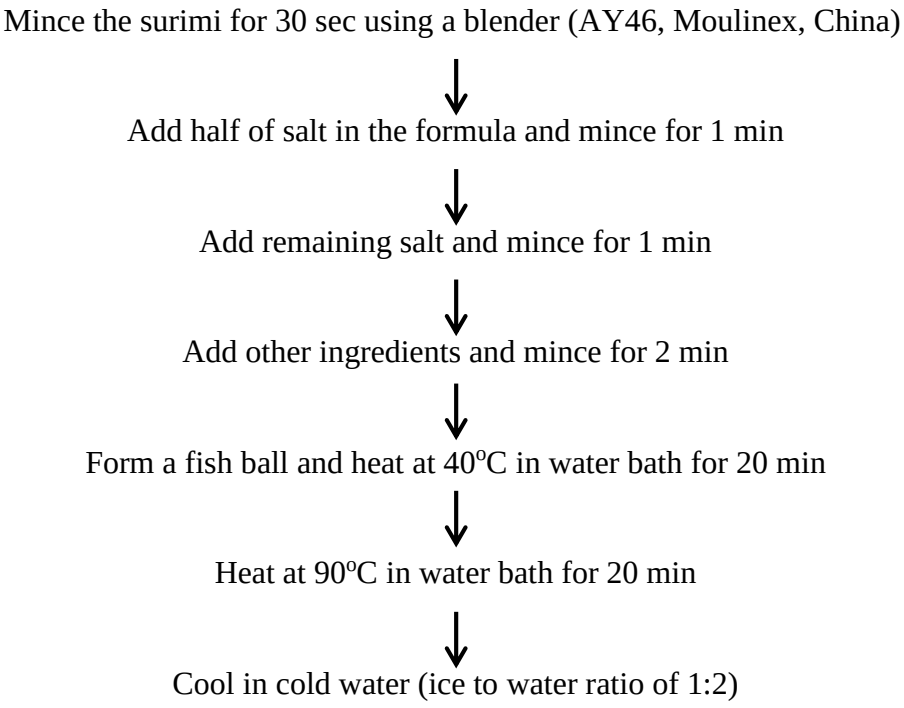


Figure 6 Preparation of fish ball added with the shrimp flavorant

Table 9 Reference samples and ratings used in descriptive analysis of shrimp flavorants

Attribute	Definition	Reference	Rating (cm)
Roasted sundried shrimp	Aromatic associated with roasted sundried shrimp, from slight to strong	The sundried shrimp was roasted at 550°C for 10 min and place in a 2 oz. cup with the lid.	
		- 1 g of roasted sundried shrimp	1.5
		- 2.5 g of roasted sundried shrimp	9.0
		- 5 g of roasted sundried shrimp	11.0
Boiled sundried shrimp	Aromatic associated with boiled sundried shrimp, from slight to strong	The sundried shrimp was soaked in distilled water with the ratio of 1:3 for 30 min before blend for 1 min. The mixture was boiled for 5 min (solution 1) and place 5 ml in a 2 oz. cup with the lid.	
		- solution 1	9.0
		- solution 1 diluted with distilled water with the ratio of 1:2	5.5
		- solution 1 diluted with distilled water with the ratio of 1:4	1.5
Boiled blue swimming crab	Aromatic associated with boiled blue swimming crab, from slight to strong	Blue swimming crab (250 g/ each) was boiled with distilled water with the ratio of 1:2 for 30 min.	
		- the crab was cut into 2 part, each part was place into 8 oz. cup with the lid	13.5
		- 5 ml of crab cooking juice was place in a 2 oz. cup with the lid	4.0
unfresh boiled shrimp juice	Aromatic associated with unfresh boiled shrimp juice, from slight to strong	Fresh shrimp (harvested within 48 hr) was storage at 4°C for 48 hr before boiled in distilled water with the ratio of 1:1, the cooking juice (solution 2) was diluted and placed in 2 oz. cup with the lid.	
		- solution 2	13.5
		- solution 1 diluted with distilled water with the ratio of 1:2	9.0
		- solution 1 diluted with distilled water with the ratio of 1:4	4.5

The samples were served according to the serving plan designed to balance first-order carry-over effects (MacFie *et al*., 1989). The intensity scales of flavor and odor were agreed on a 15 cm line scale (anchored at 1.5 cm and 13.5 cm as "low" and "high", respectively; Appendix 3).

The condition possessed the highest yield and acceptance score was selected for the next studies.

2.3.5 Effect of pretreatment on flavorant extracted from Pacific white shrimp head

Shrimp head was thawed and pretreated by soaking 2% in sodium metabisulfite solution for 15 min with the ratio of 1:5 (w/v). Pretreated and non-pretreated shrimp heads were hydrolyzed by Alcalase for 270 min as described in Part 2.3.4. The extracted solutions were subjected to analyses as follows:

- Determination of protein content as described in Part 2.3.4.1
- Determination of formaldehyde nitrogen content as described in Part 2.3.4.2
- Acceptance test of 4% flavorant solution samples as described in Part 2.3.4.3.2

2.3.6 Statistical Analysis

All experiments were run in triplicate and reported as mean ± standard deviation. Completely randomize design (CRD) was used for analysis of physical, chemical and generic descriptive analysis. A randomize complete block design (RCBD) was carried out for analysis of acceptance test. Data was subjected to analysis of variance (ANOVA). Comparison of means were carried out by Duncan's multiple range test (Steel and Torrie, 1980) at a significant level $p< 0.05$. Analysis was performed using a SPSS package (SPSS 10.0 for window, SPSS Inc, Chicago, IL).

2.4 Results and discussion

2.4.1 Determination of chemical compositions and qualities of Pacific white shrimp head

Pacific white shrimp head from Sea wealth Frozen Food Co., Ltd, Songkhla, Thailand used in this study consisted of 76.05% moisture, 12.93% protein, 3.17% fat, 3.81% ash and 4.04% carbohydrate as shown in Table 10. Shrimp head is an interesting material as it contained valuable components as protein, carotenoid pigment, chitin and chitosan along with flavor components (Ramaswamy *et al*., 1991; Klomklao *et al*., 2009). A number of studies reported that the shrimp heads from different species including *Penaeus monodo* (Babu *et al*., 2008), *Penaeus indicus* (Babu *et al*., 2008), *Metapenaeus monocerous* (Babu *et al*., 2008) and *Farfantepenaeus paulensis* (Sánchez-Camargo *et al*., 2011) consisted of 67.4 – 77.0% moisture, 34.66 – 59.57% protein, 3.68 – 7.39% fat and 26.09 – 43.39 % ash (dry weight basis). The Pacific white shrimp head used in this study had moisture and protein contents similar ranges to with the previous studies but had higher fat and lower ash contents. The variation of the compositions depended upon species, season, size, source etc. (Sriket *et al*., 2007; Liang *et al*., 2008; Rødde *et al*., 2008). Carbohydrate presented in the sample might be attribute to chitin, a structural polysaccharide (polymer of acetyl D-glucosamine) (Morganti *et al*., 2011; Ekpenyong *et al*., 2013).

Table 10 Proximate analysis of Pacific white shrimp head

Composition	Content	
	% wet basis*	**% dry basis***
Moisture	76.05 ± 0.14	-
Protein	12.93 ± 0.19	53.99 ± 0.79
Fat	3.17 ± 0.02	13.24 ± 0.08
Ash	3.81 ± 0.10	15.91 ± 0.42
Carbohydrate	4.04 ± 0.12	16.86 ± 1.29

* Mean ± SD (n=3)

Table 11 showed total volatile base nitrogen (TVBN), trimethylamine (TMA), salt content and pH of Pacific white shrimp head. Shrimp head contained 3.75 % (wet basis) salt. TVBN and TMA content were used to evaluate quality of shrimp as they were generated during postmortem handling (Bak *et al*., 1999). TVBN and TMA contents of the borderline of shrimp freshness are 40 mg nitrogen/100 g and 5 mg nitrogen/100 g, respectively. Which is it still acceptable for the consumers (Uchiyama and Kakuda, 1984; Mendess *et al*., 2002). TVBN and TMA content of Pacific white shrimp head were 6.35 and 4.05 mg nitrogen/100 g, respectively. The values were lower than the borderline indicating the acceptable freshness of the raw material used.

Table 11 Total volatile base nitrogen (TVBN), trimethylamine (TMA), salt content and pH of Pacific white shrimp head

Analysis	**value***
TVBN (mg nitrogen/ 100 g)	6.53 ± 0.49
TMA (mg nitrogen/ 100 g)	4.05 ± 0.09
Salt (% wet basis)	3.75 ± 0.31
pH	7.62 ± 0.03

* Mean ± SD (n=3)

Mu *et al.* (2011) reported that pH of fresh Pacific white shrimp was 7.04 and the pH was increased during storage and was higher than 7.8 at day 6. In addition, the slightly increasing of TVBN was occurred at the first 2 days and it sharply increased at day 3. As by-product, shrimp heads were not well treated and might be left at a room temperature before freezing. The higher temperature could allow microbial growth and resulted in the accumulation of basis compounds relating to the increasing of pH (Lopez-Caballero, *et al*., 2007) However, Mehmet *et al.* (2009) reported that shrimp with a pH 7.7 and below still remained of good quality.

2.4.2 Effect of pretreatment conditions on melanosis prevention in Pacific white shrimp head

Polyphenoloxidase (PPO) activity and melanosis scores of Pacific white shrimp head pretreated by various solutions and conditions are shown in Table 12. The PPO activity of the shrimp head was decreased with increasing soaking time regardless of soaking solution ($p<0.05$). However, the sodium metabisulfite solution was more effective than the citric acid solution. The increase in concentration and soaking time of citric acid solution had no effect on melanosis score of the shrimp head after 7 days of storage ($p\geq0.05$). In addition, the score of sample soaked in citric acid solution up to 0.5% was not different from that soaked in water with the same soaking time and the control ($p\geq0.05$). The lowest PPO activity was found in the sample soaked by 4.0% sodium metabisulfite solution for 15 and 20 min ($p<0.05$). Nevertheless, the sample soaked in 2.0% sodium metabisulfite solution for 15 and 20 min had the lowest melanosis score after storage at -20^{o}C for 7 days and thawing at room temperature for 2 hr everyday ($p<0.05$). Figure 7 represents the appearance of the Pacific white shrimp pretreated exhibiting the lowest PPO activity from each soaking solution after 7 days storage. Although, the sample soaked in 4% sodium metabisulfite solution had lower PPO activity when compared to that in 2% sodium metabisulfite solution, the color of the shrimp head was yellowish and might affect the melanosis score. PPO is a copper-containing metalloenzyme, which catalyses two basic reactions. In the presence of molecular oxygen, that includes the o-hydroxylation of monophenols to give *o*-diphenols and the subsequent oxidation of *o*-diphenols to *o*-quinones (Garcia-Molina *et al*. 2005). Nirmal and Benjakul (2012) reported that the optimal pH and temperature of PPO from Pacific white shrimp (*Litopenaeus vannamei*) were 6 and at 55°C, respectively. The activity markedly decreased in very acidic and alkaline pH ranges. The unfolding of PPO molecules might occur at the extreme pHs owing to the increase in electrostatic repulsion, leading to the losses in its activity. Citric acid was not only acts as an acidulant but it also function as chelating agent. Since PPO is a metalloprotein with copper as the prosthetic group, it can inhibited by metal chelation agents such as citric acid (Laurila *et al*., 1998; Whitaker, 1972). Bisulfite is function as a competitive inhibitor by binding to the sulphydryl group found at the enzyme's active site (Kim and Marshall, 2000).

Table 12 Polyphenoloxidase activity and melanosis scores of Pacific white shrimp head pretreated by various solutions and conditions

Soaking solution	Soaking time (min)	% PPO activity	Melanosis score*
Control	-	99.71 ± 4.18^{a}	9.50 ± 0.71 ^{a}
Water	10	85.54 ± 2.22^{b}	8.70 ± 1.57^{a}
	15	72.27 ± 1.35^{c}	9.20 ± 1.03^{a}
	20	60.76 ± 5.33^{d}	9.40 ± 1.07^{a}
0.5% citric acid solution	10	79.06 ± 12.02^{bc}	9.40 ± 0.84 ^{a}
	15	79.35 ± 8.36^{bc}	9.60 ± 0.70^{a}
	20	60.47 ± 8.36^{d}	9.50 ± 0.71^{a}
1.0% citric acid solution	10	58.70 ± 8.91^{d}	9.40 ± 0.84^{a}
	15	59.58 ± 5.33^{d}	9.40 ± 0.84^{a}
	20	60.76 ± 1.77^{d}	9.50 ± 0.71^{a}
2.0% citric acid solution	10	53.68 ± 5.89^{d}	9.60 ± 0.70^{a}
	15	39.82 ± 6.91^{ef}	9.40 ± 0.70^{a}
	20	40.41 ± 8.22^{ef}	9.50 ±0.71^{a}
1.0% sodium metabisulfite	10	43.49 ± 10.00^{e}	4.20 ± 1.87^{b}
solution	15	30.79 ± 3.85^{fg}	3.40 ± 1.35^{b}
	20	22.54 ± 1.98^{gh}	2.00 ± 0.82^{c}
2.0% sodium metabisulfite	10	39.05 ± 2.52^{ef}	1.80 ± 0.79^{c}
solution	15	21.27 ± 4.79^{gh}	0.70 ± 0.67^{d}
	20	20.32 ± 1.98^{ghi}	1.20 ± 0.92^{cd}
4.0% sodium metabisulfite	10	26.98 ± 6.12^{g}	2.10 ± 0.99^{c}
solution	15	13.33 ± 1.90^{hi}	1.80 ± 1.23^{c}
	20	10.48 ± 1.90^{i}	2.00 ± 1.25^{c}

* Melanosis score was evaluated after the sample was stored for 7 days.

Mean ± SD (n=3)

Different superscripts ($^{a-i}$) in the same column indicate the significant differences ($p<0.05$).

(a) (b) (c) (d)

Figure 7 Pacific white shrimp head pretreated by soaking in various solutions; (a) Control, (b) Water for 20 min, (c) 2.0% citric acid solution for 15 min, (d) 2.0% sodium metabisulfite solution for15 min, after storage for 7 days.

The sample soaked in 2.0% sodium metabisulfite solution for 15 min was then compared to the control and the sample pretreated by heating at 85ºC for 30 sec. The results in Table 13 showed that both sodium metabisulfite solution and heating could inhibit PPO activities and decreased melanosis score when compared with the control ($p<0.05$). PPO activities of sodium metabisulfite sample were significantly lower than that of heated sample ($p<0.05$). Which coincided with the lower melanosis score of sodium metabisulfite sample after storage of 7 days ($p<0.05$). Manheem *et al*. (2013) reported that pre-cooking at 80ºC for 30 sec had ability to inhibit PPO activities and melanosis of Pacific white shrimp. However, sulfite also functions as a bleaching agent. Thus it might bleach the melanin (black pigment) generated during transportation and storage (McEvily and Iyengar, 1992). This study revealed that 2% sodium metabisulfite solution was more effective than heating and was selected for the further studies.

Table 13 Polyphenoloxidase activity of Pacific white shrimp head after pretreated with different conditions for 3 min and their melanosis score after stored at -20°C for 7 days (thawed at room temperature for 2 hr everyday)

Pretreated condition	PPO activity (%)*	Melanosis score*
Control	99.72 ± 5.66 [a]	6.54 ± 1.20[a]
2.0% sodium metabisulfite solution, 15 min	8.39 ± 1.11[c]	0.77 ± 0.83[c]
Heat at 85°C for 30 sec	17.56 ± 0.25[b]	2.46 ± 1.39[b]

* All values are means ± standard deviation (n=3). For each run, determinations were conducted.
Different superscripts [(a-b)] in the same column indicate the significantly differences ($p<0.05$).

2.4.3 Study of enzymatic extraction of shrimp flavorant from Pacific white shrimp head

Protein and formaldehyde nitrogen content of shrimp flavorant solutions at different extraction times are shown in Figure 8 and 9, respectively. The results showed that both components were continuously increased with an increasing in extraction time for both enzymes. However, the values extracted for 270 and 300 min were not significantly different ($p\geq0.05$). The comparison also showed that the protein and formaldehyde nitrogen contents of shrimp flavorants extracted by Alcalase and Flavourzyme for 270 min were not different ($p\geq0.05$). Therefore, the flavorants extracted by both enzymes for 270 min were chosen for the next studies. Generally, the enzyme absorbs rapidly onto the insoluble protein particles, and the polypeptide chains that are loosely bound to the surface are cleaved. The more compacted core proteins are hydrolyzed more slowly. The rate of enzymic cleavage of peptide bond controls the overall rate of hydrolysis. However, available substrate for hydrolysis decreases as time of reaction increases (Benjakul and Morrissey, 1997).

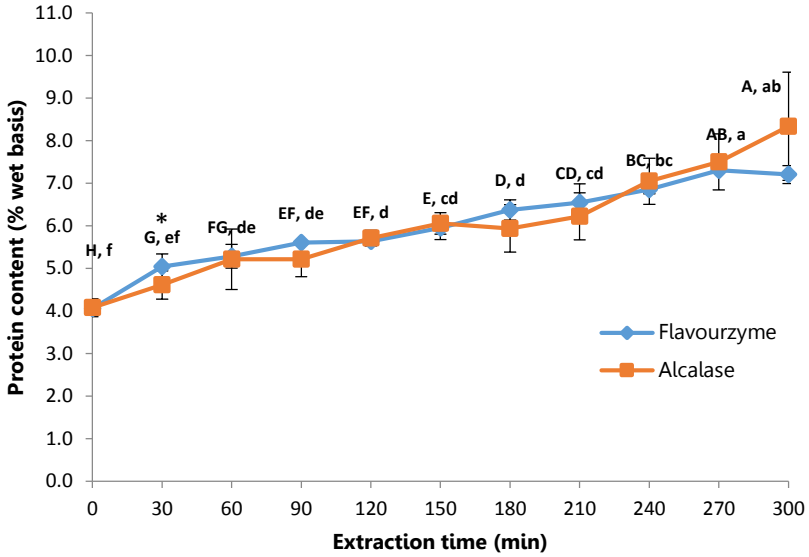


Figure 8 Protein content of shrimp flavorant solutions extracted from Pacific white shrimp head using 0.15% Flavourzyme at 55ºC, pH 7.0 and 0.15% Alcalase at 55ºC, pH 8.0 at different extraction times.

Means ± SD from triplicate determinations (n=3).

Different uppercase letters indicate the significant differences of Alcalase extraction ($p<0.05$).

Different lowercase letters indicate the significant differences of Flavourzyme extraction ($p<0.05$).

* indicates the significant difference between Alcalase and Flavourzyme extraction ($p<0.05$).

The formal nitrogen content has been used to indicate the degree of protein hydrolysis (Chaveesak *et al*. 1993). Formaldehyde can react with alpha amino group and ammonia, releasing the proton which can be titrated with alkaline solution. Thus, formal nitrogen content can be the indicator for the level of the cleavage of peptides (Angeles and Garcia-Carreno 2002). Generally, amino nitrogen content was in agreement with the formal nitrogen content (Pongsetkul *et al*, 2014). Raksakulthai and Haard (1992) reported on the correlation between the concentration of peptides and amino acids and the flavor of fish sauce. They concluded that both free glutamic acid (Glu) and peptides containing Glu were important to the flavor of fish sauce. Konosu and Yamaguchi (1982) reported that the flavor of fish and shellfish were from water soluble components with low molecular weight especially free amino acids. Glycine (Gly), proline (Pro), arginine (Arg), taurine (Tau) and alanine (Ala) were major amino acids in shrimp flavor. Therefore, it is plausible that the sample with the higher formaldehyde nitrogen content will give a stronger shrimp flavor.

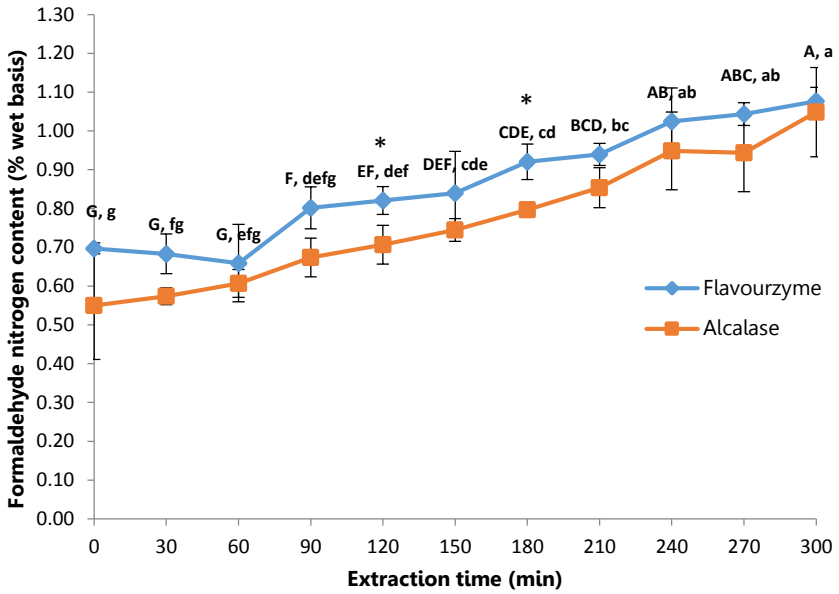


Figure 9 Formaldehyde nitrogen content of shrimp flavorant solutions extracted from Pacific white shrimp head using 0.15% Flavourzyme at 55°C, pH 7.0 and 0.15% Alcalase at 55°C, pH 8.0 at different extraction times.

Means ± SD from triplicate determinations (n=3).

Different uppercase letters indicate the significant differences of Alcalase extraction ($p<0.05$).

Different lowercase letters indicate the significant differences of Flavourzyme extraction ($p<0.05$).

* indicates the significant difference between Alcalase and Flavourzyme extraction ($p<0.05$).

The characteristics of the shrimp flavorants odor extracted from both enzymes were described as ‘boiled blue swimming crab’, ‘unfresh boiled shrimp juice’, ‘boiled sundried shrimp’ and ‘roasted sundried shrimp’. The intensity of each attribute is shown in Table 14. Alcalase extracted sample had higher intensity of ‘roasted sundried shrimp’ and ‘boiled sundried shrimp’ but lower ‘boiled blue swimming crab’ odor when compared with using Flavourzyme ($p<0.05$). However, unfresh boiled shrimp juice odor of both samples was not different ($p\geq0.05$). The differences of flavorant solutions extracted by both enzymes might be due to the specificity of the enzyme. Flavourzyme contains both endoprotease and exopeptidase activities, therefore it can be release more free amino acids than the case of serine endoprotease of Alcalase (Hrčková *et al*., 2002).

Table 14 The intensity of characterizing odors of the shrimp flavorants extracted from Pacific white shrimp head using 0.15% Flavourzyme at 55°C, pH 7.0 and 0.15% Alcalase at 55°C, pH 8.0 for 270 min.

Attributes	Intensity (cm)*	
	Alcalase	Flavourzyme
Roasted sundried shrimp	7.25 ± 1.37^{a}	5.02 ± 1.41^{b}
Boiled sundried shrimp	7.50 ± 0.85^{a}	4.22 ± 1.02^{b}
Boiled blue swimming crab	6.43 ± 0.58^{b}	7.34 ± 0.29^{a}
Unfresh boiled shrimp juice	8.77 ± 2.01^{a}	7.73 ± 1.57^{a}

* Means ± SD (n=10).

Different superscripts ($^{a-b}$) in the same column indicate the significant differences ($p<0.05$).

Color, odor and overall liking scores of flavorant extracted from Pacific white shrimp head using Alcalase and Flavourzyme are shown in Table 15. The odor and overall liking scores of both sample were not significantly different ($p\geq0.05$) but the color liking score of the flavorant extracted by Alcalase was greater than that extracted by Flavourzyme ($p<0.05$) due to lighter brown color.

The acceptance scores of fish balls added with the 4% shrimp flavorant extracted by Flavourzyme and Alcalase are presented in Table 15. The overall and color acceptance scores of the Alcalase sample were higher than the Flavourzyme sample ($p<0.05$). Color of the fish ball added with flavorant extracted by both enzymes were pale brown but the fish ball added with Alcalase flavorant was lighter than that extracted with Flavourzyme. This result was in agreement with color liking score of the flavorant solution. However, no differences in odor, flavor/taste and texture acceptance scores between fish balls added with Alcalase and Flavourzyme flavorants as well as odor and overall liking scores of extracted solutions were observed ($p\geq0.05$). Generic descriptive analysis results in Table 14 showed the differences in intensity of roasted sundried shrimp, boiled sundried shrimp and boiled blue swimming crab odors of the flavorant solutions extracted by Alcalase and Flavourzyme. However, the differences did not affect odor and flavor acceptance scores of the fish ball samples.

Table 15 Acceptance score of flavorant solution and fish balls added with the 4% flavorant extracted from Pacific white shrimp head using 0.15% Flavourzyme at 55ºC, pH 7.0 and 0.15% Alcalase at 55ºC, pH 8.0 for 270 minutes

Attributes	Alcalase	Flavourzyme
Flavorant solution		
Color	6.93 ± 0.18^{a}	6.66 ± 0.18^{b}
Odor	6.73 ± 0.18^{a}	6.96 ± 0.18^{a}
Overall	6.66 ± 0.18^{a}	6.83 ± 0.14^{a}
Fish ball		
Color	6.90 ± 0.96^{a}	5.90 ± 1.63^{b}
Odor	5.80 ± 1.29^{a}	5.77 ± 1.59^{a}
Flavor/Taste	6.60 ± 1.25^{a}	6.20 ± 1.71^{a}
Texture	7.33 ± 0.96^{a}	7.07 ± 1.11^{a}
Overall	6.80 ± 1.03^{a}	6.13 ± 1.43^{b}

* Means ± SD (n=30).

Different superscripts ($^{a-b}$) in the same row indicate the significant differences ($p<0.05$).

2.4.4 Effect of sodium metabisulfite pretreatment on flavorant extracted from shrimp head

The comparison of flavorant extracted from pretreated and non-pretreated shrimp heads was conducted in terms of protein content and formaldehyde nitrogen content as well as acceptance scores of the extracted solutions. The results in Table 16 showed that the flavorant solution from non-pretreated shrimp head had the higher protein and formaldehyde nitrogen contents than that from pretreated shrimp head ($p<0.05$). This was due to the fact that soaking in sodium metabusulfite solution and rinsing with water before protein extraction might cause protein loss via dissolving in the solution and rinsing water.

Table 16 Protein and formaldehyde nitrogen contents of shrimp flavorant solution extracted from non- and melanosis prevention pretreated (soaking in 2.0% sodium metabisulfite solution for 15 min) Pacific white shrimp head

Sample	Protein content (% wet basis)*	Formaldehyde nitrogen content (g/ml)*
Non-pretreated shrimp head	8.34 ± 1.27^{a}	1.18 ± 0.07^{a}
Pretreated shrimp head	4.98 ± 0.22^{b}	0.69 ± 0.02^{b}

* Means ± SD (n=3).

Different superscripts ($^{a-b}$) in the same row indicate the significant differences ($p<0.05$).

The color and odor acceptance scores of flavorant solution from pretreated shrimp head tended to be higher than that extracted from non-pretreated shrimp but they were not significantly different ($p\geq0.05$). However, overall acceptance score of the flavorant from pretreated shrimp head was significantly higher than that from non-pretreated sample ($p<0.05$) (Table 17). Mizani *et al*. (2005) presented that shrimp protein hydrolysate extracted with the presence of sulfite had lighter color (pink) when compared with the sample extracted without sulfite (dark brown). Maillard reaction could performed under the heating condition of extraction method. Sodium bisulfite and sulfhydryl containing compounds had ability to inhibit Maillard reaction via reduced melanosis (Freidman and Molnar-Perl, 1990; Freidman, 1996).

Table 17 Acceptance scores of flavorant solution extracted from non- and pretreated (soaking in 2.0% sodium metabisulfite solution for 15 min) Pacific white shrimp head

Sample	Color*	Odor*	Overall*
Non-pretreated shrimp head	6.70 ± 1.39^{a}	6.03 ± 1.96^{a}	6.27 ± 1.76^{b}
Pretreated shrimp head	7.10 ± 1.03^{a}	6.70 ± 1.49^{a}	6.97 ± 1.43^{a}

* Means ± SD (n=30).

Different superscripts ($^{(a-b)}$) in the same row indicate the significant differences ($p<0.05$).

2.5 Conclusion

Soaking the Pacific white shrimp head in 2.0% sodium metabisulfite solution in the ratio of 1: 5 w/v for 15 min was the most effective pretreatment for melanosis prevention of Pacific white shrimp head. The extraction of Pacific white shrimp head either using 0.15% Alcalase: pH 8.0 at 55ºC or 0.15% Flavourzyme: pH 7.0 at 55ºC for 270 min was the optimal condition for shrimp flavorant extraction. The flavorant extracted using Flavourzyme obtained higher intensity of boiled blue swimming crab odor, but lower intensity of boiled sundried shrimp and roasted sundried shrimp odor than that using Alcalase. The odor, flavor/taste and texture acceptance scores of the surimi products with addition of 4% flavorant extracted by both enzymes were not significantly different. The flavorant slightly affected the color of the product. However, the overall acceptance score of Flavourzyme sample was higher than that of Alcalase. The pretreatment for melanosis prevention by soaking in 2.0% sodium metabisulfite solution reduced flavorant extraction yield but the resulted flavorant had the higher overall liking score than that extracted from non-pretreated sample.

CHAPTER 3

CHARACTERISTIC OF ENZYMATIC EXTRACTED SHRIMP FLAVORANT FROM PACIFIC WHITE SHRIMP HEAD AS AFFECTED BY DIFFERENT DRYING METHODS

3.1 Abstract

The shrimp flavorant sample was extracted from non-pretreated Pacific white shrimp head using Alcalase. The two different flavorant solutions were prepared. The first sample was evaporated until reach 20% total solid content (20), while another was 15% total solid content with 5% maltodextrin (15+5M). Both solutions were dried with different drying methods (freeze drying (FD), tray drying (TD) and spray drying (SD)). It was found that TD20 sample had the lowest water activity ($p<0.05$). The highest solubility was obtained from SD20, TD15+5M and SD15+5M samples ($p<0.05$). Descriptive analysis revealed that the flavorant before drying had the highest intensity of roasted sundried shrimp, boiled sundried shrimp, boiled blue swimming crab and unfresh shrimp juice odors ($p<0.05$). The intensity of those odors were decreased after drying in all conditions. The highest intensity of Mungoong (concentrated shrimp extract) odor was presented in TD15+5M, due to pyrazine compounds generated during drying process as indicated by gas chromatography mass spectrometry. The shrimp flavorant odors were mostly related to nitrogen-containing and aldehyde compounds. The acceptance test indicated that drying methods had no effect on odor and flavor/taste liking scores of fish ball added with 4% shrimp flavorant ($p\geq0.05$). However, color acceptance scores of fish balls added with the TD flavorants were lower than those of other methods ($p<0.05$). The fish balls added with SD20 sample, SD15+5M and TD20 samples had the highest overall acceptance score ($p<0.05$). The storage for 2 and 4 months under air and vacuum conditions were slightly affect the volatile compounds in TD20 flavorant as pyrazine compound slightly increased and some aldehydes decreased. However, the trained panelists could not detect the

differences between the intensities of odor characteristics of the samples during storage up to 4 months ($p \geq 0.05$).

3.2 Introduction

The protein recovered in the form of hydrolysates can be used as flavorant and incorporated into fish-based foods (Teerasuntonwat and Raksakulthai, 1995; Holanda and Netto, 2006). The previous studies showed that peptides and free amino acids contributed to flavor in marine food (Konosu and Yamaguchi, 1982; Raksakulthai and Haard, 1992; Teerasuntonwat and Raksakulthai, 1995). Enzymatic hydrolysis, one of the effective approaches for protein recovery from shrimp waste has been widely studied (Simpson and Haad, 1985; Cano-Lopez *et al.*, 1987; Synowiecki and Al-Khateeb, 2000). Teerasuntonwat and Raksakulthai (1995) produced flavoring agent from shrimp (*Peneaus monodon* Fabricius) head by extraction of nitrogenous compounds with water, sodium chloride, hydrochloric acid, sodium hydroxide, bromelain, papain and neutrase. The extracted samples with 10% w/w NaCl as a binder were dried in hot air oven at 50±5 ^{o}C for 72 hr. Acceptance test results showed that crackers containing enzyme extracting flavoring agents had higher score than the control (without flavoring agent), but lower score than the sample with commercial flavorant (krill extract). Gildberg and Stengerg (2001) reported that hydrolysates prepared with bromelain had better organoleptic quality than those prepared by other proteinases. The composition of volatile compounds from several shrimp products has been published. Manley *et al*. (2005) reported some important sulfur-containing compounds occurring in seafood such as dimethyl sulfide, dimethyl disulfide, methanethiol, methyl trithiomethane, 2-methylthioethanol and methional which contributed to raw or cooked shrimp odor. Nielsen *et al*. (2007) revealed that during the processing aldehydes and ketones compounds could be generated, while hydrocarbon (saturated, unsaturated and aromatic) levels was decreased. These lipid derived oxidation products led to the presence of off-odor in marine products. In addition Park *et al*. (2014) demonstrated that aldehydes (e.g. 3-methyl butanol, 2-methyl butanol) and s-contained

compounds (e.g. dimethyl sulfide, dimethyl disulfide) were the most abundant volatile compounds in krill (*Eupausia superba*) hydrolysate concentrated and powder.

Drying is one of the key processes to maintaining the product qualities. In the case of volatile compounds, the addition of carrier agent promotes protection against the drying air temperature, degradative reaction or volatilization during process and storage, as well as controls release of flavoring during application and consumption (Breternitz *et al*., 2017). Among the various carrier agents, polysaccharide including maltodextrin, modified starch, gum and syrup are widely used (Reineccius, 2001). Teerasuntonwat and Raksakulthai (1995) compared dextrose, dextrin and sodium chloride at 10% (w/w) as binders for drying of shrimp flavor extract using hot air oven at 50 ± 5°C for 72 hr. The result showed that samples using NaCl as binder had the strongest shrimp flavor. Bueno-Solano *et al*. (2009) used spray dryer (air inlet 180^{o}C and air outlet 140^{o}C, fluid input 1 l/hr) for dehydrated protein hydrolysate from shrimp (*Penaeus* spp.) by-product (cephalothorax and exoskeleton). The dry powder protein hydrolysate had a brown color and low values for brightness (L*= 44.42, b*= 17.86 and a*= 11.93). Kurozawa *et al*. (2009) indicated that an increasing of carrier agent (maltodextrin and gun Arabic) concentration decreased moisture content and bulk density of chicken meat protein powder. The addition of carrier agents also contributed significantly to powder stability by decreased in powder hygroscopicity and increased in glass transition temperature. Nevertheless, the diverse drying methods and conditions differently influence the properties, especially flavor characteristics, of the flavorant. Therefore, the objective of this study was to investigate the sensory characteristics and related volatile compounds of shrimp flavorant as affected by different drying methods.

3.3 Meterials and methods

3.3.1 Chemicals

Alcalase (protease from *bacillus licheniformis*) were procured from Sigma, Capricorn, Singapore. Formaldehyde was obtained from Merck (Darmstadt, Germanyl). Hydrochloric acid (37%), sodium hydroxide, sulfuric acid and petrolium ether were purchased from LAB-SCAN (Thailand). Potassium sulphate, copper sulphate and boric acid were procured from Ajax Finechem (New Zealand). Maltodextrin with 9-12 DE was ordered from Shandong Xiwang Imp. & Exp Trade Co.,Ltd, China.

3.3.2 Preparation of shrimp flavorant

The shrimp flavorant solution was prepared from non-pretreated shrimp heads with the selected condition from Part 2. Frozen Pacific white shrimp heads was thawed by running water and minced for 1 min using blender (AY46, Moulinex, China). Minced shrimp head was mixed water with a ratio of 1: 2 (w/v) and was adjusted pH to 8.0 followed by incubated at 55^{o}C for 10 min in water bath (Model W350, Memmert,Schwabach, Germany). Alcalase (0.15% w/w of shrimp head) was added into the system and allowed to hydrolyzed for 270 min with the continuous stirring. NaOH solution was to maintain pH. After finish, the sample was boiled for 10 min, cooled with running water and filtered through 2 layers cheesecloth. The gained solution was evaporated to obtain 15 and 20% total solid content. The 15% solid flavorant solution was added with maltodextrin to gain the 20% total solid content. Both flavorant solutions were subjected to drying using different drying methods.

3.3.3 Effect of drying methods on characteristic of shrimp flavorant

Both flavorant solutions with and without maltodextrin were dried using different drying methods as follows:

- Freezed drying

The shrimp flavorant solution was dried using freeze dryer (Dura Top^{TM} µp, FTS system, USA). The flavorant solutions were placed in petri dish for 0.5 cm height and dried in the chamber for 20 hr.

- Tray drying

500 ml of the evaporated flavorant solution was placed in 60×30 cm^3 tray. Sample was dried in hot air oven (Model FD115, BINDER, Tuttlingen, Germany) at 50^oC until approximately 2% moisture content. Dried sample was powderized using a blender (AY46, Moulinex, China) for 30 sec, then sieved through 60 mesh (250 µm).

- Spray drying

The samples were spray-dried by Niro spray dryer with a centrifugal atomizer (Nerco-Niro, Nicolas & Research Engineering Corporation, Copenhagen, Denmark) with feeding rate of 2.2 l/ hr. The heated inlet and outlet air temperature were 180 and 75^oC, respectively (Kanpairo *et al*., 2012).

The flavorant powder was packed in laminated BOPP (Biaxially Oriented Polypropylene) /Aluminum/LLDPE (Liner Low Density Polyethylene) bag and kept at room temperature (30^oC) until subject to analyses as follows:

3.3.3.1 Determination of solubility

The solubility of shrimp flavorant was determined by the method of Kahtani and Hassan (1990). The flavorant powder of (0.5 g: W_1) was placed in a 100 ml glass beaker and added with 5 ml distilled water at 25°C. The powder was gently mixed by a spatula for 1 min or until no more fine particles was seen. The solution was then filtered through a Whatman filter paper No.4. That was known for its weight (W_2). The filter paper was dried at 100°C for 4 h in a hot air oven, cooled in a desiccator and weighed again (W_3). The powder solubility was calculated by the equation belows.

$$\% \text{ Solubility} = 100 - \left(\frac{(W_3 - W_2) \times 100}{W_1}\right)$$

3.3.3.2 Color measurement

Color of flavorant powder was measured using color meter (ColorFlex, HunterLab, Reston, VA, USA) and reported in CIE LAB color scales (L*, a* and b* value). The standard illuminant D65 and 10° standard observers as well as a white standard plate (L*=93.59, a*=−0.98, b*=0.35) were used.

3.3.3.3 Water activity measurement

Water activity of shrimp flavorant powder was measured at room temperature using a water activity meter (Model Axair AG8808, Novasina, Pfäffikon).

3.3.3.4 Determination of volatile compounds

The flavorant powder was dissolved with water to prepared 4% solution. Volatile compounds of the flavorant solution was analyzed using headspace-solid phase microextraction for gas chromatography-mass spectrometry (HS-SPME-GC-MS) technique at Scientific Equipment Center, Prince of Songkla University, Songkhla, Thailand. Sample headspace was collected at 40^{o}C using SPME holder with Divinylbenzana/Carboxen/ Polydimethylsiloxane (DVB/CAR/PDMS), diameter 50/30 µm for 5 min. SPME holder with the headspace sample was connected to the injection port of GC-MS with the injection temperature 250^{o}C and feeding time for 5 min before analysis. The analysis was run using Agilent 6890 Plus GC/HP 5973 MSD under the condition in Table 18.

Table 18 GC/MS condition for flavor analysis

Condition	Column type: HP-Innowax
Length of column (m)	30
Diameter of column (mm)	0.25
Film thickness (µm)	0.25
Type carrier gas	Helium
Rate of carrier gas (ml/min)	1.0
Injection volumn (µl)	1.0
Mode of operation	Splitless
Injection temperature (^oC)	250
Oven temperature (^oC)	40 ^oC → 230^oC, 10^oC/min
	Hold at 230^oC for 3 min
Interface temperature (^oC)	270
Mass range (amu)	20-450
Ionization energy (eV)	70
Scan rate (scans/sec)	1.43

Source: Sukkwai (2012)

3.3.3.5 Sensory evaluation

3.3.3.5.1 Generic descriptive analysis

Generic descriptive analysis was performed as previously described in Part 2.3.4.3.2. using characteristics, descriptions and references as shown in Table 19. The sensory characteristics of shrimp flavorant solutions from different drying methods were screened and evaluated as described in Chapter 2.

Table 19 Reference samples and ratings used in descriptive analysis of dried shrimp flavorants

Attribute	Definition	Reference	Rating (cm)
Roasted sundried shrimp	Aromatic associated with roasted sundried shrimp, from slight to strong	The sundried shrimp was roasted at 550°C for 10 min and place in a 2 oz. cup with the lid.	
		- 1 g of roasted sundried shrimp	1.5
		- 2.5 g of roasted sundried shrimp	9.0
		- 5 g of roasted sundried shrimp	11.0
Boiled sundried shrimp	Aromatic associated with boiled sundried shrimp, from slight to strong	The sundried shrimp was soaked in distilled water with the ratio of 1:3 for 30 min before blend for 1 min. The mixture was boiled for 5 min (solution 1) and place 5 ml in a 2 oz. cup with the lid.	
		- solution 1	9.0
		- solution 1 diluted with distilled water with the ratio of 1:2	5.5
		- solution 1 diluted with distilled water with the ratio of 1:4	1.5
Boiled blue swimming crab	Aromatic associated with boiled blue swimming crab, from slight to strong	Blue swimming crab (250 g/ each) was boiled with distilled water with the ratio of 1:2 for 30 min.	
		- the crab was cut into 2 part, each part was place into 8 oz. cup with the lid	13.5
		- 5 ml of crab cooking juice was place in a 2 oz. cup with the lid	4.0

Table 19 (continuous)

Attribute	Definition	Reference	Rating (cm)
unfresh boiled shrimp juice	Aromatic associated with unfresh boiled shrimp juice, from slight to strong	Fresh shrimp (harvested within 48 hr) was storage at 4°C for 48 hr before boiled in distilled water with the ratio of 1:1, the cooking juice (solution 2) was diluted and placed in 2 oz. cup with the lid.	
		- solution 2	13.5
		- solution 1 diluted with distilled water with the ratio of 1:2	9.0
		- solution 1 diluted with distilled water with the ratio of 1:4	4.5
Mungoong	Aromatic associated with a shrimp extract paste made by boiling shrimp head, sugar and salt, from slight to strong	Mungoong was weighted and placed in a 2 oz. cup with the lid.	
		- 1 g of Mungoong	3.5
		- 2.5 g of Mungoong	9.0
		- 5 g of Mungoong	12.0

3.3.3.5.2 Acceptance test

Fish balls added with 4% total solid content of shrimp flavorant samples were judged by 30 consumer-type panelists using a 9-point hedonic scale for color, odor, flavor/taste and overall liking scores. The samples was prepared, presented and served as mentioned in Part 2.3.4.3.3.

The samples with the most acceptance and highest solubility were selected for further study and analyzes as follows.

3.3.3.6 Proximate analysis

Shrimp flavorant powder was determined for moisture, protein, fat and ash content according to AOAC (2000).

3.3.3.7 Determination of antioxidative activities

3.3.3.7.1 Determination of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ATBS) radical scavenging activity

ABTS radical scavenging activity was determined by the ABTS assay according to the method of Arnao *et al*. (2001) with a slight modification. The stock solutions include 7.4 mM ABTS solution and 2.6 mM potassium persulfate solution. The working solution was prepared by mixing the 2 stock solutions in equal quantities and allowing them to react for 12 hr at room temperature in the dark. The solution was then diluted by mixing 1 ml ABTS solution with 50 ml methanol to obtain an absorbance of 1.1 ± 0.02 units at 734 nm using the UV-1601 spectrophotometer. Fresh ABTS solution was prepared for each assay. Sample (150 µl) was mixed with 2850 µl of ABTS solution and the mixture was left at room temperature for 2 hr in the dark. Blue/green $ABTS^{\cdot+}$ chomophore can be generated through the reaction between ABTS and potassium persulfate. The extent of decolorization in the presence of antioxidant indicates the inhibition of the $ABTS^{\cdot+}$ radical cation (Re *et al*., 1999). The absorbance was then measured at 734 nm using UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). The standard curve using Trolox ranging from 50 to 600 µM was prepared. The activity was expressed as µmol Trolox equivalents (TE)/g shrimp flavorant.

3.3.3.7.2 Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

DPPH radical scavenging activity was determined by the DPPH assay as described by Wu *et al*. (2003) with a slight modification. Sample (1.5 ml) was added to 1.5 ml of DPPH in 95% ethanol. The mixture was then mixed vigorously and allowed to stand at room temperature in the dark for 30 min. The absorbance of the resulting solution was measured at 517 nm using an UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). The blank was prepared in the same manner except that distilled water was used instead of sample. The standard curve was prepared using Trolox in the range of 10 to 60 µM. The activity was expressed as µmol Trolox equivalents (TE)/g shrimp flavorant.

3.3.3.7.3 Determination of reducing power

The reducing power was determined according to the method of Wu *et al*. (2003) with a slight modification. Diluted sample (1 ml) was mixed with 1 ml of 0.2 M phosphate buffer (pH 6.6) and 1 ml of 1% potassium ferricyanide. The mixtures was incubated at 50^{o}C for 20 min in a water bath (Model W350, Memmert,Schwabach, Germany), followed by addition of 1 ml of 10% trichloroacetic acid. To an aliquot (1 ml) of reaction mixture, 1 ml of distilled water and 200 ml of 0.1% $FeCl_3$ were added. The absorbance of the resultant solution was read at 700 nm using an UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). The blank of each sample was prepared by adding the distilled water instead of $FeCl_3$. Net increased absorbance of the reaction mixture indicates increased reducing power.

3.3.3.7.4 Determination of chelating activity

The chelating activity of Fe^{2+} was measured using the method of Boyer and McCleary (1987) with a slight modification. Diluted sample (4.7 ml) was mixed with 0.1 ml of 2 mM $FeCl_2$ and 0.2 ml of 5 mM ferrozine. The reaction mixture was allowed to stand for 20 min at room temperature. The absorbance was then read at 562 nm using an UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan); the blank used distilled water instead of the sample. The standard curve was prepared using ethylenediaminetetraacetic acid (EDTA) in the range of 0 to 80 mg/ml.

3.3.4 Storage stability of flavorant powder from Pacific white shrimp head as affected by packaging method

The selected flavorant powder samples from Part 3.3.3 were kept in laminated BOPP (Biaxially Oriented Polypropylene)/Aluminum/LLDPE (Liner Low Density Polyethylene) bag under air or vacuum conditions at room temperature for 4 months. The samples were subjected to analyses every 2 months as follows:

3.3.4.1 Determination of volatile compounds as described in Part 3.3.3.4.

3.3.4.2 Determination of thiobarbituric acid reactive substances (TBARs) value

TBARS was determined by the method of Buege and Aust (1978). The sample (0.5 g) was mixed with 2.5 ml of a solution containing 0.375% thiobarbituric acid (w/v), 15% trichloroacetic acid (w/v) and 0.25 M HCl. The mixture was heated in boiling water (95-100^{o}C) for 10 min to develop a pink color, cooled with running tap water and centrifuged at 3600×*g* at 25^{o}C for 20 min using a centrifuge (Beckman Coulter, Avanti J-E Centrifuge, Fullerton, CA, USA). The absorbance of the supernatant was measured at 532 nm using a spectrophotometer. A standard curve was prepared using 1,1,3,3-tetramethoxypropane at the concentrations ranging from 0 to 6 ppm. TBARS were calculated and expressed as mg malonaldehyde/kg lipid.

3.3.4.3 Sensory evaluation

Generic descriptive analysis was conducted as described in Part 2.3.4.3.3.

3.3.5 Statistical Analysis

All experiments were run in triplicate and reported as mean ± standard deviation. Completely randomize design (CRD) was used for analysis of physical, chemical and generic descriptive analysis. A randomize complete block design (RCBD) was carried out for analysis of acceptance test. Data was subjected to analysis of variance (ANOVA). Comparison of means were carried out by Duncan's multiple range test (Steel and Torrie, 1980) at a significant level $p<0.05$. Analysis was performed using a SPSS package (SPSS 10.0 for window, SPSS Inc, Chicago, IL).

3.4 Results and discussion

3.4.1 Physical and chemical properties of shrimp flavorant powder

The properties of shrimp flavorant powders prepared from different drying methods were presented in Table 20. The results showed that the highest solubility was obtained from SD15+5M, SD20 and TD 15+5M, while the TD15+5M showed the highest water activity ($p<0.05$). The spray dried samples had higher solubility than others because of its smaller particle size. Moreover, the presence of maltodextrin increases the solubility of atomized samples due to its high solubility (Cano-Chauca *et al*., 2005; Grabowski *et al*., 2006; Goula and Adamopoulos, 2010). Water activity of samples with maltodextrin was higher than those without maltodextrin using the same drying method, since water molecules difficulty diffused through the larger maltodextrin molecules (Adhikari *et al*., 2004).

Table 20 Solubility, a_w and color of flavorant extracted from Pacific white shrimp head using 0.15% Alcalase at 55°C, pH 8.0 for 270 minutes and drying with the difference conditions

Sample	Solubility (%)[1]	a_w[1]	Color[2]		
			L*	a*	b*
FD 15+5M	87.13 ± 3.33^{b}	0.327 ± 0.004^{c}	57.86 ± 0.00^{d}	12.70 ± 0.07^{b}	22.91 ± 0.10^{b}
FD 20	84.92 ± 3.16^{b}	0.233 ± 0.004^{d}	53.40 ± 1.19^{f}	11.88 ± 0.02^{c}	22.46 ± 0.25^{c}
SD 15+5M	91.29 ± 1.89^{a}	0.344 ± 0.004^{b}	83.18 ± 0.06^{a}	5.87 ± 0.23^{e}	18.44 ± 0.13^{f}
SD 20	88.69 ± 0.61^{ab}	0.238 ± 0.003^{d}	76.71 ± 0.00^{b}	6.99 ± 0.07^{d}	21.08 ± 0.03^{e}
TD 15+5M	91.95 ± 1.26^{a}	0.354 ± 0.004^{a}	54.77 ± 0.63^{e}	13.65 ± 0.37^{a}	24.74 ± 0.14^{a}
TD 20	86.94 ± 0.75^{b}	0.220 ± 0.002^{e}	60.62 ± 0.65^{c}	6.88 ± 0.09^{d}	22.07 ± 0.25^{d}

[1] All values are means ± standard diviation (n=3).

[2] All values are means ± standard diviation (n=10).

FD: Freeze drying, SD: Spray drying, TD: Tray drying.

15+5M: The flavorant with 15% total solid content and added with 5% maltodextrin.

20: The flavorant solution with 20% total solid content.

Different superscripts ($^{a-e}$) in the same column indicate the significant differences ($p<0.05$).

When considered on color of the samples, the SD15+5M had the lightest color as indicated by the highest L*, low redness (a*) and yellowness (b*), while the darkest sample was TD15+5M. This result was in agreement with Bueno-Solano *et al*. (2009) who found that the shrimp (*Penaeus* spp) protein hydrolysate dried by spray dryer had a brown color with low value of brightness. The dark color of tray dried sample was possibly due to Maillard's reaction. The reaction between sugar and amino acid was taken place in thermally processed food (Carabasa-Giribet and Ibarz-Ribas, 2000). During the shrimp flavorant solution (protein solution) was dried in hot air oven at 80ºC for 36 hr, amino groups in soluble proteins or hydrolyzed peptides could react with carbonyl groups of reducing sugar or lipid oxidation products and formed melanoidins and heterocycles compounds (Friedman, 1996). Moreover, particle size was reported as a factor affecting the color of the powdered product (Yu *et al*., 2000; Purohit *et al*., 2001; Prakongpan *et al*., 2002). For instance, Prakongpan *et al*. (2002) found that for the extracted pineapple dietary fiber and core cellulose, the small-size particle had lighter color than the large-size fiber.

3.4.2 Volatile compounds of shrimp flavorant powder

Approximately 44 volatile compounds in shrimp flavorant dried using different conditions were detected and identified as listed in Table 21. The compounds consisted of 12 alcohols, 4 aldehydes, 3 ketones, 11 nitrogen containing compounds, 4 aromatic compounds, 7 acids, 2 sulfur containing compounds and 1 other compound.

Numerous alcohols were found in the shrimp flavorant dried with various conditions. Giri *et al.* (2010) reported that branched-chain alcohols were formed by secondary decomposition of hydroperoxides of the n-3 and n-6 polyunsaturated fatty acids. Because of the high odor threshold values of alcohols when compared to other volatile compounds, alcohols were less effect on the odor of the product, but unsaturated alcohols such as 1-octen-3-ol, with usually lower threshold values, were expected to have a higher impact on the overall aroma (Kawai and Sakaguchi, 1996; Selli and Cayhan, 2009). In addition, some glycols found in the samples could be obtained from the Maillard reaction (Belitz *et al*., 2009). However, those compounds were less impact on the product odor.

Two branched chain (3-methylbutanal and n-pentanal) and one aromatic (benzaldehyde) aldehydes including the derivatives were identified in all samples with the different abundance. Aldehydes were more likely generated from lipid oxidation during fermentation. The high content of ω-3 fatty acid in shrimp was highly susceptible to lipid oxidation. Branched short chain aldehydes or aromatic aldehydes plausibly resulted from deamination of amino acids (Steinhaus and Schieberle, 2007). The odor thresholds of 3-methylbutanal, n-pentanal and benzaldehyde were 9-37.3, 12-42 and 350-3500 ppb, respectively (Leffingwell and Leffingwell, 1990). Those volatile compounds with the high content and low threshold contributed to the aroma of prawn, shrimp (Morita *et al*., 2001) and malty (Tachihara *et al*., 2004). Benzaldehyde and 3-methylbutanal compounds were also present in FD20 and FD15+5M samples. However, the contents of those compounds were decreased in the samples dried using thermal conditions.

Three ketones were found in the samples. 2-heptanone and 3-hydroxy-2-butanone were produced by oxidation or pyrolysis of polyunsaturated fatty acids and involved with a nasty smell in seafood (Lee *et al*., 2003). Cha *et al*. (1999) reported that ketones greatly affected the flavor of fresh fish due to low threshold values. The odor thresholds of 2-heptanone and 3-hydroxy-2-butanone were 140-3000 and 800 ppb, respectively (Leffingwell and Leffingwell, 1990). Ketones were found in all samples with the low abundance, except the FD15+5M sample consisting of high abundance of 3-hydroxy-2-butanone with related to sewage odor (Morita *et al*., 2001).

Nitrogen containing compounds, which were all pyrazine derivatives, were mostly found in the samples using thermal conditions i.e. spray drying and tray drying. Rodríguez-Bernaldo *et al*. (2001) reported that pyrazines was probably thermally generated via Maillard reaction through Strecker degradations from various nitrogen sources such as amino acids in heat processed foods. TD samples contained of more pyrazine compounds with higher contents when compared with spray drying samples, due to a long drying time (36 hr). Furthermore, it was noted that TD15+5M sample comprised more abundance of pyrazine compounds than that without maltodextrin. Maltodextrin is a group of compounds derived from acid or enzymatic hydrolysis of starch containing oligomers or/and polymers

of α(1,4) D-glucose, with a dextrose equivalent (DE) less than 20 (Zheng *et al*., 2007). Drying condition with high temperature and long drying time might cause the degradation of maltodextrin to glucose and led to increase the Maillard reaction. Major pyrazine compounds found in TD15+5M sample were trimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, 2,3,5-trimethyl-6-ethylpyrazine and tetramethylpyrazine which contributed to prawn, roasted, nutty and dried seafood like odor, respectively (Morita *et al*., 2001; Tachihara *et al*., 2004). The pyrazines with low odor detection threshold including trimethylpyrazine (400 ppb), 2-Ethyl-3,5-dimethylpyrazine (1 ppb) and tetramethylpyrazine (1000 ppb) (Leffingwell and Leffingwell, 1990), had been reported to contribute nutty, roasted and toasted characteristics to food (Maga and Sizer, 1973).

Dimethyl disulfide and dimethyl-(2,3,4-trithiapentane)-trisulfide were absent in shrimp flavorant before drying and FD15+5M sample but were present in others samples with low abundance. TD15+5M sample had the highest sulfur containing compounds content. They might be generated from the thermal breakdown of sulfur containing amino acids and commonly found in most thermally processed crustaceans such as shrimp, crab and crayfish (Buttery *et al*., 1976; Rodríguez-Bernaldo *et al*., 2001).

The acids such as acetic, valeric acid, hexanoic acid, 5,8,11,14,14-Eicosapentaenoic acid, palmitic acid, oleic acid and myristic acid with relatively low threshold values could be formed during storage and hydrolysis of shrimp head. The previous studies showed that those compounds were generally fermentation products in several fish products. In addition, they could be derived from the breakdown of fatty acid chains and also from the Maillard reaction (Giri *et al*., 2010; Montel *et al*., 1998).

For the other compounds, tridecane was a hydrocarbon odor obtained in the sample with maltodextrin. This compound found in various species of marine animal (Hesselberg and Seelye, 1982).

Table 21 Selected volatile compounds of the shrimp flavorants extracted from Pacific white shrimp head using 0.15% Alcalase at 55ºC, pH 8.0 for 270 min and dried with the different drying conditions.

Code	Compounds	RT	**Peak area** (abundance) × 10^9						
			BFD	FD 15+5M	FD 20	SD 15+5M	SD 20	TD 15+5M	TD 20
	Alcohols								
Alc1	1-pentanol	6.07	0.38	0.65	0.87	0.21	0.27	0.70	0.36
Alc2	1,5-octadien-3-ol	9.05	0.51	ND	0.93	ND	0.24	ND	0.23
Alc3	1-octanol	9.92	3.03	0.60	0.40	12.54	2.29	ND	2.83
Alc4	2-furanmethanol	11.11	ND	ND	ND	0.24	0.17	0.71	0.39
Alc5	2-octen-1-ol	13.17	ND	ND	0.41	ND	0.25	ND	0.18
Alc6	benzenemethanol	13.56	ND	0.24	ND	ND	0.33	0.05	0.15
Alc7	2-[2-(2-ethoxyethoxy)ethoxy]-ethanol	15.04	ND	ND	0.19	0.33	0.27	0.06	0.27
Alc8	2,2'-[1,2-ethanediylbis(oxy)bis-ethanol	16.06	0.39	ND	0.18	0.24	0.26	ND	0.17
Alc9	2-[2-(2-ethoxyethoxy)ethoxy]ethanol	18.86	ND	0.24	ND	0.83	1.30	0.08	0.88
Alc10	triethylene glycol	17.92	0.47	0.32	0.60	0.59	0.63	0.13	0.44
Alc11	hexaethylene glycol	19.12	0.67	ND	5.26	0.78	0.55	0.13	0.57
Alc12	pentaethylene glycol	21.79	3.91	ND	1.18	0.95	1.22	0.27	0.73
	Aldehydes								
Ald1	3-methyl-butanal	2.59	18.11	51.91	19.77	4.72	11.91	31.65	16.82
Ald2	n-pentanal	3.03	1.60	0.37	0.62	1.05	0.83	0.73	1.08
Ald3	n-hexanal	3.98	0.39	0.21	0.46	0.57	0.61	ND	ND
Ald4	hexanal	6.50	ND	ND	ND	0.22	ND	2.18	0.17
	Ketones								
K1	3-methyl-1-azidobutan-2-one	4.47	ND	ND	ND	ND	ND	0.26	0.22
K2	2-heptanone	5.16	0.54	0.36	0.61	0.92	0.75	0.12	0.36
K3	3-hydroxy-2-butanone	6.68	ND	19.51	0.17	0.23	0.17	ND	0.11

Table 21 (Continue)

Code	Compounds	RT	**Peak area** (abundance) × 10^9						
			BFD	FD 15+5M	FD 20	SD 15+5M	SD 20	TD 15+5M	TD 20
	N-containing compounds								
P1	2,5-dimethylpyrazine	7.02	2.64	0.43	0.72	1.13	1.84	2.24	1.24
P2	2-ethyl-3-methylpyrazine	7.90	2.50	0.25	0.28	1.30	3.66	ND	0.87
P3	trimethylpyrazine	8.08	ND	ND	ND	ND	0.17	7.87	0.84
P4	2-ethyl-3,5-dimethylpyrazine	8.57	0.90	ND	1.56	1.77	1.55	3.91	1.51
P5	tetramethylpyrazine	8.97	ND	ND	ND	ND	ND	3.30	ND
P6	2,3-diethyl-5-methylpyrazine	9.18	ND	ND	0.65	0.47	0.53	1.04	0.97
P7	2,3,5-trimethyl-6-ethylpyrazine	9.44	ND	ND	0.26	ND	0.26	3.52	0.40
P8	2,6-dimethyl-4-(t-butyl)pyrimidine	9.80	ND	ND	0.28	ND	1.89	0.71	2.67
P9	2,3,5-trimethyl-6-isobutulpyrazine	10.40	ND	ND	ND	ND	ND	0.15	2.08
P10	2,5-dimethyl-3-(3-methylbuthyl-)pyrazine	10.57	ND	ND	ND	0.32	1.46	0.14	0.61
P11	2,3-dimethyl-5-isopentylpyrazine	11.19	ND	ND	ND	0.15	1.01	0.48	0.15
	S-containing compounds								
S1	dimethyldisulfide	3.87	ND	ND	0.15	0.48	0.13	21.39	4.02
S2	2,3,4-drithiapentane-dimetyltrisulfide	7.75	ND	ND	0.16	ND	3.90	5.81	0.36
	Aromatic compounds								
Aro1	methylbenzene (Toluene)	3.57	ND	0.54	0.48	1.65	0.28	1.00	0.92
Aro2	benzaldehyde	9.59	47.68	12.38	49.57	55.83	53.95	6.61	53.40
Aro3	benzeneacetaldehyde	11.04	ND	0.82	0.31	0.15	0.22	0.61	0.17
Aro4	benzeneacetonitrile	14.17	ND	ND	0.17	ND	ND	0.18	0.33

Table 21 (Continue)

Code	Compounds	RT	**Peak area** (abundance) × 10^9						
			BFD	FD 15+5M	FD 20	SD 15+5M	SD 20	TD 15+5M	TD 20
	Acid								
Aci1	acetic acid	8.64	ND	4.61	0.76	ND	ND	ND	ND
Aci2	valeric acid	11.27	ND	0.77	ND	ND	0.14	0.16	0.17
Aci3	hexanoic acid	13.26	ND	ND	0.34	ND	0.12	ND	0.15
Aci4	5,8,11,14NDEicosapentaenoic acid	15.43	ND	ND	0.36	0.54	0.33	0.08	0.13
Aci5	palmitic acid	19.30	0.53	ND	4.54	0.58	1.22	0.71	0.36
Aci6	oleic acid	19.43	2.25	1.34	ND	ND	0.39	ND	ND
Aci7	myristic acid	21.63	ND	0.51	0.69	2.07	0.66	0.11	0.23
	Others								
Oth1	tridecane	6.45	0.57	ND	0.79	ND	0.27	ND	0.14

ND: non detectable.

3.4.3 Sensory characteristics and their relation to volatile compounds

Table 22 shows the intensity of characterizing odors of the shrimp flavorants extracted from Pacific white shrimp head using Alcalase and dried with the different conditions. The relationship among all samples, odor characteristics and volatile compounds in shrimp flavorant are illustrated by Principal Component Analysis (PCA). The first three components explain 70.59% (PC1: 36.19%, PC2: 19.91% and PC3: 14.49%) of the total variance as shown in Figure 10. All odor characteristics evaluated by trained panelists depended on PC1. Mungoong (MG) odor, 1 alcohol, 1 aldehyde, 1 ketone, 4 pyrazines, 2 sulfur-containing compounds and 1 aromatic compound were distributed on the negative PC1 and related to the TD15+5M sample. Mungoong is shrimp paste product obtained from boiling and evaporated shrimp head with sugar and salt (TCPS 324, 2004). Major compounds from the Mungoong product were produced by Maillard reaction taken place during processing. Amino groups in soluble proteins or hydrolyzed peptides could react with carbonyl groups of reducing sugar or lipid oxidation products formed during processing (Ogasawara *et al*., 2006). Pyrazine compounds and sulfur-containing compounds found in the flavorant sample with maltodextrin were positively related to Mungoong odor. The high correlation value of TD20 and 2,6-dimethyl-4-(t-butyl) pyrimidine as well as 2,5-dimethyl-3-(3-methylbuthyl-) pyrazine was depended on PC5 (data not shown).

FD15+5M and BFD were located on negative PC2 which were associated to with 3-hydroxy-2-butanone (sewage odor; Morita *et al*., 2001), 3-methylbutanal (shrimp, malty odors; Morita *et al*., 2001; Tachihara *et al*., 2004), acetic acid (Sour, vinegar, pungent; Morita *et al*., 2001) and valeric acid.

Table 22 The intensity of characterizing odors of the shrimp flavorants extracted from Pacific white shrimp head using 0.15% Alcalase at 55°C, pH 8.0 for 270 min and dried with the different conditions.

Attributes	Intensity (cm)						
	BFD	FD 15+5M	FD 20	SD 15+5M	SD 20	TD 15+5M	TD 20
Roasted sundried shrimp	7.10 ± 0.82^{a}	3.34 ± 0.94^{b}	2.52 ± 1.54^{c}	2.75 ± 1.39^{bc}	3.25 ± 1.06^{b}	0.92 ± 0.76^{d}	3.07 ± 1.27^{bc}
Boiled sundried shrimp	3.72 ± 1.42^{a}	2.67 ± 1.30^{bc}	3.07 ± 1.05^{ab}	3.04 ± 1.29^{ab}	3.63 ± 1.21^{a}	2.05 ± 1.43^{d}	2.69 ± 1.64^{bc}
Boiled blue swimming crab	6.53 ± 0.58^{a}	2.64 ± 0.85^{c}	2.53 ± 1.10^{c}	2.35 ± 0.80^{c}	4.91 ± 0.93^{b}	0.99 ± 0.65^{d}	2.29 ± 0.97^{c}
Unfresh shrimp juice	8.13 ± 1.09^{a}	3.11 ± 1.25^{c}	4.46 ± 1.20^{b}	3.27 ± 0.57^{c}	4.71 ± 1.08^{b}	1.39 ± 0.65^{d}	2.99 ± 0.83^{c}
Mungoong	0.00 ± 0.00^{e}	0.78 ± 0.72^{d}	0.96 ± 0.89^{cd}	0.73 ± 0.79^{d}	1.28 ± 0.98^{c}	8.56 ± 1.40^{a}	1.96 ± 0.82^{b}

Note BFD mean the flavorant sample before dried, FD mean Freeze drying, SD mean Spray drying, TD mean Tray drying.

15+5M mean flavorant solution was evaporated to obtain 15% total solid content and adjusted to gain 20% total solid content using maltodextrin before drying

20 mean flavorant solution was evaporated to obtain 20% total solid content before drying

Means ± SD (n=10).

Different superscripts $^{(a\text{-}d)}$ in the same row indicate the significant differences ($p<0.05$).

SD20, SD15+5M and FD20 samples were appeared on positive PC1 and positive PC2 and related to boiled sun dried shrimp odor and numerous alcohols, aldehyde, acid, aromatic compounds, 2 pyrazine and tridecane. However, FD 20 sample had high correlation with PC4 (data not shown) with the positive relation to 1-pentanol, 1,5-octadien-3-ol, hexaethylene glycol, hexanoic acid and palmitic acid and negative relation to 2-[2-(2-ethoxyethoxy) ethoxy]ethanol. The odor of alcohol and acid compounds were reported as fruity, green, wax (Tachihara *et al*., 2004) and sweet, pungent, cheesy (Tachihara *et al*., 2004), goat-like, rancid (Ishizaki *et al*., 2005), respectively. Acids were formed during storage and hydrolysis of shrimp head as well as the breakdown of fatty acid (Montel *et al*., 1998; Giri *et al*., 2010).

The plot of positive PC1 and positive PC3 show the relation of roasted sundried shrimp, boiled sundried shrimp, boiled blue swimming crab and unfresh shrimp juice odors with n-pentanal, 2-ethyl-3-methylpyrazine, 2,2'-[1,2-ethanediylbis(oxy) bis-ethanol, pentaethylene glycol and oleic acid. Those odor characteristics and volatile compounds were related to the flavorant before drying sample. 2-ethyl-3-methylpyrazine was described as prawn and roast, nutty odors, respectively (Morita *et al.*, 2001; Tachihara *et al.*, 2004). The intensity of roasted sundried shrimp, boiled sundried shrimp, boiled blue swimming crab and unfresh shrimp juice odors were decreased after drying for all conditions in the descending order of freeze drying, spray drying and tray drying, respectively.

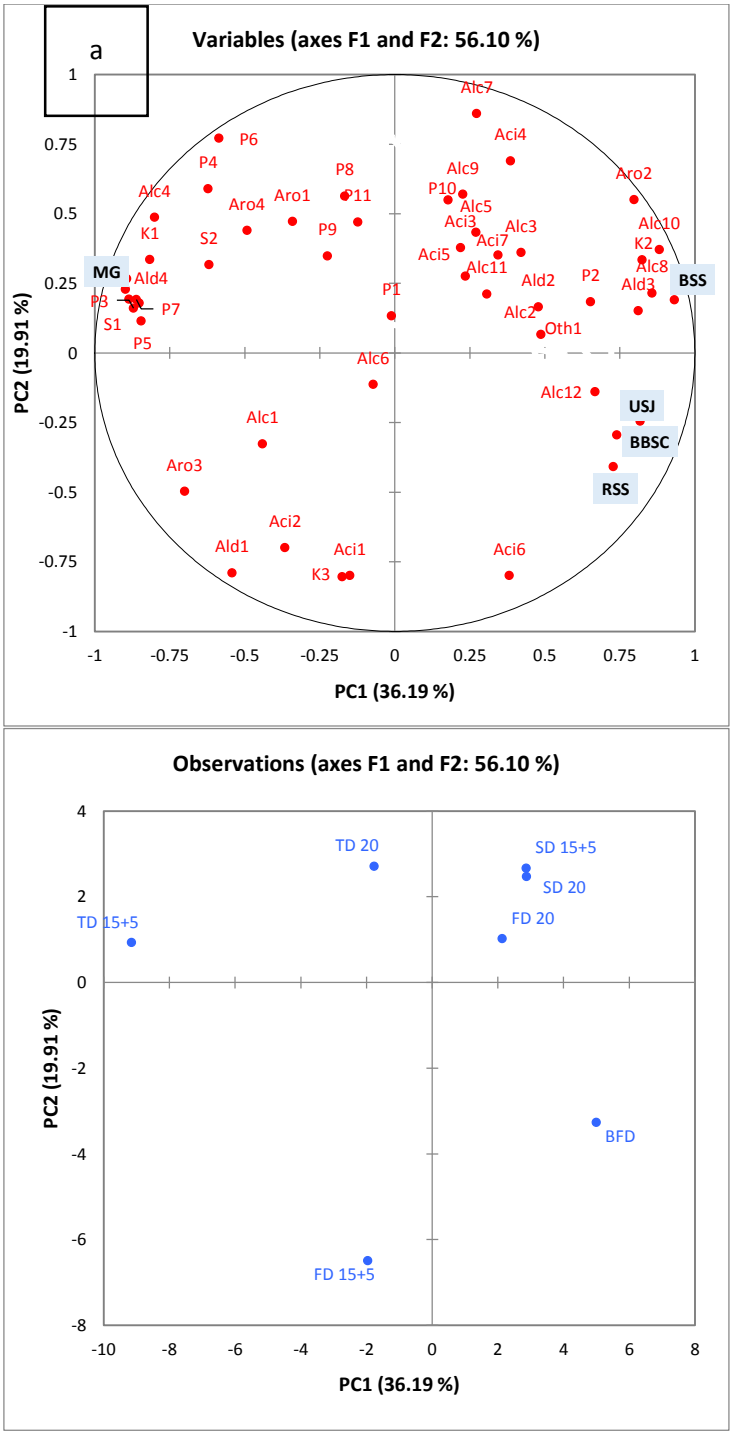
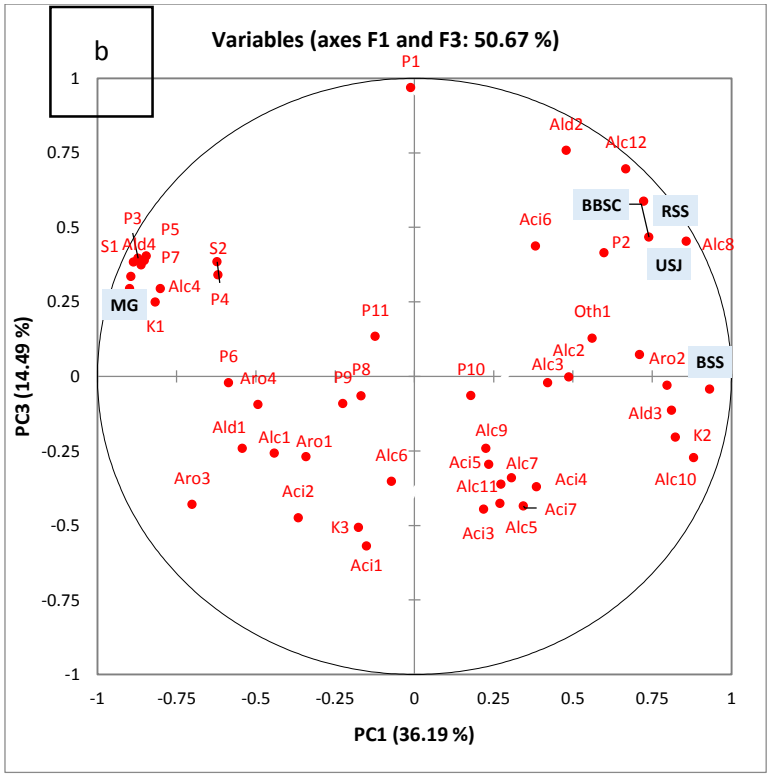


Figure 10 PCA plots of odors and volatile compounds of flavorant extracted from Pacific white shrimp head on (a) PC1 and PC2, (b) PC1 and PC3



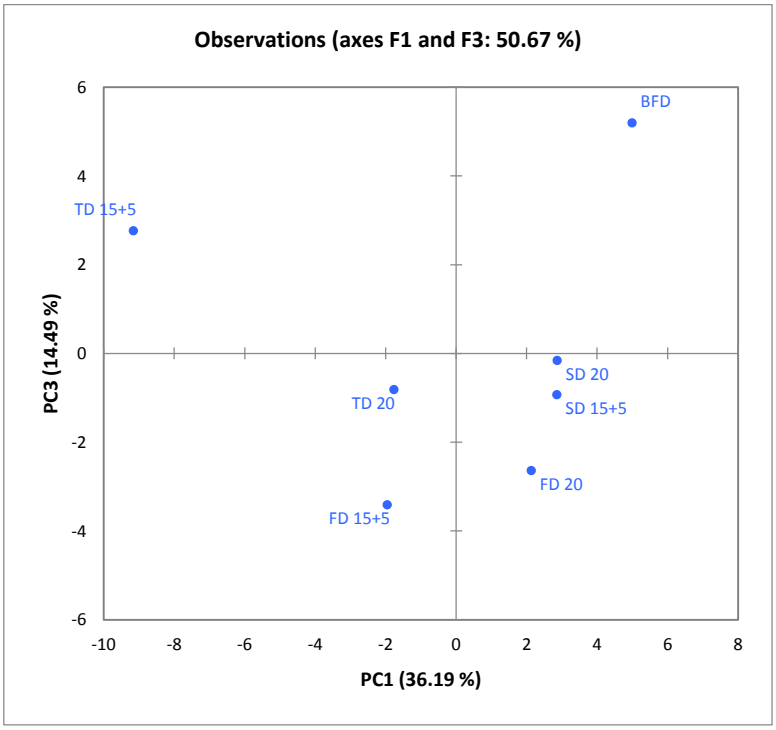


Figure 10 (Continued)

The Acceptance score of fish balls added with the 4% flavorant powder using the various drying methods are shown in Table 23. Odor and taste/flavor acceptance scores of all samples were not significantly different ($p \geq 0.05$). However, the highest color acceptance score was obtained from FD15+5M, FD20, SD15+5M, SD20 ($p < 0.05$). This result was related with the color of the flavorant samples shown in Table 20. The fish ball with freeze and spray dried flavorants had pale pink color while the fish balls added with TD flavorants obtained a pale brownish color, which affected on the color acceptance score from the consumer-type panelists. The highest overall acceptance score was obtained from SD15+5M, SD20 and TD20, while the sample with FD flavorant added obtained the lowest score ($p < 0.05$). During the shrimp flavorant solution was drying in the thermal condition, some volatile compounds including the compounds that represent 'off odor' including 3-hydroxy-2-butanone (sewage odor), dimethyldisulfide (sulfer), 2,3,4-drithiapentane-dimetyltrisulfide (fermented), acetic acid (pungent), hexanoic acid (rancid) (as shown in Table 21) could be evaporated. Refer to Manley and others (2005), the volatile aroma components are susceptible to loss during thermal treatments, depending on the degree of volatility. Thus, the freeze dried samples might contained more volatile compounds relating off odor when compared with the samples dried using thermal process (spray and tray drying) and affected on the overall acceptance score of the fish ball samples.

Although, the SD15+5M, SD20 and TD20 samples showed the highest overall acceptance score, TD20 was selected for the further studies due to the higher yield (data not shown).

Table 23 Acceptance score of fish balls added with the 4% flavorant extracted from Pacific white shrimp head using 0.15% Alcalase at 55ºC, pH 8.0 for 270 minutes and drying with the different conditions

Sample	Hedonic score (9-point)			
	Color	Odor	Taste/ Flavor	Overall
FD 15+5M	6.30 ± 1.49^{a}	5.63 ± 1.52^{a}	5.60 ± 1.38^{a}	5.57 ± 1.41^{b}
FD 20	6.43 ± 1.17^{a}	5.57 ± 1.45^{a}	5.83 ± 1.29^{a}	5.37 ± 1.33^{b}
SD 15+5M	6.03 ± 1.67^{ab}	5.77 ± 1.63^{a}	5.57 ± 1.48^{a}	5.90 ± 1.37^{ab}
SD 20	6.40 ± 1.52^{a}	5.80 ± 1.65^{a}	5.97 ± 1.65^{a}	6.23 ± 1.45^{a}
TD 15+5M	5.10 ± 1.42^{c}	5.47 ± 1.96^{a}	5.70 ± 1.86^{a}	5.57 ± 1.72^{b}
TD 20	5.63 ± 1.30^{b}	5.57 ± 1.81^{a}	6.13 ± 1.25^{a}	6.13 ± 1.33^{a}

1 All values are means ± standard diviation (n=30).

FD: Freeze drying, SD: Spray drying, TD: Tray drying.

15+5M: The flavorant with 15% total solid content and added with 5% maltodextrin.

20: The flavorant solution with 20% total solid content.

Different superscript ($^{a-e}$) in the same column indicate the significant differences ($p<0.05$).

The proximate analysis and antioxidative activity of flavorant powder extracted from non-pretreated shrimp head using Alcalase for 270ºC and dried by tray drying method are shown in Table 24. The main component of the flavorant was protein with 76.35% followed by ash, moisture and fat for 16.22, 5.75 and 4.47%, respectively. Amino acids and peptides were classified as flavorant compounds by Manley *et al.* (2005) and Vandanjon *et al*. (2002). In this study, Alcalase was used to recover those compounds from shrimp head with continuous stirring. When compared with raw Pacific white shrimp head (Table 10) (53.99% protein, 13.24% fat and 15.91% ash) the flavorant had much lower fat and higher ash contents. It was possible that fat could be liberated to the extracted solution by mechanical force during enzymatic extraction. In addition, ash content might be generated from NaOH used for maintaining pH in enzymatic extraction (Nielsen, 1997).

Table 24 Proximate analysis and antioxidative activities of flavorant extracted from Pacific white shrimp head using 0.15% Alcalase at 55ºC, pH 8.0 for 270 min and dried by tray drying method without maltodextrin

Compositions and antioxidative activities	Values
Moisture content (%)	5.75 ± 0.13
Protein content (% dry basis)	76.35 ± 0.19
Fat content (% dry basis)	4.47 ± 0.19
Ash content (% dry basis)	16.22 ± 0.16
DPPH radical scavenging activity (µmol TE eq/g)	76.11 ± 0.25
ABTS radical scavenging activity (µmol TE eq/ g)	247.58 ± 2.13
Ferric reducing power (µmol TE eq/ g)	71.15 ± 0.45
Metal chelating activity (µg EDTA eq/ g)	88.99 ± 2.33

All values are means ± standard diviation (n=3).

As reported by the previous studies, protein hydrolysate was found to be responsible for antioxidant activity (Amarowicz and Shahidi, 1997; Jeon *et* al., 1999; Kristinsson and Rasco, 2000; Gildberg and Stenberg, 2001; Binsan *et al*., 2008; Benjakul *et al*., 2009; Dey and Dora, 2014). Therefore, the antioxidative activities of the flavorant were performed and the results are shown in Table 24. This result was similar to Mungoong product produced by different Flavourzyme concentration which obtained DPPH radical scavenging activities, ABTS radical scavenging activities and ferric reducing power of 18-28, 130-240 and 28-48 µmol TE/g, respectively (Benjakul *et al*., 2009)

3.4.4 Effect of packaging on stability of flavorant from shrimp head

The stability of flavorant powder was tested under air and vacuum conditions. The differentiations of volatile compounds of the samples are shown in Table 25. Pyrazine compounds were the most abundance of the volatile compounds found in the flavorant powder extracted from Pacific white shrimp head and dried using tray drying method. These compounds were generated during the drying at high temperature as described in Part 3.4.2 and 3.4.3. A slightly increasing of the compounds including as 3-ethyl-2,5-dimethylpyrazine, 2,3-diethyl-5-methylpyrazine, 2,3,5-trimethyl-6-ethylpyrazine and 2,5-dimethyl-3-(3-methylbuthyl-)pyrazine was observed. While 2-methyl butanal contributed to shrimp (Morita *et al*., 2001) or malty odors (Tachihara *et al*., 2004) decreased for both vacuum and air conditions. It was noticeable that the samples kept under vacuum had the higher abundance of 2-methyl butanal than those kept in air. The result also showed that samples kept in air contained n-pentanal after storage for 2 and 4 months. The result was in agreement with Nielsen *et al*. (2017) who showed that krill powder had lower abundance of pentanal in the vacuum sample when compared with that kept in air. The aldehyde compounds were generated from the lipid oxidation (Steinhaus and Schieberle, 2007), therefore, under condition with air, fat in flavorant powder could reacted with the oxygen within the package.

Table 25 Selected volatile compounds of the shrimp flavorants powder extracted from Pacific white shrimp head packed under air and vacuum.

No.	Compounds	RT	**Peak area** (abundance) × 10^9				
			0 month	Air		Vacuum	
				2 months	4 months	2 months	4 months
1	2-methyl-butanal	2.97	3.09	1.48	1.45	2.10	2.09
2	n-pentanal	3.48	-	0.69	0.56	-	-
3	unidentified	3.58	1.31	0.38	0.82	1.27	1.18
4	dimethyldisulfide	4.41	7.01	6.27	7.00	7.44	5.84
5	3-methyl-1-azidobutan-2-one	4.95	0.26	0.24	0.19	0.22	0.37
6	2-heptanone	5.68	0.61	0.38	0.43	0.42	0.41
7	1-decanol	6.38	0.34	0.39	0.50	0.36	0.45
8	2,3,4-drithiapentane-dimetyltrisulfide	7.12	0.56	0.58	0.45	0.46	0.68
9	2,5-dimethylpyrazine	7.47	3.82	3.48	3.71	3.46	4.05
10	2,3-dimethyl-pyrazine	7.78	0.21	0.19	0.20	0.20	0.20
11	dimethyltrisulfide	8.35	3.63	5.14	5.64	4.67	4.81
12	trimethylpyrazine	8.53	2.04	2.00	2.20	2.01	2.01
13	3-ethyl-2,5-dimethylpyrazine	9.04	3.13	3.27	3.71	3.42	3.54
14	tetramethylpyrazine	9.23	1.41	1.35	1.43	1.36	1.39
15	2,6-dimethyl-4-(t-butyl)pyrimidine	9.41	0.22	0.18	0.26	0.14	0.22
16	2,3-diethyl-5-methylpyrazine	9.65	1.46	1.50	1.70	1.63	1.50
17	2,3,5-trimethyl-6-ethylpyrazine	9.90	0.57	0.58	0.67	0.65	0.63
18	benzaldehyde	10.13	12.11	13.48	14.10	14.42	15.27
19	2,3-dimethyl-5-isopentylpyrazine	11.18	0.44	0.36	0.41	0.37	0.42
20	2,5-dimethyl-3-(3-methylbuthyl-)pyrazine	11.63	0.16	0.18	0.20	0.18	0.21

2-heptanone, the product of fatty acid, was decreased after storage for 2 and 4 months. The decreasing in lipid derived volatiles was due to quick reaction with the amine group from phosphatidylethanolamine or residual amino (Baek and Cadwallader, 1996; Thanonkaew *et al*., 2006; Lu *et al*., 2011; Lu *et al*., 2012, Lu *et al*., 2013).

The results in Table 26 demonstrated that the intensity of odor characteristics on shrimp flavorant powder storage with and without vacuum condition for 2 and 4 months. All odor characteristics intensities (roasted sundried shrimp, boiled sundried shrimp, boiled blue swimming crab, unfresh shrimp juice and Mungoong) were not significantly different ($p \geq 0.05$). This result was related to the volatile compounds shown in Table 25 which a slightly differentiation between the samples was observed. The TBARs values were also reported in Table 26. It was noticeable that the values were continuously decreased during the storage for 2 and 4 months for all conditions ($p < 0.05$). At the first 2 months, the sample storage under vacuum condition (79.57 mg malonaldehyde/kg lipid) had the higher TBARs value than those stored in air (55.26 mg malonaldehyde/kg lipid). TBARS value was used as the indicator to determine the oxidative changes resulted from formation of the unpleasant off flavor in the product (Sharma *et al*., 1995). Many previous studies revealed the decreasing of TBARs values in the initial on storage time followed by the increasing during the storage for the longer time (Kanpairo *et al*., 2012; Takeungwongtrakul *et al*., 2012). Marine lipids contained high content of polyunsaturated fatty acid (Tocher and Sargent, 1984) those prone to oxidation and TBARS value could indicate the secondary lipid oxidation products, especially aldehydes (Chaijan et al., 2006; Nawar, 1996). Those products could decrease when the oxidation was conducted to the termination step (Shahidi and Zhong, 2005).

Table 26 TBARs and the intensity of characterizing odors of shrimp flavorant powder extracted from Pacific white shrimp head packed with the different conditions

Sample		TBARs[1] (mg malonaldehyde/ kg sample)	Intensity[2] (cm)				
			RSS	BSS	BBSC	USJ	MG
0 month		1.00 ± 0.24[a]	2.76 ± 1.28 [a]	3.80 ± 1.44 [a]	2.59 ± 1.53 [a]	3.04 ± 1.81 [a]	3.77 ± 1.21 [a]
Vacuum	**2 month**	0.80 ± 0.08[ab]	2.28 ± 1.25 [a]	3.43 ± 1.08 [a]	2.06 ± 0.93 [a]	3.63 ± 2.44 [a]	3.51 ± 1.04 [a]
	4 month	0.24 ± 0.08[d]	4.30 ± 2.34 [a]	3.06 ± 0.76 [a]	2.29 ± 1.97 [a]	3.19 ± 2.81 [a]	4.30 ± 1.30 [a]
Non-vacuum	**2 month**	0.55 ± 0.05[bc]	3.69 ± 2.91 [a]	3.27 ± 1.45 [a]	2.26 ± 1.65 [a]	3.06 ± 2.86 [a]	4.50 ± 1.66 [a]
	4 month	0.34 ± 0.13[cd]	2.85 ± 0.45 [a]	3.36 ± 1.19 [a]	3.09 ± 1.19 [a]	3.41 ± 2.99 [a]	3.03 ± 0.93 [a]

[1] All values are means ± standard diviation (n=3).

[2] All values are means ± standard diviation (n=3).

Different superscripts [(a-d)] in the same column indicate the significant differences ($p<0.05$).

RSS: roasted sundried shrimp odor., BSS: boiled sundried shrimp odor., BBSC: boiled blue swimming crab odor., USJ: unfresh shrimp juice odor., MG: Mungoong odor.

3.5 Conclusion

Drying methods affected on flavor characteristics and color of flavorant powder. TD sample was the darkest when compared with others. The shrimp flavorant solution extracted by Alcalase and FD sample had the high intensity of roasted sundried shrimp and unfresh shrimp juice odors relating to 3-methylbutanal and benzaldehyde, the major volatile compounds of the samples' head space. While pyrazine compounds was mainly found in the dried shrimp flavorant using SD and TD especially the samples with maltodextrin. These compound related to mungoong odor. The highest overall acceptance score was obtained from the SD20, SD15+5M and TD15+5M. The storage under vacuum condition had less effect on the volatile compounds than that under air condition. However, both conditions did not affect the odor characteristics after storage up to 4 months.

CHAPTER 4

STUDY OF PACIFIC WHITE SHRIMP HEAD COLORANT EXTRACTION

4.1 Abstract

To produce a natural colorant from Pacific white shrimp (*Litopenaeus vanamei*) head, the optimized mixture proportions of isopropanol and hexane for carotenoid extraction was studied by applying the simplex lattice design. The prediction model showed that the proportion gaining the highest carotenoid yield was 40.731: 59.269 (v/v). The chemical compositions and carotenoid yield of colorant extracted from non-pretreated Pacific white shrimp head were compared to those extracted from the shrimp head pretreated by soaking in 2% sodium metabisulfite solution with the ratio of 2:5 (weight of shrimp head: volume of solution) for 15 min to prevent blackening. The result demonstrated that the colorant extracted from pretreated shrimp head had the higher carotenoid yield, protein and ash content than that from non-pretreated shrimp head ($p<0.05$). However, moisture and fat content of both samples were not different ($p\geq0.05$). The antioxidative activity results showed that the colorant extracted from non-pretreated shrimp head exhibited the higher DPPH and ABTS radical scavenging activities. However, no differences in ferric reducing power and metal chelating activity were observed between both samples ($p\geq0.05$).

4.2 Introduction

Carotenoid, a red-orange pigment, is one for the most interested component in shrimp waste. Astaxanthin, a major carotenoid found in shrimp, is a powerful biological antioxidant that occurs naturally in a wide variety of living organisms (Hussein *et al*., 2006). The extracted carotenoid can be used as natural colorant fortified foods in various industries such as beverage, ice cream, candy, confectionary, meat product, pet food and aquaculture food (Pu *et al*., 2010). A number of studies have been reported methods on carotenoid extraction from shrimp by-product using various methods. Sachindra and Mahendrakar (2005) applied vegetable oils to extract carotenoids from shrimp by-products and found that the sun flower oil exhibited the highest yield when compared among groundnut oil, gingelly oil, mustard oil, coconut oil, and rice bran oil.

Sachindra *et al.* (2006) also showed that a 50:50 mixture of isopropyl alcohol and hexane gave the highest carotenoid extraction yield (43.9 µg/g waste) compared to individually polar solvents (acetone, methanol, ethyl methyl ketone, isopropanol, ethyl acetate and ethanol) and non-polar solvent (petroleum ether and hexane) as well as a mixture of acetone and hexane ($p<0.05$). Moreover, Sachindra *et al*. (2006) demonstrated that the present of 60% hexane in solvent mixture could yield the maximum extracted carotenoids content from shrimp waste from processing of *Penaeus indicus*, comprising of head and carapace with solvent mixture to waste ratio of 5:1 and repeat three times for each extraction. Takeungwongtrakul *et al.* (2013) compared among single solvents (acetone, isopropanol and hexane) and their mixtures for carotenoids extraction from hepatopancreas of Pacific white shrimp (*Litopenaeus vannamei*). A mixture of hexane and isopropanol (50:50, v/v) rendered lipids with the highest carotenoid yield (336.40 mg/kg hepatopancreas). From previous studies, the extraction using solvent mixtures (polar and non-polar) seemed to be a promising method for carotenoids extraction. However, no information on optimization of the solvent mixture for the colorant extraction from Pacific white shrimp by-product has been reported. The aims of this study was to optimize solvent mixture for colorant extraction from Pacific white shrimp head and to study the effects of sodium metabisulfite pretreatment for blackening prevention of shrimp head on the extracted colorant.

4.3 Meterials and methods

4.3.1 Chemicals

Alcalase (protease from *bacillus licheniformis*) was procured from Sigma, Capricorn, Singapore. Hydrochloric acid (37%), sodium hydroxide, sodium chloride, sulfuric acid, isopropanol, hexane and petroleum ether were purchased from LAB-SCAN (Thailand). Potassium sulfate, copper sulfate, sodium metabisulfite and boric acid were procured from Ajax Finechem (New Zealand). Astaxanthine was ordered from Dr. Ehrenstorfer GmbH (Augsburg, Germany).

4.3.2 Shrimp head preparation

Frozen Pacific white shrimp heads prepared from shrimp harvested within 48 hr was thawed by running water and used as 'non-pretreated shrimp head'. The pretreated shrimp head was prepared by soaking the thawed shrimp head in 2% sodium metabisulfite solution with a ratio of 1:5 (w/v) for 15 min, rinsing with water for 5 min and draining through strainer for 10 min. Both samples were minced for 1 min using blender (AY46, Moulinex, China) before used.

4.3.3 Optimization of extraction of shrimp colorant from Pacific white shrimp head

Minced shrimp head was extracted in solvent and mixtures of isopropanol and hexane with various ratios using augmented simplex lattice design. The experimental design contained 5 design points and 3 replicated points used for estimation a pure error sum of squares as shown in Table 27.

Extraction method was modified from Sachindra *et al*. (2006). Minced non-pretreated shrimp head was mixed with solvent with a ratio of 2:5 (w/v) before shaking for 15 min using a shaking incubator (LMS, VS-8480-SR-4, Korea). The sample was filtered through cheese cloth and the extracted solution was collected.

Table 27 Experimental design of mixture solvent for shrimp colorant extraction

Treatment	Isopropanal (%)	Hexane (%)
1*	100	0
2	75	25
3*	50	50
4	25	75
5*	0	100

Note: * the replicated design point treatments.

The colorant extracts in solvent were pooled together and in the case of extracts in polar solvents, they were phase separated with an equal quantity of petroleum ether. The petroleum ether extract was repeatly washed with an equal quantity of 0.1% saline to remove traces of polar solvents, if any, then dried with 25 g of sodium sulfate, filtered, flushed with nitrogen for 5 min, and then evaporated under vacuum at 40^{o}C using a rotary evaporator (Buchi rotavapor, Switzerland). In the case of colorant extract in hexane and solvent mixture, the addition of petroleum ether for phase separation was avoided and the extracts were directly washed with saline, dried and concentrated as described previously (Sachindra *et al*., 2006).

4.3.3.1 Carotenoid yield

The resulting colorant was taken up in petroleum ether and made up to 100 ml. The absorbance of the appropriately diluted extract was measured at 468 nm using a spectrophotometer. The yield of the colorant was expressed as carotenoids and calculated as astaxanthin (Simpson and Haard, 1985) using the following equation:

$$\text{Carotenoid yield (µg astaxanthin/ g sample)} = \frac{A_{468\,nm} \times V_{extracted} \times \text{Dilution factor}}{0.2 \times W_{sample}}$$

Where A is absorbance, V is volume of extract, 0.2 is the A_{468} of 1 µg/ml of standard astaxanthin and W is weight of sample in grams.

The optimal hexane and isopropanol mixture was achieved with the highest yield.

4.3.4 Effects of sodium metabisulfite pretreatment on colorant extracted from Pacific white shrimp head

Colorants were extracted from non-pretreated and sodium metabisulfite pretreated shrimp head using the selected solvent mixture from Part 4.3.3. Both samples were subjected to analyze as follows.

4.3.4.1 Carotenoid yield of samples as described in part 4.4.3.1

4.3.4.2 Proximate analysis

Determinations of moisture, protein, fat and ash content were carried out according to AOAC (2000).

4.3.4.3 Determination of antioxidative activities

The antioxidative activities of colorant including ATBS radical scavenging activity, DPPH radical scavenging activity, reducing power and chelating activity were analyzed as described in Part 3.3.3.7.

4.3.5 Statistical Analysis

Mixture design experiments were analyzed using Design Expert Statistical Package version 7.0 (Stat-Ease, Inc., Minneapolis, MN).

Experimental data was fitted to a linear (Equation (1)), quadratic (Equation (2)) or cubic (Equation (3)) depending on the degree of fit and predictive power of the model.

$$Y = \sum_{i=1}^{q} \beta_i x_i \quad (1)$$

$$Y = \sum_{i=1}^{q} \beta_i x_i + \sum \sum_{i<j}^{q} \beta_{ij} x_i x_j \quad (2)$$

$$Y = \sum_{i=1}^{q} \beta_i x_i + \sum \sum_{i<j}^{q} \beta_{ij} x_i x_j + \sum \sum_{i<j}^{q} \delta_{ij} x_i x_j \left(x_i - x_j\right) \quad (3)$$

Where Y is carotenoid yield; β_i is the equation coefficient and X is proportion of the pseudo-component.

The dependent variable (carotenoid yield) was analyzed and the model of isopropanol and hexane ratio for carotenoid extraction was subjected to analysis of variance (ANOVA) to determine the significant ($p<0.05$), coefficient of determination (R^2) and lack of fit. The maximum yield was determined using the software.

To validate the optimum solvent mixture, the experimental error for the predicted model was carried out by comparing observed values with the predicted values as follow.

$$\text{Error (\%)} = \frac{\text{observed values - predicted value}}{\text{observed value}} \times 100$$

Paired sample t-test was performed to compare the results from chemical analysis of the colorants extracted from pretreated and non-pretreated at a significant level $p<0.05$. Analysis was performed using a SPSS package (SPSS 10.0 for window, SPSS Inc, Chicago, IL).

4.4 Results and discussion

4.4.1 Optimization of shrimp colorant solvent extraction

Based on mixture design experiment, the carotenoid yields extracted from Pacific white shrimp head in solvents mixtures of isopropanol and hexane with various ratios are shown in Table 28. The predicted response (Y) for the carotenoid yield could be expressed by the following equation.

Carotenoid content (Y) = 28.56 (isopropanol) + 11.52 (hexane) + 42.68 (isopropanol* hexane) – 5.35 (isopropanol * hexane) (isopropanol – hexane)

Table 28 Yield of carotenoid extracted from Pacific white shrimp head using solvents mixtures of isopropanol and hexane with various ratios

Treatment	Isopropanol: Hexane ratio	Crude oil (g/ 100 g of shrimp head)	Carotenoid content (µg carotenoid/ g crude oil)	Carotenoid yield (µg carotenoid/ g shrimp head)
1*	100: 0	6.22 ± 1.54^{a}	450.32 ± 21.15^{b}	28.39 ± 0.56^{c}
2*	0: 100	8.88 ± 2.04^{a}	472.43 ± 83.07^{b}	11.35 ± 0.21^{d}
3*	50: 50	9.72 ± 2.32^{a}	204.41 ± 25.83^{c}	29.72 ± 1.54^{ab}
4	75: 25	7.85 ± 2.87^{a}	427.75 ± 11.76^{b}	28.61 ± 0.15^{bc}
5	25: 75	7.32 ± 2.47^{a}	701.13 ± 12.50^{a}	30.12 ± 0.36^{a}

* The design point with replication.

Means ± SD (n=3 for each replication).

Different superscripts ($^{a-d}$) in the same column indicate the significant differences ($p<0.05$).

The contour plot represented the regression equation are shown in Figure 11. The ANOVA was performed to evaluate the adequacy of the generated mathematical models as shown in Table 29. Myers and Montgomery (2002), Koocheki *et al*. (2009) proposed that a good predictive model should have determination coefficient (R^2) and adjusted $R^2 > 0.80$, significant level of $p<0.05$, coefficient of variance (C.V.) values ≤ 10% and lack of fit value > 0.1. Our study showed that the R^2 was 0.9794, implying that only 2.06 % of the total variance was not explained by the model. The C.V. of the response was lower than 10%, this value measures the dispersion of a probability distribution, which high C.V. indicate high variation in the mean value and sufficient response model cannot be developed (Idrus *et al*., 2013). In addition, the p-value regarding the lack of fit (0.1445) indicated that the lack of fit was not significant in relation to pure error, implying the model is fairly appropriate (Huang and Ma, 2016). The F value and p-value were considered to determine the significance of each coefficient. The higher F and lower p values, the more significant of the coefficient is.

Table 29 Analysis of variance for the regression model for carotenoid extraction of shrimp head

Source	Sum of squares	Degree of freedom	Mean square	F value	p-value Prob>F
Model	472.78	3	157.59	63.38	0.0008
Residual	9.95	4	2.49		
Lack of Fit	5.59	1	5.59	3.85	0.1445
Pure error	4.36	3	1.45		
Cor Total	482.73	7			
R^2=0.9794	R^2_{adj}=0.9639	CV%=6.38			

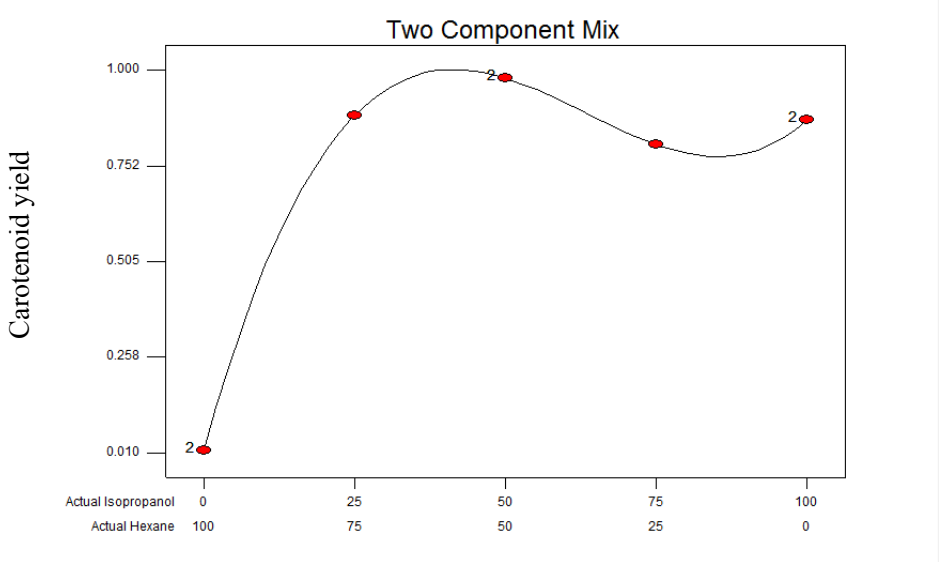


Figure 11 Graphical result of the matrix DOE for Pacific white shrimp head colorant extraction

Table 29 demonstrated that the linear coefficient (X_1, X_2), quadratic term coefficients (X_1X_2) and cubic coefficient [$X_1X_2(X_1 - X_2)$] were significant, with p-value lower than 0.05. This result indicated that the components had significant positive effect on yield. The highest predicted carotenoid yield was found at the mixture of isopropanol: hexane ratio of 40.731: 59.269 (v/v). The validation of the regression equation of the optimal solvent mixture (isopropanol: hexane ratio of 40.731: 59.269 (v/v)) was performed. The prediction and observed values under optimal condition were 31.1559 and 29.99 ± 0.71 µg carotenoid/ g shrimp head, respectively. The experimental error was 3.74% which was lower than 10% indicating the acceptability and validity of statistical model for the optimized solvent mixture of colorant extraction from shrimp head. The result was in agreement with Sachindra *et al.* (2006) who found that the highest carotenoid yield was obtained from the extraction of *Penaeus indicus* head and carapace by the solvent mixture of isopropanol: hexane in the ratio of 40: 60 % (v/v) with the shrimp waste: solvent ratio of 1:5 in each extraction and 3 extractions. Takeungwongtrakul *et al.* (2013) also reported that the extraction of carotenoid from hapatopancreas of Pacific white shrimp by solvent mixture of isopropanol: hexane in the ratio of 50: 50 % (v/v) and sample: solvent ratio of 2:5 for 3 times yielded the highest carotenoid extracted. Carotenoids are generally soluble in nonpolar solvent (Sachindra *et al*., 2006) but polar solvent and the mixture of polar and nonpolar solvent were used to improve the efficiency of carotenoid extraction (Nègre-Sadargues *et al*., 2000; Sachindra *et al*., 2006). In the state that tissues contain a large amount of water, the presence of polar solvent in the extraction may remove little pigment, but they could remove water from the tissues that will treat the sample for the subsequent nonpolar solvent or mixture solvent extraction (Sachindra *et al*., 2006).

4.4.2 Effect of sodium metabisulfite pretreatment on shrimp colorant extraction

Sodium metabisulfite solution was used to prevent melanosis reaction in shrimp head. The comparison of carotenoid yield and chemical composition of colorants extracted from pretreated and non-pretreated shrimp head are shown in Table 30. The colorant extracted from pretreated shrimp head had the higher carotenoid yield and protein content but lower ash content ($p<0.05$) when compared with those extracted from non-pretreated. However, moisture and fat content of both samples were not different ($p\geq0.05$). The higher protein content in the sample extracted from pretreated shrimp head might be due to the effect of sodium metabisulfite as Mizani *et al*. (2005) reported that the presence of sodium sulfite (200 mmol/L) with the Alcalase increased the protein extraction from shrimp waste (*P. semisulcatus*) to 62% when compared to the extraction by enzyme alone (45.1%). It seemed that sodium sulfite could reduce disulfide bonds of the shrimp waste protein and made enzyme more accessible.

Table 30 Carotenoid yield and chemical compositions of colorant extracted from pretreated and non-pretreated Pacific white shrimp head

Composition	Non-pretreated	Pretreated
Carotenoid yield (mg carotenoid/ kg shrimp waste)	29.99 ± 0.71^{b}	35.47 ± 0.32^{a}
Moisture content (%)	1.98 ± 0.53^{a}	2.03 ± 0.71^{a}
Fat content (% dry basis)	98.60 ± 2.98^{a}	99.32 ± 2.26^{a}
Protein content (% dry basis)	2.89 ± 0.06^{b}	4.03 ± 0.10^{a}
Ash content (% dry basis)	3.03 ± 0.07^{a}	2.51 ± 0.25^{b}

Means ± SD (n=3).

Different superscripts ($^{a-b}$) in the same row indicate the significant differences ($p<0.05$).

The extracted colorant in our study was carotenoid-containing oil which astaxanthin, a major carotenoid found in shrimp, has been reported as a powerful biological antioxidant (Hussein *et al.*, 2006; Takeungwongtrakul *et al.*, 2013). The antioxidative activities of the colorant extracted from pretreated and non-pretreated Pacific white shrimp head are descripted in Table 31. The result showed that DPPH and ABTS radical scavenging activities of the colorant extracted from non-pretreated

shrimp head were higher when compared with those extracted from pretreated shrimp head ($p<0.05$) due to the higher carotenoid concentration contained in the non-pretreated sample. However, no differences in ferric reducing power and metal chelating activity were observed between both samples ($p\geq0.05$). That might be the effect of residue sodium metabisulfite from pretreatment process. Sulfites are the reducing agent with highly effective in controlling browning in food products but are known to cause adverse health effects such as asthma (McEvily *et al*., 1992). Basically, if the food contains ≥ 10 ppm total SO_2, then sulfite must be declared on the label (US Food and Drug Administration, 2015). Both sample did not show the different in metal chelating activity ($p\geq0.05$). The colorant sample in this study had 2-fold metal chelating activity when compared to Sowmya and Sachindra (2012), who studied the antioxidative activity of carotenoid-containing oil from head and carapace of *Penaeus indicus* (21.1 EDTA equivalent μg/mg extract). Senphan *et al*. (2013) reported that carotenoprotein from Pacific white shrimp shell had antioxidative activities including ABTS, DPPH radical scavenging activities, FRAP and metal chelating activity. Moreover, the antioxidative activities of the sample were in the concentration-dependent manner as the increasing of all activities were occurred with the concentrations increased up to 5 mg/ml.

Table 31 Antioxidative activities of shrimp colorant extracted from pretreated and non-pretreated shrimp head

Antioxidative activities	Non-pretreated	Pretreated
DPPH radical scavenging activity (mmol TE/g colorant)	7.90 ± 0.12^{a}	7.57 ± 0.04^{b}
ABTS radical scavenging activity (mmol TE/g colorant)	4.25 ± 0.13^{a}	3.49 ± 0.26^{b}
Ferric reducing power (mmol TE/g colorant)	2.57 ± 0.19^{a}	3.06 ± 0.23^{a}
Metal chelating activity (mg EDTA eg/g colorant)	5.08 ± 0.58^{a}	4.62 ± 0.97^{a}

Means ± SD (n=3).

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

4.5 Conclusion

The highest yield of colorant extracted from Pacific white shrimp head was obtained from the extraction using solvent mixture of isopropanol and hexane in the ratio of 40.7: 59.3 (v/v) and the shrimp head to solvent ratio of 2:5 (w/v). Pretreated the Pacific white shrimp head by 2.0% sodium metabisulfite solution for 15 min led to the higher carotenoid yield and protein content but lower ash content when compared to the sample extracted from non-pretreated shrimp head. However, the colorant from pretreated shrimp head had the lower DPPH and ABTS radical scavenging activities. The ferric reducing power and metal chelating activity of both samples were not different.

CHAPTER 5

APPLICATION OF FLAVORANT AND COLORANT FROM PACIFIC WHITE SHRIMP HEAD

5.1 Abstract

The applications of flavorant and colorant extracted from Pacific white shrimp head in foods were conducted. Central composite design was used to optimized the concentration of shrimp flavorant (0-3% w/w) and colorant (0-0.4% w/w) in a shrimp shumai. It was noticeable that the shrimp flavorant decreased liking scores of color, flavor, texture and overall ($p<0.05$), while color liking score was increased with the shrimp colorant added ($p<0.05$). The colorant concentration was further studied by adding in a shrimp shumai filling at the levels of 0, 0.2, and 0.4% (w/w). The result from acceptance test indicated that addition of the colorant at 0.2% increased color liking score of the shumai sample (7.63 ± 0.89) when compared with that without the colorant (6.73 ± 1.20) and added at 0.4% (7.07 ± 0.60)($p<0.05$).

Another application, the shrimp colorant was applied to enhance color of mayonnaise-based dipping sauce. The colorant from Pacific white shrimp head (NC: 0, MC: 1.2 and HC: 3.6%, w/w) and salts (RS: NaCl, ReS: KCl and NS: no salt added) were added to mayonnaise-based dipping sauce to see the effects on consumer's liking, emotions, saltiness perception and purchase intent. The results showed that when colorant concentration increased a* and b* values were also increased (0.07 to 27.22 and 10.13 to 36.50, respectively) while L* values (88.39 to 70.22) and saltiness liking score decreased ($p<0.05$). Too intense color (HC) affected the decreasing of color liking score ($p<0.05$). Visual saltiness expectation did not directly be affected by color but samples with the higher color liking scores gained the higher percentage to be just-about-right samples for expected saltiness. At the given colorant concentration, the highest saltiness liking score was occurred in RS followed by ReS and NS, respectively ($p<0.05$). Scores of emotions elicited by dipping sauces were highly affected by colorant concentration as scores of

positive emotions (*good*, *interested* and *satisfied*) were decreased while negative emotions (*guilty*, *unsafe* and *worried*) were increased with the increasing of colorant concentration ($p<0.05$). Statements of 'colorant from a natural source' and 'sodium content' had a few effects on elicited emotions and consumer's purchase intent.

5.2 Introduction

Shrimp waste which generated during shrimp processing was the rich source of valuable components such as protein, carotenoid pigments, chitin and chitosan (Ramaswamy *et al*., 1991; Klomklao *et al*., 2009). In the previous, a number of studies were conducted to recover and use those components. The extracted protein from shrimp waste in form of protein hydrolysate could be used as a flavorant fortified in food (Teerasuntonwat and Raksakulthai, 1995; Holanda and Netto, 2006). Teerasuntonwat and Raksakulthai (1995) produced flavoring agents from shrimp (*Peneaus monodon* Fabricius) head by extraction with water, sodium chloride, hydrochloric acid, sodium hydroxide, bromelain, papain or neutrase. The cracker incorporated with extracted flavoring agents obtained the higher acceptance score (color, flavor, odor and overall) when compared to control (without flavoring agent) but lower than the commercial flavoring agent. In addition, Kim *et al*. (2014) reported that the enzymatic hydrolysate of krill (*Euphausia superba*) was successful applied in ramen sauces.

Astaxanthin or carotenoid pigments from shrimp waste with red-orange color can be used as a natural food colorant in various industries as beverage, ice cream, candy, confectionary, meat product, pet food and aquaculture food (Pu *et al.*, 2010). Color is an important sensory attribute that consumers used to evaluate foods before they decide to consumes or make purchase decision. Wadhera and Capaldi-Phillips (2014) stated that visual cues, such as color, can affect sensory acceptance of a food by affecting expectation of palatability of such food, which can further influence food choice and consumption. Within the trend of health eating, 'health labels' and 'clean labels' were become interested by consumers and were studied for the effects on consumer's perceptions, acceptability and purchase intent (Bower *et al.*, 2003; Kleef *et al.*, 2005; Roe and Teisl, 2007; Sabbe *et*

al., 2009; Leim *et al*., 2012; Chareonthaikij *et al*., 2016, Poonnakasem *et al.*, 2016). Consumers are increasingly demanding products that made from natural ingredients (Chareonthaikij *et al.*, 2016) including flavorant and colorant (Nachay, 2016). Many researchers developed and applied natural colorant into their food as fresh-cut apple (Yilmaz and Bilek, 2017), On the other hand, health labels may cause the negative results for the consumers who worried for taste of products, rather than healthiness (Leim *et al*., 2012).

Spence (2015) demonstrated that changing of food or beverage color, hue and intensity could affect consumer's expectations regarding the likely taste and flavor of food and drink. Moreover, some previous studies reported the influencing of food color on perception of taste and their intensities (Chan and Kane-Martinelli, 1997; Hoegg and Alba, 2007; Wei *et al*., 2012, Lynch *et al*., 2017). Nevertheless, there are just a few studies those focused on the association of food color with salty taste as Maga (1974) revealed that salty foods were associated to many different colors and had no particular color could related to food's saltiness. However, Wan *et al*. (2014) with their more recently study showed the different results and concluded that salty taste was associated salt with white color. Food color was often used to inspect foods by consumers to make the decisions on consumption or purchase (Cardello, 1996) as its effects on product's acceptability (Wadhera and Capaldi-Phillips, 2014). Additionally to overall acceptance, food elicited emotions also affect consumer purchase decision as they could define the differentiation of products those were produced with the similar characteristics (Poonnakasem *et al*., 2016). A number of studies revealed the relationships between food elicited emotion and product characteristics as dark chocolate (Thomson *et al*., 2010), food odorants (Porcherot *et al*., 2012), chicken egg (Wardy *et al*., 2015), sponge cake (Poonnakasem *et al*., 2016).

Reduced or low-sodium foods were attended as consuming excessive sodium is a risk of hypertension that would leads to chronic heart and kidney-related disease (Chokumnoyporn *et al*., 2015). Salt (NaCl) that commonly used as salting agent is an importance source of sodium in foods (Torrico *et al*., 2015). Variety techniques were studied to reduce sodium content in foods without the reduction of consumer's acceptability such as using flavor to enhance salty taste (Kremer *et al*., 2009; Seo *et al*., 2013; Chokumnoyporn *et al*., 2015) , use of foammat salt (Chokumnoyporn *et al*., 2016), salting processes (Almli and Hersleth, 2013) and utilization of salt substitutes (Guàrdia *et al*., 2008; Mitchell *et al*., 2009; Mitchell *et al*., 2011; Feltrin *et al*., 2015; Ambra *et al*., 2017). Potassium chloride (KCl) was widely used as NaCl substitute due to the ability to import salty taste (Albarracìn *et al*., 2011). However, Buren *et al*. (2016) demonstrated that KCl could not be used unlimited quantities in food as it provide low salty taste and bitter taste, chemical flavor including metallic flavor will be occurred and affect to consumer's acceptability. For instance, olive fermented in NaCl solutions substituted by 50 and 75% KCl were evaluated to be less salty and bitter than that fermented in brine (Ambra *et al*., 2017) or Torrico *et al*. (2015) who reported that consumers could perceived bitter taste from oil-in-water emulsion with KCl added.

The objectives of this study were to optimize the concentration of shrimp flavorant and colorant applied in a shrimp shumai and to investigate the effects of shrimp colorant concentrations with salts on consumer's liking, food elicited emotions, purchase intent and perceptions of reduced sodium mayonnaise-based dipping sauce.

5.3 Materials and methods

5.3.1 Materials

For shrimp shumai preparation, fresh shrimp was purchased from local market in Songkhla province, Thailand and used within 2 days. Wrapping sheet (Shimakyu C., Ltd, Thailand), surimi (Man A Frozen Foods Co., Ltd, Thailnd) and vegetable powder (International Ingredients Supply Co., Ltd, Thailand) were supported by Sea Wealth Frozen Food Co., Ltd (Songkhla, Thailand). Other ingredients consisted of sesame oil (Double Dragon, Union Food Industry Co., LTD, Thailand), sugar (Wangkanai, Wangkanai Corp., Ltd., Thailand), all-purpose wheat flour (Kite, UFM Food Center LTD., Thailand), soybean oil (A-Ngoon, Thai Vegetable Oil Public Company LTD., Thailand), salt (Prung Thip, Thai Refined Salt Co.,LTD., Thailand) and white pepper (Nguan Soon, Artchit International Pepper and Spice Co., Ltd., Thailand).

The ingredients for dipping sauce preparation including: mayonnaise (Kraft®, Kraft Food Group, Inc., Northfield, Il., U.S.A.), sour cream (Daisy®, Daisy Brand, Dallas, TX., U.S.A.) , cream cheese (Philadelphia, Kraft Food Group, Inc., Northfield, Il., U.S.A.), salt (sodium chloride; Morton®, Morton Salt, Inc., Chicago, Il., U.S.A.), black pepper (McCormick, McCormick and Co. Inc., Hunt Valley, MD., U.S.A.), and dried chive (ADAMS® since 1888, ADAMS Flavors, Foods and Ingredients, LLC, Gonzales, T.X., U.S.A.).

5.3.1 Application of flavorant and colorant in shrimp shumai

5.4.1.1 Shrimp shumai preparation

The flavorant powder from Part 3 and colorant oil from Part 4 with the different concentrations were added into shrimp shumai. The formulation and preparation of the shumai modified from Sea Wealth Frozen Food Co., Ltd (Songkhla, Thailand) are shown in Table 32 and Figure 12, respectively. The flavorant was added into the filling by replacing to the vegetable extract powder. In addition, the vegetable oil (soybean oil) was replaced by the colorant. A central composite design of flavorant and colorant was used in this study. A total of 11 experiments were designed based on the three replications of the midpoint in order to calculate the experimental error. Both actual and coded forms of the

level of variables are shown in Table 33. The maximum level of flavorant was 3% and colorant was 0.4% (related to total formulation).

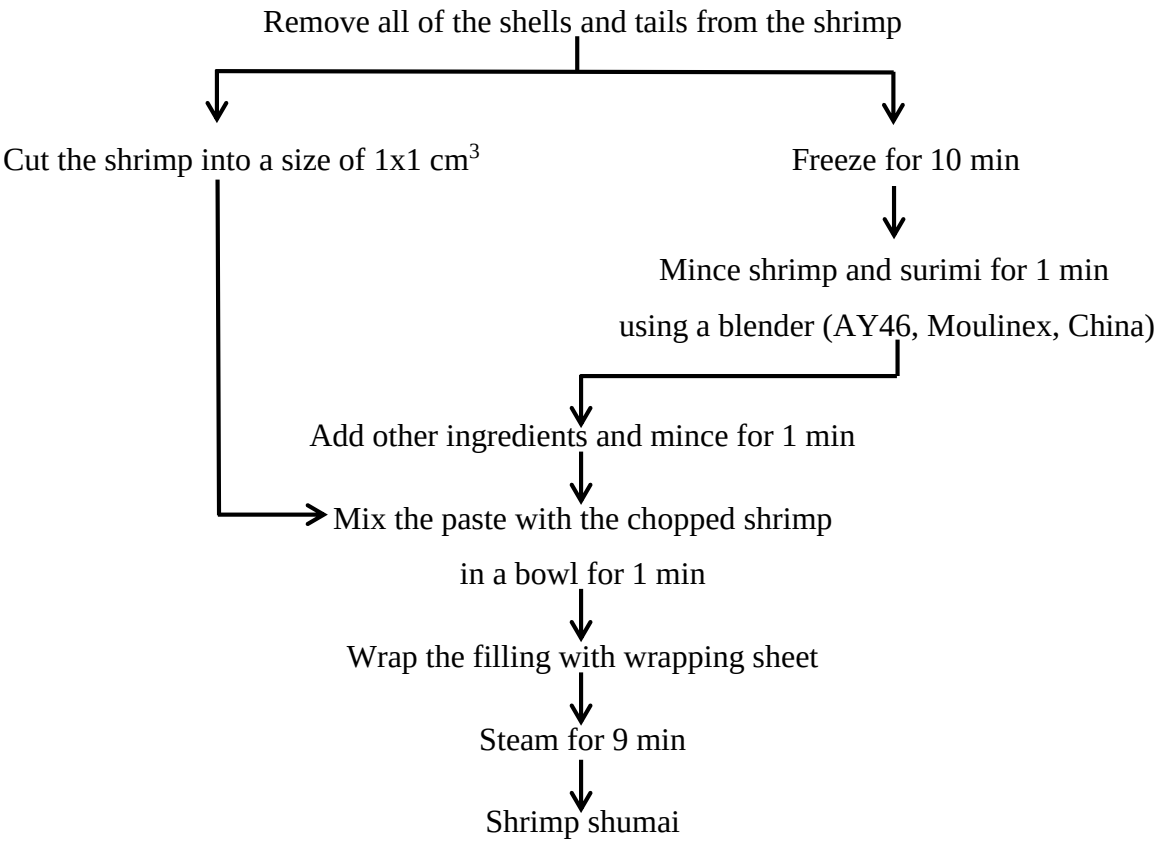


Figure 12 Preparation of shrimp Shumai

The acceptance of shrimp shumais with the different flavorant and colorant concentrations was judged by 50 consumer-type panelists recruited from Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Thailand. The panelists were asked to evaluate for color, odor, flavor and overall liking using 9-point hedonic scale. The samples were served to the panelists within 5 min after steaming. The samples were labeled with random three-digit codes and the order of presentation of the samples was randomized according to "balance order and carry-over effects design" (Macfie *et al*., 1989).

Table 32 Ingredients of shrimp shumai

Ingredients	**%**
Wrapping Sheet (20%: Wheat starch, Tapioca modified starch, Tapioca starch, Sodium chloride)	
Filling (80%)	
Shrimp (cut)	50-60
Shrimp (paste)	10-15
Surimi from bigeye snapper	5-10
Fish	2-5
Sugar	0.1-0.5
Mineral Salt (E451)	0.01-0.05
Sesame Oil	2-5
Sugar	2-5
Wheat flour	2-5
Vegetable oil (soy bean oil)	2-5
Vegetable powder (VPC)	1-3
Salt	0.5-1.0
White pepper	0.1-0.3

Table 33 Experimental design of shrimp flavorant powder and shrimp colorant oil for shrimp shumai

Treatment	Coded levels		Actual levels	
	Shrimp flavorant powder (X_1)	**Shrimp colorant oil** (X_2)	**Shrimp flavorant powder** (%)	**Shrimp colorant oil** (%)
1	-1	-1	0.44	0.06
2	1	-1	2.56	0.06
3	-1	1	0.44	0.34
4	1	1	2.56	0.34
5	-1.414	0	0	0.2
6	1.414	0	3	0.2
7	0	-1.414	1.5	0
8	0	1.414	1.5	0.4
9	0	0	1.5	0.2
10	0	0	1.5	0.2
11	0	0	1.5	0.2

5.3.2 Effect of colorant concentration on shrimp shumai

To study the effect of shrimp colorant in food, the shrimp shumai was prepared by the method and formulation as shown in part 5.3.1. The colorant extracted from non-pretreated Pacific white shrimp head was added into the shrimp shumai for 0 (control), 0.2 and 0.4% w/w of shrimp shumai filling by substitution to the vegetable oil in the formula. The shrimp shumai samples were subjected to analyses as follow.

5.3.2.1 Acceptance test

Color liking scores of all samples were evaluated by 50 consumer-type panelists using 9-point hedonic scale as described in part 5.3.1.

5.3.2.2 Color measurement

The shrimp shumai filling was cut into two pieces before determination using color meter (ColorFlex, HunterLab, Reston, VA, USA) and was reported in CIE LAB color scales (L*, a* and b* value).

5.3.3 Application of colorant in mayonnaise-based dipping sauce

5.3.3.1 Dipping sauce preparation

To study the effect of colorant concentration and salt in dipping sauce, 9 samples were prepared with 3 different colorant concentrations (NC: no colorant added, MC: moderate colorant added and HC: high colorant added (0, 1.2 and 3.6% w/w of colorant/dipping sauce, respectively) and 3 different salts (RS: 0.4 g sodium chloride added, ReS: 0.4 g potassium chloride added and NS: no salt added). RS, ReS and NC contained 146.87, 103.60 and 103.76 mg sodium per serving, respectively.

Cream cheese was blended with hand blender (Hamilton Beach, HB08, Hamilton Beach Brand, Inc., V.A., U.S.A.) at low speed for 2 min then mayonnaise, sour cream, salt, black pepper and dried chive were added. In this step colorant was also added into the mix with the different concentrations. Mix with all ingredients was blended with the highest speed for 2 min and keep at 4^{o}C until used.

5.3.3.2 Color measurement

Color measurement was carried out by color meter (model CM-5 Spectrophotometer, Konica Minolta, Jakarta Raya, Indonesia) for triplicate and reported as L*, a*, b* values.

5.3.3.3 Online survey for emotion term screening and selection

Use of human subjects in this research was approved by the Louisiana State University Agricultural Center Institutional Review Board (IRB# HE15-9). To screen and select emotion terms, the 39 food-elicited emotion terms (*Active, Adventurous, Affectionate, Aggressive, Bored, Calm, Daring, Disgusted, Eager, Energetic, Enthusiastic, Free, Friendly, Glad, Good, Good-natured, Guilty, Happy, Interested, Joyful, Loving, Merry, Mild, Nostalgic, Peaceful, Pleasant, Pleased, Polite, Quiet, Satisfied, Secure,*

Steady, *Tame*, *Tender*, *Understanding*, *Warm*, *Worried*, *Whole* and *Wild*) from the Essense Profile® (King and Meiselman, 2010; Poonnakasem *et al*., 2016) were used with slight modification. According to Wardy *et al*. (2015, 2017) and Poonnakasem *et al*. (2016), '*secure*' was subjectively modified to '*safe*' as it was more relevant to food. In this study, the term '*safe*' was further modified to '*unsafe*' to reflect the elicited emotion related to the survey question on consumption of dipping sauces containing natural or synthetic colorant. Four photographs (as shown in Figure 13) and their brief descriptions were used in the online survey. Brief description of each photograph was as follows: C = a colorant from a natural source (shrimp head). #1 = a dipping sauce without added colorant and containing the amount of sodium comparable to that found in commercial products. It was made with vegetable oil, vinegar, sour cream, cream cheese, black pepper, green onion, sodium chloride (salt) and gums. #2 = a dipping sauce #1 that was colored with a colorant from a natural source (shrimp head). #3 = a dipping sauce #1 that was colored with a synthetic colorant approved by the United States Food and Drug Administration (US FDA). The photographs were taken by a camera (Samsung Galaxy A5-2016, Samsung Electronics Co., Ltd, Suwon, Korea) under fluorescence light. The same fifteen computer monitors (Dell Optiplex 3020 X16, Dell Inc., Round Rock, TX, USA) were used by consumers for consistency purpose. Consumers (N = 111) viewed each photograph and its description on a computer screen administered via the 2016 Qualtrics Survey Software© (Qualtrics LLC, Provo, UT, USA). Afterwards, they responded to the online question associated with photograph C 'Please select the emotion terms (check-all-that-apply) that are elicited when thinking of using this colorant from a natural source in food formulations', as well as photograph #1, #2, and #3 'Please select the emotion terms (check all that apply) that are elicited when thinking of consuming this dipping sauce' (Appendix 4).

Figure 13 Photographs and their brief descriptions that were used in questionnaire.

C = a colourant from a natural source (shrimp head).

#1 = a dipping sauce without added colourant and containing the amount of sodium comparable to that in commercial products. It is made with vegetable oil, vinegar, sour cream, cream cheese, black pepper, green onion, sodium chloride (salt) and food gums.

#2 = a dipping sauce #1 that was coloured with a colourant from a natural source (shrimp head).

#3 = a dipping sauce #1 that was coloured with a synthetic colourant approved by the United States Food and Drug Administration (US FDA).

5.3.3.3 Consumer liking scores, emotion responses, purchase intent, expected saltiness intensity and JAR evaluation

The research protocol approved by the Louisiana State University Agricultural Center Institutional Review Board (IRB# HE15-9) was used for human subjected in this research. Dipping sauces with the different colorant concentrations and sodium contents were prepared and served to 216 consumers, that were recruited from a faculty, staff and student of Louisiana State University, Baton Rouge, L.A., U.S.A. All samples were served by a Balanced Incomplete Block (BIB) design (t=9, k=3, r=8, b=24, λ=9, E=0.75; generated by PROC OPTEX (SAS Int. 2012)). Samples were evaluated in partitioned sensory booth with cool, natural, fluorescent lights. The Compusense® *five* (Compusense Inc., Guelph, Canada) software was used to develop questionnaire and collect data. The questionnaire was shown in Appendix 5.

All liking scores for color and saltiness (before and after statement) were evaluated using 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely). Expectation of saltiness intensity by visual was rated by 9-point scale. Five-point scale was used to rate score for emotion responses (1 = not at all, 3 = moderately, 5 = extremely; King and Meiselman, 2010) including *good*, *guilty*, *interested*, *satisfied*, *unsafe* and *worried*. The Just-About-Right (JAR) scale was applied for saltiness intensity expected by visual (1 = too weak, 2 = just about right, 3 = too much; Stone and Sidel, 1993). For purchase intent, a binomial (yes/no) scale was used (Sae-Eaw *et al.*, 2007).

A tablespoon of dipping sauce was served in the cup, 3 samples per each consumer. All samples were coded with 3-digit random numbers.

Before tasting, consumers read and electronically signed a consent form. First, consumers were informed "Please, do not taste the sample yet" and evaluated color liking score, expectation of saltiness intensity and JAR-score for saltiness just by visual. Then consumers were asked to dip corn chip into the dipping sauce and taste it before rate liking score for saltiness liking as well as purchase intent. Emotion responses scores after consuming the dipping sauce were also evaluated.

After tasting, consumers were informed for colorant statement ("This dipping sauce has no colorant added." for NC, "This dipping sauce contained colorant from a natural source." for MC and HC) and asked to rate scores for emotion responses and purchase intent again.

Between the samples, consumers were asked to clean their palate by eating unsalted plain crackers and drinking water.

5.3.4 Statistical Analysis

The study of Part 5.3.1, central composite design was applied to determine the optimized concentrations of shrimp flavorant and colorant using the Design-Expert Software (version 7.0.5, Stat-Ease, Inc., Minneapolis, MN.). The quality of the fit of determination R^2, and the significances of the regression coefficient were checked by F-test and p-value of 0.05. A randomize complete block design (RCBD) was carried out for analysis of shrimp shumai's acceptance test. Color measurement of shrimp shumai was run for triplicate and analyzed by completely randomize design (CRD). Data was subjected to analysis of variance (ANOVA) and comparison of means carried out by Duncan's multiple range test (Steel and Torrie, 1980) at a significant level $p< 0.05$ using SPSS package (SPSS 10.0 for window, SPSS Inc, Chicago, IL).

For Part 5.3.3, correspondences analysis (CA) was applied to identify attributes largely accounted for overall product differences among dipping sauces and colorant from a natural source when all emotion terms were considered simultaneously. Analysis of variance (ANOVA) was performed to determine whether differences existed among the 9 dipping sauces in terms of L*, a* and b* values. Sensory liking scores and emotion responses were analyzed by randomized completely block design (RCRD). The Duncan's multiple range test was performed for *post hoc* multiple comparisons. The JAR data was presented as frequency. The 2-related sample-dependent *t*-test was used to determine significant differences in saltiness liking scores comparing before and after statement of sodium was provided to consumers. Analysis of covariance (ANCOVA) was conducted to analyse the effect of colorant concentration, color liking score and their interaction on expectation of saltiness. SPSS 11.5 software (SPSS Inc., Chicago, Ill., U.S.A.) was used to analyse for ANCOVA, ANOVA, Duncan's multiple range test and paired sample *t*-test. Frequency and spider web plot were performed using Excel 2010 (Microsoft Corporation, Redmond, USA). CA was carried out using the XLstat2007 software (Addinsoft, Paris, France).

5.4 Results and discussion

5.4.1 Applications of flavorant and colorant from Pacific white shrimp head in shrimp shumai

5.4.1.1 Effects of shrimp flavorant and colorant mixture on shrimp shumai

A central composite design (CCD) was used to study the effects of flavorant and colorant from shrimp head on shrimp shumai. From the preliminary experiment, it was found that the highest concentrations of shrimp flavorant and colorant that could be added into shrimp shumai were 3 and 0.4% (w/w), respectively. The color, flavorant, texture as well as overall liking scores of shrimp shumai with the various flavorant and colorant concentrations are shown in Table 34. It was found that the p-value of the models from all responses were greater than 0.05 indicating that the test of variable (flavorant and colorant concentrations) had no effect on liking scores (the analysis of variance for the response surface regression model is presented in Appendix 6). Therefore, the predicted models could not be used to estimate the response for the purpose of optimization. However, it was noticed that the shrimp shumai with 0.2% colorant without flavorant had the highest liking scores for all attributes ($p<0.05$). The decrease in color liking score of the flavorant added sample with regardless of colorant concentration was due to the impact from brown color of flavorant ($p<0.05$) as it impart darker color. There were slightly different among flavor, texture and overall liking scores of the samples ($p<0.05$). Sample with the higher flavorant concentration tended to obtain the lower liking scores for all attributes. The results from Part 5 indicated that shrimp head flavorant contained many volatile compounds affecting the odor and flavor. The flavorant was previously described as roasted sundried shrimp, boiled sundried shrimp, boiled blue swimming crab, unfresh shrimp juice and Mungoong odors which were different from flavor of cooked fresh shrimp as expected in shrimp shumai.

Table 34 Mean liking scores of shrimp shumais added with the different flavorant and colorant concentrations

Flavorant (%)	Colorant (%)	Hedonic Score (9-point)			
		color	flavor	texture	overall
0.44	0.06	7.67 ± 0.88^{ab}	7.20 ± 1.03^{ab}	7.13 ± 0.97^{bc}	7.27 ± 0.78^{abc}
2.56	0.06	7.40 ± 0.89^{bc}	7.13 ± 0.78^{ab}	7.37 ± 0.96^{abc}	7.07 ± 1.01^{bcd}
0.44	0.34	7.87 ± 0.90^{a}	7.57 ± 0.97^{a}	7.40 ± 1.00^{ab}	7.53 ± 0.86^{ab}
2.56	0.34	7.20 ± 0.85^{bc}	7.30 ± 1.18^{ab}	7.27 ± 1.11^{abc}	7.20 ± 1.06^{bcd}
0	0.2	7.63 ± 0.89^{ab}	7.60 ± 0.81^{a}	7.63 ± 0.76^{a}	7.70 ± 0.70^{a}
3	0.2	7.00 ± 0.91^{c}	7.33 ± 1.21^{ab}	7.23 ± 1.01^{abc}	7.13 ± 0.97^{bcd}
1.5	0	6.93 ± 0.78^{c}	7.17 ± 0.91^{ab}	7.33 ± 0.71^{abc}	7.27 ± 0.74^{abc}
1.5	0.4	7.30 ± 1.26^{bc}	6.93 ± 1.31^{bc}	7.10 ± 1.09^{bc}	6.97 ± 1.16^{bd}
1.5	0.2	7.00 ± 1.05^{c}	6.60 ± 1.25^{c}	6.93 ± 0.94^{c}	6.77 ± 1.19^{d}
1.5	0.2	7.27 ± 0.94^{bc}	6.97 ± 1.10^{bc}	7.00 ± 1.05^{bc}	7.03 ± 1.07^{bd}
1.5	0.2	7.23 ± 0.86^{bc}	7.30 ± 0.79^{ab}	7.27 ± 0.87^{abc}	7.23 ± 0.97^{abcd}

Means ± SD (n=30)

Different superscripts ($^{a-d}$) in the same column indicate the significant differences ($p<0.05$).

In conclusion, the shrimp head flavorant was not suitable to apply in the shrimp shumai due to the dark color and its flavor characteristics. Therefore, the application of shrimp flavorant in other products types such as snack, soup, seasoning may be more appropriated and is suggested for further study. However, the shrimp head colorant effectively enhanced the color of shrimp shumai and led to increase the color liking score of the product.

5.4.1.2 Effects of shrimp colorant concentration on shrimp shumai

Table 35 shows the color liking score of shrimp shumai added with various concentrations of shrimp colorant extracted from Pacific white shrimp head and figure of shrimp shumai samples was shown in Figure 14. The color measurement of shrimp shumai sample are shown in Table 4. L* value was not different among all samples ($p \geq 0.05$), while a* and b* values were increased with the increasing in colorant concentrations ($p < 0.05$). The acceptance test indicated that the samples added with 0.2 and 0.4% of colorant had the higher liking score when compared to control (without the colorant). However, the panelists commented that the sample with 0.4% colorant was too intense and similar to synthetic colorant. DuBose *et al*. (1980) reported that color acceptability of low-fat cheese was affected by color concentration. The color liking score was decreased, when color was too orange. The similar results were found in other studies such as bread containing various concentrations of crude malva nut gum with too intent color (Phimolsiripol *et al*., 2017) and the decreasing of consumer's acceptability as color acceptance score of lemon cake decreased when the colorant concentration was higher than 0.003% (Wadhwani and McMahon, 2012).

Table 35 Hedonic score and L*, a*, b* values of shrimp shumai added with various concentrations of shrimp colorant

Sample	**Color liking score[1]**	**Color measurement[2]**		
		L*	**a***	**b***
Control	6.73 ± 1.20^{b}	65.47 ± 1.26^{a}	7.92 ± 0.14^{c}	23.33 ± 0.56^{b}
0.2% colorant	7.63 ± 0.89^{a}	64.75 ± 1.44^{a}	11.59 ± 0.19^{b}	23.02 ± 1.03^{b}
0.4% colorant	7.07 ± 0.60^{ab}	65.03 ± 0.39^{a}	14.90 ± 0.15^{a}	25.74 ± 0.51^{a}

1 Means ± SD (n=30), **2** Means ± SD (n=5).

Different superscripts ($^{a-c}$) in the same column indicate the significant differences ($p < 0.05$).

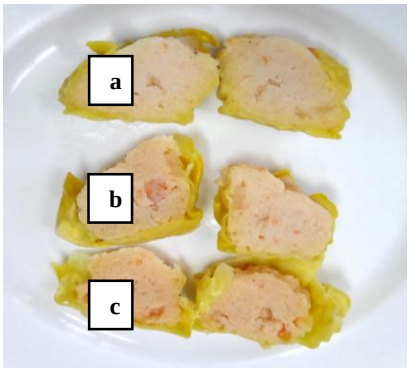


Figure 14 Shrimp shumai filling with the different shrimp colorant concentrations (a) control (without colorant), (b) 0.2% colorant and (c) 0.4% colorant

5.4.2 Applications of colorant from Pacific white shrimp head in mayonnaise-based dipping sauce

5.4.2.1 Screening for emotion terms for further testing

In this study, consumers (N = 111) were asked to select the emotion terms elicited by thinking of using the colorant from a natural source in food formulations or thinking of consuming one of the dipping sauces. Large differences in the emotion profiles of (i) colorant from a natural source, (ii) dipping sauce containing colorant from a natural source, (iii) dipping sauce containing a synthetic colorant and (iv) dipping sauce without added colorant were observed. The emotion profiles of the dipping sauce with a natural colorant and the one without added colorant were similar, both having high frequencies (40.78– 65.05%) of selected emotion terms including *friendly*, *good*, *good-natured*, *happy*, *satisfied* and *pleasant*. The dipping sauce without colorant also had high frequencies (40.78–55.34%) for *bored*, *calm*, *free*, *mild* and *quiet* emotions while the sample with a natural colorant had high frequencies (41.45–60.19%) for *enthusiastic*, *glad*, *interested*, *pleased* and *warm* emotions. Colorant from a natural source exhibited an emotional profile similar to that of the dipping sauce with a natural colorant, however, with much lower frequencies. The dipping sauce with a synthetic colorant was more associated with negative emotion terms with the five highest frequencies observed for *unsafe* (63.11%), *worried* (54.37%), *guilty* (47.57%), *aggressive* (47.56%) and *disgusted* (39.81%) emotions (see Table 36).

Table 36 Percentage of emotion terms selected by 111 respondents

Emotion terms	Percentage of selected			
	Colorant	No color	Natural colorant	Synthetic colorant
Active	17.48	25.24	39.81	20.39
Adventurous	12.62	11.65	33.01	33.01
Affectionate	2.91	27.18	36.89	11.65
Aggressive	20.39	5.83	6.80	47.56
Bored	4.85	54.37	15.53	8.74
Calm	8.74	46.60	36.89	2.91
Daring	7.77	8.74	24.27	37.86
Disgusted	5.83	6.80	2.91	39.81
Eager	8.74	15.53	35.92	20.39
Energetic	13.59	20.39	35.92	19.42
Enthusiastic	8.74	12.62	41.75	16.50
Free	6.80	47.57	21.36	7.77
Friendly	10.68	43.69	43.69	9.71
Glad	17.48	29.31	48.54	8.74
Good	36.89	52.43	65.05	15.53
Good-natured	24.27	44.66	44.66	4.85
Guilty	3.88	14.56	8.74	47.57
Happy	20.39	40.78	52.43	11.65
Interested	33.98	22.33	60.19	20.39
Joyful	5.83	25.24	38.83	12.62
Loving	3.88	20.39	35.92	3.88
Merry	1.94	18.45	35.92	11.65
Mild	6.80	55.34	30.10	5.83
Nostalgic	0.97	24.27	15.53	8.74
Peaceful	3.88	45.63	22.33	3.88
Pleasant	15.53	47.57	52.43	11.65
Pleased	24.27	38.83	51.46	12.62
Polite	0.00	39.81	20.39	6.80
Quiet	0.97	40.78	12.62	6.80
Satisfied	23.30	43.69	64.08	12.62
Steady	5.83	34.95	30.10	5.83
Tame	1.94	35.92	19.42	1.94
Tender	1.94	27.81	26.21	7.77
Understanding	6.80	18.45	29.13	5.83
Unsafe (pertaining to health)	15.53	6.80	2.91	63.11
Warm	16.50	13.59	47.57	18.45
Whole	4.85	30.10	27.18	4.85
Wild	10.68	9.71	18.45	34.95
Worried	3.88	5.83	9.71	54.37

To identify association of the three dipping sauces and emotion terms, the correspondence analysis (CA) was performed. The CA plot showed that dipping sauces with and without colorant from a natural source were more associated with positive emotions, while the dipping sauce with a synthetic colorant was more associated with negative emotions (Figure 15). Because of the length of the survey and the nature of the samples used in this study, we had to minimize the numbers of emotion terms to be further evaluated by consumers. According to King and Meiselman (2010), one of the criteria for term selection was frequency of use; they recommended the terms with ≥20% frequency of use to be selected. In our study, therefore, six positive emotion terms (*good*, *good-natured*, *interested*, *happy*, *satisfied* and *pleased*), all with ≥20% frequency of use, were initially selected. However, only *good*, *interested* and *satisfied* were arbitrarily further chosen as their highest possible frequencies were >60%. Five negative emotion terms (*aggressive, disgusted, guilty, unsafe* and *worried*) with ≥20% frequency of use were initially considered but only three terms (*guilty*, *unsafe* and *worried*) with the highest frequencies (>47%) were chosen. Even though six emotion terms were selected for further evaluation using a 5-point rating scale, it was not our intention to imply that only these six emotions were evoked or associated with the above described dipping sauces. Wardy *et al*. (2017) also used similar criteria to screen emotion terms from the Essense Profile® and selected 12 terms from 39 terms in their study to minimize respondent fatigue during testing of the sweetened ice tea products.

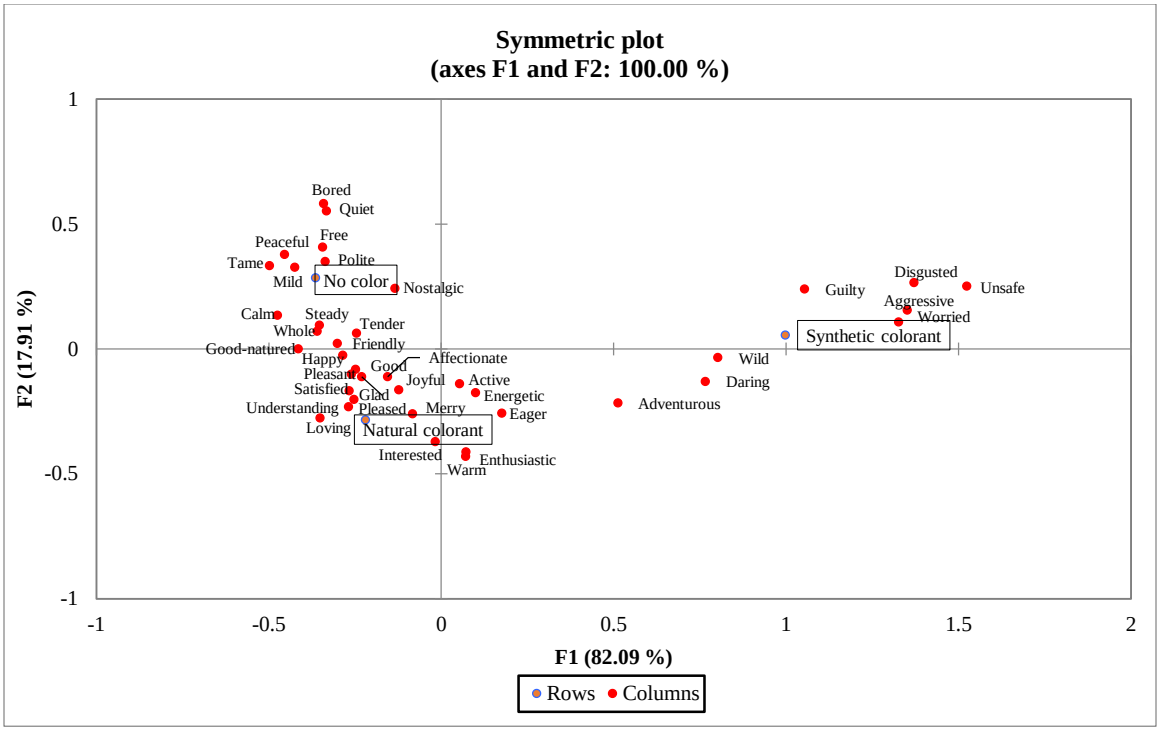


Figure 15 Correspondence analysis of Factor (F) 1 and 2 visualizing among dipping sauces with different types of colorant and emotion terms. No color = a dipping sauce without added colorant; natural colorant = a dipping sauce with colorant from a natural source; synthetic colorant: a dipping sauce with synthetic colorant.

5.4.2.2 Effect of colorant concentration on consumer's color liking score and saltiness intensity expectation

Various sodium content dipping sauces were added with colorant from shrimp head with the different concentrations. The results in Table 37 showed that colorant with reddish orange color affected on the decreasing of L* value (88.39 to 70.22) and increasing of a* and b* values (0.07 to 27.22 and 10.13 to 36.50, respectively; $p<0.05$). Consumers used color of food as visual queue for decide quality, freshness, flavors, and possible ingredients of products (Barham *et al.*, 2010). Millar *et al.* (2017) found the positive correlation of color and overall acceptance score of crackers with and without broad-bean and yellow-pea flour, and concluded that samples with the colored ingredients

were more preferred than control. In our study, the increasing of colorant concentration decreased color liking score when compared regardless with sodium content ($p<0.05$). "Too intense color" could decrease color liking scores, for instance, color liking score of bread contained 2.5% crude malva nut gum (CMG) with brown color was not different from score of control with white color but when CMG was added to the higher concentration (5, 7.5 and 10%) that affected to the darker color, color liking score of breads were decreased (Phimolsiripol *et al*., 2017). Wadhwani and McMahon (2012) also found the decreasing of acceptance score of lemon cake when yellow colorant was added higher than 0.003%. Color liking score of low-fat cheese was increased when color was changed from white to orange in DuBose *et al*. (1980) study but the decreasing was occurred for too orange sample. In our study, color liking scores of dipping sauces with off-white color were not different from pale orange color samples (MC; 1.2% colorant added) ($p\geq0.05$) but for HC samples those contained higher colorant concentration (3.6% HC) the decreasing of color liking scores were occurred ($p<0.05$).

Table 37 L*, a*, b* values, color liking score and saltiness intensity expectation of dipping sauces with the different colorant concentrations and salts.

Sample	Color measurement[1]			Visual color liking[2]	Visual saltiness expectation[2]	Saltiness intensity (%)[3]		
	L*	a*	b*			Too less	JAR	Too much
RS/NC	87.90 ± 0.39[b]	0.07 ± 0.02[e]	10.14 ± 0.09[e]	6.33 ± 1.73[a]	5.37 ± 1.58[a]	22.22	73.61	4.17
RS/MC	77.85 ± 0.19[d]	16.33 ± 0.27[c]	24.20 ± 0.53[c]	6.24 ± 1.72[a]	5.24 ± 1.46[a]	22.22	72.22	5.56
RS/HC	70.94 ± 0.20[e]	26.73 ± 0.20[b]	35.75 ± 0.38[b]	4.65 ± 1.71[c]	5.01 ± 1.58[a]	9.72	63.89	26.39
NS/NC	88.36 ± 0.24[a]	0.13 ± 0.04[e]	10.13 ± 0.15[e]	5.58 ± 1.82[b]	5.28 ± 1.49[a]	19.44	73.61	6.94
NS/MC	79.03 ± 0.24[c]	15.56 ± 0.35[d]	23.21 ± 0.36[d]	5.81 ± 1.77[ab]	5.07 ± 1.57[a]	16.67	63.89	19.44
NS/HC	70.81 ± 0.06[e]	26.50 ± 0.42[b]	35.33 ± 0.40[b]	5.42 ± 1.75[b]	4.79 ± 1.59[a]	12.50	56.94	30.56
ReS/NC	88.39 ± 0.13[a]	0.08 ± 0.03[e]	10.17 ± 0.11[e]	6.29 ± 1.73[a]	5.40 ± 1.34[a]	12.50	80.56	6.94
ReS/MC	78.03 ± 0.22[d]	16.12 ± 0.09[c]	24.02 ± 0.21[c]	5.39 ± 1.74[b]	5.01 ± 1.51[a]	11.11	76.39	12.50
ReS/HC	70.22 ± 0.17[f]	27.22 ± 0.23[a]	36.50 ± 0.48[a]	5.14 ± 1.76[bc]	5.10 ± 1.52[a]	23.61	58.33	18.06

[1] Mean ± standard deviation from 3 replications.

[2] Mean ± standard deviation from 216 consumer responses based on a 9-point hedonic scale. (72 data for each sample)

[3] Percentages based on 72 consumer responses and 3 JAR scale.

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

RS: Dipping sauce with NaCl added, NS: Dipping sauce without salt, ReS: Dipping sauce with KCl added

NC: Dipping sauce without colorant, MC: Dipping sauce with 1.2% colorant added, HC: Dipping sauce with 3.6% colorant added

Visual saltiness expectation was not significantly different for all samples ($p \geq 0.05$). It indicated that colorant concentration did not directly affect expectation of saltiness intensity. At the time, Maga (1974) revealed that salt was not associated with a particular color as salty foods were related to many different color. That meant color that could affect on saltiness was depended on type of food, for instance, pretzels, popcorn and olive saltiness were associated to brown, white and black color, respectively. However, more recently study demonstrated that many people associated salt with white color (Wan *et al*., 2014).

It was noticeable that when color liking scores of dipping sauces increased, percentages of Just-About-Right (JAR) for saltiness intensity were also increased in every sodium concentrations. This caused by halo effect which was defined by Lawless and Heymann (1998) as "the tendency for an attribute to be viewed more positively than normal due to one or more positive attributes within the product and/or the tendency to rate an attribute as more intense or positive due to logically unrelated attributes within the product." ANCOVA was conducted to confirm and the results showed that colorant concentration and its interaction with color liking score had no effect on saltiness expectation ($p \geq 0.05$) while color liking score significantly affected on saltiness expectation ($p < 0.05$).

5.4.2.3 Effect of colorant concentration, salt and sodium statement on saltiness liking score

Saltiness liking scores of dipping sauces with the different salt and colorant concentrations before and after statement of sodium was provided were shown in Table 38. At the given salt concentration, dipping sauce with the higher colorant concentration had the lower saltiness liking score for both before and after statement was shown ($p < 0.05$). This substantiates that color liking had effect on saltiness expectation as discussed above.

Table 38 Saltiness liking scores before and after sodium statement

sample	Saltiness liking	
	Before	**After**
RS/NC*	6.38 ± 1.88[a]	6.08 ± 1.85[a]
RS/MC	5.43 ± 1.93[b]	5.22 ± 1.86[bc]
RS/HC	3.97 ± 1.92[d]	3.83 ± 1.85[f]
NS/NC	5.40 ± 1.90[b]	5.54 ± 1.90[ab]
NS/MC	4.96 ± 1.88[bc]	4.83 ± 1.85[cd]
NS/HC	4.60 ± 1.88[cd]	4.43 ± 1.84[de]
ReS/NC*	6.13 ± 1.93[a]	5.83 ± 1.87[ab]
ReS/MC	4.51 ± 1.93[cd]	4.58 ± 1.86[de]
ReS/HC	4.31 ± 1.95[d]	4.17 ± 1.86[ef]

Mean ± standard deviation from 216 consumer responses based on a 9-point hedonic scale. (72 data for each sample).

* means significant of different saltiness liking score between before and after sodium statement.

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

RS: Dipping sauce with NaCl added, NS: Dipping sauce without salt, ReS: Dipping sauce with KCl added

NC: Dipping sauce without colorant, MC: Dipping sauce with 1.2% colorant added, HC: Dipping sauce with 3.6% colorant added

The results also showed that before sodium statement was given, dipping sauces with NaCl (RS) had the highest saltiness liking score followed by dipping sauces with KCl (ReS) and without salt (NS), respectively ($p<0.05$) regardless with colorant concentration. From the previous studies, potassium chloride was used as salt substitute to develop low/reduced sodium foods as it could import saltiness taste. An important limitation of KCl is it provides less salty taste in food products when compared with NaCl (Albarracìn *et al.*, 2011). That caused the lower saltiness liking score of ReS samples when compared with RS samples. The addition limitations of KCl utilization in foods are the presence of bitterness and metallic attribute that affect the

lower liking score (Siponoli and Lawless, 2012). Ambra *et al*. (2017) evaluated bitterness and saltiness of table olive fermented in brine that prepared using 0, 50 and 75% KCl to replace NaCl by trained panelists and a non-structured continuous scale. They reported that the olive with the higher KCl content had the higher bitterness and lower saltiness. Torrico *et al*. (2015) also showed that consumers could perceive bitter taste from oil-in-water emulsion.

The significantly decreasing of saltiness liking score of RS formulation for NC was observed ($p<0.05$). However, statement of sodium did not significantly affected saltiness liking score overall ($p\geq0.05$). Similarly to Leim *et al*. (2012) who reported that chicken soup with 'reduced salt' label was expected to be less salty than control even they were the same sample. Nevertheless, after tasting there was no different for liking or desire between samples.

5.4.2.4 Effect of colorant concentration, salt and their statements on emotions

Emotion scores of 3 positive (*good*, *interested* and s*atisfied*) and 3 negative (*guilty*, *unsafe* and *worried*) emotions of dipping sauces were evaluated for 3 steps. The first one was evaluated after consumer's tasting, followed by after statement of sodium was provided and the last one was rated after statement of colorant was given. Scores from all 3 steps were present in the same figure for each emotion term to compare the differentiation as shown in Figure 16. The higher scores were observed for positive emotions (1.69 – 3.10) when compared with negative emotions (1.46 – 1.99). Similar trend was also presented in the previous studies (King *et al*., 2010; King *et al*., 2013; Wardy *et al*., 2015; Poonnakasem *et al*., 2016).

Desmet and Schifferstein (2008) demonstrated that sources of food emotions included sensory attributes, experienced consequences, anticipated consequences, personal or cultural meanings, and actions of associated agents. In our study, color of dipping sauce was the strongest parameter as the emotion scores after consumer's tasting revealed that colorant concentration highly impacted on the increasing of negative emotion scores and the decreasing of positive emotion scores ($p<0.05$). As positive emotion scores were 0.61 – 1.39 unit decreased and negative emotion scores were 0.12 – 0.49 unit increased when compared NC and HC. However, it did not affect

score of unsafe ($p \geq 0.05$). While salt concentration had a few effect on good and satisfied ($p < 0.05$). The results were according to Spence (2015) who reviewed that color is the single most important product-intrinsic sensory cue when it came to setting people's expectations regarding the likely taste and flavor of food and drink.

Consumers were informed that how sodium intake could affect their health and sodium content in each sample then they were asked to rate emotion scores again. The results indicated that sodium statement did not affect scores of positive emotions (*good*, *interested* and *satisfied*; $p \geq 0.05$). NS formula with colorant had lower guilty scores ($p < 0.05$) while unsafe and worried scores also decreased in NS and ReS formulas with HC ($p < 0.05$). In the last step, statement of colorant was presented before emotion scores rating. The results showed that colorant statement did not affect *interested*, *worried* and *unsafe* ($p \geq 0.05$). However, scores of *guilty* were decreased especially for HC samples (0.07 – 0.21 unit; $p < 0.05$). Food labelings were used to provide the informations in order for making decision to purchase and rational of consumers (O'Fallon *et al*., 2007). The statements of health benefits (sodium statement) and food choices (colorant from a natural source) could impact consumer's emotions, liking as well as purchase intent as reported in many previous studies (Bower *et al*., 2003; Kleef *et al*., 2005; Chen, 2007; Sabbe *et al*., 2009; Yao and Wang, 2012; Poonnakasen *et al*. 2016). Poonnakasem *et al*. (2016) demonstrated that giving information of oil health benefits used in sponge cake significantly increased overall liking, positive emotion, and purchase intent scores while decreased negative emotion scores. In our presence study, emotions elicited by dipping sauces were strongly associated to colorant concentration that affected the low impact of statements. However, Leim *et al*. (2012) reported that health label as 'reduced in salt' of 'health choices' logo also affected to consumer's expectation and made they are more worried about taste of the products, rather than healthiness.

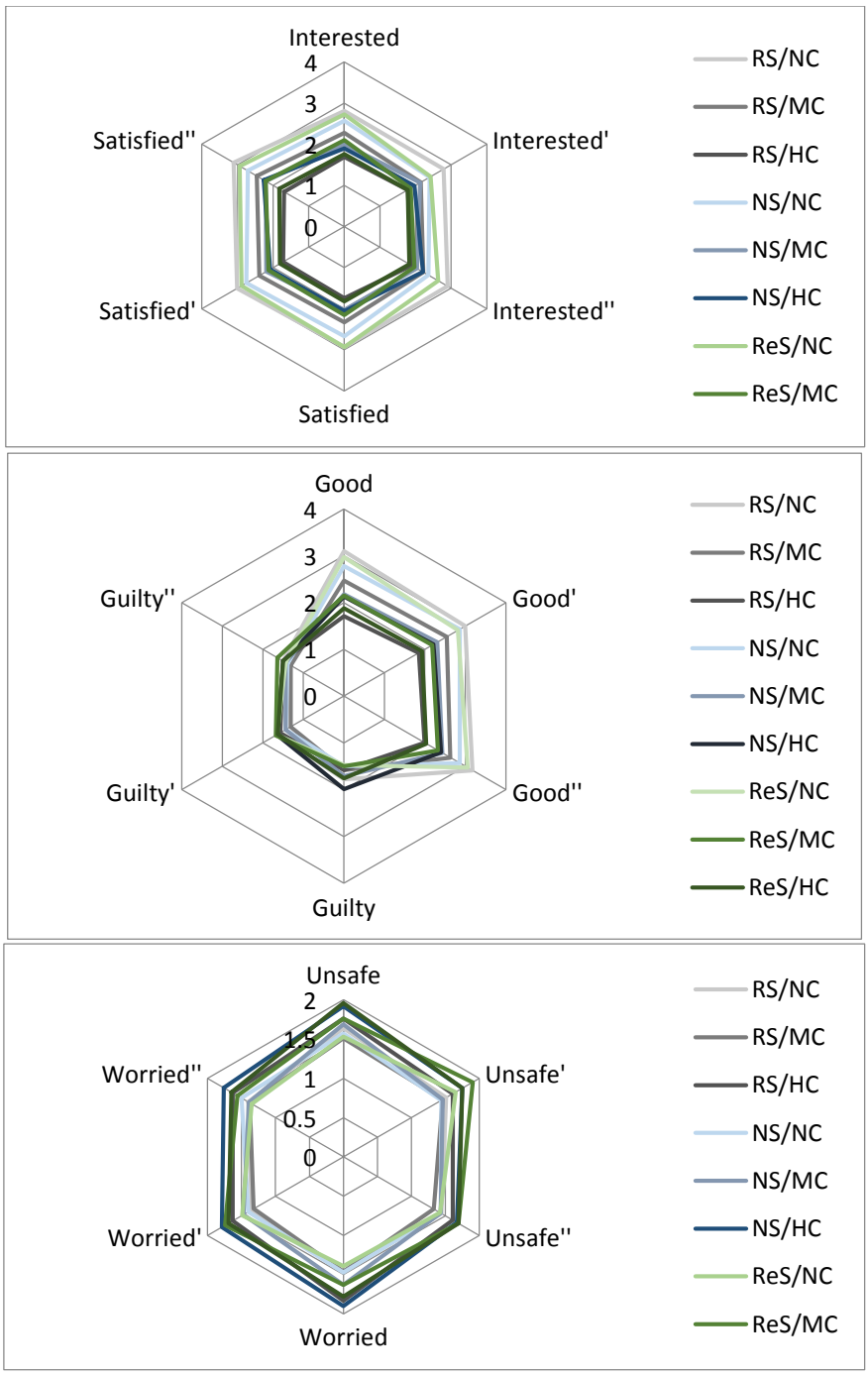


Figure 16 Emotion scores after tasting, after colorant statement (') and after sodium statement (") of dipping sauces with the different colorant concentrations and salts. A: scores for *good* and *guilty*, B: scores for *interested* and satisfied, C: scores for *unsafe* and *worried*

Based on 216 consumer responses (72 data for each sample) and 5-point scale.

5.4.2.5 Effect of colorant concentration, salt and their statement on purchase intent

Purchase intents of dipping sauces were evaluated using binomial (yes/no) scale for 3 steps as same as emotion scores. The results in Table 39 showed that colorant addition had high effect on purchase intent, it was found that when colorant concentration increase from NC (0%) to MC (1.2%) and from MC to HC (3.6%), purchase intent was decreased for 18.05–33.34% and 11.11-25.00%, respectively. Effect of salt concentration was presented as dipping sauce with RS formula had the highest purchase intent (34.72–68.06%) followed by ReS (23.61–56.94%) and NS (26.39-44.44%), respectively for NC and MC. For HC samples, RS had the lowest purchase intent (9.72%). Both colorant concentration and salt affected liking scores and elicited emotions as described above and influenced to purchase intent. Poonnakasem *et al*. (2016) and Prinyawiwatkul and Chompreeda (2007) were also reported the association of sensory acceptance scores and elicited emotions on consumer's purchase intent.

After statement of sodium was given, the slightly increasing of purchase intents for all dipping sauces were occurred (1.39-9.72 %) but purchase intent of RS/MC, ReS/NC and ReS/MC were not affected by sodium statement. While colorant statement scarcely affected on purchase intent as after colorant statement was given, only 1.39-2.78% purchase intents were increased, except NS/MC, RS/NC, ReS/NC and ReS/HC that were not different. The results were presented the similar trend with the effects of statements on elicited emotions.

Table 39 Purchase intent of dipping sauces before and after statements of sodium and colorant were provided

Sample	Purchase intent (%)		
	Before Statement	After statement of	
		Sodium	Colorant
RS/NC	68.06	70.83	70.83
RS/MC	34.72	34.72	37.50
RS/HC	9.72	15.28	18.06
NS/NC	44.44	50.00	51.39
NS/MC	26.39	31.94	31.94
NS/HC	15.28	25.00	26.39
ReS/NC	56.94	56.94	56.94
ReS/MC	23.61	23.61	25.00
ReS/HC	12.50	13.89	13.89

Percentage from 216 consumer responses based on yes/no question (72 data for each sample).

RS: Dipping sauce with NaCl added, NS: Dipping sauce without salt, ReS: Dipping sauce with KCl added

NC: Dipping sauce without colorant, MC: Dipping sauce with 1.2% colorant added, HC: Dipping sauce with 3.6% colorant added

5.5 Conclusion

Flavorant extracted from Pacific white shrimp head using Alcalase for 270 min and dried by tray drying method was not suitable to apply in the shrimp shumai as it imparted brown color and led to the decreasing of liking score of the shumai product. However, the addition of 0.2% colorant increased color liking score of shrimp shumai and gained the highest color liking score, while sample with 0.4% colorant added was too intense color.

The concentration of colorant from shrimp heads strongly affected the color liking, saltiness liking, elicited emotions and purchase intent of mayonnaise-based dipping sauce. The increasing colorant concentration decreased both color and saltiness liking scores as well as scores of positive emotions (*good*, *interested* and *satisfied*) and consumer's purchase intent while the increase of negative emotions (*guilty*, *unsafe* and *worried*) scores occurred. Regard with salt, dipping sauces with NaCl (RS) had the highest saltiness liking scores followed by samples with KCl (ReS) and without salt (NS), respectively. Salt had a few effects on *good* and *satisfied* emotion scores including purchase intent. Statements of sodium content and source of colorant almost did not affect emotions scores and purchase intent as the effect of colorant was much stronger. It was noted that colorant concentration did not directly affect the expectations of saltiness of the dipping sauce but the dipping sauce with the higher color liking scores always got the higher percentages of just-about-right for saltiness intensity.

CHAPTER 6

CONCLUSION AND SUGGESTION

6.1 Conclusions

1. Soaking Pacific white shrimp head in 2.0% sodium metabisulfite solution for 15 min was the most effective pretreatment for melanosis prevention of shrimp head.

2. The protein extraction using Alcalse (pH 8.0) or Flavourzyme (pH 7.0) at 55^{o}C for 270 min yielded the highest protein content and the extracted protein using different enzyme had the different intensity of odor characteristics.

3. The optimal proportion of isopropanol: hexane for carotenoid extraction from Pacific white shrimp head was 40.7: 59.3 (v/v) due to the highest carotenoid extraction yield.

4. Sodium metabisulfite solution soaking for melanosis prevention decreased flavorant extraction yield, but increased colorant extraction yield.

5. Drying method had highly effect on the odor of the flavorant as pyrazine compounds could be generated during the process and some volatile compounds were lose. However, the tray dried sample without maltodextrin had the highest consumers' acceptant score.

6. The flavorant stored under air and vacuum conditions at room temperature for 4 months slightly different volatile compounds proportion. However, the trained panelists could not detect the differences of the odors after the flavorant kept under both conditions for 4 months.

7. The shrimp colorant up to 0.2% could enhance color and acceptability of shrimp shumai.

8. The increasing shrimp colorant concentration decreased both color and saltiness liking scores and the sample with the higher color liking score had the higher percentages of just-about-right for saltiness intensity.

6.2 Suggestions

1. The flavorant extracted from shrimp head by enzymatic method and dried with tray drying had strong roasted sundried shrimp, boiled sundried shrimp, boiled blue swimming crab, unfresh shrimp juice and Mungoong odors and flavors. Therefore, the application in appropriate product and flavorant concentration should be further studies.

2. The colorant was carotenoid-containing oil and contained raw shrimp flavor led to the limitation for applying into food.

REFERENCES

Adhikari, T.B., Cavaletto, J.R., Dubcovsky, J., Gieco, J.O., Schlatter, A.R. and Goodwin, S.B., 2004. Molecular mapping of the Stb4 gene for resistance to septoria tritici blotch in wheat. Phytopathology. 94: 1198–206.

Albarracìn, W., Sánchez, I.C., Grau, R. and Barat, J.M. 2011. Salt in food processing; usage and reduction: a review. Int. J. Food Sci. Technol. 46: 1329–1336.

Almli, V.L. and Hersleth, M. 2013. Salt replacement and injection salting in smoked salmon evaluated from descriptive and hedonic sensory perspectives. Aquac. Int. 21: 1091–1108.

Amarowicz, R. and Shahidi, F. 1997. Antioxidant activity of peptide fractions of capelin protein hydrolysates. Food. Chem. 58: 355–559.

Ambra, R., Lucchetti, S., Moneta, E., Peparaio, M., Nardo, N., Baiamonte, I., Costanzo, M.G.D., Civitelli, E.S. and Pastore, G. 2017. Effect of partial substitution of sodium with potassium chloride in the fermenting brine on organoleptic characteristics and bioactive molecules occurrence in table olives debittered using Spanish and Castelvetrano methods. Int. J. Food Sci. Technol. 52: 662–670.

Angeles Navarrete del Toro, M. and Garcia-Carreno, F.L. 2002. Evaluation of the progress of protein hydrolysis. *In* Current protocols in food analytical chemistry (Wrolstad R.E., *et al*., eds). Wiley, New York.

Anon. 1992. Techniques for biological assessment in fisheries management. Report of the Workshop Jülich, July 17–24, 1991. Berichte ans der Ökologischen Forschung, Forschungszentrum Jülich GmbH. 9: 63 p.

Anonymous. 1988. Aquaculture drugs. *In* Fish and Fishery Products Hazards and Control Guide, FDA, Center for Food Safety and Applied Nutrition. p. 115-132. Office of Seafood. Washington, DC.

Anonymous. 1992. Code of Federal Regulation, Food and Drug 21 Parts 170.199. Office of the Federal Register National Archives and Records Administration: Washington, DC.

AOAC. 2000. Official method of analytical chemists. 16th ed. The Association of Official Analytical Chemists, Inc, Washington, DC.

Arnao, M.B., Cano, A. and Acosta, M. 2001. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chem. 73: 239–244.

Babu, C. M., Chakrabarti, R. and Sambasivarao, K. R. S. 2008. Enzymatic isolation of carotenoid-protein complex from shrimp head waste and its use as a source of carotenoids. LWT- Food Sci. Technol. 41: 227–235.

Baek, H. H and Cadwallader, K. R. 1995. Enzymatic hydrolysis of crayfish processing by-products. J. Food Sci. 60: 929–935.

Bak, L. S., Jacobsen, L., and Jørgensen, S. S. 1999. Characterisation of qualitative changes in frozen, unpeeled cold-water shrimp (*Pandalus borealis*) by static headspace gas chromatography and multivariate data analysis. Food Res. Technol. 208: 10–16.

Banik, R. M. and Prakash, M. 2006. Purification and characterization of laundry detergent compatible alkaline protease from *Bacillus cereus*. Ind. J. Biotechnol. 5: 380-384.

Barham, P., Skibsted, L.H., Bredie, W.L.P. et al. 2010. Molecular gastronomy: a new emerging scientific discipline. Chem. Rev. 110: 2313–2365.

Belitz, H.D., Grosch, W., and Schieberle, P. 2009. Food chemistry. *In* H.D. Belitz, W. Grosch & P. Schieberle. Meat (^{4th} ed., Vol. 12, pp.605-608). Berlin: Springer-Verlag.

Benjakul, S. and Morrissey, M.T. 1997. Protein hydrolysates from Pacific whiting solid wastes. J. Agric. Food Chem. 45: 3423–3430.

Benjakul, S., Binsan, W., Visessanguan, W., Osako, K. and Tanaka, M. 2009. Effects of flavourzyme on yield and some biological activities of Mungoong, an extract paste from the cephalothorax of white shrimp. J. Food Sci. 74: s73-s80.

Benjakul, S., Lertittikul, W. and Bauer, F. 2005. Antioxidant activity of Maillard reaction products from a porcine plasma protein-sugar model system. Food Chem. 93: 189–196.

Benjakul, S., Seymour, T.S., Morrissey, M.T. and An, H. 1997. Protein hydrolysates from Pacific whiting solid wastes. J. Agric. Food Chem. 45: 3423–3430.

Benjakul, S., Visessanguan, W. and Tanaka, M. 2006. Inhibitory effect of cysteine and glutathione on phenoloxidase from kuruma prawn (*Penaeus japonicus*). Food Chem. 98: 158–163.

Binsan, W, Benjakul, S., Visessanguan, W., Roytrakul, S., Tanaka, M. and Kishimura, H. 2008. Antioxidative activity of Mungoong, an extract paste, from the cephalothorax of white shrimp (*Litopenaeus vannamei*). Food Chem. 106: 185–193

Boran, G., Karacam, H. and Boran, M. 2006. Changes in the quality of fish oils due to storage temperature and time. Food Chem. 98: 693–698.

Bower, J.A., Saadat, M.A. and Whitten, C. 2003. Effect of liking, information and consumer characteristics on purchase intention and willingness to pay more for a fat spread with a proven health benefit. Food Qual. Prefer. 14: 65–74.

Boyer, R. F. and McCleary, C. J. 1987. Superoxide ion as a primary reductant in ascorbate-mediated ferritin iron release. Free Radical Biol. Med. 3: 389–395.

Boyle, J. L., Lindsay, R. C. and Stuiber, D. A. 1993. Occurance and properties of flavor related bromophenols found in the marine environment: a review. J. Aquat. Food Product Technol. 2: 75–82.

Bremner, A. 1998. If freshness is lost, where dose it go? *In* Method to Determine the Freshness of Fish in Research and Industry. (Ólafsdóttir, G., Luten, J., Dalgaard, P., Careche, M. Verrez, B. V., Martinsdóttirr. E. and Heia K. eds.). p. 3651. Int Inst. Of Refrigeration: Paris.

Buege, J. and Aust, S. 1978. Microsomal lipid peroxidation. Method Enzymol. 52: 302-310.

Bueno-Solano, C., López-Cervantes, L., Campas-Baypoli, N. O., Lauterio-García, R., Adan-Bante, N. P. and Sánchez-Machado, D. I. 2009. Chemical and biological characteristics of protein hydrolysates from fermented shrimp by-products. Food Chem. 112: 671–675.

Buren, L., Dötsch-Klerk, M., Seewi, G. and Newson, R.S. 2016. Dietary Impact of Adding Potassium Chloride to Foods as a Sodium Reduction Technique. *Nutrients*, 8, 235; doi:10.3390/nu8040235.

Buttery, R.G., Guadagni, D.G., Ling, L.C., Seifert, R.M. and Lipton, W. 1976. Additional volatile components of cabbage, broccoli and cauliflower .J. Agric. Food Chem. 24:829–832.

Cahú, T.B., Santos, S.D., Mendes, A., Córdula, C.R., Chavante, S.F., Carvalho Jr., L.B., Nader, H.B. and Bezerra, R.S. 2012. Recovery of protein, chitin, carotenoids and glycosaminoglycans from Pacific white shrimp (*Litopenaeus vannamei*) processing waste. Process Biochem. 47: 570–577.

Cano-Chauca, M., Stringheta, P.C., Ramos, A.M. and Cal-Vidal, J. 2005. Effect of the carriers on the microstructure of mango powder obtained by spray drying and its functional characterization. Innov. Food Sci. Emerg. Technol. 5: 420–428.

Cano-Lopez, A., Simpson, B. K. and Haard, N. F. 1987. Extraction of carotenoprotein from shrimp process wastes with the aid of trypsin from Atlantic cod. J. Food Sci. 52: 503–504.

Carabasa-Giribet, M., and Ibarz-Ribas, A. 2000. Kinetics of colour development in aqueous glucose systems at high temperatures. J. Food Eng. 44: 181–189.

Cardello, A.V. 1996. The role of the human senses in food acceptance. In: Food choice, acceptance and consumption. (edited by H.L. Meisalman & H.J.H MacFie). Pp. 1-84. New York, USA: Blackie Academic and Professional.

Cha, Y.J., Kim, H., Jang, S.M. and Park, J.Y. 1999. Identification of aroma-active compounds in Korean salt-fermented fishes by aroma extract dilution analysis, 2. aroma-active components in salt-fermented shrimp on the market. J. Korean Soc. Food Sci. Nutr. 28: 319-325.

Chaijan, M., Benjakul, S., Visessanguan, W. and Faustman, C. 2006. Changes of lipids in sardine (*Sardinella gibbosa*) muscle during iced storage. Food Chem. 99: 83–91.

Chakrabarti, R.2002. Carotenoprotein from tropical brown shrimp shell waste by enzymatic process. Food Biotechnol. 16: 81–90.

Chan, M.M. and Kane-Martinelli, C. 1997. The effect of color on perceived flavor intensity and acceptance of foods by young adults and elderly adults. J. Am. Diet. Assoc. 97: 657-659.

Chareonthaikij, P., Uan-On, T. and Prinyawiwatkul, W. 2016. Effects of pineapple pomace fibre on physicochemical properties of composite flour and dough, and consumer acceptance of fibre-enriched wheat bread. Int. J. Food Sci. Technol. 51: 1120–1129.

Chaveesak, R., Smith, J.P. and Simpson, B.K. 1993. Production of fish sauce and acceleration of sauce fermentation using proteolytic enzymes. J. Aquat. Food Prod. Technol. 2: 59–77.

Chen, J. S., Balaban, M. O., Wei, C. I., Gleeson, R. A. and Marshall, M. R. 1993. Effect of carbon dioxide on the inactivation of Florida spiny lobster polyphenol oxidase. J. Sci. Food Agric. 61: 253-259.

Chen, J. S., Balaban, M. O., Wei, C. I., Marshall, M. R. and Wei, Y. 1992. 'Inactivation of polyphenol oxidase by high-pressure carbon dioxide. J. Agric. Food Chem. 40: 2345-2349.

Chen, J. S., Wei, C. I. and Marshall, M. R. 1991. Inhibition mechanism of kojic acid on polyphenol oxidase. J Agric. Food Chem. 39:1897–1901.

Chen, M.F. 2007. Consumer attitudes and purchase intentions in relation to organic foods in Taiwan: Moderating effects of food-related personality traits. Food Qual. Prefer. 18: 1008–1021.

Chersman, D. F., Lee, W. L. and Zorgalsky. 1967. Carotenoproteins in invertebrates. Biol. Rev. 42: 132-160.

Chokumnoyporn, N. Sriwattana, S. and Prinyawiwatkul, W. 2016. Saltiness enhancement of oil roasted peanuts induced by foammat salt and soy sauce odour. Int. J. Food Sci. Technol. 51: 978–985.

Chokumnoyporn, N., Sriwattana, S., Phimolsiripol, Y., Torrico, D.D. and Prinyawiwatkul, W. 2015. Soy sauce odour induces and enhances saltiness perception. Int. J. Food Sci. Technol. 50: 2215–2221.

Cobb, B. F., Conte, F. S. and Edwards, M. A. 1979. Free amino acids and osmoregulation in penaeid shrimp. J. Agric. Food Chem. 23: 1172–1174.

Conway, E. J. and Byrne, A., 1936. An absorption apparatus for the microdetermination of certain volatile substances I. The micro-determination of ammonia. J. Biochem. 27: 419–429.

D'Aniello, A. 1980. Free amino acids in some tissues of marine crustacean. Experientia. 36: 392–393.

Desmet, P., M.A. and Schifferstein, H.N.J. 2008. Sources of positive and negative emotions in food experience. Appetite. 50: 290–301.

Dey, S.S. and Dora, K.C. 2014. Antioxidative activity of protein hydrolysate produced by alcalase hydrolysis from shrimp waste (*Penaeus monodon* and *Penaeus indicus*). J. Food Sci. Technol. 51:449–457.

Diaz-Tenorio, L. M., Garcia-Carreno, F. L., and Pacheco-Aguilar, R. 2007. Comparison of freezing and thawing treatments on muscle properties of white leg shrimp (*Litopenaeus vannamei*). J. Food Biochem. 31: 563–576.

DuBose, C.N., Cardello, A.V. and Maller, O. 1980. Effects of colorants and flavorants on identification, perceived flavor intensity, and hedonic quality of fruit-flavored beverages and cake. J. Food Sci. 45: 1393-1399.

Dziezak, J. D. 1986. Preservatives: Antioxidants-The ultimate answer to oxidation. Food Technol. 40(9): 94-102.

Edge, R., McGarvey, D. J., and Truscott, T. G. 1997. The carotenoids as anti-oxidants - a review. J. Photochem. Photobiol. B-Biology. 41: 189-200.

Ekpenyong, E., Williams, I.O, and Osakpa, U.U. 2013. Variation in the proximate, energy and mineral compositions of different body parts of *Macrobrachium macrobranchion* (prawn). J. Food Res. 2: 150-156.

Erickson, E. C., Bulgarelli, M. A., Resurreccion, A. V. A., Vendetti, R. A. and Gates, K. A. 2007. Sensory differentiation of shrimp using a trained descriptive analysis panel. LWT- Food Sci. Technol. 40: 1774-1783.

Fassett, R. G. and Coombes, J. S. 2012. Astaxanthin in Cardiovascular Health and Disease. Molecules. 17: 2030-2048.

Feltrin, A.C., Souza, V.R., Saraiva, C.G., Nunes, C.A. and Pinheiro, A.C.M. 2015. Sensory study of different sodium chloride substitutes in aqueous solution. Int. J. Food Sci. Technol. 50: 730–735.

Finne, J. 1982. Occurrence of unique polysialosyl carbohydrate units in glycoproteins of developing brain. J. Biol. Chem. 257: 11966-11970.

Food Standards Australia New Zealand. 2004. Food Standards code. Available: [http://www.foodstandards.gov.au/foodstandardscode/].

Francis, F.J. and Clydesdale, F.M. 1975. Food Colorimetry. In: Theory and applications. Westport, CT, USA: AVI Publishing Company Inc.

Friedman, M. 1996. Food browning and its prevention: An overview. J. Agric. Food Chem. 44: 631-653.

Friedman, M. and Molnar-Perl, I. 1990. Inhibition of browning by sulfur amino acids. 1. Heated amino acid-glucose systems. J. Agric. Food Chem. 38: 1642–1647.

Friedman, M., 1996. Nutritional value of protein from different food sources. J. Agric. Food Chem. 44: 6-29.

Garcia-Molina, F., Penalver, M. J., Rodriguez-Lopez, J. N., Garcia-Canovas, F., and Tudela, J. 2005. Enzymatic method with polyphenol oxidase for the determination of cysteine and *N*-acetylcysteine. J. Agric. Food Chem. 53: 6183–6189.

Gentles, A., and Haard, N. F. 1991. Pigmentation of rainbow-trout with enzyme-treated and spray-dried phaffia-rhodozyma. Prog. Fish Cult. 53: 1-6.

Gildberg, A. 1993. Enzymatic processing of marine raw materials. Process Biochem. 28: 1–15.

Gildberg, A. and Stenberg, E. 2001. A new process for advanced utilization of shrimp waste. Process Biochem. 36: 809–12.

Giri, A., Osako, K. and Ohshima. T. 2010. Identification and characterisation of headspace volatiles of fish miso, a Japanese fish meat based fermented paste, with special emphasis on effect of fish species and meat washing. Food Chem. 120: 621–631.

Gonçalves, A.C., Lopez-Caballero, M.E. and Nunes, M.L. Quality changes of deep water pink shrimp (*Parapenaeus longirostris*) packed in modified atmosphere. J. Food Sci. 68: 2586-2590.

Goodwin, T.W. 1954. Carotenoids, Their Comparative Biochemistry. Chemical Publishing Co. Inc. New York, 356 p.

Gordon, R. J. and Josephson, D. B. 2000. Surimi seafood flavors, creation and evaluation. *In* Surimi Seafood. 1st ed. (Park, J. W. ed.). p. 393–416. Marcel Dekker. New York.

Goula, A.M. and Adamopoulos, K.G. 2010. A new technique for spray drying orange juice concentrate. Innov. Food Sci. Emerg. Technol. 11: 342–351.

Grabowski, J.A., Truong, V.D. and Daubert, C.R. 2006. Spray-drying of amylase hydrolyzed sweet potato puree and physicochemical properties of powder. J. Food Sci. 71: 209–217.

Guàrdia, M.D., Guerrero, L., Gelabert, J., Gou, P. and Arnau, J. 2008. Sensory characterisation and consumer acceptability of small calibre fermented sausages with 50% substitution of NaCl by mixtures of KCl and potassium lactate. Meat Sci. 80: 1225–1230.

Guerrero-beltrán, J. A., Swanson, B. G. and Barbosacánovas, G. V. 2005. Inhibition of polyphenoloxidase in mango puree with 4-hexylresorcinol, cysteine and ascorbic acid. LWT-Food Sci. Technol. 38: 625–630.

Handayani, A. D., Sutrisno, Indraswati, N. and Ismadji, S. 2008. Extraction of astaxanthin from giant tiger (*Panaeus monodon*) shrimp waste using palm oil: Studies of extraction kinetics and thermodynamic. Biores. Technol. 99: 4414–4419.

Hendrickx, M., Ludikhuyze, L., Van Den Broeck, I. and Weemaes, C. 1998. Effects of high pressure on enzymes related to food quality. Trends Food Sci. Technol. 9: 197–203.

Hesselberg, R.J. and Seelye, J.G. 1982. Identification of organic compounds in Great Lakes fishes by gas chromatography/mass spectrometry: 1977. Great Lakes Fishery aboratory, Ann Arbor, Michigan, Administrative Report No 82-1, p 49.

Heu, M.S., Kima, J.S. and Shahidib, S. 2003. Components and nutritional quality of shrimp processing by-products. Food Chem. 82: 235-242.

Higuera-Ciapara, I., Felix-Valenzuela, L. and Goycoolea, F.2006. Astaxanthin: a review of its chemistry and applications. Crit. Rev. Food Sci. and Nutri. 46: 185–196.

Hoegg, J. and Alba, J.W. 2007. Taste perception: more than meets the tongue. J. Consum. Res. 33: 490-498.

Holanda, H. D. and Netto, F. M. 2002. Optimization of the conditions for the enzyme hydrolysis of shrimp residue, using response surface methodology (RSM). *In* Book of Abstracts, 2002 IFT Annual Meeting. p. 194. Anaheim.

Holanda, H. D. and Netto, F. M. 2006. Recovery of components from shrimp (*Xiphopenaeus kroyeri*) processing waste by enzymatic hydrolysis. J. Food Sci. 71: C298-C303.

Hrčková, M., Rusňáková, M. and Zemanovič, J. 2002. Enzymatic hydrolysis of defatted soy flour by three different proteases and their effect on the functional properties of resulting protein hydrolysates. Czech J. Food Sci. 20: 7–14.

Huang, Y.L. and Ma, Y.S. 2016. The effect of extrusion processing on the physiochemical properties of extruded orange pomace. Food Chem. 192: 363-369. J. Sens. Stud. 4: 129-148.

Huss, H.H. 1995. Quality and quality changes in fresh fish. FAO Fish. Tech. Pap. 348: 35-67.

Hussein, G., Sankawa, U., Goto, H., Matsumoto, K. and Watanabe. H. 2006. Astaxanthin, a Carotenoid with potential in human health and nutrition. J. Nat. Prod. 69: 443-449.

Idrus, N.F.M., Febrianto, N.A., Zzaman, W., Cuang, T.E. and Yang, T.A. 2013. Optimization of the Aqueous Extraction of Virgin Coconut Oil by Response Surface Methodology. Food Sci. Techol. Res. 19: 729-737.

Iida, H. 1998. Studies of the accumulation of dimethyl-beta-propiothetin and the formation of dimethyl sulfide in aquatic organisms. Bull.Tokai Regional Fisheries Res. Lab. 35-111.

International Standard ISO 8586-1. 1993. Sensory Analysis-General guidance for the selection and monitoring of assessors. International Organization for Standardization, Geneva. Switzerland, pp 1-14.

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

Jeyakumari, A., Janarthanan, G., Chouksey, M. and Venkateshwarlu, G. 2016. Effect of fish oil encapsulates incorporation on the physico-chemical and sensory properties of cookies. J. Food Sci. Technol. 53: 856–863.

Kahn, V., Lindner, P. and Zakin, V. 1995. Effect of kojic acid on the oxidation of *o*-dihydrohyphenols by mushroom tyrosinase. J. Agric. Food Chem. 18: 253-271.

Kahtani, H. A. and Hassan, B. H. 1990. Spray drying of roselle (*Hibiscus sabdariffa*, L.) extract. J. Food Sci. 55: 1073-1076.

Kanpairo, K., Usawakesmanee, W., Sirivongpaisal, P. and Siripongvutikorn, S. 2012. The compositions and properties of spray dried tuna flavor powder produced from tuna precooking juice. Int. Food Res. J. 19: 893-899.

Kawai, T. and Sakaguchi, M. 1996. Fisg flavor. Crit. Rev. Food Sci. Nutri. 36: 257-298.

Kim, J. and Marshall, M. R. 2000. Polyphenoloxidase. *In* Seafood Enzymes: Utilization and Influence on Postharvest Seafood Quality. (Haard, N. F. and Simpson, B. K. eds.). p. 271-315. Marcel Dekker Inc, New York.

Kim, S. H., Ben-gigirey, B., Baeeos-Velazquez, J., Price, R. J. and An, H. 2000. Histamine and biogenic amine production by *Morganella morganii* isolated from temperature-abuse albacore. J. Food Prot. 63: 244–251.

Kim, Y.J., Park, J.Y., Park, H.J., Kim, S.B., Chun, B.S. and Lee, Y.B. 2014. Development of Reaction Flavors with Enzymatic Hydrolysate of Krill *Euphausia superba* in Ramen Sauce. Fish Aquat. Sci. 17: 403-408.

King, S.C. and Meiselman, H.L. 2010. Development of a method to measure consumer emotions associated with foods. Food Qual. Prefer. 21: 168-177.

King, S.C., Meiselman, H.L. and Carr, B.T. 2010. Measuring emotions associated with foods in consumer testing. Food Qual. Prefer. 21: 1114-1116.

King, S.C., Meiselman, H.L. and Carr, B.T. 2013. Measuring emotions associated with foods: important elements of questionnaire and test design. Food Qual. Prefer. 28: 8-16.

Kleef, E.V., Trijpa, H.C.M.V. and Luning, P. 2005. Functional foods: health claim-food product compatibility and the impact of health claim framing on consumer evaluation. Appetite. 44: 299–308.

Klomklao, S., Benjakul, S. Visessanguan, W., Kishimura, H. and Simpson, B. K. 2009. Extraction of carotenoprotein from black tiger Shrimp shells with the aid of bluefish trypsin. J. Food Biochem. 33: 201–217.

Konosu, S. K. and Yamaguchi, K. 1982. The flavor components in fish and shellfish. *In* Chemistry and Biochemistry of Marine Food Products. (Martin, R. E., ed.). Avi Publishing Co., Westport.

Konosu, S. K. and Yamaguchi, K. 1982. The flavor components in fish and shellfish. *In* Chemistry and Biochemistry of Marine Food Products. (Martin, R. E., ed.). Avi Publishing Co., Westport.

Koocheki, A., Taherian, A.R., Razavi, S.M.A. and Bostan, A. 2009. Response surface methodology for optimization of extraction yield, viscosity, hue and emulsion stability of mucilage extracted from *Lepidium perfoliatum* seeds. Food Hydrocoll. 23: 2369–2379.

Koza, B. J., Cilmi, A., Dolese, M. and Zellner, D.A. 2005. Color enhances orthonasal olfactory intensity and reduces retronasal olfactory intensity. Chem. Sens. 30: 643–649.

Krause, W. and Freimuth, U. 1985. Alkali treatment of proteins. VII. Racemization and enzymic hydrolysis. Nahrung. 29: 957–68.

Kremer, S., Mojet, J. and Shimojo, R. 2009. Salt reduction in foods using naturally brewed soy sauce. J. Food Sci. 74: s255-s262.

Kristinsson, H.G. and Rasco, B.A. 2000. Fish protein hydrolysates: Production, biochemical and functional properties. Crit. Rev. Food. Sci. Nutr. 40: 43–81.

Kurozawa, L. E., Park, J. K. and Hubinger, M. D. 2009. Effect of carrier agents on the physicochemical properties of a spray dried chicken meat protein hydrolysate. J. Food Eng. 94: 326–333.

Lannelongue, M., Finne, G., Hanna, M.O., Nickelson II, R. and Vanderzant, C. 1982. Microbiological and chemical changes during storage of swordfish (*Xiphias gladius*) steaks in retail packages containing CO_2-enriched atmospheres. J. Food Prot. 45: 1197-1203.

Latscha, T. 1989. The role of astaxanthin in shrimp pigmentation. Advances In Tropical Aquaculture Tahrh. Feb 20 - March 4. AQUACOP IFREMER Actes de Colloque. 9: 319-325.

Laurila, E., Kervinen, R. and Ahvenainen, R. 1998. The inhibition of enzymatic browning in minimally processed vegetables and fruits. Posthavest News Inform. 9: 53–66.

Lawless, H.T. and Heymann, H. 1998. Principles and practices. In: Sensory evaluation of food. New York: Chap-man and Hall. 819 p.

Lee, H., Kizito, S.A., Weese, S.J., Craig-Schmidt, M.C., Lee, Y., Wei, C.I. and An, H. 2003. Analysis of headspace volatile and oxidized volatile compounds in DHA-enriched fish oil on accelerated oxidative storage. J. Food Sci. 68: 2169–2177.

Leffingwell, J. C. and Leffingwell, D. 1990. Odor Detection Thresholds of GRAS Flavor Chemicals. Perfum. Flavor. 16: 1-10.

Liang, M., Wang, S., Wang, J., Chang, Q. and Mai, K. 2008. Comparison of flavor component in shrimp *Litopenaeus vannamei* cultured in sea water and low salinity water. Fisheries Sci. 74: 1173–1179.

Liem, D.G., Aydin, NT. & Zandstra, E.H. 2012. Effects of health labels on expected and actual taste perception of soup. Food Qual. Prefer. 25: 192–197.

Loffler, A. 1986. Proteolytic enzymes: sources and applications. Food Technol. 40: 63–70.

Lopez-Caballero, M.E., Martinez-Alvarez, O., Gomez-Guillen, M.C., and Montero, P. 2007. Quality of thawed deepwater pink shrimp (*Parapenaeus longirostris*) treated with melanosis-inhibiting formations during chilled storage. Int. J. Food Sci. Technol. 42: 1029–1038.

Lu, F.S.H., Nielsen, N.S., Baron, C., and Jacobsen, C. 2012. Oxidative degradation and non-enzymatic browning between oxidized lipids and primary amine groups in different marine PL emulsions. Food Chem. 135: 2887–2896

Lu, F.S.H., Nielsen, N.S., Baron, C.P., Diehl, B.W.K. and Jacobsen, C. 2013. Impact of primary amine group from aminophospholipids and amino acids on marine phospholipids stability: Non-enzymatic browning and lipid oxidation. Food Chem. 141: 879–888.

Lu, F.S.H., Nielsen, N.S., Timm-Heinrich, M., and Jacobsen, C. 2011. Oxidative stability of marine phospholipids in the liposomal form and their applications. Lipids. 46: 3–23.

Luisa, D. M., Sanabria, D. and Spence, C. 2006. Cross-modal associations between odors and colors. Chem. Sens. 31: 531–538.

Lynch, C., Koppel, K. and Reid, W. 2017. Sensory characteristics and consumer acceptance of black walnut gelato. Int. J. Food Sci. Technol. doi:10.1111/ijfs.13432.

Macfie, H. J., Bratchell, N., Greenhoff, K. and Vallis, L. V. 1989. Designs to balance the effect of order of presentation and first-order carry-over effects in hall tests. J. Sens. Stud. 4, 129-148.

Maga, J. A., and Sizer, C. E. 1973. Pyrazines in foods, A review. J. Agric. Food Chem. 21: 22–30.

Maga, J.A. 1974. Influence of color on taste thresholds. Chem. Sens. 1: 115-119.

Manheem, K., Benjakul, S., Kijroongrojana, K. Faithong, N. and Visessanguan, W. 2013. Effect of pre-cooking times on enzymes, properties, and melanosis of Pacific white shrimp during refrigerated storage. Int. Aqua. Res. 5: 1.

Manley, C., Mankoo, A. and Dubosc, V. 2005. Surimi seafood flavors. *In* Surimi and Seafood. 2nd ed. 709-748. CRC Press, Taylor & Francis Group, Boca Raton.

Mayers, S. P. 1986. Utilization of shrimp processing waste. InfofishMark Digest. 4: 18–9.

Mayne, S. T. 1996. Beta-carotene, carotenoids, and disease prevention in humans. FASEB Journal. 10: 690-701.

McEvily, A. J. and Iyengar, R. 1992. Inhibition of enzymatic browning in foods and beverages. Crit. Rev. Food Sci. Nutri. 32: 253-273.

McEvily, A. J., Iyengar, R. and Otwell, S. 1991. Sulfite alternative prevents shrimp melanosis. Food Technol. 45: 80-86.

Mehmet, B., Faruk, B. and Hami, A. 2009. Preservation and shelf-life extension of shrimps and clams by high hydrostatic pressure. Int. J. Food Sci. Technol. 44: 1495–1502.

Mendes, R., Huidobro, A. and Lopez-Caballero, E. 2002. Indole levels in deep water pink shrimp (*Parapenaeus longirostris*). Eur. Food Res. Technol. 214: 125-130.

Miki, W. 1991. Biological functions and activities of animal carotenoids. Pure App. Chem. 63: 141-146.

Millar, K.A., Barry-Ryan, C., Burke, R., Hussey, K., McCarthy, S. and Gallagher, E. 2017. Effect of pulse flours on the physiochemical characteristics and sensory acceptance of baked crackers. Int. J. Food Sci. Technol. 52: 1155–1163.

Mitchell, M., Brunton, N.P. and Wilkinson, M.G. 2009. Sensory acceptability of a reformulated reduced salt frozen ready meal. J. Foodserv. 20: 298–308.

Mitchell, M., Brunton, N.P. and Wilkinson, M.G. 2011. Current salt reduction strategies and their effect on sensory acceptability: a study with reduced salt ready-meals. Eur. Food Res. Technol. 232: 529–539.

Mizani, M., Aminlari, M. and Khodabandeh, M. 2005. An effective method for producing a nutritive protein extract powder from shrimp-head waste. Food Sci. Technol. Int. 11: 49–54.

Montel, M.C., Masson, F., and Talon, R. 1998. Bacterial role in flavour development. Meat Sci. 49: 111–123.

Montero, P., Avalos, A. and Perez-mateos, M. 2001b. Characterization of polyphenoloxidase of prawns (*Panaeus japonicus*). Alternatives to inhibition: additives and high pressure treatment. Food Chem. 75: 317- 324.

Montero, P., Lopez-Caballero, M. E. and Perez-Mateos, M. 2001a. The effect of inhibitors and high pressure treatment to prevent melanosis and microbial growth on chilled prawns (*Penaeus japonicus*). J. Food Sci. 66: 1201-1206.

Moretti, V. M., Mentasti, T., Bellagamba, F., Luzzana, U., Caprino, F., Turchini, G. M., Gianil, I. and Valfre, F. 2006. Determination of astaxanthin stereoisomers and colour attributes in flesh of rainbow trout (*Oncorhynchus mykiss*) as a tool to distinguish the dietary pigmentation source. Food Addit. Contam. 23: 1056–1063.

Morganti, P., Morganti, G., and Morganti, A. 2011. Transforming nanostructured chitin from crustacean waste into beneficial health products: a must for our society. Nanotechnology. Sci. Appl. 4: 123-129.

Morita, K., Kubota, K. and Aishima, T. 2001. Sensory characteristics and volatile components in aromas of boiled prawns prepared according to experimental designs. Food Res. Int. 34: 473–481.

Mu, H., Chen, H., Fang, X., Mao, J., and Gao, H. 2012. Effect of cinnamaldehyde on melanosis and spoilage of Pacific white shrimp (*Litopenaeus vannamei*) during storage. J. Sci. Food Agric. 92: 2177-2182.

Myers, R. H., and D. C. Montgomery. 2002. Response surface methodology. Willey, New York (2001).

Nachay, K. 2016. Nine flavour and color developments for product formulators. Food Technol. 70: 49-61.

Nawar, W.W. 1996. Lipids. *In* O. R. Fennema (Ed.), Food chemistry (pp. 225–314). New York, USA: Marcel Dekker, Inc.

Nègre-Sadargues, G., Castillo, R. and Segonzac, M. 2000. Carotenoid pigments and trophic behavior of deep-sea shrimps (Crustacea, Decapoda, Alvinocarididae) from a hydrothermal area of the mid-atlantic ridge. Comp. Biochem. Physiol. A., 127: 293-300.

Nielsen, I.S., Taarning, E., Egeblad, K., Madsen, R. and Christensen, C.H. 2007. Direct aerobic oxidation of primary alcohols to methyl esters catalyzed by a heterogeneous gold catalyst. Catal. Lett. 116: 35-40.

Nielsen, K.J. 1997. Genetics of the ability of Phyllotreta nemorum larvae to survive in an atypical host plant, *Barbarea vulgaris* ssp. arcuata. Entomol. Exp. Appl. 82: 37–44.

Nilsang, S., Lertsiri, S., Suphantharika, M. and Assavanig, A. 2005. Optimization of enzymatic hydrolysis of fish soluble concentrate by commercial proteases. J. Food Eng. 70:571–8.

Nirmal, N. P. and Benjakul, S. 2012. Biochemical properties of polyphenoloxidase from the cephalothorax of Pacific white shrimp (*Litopenaeus vannamei*). Int. Aquat. Res. doi:10.1186/2008-6970-4-6.

O'Fallon, M.J., Gursoy, D. and Swanger, N. 2007. To buy or not to buy: Impact of labeling on purchasing intentions of genetically modified foods. Hosp. Manag. 26: 117–130.

Ogasawara, M., Katsumata, T., and Egi, M. 2006. Taste properties of Maillard reaction products prepared from 1000 to 5000 da peptide. Food Chem. 99: 600-604.

Ogawa, M. 1984. On physiological aspects of black spot appearance in shrimp. Bull. Japan. Soc. Sci. Fish. 50: 1763-1769.

Ogawa, M. 1985. Influence of freezing, storing and thawing on melanin formation in lobster tails. B. Jpn. Soc. Sci. Fish. 51: 127-131.

Okpala, C.O.R. 2014. Investigation of quality attributes of ice-stored Pacific white shrimp (*Litopenaeus vannamei*) as affected by sequential minimal ozone treatment. LWT - Food Sci. Technol. 57: 538-547.

Otwell, S. and Marshall, M. R. 1986. Screening alternatives to sulfiting agents to control shrimp melanosis. Proceedings of the Eleventh Annual Tropical and Subtropical Fisheries Conference of the Americas. January 13-16, 1986, Florida, pp. 35-44.

Palozza, P., and Krinsky, N. I. 1992. Astaxanthin and canthaxanthin are potent antioxidants in a membrane model. Arch. Biochem. Biophys. 297: 291-295.

Pérez-Gilabent, M. and García-Carmona, F. 2000. Characterization of catecholase and cresolase activities of eggplant polyphenol oxidase. J. Agric. Food Chem. 48: 695-700.

Phimolsiripol, Y., Siripatrawan, U., Teekachunhatean, S., Wangtueai, S., Seesuriyachan, P., Surawang, S., Laokuldilok, T., Regenstein, J.M. and Henry, C.J. 2017. Technological properties, in vitro starch digestibility and in vivo glycaemic index of bread containing crude malva nut gum. Int. J. Food Sci. Tech. doi:10.1111/ijfs.13369.

Pongsetkul, J., Benjakul, S., Sampavapol, P., Osako, K. and Faithong, N. 2014. Chemical composition and physical properties of salted shrimp paste (Kapi) produced in Thailand. Int. Aquat. Res. 6: 155–166.

Poonnakasem, N., Pujols, K.D., Chaiwanichsiri, S., Laohasongkram, K. and Prinyawiwatkul, W. 2016. Different oils and health benefit statements affect physicochemical properties, consumer liking, emotion, and purchase intent: a case of sponge cake. J. Food Sci. 81: s165-173.

Porcherot, C., Delplanque, S., Planchais, A., Gaudreau, N., Accolla, R. and Cayeux, I. 2012. Influence of food odorant names on the verbal measurement of emotions. Food Qual. Prefer. 23, 125–133.

Prakongpan, T., Nitithamyong, A. and Luangpituksa, P. 2002. Extraction and application of dietary fiber and cellulose from pineapple cores. J. Food Sci. 67: 1308-1313.

Prinyawiwatkul, W. and Chompreeda, P. 2007. Applications of discriminant and logistic regression analysis for consumer acceptance and consumer-oriented product optimization study (chapter 16). In: Accelerating new food product design and development. (edited by J.H. Beckley, M. Foley, E.J. Topp, J.C. Huang & W. Prinyawiwatkul). Ames, Iowa: Blackwell Publishing Professional.

Pu, J., Bechtel, P. J. and Sathivel, S. 2010. Extraction of shrimp astaxanthin with flaxseed oil: Effects on lipid oxidation and astaxanthin degradation rates. Biosyst. Eng. 107: 364-371.

Purohit, R.D., Sharma, B.P., Pillai, K.T. and Tyagi, A.K. 2001. Ultrafine ceria powders via glycine-nitrate combustion. Mater. Res. Bull. 36: 2711–2721.

Quaglia, F. B. and Orban, E. 1987. Influence of the degree of hydrolysis on the solubility of the protein hydrolysates fromsardine (*Sardina pilchardus*). J. Sci. Food Agric. 38: 271–6.

Raksakulthai, N. and Haard, N. F. 1992. Correlation between the concentration of peptides and amino acids and the flavour of fish sauce. ASEAN Food J. 7: 86-90.

Ramaswamy, H. S., Simpson, B. K., Ya, T. U. and Yaylayan, V. 1991. Tray-drying of carotenoproteins recovered from lobster waste. J. Food Process Preserv. 15: 273–284.

Rapeanu, G., Loey, A. V., Smout, C. and Hendrickx, M. 2005. Effect of pH on thermal and/or pressure inactivation of Victoria grape (*Vitis vinifera sativa*) polyphenol oxidase: A kinetic study. J. Food Sci. 70: E301–E307.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biol. Med. 26: 1231-1237.

Rebeca, B., Pena-Vera, M. T. and Diaz-Castaneda, M. 1991. Production of fish protein hydrolysates with bacterial proteases: yield and nutritional value. J. Food Sci. 56: 309–14.

Reineccius, G. 1994. Flavoring Materials Contributing to Taste. 2nd ed. 626–654. Chapman & Hall, New York.

Rengel, D., Diez-Navajas, A., Serna-Rico, A., Veiga, P., Muga, A., and Milicua, J. C. G. 2000. Exogenously incorporated ketocarotenoids in large unilamellar vesicles. Protective activity against peroxidation. Biochimica Et Biophysica Acta- Biomembranes. 1463: 179-187.

Richardson, T. and Hyslop, D. B. 1985. Enzymes. *In* Food Chemistry. (Fennema, O. R. ed). Marcel Dekker, New York.

Rødde, R., Einbu, A., Varum, K., 2008. A seasonal study of the chemical composition and chitin quality of shrimp shells obtained from northern shrimp (*Pandalus borealis*). Carbohydr. Polym. 71: 388–393.

Rodríguez-Bernaldo De Quirós, A., López-Hernández, J., González-Castro, M., Cruz-García, C. and Simal-Lozano, J. 2001. Comparison of volatile components in fresh and canned sea urchin (*Paracentrotus lividus*, *Lamarck*) gonads by GC–MS using dynamic headspace sampling and microwave desorption. Eur. Food Res. Technol. 212: 643–647.

Roe, B. and Teisl, M.F. 2007. Genetically modified food labeling: The impacts of message and messenger on consumer perceptions of labels and products. Food Policy. 32: 49–66.

Rolle, R. S., Guizani, N., Chen, J. S., Marshall, M. R., Yang, J. S. and Wei, C. I. 1991. Purification and characterization of phenoloxidase isoforms from Taiwanese black tiger shrimp (*Penaeus monodon*). J. Food Biochem. 15: 17–32.

Sabbe, S., Verbeke, W., Deliza, R., Matta, V. and Dammea, P.V. 2009. Effect of a health claim and personal characteristics on consumer acceptance of fruit juices with different concentrations of açaì (*Euterpe oleracea* Mart.). Appetite. 53: 84–92.

Sachindra, N. M. and Mahendrakar, N. S. 2005. Process optimization for extraction of carotenoids from shrimp waste with vegetable oils. Bioresource. Technol. 96: 1195-1200.

Sachindra, N. M., Bhaskar, N. and Mahendrakar, N. S. 2006. Recovery of carotenoids from shrimp waste in organic solvents. Waste Manage. 26: 1092-1098.

Sachindra, N. M., Sato, E., Maeda, H., Hosokawa, M., Niwano, Y., Kohno, M., et al. 2007. Radical scavenging and singlet oxygen quenching activity of marine carotenoid fucoxanthin and its metabolites. J. Agri. Food Chem. 55: 8516–8522.

Sadighara, P., Moghadam, H.T., Eskandari, S. and Salehi, A. 2015. Optimization of extraction of chitosan and carotenoids from shrimp waste. Int. J. Fish. Aquat. Stud. 2: 36-38.

Sae-Eaw, A., Chompreeda, P., Prinyawiwatkul, W., Haruthaithanasan, V., Suwansichon, T., Saidu, J.E. and Xu, Z. 2007. Acceptance and purchase intent of the US consumers for the non-wheat rice butter cake. J. Food Sci. 72: s92-97.

Sánchez-camargo, A. P., Almeida meireles, M. Â., Lopes, B. L. F. and Cabral, F. A. 2011. Proximate composition and extraction of carotenoids and lipids from Brazilian redspotted shrimp waste (*Farfantepenaeus paulensis*). J. Food Eng. 102: 87–93.

Schiedt K., 1987. Absorption, retention and metabolic transformations in chicken, salmonids and crustaceans. Ph. D. Thesis, University of Trondheim, Norway.

Schoffeniels, E. and Gilles, R. 1970. Osmoregulation in aquatic arthropods. *In* Chemical Zoology, Vol. 5. (Florkin, M. and Scheer, B. T., eds). p. 255–282, Academic Press, New York.

Selli, S. and Cayhan, G.G. 2009. Document Analysis of volatile compounds of wild gilthead sea bream (*Sparus aurata*) by simultaneous distillation-extraction (SDE) and GC-MS. Microchem. J. 92: 232-235.

Senphan, T. and Benjakul, S. 2012. Compositions and yield of lipids extracted from hepatopancreas of Pacific white shrimp (*Litopenaeus vannamei*) as affected by prior autolysis. Food Chem. 134: 829–835.

Seo, H.S., Iannilli, E., Hummel, C., Okazaki, Y., Buschhüter, D., Gerber, J., Krammer, G.E, Lengerich, B. and Hummel, T. 2013. A salty-congruent odor enhances saltiness: functional magnetic resonance imaging study. Hum. Brain Mapp. 34: 62–76.

Shahidi, F. and Zhong, Y. 2005. Lipid oxidation: Measurement methods. *In*: Shahidi, F. (ed) Bailey's industrial oil and fat products, Wiley and Sons.

Shahidi, F. and Brown, J. A. 1998. Carotenoid pigments in seafoods and aquaculture. Crit. Rev. Food Sci. 38: 1–67.

Shahidi, F. and Synowiecki, J. 1991. Isolation and characterization of nutrients and value-added products from snow crab (*Chionoecetes opilio*) and shrimp (*Pandalus borealis*) processing discards. J. Agri. Food Chem. 39: 1527-1532.

Shahidi, F., Han, X. Q. and Synowiecki, J. 1995. Production and characteristics of protein hydrolysates from capelim (*Mallotus villosus*). Food Chem. 53: 285–93.

Sila, A., Nasri, M. and Bougatef, A. 2012. Isolation and characterisation of carotenoproteins from deep-water pink shrimp processing waste. Int. J. Biol. Macromol. 51: 953-9.

Simpson, B. K. and Haard, N. F. 1985. The use of proteolytic enzymes to extract carotenoproteins from shrimp wastes. J. Appl. Biochem. 7: 212–22.

Simpson, B. K., Marshall, M. R. and Otwell, W. S. 1987. Phenoloxidases from shrimp (*Penaeus setiferus*): purification and some properties. J. Agric. Food Chem. 35: 918–921.

Simpson, B.K., Marshall, M.R. and Otwell, W.S. 1987. Phenoloxidases from shrimp (*Penaeus setiferus*): purification and some properties. J. Agric. Food Chem. 35: 918–921.

Simpson, B. K., Marshall, M. R. and Otwell, W. S. 1988. Phenoloxidases from pink and white shrimp: kinetic and other properties. J. Food Biochem. 12: 205–217.

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

Simpson, J. W., Allen, K. and Awapara, J. 1959. Free amino acids in some aquatic invertebrates. Biol. Bull. 117: 371–381.

Sinopoli, D.A. and Lawless, H.T. 2012. Taste properties of potassium chloride alone and in mixtures with sodium chloride using a check-all-that-apply method. J. Food Sci. 77: s319-s322.

Šlizyte, R., Dauksas, E., Falch, E., Storrø1, I. and Rustad, T. 2005. Yield and composition of different fractions obtained after enzymatic hydrolysis of cod (*Gadus morhua*) byproducts. Process Biochem. 40: 1415–24.

Sowmya, R., and Sachindra, N.M. 2012. Evaluation of antioxidant activity of carotenoid extract from shrimp processing by-products by in vitro assays and in membrane model system. Food Chem. 134: 308–314.

Sowmya, R., Ravikumar, T.M., Vivek, R., Rathinaraj, K. and Sachindra, N.M. 2014. Optimization of enzymatic hydrolysis of shrimp waste for recovery of antioxidant activity rich protein isolate. J. Food Sci. Technol. 51: 3199–3207.

Spence, C. 2015. On the psychological impact of food colour. Spence flavour. 4: 21.

Sriket, P., Benjakul, S., Visessanguan, W. and Kijroongrojana, K. 2007. Comparative studies on chemical composition and thermal properties of black tiger shrimp (*Penaeus monodon*) and white shrimp (*Penaeus vannamei*) meats. Food Chem. 103: 1199–1207.

Stansby, M. E. 1967. Fish oils: Their chemistry, technology, stability, nutritional properties and uses. The Avi Publishing Company, Inc, Westport.

Steel, R. G. D. and Torrie, J. H. 1980. Principle and procedure of statistics. 2nd ed. McGraw-Hill, New York.

Steinhaus, P. and Schieberle, P. 2007. Characterization of the Key Aroma Compounds in Soy Sauce Using Approaches of Molecular Sensory Science. J. Agric. Food Chem. 55: 6262−6269.

Stone, H. and Sidel, J.L. 1993. Sensory evaluation practices. Calf: Academic Press, Inc. 338 p.

Suh, H. J., Whang, J. H., Kim, Y. S., Bae, S. H. and Noh, D. O. 2003. Preparation of angiotensin I converting enzyme inhibitor from corn gluten. Process. Biochem. 38:1239–44.

Suhnel, S., Lagreze, F., Ferreira, J. F., Campestrini, L. H. and Maraschin, M. 2009. Carotenoid extraction from the gonad of the scallop Nodipecten nodosus (Linnaeus, 1758) (Bivalvia: Pectinidae). Braz. J. Biol. 69: 209-215.

Sukkwai, S., 2012. Production of Gelatin and Gelatin Hydrolysate from Fish Skin for Development of Healthy drinks. Ms. D. Thesis, Prince of Songkla University, Songkhla.

Synowiecki, J. and Al-Khateeb, N. 2000. The recovery of protein hydrolysate during enzymatic isolation of chitin from shrimp *Crangon crangon* processing discards. Food Chem. 68:147–52.

Tachihara, T., Ishizaki, S., Ishikawa, M. and Kitahara, T. 2004. Studies on volatile compounds of roasted spotted shrimp. Chem. Biodivers. 1: 2024-2033.

Takeungwongtrakul, S., Benjakul, S. and H-kittikun, A. 2012. Lipids from cephalothorax and hepatopancreas of Pacific white shrimp (*Litopenaeus vannamei*): Compositions and deterioration as affected by iced storage. Food Chem. 134: 2066–2074.

Takeungwongtrakul, S., Benjakul, S., Santoso, J., Trilaksani, W. and Nurilmala, M. 2013. Extraction and stability of carotenoid-containing lipids from hepatopancreas of pacific white shrimp (*Litopenaeus vannamei*). J. Food Process. Preserv. 39: 10-18.

Taoukis, P. S., Labuza, T. P., Lillemo, J. H. and Lin, S.W. 1990. Inhibition of shrimp melanosis (Black Spot) by Ficin. Wiss. Technol. Food Sci. Technol. 23: 52-54.

TCPS 324. 2004. Thai community Product standard.

Teerasuntonwat, P. and Raksakulthai, N. 1995. Production of flavouring agent from shrimp heads. ASEAN Food J. 10: 131-138.

Thai Industrial Standards Institute. 1983. Standard of local fish sauce. Ministry of Industry, Bangkok.

Thanonkaew, A., Benjakul, S., Visessanguan, W., and Decker, E. A. (2006). Development of yellow pigmentation in squid (*Loligo peali*) as a result of lipid oxidation. J. Agric. Food Chem. 54: 956–962.

Thiansilakul, Y., Benjakul, S. and Shahidi, F. 2007. Antioxidative activity of protein hydrolysate from round scad muscle using Alcalase and Flavourzyme. J. Food Biochem. 31: 266-87.

Thomson, D.M.H., Crocker, C. and Marketo, C.G. 2010. Linking sensory characteristics to emotions: an example using dark chocolate. Food Qual. Prefer. 21: 1117–1125.

Tocher, D.R. and Sargent, J.R. 1984. Analyses of lipids and fatty acids in ripe roes of some Northwest European marine fish. Lipid. 19: 492-499.

Vandanjon, L., Cros, S., Jaouen, P., Quéméneur, F. and Bourseau, P. 2002. Recovery by nanofiltration and reverse osmosis of marine flavours from seafood cooking waters. Desalination. 144: 379-385.

Torrico, D.D., Carabante, K.M., Pujols, K.D., Chareonthaikij, P. and Prinyawiwatkul, W. 2015. Oil and tastant concentrations affect saltiness and bitterness perception of oil-in-water emulsions. Int. J. Food Sci. Technol. 50: 2562–2571.

Toussaint, O. and Lerch, K. 1987. Catalytic oxidation of 2-aminophenols and ortho hydroxylation of aromatic amines by tyrosinase. Biochem. 26: 8587-8571.

U.S. Food and Drug Administration. 2015. Sulfite in standardized food. *In* Code of Federal Regulations, Title 21(2). Department of Health and Human Services, Food and Drug Administration, Washington, D.C., United states of America.

Uchiyama H, Kakuda K. 1984. A simple and rapid method for measuring K-value, a fish freshness index. Bull. Jap. Soc. Sci. Fish. 50:263-265.

Vandanjon, L., Cros, S., Jaouen, P., Quéméneur, F. and Bourseau, P. 2002. Recovery by nanofiltration and reverse osmosis of marine flavours from seafood cooking waters. Desalination. 144: 379-385.

Venugopal, V. and Shahidi, F. 1995. Value-added products from underutilized fish species. Crit. Rev. Food Sci. 35: 431–53.

Wadhera, D. and Capaldi-Phillips, E.D. 2014. A review of visual cues associated with food on food acceptance and consumption. Eat. Behav. 15: 132–143.

Wadhwani, R. and McMahon, D.J. 2012. Color of low-fat cheese influences flavor perception and consumer liking. J. Dairy Sci. 9: 2336–2346.

Wan, X., Woods, A.T., Bosch, J., Mckenzie, K.J., Velasco, C. and Spence, C. 2014. Cross-cultural differences in cross-modal correspondences between tastes and visual features. Front. Psychol. 5: 1365.

Wardy, W., Chonpracha, P., Chokumnoyporn, N., Sriwattana, S., Prinyawiwatkul, W. & Jirangrat, W. (2017). Influence of package visual cues of sweeteners on the sensory-emotional profiles of their products. J. Food Sci. 82: 500–508.

Warndy, W., Sae-Eaw, A., Sriwattana, S., No, H.K. and Prinyawiwatkul, W. 2015. Assessing consumer emotional responses in the presence and absence of critical quality attributes: a case study with chicken eggs. J. Food Sci. 80: s1574-1582.

Wei, S.T., Ou, L.C., Luo, M.R. and Hutchings, J.B. 2012. Optimization of food expectations using product colour and appearance. Food Qual. Prefer. 23: 49-62.

Whitaker, J. R. 1972. Polyphenol oxidase. *In* Principle of Enzymology for the Food Science. (Whitaker, J. R. ed). p. 571-582. Marcel Dekker: New York.

Williams, H. G., Davidson, G. W., and Mamo, J. C. 2003. Heat-induced activation of polyphenoloxidase in western rock lobster (*Panulirus cygnus*) hemolymph: Implications for heat processing. J. Food Sci. 68: 1928–1932.

Wu, H. C., Chen, H. M.,and Shiau, C. Y. 2003. Free amino acid and peptide as related to antioxidant properties in protein hydrolysates of mackerel (*Scomber austriasicus*). Food Res. Int. 36: 949–957.

Ya, T., Simpson, B. K. Ramaswamy, H., Yaylayan, V., Smith, J. P. and Hudon, C. 1991. Carotenoproteins from lobster waste as a potential feed supplement for culterd salmonids. Food Biotechnol. 5: 87-93.

Yao, Q. and Wang, L. 2012. Consumer purchase intention towards genetically modified food: beneficial, price, socio-demographic and label determinants. Int. J. Trade Econ. Finan. 3: 176-182.

Yazawa, K. and Kageyama, H. 1991. Physiological activity of docosahexaenoic acid. Yukagaku. 40: 202-206.

Yılmaz, F.M. and Bilek, S.E. 2017. Natural colorant enrichment of apple tissue with black carrot concentrate using vacuum impregnation. Int. J. Food Sci. Technol. 52: 1508–1516.

Yu, X., Li, F., Ye, X., Xin, X. and Xue, Z. 2000. Synthesis of cerium (IV) oxide ultrafine particales by solid-state reactions. J. Am. Ceram. Soc. 83: 964-966.

Zheng, M., Jin, Z. and Zhang, Y. 2007. Effect of cross-linking and esterification on hygroscopicity and surface activity of cassava maltodextrins. Food Chem. 103: 1375–1379.

APPENDIXES

Appendix 1

Ten-points scale for melanosis evaluation

กรุณาดูสีของหัวกุ้งและให้คะแนนการเกิดสีดำโดยเฉลี่ยของหัวกุ้งทั้งหมดในแต่ละถาด โดย

0 คะแนน หมายถึง ไม่มีสีดำปรากฏเลย

2 คะแนน หมายถึง เริ่มมีสีดำปรากฏขึ้นเล็กน้อยในหัวกุ้งบางหัว

4 คะแนน หมายถึง เริ่มมีสีดำปรากฏขึ้นเล็กน้อยในหัวกุ้งส่วนใหญ่

6 คะแนน หมายถึง สังเกตเห็นสีดำปรากฏขึ้นในหัวกุ้งส่วนใหญ่

8 คะแนน หมายถึง สังเกตเห็นสีดำในหัวกุ้งส่วนใหญ่อย่างชัดเจน

10 คะแนน หมายถึง หัวกุ้งทั้งหมดมีสีดำ

คุณลักษณะ	**รหัส . . . . . .**	**รหัส . . . . . .**	**รหัส . . . . . .**	**รหัส . . . . . .**	**รหัส . . . . . .**
คะแนนการเกิดสีดำ					

Appendix 2

Sample of 'Hedonic scale'

หมายเลขแบบทดสอบ.....

วันที่.....

แบบสอบถามคุณลักษณะทางประสาทสัมผัส "ขนมจีบกุ้ง"

คำแนะนำ : กรุณาทดสอบตัวอย่างที่เสนอให้แล้วให้คะแนนความชอบในแต่ละคุณลักษณะ โดยกำหนดให้

9 = ชอบมากที่สุด	8 = ชอบมาก	7 = ชอบปานกลาง
6 = ชอบเล็กน้อย	5 = บอกไม่ได้ว่าชอบหรือไม่ชอบ	4 = ไม่ชอบเล็กน้อย
3 = ไม่ชอบปานกลาง	2 = ไม่ชอบมาก	1 = ไม่ชอบมากที่สุด

********* กรุณาให้คะแนนจากส่วนของไส้ขนมจีบเท่านั้น *********

คุณลักษณะ	รหัส	รหัส	รหัส	รหัส	รหัส . . . . .
สี					
กลิ่น					
กลิ่นรส/ รสชาติ					
เนื้อสัมผัส					
ความชอบโดยรวม					

ข้อเสนอแนะ

.....

.....

.....

Appendix 3

Sample of 'Generic Descriptive Analysis'

การทดสอบเชิงพรรณนา (เชิงปริมาณ)

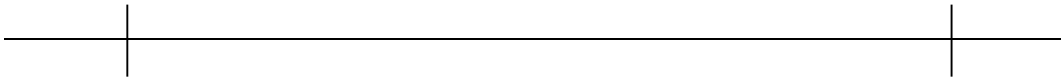
ชุดที่.....

ชื่อผู้ตัดสิน..... วันที่..... เวลา.....

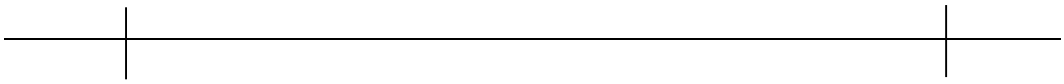
คำแนะนำ กรุณาทดสอบกลิ่นของตัวอย่างที่เสนอจากซ้ายไปขวาแล้วขีดเส้นตั้งฉากกับเส้นคะแนนของแต่ละคุณลักษณะทางประสาทสัมผัสตาม **ความเข้ม** ที่ตรงกับความรู้สึกของท่าน พร้อมระบุรหัสตัวอย่างเหนือเส้น และดมกระดาษทิชชูทุกครั้งก่อนทดสอบตัวอย่างถัดไป

รหัสตัวอย่าง ....

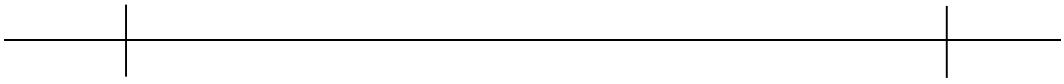
1. กลิ่นกุ้งแห้งคั่ว



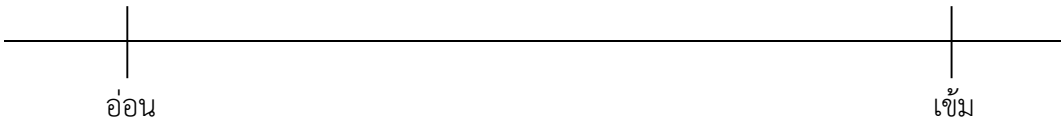
2. กลิ่นกุ้งแห้งต้ม



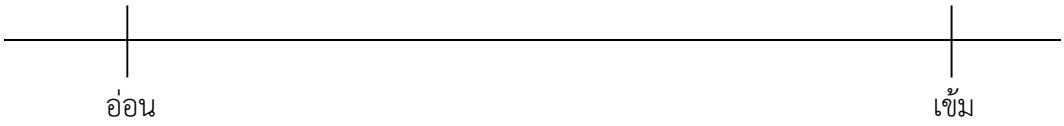
3. กลิ่นปูม้าต้ม



4. กลิ่นกุ้งไม่สดต้ม



5. กลิ่นมันกุ้ง



Appendix 4

Online questionnaire for emotion term screening

Research Consent Form

Thank you for your interest in this survey. Please read the below consent form before proceeding to the survey.

This is a consent form for research participation in the research entitled “Effects of colorant on human perception of dipping sauce” which is being conducted by Sineenath Sukkwai, a PhD Exchange Student of the School of Nutrition and Food Sciences at Louisiana State University Agricultural Center, (225) 578-5188.

I understand that participation is entirely voluntary and whether or not I participate will not affect how I am treated on my job. I can withdraw my consent at any time without penalty or loss of benefits to which I am otherwise entitled and have the results of the participation returned to me, removed from the experimental records, or destroyed. Two hundred consumers will participate in this research. For this particular research, about 5 minute participation will be required for each consumer.

Participant's Statement

“I have read and understand the above information provided about this study (consent document). I volunteer to take part in this research. I know I can ask questions at any time by contacting the research staff via email ssukkwai@lsu.edu. I understand that I can change my mind and withdraw my consent to participate by closing the website or contacting the research staff by email without penalty.”

If you agree with this statement, please select 'I AGREE' below. If you do not agree with this statement, please exit the study now or click on 'I DO NOT AGREE' and you will be redirected away from this website.

1. Please note that you cannot proceed with the survey until you agree.

❍ I agree
❍ I do not agree

2. Please indicate your **gender**:

❍ Male
❍ Female

In this questionnaire you will see 4 samples as follows:

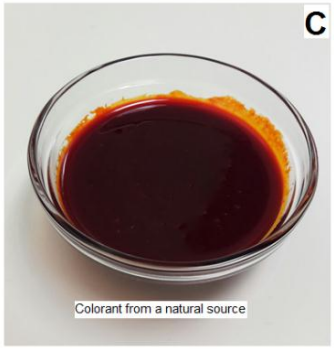
Sample C a colorant comes from a natural source.

Sample #1 a dipping sauce contains the amount of sodium comparable to that in commercial products. It is made with vegetable oil, vinegar, sour cream, cream cheese, black pepper, green onion, sodium chloride (salt), and food gums.

Sample#2 a dipping sauce colored with a colorant from a natural source

Sample#3 a dipping sauce colored with a synthetic colorant approved by the United States Food and Drug Administration (US FDA).

Please click >> to continue



Please select the ***emotion terms*** (check all that apply) that are elicited when thinking of using this colorant from a natural source in food formulations.

* (39 food-elicited emotion terms were shown as choices (*Active*, *Adventurous*, *Affectionate*, *Aggressive*, *Bored*, *Calm*, *Daring*, *Disgusted*, *Eager*, *Energetic*, *Enthusiastic*, *Free*, *Friendly*, *Glad*, *Good*, *Good-natured*, *Guilty*, *Happy*, *Interested*, *Joyful*, *Loving*, *Merry*, *Mild*, *Nostalgic*, *Peaceful*, *Pleasant*, *Pleased*, *Polite*, *Quiet*, *Satisfied*, *Secure*, *Steady*, *Tame*, *Tender*, *Understanding*, *Warm*, *Worried*, *Whole* and *Wild*))

Please select the sample *(check all that apply)* that elicits an emotion "**.....**" when consuming this dipping sauce.

* "**.....**" Was filled with 39 food-elicited emotion terms for each question and choices were: Sample #1, Sample #2, Sample #3 and None of the above.

Appendix 5

Questionnaire for Compusense® *five*

Please read and sign the following Consent form:

Research Consent Form

I, _____, agree to participate in the research entitled "Effects of colorant on human perception of dipping sauce" which is being conducted by Witoon Prinyawiwatkul of the School of Nutrition and Food Sciences at Louisiana State University Agricultural Center, (225) 578-5188.

I understand that participation is entirely voluntary and whether or not I participate will not affect how I am treated at my job. I can withdraw my consent at any time without penalty or loss of benefits to which I am otherwise entitled and have the results of the participation returned to me, removed from the experimental records, or destroyed. Two hundred sixteen consumers will participate in this research.

The following points have been explained to me:

1. In any case, it is my responsibility to report prior to participation to the investigator any food allergies I may have.
2. The reason for the research is to gather information on the effect of colorant from natural source on saltiness perception of dipping sauce. The benefit that I may expect is satisfaction that I have contributed to a solution and evaluation of problems relating to such examinations.
3. The procedures are as follows: three coded samples will be placed in front of me, and I will evaluate them by normal standard methods and indicate my evaluation on score sheets. All procedures are standard methods as published by the American Society for Testing and Materials and the Sensory Evaluation Division of the Institute of Food Technologists.
4. Participation entails minimal risk: **The only risk may be an allergic reaction to shrimp products, vegetable oil, vinegar, sour cream, cream cheese, black pepper, garlic, onion, sodium chloride (salt), and/or food gums**. However, because it is known to me beforehand that the above mentioned foods and ingredients are to be tested, the situation can normally be avoided.
5. The results of this study will not be released in any individual identifiable form without my prior consent unless required by law.
6. The investigator will answer any further questions about the research, either now or during the course of the project.

The study has been discussed with me, and all of my questions have been answered. I understand that additional questions

1. Please type your name stating your agreement with the consent form:

_____

2. Please indicate your **gender**:

❍ Male
❍ Female

Please, do not taste the sample yet.

1. How would you like the color of this dipping sauce?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
1	2	3	4	5	6	7	8	9

2. Please rate your expectation of saltiness intensity based only on the orange color of this dipping sauce.

Extremely worse than expected	Very Much worse than expected	Moderately worse than expected	Slightly worse than expected	Same as expected	Slightly better than expected	Moderately better than expected	Very Much better than expected	Extremely better than excpected
1	2	3	4	5	6	7	8	9

3. Please rate your expectation of the saltiness intensity based on the orange color of this dipping sauce.

Too weak	Just about right	Too strong
1	2	3

Please dip corn chips into the dipping sauce.

How would you rate the following attributes of this dipping sauce?

4. Odor/Aroma liking

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
1	2	3	4	5	6	7	8	9

5. Viscosity liking

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
1	2	3	4	5	6	7	8	9

6. Please rate your perception of the viscosity of this dipping sauce.

Too thin	Just about right	Too thick
1	2	3

7. Saltiness liking

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
1	2	3	4	5	6	7	8	9

8. Overall liking

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
1	2	3	4	5	6	7	8	9

9. How would you emotionally feel about this dipping sauce?

9.1 Good

Not at all	Slightly	Moderately	Very much	Extremely
1	2	3	4	5

9.2 Guilty

Not at all	Slightly	Moderately	Very much	Extremely
1	2	3	4	5

9.3 Interested

Not at all	Slightly	Moderately	Very much	Extremely
1	2	3	4	5

9.4 Satisfied

Not at all	Slightly	Moderately	Very much	Extremely
1	2	3	4	5

9.5 unsafe (pertaining to health)

Not at all	Slightly	Moderately	Very much	Extremely
1	2	3	4	5

9.6 Worried

Not at all	Slightly	Moderately	Very much	Extremely
1	2	3	4	5

10. How likely would you purchase this dipping sauce?

Yes	No
1	2

Statement sodium: Excessive dietary sodium intake is linked to heart disease and other illnesses.

Statement for regular salt added sample: This product contain similar amount of sodium compared with commercial products.

Statement for no salt added sample: This dipping sauce has no salt added.

Statement for KCl added sample: This dipping sauce is considered "Reduced Sodium" according to The United States Food and Drug Administration

* The different statement was showed depend on the served sample.

11. How would you rate the following attributes of this dipping sauce?

11.1 Saltiness liking

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
1	2	3	4	5	6	7	8	9

11.2 Overall liking

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
1	2	3	4	5	6	7	8	9

12. How would you emotionally feel about this dipping sauce?

12.1 Good

Not at all	Slightly	Moderately	Very much	Extremely
1	2	3	4	5

12.2 Guilty

Not at all	Slightly	Moderately	Very much	Extremely
1	2	3	4	5

12.3 Interested

Not at all	Slightly	Moderately	Very much	Extremely
1	2	3	4	5

12.4 Satisfied

Not at all	Slightly	Moderately	Very much	Extremely
1	2	3	4	5

12.5 unsafe (pertaining to health)

Not at all	Slightly	Moderately	Very much	Extremely
1	2	3	4	5

12.6 Worried

Not at all	Slightly	Moderately	Very much	Extremely
1	2	3	4	5

13. How likely would you purchase this dipping sauce?

Yes	No
1	2

Statement colorant:

Statement for sample without colourant: This dipping sauce has no colorant added.

Statement for sample with colourant: This dipping sauce contained colorant from natural source.

* The different statement was showed depend on the served sample.

14. How would you emotionally feel about this dipping sauce?

14.1 Good

Not at all	Slightly	Moderately	Very much	Extremely
1	2	3	4	5

14.2 Guilty

Not at all	Slightly	Moderately	Very much	Extremely
1	2	3	4	5

14.3 Interested

Not at all	Slightly	Moderately	Very much	Extremely
1	2	3	4	5

14.4 Satisfied

Not at all	Slightly	Moderately	Very much	Extremely
1	2	3	4	5

14.5 unsafe (pertaining to health)

Not at all	Slightly	Moderately	Very much	Extremely
1	2	3	4	5

14.6 Worried

Not at all	Slightly	Moderately	Very much	Extremely
1	2	3	4	5

15. How likely would you purchase this dipping sauce?

Yes	No
1	2

Appendix 6

The analysis of variance for the response surface regression model

1. Color

Sequential Model Sum of Squares [Type I]

Source	Sum of aquares	df	Mean square	F value	p-value Prob > F
Mean vs Total	589.11	1	589.11		
Linear vs Mean	0.45	2	0.23	3.97	0.0634
2FI vs Linear	0.040	1	0.040	0.67	0.4393
Quadratic vs 2FI	0.13	2	0.067	1.20	0.3765
Cubic vs Quadratic	0.035	2	0.017	0.21	0.8220
Residual	0.25	3	0.082		
Total	590.02	11	53.64		

Lack of Fit Tests

Source	Sum of aquares	df	Mean square	F value	p-value Prob > F
Linear	0.41	6	0.069	3.25	0.2539
2FI	0.37	5	0.075	3.52	0.2358
Quadratic	0.24	3	0.080	3.76	0.2173
Cubic	0.20	1	0.20	9.65	0.0899
Pure error	0.042	2	0.021		

Model Summary Statistics

Source	Std. Dev.	R-square	Adjusted R-square	Predicted R-square	Press
Linear	0.24	0.4982	0.3728	-0.0214	0.93
2FI	0.24	0.5422	0.3460	-0.7492	1.59
Quadratic	0.29	0.6903	0.3805	-0.9757	1.80
Cubic	0.24	0.7282	0.0940	-13.5123	13.20

2. Flavor

Sequential Model Sum of Squares [Type I]

Source	Sum of aquares	df	Mean square	F value	p-value Prob > F
Mean vs Total	568.80	1	568.80		
Linear vs Mean	0.070	2	0.035	0.37	0.7007
2FI vs Linear	0.010	1	0.010	0.094	0.7680
Quadratic vs 2FI	0.40	2	0.20	2.88	0.1471
Cubic vs Quadratic	0.097	2	0.048	0.58	0.6107
Residual	0.25	3	0.083		
Total	569.63	11	51.78		

Lack of Fit Tests

Source	Sum of aquares	df	Mean square	F value	p-value Prob > F
Linear	0.81	6	0.085	0.69	0.6927
2FI	0.50	5	0.100	0.81	0.6319
Quadratic	0.10	3	0.034	0.27	0.8433
Cubic	3.613E-003	1	3.613E-003	0.029	0.8795
Pure error	0.25	2	0.12		

Model Summary Statistics

Source	Std. Dev.	R-square	Adjusted R-square	Predicted R-square	Press
Linear	0.31	0.0851	-0.1436	-0.5633	1.29
2FI	0.33	0.0972	-0.2897	-0.9633	1.62
Quadratic	0.26	0.5806	0.1613	-0.5361	1.27
Cubic	0.29	0.6981	-0.0062	0.0503	0.78

3. Texture

Sequential Model Sum of Squares [Type I]

Source	Sum of aquares	df	Mean square	F value	p-value Prob > F
Mean vs Total	576.88	1	576.88		
Linear vs Mean	0.029	2	0.014	0.32	0.7344
2FI vs Linear	0.034	1	0.034	0.73	0.4202
Quadratic vs 2FI	0.17	2	0.086	2.80	0.1525
Cubic vs Quadratic	0.088	2	0.044	1.99	0.2823
Residual	0.066	3	0.022		
Total	577.27	11	52.48		

Lack of Fit Tests

Source	Sum of aquares	df	Mean square	F value	p-value Prob > F
Linear	0.30	6	0.049	1.53	0.4458
2FI	0.26	5	0.052	1.63	0.4227
Quadratic	0.090	3	0.030	0.93	0.5567
Cubic	1.800E-003	1	1.800E-003	0.056	0.8352
Pure error	0.064	2	0.032		

Model Summary Statistics

Source	Std. Dev.	R-square	Adjusted R-square	Predicted R-square	Press
Linear	0.21	0.0743	-0.1571	-0.6928	0.66
2FI	0.22	0.7621	-0.1971	-1.0007	0.78
Quadratic	0.18	0.6051	0.2102	-1.0046	0.78
Cubic	0.15	0.8301	0.4336	0.3326	0.26

4. Overall

Sequential Model Sum of Squares [Type I]

Source	Sum of aquares	df	Mean square	F value	p-value Prob > F
Mean vs Total	569.81	1	569.81		
Linear vs Mean	0.22	2	0.11	2.05	0.1905
2FI vs Linear	4.225E-003	1	4.225E-003	0.069	0.8008
Quadratic vs 2FI	0.23	2	0.12	2.91	0.1449
Cubic vs Quadratic	0.092	2	0.046	1.30	0.3915
Residual	0.11	3	0.035		
Total	570.47	11	51.86		

Lack of Fit Tests

Source	Sum of aquares	df	Mean square	F value	p-value Prob > F
Linear	0.33	6	0.055	1.03	0.5692
2FI	0.32	5	0.065	1.22	0.5082
Quadratic	0.092	3	0.031	0.58	0.6831
Cubic	1.110E-016	1	1.110E-016	2.087E-015	1.0000
Pure error	0.11	2	0.053		

Model Summary Statistics

Source	Std. Dev.	R-square	Adjusted R-square	Predicted R-square	Press
Linear	0.23	0.3393	0.1742	-0.1697	0.77
2FI	0.25	0.3458	0.0654	-0.5229	1.00
Quadratic	0.20	0.6979	0.3957	-0.3625	0.90
Cubic	0.19	0.8383	0.4610	0.6362	0.24

VITAE

Name Miss Sineenath Sukkwai

Student ID 5511030015

Education Attainment

Degree	Name of Institution	Year of Graduation
Bachelor of Science (Agro-Industry)	Prince of Songkla University	2007
Master of Science (Agro-Industry)	Prince of Songkla University	2011

Scholarship Awards during Enrolment

- Government budget (AGR560041S) of Prince of Songkla University
- Ph. D. Student Research Scholarship by Thailand Research Fund under the Royal Golden Jubilee Ph.D. Program (PHD/0228/2553)

List of Publication and Proceedings

Publications

Sukkwai, S., Chonpracha, P., Kijroongrojana, K. and Prinyawiwatkul, W. 2017. Influences of a natural colourant on colour and salty taste perception, liking, emotion and purchase intent: a case of mayonnaise-based dipping sauces. Int. J. Food Sci. Technol. doi:10.1111/ijfs.13506.