PRODUCT DEVELOPMENT OF PASTEURIZED READY-TO-COOK MARINATED WHITE SHRIMP *Litopenaeus vannamei* IN GREEN CURRY PASTE

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การพัฒนาผลิตภัณฑ์กุ้งขาว *Litopenaeus vannamei* ในเครื่องแกงเขียวหวานพร้อมปรุงที่ผ่านการพาสเจอไรส์

นางนอร์ ซาลาเซีย บินติ โมฮัมเม็ด

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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Thesis Title	PRODUCT DEVELOPMENT OF PASTEURIZED READY-TO-COOK MARINATED WHITE SHRIMP *Litopenaeus vannamei* IN GREEN CURRY PASTE
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นอร์ ซาลาเซีย บินติ โมฮัมเม็ด : การพัฒนาผลิตภัณฑ์กุ้งขาว *Litopenaeus vannamei* ในเครื่องแกงเขียวหวาน พร้อมปรุงที่ผ่านการ พาสเจอไรส์. (PRODUCT DEVELOPMENT OF PASTEURIZED READY-TO-COOK MARINATED WHITE SHRIMP *Litopenaeus vannamei* IN GREEN CURRY PASTE) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: รศ.ดร. จิรารัตน์ อนันตกูล, 105 หน้า

ส่วนแรกของการวิจัยมีวัตถุประสงค์เพื่อศึกษาผล ของการแช่กุ้งในสารละลายต่อ คุณภาพของกุ้ง ขาว (*Litopenaeus vannamei*) โดยใช้ NaCl 2% ร่วมกับสาร 3 ชนิด ได้แก่ โซเดียมไตรโพลีฟอสเฟต (STPP) โซเดียมไบคาร์บอเนต (NaHCO3) และเอนไซม์ทรานสกลูกามิเนสที่ผลิตจากจุ ลินทรีย์ (MTGase) ที่ระดับความเข้มข้น (2, 5 และ 8%) และเวลาแช่ที่แตกต่างกัน (10, 30 และ 60 นาที) โดยแช่กุ้งที่สารละลายที่อุณหภูมิ 4 ± 2 ° C พบว่า เมื่อใช้สาร ทั้งสามชนิดร่วมกับ NaCl 2% การอุ้มน้ำ (WHC) การสูญเสียน้ำ หนักจากการทำสุก (CL) น้ำหนักที่เพิ่มขึ้น (WG) ความชื้น (MC) และปริมาณจุลินทรีย์ (TVC) ของกุ้ง แตกต่างจากกุ้งควบคุม อย่างมีนัยสำคัญ การแช่กุ้งใน MTGase 5%+NaCl 2% เป็นเวลา 30 นาที สามารถปรับปรุงคุณภาพของกุ้งขาวได้ดีเมื่อเทียบกับกุ้งควบคุม โดยสามารถ เพิ่ม WG ได้ 15.7% เพิ่ม WHC ถึง 94.5% ลด CL ลง 10.1% และลด TVC ลง 1.44 log CFU/g ส่วนที่สองศึกษาผลกระทบของการ พาสเจอไรส์ต่อ กุ้งที่หมักใน เครื่อง แกงเขียวหวาน ใน 5 สภาวะที่ใช้อุณหภูมิและเวลาต่างกัน ได้แก่ (T1) 65 °C 32.5 นาที (T2) 67 °C 18.5 นาที (T3) 68 °C 17 นาที (T4) 69°C 7 นาที (T5) 70 °C 7 นาที เทียบกับตัวอย่าง ควบคุม (กุ้งที่หมักในเครื่อง แกงเขียวหวาน ไม่ผ่านการพาสเจอไรส์) พบว่า ผลิตภัณฑ์ที่ผ่านการ พาสเจอไรส์ (T1-T5) มี TVC น้อยกว่า 10 CFU/g และไม่พบ *Listeria* spp. ขณะที่ตัวอย่างควบคุมมี TVC 3.67 log CFU/g และพบ *Listeria* spp. 1.18 log CFU/g ค่าความเป็นกรดด่างของผลิตภัณฑ์ที่ผ่านการพาสเจอไรส์ทั้ง 5 สภาวะ (T1-T5) มีค่าต่ำกว่าตัวอย่างควบคุม ($p \leq 0.05$) แรงเฉือนของกุ้งในตัวอย่างที่พาสเจอไรส์ในสภาวะ T3 T4 และ T5 ไม่แตกต่างกับตัวอย่างควบคุม และพบว่าผลิตภัณฑ์ที่พาสเจอไรส์ที่อุณหภูมิ 70 °C เป็นเวลา 7 นาที (T5) และตัวอย่างควบคุม มีคะแนนการยอมรับรวม 5.75 ดังนั้นจึงได้เลือกการพาสเจอไรส์ในสภาวะ 70 °C เป็นเวลา 7 นาที เพื่อเตรียมผลิตภัณฑ์พาสเจอไรส์สำหรับการศึกษาอายุการเก็บรักษา ซึ่งพบว่า ผลิตภัณฑ์พาสเจอไรส์มีความปลอดภัยสำหรับการบริโภคจนสิ้ นสุดระยะเวลาการเก็บรักษาในตู้เย็น (0-3°C) เป็นเวลา 15 วัน โดยไม่พบความแตกต่าง ทางด้านประสาทสัมผัสอย่าง มีนัยสำคัญ ระหว่างผลิตภัณฑ์พาสเจอไรส์ที่เก็บไว้ที่ 0-3° C กับผลิตภัณฑ์ปรุงเสร็จใหม่ ๆ ตลอดระยะเวลาเก็บเป็นเวลา 15 วัน

ภาควิชา เทคโนโลยีทางอาหาร ลายมือชื่อนิสิต.....

สาขาวิชา วิทยาศาสตร์และเทคโนโลยีทางอาหาร ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

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KEYWORDS : WHITE SHRIMP, SODIUM TRIPOLYPHOSPHATE (STPP), MICROBIAL TRANSGLUTAMINASE (MTGASE), SODIUM BICARBONATE, PANAEUS VANNAMEI, THAILAND.

NOR SALASIAH BINTI MOHAMED: PRODUCT DEVELOPMENT OF PASTEURIZED READY-TO-COOK MARINATED WHITE SHRIMP *Litopenaeus vannamei* IN GREEN CURRY PASTE. ADVISOR: ASSOC. PROF. JIRARAT ANUNTAGOOL, Ph.D., 105 pp

The first part of this research aimed to study the effect of treated shrimp using 2% NaCl in combination with three additives: sodium tri-polyphosphate (STPP), sodium bicarbonate ($NaHCO_3$) and microbial transglutaminase (MTGase) at different concentrations (2, 5 and 8%) and immersion times (10, 30 and 60 minutes) on the quality of white shrimp (*Litopenaeus vannamei*). In average, the three additives with 2% NaCl significantly affected the water holding capacity (WHC), cooking loss (CL), weight gain (WG), moisture content (MC) and total viable count (TVC) of white shrimps. Immersions in 5% MTGase with 2% NaCl for 30 minutes at 4±2°C improved the quality of white shrimp when compared with control. It increased weight gain (15.7%), maximizes WHC up to 94.5%, lowered CL to 10.1% and reduced 1.44 log CFU/g of total viable count (TVC). The second part studied the effects of pasteurization on marinated shrimp in green curry paste at 5 conditions (T1) 65°C for 32.5 minutes, (T2) 67°C for 18.5 minutes, (T3) 68°C for 17 minutes, (T4) 69°C for 7 minutes, (T5) 70°C for 7 minutes in comparison with the control (non-pasteurized) sample. TVC of pasteurized marinated shrimp (T1 to T5) was lower than 10 CFU/g and *Listeria* spp. was not detected in 25g sample, while the TVC for control was 3.67 log CFU/g and *Listeria* spp. was 1.18 log CFU/g. The pH value of pasteurized marinated shrimp (T1 to T5) was significantly lower than the non-pasteurized marinated shrimp. The shear force for samples pasteurized at T3, T4 and T5 was not significantly different from control. Sensory evaluation result showed that the highest score for overall acceptability was 5.75 for the product pasteurized at 70°C for 7 minutes (T5). Treatment 5 (70°C for 7 minutes) was selected to prepare pasteurized sample for the shelf life study. From shelf life study, the pasteurized marinated shrimp in green curry paste was safe for consumption until the end of storage period for 15 days at 0-3°C. There was no significant difference between pasteurized products stored at 0-3°C with freshly prepared product up to 15 days for all attributes that were sensorial tested.

Department : Food Technology Student's Signature……...……..

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NOMENCLATURE

a* = Redness

AOAC= Association of the Official Analytical Chemists.

ANOVA = Analysis of variance

a_w = Water activity

b* = Yellowness

CRD = Completely randomized design

CL = Cooking loss

DMRT = Duncan's multiple range test

FAO= Food and Agriculture Organization of the United Nations

FDA = Food and Drug Administration

ICMSF= International Commission on Microbiological Specifications for Foods

IFST = Institute of Food Science and Technology

ISO = International Standard of Organization

L* = Lightness

MC = Moisture content

RCBD = Randomized complete block design

TIS = Thai Industrial Standard Institute

TVC = Total viable count

WHO = World Health Organization

WHC = Water holding capacity

CHAPTER 1

INTRODUCTION

White shrimps *(Litopenaeus vannamei*) are one of the dominant economically important products of Thailand. Lindner *et al*. (1989) reported that the softening of freshwater prawn tissue was occurred on 7-9 days of storage at 0°C because of degradation of muscle components, due to the proteolytic activities. The changes in texture of thawed shrimp were negative economic factor. Thus the processing of shrimp products using frozen shrimp raw material requires wide array of pre-treatment to create higher quality end-products and, as a consequence, higher value addition. Sodium tri-polyphosphate (STPP) was the most popular chemical used in the pre-treatment of seafood products to improve water holding capacity, texture, stabilise colour and reduce cooking loss. Unfortunately STPP is limited to maximum 0.5g/100g sample in final product according to the EU, Canadian and Brazilian regulations for seafood products (Gonçalves and Ribeiro, 2008). The use of non-phosphate additives could be an alternative way to improve the quality and physical properties of frozen thawed shrimp.

Current trends show increasing consumer demand for consumer ready products with smaller portion sizes and less fat, as well as being ready-to-cook. In modern lifestyle, there has been an increasing number in smaller families with less cooking activity at home. They also keep demanding for fresh foods with ease of preparation. Convenient chilled foods including ready-to-cook shrimp green curry paste is suitable for modern consumers for the mentioned reasons.

Green curry is the second most favourite dish among consumers in Thailand because of its taste, flavour and appearance. Many fresh ingredients used in the formulation of green curry paste have been found to possess antimicrobial and antioxidant activity as well as medicinal values. Marinades using these ingredients can help improving the shelf-life of the products. However, to completely ensure the safety of the fresh ready-to-cook product, pasteurization should be employed to minimize

microbial load. So far, very few studies have been carried out for the development of ready-to-cook products.

This study aimed to develop pasteurized ready-to-cook marinated shrimps in green curry paste. The first part of this research aimed to study the effect of treated shrimp using 2% NaCl in combination with three additives: sodium tri-polyphosphate (STPP), sodium bicarbonate ($NaHCO_3$) and microbial transglutaminase (MTGase) at different concentrations (2, 5 and 8%) and immersion times (10, 30 and 60 minutes) at 4±2°C on the quality of white shrimp (*Litopenaeus vannamei*). The pre-treatment was important to maintain the quality of untreated frozen-thawed white shrimp to maximize the water holding capacity (WHC), increase weight gain (WG) and moisture content (MC), improve texture, and reduce cooking loss (CL) of the shrimp. As the ingredients of ready-to-cook shrimp in green curry paste was in the raw state, to reduce the microbial load and prolong the shelf life, pasteurization at 5 conditions (T1) 65°C for 32.5 minutes, (T2) 67°C for 18.5 minutes, (T3) 68°C for 17 minutes, (T4) 69°C for 7 minutes, (T5) 70°C for 7 minutes were studied with comparison to the control (non-pasteurized) sample. The condition that was suitable for the product to achieve a 6 log reduction of *Listeria* spp. and maintain the quality of the white shrimp was determined.

Lastly, the shelf-life study for non-pasteurized and pasteurized marinated shrimp in green curry paste was carried out for 15 days to obtain the data on microbial, sensory evaluation, physical and chemical changes.

CHAPTER II

LITERATURE REVIEW

2.1 White shrimp

White shrimp (*Litopenaeus vannamei*), also known as Pacific white shrimp, is a prawn commonly caught or farmed. White shrimps are decapods crustaceans of the sub-order Dendrobranchiata. FAO (2013) reported that rostrum moderately long with 7-10 dorsal and maximum size 23 cm. Females commonly faster growing and larger than males. *Penaeus vannamei* live in tropical marine habitats. Males become mature from 20 g and females from 28 g onwards at the age of 6–7 months.

Fresh shrimps are highly perishable, thus predominantly appear in the form of frozen raw, ready-to-cook or ready-to-eat products. In-store market, shrimps products are found as a whole piece, peeled-off, individually quick frozen (IQF) breaded prawns and shelf stable cooked products in cans. The main countries that produce *Penaeus vannamei* include China, Thailand, Indonesia, Brazil and others. FAO (2013) reported that the total farmed production of *P. vannamei* steadily increased to over 1.386 million tonnes in 2004, due to the rapid spread of this species in Asia (Figure 2.1). In 2004, the main producer was China (700,000 tonnes), Thailand (400,000 tonnes), Indonesia (300,000 tonnes) and Vietnam (50,000 tonnes).

Frozen shrimp products like frozen head-on and head-less peeled un-deveined shrimp are the major products for export to United States of America, Japan and European Union. The value-added products have also become one of the popular trends. This may be due to lack of anti-dumping tariffs for processed products in United

States of America market (FAO, 2013). United States of America was the major market for exported shrimp; this country imported 477,000 tonnes worth USD 3.1 billion in 2005, 1.8 times more than that imported in 2000 that was 264,000 tonnes. United States of America needed Asia to supply its increasing demand 1.9 kg/capita in 2004.

However, shrimp price was decreased due to rapidly increasing production of *P. vannamei*. The price of white shrimp (15–20 g/piece) was decreased from USD 5/kg in 2000 to only USD 3.0–3.5/kg in 2005 (FAO, 2013).

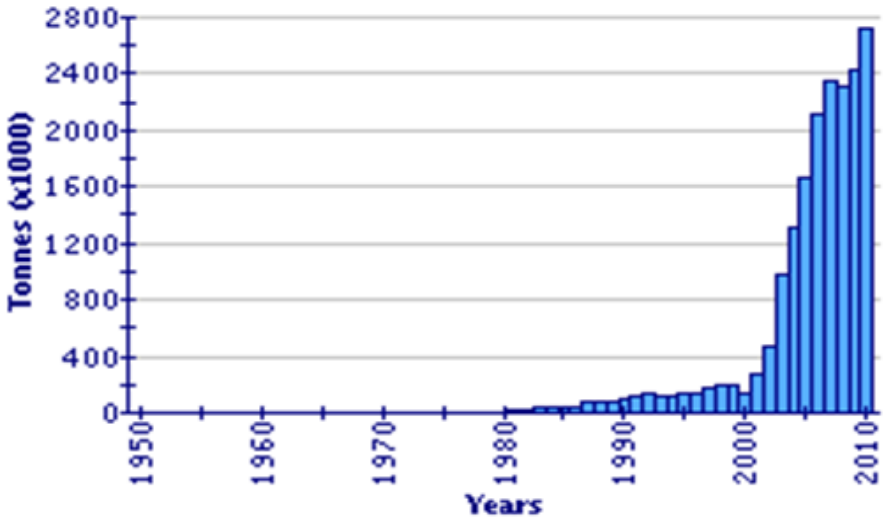


Figure 2.1 Global aquaculture production of *Penaeus vannamei*

Source: FAO (2013)

Thailand is one of the world's producers and exporters of processed food products. For shrimp products, Thailand exports over 20% of the world trade in shrimps and prawns. Thailand food processors increased developing frozen food products to meet the demand of Thai people that have changed their preferences to frozen food. Thailand, which is rich with agricultural roots and resources, applied international standard of quality, technology, and research and development (R&D) for food technology and food safety. Thailand board of investment (BOI), (2008) reported that the shrimp export quantity was 361,650 tonnes valued 2,584 million USD.

Thailand exported 307, 492 tonnes of ready-to-eat foods valued USD 483 million in 2007; the value was increased 20% from 2006 (Thailand board of investment, 2008). Thai ready-to-eat (RTE) and ready-to-cook (RTC) foods are popular in oversea market as other countries have increased their preferences on Thai quality, nutritious and tasty foods. The development of RTE and RTC products that are targeted for developed countries such as Japan, United States of America and European Union (EU) will promote the high quality products and strengthen the sanitation standards for Thai food producers.

Sriboonchitta *et al.* (2000) reported that Thailand exports various forms of shrimp products such as frozen, fresh chilled, dried, boiled, canned, ready-to-eat and ready-to-cook products. Fresh chilled and frozen are the largest form of exported products which is about 70% of all shrimp exports in each year.

2.2 Chilled food

Chilled foods are perishable food which to extend their shelf-life, the food are kept within specified ranges of temperature above -1°C and below 8°C (IFST, 1990). Normally chilled foods are kept at temperature between 0°C to 4°C, but it depends on the standard and regulations of the countries. According to FDA (1997) food hold at 1.7°C was safe for consumption until 19 days and IFST (1990) reported that the shelf-life of the food pasteurized at 70°C for 2 minutes and kept at -1 to 8°C, the shelf-life was 1 to 2 weeks. Cooked chilled foods are often erroneously referred as 'cook-chill' products. These foods need to be cooked followed by fast chilling and stored at the temperature range of 0 to 3ºC. They are subsequently reheated before consumption (Dennis and Stringer, 2000). The short shelf-life products or perishable products can have their shelf-life extended by means of pasteurization. The shelf-life can be prolonged up to 10-14 days after pasteurization. Food with neutral pH and high water activity, pasteurization process should attain 6 log reductions of pathogens (*Salmonella* spp. and *Listeria* spp.), a thermal treatment of at least 70°C for 2 minutes was adequate for this reduction (Brown and Gould, 1992).

Food producers had produced many kinds of value-added chilled food products that are either ready-to-eat or ready-to-cook. This serves the needs of modern consumers who, for the majority, live in big city and are engaged in hard-working and rushing daily live. It was reported that for 40% of eating occasions, convenience is the most important factor. The average home-cooked meal is estimated to take about 30 minutes to prepare. Convenience is a specific need of the cash-rich time-poor consumers. This development is in part the result of the increase in the number of working women, single-parent and single-person households with limited time available for home cooking.

Seafood has always gained higher popularity due to their taste and values. Further processing into a more convenient product makes seafood more attractive. Dennis and Stringer (2000) wrote that chilled prepared foods can be manufactured from many kind of raw materials in terms of level of processing (Table 2.1) and can be designed to be ready to eat, to be reheated (minimal heat application before serving, for organoleptic purposes) or to be cooked (thorough and prolonged heating before serving). The raw materials for these foods are either used in their raw state or they are subjected to various treatments, e.g. blanching, freezing, and cooking (i.e. equivalent to a time temperature combination of 70ºC for 2 minutes). Cross-contamination during manufacturing is avoided by the use of Good Hygienic Practice, as set out in the European Chilled Food Federation Guidelines (ECFF, 2006).

Table 2.1 Chilled prepared food according to level of processing

Ingredients	Further Processing
Raw	None or reheated
Raw + cooked	None or reheated
Raw and/or cooked	Cooked, then packed
Raw and/or cooked	Cooked in package

Source: Dennis and Stringer (2000)

As known, the chill storage only slows down the rate of microbial growth and does not reduce the microbial count during chilled storage. Reducing the temperature does not kill all microorganisms, but it retards their growth. At chilled temperatures food spoilage and deterioration reactions are inhibited to such an extent that food safety and quality is preserved for extended periods. Hence, by controlling finished products at appropriate chill temperatures, chilling can ensure the safety of the foods.

Venugopal (2006) reported that the term "pasteurized chilled food'' is included in the context of "cook-chill'' or "minimally processed product'', which the food received a heat treatment to reduce their initial microbial population and was stored at 0 to 3°C range to maintain the shelf-life for a few days. The cook-chill process was introduced to market as precooked, ready-to-cook and convenient foods. Cook-chill products are categorized as minimally processed food that is not fully inactivated all the pathogenic bacteria, means that this product is not sterile. Appropriate refrigerated storage for specified periods is needed to prevent the growth of any photogenic microorganisms that can jeopardize food safety.

The temperatures for pasteurization is usually set in the range between 65 and 95°C for a suitable range of time that is sufficient for the destruction of 6 log cycles of the target microorganism. After heat treatment, the products should be rapidly chilled to 0 to 3°C within 30 minutes and they should be reheated to at least 70°C for consumption (Venugopal, 2006).

2.3 Marinated shrimp in green curry paste

Green curry made from a variety of fresh spices and it is the second most favourite dish for consumers in Thailand because of its taste, unique flavour and appearance (Office of the National Culture Commission, 1999). The basic ingredients of green curry consist of green chili, galangal, lemon grass, shallot, cumin powder, kaffir lime peel, garlic, coriander seed and black pepper. The ingredients listed in the green curry recipe may slightly differ from one to another to suite consumer tastes.

Many ingredients used in the curry paste contain antimicrobial, antioxidant, and medicinal value. Nishimura *et al*. (2000) reported that garlic contains alicin which has antimicrobial and antioxidant compounds for health benefits. Kaffir lime peel; lemon grass and galangal have been reported to be effective in inhibiting tumors in the digestive tracts (Murakami *et al*., 1994). Thai green curry demonstrated antibacterial activity against seafood spoilage organism and foodborne pathogens (Ifesan *et al*., 2010).

The major antimicrobial compound in garlic is alicin, garlic extracts have been found to possess antibacterial property against *Salmonella* Typhimurium*, Escherichia coli, Bacillus cereus* and *Staphylococcus aureus* (Nanasombat and Lohasupthawee, 2005)

Kaffir lime (*Citrus hystrix* DC) peels contain antimicrobial compounds. Nanasombat and Lohasupthawee (2005) reported that the main compound in kaffir lime leaves is citronellal (65.4%), whereas the major constituents in essential oil of kaffir lime peels are β-pinene (30.6%), limonene (29.2%), sabinene (22.6%). β-pinene and limonene had greater inhibitory activity against *Salmonella* Enteritidis than citronellal with minimum inhibitory concentrations for kaffir lime peels was 41.7mg/ml whereas kaffir lime leaves need 166.7mg/ml of the crude ethanolic extract (Nanasombat and Lohasupthawee, 2005).

The ethyl acetate extract from kaffir lime peel showed a broad spectrum of inhibition compare with essential oil (EO) against all gram negative bacteria namely *Escherichia coli, Salmonella* spp., yeast and mould, gram positive bacteria namely *Bacillus cereus, Staphylococcus aureus, Listeria monocytogenes* and *Saccharomyces cerevisiae var. sake* (Chanthapon, Chanthachum and Hongpattarakere, 2008). Kaffir lime peel EO could reduce the total viable count and significantly retard the lipid oxidation in Chinese sausages (Kingchaiyaphum and Rachtanapun, 2012).

The term ‘‘marination’’ is a process of soaking foods or semi-preserved meat in acidic solution (organic acids, vinegar, lemon juice or wine) or enzymatic (made with ingredients such as fresh spice and herb or pineapple). In Indian cuisine the marinade

is usually prepared with a mixture of herb and spices to give flavour to the foods. Marinades may extend the shelf-life and maintain texture of the shrimp particularly when the curry paste has a kaffir lime peel and garlic content.

Xiong *et al.* (2002) reported that high acid food like marinated food in acidic solutions (citric acid, lemon juice) caused protein denaturation, resulting in decreased water binding ability of myosin, actin and other myofibrillar proteins. The SDS-PAGE of raw muscle of marinated prawn in acidic solutions revealed myosin and actin losses.

Xiong *et al.* (2002) wrote that prawns marinated in citrate solutions and sodium chloride (pH 7.0) were more tender when cooked ($p<0.05$). Marination in tri-polyphosphate (pH 7.0) solution did not affect the muscle toughness, but immersion in $CaCl_2$ (pH 7.0), citric acid and lemon juice (pH 3.0) increased the muscle toughness of marinated prawn ($p<0.05$).

2.4 Pasteurized chilled foods

Thermal process increased the toughness of muscle texture due to protein denaturation, shrinkage of muscle fibers and water losses from muscle structure (Benjakul *et al*., 2008). Snyder (2003) reported that if pasteurized-chilled foods are kept at a temperature lower than 3.3°C, there is absolutely no hazard and it can be held until spoiled. There can even be food temperature fluctuations above 3.3°C for a few days to a maximum value of 7°C, the spores do not grow and produce toxin instantly, the food will remains safe. At 4°C, it will take about 43 days for toxin to be produced.

For products not packaged under reduced oxygen atmosphere, *Listeria monocytogenes* is usually the target pathogen to be controlled (FDA, 2011). Mizuta *et al*. (1999) reported that when the heating temperature exceeds 70°C, the texture and taste of the product become rapidly undesirable with an excessive firmness and a lack of juiciness. Therefore, to prolong the shelf-life of ready-to-cook marinated shrimp, pasteurization parameter at low temperature to achieve a 6 log reduction (6D) of *L. monocytogenes* was important to be studied to keep the texture properties of the product pleasing to the consumer. FDA (2011) suggested that, for safety of seafood

product packaged with presence of oxygen, *L. monocytogenes* was the target pathogen. It is important to bring the core temperature of the food to 70°C and hold for 2 minutes (with z-value of 7.5°C).

FDA (2011) reported that *L. monocytogenes* risk assessment indicated that approximately 8% of raw seafood are contaminated with 1 to 10^3 colony forming unit (CFU)/g and approximately 91% are contaminated at less than 1 CFU/g. FDA's limit for *L. monocytogenes* in ready-to-eat products was non-detectable which the level is less than 1 CFU/25g.

Ifesan *et al.* (2010) found pasteurization of the green curry ingredients at 90°C for 2 minutes and the dry ingredients was added after the pasteurization, stored at 4°C reduced the total viable count (TVC) for 0.78 log CFU/g on the first day and made the antibacterial activity of the green curry more stable. There was no lactic acid bacteria growth found throughout the period of 30 days.

pH values of pasteurized green curry decreased during 6 days storage period and the acid concentration was increased as the storage days increased (Ifesan *et al.*, 2010).

2.5 Packaging

Venugopal (2006) reported that packaging is an important part of the cook-chill process. Flexible packaging is usually used for the product. It performs three functions, namely: (i) prevent moisture vapor transfer from the product and microbial contamination to enhance storage life (ii) improve product display attraction (iii) protect the product during transportation and storage. Actually, a single layer of materials can cover all the required functions of the packaging material. Sometimes several layers packages are required for different purposes.

Majority of chilled foods in the market employs plastics as the packaging material. Commonly chilled ready-to-eat, meats, dairy products, seafood, dessert, poultry, fruit and vegetables are packed in plastics or plastic-based materials. Semi rigid plastic containers for chilled foods are predominantly made from polyethylene (PE),

polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyethylene terephthalate (PET) and acrylonitrile-butadiene-styrene (ABS) (Dennis and Stringer, 2000).

Mohan *et al.* (2008) reported that 'kuruma' prawns packed in pouches gave superior results than cans due to textural and sensory attributes such as color, hardness, firmness, chewiness, and overall acceptability. The use of 16 cm x 20 cm and 17 cm x 30 cm retortable pouches resulted in 35.67% and 56.56% reduction in process time compared with 301 x 206 and 401 x 411 cans, respectively, with equal F_0 of 8.0 minutes and pack weight.

2.6 Food additives

2.6.1 Salt

Sodium chloride (NaCl) known as salt or refined salt (table salt) is an ionic compound. It is commonly used as food additives. NaCl is considered as generally recognized as safe (GRAS) by the US FDA (US Food and Drug Administration) and listed as permitted food additives (FDA, 2012a).

Sodium tri-polyphosphate (STPP), with formula $Na_5P_3O_{10}$, is a polyphosphate of sodium. It is the sodium salt of tri-phosphoric acid. STPP is used to increased water holding capacity in food by retaining the moisture in foods. Shrimp muscle become too solid, unpalatable and increased in firmness by heat processing when its inner temperature was above 100°C (Mizuta *et al*., 1999). This was the reason for some entrepreneurs that used STPP to increase the quality and sale weight of their products. STPP has been widely used as an additive in seafood product and the most popular choice to improve water holding capacity, texture and reduce cooking weight loss of the shrimp.

Faithong, Raksakulthai and Chaiyawat (2006) reported that combined effect of 2% salt with all phosphate namely tetra sodium pyrophosphate (TSPP), STPP and sodium hexametaphosphate (SHMP) yielded a good quality cooked shrimp with high

acceptability ($p<0.05$). The bacterial load was reduced by immersion of frozen-thawed fish in 5% STPP for 10 minutes at 4°C when compared with other phosphate groups (Kilinc *et al*., 2009).

Although phosphate has an important role to enhance water-binding capacity and reduced shrinkage, the presence of excessive amount in diet may influence the calcium, iron and magnesium balance in human body and can increase the risk of bone disease (Shahidi and Synowiecki, 1997). STPP adds an unpleasant taste, particularly to delicate seafood. The taste tends to be slightly sharp and soapy and is particularly detectable in mild-tasting foods. Craig, Bowers and Seib (1991) reported that soapy flavour was higher in frozen cooked turkey containing STPP or sodium ascorbate monophosphate (SAsMP) in water solutions (0.3 and 0.5% levels).

STPP usage is limited to a maximum of 0.5g/100g sample in final product according to the EU, Canadian and Brazilian regulations for seafood products (Gonçalves and Ribeiro, 2008). FAO and WHO (1968) reported that the delegation of the Federal Republics of Germany informed that the use of phosphates was not permitted in their country. Sodium citrate was used in some countries as an alternative to phosphates. The use of non-phosphate ingredient such as sodium bicarbonate ($NaHCO_3$), citric acid and microbial transglutaminase (MTGase) has an advantage over using phosphate because it affirmed as GRAS (generally recognized as safe) status according to US FDA (FDA, 2001 and FDA, 2012a) and this food additives will not give a bitter taste or a soapy flavour to the foods.

Sodium bicarbonate ($NaHCO_3$) has GRAS (GRN 000003) status according to US FDA with CAS register number 144-55-8 and maximum daily dosage limit 200 mEq/kg (FDA, 2012a). $NaHCO_3$ is a chemical compound that is able to increase the weight gain of fresh shrimp up to 10% and lowered cooking weight loss to 20% by dipping at 8% concentration (Henderson, Kaiser and Montville, 1990).

Citric acid affirmed as GRAS (GRN 000222) status according to US FDA with CAS register number 77-92-9 (FDA, 2012a). The maximum daily dosage limit was 8 grams (FDA, 2012b). Lopkulkiaert, Prapatsornwattana, and Rungsardthong (2009) reported

that treated white shrimps with $NaHCO_3$ had a similar effect with $NaHCO_3$ containing citric acid. The sample treated with 4% $NaHCO_3$ combination with 3% salt at 5°C showed an increased yield after freeze-thaw, increased water holding capacity, reduced freezing loss and toughening of thawed samples.

Usually, amount of salt added in the product was range from 0.5 to 2%. Sun (2009) reported that when the salt level used lower than 2% there is a negative effect on functional and mechanical properties of meat products. The combination of phosphate with salt in restructured meats play an important role to reduce shrinkage, increase shear values, water holding capacity, reduce amount of oxidation, improve texture with no effect on colour.

There was less than 50% of the panellists accepted the sample treated with 3% salt, therefore immersing in 2% STPP solution with 2% salt was the most appropriate treatment (Faithong *et al.,* 2006). For the healthy reason, addition of 2% salt in ready-to-cook product may be considered as the maximum limit amount added without additional salt in the cooking process before consumption.

The development of rancidity was delayed by adding 0.25% STPP for adequate protein extraction and flavour development (Pearson and Gillet, 1996). Pietrasik and Li-Chan (2002) reported that in cooked restructured meat, gel firmness and water holding capacity (WHC) significantly increased by combination of 2% salt with 0.5% MTGase, but not in low salt products. Faithong *et al.* (2006) reported that the combined effect of 2% STPP with 1-3% salt used for an immersion for 10 hours at <5°C showed a synergistic effect on reducing cooking weight loss and increasing raw weight gain of white shrimp ($p<0.05$) compare with sample treated with only phosphate.

Lee, Hendricks and Cornforth (1998) study the effects of 0.5% sodium phytate (SPT), sodium pyrophosphate (SPP) and sodium tripolyphosphate (STPP) in combination with 1% NaCl, on physico-chemical properties of restructured cooked beef. SPT, SPP, and STPP increased the cook yield, binding strength, moisture level, pH, and decreased thiobarbituric acid reactive substances (TBARS) compare with control (salt alone) ($p<0.05$).

2.6.2 Microbial Transglutaminase

The food-grade enzyme commonly used "Activa®TG'' or commercially known as "MTGase" (microbial transglutaminase) or "TG" (Transglutaminase) is a product which contains enzyme made by fermentation process from several microorganisms such as *Streptoverticullum*, *Bacillus subtilis* and a variant *Streptoverticullium mobaraense* produced by Ajinomoto's company. Various forms of transglutaminase are found in animal, plant and microbial sources.

Activa®TG is a calcium independent form of the enzyme and this characteristic gives it certain advantages in many food systems. This calcium independent form of the enzyme consists of 331 amino acids, with cysteine as the active center. TG is an enzyme with the ability to cross-link proteins through covalent bonds by forming hydrophobic interactions. Two amino acids used to cross-link are glutamine and lysine (Moreno, Carballo and Borderías, 2010).

TG (Glutaminyl-peptide: amine γ-glutaminyl-transferase, EC 2.3.2.13) modify proteins by catalyzing acyl transfer between a γ-carboxyamide of a peptide/protein bound glutamine and lysine forming an ε-(γ-glutamyl) lysine [ε-(γ-Glu)Lys] cross-link (G-L bond) (Kuraishi, Yamazaki and Susa, 2001). This bond is stable against heat treatment or physical stress. TG, catalyses conversion of soluble proteins to insoluble high-molecular weight polymers through formation of covalent crosslinks (Motoki *et al.,* 1987).

Electrophoretic pattern SDS-page of white shrimp reported by Sriket *et al*. (2007) showed that myosin heavy chain (MHC) was the dominant protein component (56.8-64.3%) Actin was found as a second dominant protein. Actin and myosin react as substrate, (actomyosin) gelled by enzyme (MTGase) through the cross-link protein and result in thermal stability of protein (Motoki and Seguro, 1998). Improvement of water holding capacity increased the gel forming ability, and resulted in a good texture of shrimp with reduced toughness, shrinkage, increased firmness, juiciness and elastic texture that provided fibrous characteristic.

TG is widely used in food industry to provide variety of food choices with a good taste, improved the physical properties of various foods containing proteins. TG does not affect the taste of food (Ajinomoto, 2013). TG treatment improves and maintains the texture-quality of fish products, which strictly depends on freshness of raw materials (Kumazawa *et al*., 1996). The US FDA affirmed TG as generally recognized as safe GRAS GN 000095 (FDA, 2001). The US FDA expanded the use of this material to all foods. TG was allowed to be contained in seafood products at 65 ppm, approved by FDA on 21 December 2001 (FDA, 2001).

Moreno, Carballo and Borderías (2010) reported that TG is active at the pH range of 4-9, with an optimal pH of 5-8, and temperature range of 0–70°C with the optimum of 50-55°C, with fully sustained activity for 10 minutes at 50°C. TG was inactivated during cooking processes above 70°C for few minutes; the inactivation varies with the conditions and composition of the food system. Thawing and cooking loss of frozen fish products was reduced by combination of MTGase in tumbling or injection of frozen fish products (Motoki and Seguro, 1998).

Fish muscle needs enough calcium ions to activate transglutaminase and setting was induced at low or moderate temperatures of 25–30°C (Moreno, Carballo and Borderías, 2010). A stronger gel network was associated with the water holding capacity. Tammatinna *et al*. (2007) revealed that lowered expressible moisture was noticeable and the breaking force of white shrimp gel increased with increasing MTGase (Activa® TG-K) amount added from 0.2-0.8 unit/g sample ($p<0.05$).

2.7 Microbial hazards in chilled foods

Venugopal (2006) wrote that contamination of fishery products with *Listeria monocytogenes* is common and could lead to health hazard. The survey of fish processing industry showed that this bacterium was present in 16.7% of 234 raw fish samples, 9% of 253 finished products, and 27.3% of 553 environmental samples. 18% of retailed chilled meals contain *L. monocytogenes*, possibly through contamination. Venugopal (2006) reported that Public Health Laboratory Service (PHLS) examined 1301

samples of cooked poultry and chilled meals in 1988 and 1989, *L. monocytogenes* was isolated from 63 of 527 (12%) samples of ready-to-eat poultry, 13 of 74 (18%) chilled meals, and 10 of 627 (2%) main course items from cook-chill catering units.

L. monocytogenes can grow at chilled conditions (0-4°C). It cannot survive the thermal process with the temperature regimes such as 70°C for 2 minutes. The presence of *L. monocytogenes* in cooked product can be used as an indicator to assess the hygienic status of a food product either because of cross contamination after cooking or others (FDA, 2011)

FDA (2011) reported that *L. monocytogenes* is facultative anaerobe and most heat-resistant vegetative pathogen for the chilled food product with oxygen presence. The D value for this infectious pathogen at 70°C was 0.3 minutes. *L. monocytogenes* was reported to grow at temperatures between -0.4 to 45°C. It needs at least 7 days exposure time to produce toxin at -0.4 to 5°C. The condition which this bacterium can survive is in the pH range of 4.4-9.4. Minimum water activity for growth is 0.92. This pathogenic microorganism can grow in 10 % salt.

Growth characteristics of food-poisoning bacteria important in seafood processing are shown in Table 2.2. There are many pathogens that are capable of growth at pH values as low as 4.6 and tolerate salt. *L. monocytogenes* and *Vibrio* spp. tolerate salt up to 30% and 10%, respectively. Many pathogens are capable of growth in the products during chilled storage, if they are not eliminated during the processing stage (Venugopal, 2006).The survival of the bacteria at low pH and high salt concentration depends on temperature.

Yeast and mould require oxygen to grow, optimum condition for growth for yeast pH range 4.5 to 6.0, mould pH range 3.5-4.0, temperature range -8°C to 30°C, optimum temperature 25 to 30°C, the water activity for mould more than 0.62 and yeasts require a higher water activity with a minimum of 0.88 (Adams and Moss, 2006). *Escherichia coli* are facultative anaerobe, recognized as hygienic indicator. This pathogenic microorganism can grow in temperature range of 6.5 to 49.4°C with minimum water activity of 0.95, 4-10 pH range and 6.5% water phase salt (ECFF, 2006; FDA, 2011).

Table 2.2 Growth characteristics of food-poisoning bacteria important in seafood processing

Bacteria	Growth temperature (°C)			Lowest pH for growth	Maximum tolerate NaCl (%)
	Minimum	Optimum	Maximum		
C. botulinum types A&B	10.0	35.0	48.0	4.6	10.0
C. botulinum type E	3.3	30.0	45.0	4.8	6.0
Vibrio spp.	5.0-7.0	35.0-35.2	42.2	5.0	9-10
Salmonella and Shigella spp.	5.5	37.2	49.1	5.0	8.0
S. aureus	5.6	35.0	47.2	4.8	17.0
C. perifringes	15.0	49.0	50.0	5.0	5.0
L. monocytogenes	2.8	30.0-37.2	45.0	4.9	30.0

Source: Lee and Hilderbrand (1997)

CHAPTER III

MATERIALS AND METHODS

3.1 Food additives treatment of white shrimp

White shrimps (90-100 count/kg) were procured from Charoean Pokphand Food Public Company, Thailand. The untreated white shrimps were dressed in headless, peeled and deveined style and frozen prior to transportation and treatments. The untreated frozen white shrimp were thawed in chill storage (0-4°C) for overnight (12 hours) before the treatments (Figure 3.1).

The treatments was done using 2% sodium chloride (NaCl) in combination with three additives: sodium tri-polyphosphate (STPP), sodium bicarbonate ($NaHCO_3$) and microbial transglutaminase (MTGase; Activa® TG-HP) at different concentrations (2, 5 and 8%) and different immersion times (10, 30 and 60 minutes) at 4±2°C with volume to weight ratio of the solution to the shrimp was approximately 2:1 (v/w). The experiment was designed using 3^3 factorial in Completely Randomized Design (CRD) with two replicates (Table 3.1). Food grade NaCl, $NaHCO_3$ and STPP was procured from CT Chemichal Co., Ltd and MTGase (Activa® TG-HP) was supplied by Ajinamoto Co., (Thailand) Ltd.

The qualities of raw shrimps were analyzed according to procedure in 3.3.1 to 3.3.4, 3.4.1 to 3.4.5 and 3.5. Treated shrimps were analyzed according to procedure 3.3.1(Moisture analysis), 3.3.4, 3.4.1 to 3.4.5 and 3.5.

Table 3.1 Pre-treatment using 3^3 Factorial in Completely Randomized Design (CRD)

Run	Batch 1	Batch 2	Batch 3
1	8% MTGase for 10 min	8% MTGase for 60 min	8% STPP for 60 min
2	2% STPP for 60 min	5% MTGase for 30 min	5% STPP for 10 min
3	2% STPP for 30 min	5% STPP for 30 min	5% STPP for 60 min
4	8% $NaHCO_3$ for 30 min	2% MTGase for 60 min	8% MTGase for 30 min
5	8% $NaHCO_3$ for 60 min	2% $NaHCO_3$ for 10 min	2% $NaHCO_3$ for 60 min
6	5% $NaHCO_3$ for 10 min	8% $NaHCO_3$ for 10 min	8% STPP for 30 min
7	2% STPP for 10 min	8% STPP for 10 min	2% $NaHCO_3$ for 30 min
8	5% $NaHCO_3$ for 30 min	2% MTGase for 30 min	2% MTGase for 10 min
9	5% $NaHCO_3$ for 60 min	5% MTGase for 60 min	5% MTGase for 10 min
	Control (un-treated shrimp)	Control (un-treated shrimp)	Control (un-treated shrimp)

min: minutes. The pre-treatment was done using the same raw material for every batch in the same replicates, the pre-treatment was replicated two times.

Factor A : Percentage of preservative : a1= 2% , a2 = 5% and a3 = 8%

Factor B : Time immersion: b1 = 10 minutes, b2 = 30 minutes and b3 = 60 minutes

Factor C : Additives: C1= STPP and 2%NaCl, C2 = $NaHCO_3$ (sodium bicarbonate) and 2% NaCl and C3= MTGase (Activa® TG-HP) and 2% NaCl.

Untreated frozen white shrimps (headless and peeled)

↓

Shrimp thawed at chiller 0-4°C for overnight (12 hours)

↓

Pre-treatment at 4±2°C using three food grade chemicals

Figure 3.1 Pre-treatment process

3.2 Pasteurization and shelf-life study

The treated shrimp was mixed with green curry paste with shrimp to green curry paste ratio 2:1 (w/w). About 80±2g of shrimps was packed along with 40±2g curry in pouch to maintain a pack weight of about 120g (Figure 3.2). The green curry paste developed by Charoean Pokphand Food Public Company from Thailand was used in this study.

The products in stand up pouch were pasteurized using hot water bath to achieve 6 log reductions of *L. monocytogenes* according to recommendations from FDA (2011) for fish and seafood products and ECFF (2006). The pasteurization conditions

employed were: (T1) 65°C for 9.3 minutes, (T2) 67°C for 5 minutes, (T3) 68°C for 3.7 minutes, (T4) 69°C for 2.7 minutes and (T5) 70°C for 2 minutes with comparison to the control (non-pasteurized) sample, the experiments was done in two replicates using Completely Randomized Design (CRD) (Figure 3.2). Cooling process was done rapidly after the pasteurization process by immersing the pouch in an ice water bath (10±2°C) for 2 minutes at control room temperature (25±2°C). The cooled pack was dried, labeled and stored at 0-3°C for 15 days in a chiller (Karel and Lund, 2003; FDA, 1997; Snyder, 2003). The temperature profile analysis for pasteurization in package was done by inserting the thermocouple in the pouch and the accumulated lethality (F_0) was calculated (Appendix C). Five treatments of pasteurized sample and non-pasteurized sample were analyzed according to the procedure in 3.3.4, 3.4.1, 3.4.2 and 3.5.

The analysis of physical, chemical and microbiology result was done using Completely Randomized Design (CRD). The analysis of sensory evaluation result was done using Randomized Complete Block Design (RCBD). One optimum pasteurization condition that yielded the product with the highest score for sensory evaluation and good characteristics was used in the shelf-life study.

For shelf-life study, two samples prepared were (T1) control; non-pasteurized marinated shrimp in green curry paste (120g/pack) and (T2) pasteurized marinated shrimp in green curry paste at 70°C for 7 minutes. The experiment was done using Completely Randomized Design (CRD) with two replicates stored at 0-3°C for 15 days. Packaging material, polyethylene (PE/nylon/PE) stand up pouch with packing size of 120g was used. The analysis of physical, chemical and microbiology result was done using Completely Randomized Design (CRD). The analysis of sensory evaluation result was done using Randomized Complete Block design (RCBD).

Samples were analyzed every three days until 15 days of storage (6 times sampling). The products was analyzed according to the procedures in 3.3.2, 3.3.4, 3.4.1, 3.4.2, 3.4.6, 3.4.8 and 3.5. For green curry (gravy) the analysis was done according to the procedures in 3.4.1 and 3.4.7.

Frozen white shrimps (headless and peeled)

↓

Thaw in a chiller (0-4°C) for overnight (12 hours)

↓

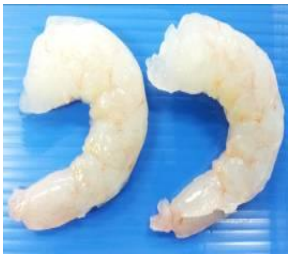
Wash the shrimp using running tap water and immerse in 5% MTGase (Activa® TG-HP) with 2% NaCl for 30 minutes at 4±2°C

↓

Drain and dip again in water for 30 seconds before immersing in 0.5% citric acid for 5 minutes at 4±2°C to reduce the pH value

↓

80g of treated shrimp (5% MTGase + 2% NaCl for 30 minutes)



↓

Mixed with 40g green curry paste

↓

Pack the marinated shrimp in green curry paste (120g) in a stand up pouch (PE/nylon/PE) and seal using an impulse sealer

↓

Pasteurized marinated shrimp green curry in pouch at 70°C for 7 minutes in hot water bath

↓

Shelf-life study stored at 0-3°C for 15 days

Figure 3.2. The marination and pasteurization process

3.3 Chemical analysis

3.3.1 Proximate analysis

Sample was homogenized before the proximate analysis. Moisture content was determined by drying sample in a hot-air oven at 100±2°C for 5-12 hours to constant weight following the method of AOAC 950.46 (AOAC, 2005). Protein was determined by the Kjeldahl Method AOAC 928.08 (AOAC, 2005). Crude fat content of the sample was determined using Solvent Extraction Method AOAC 991.36 (AOAC, 2005). Ash content was determined by heating at 550°C for 12 hours using a muffle furnace following the method in AOAC 938.08 (AOAC, 2005). All analyses were done in two replicates, in each replicate the analysis were done three times (Appendix B.3 – B.6).

3.3.2 Thiobarbituric acid (TBA) value determination

Oxidative rancidity, measured as thiobarbituric acid reactive substances (TBA) in mg malonaldehyde/kg sample was determined according to the method described by Vyncke (1970) (Appendix B.1). The analysis was done in two replicates, each replicate: three readings.

3.3.3 Total volatile base nitrogen (TVB-N)

Total volatile basic nitrogen (mg TVB-N/100 g) was determined according to the method of Pearson (1981) (Appendix B.2). The analysis was done in two replicates, each replicate: three readings.

3.3.4 pH analysis

The pH value was recorded using a pH meter Consort C860 (Consort, Turnhout, Belgium). Each measurement was replicated three times with two replicates. Thirty

grams of shrimp was homogenized with 150 ml distilled water at sample to water ratio 1:5 (w/v) and allowed to dissolve for 2 minutes before the analysis. The pH meter was calibrated with standard buffers of pH 4.0 and 7.0 before the measurement (Siripongvutikorn *et al.*, 2008).

3.4 Physical analysis

3.4.1 Colour analysis

Colour of the raw and treated shrimps, the gravy (green curry) and the homogenized sample (marinated shrimp in green curry) were measured using a Chroma meter CR-400 (Minolta, Osaka, Japan). The color was expressed in CIE Lab system *L**, *a**, and *b** values, where L* denotes lightness on a 0-100 scale from black to white, a* (+) red or (-) green and b* (+) yellow or (-) blue (Schubring *et al.*, 2003). This instrument was calibrated with white reference tiles (Y=93.5; x = 0.3132; y = 0.3198) before the analysis. The shrimp was placed on white tray above the light sources and will be measure directly. Glass cell containing the green curry and homogenized sample of shrimp with green curry was placed above the light sources and *L**, *a**, *b** values were then recorded. Five readings were done for each sample with two replicates (Mallick *et al.,* 2010).

3.4.2 Texture analysis

Shear force determination was carried out at room temperature 25±2°C. The maximum force required to cut the sample (Newton) was recorded. The shear force of samples was measure using a texture analyser (Stable Micro System; TA.XT2*i*, England) with a Warner-Bratzler blade. The operating parameters used are cross-head speed of the machine 10 mm/s. The maximum force to cut at the centre of the second abdominal segment about 1 mm at 45° angle of the shrimp was recorded as the shear force as

described by (Benjakul *et al*., 2008) and (Mallick *et al.,* 2010). The measurement was replicated ten times for each sample with two replicates.

3.4.3 Water holding capacity (WHC)

WHC was characterized by measuring expressible moisture (EM). EM was determined using a modification of the filter paper press method as described by Schubring *et al*. (2003). The sliced samples (5g) were pressed between paired filter sheets (Whatman™ no. 1, 150mm) and parallel plates using a texture analyser (Stable Micro Systems; TA.XT2*i*, England). A 25 kg load cell and a crosshead speed of 1.7 mm/s were used. Samples were pressed to 75% deformation and held at that point for 15s. WHC was defined as the expressible moisture, calculated as %EM = 100 (initial weight - final weight)/ initial weight. WHC was also calculated as %WHC = {1-[initial weight-final weight)/initial weight]} x 100 (Diaz-Tenorio *et al*., 2007). The measurement was replicated four times for each sample with two replicates.

3.4.4 Determination of cooking loss

Cooking losses determination was followed the method reported by Erdogdu, Erdogdu and Ekiz (2007). The treated shrimp were cooked in boiling water for 5 minutes with 1:5 (shrimp: water ratio). After cooking, the shrimp were place on the sieve and for the cooling process; the sample was left at room temperature 25±2°C for 5 minutes. The shrimp weight of the raw shrimp and cooked shrimp (after cooling process) was recorded. Cooking losses were calculated from the weight changes as percentage of the weight difference between the raw and cooked shrimp based on the weight of the raw shrimp. The measurement was replicated three times for each sample with two replicates. The calculation for cooking loss was done using the following equation:

$$\text{Cooking loss, (\%)} = \frac{W1 - W2}{W1} \times 100$$

Where W1 is the weight before cooking (raw) and W2 is the weight after cooking.

3.4.5 Weight gain

Weight gain was calculated from the weight changes as percentage of the weight difference between the raw and treated shrimp based on the weight of the raw shrimp (AOAC, 2005). Two readings were done for each sample with two replicates. Equation used was:

$$\text{Weight gain, (\%)} = \frac{W1 - W2}{W1} \times 100$$

Where W1 is the weight of raw shrimp and W2 is the weight of treated shrimp.

3.4.6 Drained weight

Drained weight was measured for proportion of the shrimp in the pouch according to AOAC 976.17 (AOAC, 2005). Drained weight is defined as the weight of the retained material after placing sample of shrimp on the no.8 sieve (0.24 cm), rinse with water and cover with aluminium foil, after that the shrimp was drained for 2 minutes. Three readings were done for each sample with two replicates. The weight of the shrimp was recorded and the percentage of drained weight was calculated as:

$$\text{Drained weight, (\%)} = \frac{\text{Weight shrimp}}{\text{Declared weight total contents}} \times 100$$

3.4.7 Viscosity analysis

The viscosity measurements for the gravy (green curry paste) were carried out using a rotational concentric cylinder viscometer (Fungilab viscometer, Spain). For the viscosity of green curry paste, the green curry paste was dilute to 10% solid content with water before torque measurements using spindle number 3 with 100 rpm speed. The temperature of samples was maintained at room temperature (25-30°C) during the measurements. All readings were taken after 1 minute. Ten readings were done for each sample with two replicates (Ibanoglu and Ainsworth, 2004).

3.4.8 Sensory analysis

The sensory evaluation to determine the quality of shrimp in green curry was evaluated by 10 trained panelists with two replicates. The sensory characteristics for non-pasteurized and five pasteurized samples were assayed by Quantitative Descriptive Analysis (QDA), simple descriptive using nine category scales (numeric 0 to 8) to know the intensity of every sensory attributes from weaker to stronger. The scale was categorized as 0: None, 2: Slightly, 4: Moderate, 6: Very, 8: Extremely like (Appendix A.1). A score above 4 for overall acceptability will be considered as the margin for the selection of the highest score for all of attributes of the product. The eleven sensory attributes cover under the taste panel is 1) Colour; colour of green curry, colour of shrimp, 2) Odour, 3) Flavours: sweetness, saltiness and off flavour, 4) Texture: juiciness, toughness, tenderness and firmness and 5) Overall acceptability. The statistical analysis was performed according to Randomized Complete Block Design (RCBD).

For the shelf-life study, the stored products were assayed by Discriminative test for Difference from control test also using nine category scales (numeric: 0 to 8). The scale was categorized as 0 means no difference, 2: slightly difference, 4: moderate, 6: very difference and 8: extremely difference (Appendix A.2). The cooked products (non-pasteurized and pasteurized marinated shrimp in green curry paste stored at 0-3°C) with blind control (freshly prepared product) were compared with the freshly prepared

shrimp in green curry to know the acceptance of the product during storage of 15 days. The nine sensory attributes covered under the taste panel is 1) Colour; colour of green curry, colour of shrimp, 2) Odour, 3) Flavour, 4)Texture: juiciness, toughness, tenderness and firmness and 5) Overall difference. The statistical analysis was performed according to Randomized Complete Block Design (RCBD).

3.4.8.1 Selection and training of sensory panels

The sensory evaluation training session was followed the method of training panels from Watts *et al*. (1989); Aminah (2000); Rehbein and Oehlenschlager (2009). For the training of sensory panels, questionnaire was distributed to find the panelists that eat shrimp and did not follow any diet. The panelists were selected among Food Technology officers and master students who were willing to do the sensory evaluation. The training of sensory panels begins by describing the procedures of the sensory evaluation and what is expected of the panelists. The nature and limits of the sense organs are described, such as importance of breathing deeply and resting between samples during odour evaluation. In the first training session for basic taste recognition test, the sensory panels were given questionnaire with samples (salt, sugar, citric acid and caffeine) solution to test the sensitivity of their taste bud (Table 3.2). The answer was given on the same day. If the panels were failed in the test, they could repeat it for the second time.

Second training session was for the QDA training session, 4 hours of training session was conducted for two times in different days. In the training session the attributes was elaborated to the panels and the panels was asked to evaluate 4 samples for one session and total 8 samples of cooked shrimp in green curry was given to the panels in the training session.

The panels gave their feedback on what they understood about every attributes in the sensory evaluation score sheet. After that the sensory evaluation form was developed according to the attributes that the panels understood and suitable to evaluate the marinated shrimp in green curry. The sensory analysis was done by 10

trained panels with the age from 25 to 45 years old, 5 panelists were married and the rest were single.

Table 3.2 Basic taste for recognition test

Basic taste	Substance	Concentration
Sweet	Sucrose	1.0%
Salty	Sodium Chloride	0.2%
Sour	Citric acid	0.04%
Bitter	Caffeine	0.05%

Source: Watts *et al*. (1989)

3.4.8.2 Method of cooking marinated shrimp in green curry paste.

One hundred and fifty millilitre pure coconut milk (100%) and 5ml of oil (1 tsb spoon) was poured in the pot and cook at 100°C. After that 350 ml water was added and the mixture was brought to boil for 6 minutes. Two packs of marinated shrimp in green curry (80g shrimp and 40g green curry paste/pack) were added, the mixture was stirred and cooked for about 2 minutes. The sample for sensory evaluation was served warm (45-50°C) in a coded plate after cooked. The percentage of ingredient used was green curry paste 13.7%, water 59.8%, coconut milk 25.6% and oil 0.85%.

3.5 Microbiological analysis

The microbiology analysis of raw shrimp that had been done included total plate count (McLandsborough, 2005), *Staphylococcus aureus* tested using 3M™ Petrifilm™ Staph Express Count Plates (AOAC Official Method 2003.11) and *Escherichia coli* using 3M™ Petrifilm™ E.coli/Coliform Count Plates (AOAC Official Method 998.08 for *E.coli* and AOAC Official Method 991.14 for Coliform) with two replicates.

Treated shrimps with the three different chemicals (27 treatments and control) was analysed for total plate count according to McLandsborough method (2005) with

two replicates. Non-pasteurized sample and five pasteurized samples for the selection of pasteurization parameter were analyzed for the total plate count using Compact Dry Total Count, AOAC. No. 010404 (Mizuochi and Kodaka, 2000) and *Listeria* spp. was analyzed using Compact Dry LS 'Nissui', ISO 11290 (Teramura *et al*., 2011). The analysis was replicated two times.

Processed chilled shrimp green curry paste (pasteurized and non-pasteurized samples) for shelf-life study was analyzed for *E.coli* using 3M™ Petrifilm™ E.coli/Coliform Count Plates (AOAC Official Method 998.08 for *E.coli* and AOAC Official Method 991.14 for Coliform). Total plate count was analyzed using Compact Dry Total Count 'Nissui', AOAC. No. 010404 (Mizuochi and Kodaka, 2000), *Listeria* spp. was analyzed using Compact Dry LS 'Nissui', ISO 11290 (Teramura *et al*., 2011), and yeast and mould using Compact Dry YM 'Nissui' (AOAC. No. 100401). The microbiology analysis was done with two replicates.

3.5.1 Sample preparation for microbiology analysis

Total viable count (TVC) was determined according to McLandsborough (2005). 25g of sample was aseptically weighed and transferred to a sterile stomacher bag, 225ml of 0.1% sterile peptone water (Merck, Germany) was added to make a 10^{-1} dilution and homogenized for 30s at 230 rpm using paddle blender (Seward Stomacher model 400, England). Homogenized samples were then subjected to determine the total viable count. Serial dilutions were made and one milliliter of each appropriate dilution was pour plated using plate count agar (PCA, HiMedia Laboratories Pvt. Ltd. India) and sterile petri plate (Hycon disposable petri plate), allowed the agar to solidify. All the plates were inverted and incubated at 35±2°C for 48 hours. Plates showing 25–250 colonies were counted.

For samples analyzed using Compact Dry 'Nissui' and 3M™ Petrifilm method, 25g of sample was aseptically weighed and transferred to a sterile stomacher bag, 225ml of 0.1% sterile peptone water (Merck, Germany) was added to make a 10^{-1} dilution and homogenized for 30s at 230 rpm using paddle blender (Seward Stomacher model

400, England). Homogenized samples were then subjected to determine the total viable count and other pathogens. One milliliter of specimen was pipetted and perpendicular on the middle of the dry sheet of the Compact Dry plate or 3M™ Petrifilm. Specimen was diffused automatically and evenly into the sheet. Dilution was done when necessary, some information was written on the memorandum section. The 3M™ Petrifilm and Compact Dry plate were incubated according to the setting temperature and time varies to the type of the microbiology analysis. The number of the colonies was count from 25-250 colonies.

For total viable count, the Compact Dry Total Count plate was inverted and incubated for 48 hours at 35±2°C, for *E.coli* (for meat, poultry and seafood AOAC 998.08) the 3M™ Petrifilm™ E.coli/Coliform Count Plates was incubated for 24±2 hours at 35±1°C, the blue colonies with gas was count as *E.coli* and red or blue colonies with gas was count as coliform. For *Listeria* spp. analysis the Compact Dry LS plate was inverted and incubated for 24-48 hours at 35±2°C, light yellow and blue-green colonies was count as *Listeria* spp. Compact Dry YM for yeast and mould was incubated for 3-7 days at 20-25°C, on day 3 of incubation, blue, white or cream with clear boundaries was observed for yeast and on day 7 the cottony colonies with a characteristic colour (large colonies) was observed for mould.

The microbiological limit was based on the guideline for ready-to-eat or ready-to-cook shrimp at the point of sale (Table 3.3) and recommendation of microbiology limit for seafood products (Table 3.4). The total viable count was limited to $<10^5$ for ready-to-cook shrimp and <20 cfu/g for *Listeria spp.* under satisfactory category at the point of sale.

Table 3.3 Guideline of microbiological quality ready-to-eat or to be cooked shrimp for chilled and frozen storage at the point of sale

	Microbiology quality (CFU per gram)		
Criteria	Satisfactory	Acceptable	Unsatisfactory
Total viable count	$<10^5$	10^5- $<10^6$	$>10^6$
Listeria spp. (total)	<20	20 - $<10^3$	$\geq 10^3$
Escherichia coli (total)	<20	20 - <100	$\geq 10^2$
Faecal Coliform	10^2	10^2- 10^3	$> 10^3$
Staphylococcus aureus	<20	20 - <100	10^2– $<10^4$
Yeast	$<10^4$	10^4 - 10^6	$>10^6$

Source: Stannard (1997); Gilbert, Roberts and Bolton (2000)

Table 3.4 Recommended microbiological limits for seafoods

				Limit per gram	
Product	Test	n	c	m	M
Prawns and shrimps (raw, frozen)	Aerobic plate count at 35°C (/g)	5	2	$5x10^5$	$5x10^6$
	Coagulase producing *Staphylococcus* (/g)	5	2	10^2	10^3
	Faecal coliform (/g)	5	2	10^2	10^3
	Salmonella (/25g)	5	0	0	0

n = Number of representative sample units.

c = Maximum number of acceptable sample units with bacterial counts in m and M.

m = Maximum recommended bacterial counts for good quality products.

M = Maximum recommended bacterial counts for marginally acceptable quality products.

Plate counts below "m" are considered good quality. Plate counts between "m" and "M" are considered marginally acceptable quality, but can be accepted if the number of samples does not exceed "c." Plate counts at or above "M" are considered unacceptable quality.

Source: ICMSF (1986), Food Administration Manual (1995)

3.6 Statistical analysis

Statistical analyses of the data from the experimental result were performed using the Statistical Package for Social Science (SPSS 11.5 for windows, SPSS Inc., Chicago IL, USA). Results were calculated as mean $\pm$ standard deviation. Data was subjected to analysis of variance (ANOVA) and mean comparison was carried out using Duncan's multiple range test (DMRT) with a significant level of 95%.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Microbial load of raw white shrimp

Table 4.1 shows that the microbial load of frozen raw white shrimp did not exceed the recommended maximum limit of aerobic plate count ($5x10^5$) for a good quality of raw or frozen shrimp according to ICMSF (1986) and Food Administration Manual, 1995 (Table 3.4). The total viable count of frozen raw white shrimp (headless and peeled style) was 4.23 log CFU/g. *Escherichia coli* was not detected in 25g sample and the coliform count was 1.57 log CFU/g, which did not exceed the limit of maximum recommended coliform count (10^3) for marginally acceptable quality frozen shrimp (Table 3.4). The *Staphylococcus aureus* count was 2.14 log CFU/g and not exceeds the maximum limit of *Staphylococcus* count (10^3) for marginally acceptable quality of frozen shrimp in Table 3.4.

Table 4.1 Microbial load of frozen raw white shrimp

Total viable count (Log CFU/g)	Coliform (Log CFU/g)	*Escherichia coli*	*Staphylococcus aureus* (Log CFU/g)
4.23±0.06	1.57±0.12	Not detected	2.14±0.01

4.2 Effect of pre-treatment on frozen-thawed white shrimp

Pearson's correlation coefficients of physiochemical and microbiology analysis are summarized in Table 4.2. Weight gain (WG) correlated positively with pH, water holding capacity (WHC) and moisture content (MC) with a correlation coefficient (CC) of 0.60, 0.58 and 0.59, respectively ($p \leq 0.01$). Shear force significantly correlated negatively with WG (CC = -0.54; $p \leq 0.01$) and WHC (CC = -0.411; $p \leq 0.05$). Cooking loss

correlated positively with shear force (CC = 0.44 at $p \leq 0.05$) and correlated negatively with WHC, MC, WG and pH at CC = -0.54, -0.46, -0.84 and -0.49, respectively ($p \leq 0.01$). Total viable count correlated negatively with pH at CC = -0.73 ($p \leq 0.01$).

Effects of concentration and immersion time of each type of additives on physical properties and microbiology analysis are shown in Tables 4.3 to 4.6. In average, the results showed that both concentration and immersion time had an effect with weight gain (WG), total viable count (TVC), water holding capacity (WHC), cooking loss (CL) and moisture content (MC) of the treated shrimp when compared with control. Among all treatments using three types of additives in combination with 2% NaCl, MTGase gave better results in WHC, CL, WG, MC and TVC. Immersion white shrimp in 5% MTGase for 30 minutes increased the WG (15.68%), MC (84.00%) and WHC (94.50%), lowered CL to 10.15% and reduced TVC for about 1.44 log CFU/g. The shear force value for this sample was not significantly different with control ($p>0.05$). For $NaHCO_3$ and STPP, when compared within their own group, 5% of concentration and 60 minutes immersion reduced TVC and CL, increased WG, WHC and MC significantly.

Table 4.2 Correlations between physiochemical and microbiology properties of treated white shrimp

	WG	pH	Shear	CL	WHC	Moisture	TVC
WG	1						
pH	0.60(**)	1					
Shear	-0.54(**)	-0.64(**)	1				
CL	-0.84(**)	-0.49(**)	0.44(*)	1			
WHC	0.58(**)	0.74(**)	-0.411(*)	-0.54(**)	1		
Moisture	0.59(**)	0.15	-0.10	-0.46(**)	0.33	1	
TVC	-0.52(**)	-0.73(**)	0.62(**)	0.51(**)	-0.53(**)	-0.14	1

Pearson correlation, **Correlation at 0.01 and *Correlation at 0.05. * Significant at $p \leq 0.05$ and ** significant at $p \leq 0.01$. WG: Weight gain, Shear: Shear force (Newton), CL: Cooking loss, WHC: Water holding capacity and TVC: Total viable count.

4.2.1 Weight gain (WG)

Weight gain of the treated shrimp increased up to 15.77% compared with control (WG = 0%; Table 4.3) ($p \leq 0.05$). Treated shrimps (treatments number 4-8, 12, 15-18, 22-23 and 25-27) did not show an increase in WG as the concentration and immersion time increased (Table 4.3). Higher concentration of the additives could lead to yield loss due to excessive solubilisation or disruption of protein filaments that increases expressible moisture and reduces water holding capacity.

4.2.2 Texture

The shear force value for treated shrimp was not significantly different from control (Table 4.3). When toughening of the treated shrimp was reduced, firmness and juiciness increased and the shrimp texture become more elastic (chewy). The elastic texture was dominant and provided fibrous characteristic on the sample treated with MTGase+2% NaCl. Faithong, Raksakulthai and Chaiyawat (2006) reported that the shrimp immersed in higher concentration of phosphate (2-3%) and salt (2-3%) for 8 and 10 hours at 5°C resulted in higher shear force.

According to physical observation, the texture of the raw shrimp muscle (control) was softer than the treated shrimp, but the shear force value for control was higher because of the ventral nerve code at below abdominal segment inside the shrimp meat. The ventral nerve code was toughed and stronger than the ventral nerve code in treated shrimp. This ventral nerve code usually was not removed by the manufacturer. The graph of the shear force was increased when the Warner-Bratzler blade cut at the ventral nerve code inside the shrimp meat and gave a higher value of the shear force.

Electrophoretic pattern SDS-PAGE of white shrimp reported by Sriket *et al*. (2007) showed that myosin heavy chain (MHC) was the dominant protein component (56.8-64.3%), actin was found as a second dominant protein. The actin and myosin (actomyosin) react as substrate, gelled by enzyme (MTGase) through the cross-link protein and result in thermal stability of protein (Motoki and Seguro, 1998).

Improvement of water holding capacity in treated shrimp with MTGase resulted in a good texture of shrimp which reduced toughness (shear force range 15.35-18.09 newton) and increased firmness, juiciness and gel forming ability (elastic texture) that provided fibrous characteristic. Motoki and Seguro (1998) reported that the activity of MTGase was inactivated after heating to above 70°C.

4.2.3 Total viable count (TVC)

The treatment with 5% MTGase+2%NaCl for 30 minutes resulted in the lowest microbial load (2.92 log CFU/g) compared with the other treatments ($p \leq 0.05$). This treatment lowers the microbial load for about 1.44 log cycle compared with control sample (4.36 log CFU/g) (Table 4.3). Result showed that only sample treated with MTGase had a significant lower TVC value compared with control. This might be due to the pH value range from 8.17 to 11.15, which was not suitable for microbial growth. Total viable count correlated negatively with pH at CC = -0.73 ($p \leq 0.01$) (Table 4.2). Carballo *et al*. (2006) reported that MTGase/caseinate (1.5 g/100 g) using cold binding systems for restructured meat inhibited the rapid growth of bacteria.

4.2.4 Cooking loss (CL)

The lowest CL value was found in sample treated with 5% MTGase+2%NaCl for 30 minutes (10.15%) that was much lower than the control sample which had 51.13% CL (Table 4.4). It was also found that, treated shrimp had lower CL when the concentration of the additives and immersion time was increased ($p \leq 0.05$). Only the sample treated with 8% MTGase+ 2% NaCl for 60 minutes and 2%STPP + 2%NaCl for 30 and 60 minutes had a higher CL value as the immersion time increased. Treatments with longer immersion time reduced the water retention and increased water loss during cooking. This maybe due to an excessive solubilisation and disruption of protein filaments resulting an increase in CL value.

Benjakul *et al*. (2008) reported that the CL of black tiger shrimp and white shrimp increased sharply after being heated for a longer time for more than 0.5 to 3 minutes ($p<0.05$). Cooking loss of frozen fish products after thawing was reduced by combination of MTGase in tumbling or injection of frozen fish products (Motoki and Seguro, 1998). Tammatinna *et al*. (2007) reported that more water retained in the gel network of white shrimp with the increasing MTGase (Activa® TG-K) amount added (0.2-0.8 unit/g sample).

Erdogdu, Erdogdu and Ekiz (2007) explained that cook losses was highly depending on the concentration and time of immersion in sodium tri-polyphosphate (STPP) solution ($p<0.05$). The diffused amount of STPP was around 0.25% in the meats dipped in the 2% STPP solution after 30 min of dipping time which made 2% STPP not effective to reduce cook losses.

Faithong *et al*. (2006) reported that the combined effect of 2% STTP with 1-3% salt for 10 hours at <5°C showed a synergistic effect on reducing cooking loss and increase raw weight gain of white shrimp ($p<0.05$) when compared with sample treated with only phosphate. There was no significant effect with tetra sodium pyrophosphate (TSPP) and sodium hexametaphosphate (SHMP) in reducing cooking weight loss.

4.2.5 Water holding capacity (WHC)

Overall, the WHC of treated shrimp was increased ($p\leq0.05$) from 87.50% for the control sample up to 94.68% (Table 4.4). The result showed that when pH increased the WHC value was also increased (Table 4.4 and 4.6). WHC correlated positively with pH with CC = 0.74 ($p\leq0.05$) in Table 4.2. This means that when pH increased the ionic strength was also increased and may lead to increase in WHC. Only three samples (treatment number 11, 24 and 27) did not show an increase in WHC compared with control ($p>0.05$). For treatments with MTGase the WHC was increased when the concentration and immersion time increased. Tammatinna *et al*. (2007) reported that lowered expressible moisture was noticeable with increasing MTGase (Activa® TG-K)

amount added from 0.2 to 0.8 unit/g sample ($p<0.05$). For $NaHCO_3$ and STPP at higher concentration and longer immersion time the WHC was not increased. This is due to the absorption of too much water that increased the muscle protein solubility and thus, lowered WHC.

Pietrasik and Li-Chan (2002) reported that in cooked restructured meat products, gel firmness and water-holding capacity (WHC) significantly ($p<0.01$) increased by addition of 0.5% MTGase in high salt (2%) compare to the sample without MTGase.

4.2.6 Moisture content (MC)

Compared with control, MC of treated shrimp was increased when the concentration and immersion time increased ($p\leq0.05$). The MC increased from 81.41% (control) to 84.52% (Table 4.4). Only five samples (treatments number 8, 9, 22, 26 and 27) were not significantly different from control. Higher concentration and longer immersion time might cause protein denaturation and lower the moisture content. Faithong *et al*. (2006) reported that moisture content of the sample treated with 1-3% salt and 2% STPP for 10 hours at <5°C was significantly lower than that of the control (without salt).

Table 4.3 Effects of three additives in combination with 2% NaCl on weight gain (WG), total viable count (TVC) and shear force of treated white shrimps

Treatment	Level (%)	Time (minute)	Additives +2% NaCl	Weight gain (%)	Shear force (N)	TVC (log cfu/g)
Control	0	0	Un-treated	0.00^{l}	18.81^{ab}±2.01	4.36^{ab} ±0.28
1	2	10	MTGase	11.26^{efg}±0.51	16.76^{ab} ±3.23	3.30^{efghi} ±0.03
2	2	30	MTGase	11.22^{efgh}±0.35	16.73^{ab} ±1.18	3.46^{defghi} ±0.40
3	2	60	MTGase	14.45^{b}±0.10	17.75^{ab}±1.84	3.26^{fghi} ±0.20
4	5	10	MTGase	15.65^{a}±0.13	15.88^{b} ±4.71	3.24^{fghi} ±0.07
5	5	30	MTGase	15.68^{a}±0.02	17.14^{ab} ±2.88	2.92^{i} ±0.01
6	5	60	MTGase	15.77^{a}±0.09	15.35^{b} ±2.17	3.05^{ghi} ±0.01
7	8	10	MTGase	11.28^{ef}±0.42	18.09^{ab} ±3.21	3.22^{fghi} ±0.08
8	8	30	MTGase	11.62^{de}±0.26	16.19^{b} ±0.37	2.97^{hi} ±0.12
9	8	60	MTGase	13.10^{c}±0.82	15.35^{b} ±0.81	3.07^{ghi} ±0.60
10	2	10	$NaHCO_3$	8.00^{ij}±0.78	17.75^{ab} ±1.14	$3.60^{bcdefghi}$ ±0.58
11	2	30	$NaHCO_3$	13.28^{c}±0.28	17.43^{ab}±0.49	4.22^{abcd} ±0.08
12	2	60	$NaHCO_3$	11.07^{efgh}±0.60	17.27^{ab} ±0.12	$3.68^{bcdefghi}$ ±0.34
13	5	10	$NaHCO_3$	11.11^{efgh}±0.56	19.87^{a} ±1.01	4.21^{abcd} ±0.24
14	5	30	$NaHCO_3$	13.25^{c}±0.25	16.77^{ab} ±1.50	4.31^{abc} ±0.10
15	5	60	$NaHCO_3$	12.60^{cd}±0.99	17.92^{ab} ±4.43	$3.49^{cdefghi}$ ±0.39
16	8	10	$NaHCO_3$	8.55^{i}±0.21	16.89^{ab}±1.54	3.41^{defghi} ±0.42
17	8	30	$NaHCO_3$	10.35^{fgh}±0.09	17.93^{ab}±3.16	4.03^{abcdef} ±0.15
18	8	60	$NaHCO_3$	13.62^{bc}±0.73	17.70^{ab} ±3.22	$3.68^{bcdefghi}$ ±0.46
19	2	10	STPP	10.90^{efgh}±0.38	17.78^{ab} ±3.59	4.17^{abcd} ±0.24
20	2	30	STPP	10.93^{efgh}±0.48	18.20^{ab} ±2.14	4.56^{a} ±0.40
21	2	60	STPP	11.64^{de}±0.75	17.94^{ab} ±5.13	4.09^{abcde} ±0.08
22	5	10	STPP	6.74^{k}±0.79	17.34^{ab} ±0.72	$3.80^{abcdefg}$ ±0.45
23	5	30	STPP	8.81^{i}±0.80	18.24^{ab} ±1.42	$3.79^{abcdefgh}$ ±0.97
24	5	60	STPP	11.56^{def}±0.92	18.07^{ab} ±1.10	3.44^{defghi} ±0.10
25	8	10	STPP	7.07^{jk}±0.39	17.53^{ab}±2.02	3.42^{defghi} ±0.44
26	8	30	STPP	9.98^{h}±0.74	18.02^{ab}±1.10	4.11^{abcde} ±0.23
27	8	60	STPP	10.02^{gh}±1.05	17.36^{ab}±0.64	3.95^{abcdef} ±0.13

Means with different superscripted letters in the same column are significantly different ($p \leq 0.05$).

Table 4.4 Effects of three additives with 2% NaCl on cooking loss (CL), water holding capacity (WHC), and moisture content (MC) of treated white shrimps

Treatment	Level (%)	Time (minute)	Additives +2% NaCl	Cooking loss (%)	WHC (%)	Moisture content (%)
Control	0	0	Un-treated	$51.13^{a}\pm1.27$	$87.50^{kl}\pm1.21$	$81.41^{l}\pm0.79$
1	2	10	MTGase	$17.30^{d}\pm0.13$	$89.93^{hi}\pm0.65$	$83.06^{defgh}\pm1.06$
2	2	30	MTGase	$15.74^{def}\pm0.68$	$91.09^{gh}\pm0.59$	$84.52^{a}\pm1.37$
3	2	60	MTGase	$16.43^{de}\pm0.08$	$92.68^{ef}\pm1.71$	$84.27^{abc}\pm1.48$
4	5	10	MTGase	$14.93^{efg}\pm1.17$	$92.91^{def}\pm0.88$	$82.72^{fghij}\pm0.70$
5	5	30	MTGase	$10.15^{k}\pm0.11$	$94.50^{ab}\pm1.42$	$84.00^{abcd}\pm0.97$
6	5	60	MTGase	$10.59^{jk}\pm0.27$	$94.04^{abcd}\pm1.32$	$83.50^{abcdefg}\pm1.28$
7	8	10	MTGase	$11.69^{ijk}\pm1.33$	$93.49^{abcde}\pm1.90$	$82.96^{defghi}\pm1.80$
8	8	30	MTGase	$10.58^{jk}\pm0.06$	$94.40^{abc}\pm1.39$	$81.17^{l}\pm0.90$
9	8	60	MTGase	$15.18^{ef}\pm0.60$	$94.68^{a}\pm0.21$	$82.12^{hijkl}\pm1.91$
10	2	10	$NaHCO_3$	$19.03^{c}\pm1.55$	$89.31^{ij}\pm1.39$	$83.99^{abcd}\pm1.19$
11	2	30	$NaHCO_3$	$14.00^{fgh}\pm0.28$	$87.21^{l}\pm1.99$	$83.11^{defgh}\pm0.67$
12	2	60	$NaHCO_3$	$15.63^{def}\pm0.52$	$85.96^{m}\pm1.65$	$83.80^{abcde}\pm0.08$
13	5	10	$NaHCO_3$	$12.49^{hi}\pm0.19$	$93.26^{bcde}\pm1.33$	$83.64^{abcdef}\pm0.79$
14	5	30	$NaHCO_3$	$12.59^{hi}\pm0.28$	$93.69^{abcde}\pm1.24$	$84.33^{ab}\pm0.94$
15	5	60	$NaHCO_3$	$12.57^{hi}\pm0.28$	$93.17^{cde}\pm1.37$	$83.73^{abcdef}\pm0.74$
16	8	10	$NaHCO_3$	$14.39^{fg}\pm0.44$	$92.91^{def}\pm1.42$	$82.87^{efghi}\pm1.09$
17	8	30	$NaHCO_3$	$12.22^{hij}\pm0.09$	$93.45^{abcde}\pm1.73$	$83.46^{bcdefg}\pm1.29$
18	8	60	$NaHCO_3$	$12.32^{hij}\pm0.07$	$93.50^{abcde}\pm1.28$	$83.36^{bcdefg}\pm1.26$
19	2	10	STPP	$13.24^{ghi}\pm0.18$	$91.09^{gh}\pm0.32$	$83.01^{defghi}\pm1.31$
20	2	30	STPP	$23.22^{b}\pm0.22$	$91.00^{gh}\pm0.30$	$83.30^{cdefg}\pm1.35$
21	2	60	STPP	$23.47^{b}\pm0.35$	$89.56^{ij}\pm0.56$	$83.78^{abcde}\pm1.27$
22	5	10	STPP	$17.37^{d}\pm0.10$	$89.52^{ij}\pm0.56$	$82.04^{ijkl}\pm0.83$
23	5	30	STPP	$14.93^{efg}\pm0.36$	$91.71^{fg}\pm0.93$	$83.26^{cdefg}\pm1.41$
24	5	60	STPP	$11.76^{ijk}\pm0.83$	$88.61^{jk}\pm1.48$	$82.57^{ghijk}\pm0.25$
25	8	10	STPP	$13.29^{ghj}\pm0.14$	$91.75^{fg}\pm0.88$	$82.53^{ghijk}\pm1.29$
26	8	30	STPP	$12.24^{hij}\pm1.27$	$88.82^{ij}\pm1.61$	$81.75^{jkl}\pm0.73$
27	8	60	STPP	$12.29^{hij}\pm1.20$	$88.42^{jk}\pm1.55$	$81.69^{kl}\pm0.36$

Means with different superscripted letters in the same column are significantly different ($p \leq 0.05$).

4.2.7 Colour (L* a* b*)

Table 4.5 showed that the colour (L* a* and b*) value for treated shrimp was significant different from control ($p \leq 0.05$). Treatments with MTGase increased a* value (+ = red) of treated shrimp, while treatments with STPP and $NaHCO_3$ increased b* value (+ = yellow). The a* and b* value increased in treated shrimp might be due to the interaction of pigments with the food additives. Pietrasik and Li-Chan (2002) reported that MTGase addition had no significant influence on the b* value of restructured meat, the addition of 0.5% MTG increased lightness and redness of gels containing 8% meat protein and 2% egg albumin with 0.5% K-carragenan, respectively.

Benjakul *et al*. (2008) reported that the L* a* b* value of white shrimp was increased when heating time was longer than 0.5 to 1 minutes ($p>0.05$).

4.2.8 pH analysis

Treated shrimp with MTGase and $NaHCO_3$ significantly increased the pH value of white shrimp up to 11.15 and 8.72 respectively (Table 4.6). The pH value was increased when the concentration of the additives and immersion time increased. For shrimp treated with 2% MTGase+2% NaCl for 10 minutes, 2% $NaHCO_3$ for 10, 30 and 60 minutes and all treated shrimp using STPP+2% NaCl, the pH value was not significant different from control ($p>0.05$).

Unal *et al.* (2004) reported that there were two diffusion mechanisms taking place. Where the meat samples naturally had high amounts of orthophosphates the compound diffuses into the solutions, while STPP diffuses into the meat samples. The STPP diffusion into the meat samples was relatively slower compared with the orthophosphate diffusion out of the meat samples until the water-protein-STPP complex barrier formation on the surface of the meat samples was completed (Tenhet *et al.,* 1981). Moreno, Carballo and Borderías (2010) reported that MTGase is active and stable at wide pH range of 4-9 and the optimum pH was at 5-8.

Table 4.5 Effects of three additives combination with 2% NaCl on colour L* a* b*

Treatment	Level (%)	Time (minute)	Additives +2% NaCl	L*	a*	b*
Control	0	0	Un-treated	51.67^{a}±2.01	-0.10^{de}±0.92	-3.55 ^{bcdef} ±1.42
1	2	10	MTGase	46.98 ^{bcde}±1.24	3.76 ^{ab}±0.22	-2.46 ^{abc}±4.11
2	2	30	MTGase	47.42 ^{bcd} ±1.09	4.35 ^{a}±2.06	-1.32^{ab}±1.29
3	2	60	MTGase	46.16 ^{bcdef}±0.99	3.95 ^{ab}±1.32	-1.35 ^{ab}±2.12
4	5	10	MTGase	45.88^{cdef}±1.48	4.04 ^{ab}±1.28	-2.97^{abcde} ±2.03
5	5	30	MTGase	45.35^{cdef}±0.19	5.41^{a}±3.68	-1.86 ^{abc}±2.10
6	5	60	MTGase	46.65 ^{bcdef}±1.46	4.79 ^{a}±2.85	-1.36 ^{ab}±2.40
7	8	10	MTGase	46.25 ^{bcdef}±1.40	4.23 ^{a}±1.65	-0.89 ^{a}±2.61
8	8	30	MTGase	47.45 ^{bcd}±0.86	4.94 ^{a}±1.65	-2.77 ^{abcd}±1.36
9	8	60	MTGase	46.72 ^{bcdef}±0.69	4.08 ^{ab}±2.71	-2.48 ^{abc}±0.93
10	2	10	$NaHCO_3$	46.60 ^{bcdef}±4.33	1.80 ^{cd}±1.94	-4.08^{cdefg}±1.77
11	2	30	$NaHCO_3$	46.41 ^{bcdef}±4.25	0.49 ^{cde}±1.17	-5.60^{fghijk}±3.20
12	2	60	$NaHCO_3$	46.83 ^{bcdef}±1.36	0.33 ^{de}±0.55	-6.10 ^{ghijkl}±2.43
13	5	10	$NaHCO_3$	45.98^{cdef}±1.54	0.83 ^{cde}±1.00	-5.36^{fghij}±1.48
14	5	30	$NaHCO_3$	44.73 ^{def}±3.51	1.30 ^{cde}±2.16	-7.72^{jkl} ±1.12
15	5	60	$NaHCO_3$	45.48^{cdef}±3.10	0.73 ^{cde}±1.76	-7.59 ^{ijkl}±1.66
16	8	10	$NaHCO_3$	43.59 ^{ef}±1.96	2.31^{bc}±1.63	-6.55 ^{ghijkl}±2.75
17	8	30	$NaHCO_3$	44.87^{def}±2.83	0.92 ^{cde}±1.29	-7.77 ^{jkl}±1.07
18	8	60	$NaHCO_3$	43.22^{f}±1.00	1.04 ^{cde}±1.60	-8.07 ^{kl} ±1.12
19	2	10	STPP	47.88 ^{bcd}±1.21	-0.13 ^{de}±0.01	-5.61 ^{fghijk}±0.81
20	2	30	STPP	48.63 ^{abc}±0.49	-0.28 ^{e}±0.28	-5.17^{fghi}±0.13
21	2	60	STPP	49.81^{ab}±2.68	-0.47^{e}±0.52	-5.00^{defgh}±0.56
22	5	10	STPP	46.58 ^{bcdef}±2.67	0.05 ^{de}±0.72	-6.17 ^{ghijkl}±2.79
23	5	30	STPP	46.40 ^{bcdef}±2.18	0.96 ^{cde}±1.22	-6.06 ^{ghijkl}±2.96
24	5	60	STPP	46.00^{cdef}±0.13	0.11 ^{de}±0.71	-8.12^{l}±1.14
25	8	10	STPP	44.38 ^{def}±0.69	0.49 ^{cde}±1.33	-6.60 ^{hijkl}±2.86
26	8	30	STPP	45.19^{cdef}±1.43	0.23 ^{de}±0.95	-7.28 ^{hijkl}±1.00
27	8	60	STPP	44.76 ^{def}±3.70	0.23^{de}±1.11	-8.51^{l}±2.40

Means with different superscripted letters in the same column are significantly different ($p \leq 0.05$).

Table 4.6 Effects of three additives with 2% NaCl on pH of white shrimp

Treatment	Level (%)	Time (minute)	Additives +2% NaCl	pH
Control	0	0	Un-treated	7.16^{i} ±0.33
1	2	10	MTGase	8.17^{efghi}±0.24
2	2	30	MTGase	8.80^{cdefg}±0.05
3	2	60	MTGase	9.24^{cdef} ±0.49
4	5	10	MTGase	9.30^{cde}±0.12
5	5	30	MTGase	9.29^{cde} ±1.36
6	5	60	MTGase	9.54^{bcd}±1.05
7	8	10	MTGase	9.90^{bc}±0.74
8	8	30	MTGase	10.55^{ab}±0.81
9	8	60	MTGase	11.15^{a}±0.12
10	2	10	$NaHCO_3$	8.02^{ghi} ±0.02
11	2	30	$NaHCO_3$	8.06^{fghi} ±0.37
12	2	60	$NaHCO_3$	8.09^{efghi} ±0.40
13	5	10	$NaHCO_3$	8.42^{defgh}±0.01
14	5	30	$NaHCO_3$	8.68^{defg}±0.11
15	5	60	$NaHCO_3$	8.71^{defg}±0.10
16	8	10	$NaHCO_3$	8.64^{defg}±0.04
17	8	30	$NaHCO_3$	8.71^{defg}±0.08
18	8	60	$NaHCO_3$	8.72^{defg}±0.09
19	2	10	STPP	7.20^{i} ±0.12
20	2	30	STPP	7.33^{hi} ±0.15
21	2	60	STPP	7.38^{hi} ±0.20
22	5	10	STPP	7.38^{hi} ±0.53
23	5	30	STPP	7.69^{ghi} ±0.71
24	5	60	STPP	7.69^{ghi} ±0.87
25	8	10	STPP	7.68^{ghi} ±0.61
26	8	30	STPP	7.79^{ghi} ±0.76
27	8	60	STPP	7.90^{ghi} ±0.76

Homogenized sample was used in the pH analysis. Means with different superscripted letters in the same column are significantly different ($p \leq 0.05$).

4.3 Effect of 2% NaCl combination with MTGase, STPP and $NaHCO_3$

When the frozen-thawed shrimp were submerged in the additives solution with 2% NaCl, water molecules started diffusing into the shrimp meat. The role of the 2% NaCl was to enhance protein-water interaction. Part of the additives and 2% NaCl molecule anchored to positively charged groups of proteins, while the rest scavenged for free water molecule and presumably created a gradient concentration to allow more water to propagate into shrimp muscle. Protein composition and conformation have significant effects on water holding capacity (WHC). Combination of 2% NaCl with the three types of additives have a synergistic effect to enhance the WHC and significantly increased the weight gain, moisture content and reduced cooking loss ($p \leq 0.05$) (Table 4.3 and 4.4).

Shults and Wierbicki (1973) reported that the combination of 1-5 % salt to 0.5% phosphates namely; tetra sodium phyrophosphate (TSPP), sodium tri-polyphosphate (STPP) and sodium hexametaphosphate (SHMP) solution reduce the shrinkage of chicken breast after cooking and increased the water holding capacity compared to sample treated with phosphate without salt. Phosphate and salt were found to have a synergistic effect on reducing cooking loss. When WHC increased, the ionic strength was also increased which caused the swelling of muscle fibre and increased extractability and solubility of myofibrillar protein (Liu and Xiong, 1997).

Damodaran and Kinsella (1982) reported that salt increased the functional properties of protein due to hydrophobic and electrostatic interactions which resulted in thermal-stability of proteins. Faithong *et al*. (2006) reported that the effect of 1-3% salt combination with 2% tetra sodium pyrophosphate (TSPP), sodium tripolyphosphate (STPP) and sodium hexametaphosphate (SHMP) on shear force was found to depend on salt concentration; the higher shear force value was with the shrimp immersed in a higher salt concentration. In cooked restructured meat, the water-holding capacity (WHC) was increased by addition of 0.5% MTGase in high salt (2%), but not in low salt products (Pietrasik and Li-Chan, 2002).

4.4 Selection of suitable food additives for the pre-treatment

The most suitable food additive that had been selected was the treatment with MTGase (Activa® TG-HP) at 5% concentration with 2% NaCl for 30 minutes. Immersion white shrimp in 5% MTGase with 2% NaCl for 30 minutes increased the WG (15.68%), MC (84.00%) and WHC (94.50%), lowered CL to 10.15% and reduced TVC for about 1.44 log CFU/g compared with control. The shear force value for this sample was not significantly different from control. Only the pH value of treated shrimp (5% MTGase with 2% NaCl for 30 minutes) needed to be adjusted because the pH of treated shrimp was 9.30. If the beginning of pH value of marinated shrimp in green curry was higher the final pH of the product after storage will be too high. Dziezak (1990) reported that higher pH value will shorten the shelf-life of the product and failures as sliminess, translucency and fat decomposition will be observed.

Decision had been made to reduce the pH value of the treated shrimp before it was mixed with green curry. The raw shrimp was rinsed using running tap water before the pre-treatment with the 5% MTGase and 2% NaCl for 30 minutes at 4±2°C. For the second step, after the pre-treated shrimp was drained, it was rinsed with water again for 30 seconds and drained before immersion in 0.5% citric acid for 5 minutes at 4±2°C. After that, the treated shrimp was drained and mixed with green curry. The pH of the green curry was 5.10±0.03.

Finally, the average pH value of 120g marinated shrimp with green curry was 7.68. This product was categorized as a low acid food, which is suitable to maintain a good quality of shrimp texture. Citric acid was certified as GRAS (Generally Recognized as Safe) status according to US FDA (Food and Drug Administration) and maximum daily dosage limit is 8 grams (FDA, 2012b).

Low acid food products were important for product like marinated shrimp in green curry to improve the quality of the shrimp by increasing the WHC, MC, WG and improve the texture. Xiong *et al.* (2002) reported that high acid food like marinated food in acidic solutions (citric acid, lemon juice) caused protein denaturation, resulting in decreased water binding ability of myosin, actin and other myofibrillar proteins. Prawns

marinated in sodium tri-polyphosphate solution (pH 7.0) did not show a significant change in muscle toughness. But immersion in calcium chloride ($CaCl_2$) at pH 7.0, citric acid (pH 3.0) and lemon juice (pH 3.0) increased ($p<0.05$) the muscle toughness of marinated prawn (Xiong *et al.,* 2002).

4.5 Optimum pasteurization parameter

For pasteurization in package, temperature profile analysis (Table 4.7) was done to get the actual time, F value and lethal rate for the marinated shrimp in green curry paste. The calculations for accumulated lethality using general method are shown in Appendix C. The temperature profile analysis was done using 5 conditions according to the recommendations from FDA (2011) for fish and seafood products and ECFF (2006) to obtain 6 log reductions of *Listeria monocytogenes*. The product was pasteurized at 5 conditions: (T1) 65°C for 9.3 minutes, (T2) 67°C for 5 minutes, (T3) 68°C for 3.7 minutes, (T4) 69°C for 2.7 minutes and (T5) 70°C for 2 minutes in two replicates using Completely randomized design (CRD) (Table 4.7).

The average of the actual time was significant different ($p\leq0.05$) between treatment (T1to T5) and there was no significant different between replicates ($p>0.05$). The average of the actual time from two replicates was as follow: T1) 65°C for 32.0 minutes (T2) 67°C for 18.0 minutes, (T3) 68°C for 16.5 minutes, (T4) 69°C for 6.5 minutes and (T5) 70°C for 6.5 minutes (Table 4.7).

The pasteurized marinated shrimp in green curry paste were reproduced at 5 conditions using the conditions in replicate two: (T1) 65°C for 32.5 minutes, (T2) 67°C for 18.5 minutes, (T3) 68°C for 17 minutes, (T4) 69°C for 7 minutes and (T5) 70°C for 7 minutes in comparison with the control (non-pasteurized) sample for two replicates using Completely randomized design (CRD) (Table 4.7). Initial product temperature was set at 25°C. The condition that yielded pasteurized product with the highest score for sensory evaluation and good characteristics from the other analysis was chosen for the shelf-life study.

The results on total viable count, *Listeria* spp., shear force, pH and colour of pasteurized marinated shrimp in green curry paste is shown in Table 4.8. The pH value of pasteurized marinated shrimp in green curry (T1 to T5) was significantly lower than the non-pasteurized marinated shrimp (control). Total viable count (TVC) of pasteurized marinated shrimp (T1 to T5) was <10 CFU/g and *Listeria* spp. was not detected in 25g sample while the TVC for non-pasteurized marinated shrimp was 3.67 log CFU/g and *Listeria* spp. 1.18 log CFU/g. The TVC in non-pasteurized sample was reduced 0.6 log CFU/g and the count of *Listeria* spp. was reduced about 1.58 log CFU/g compared with raw shrimp.

Ko, Mendonca and Ahn (2008) reported that addition of 0.5% citric acid in ham in vacuum-packaged resulted in synergistic effect against *L. monocytogenes* and *E.coli* 0157:H7. After 8 days storage at 4°C, the count of *L. monocytogenes* and *E.coli* 0157:H7 was <1 log CFU/g. Gonzalez-Fandos, Herrera, and Maya (2009) reported that chicken leg washed with 0.156 M (3%) citric acid for 5 minutes showed a significant ($p<0.05$) inhibitory effect on *L. monocytogenes* compared with control leg, being about 1.55 log CFU/g unit lower than control legs after 1 day of storage.

The shear force value for sample (T3, T4 and T5) was not significantly different from control ($p>0.05$) (Table 4.8). This finding was good for a better texture quality of shrimp products after the pasteurization process. Benjakul *et al*. (2008) reported that the shear force of black tiger shrimp and white shrimp meat increased markedly when the samples was heated for longer than 0.5 minutes ($p<0.05$).

The L*, a* and b* value of homogenized samples (non-pasteurized and pasteurized marinated shrimp in green curry paste) is shown in table 4.8. L* denote lightness on 0-100 scale from black to white, a* denote (+) red and (-) green; b* denote (+) yellow and (-) blue (Scrubring *et al.,* 2003). The L* value for pasteurized sample (treatment T1 to T3) was higher than non-pasteurized sample with a significant difference. The a* value for pasteurized sample for treatment T1 was increased, which means that it was more reddish. The b* value for T1 to T5 was not significant different

from non-pasteurized sample. Benjakul *et al*. (2008) reported that the L* a* b* value of white shrimp was increased when heating time is more than 0.5 to 1 minutes ($p<0.05$).

Sensory evaluation result in Table 4.9 showed that the highest score for overall acceptability was 5.75 (T5). The sensory evaluation was done by 10 trained panelists using nine category scales 0-8 (0: None, 2: Slightly, 4: Moderate, 6: Very, 8: Extremely like). The firmness score was not significantly different from control and the highest score was 5.15 (T5), this was related to the lowest score for the tenderness of the texture (3.68). The decrease of shrimp tenderness was attributed to decrease in protein solubility during marinating and pasteurization.

The juiciness score was not significantly different between samples and the highest score was 5.40 (T5) and the toughness score was also increased to 4.38. Overall, pasteurization condition in treatment T5 (70°C for 7 minutes) was selected as the optimum pasteurization parameter for marinated shrimp in green curry paste for the shelf-life study.

Table 4.7 Thermal processing time needed to obtain 6 log reduction of *Listeria monocytogenes* (Z = 7.5) based on recommended condition by FDA (2011) and accumulated lethality from temperature profile analysis

Condition	Internal product temperature (°C)	Time required for 6D Process (minute)	Actual Time (minute) Replicate 1	Actual Time (minute) Replicate 2	Average Actual Time (minute)	Accumulated lethality at 70°C (minute) Replicate 1	Accumulated lethality at 70°C (minute) Replicate 2
T1	65	9.3	31.5	32.5	32.0[a]±0.7	2.064	2.016
T2	67	5.0	17.5	18.5	18.0[b]±0.7	2.109	2.013
T3	68	3.7	16.0	17.0	16.5[c]±0.7	2.041	2.106
T4	69	2.7	6.0	7.0	6.5[d]±0.7	2.003	2.020
T5	70	2.0	6.0	7.0	6.5[d]±0.7	2.067	2.174

Initial product temperature 25±2°C. Means with different superscripted letters in the same column are significantly different ($p \leq 0.05$).

Table 4.8 Total viable count, *Listeria* spp., shear force, pH and colour of non-pasteurized and pasteurized marinated shrimp in green curry paste

Sample	TVC (log cfu/g)	*Listeria* spp. (log cfu/g)	Shear Force (Newton)	pH	L*	a*	b*
Control	3.67±0.10	1.18±0.0	17.94^{a}±0.45	7.68^{a}±0.02	44.80^{b}±2.23	1.61^{b}±1.05	15.10^{a}±2.31
T1	<10 cfu/g	ND	15.27^{c}±0.57	7.42^{e}±0.56	48.73^{a}±0.44	2.48^{a}±0.47	16.86^{a}±2.15
T2	<10 cfu/g	ND	16.74^{b}±0.08	7.46^{d}±0.42	48.12^{a}±1.39	2.08^{ab}±0.88	17.14^{a}±2.96
T3	<10 cfu/g	ND	17.77^{a}±0.31	7.49^{c}±0.42	48.98^{a}±0.44	2.12^{ab}±0.90	16.78^{a}±3.17
T4	<10 cfu/g	ND	18.14^{a}±0.23	7.52^{b}±0.42	47.40^{ab}±2.19	1.77^{b}±0.81	16.39^{a}±4.57
T5	<10 cfu/g	ND	18.33^{a}±0.44	7.54^{b}±0.03	47.83^{ab}±1.13	1.70^{b}±0.48	15.75^{a}±3.67
Raw shrimp	4.27±0.01	NT	NT	6.44±0.04	46.93±2.31	-0.68±0.29	-4.90±1.08
Green curry	NT	NT	NT	5.10±0.03	34.47±0.17	-0.40±0.04	14.51±0.10

Mean value ± standard deviation. Initial product temperature was set at 25°C. ND= Not detected in 25g sample.

Control= Non-pasteurized marinated shrimp in green curry. T1-T5= Pasteurized marinated shrimp in green curry paste; T1= 65°C for 32.5 minutes, T2 = 67°C for 18.5 minutes, T3 = 68°C for 17 minutes, T4 = 69°C for 7 minutes and T5 =70°C for 7 minutes. Homogenized samples were used for microbiology, pH and colour analysis. Means with different superscripted letters in the same column are significantly different ($p \leq 0.05$).

Table 4.9 Sensory evaluation of non-pasteurized and pasteurized marinated shrimp in green curry paste using Quantitative descriptive analysis

Sample	Colour of green curry ns	Colour of shrimp ns	Odour ns	Sweetness ns	Saltiness	Off Flavour	Texture: Juiciness	Texture: Toughness ns	Texture: Tenderness	Texture: Firmness	Overall acceptability
Control	4.38 ±0.92	4.89 ±1.13	4.20 ±1.11	3.24±1.09	4.17^{a}±0.93	0.0	5.03^{ab}±0.73	4.11±1.10	3.86^{ab}±1.23	4.85^{ab}±0.70	5.09^{b}±1.23
T1	4.77±1.10	5.09±1.06	4.08±0.89	3.41±0.83	3.89^{ab}±0.84	0.0	4.94^{b}±1.00	4.21±1.18	4.22^{a}±1.21	4.57^{b}±0.66	4.71^{b}±1.11
T2	4.60±1.03	5.09±1.10	4.48±0.74	3.20±0.80	3.96^{ab}±0.75	0.0	4.87^{b}±0.72	3.97±0.93	4.07^{ab}±0.97	4.53^{b}±0.90	4.81^{b}±0.97
T3	4.43±0.67	4.99±1.18	4.31±0.85	3.35±1.04	3.76^{b}±1.02	0.0	4.95^{b}±0.71	3.92±0.91	4.09^{ab}±1.02	4.66^{ab}±0.67	4.84^{b}±1.10
T4	4.31±0.71	5.00±0.72	4.13±1.13	3.24±1.02	3.95^{ab}±0.72	0.0	5.10^{ab}±0.83	4.23±0.75	4.07^{ab}±0.87	4.80^{ab}±0.64	4.94^{b}±1.36
T5	4.65±0.78	5.27±1.00	4.49±1.12	3.31±0.83	3.75^{b}±0.74	0.0	5.40^{a}±1.24	4.37±0.77	3.68^{b}±0.91	5.15^{a}±1.17	5.75^{a}±1.14

Mean ± standard deviations were evaluated by 10 trained panelists using nine category scales 0-8 (0: None, 2: Slightly, 4: Moderate, 6: Very, 8: Extremely like). Control = Non-pasteurized, T1= 65°C for 32.5 minutes, T2 = 67°C for 18.5 minutes, T3 = 68°C for 17 minutes, T4 = 69°C for 7 minutes and T5 =70°C for 7 minutes. ns= not significantly different ($p>0.05$). Means with different superscripted letters in the same column are significantly different ($p\leq0.05$).

4.6 Shelf-life study

4.6.1 Chemical analysis

Chemical composition of frozen white shrimp (raw) and pasteurized marinated shrimp in green curry paste is shown in Table 4.10. Frozen white shrimp had high moisture content of 81.59% while pasteurized marinated shrimp in green curry paste contained 70.77% moisture. The moisture content decreased for 13% in pasteurized marinated shrimp in green curry paste. Mohan *et al*. (2006) reported that the moisture content of Indian white shrimp in pouch after thermally processed at 100°C was 71% while raw shrimp contained 80.98% moisture.

The fat content of frozen white shrimp was 0.46% and the fat content of pasteurized marinated shrimp in green curry paste was 3.57%. There was a significant difference between the ash of raw shrimp (1.0%) and pasteurized marinated shrimp in green curry paste (1.3%). The amount of ash showed the richness of food in term of element composition, the ash in raw shrimp usually was in the range of 1.0-1.6%.

Raw shrimp contained protein as the major constituent (15.5%), and pasteurized marinated shrimp (10.9%), indicating that shrimp can be a good source of amino acids (Table 4.10). Protein in raw shrimp meat was normally in range of 15-20% wet weight. The water activity (a_w) of pasteurized (70°C for 7 minutes) marinated shrimp in green curry paste was 0.95±0.01.

Sriket *et al.* (2007) reported that the proximate composition of raw white shrimp cultured in Songkla, Thailand was 77.21% moisture, 18.8% protein, 1.30% fat and 1.47% ash in wet weight basis. While Boonsumrej *et al.* (2007) reported that the proximate composition of raw tiger shrimp (*Penaeus monodon*) was 79.75% moisture, 17.70% protein, 0.86% fat and 0.99% ash.

Proximate composition in shrimp muscles is governed by many factors, including species, growth stage, feeding period, spawning and the season.

Karakoltsidis, Zotos and Constantinide (1995) reported that the chemical composition of shrimp (*Aristeus antennatus*) was seasonal dependent (spring, fall and winter). Cadun, Kisla and Cakli (2008) reported that the moisture content of raw deep-water pink shrimp was decreased after marinating with rosemary extract from 84.7% to 82.3%. Fat content of raw shrimp was 0.9% and after marinating, fat content increased to 1.4%.

TVB-N and TBA values were determined to investigate the chemical quality changes in raw and marinated shrimp (Table 4.10). The TVB-N value of raw white shrimp was 4.37mgN/100g sample and TBA value was 0.49 mg malonaldehyde/kg sample. Nirmal and Benjakul (2010) reported that the initial TVB-N value of Pacific white shrimp from Thailand with size of 55-60/kg was 4.11mgN/100g sample and TBA value was 0.73 mg malonaldehyde/kg sample. Siripongvutikorn *et al*. (2008) wrote that TVB-N value of raw white shrimp from Thailand was 5.4-5.6 mgN/100g sample. Cadun *et al*. (2008) found that the TBA value of deep-water pink shrimp was 0.26 mg malonaldehyde/kg sample and TVB-N value was 13.3 mg N/100g sample.

Table 4.10 Chemical composition of frozen white shrimp and pasteurized marinated shrimp in green curry paste

Sample	Moisture (% wb)	Protein (% wb)	Fat (%wb)	Ash (%wb)	TBA (mg.malo./kg)	TVB-N (mgN/100g)
Raw shrimp	81.6^{a}±0.3	15.5^{a}±0.5	0.46^{b}± 0.01	1.00^{b}±0.00	0.49^{b}± 0.03	4.37±0.06
Trt	70.8^{b}±0.2	10.9^{b}±0.1	3.57^{a} ±0.03	1.31^{a}±0.02	2.26^{a} ± 0.06	NT

n=3; mean value ± standard deviation, malo; malonaldehyde, wb; wet weight. NT; Not Tested. Trt: Pasteurized marinated shrimp in green curry paste at 70°C for 7 minutes. Homogenized samples were used for proximate, TBA and TVB-N analysis. Means with different superscripted letters in the same column are significantly different ($p \leq 0.05$).

Chemical quality analysis (TVB-N, TBA) is used for quality assessment of seafood products. Fish decomposition is a progressive proteolysis of muscle tissue brought about primarily by the action of microorganisms, and to a lesser extent by autolytic enzymes. TVB-N is well documented as an index of the quality of fresh or frozen fish because its increase is related to spoilage by bacteria and the activity of endogenous enzymes. Lipid oxidation is a major quality problem. It leads to the development of off-odours and off-flavours, called oxidative rancidity, in edible oils and fat containing foods (Ashie, Smith and Simpson, 1996).

A level of 30 mgN/100g sample for TVB-N is considered to be the upper limit (normally considered as the spoilage level), above which the fishery products are thought to be unfit for human consumption. The TVB-N value of $\leq$20 mg N/100g sample was considered fresh, $\leq$30 mgN/100g sample was acceptable and >40 mgN/100g sample was not suitable for consumption. According to the Thai standard for frozen shrimps and prawns the acceptable limit for TVB-N value was 30 mgN/100 g sample (TIS, 1986).

TBA (mg malonaldehyde/kg sample) analysis is an important quality index indicating secondary lipid oxidation. The TBA analysis is usually done for frozen, chilled or stored seafood product. It was suggested that a maximum level of TBA value indicating the good quality of frozen fish, chilled or stored with ice is 5 mg malonaldehyde/kg sample, while the consumption limit was 7-8 mg malonaldehyde/kg sample TBA value (Schormüller, 1969).

Changes in pH values of pasteurized and non-pasteurized marinated shrimp in green curry paste during storage at 0-3°C for 15 days are shown in Table 4.12. The pH analysis was done using homogenized samples. The pH value of raw shrimp was 6.90. The pH value of non-pasteurized and pasteurized samples was decreased during storage from 7.70 and 7.42 (day 0) to 7.47 and 7.36 for stored samples of 15 days respectively ($p<0.05$). The pH value of non-pasteurized samples was significantly higher than pasteurized samples. The pH value of both pasteurized and non-pasteurized

samples were decreased starting from day 3 until day 6 and continued to increase from 9 to 15 days ($p \leq 0.05$) (Table 4.12).

The decrease in pH of marinated shrimp during the first 6 days may be because the acid in green curry paste with pH value 5.3±0.03 diffused into the shrimp. Vivar-Quintana *et al*. (1999) reported that the acid concentration increased as the storage proceeded attributed to the buffering capacity of the curry ingredients due to the presents of Vitamin C and phenolic compounds.

The pH increased from 9 to 15 days maybe due to the growth of microorganism in marinated products. Table 4.11 shows that the pH value of non-pasteurized and pasteurized marinated shrimp in green curry paste correlated negatively at -0.56 and -0.72, respectively with TVC from 0 to 15 days ($p \leq 0.05$). Ammonia production by bacterial deamination of amino acids has been assumed to be a reason for the increase of pH observed in the spoiled sample (ICMSF, 1986). Okuma and Abe (1992) reported that the pH increase is attributed to buffering capacity of some compounds produced in the shrimp, for example, histidine, amine, phosphate and nucleotide.

During storage, the pH value was increased from 9 to 15 days but pH value is not the criterion for spoilage. It has to be supported by other microbiological, chemical and sensory analyses. The findings were similar with the study by Siripongvutikorn *et al.* (2008)., The pH value of marinated shrimp tended to decreased for the first 6 days, increased from 9 to 10 days and decreased again from 10 to 12 days, finally increased from 12 to 15 days of storage.

Table 4.11 Pearson correlation for pH value and TVC count of non-pasteurized marinated shrimp in green curry paste stored at 0-3°C for 15 days

Sample stored for 15 days		pH
Non-pasteurized		
TVC	Pearson Correlation	-0.56(*)
Pasteurized		pH
TVC	Pearson Correlation	-0.72(**)

*Correlation at 0.05 ($p \leq 0.05$)

TBA value (mg malonaldehyde/kg sample) indicates the lipid oxidation during storage (Table 4.12). At the beginning of storage, the TBA value of non-pasteurized and pasteurized marinated shrimp were 1.61 and 2.16 mg malonaldehyde/kg sample, respectively. Whereas, at the end of storage, TBA value of non-pasteurized and pasteurized samples reached 3.61 and 3.11 mg malonaldehyde/kg sample, respectively ($p \leq 0.05$). In good quality material, TBA values should not be more than 5 mg malonaldehyde/kg sample at the point of sale. The consumption limit is 7–8 mg malonaldehyde/kg sample (Schormüller, 1969).

The TBA value of non-pasteurized marinated shrimp was significantly increased during storage for 15 days. At the end of storage, the TBA value was higher than pasteurized marinated shrimp ($p \leq 0.05$). The increase in TBA value of pasteurized sample was more slowly and the TBA value of pasteurized sample on 12 and 15 days was not significant different ($p > 0.05$) (Table 4.12).

Kingchaiyaphum and Rachtanapun (2012) reported that kaffir lime peel essential oil 5 to 20% could significantly retard the lipid oxidation in Chinese Sausages. Cadun *et al.* (2008) reported that at the beginning of the storage, TBA values of the control (not marinated) and marinated deep-water pink shrimp were 0.9 and 0.4 mg malonaldehyde/kg sample, respectively, whereas at the end of storage, they reached 6.6 and 2.4 mg malonaldehyde/kg sample, respectively.

Table 4.12 The chemical changes of non-pasteurized and pasteurized marinated shrimp in green curry paste during storage at 0-3°C

	Storage period (days)					
pH	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
Raw	6.90±0.10					
Non-pasteurized	7.70^{a}±0.01	7.45^{bc}±0.01	7.40^{d}±0.02	7.42^{cd} ±0.01	7.44^{bc} ±0.01	7.47^{b} ±0.01
Pasteurized	7.42^{cd}±0.04	7.30^{f}±0.01	7.25^{g}±0.01	7.32^{ef} ±0.01	7.34^{e} ±0.01	7.36^{e} ±0.01
TBA mg malonaldehyde/ kg sample						
Non-pasteurized	1.61^{h}±0.04	2.22^{g}±0.04	2.57^{e}±0.11	2.79^{d}±0.01	3.20^{b}±0.05	3.61^{a}±0.01
Pasteurized	2.16^{g}±0.23	2.40^{f}±0.10	2.65^{de}±0.04	2.76^{d}±0.01	3.01^{c}±0.02	3.11^{bc}±0.01

Mean value ± standard deviation. Pasteurized: Pasteurized marinated shrimp in green curry paste at 70°C for 7 minutes. Non-pasteurized: marinated shrimp in green curry paste. Homogenized samples were used for pH and TBA analysis. Means with different superscripted letters are significantly different ($p \leq 0.05$).

4.6.2 Physical analysis

Prior to consumption, shrimp stored for a certain period in frozen or chilled conditions normally needs cooking. Thermal process causes the denaturation of muscle protein and degree of denaturation varies with shrimp species. The shrinkage of muscle fibers after heating might be associated to tougher texture. The texture and taste of the product become rapidly undesirable with an excessive firmness and lack of juiciness when the heating temperature exceeds 70°C (Mizuta *et al.,* 1999). The texture analysis was carried out to measure the shear force value of non-pasteurized and pasteurized marinated shrimp in green curry paste during storage for 15 days at 0-3°C.

Shear force of treated white shrimp (5% MTGase with 2% NaCl for 30 minutes and 0.5% citirc acid, 5 minutes) marinated in green curry paste was reported in Table 4.13. At the beginning of storage, the shear force for non-pasteurized and pasteurized marinated shrimp in green curry (70°C, 7 minutes) was 18.34 and 19.93 Newton, respectively. The shear force of pasteurized sample was higher than the non-pasteurized sample ($p \leq 0.05$). The shear force increased after the sample was heated for 7 minutes at 70°C and the difference was 1.59 Newton on day 0 compare with non-pasteurized sample. There was no significant different in shear force value of pasteurized sample from the beginning of storage to 15 days ($p > 0.05$).

Shear force of non-pasteurized marinated shrimp was significantly decreased on day 9 of storage when compared with day 0 and the value was not significantly different from 9 to 15 days (Table 4.13). At the end of storage 15 days, the shear force for non-pasteurized sample was not significant different from the value on the beginning of storage (day 0).

Benjakul *et al.* (2008) reported that the shear force of white shrimp increased when the samples were heated for more than 0.5 minute, there was no significant different in shear force observed in all part of shrimp when sample was heated to 2-3 minutes ($p > 0.05$).

The shear force of raw white shrimp from Thailand ranged from 15-20 Newtons and shear force of steamed white shrimp for 0.5-3.0 minutes was from 17-28 Newtons at the front and middle part of the shrimp (Benjakul *et al*., 2008).

Viscosity is the measure of the resistance to flow or internal friction of the fluid. Viscosity changes with the temperature at which the measurement was made, therefore the temperature of the product was set at control room temperature of 25±2°C for the whole viscosity analysis. Table 4.13 shows the viscosity of the green curry gravy from pasteurized and non-pasteurized marinated shrimp in green curry from the beginning of storage until the end of storage period for 15 days.

Pasteurization caused the viscosity of the green curry gravy to decrease on day 0 (0.44 Pa.s), while the viscosity of non-pasteurized green curry was 0.74 Pa.s. The viscosity of green curry gravy in pasteurized sample continued to decreased until day 6 and increased on day 9 to 15 ($p \leq 0.05$). For non-pasteurized sample the viscosity continued to increase from the beginning of storage until the end of storage for 15 days ($p \leq 0.05$).

Drained weight of the shrimp was evaluated during storage for 15 days (Table 4.13). The initial shrimp weight was 80g from total net content of 120g marinated shrimp in green curry paste; the initial drain weight was 66.6%. The drained weight of shrimp in non-pasteurized marinated shrimp increased 3.66% from the beginning of storage until the end of storage from 66.6% up to 70.26% ($p \leq 0.05$).

The drained weight of shrimp in pasteurized marinated shrimp significantly decreased for 4.1% after pasteurization (62.50%) on the beginning of storage compare with non-pasteurized sample (66.6%) in Table 4.13. The drained weight of pasteurized marinated shrimp continued to decreased until day 6 (59.97%), but the value significantly increased on day 9 (60.82%) until the end of storage (63.61%).

Table 4.13 The physical changes of pasteurized and non-pasteurized marinated shrimp in green curry paste during storage at 0-3°C

	Storage period (days)					
	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
Shear Force (Newton)						
Non-pasteurized	18.34^{abcd}±0.38	17.76^{bcde}±0.01	17.19^{de}±0.01	16.13^{e}±1.34	17.24^{de}±0.23	17.42^{cde}±0.81
Pasteurized	19.93^{a}±1.24	19.57^{ab}±0.94	19.30^{abc}±1.02	18.80^{abcd} ±1.42	18.64^{abcd} ±1.47	18.31^{abcd}±1.26
Viscosity (Pa.s)						
Non-pasteurized	0.74^{e} ±0.07	0.80^{d}±0.01	0.84^{c}±0.01	0.86^{b}±0.02	1.02^{a}±0.01	1.04^{a}±0.01
Pasteurized	0.44^{g} ±0.01	0.37^{i}±0.01	0.29^{j} ± 0.01	0.40^{h}±0.01	0.44^{g}± 0.01	0.48^{f} ±0.01
Drain weight shrimp (%)						
Non-pasteurized	66.60^{f}±0.03	67.65^{e}±0.23	69.20^{d}±0.01	69.48^{c} ±0.01	69.95^{b} ±0.01	70.26^{a}±0.02
Pasteurized	62.50^{h}±0.11	60.86^{i}±0.23	59.97^{j}±0.19	60.82^{i} ±0.35	62.56^{h} ±0.02	63.61^{g} ±0.01

Mean value ± standard deviation. Means with different superscripted letters are significantly different ($p \leq 0.05$). Pa.s: pascal-second. Pasteurized: Pasteurized marinated shrimp in green curry paste at 70°C for 7 minutes. Non-pasteurized: marinated shrimp in green curry paste. Initial shrimp weight = 80 g; Total content 120 g marinated shrimp in green curry paste.

The colour of marinated shrimp in green curry paste during 15 days of storage is shown in Table 4.14. Significant difference between the L*, a* and b* value of non-pasteurized and pasteurized samples was notice on the beginning of storage until the end of storage for 15 days.

L* value of the non-pasteurized marinated shrimp in green curry paste was 39.87 at the beginning and 46.73 at the end of storage for 15 days, respectively ($p \leq 0.05$). The lightness L* value increased from the beginning of storage until day 3, decreased on day 9, increased on day 12 until the end of storage ($p \leq 0.05$). The a* value of the same group was 0.65 at the beginning and 0.78 at the end of storage. The a* value significantly increased on day 3 of storage and decreased on day 9 until the end of storage. When a* value increased means that the sample was more reddish. There was a significant difference between the b* value from the beginning of storage 12.98 and at the end of storage 18.37. The b* value increased on the first 6 days, but decreased on day 9, increased on day 12 until the end of storage ($p \leq 0.05$). It increased up to 29% at the end of storage. The b* value increased means that the sample was more yellowish.

The L* value of pasteurized marinated shrimp at the beginning of storage was 45.55 which was higher than the L* of non-pasteurized sample which was 39.88 ($p \leq 0.05$). The L* value decreased on day 6 and increased on day 9 to 15 (47.18). The a* and b* of pasteurized samples were higher than non-pasteurized samples (43.9% and 24.5%) respectively, at the beginning of storage period ($p < 0.05$). When a* value increased means that the sample was more reddish and b* value increased means that the pasteurized samples was more yellowish.

The a* value of the pasteurized sample were 1.16 at the beginning and 1.61 at the end of storage period ($p \leq 0.05$). This value was significantly increased on the first three days, decreased on day 6 to 9 and increased on day 12 until the end of the storage. There was a significant difference between the b* value from the beginning 17.19 and at the end of storage period 17.94. The b* value increased on the first three days and decreased on day 6 to 12 and increased at the end of storage day 15 ($p \leq 0.05$).

According to the sensory evaluation result, the L* a* b* changes of non-pasteurized and pasteurized samples during storage for 15 days at 0-3°C (Table 4.14) did not affect the colour of the products after cooked. Result from sensory evaluation for Difference from control test (Table 4.17) show that there was no difference in colour of the shrimp and colour of the green curry between the cooked marinated shrimp in green curry that had been stored with freshly prepared shrimp in green curry. The average of sensory score for colour of shrimp and colour of green curry was below 1.5. According to the sensory evaluation form (Appendix A.2), the score below 2 means that the samples was not different from freshly prepared products (scale 2: slightly difference). This means that, 10 trained panelists did not detect any colour changes in cooked products of non-pasteurized and pasteurized marinated shrimp in green curry.

Table 4.14 Changes in colour of pasteurized and non-pasteurized marinated shrimp in green curry paste during storage at 0-3°C

Sample	Day	L*	a*	b*
Non-pasteurized	0	39.87^{g} ±0.03	0.65^{f}±0.10	12.98^{g}±0.04
	3	46.24^{bcd}±0.66	1.48^{bc}±0.32	19.01^{ab}±0.11
	6	44.89^{def} ±0.20	1.89^{a}±0.26	19.71^{a}±0.05
	9	43.69^{f} ±0.61	1.24^{cd}±0.04	16.74^{def}±0.46
	12	47.90^{a} ±0.01	1.04^{de}±0.01	19.25^{ab}±0.01
	15	46.73^{abc}±0.99	0.78^{ef}±0.25	18.37^{abc}±1.44
Pasteurized	0	45.55^{cde}±0.63	1.16^{cd}±0.04	17.19^{cde}±0.16
	3	45.75^{bcd} ±0.97	1.49^{bc}±0.18	18.32^{abc}±0.42
	6	44.22^{ef}±1.02	1.11^{de}±0.07	16.01^{ef}±1.36
	9	45.99^{bcd}±0.01	1.14^{cd}±0.02	16.16^{ef}±0.01
	12	45.78^{bcd}±0.09	1.49^{bc}±0.01	15.62^{f}±0.01
	15	47.18^{ab}±0.06	1.61^{ab}±0.01	17.94^{bcd}±0.01

Mean value ± standard deviation. Non-pasteurized: marinated shrimp in green curry paste. Pasteurized: Pasteurized marinated shrimp in green curry paste at 70°C for 7 minutes. Means with different superscripted letters in the same column are significantly different ($p \leq 0.05$).

4.6.3 Microbiology analysis

The storage temperature for pasteurized marinated shrimp in green curry paste was 0-3°C for 15 days. Snyder (2003) wrote that natural contaminating spores of *Bacillus cereus* survive pasteurization in not-reduce oxygen package. However the outgrowth of these pathogenic spores in chilled food products during storage is prevented by maintaining the appropriate refrigeration temperatures below 3.3°C. If pasteurized-chilled foods are kept at a temperature lower than 3.3°C, there is absolutely no hazard and it can be held until spoiled (Snyder, 2003). According to Table 4.15 the most concern pathogenic bacteria that can grow at very low temperature is *Listeria monocytogenes*. For product packaged with oxygen, *L. monocytogenes* is usually target pathogen to be controlled (FDA, 2011). This pathogenic bacterium is most heat-resistant vegetative pathogen in chilled food. The D value for this infectious pathogen at 70°C was 0.3 minutes.

Table 4.15 Minimum growth temperatures of some pathogen in foods

Pathogen	Product temperature	Maximum cumulative exposure time
Growth and toxin formation *Bacillus cereus*	4-6°C	5 days
Growth of *Escherichia coli*	6.6-10°C	2 days
Growth of *Listeria monocytogenes*	-0.4-5°C	7 days
Growth of *Salmonella* species		
Growth of *Yersinia enterocolitica*	5.2-10°C	2 days
Growth and toxin formation	-1.3-10°C	1 days
Staphylococcus aureus	7-10°C	14 days

Source: FDA (2011)

The microbiology analysis was carried out to measured the safety limit of the marinated shrimp in green curry paste for the consumption and determine the shelf-life of the product according to the safety level of microbiological quality of seafood products recommended by ICMSF (1986) and Food administration manual (1995) (Table 3.4) and the microbiological limit of ready-to-cook shrimp reported by Stannard (1997) and Gilbert, Roberts and Bolton (2000) (Table 3.3).

The microbial load of thawed frozen raw white shrimp and the microbial changes of pasteurized and non-pasteurized marinade shrimp in green curry paste during storage are shown in Table 4.15. The total viable count and *Listeria* spp. of frozen-thawed white shrimp were 4.52 and 2.37log CFU/g, respectively. The microbial load of non-pasteurized marinated shrimp in green curry paste was decreased ($p \leq 0.05$) after the marinating. *Escherichia coli* were not detected in 25g sample. The total viable count (TVC) of non-pasteurized marinated shrimp was 3.57 log CFU/g at the beginning of storage and 6.47 log CFU/g at the end of storage for 15 days (Table 4.16). According to Stannard (1997) and Gilbert *et al*. (2000) (Table 3.3) the microbiological limit for good quality ready-to-cook sample was $<10^5$, this makes the non-pasteurized sample not acceptable as a good quality products and not safe for consumption on day 9 because it contained 5.42 log CFU/g of TVC (Table 4.16).

The TVC value of the non-pasteurized sample on day 6 was 6.01log CFU/g which exceeded the microbiology limit for unsatisfactory sample ($> 10^6$) (Stannard, 1997 and Gilbert *et al*. (2000); Table 3.3).

This finding was consistent with other studies. After white shrimp was marinated the microbial load was decreased (Cadun *et al*., 2008). This microorganism was not completely killed by marinating and it was still viable in the semi-sterile medium, they are able to continue their activity more or less rapidly according to their ability to adapt to the medium during storage time (Fuselli *et al*., 1994).

Ifesan *et al*. (2010) stated that Thai green curry demonstrated antibacterial activity against seafood spoilage organism and foodborne pathogens. The microbial load of non-pasteurized marinated shrimp in green curry paste reduced for about 0.95

log CFU/g on day 0 (Table 4.16). This may be due to the antimicrobial property of green curry paste. Marinating shrimp with green curry paste prolongs the shelf-life of the product particularly when the green curry contains higher kaffir lime peel and garlic. However, higher garlic in the recipe may alter the product acceptability (Siriponngvutikorn *et al*., 2008).

Kaffir lime (*Citrus hystrix* DC) peels contain antimicrobial compounds. The ethyl acetate extract from kaffir lime peel showed a broad spectrum of inhibition compare with essential oil (EO) against all gram negative bacteria namely *Escherichia coli, Salmonella* spp., yeast and mould, gram positive bacteria namely *Bacillus cereus, Staphylococcus aureus*, *Listeria monocytogenes* and *Saccharomyces cerevisiae var. sake* (Chanthapon, Chanthachum and Hongpattarakere, 2008). Kaffir lime peel EO reduces the total viable count and significantly retards the lipid oxidation in Chinese sausages (Kingchaiyaphum and Rachtanapun, 2012).The major antimicrobial compound in garlic is alicin, garlic extracts have been found to possess antibacterial property against *Salmonella* Typhimurium*, Escherichia coli, Bacillus cereus* and *Staphylococcus aureus* (Nanasombat and Lohasupthawee, 2005).

After pasteurization, the safety level of marinated shrimp in green curry paste was increased, where the TVC was <10 CFU/g at the beginning of storage and 1.79 log CFU/g at the end of storage (Table 4.15). According to the microbiological limit of ready-to-cook shrimp reported by Stannard (1997) and Gilbert *et al*. (2000) (Table 3.3) the pasteurized marinated shrimp was categorized in a good quality products with satisfactory microbiology quality $<10^5$, the TVC did not exceed 10^5 until the end of 15 days (Table 4.16). The other pathogenic microorganism *Listeria* spp.*, E. coli,* yeast and mould was not detected in 25g sample.

Listeria spp. count in non-pasteurized sample at the beginning of storage was 1.09 log CFU/g and was reduced about 1.28 log CFU/g compare with frozen-thawed white shrimp at the beginning of storage (Table 4.16). This might be due to the antimicrobial property of green curry paste. The count was increased on day 3 (1.53 log CFU/g), but decreased on day 12 (1.09 log CFU/g) and at the end of storage *Listeria*

spp. was not detected in 25g sample. *E.coli* was not detected in 25g sample in both non-pasteurized and pasteurized samples.

The coliform count in non-pasteurized sample was 0.85 log CFU/g at the beginning of the storage and 0.70 log CFU/g on day 6 and it was not detected on the rest 9 days of storage (Table 4.16).

For mould and yeast analysis for non-pasteurized sample, mould was not detected in 25g sample but yeast was detected at the beginning of storage (1.46 log CFU/g) and the count significantly increased until the end of storage on day 15 (2.76 log CFU/g). Morgan (1999) reported that yeasts grow best in a neutral or slightly acidic pH environment; yeasts vary in temperature range that it does grow best. For example, *Aspergillus niger* grow at 35-40°C and *Penicillium expansum* grow at 25 to 30 °C.

Adams and Moss (2006) reported that mould requires oxygen to grow. The pH, optimum temperature, and water activity range for growth is 3.5 to 4.0, 25 to 30°C, and >0.62, respectively. The pH value for non-pasteurized marinated shrimp on day 1 was 7.7. Mould cannot grow in the chilled temperature of 0-3°C. Few moulds can begin growing at 4 °C, the temperature within a typical refrigerator or less (Morgan, 1999).

The result is consistent with Ifesan *et al*. (2010) who found that pasteurization of green curry ingredients at 90°C for 2 minutes and adding dry ingredients after the pasteurization, stored at 4°C, reduced the total viable count (TVC) for 0.78 log cfu/g on the first day. This made the antibacterial activity of the green curry more stable. There was no lactic acid bacteria growth found throughout the period of 30 days.

Table 4.16 The microbiological changes of pasteurized and non-pasteurized marinated shrimp in green curry paste during storage at 0-3°C

	Storage period (days)						
	Raw Shrimp	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
Total viable count (log CFU/g)	4.52±0.06 (Acceptable)	Satisfactory	Satisfactory	Acceptable	Acceptable	Unsatisfactory	Unsatisfactory
Non-pasteurized		3.57^{d} ±0.11	3.94^{d} ±0.21	5.05^{c}±0.25	5.42^{c}±0.64	6.01^{b}±0.06	6.47^{a}±0.38
		Satisfactory	Satisfactory	Satisfactory	Satisfactory	Satisfactory	Satisfactory
Pasteurized		<10 cfu/g	1.82^{e}±0.11	2.00^{e}±0.03	2.08^{e}±0.03	1.97^{e}±0.18	1.79^{e}±0.13
***Listeria* spp. count (log CFU/g)**	2.37±0.04 (Acceptable)	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	
Non-pasteurized		1.09^{b}±0.13	1.53^{a}±0.17	1.35^{ab}±0.07	1.30^{ab}±0.00	1.09^{b}±0.13	ND
Pasteurized		ND	ND	ND	ND	ND	ND
Coliform count (log CFU/g)		Acceptable	Acceptable	Acceptable			
Non-pasteurized		0.85^{a}±0.21	0.94^{a}±0.34	0.70^{a}±0.00	ND	ND	ND
Pasteurized		ND	ND	ND	ND	ND	ND
***E.coli* (log CFU/g)**							
Pasteurized and Non-pasteurized		ND	ND	ND	ND	ND	ND
Yeast count (log CFU/g)		Satisfactory	Satisfactory	Satisfactory	Satisfactory	Satisfactory	Satisfactory
Non-pasteurized		1.46^{e} ±0.11	1.97^{d} ±0.27	2.17^{cd}±0.31	2.43^{bc}±0.14	2.60^{ab}±0.09	2.76^{a} ±0.01
Pasteurized		ND	ND	ND	ND	ND	ND
Mould count (log CFU/g)							
Pasteurized and Non-pasteurized		ND	ND	ND	ND	ND	ND

Mean value ± standard deviation. Means with different superscripted letters are significantly different ($p \leq 0.05$). ND; Not detected in 25g sample.

4.6.4 Sensory evaluation

The sensory quality of the products was evaluated by 10 trained panelists with two replicates. The sensory characteristic for shelf-life study of processed marinated shrimp in green curry paste was assayed by Discriminative test for Difference from control test using nine category scales (0 to 8), where 0 means not different, 2: slightly different, 4: moderately different, 6: very different and 8: extremely different (Appendix A.2). The cooked products (non-pasteurized and pasteurized marinated shrimp in green curry paste stored at 0-3°C) with blind control (freshly prepared) were compared with the freshly prepared shrimp in green curry to know the acceptance of the product during storage for 15 days.

The results for sensory evaluations of non-pasteurized and pasteurized marinated shrimp in green curry paste during storage are shown in Tables 4.17. The sensory evaluation for non-pasteurized marinated shrimp was carried out until day 6 because the sample contained 5.05 log CFU/g TVC (Table 4.16) which exceeded the microbiological limit for satisfactory ready-to-cook shrimp at the point of sale.

The average of sensory score for 9 attributes tested for non-pasteurized sample compared with freshly prepared product was lower than 1. While the average of sensory score for pasteurized and blind samples was below 1.5 when the sample was compared with freshly prepared sample. According to the sensory evaluation form (Appendix A.2) the score below 2 means that the non-pasteurized and pasteurized samples was not different from freshly prepared products (scale 2: slightly different). The 10 trained panelists did not detect any difference for stored samples after cooked compared with freshly prepared shrimp in green curry. Further, there was no significant difference between non-pasteurized, pasteurized and blind samples ($p>0.05$) for 9 attributes tested in the sensory evaluation for shelf-life study (Table 4.17).

Table 4.17 Sensory evaluation using Difference from control test for marinated shrimp in green curry during storage at 0-3°C

Sample	Day	Colour of green curry	Colour of shrimp	Odour	Flavour	Juiciness	Toughness	Tenderness	Firmness	Overall difference
NP	0	0.45^{abc} ±0.44	0.72^{abcd} ±0.85	0.43^{ab} ±0.67	0.44^{ab} ±0.88	0.63^{abc} ±0.87	0.53^{bc} ±0.80	0.85^{a} ±1.08	0.58^{abc} ±0.74	0.84^{ab} ±0.98
	3	0.50^{abc} ±0.53	0.78^{abcd} ±1.05	0.80^{a} ±1.19	0.74^{ab} ±0.85	0.78^{ab} ±1.05	0.85^{ab} ±1.07	0.86^{a} ±0.80	0.76^{abc} ±0.84	0.83^{ab} ±0.87
	6	0.53^{abc} ±0.65	0.60^{bcd} ±0.93	0.54^{ab} ±0.81	0.59^{ab} ±0.80	0.63^{abc} ±0.87	0.61^{abc} ±0.67	0.52^{a} ±0.68	0.70^{abc} ±0.86	0.71^{ab} ±0.84
	9	Not Tested	Not Tested	Not Tested	Not Tested	Not Tested	Not Tested	Not Tested	Not Tested	Not Tested
	12	Not Tested	Not Tested	Not Tested	Not Tested	Not Tested	Not Tested	Not Tested	Not Tested	Not Tested
	15	Not Tested	Not Tested	Not Tested	Not Tested	Not Tested	Not Tested	Not Tested	Not Tested	Not Tested
P	0	0.46^{abc} ±0.49	0.80^{abcd} ±0.94	0.51^{ab} ±0.75	0.34^{b} ±0.73	0.93^{a} ±0.83	0.87^{ab} ±0.97	0.98^{a} ± 1.02	0.98^{ab} ± 1.02	1.11^{a} ± 1.21
	3	0.25^{bc} ±0.35	1.06^{abc} ±1.23	0.53^{ab} ±0.87	0.63^{ab} ±0.98	0.65^{abc} ±0.81	0.90^{ab} ±1.12	0.85^{a} ± 1.04	0.70^{abc} ± 0.85	0.90^{ab} ±1.11
	6	0.45^{abc} ±0.54	0.72^{abcd} ±0.62	0.44^{ab} ±0.72	0.48^{ab} ±0.49	0.96^{a} ±0.88	0.85^{ab} ±0.72	1.05^{a} ± 0.93	1.00^{ab} ±0.90	0.98^{ab} ± 0.65
	9	0.52^{abc} ±0.74	0.55^{bcd} ±0.81	0.66^{ab} ±0.93	0.91^{a} ±1.15	0.86^{ab} ±1.26	1.10^{a} ±1.24	0.91^{a} ± 1.24	1.11^{a} ±1.24	1.05^{ab} ±1.24
	12	0.46^{abc} ± 0.48	1.09^{ab} ±1.20	0.58^{ab} ±0.81	0.86^{ab} ±0.80	1.01^{a} ±1.02	0.98^{ab} ±1.03	0.86^{a} ± 1.10	0.93^{abc} ±1.05	1.01^{ab} ±1.24
	15	0.73^{a} ±0.72	0.63^{bcd} ±0.65	0.68^{ab} ±0.79	0.75^{ab} ±1.06	0.65^{abc} ±0.73	0.63^{abc} ±0.67	0.70^{a} ± 0.62	0.65^{abc} ± 0.65	0.83^{ab} ±0.74
B	0	0.56^{ab} ±0.63	1.34^{a} ±1.72	0.67^{ab} ±0.92	0.45^{ab} ± 0.82	0.85^{ab} ± 0.87	0.85^{ab} ±0.99	0.75^{a} ± 0.91	0.85^{abc} ±0.99	1.11^{a} ±1.01
	3	0.42^{abc} ±0.50	0.69^{bcd} ±0.71	0.23^{b} ±0.41	0.51^{ab} ± 0.68	0.36^{bc} ± 0.67	0.50^{bc} ±0.76	0.55^{a} ±0.77	0.70^{abc} ±0.90	0.66^{ab} ±0.80
	6	0.16^{c} ±0.33	0.75^{abcd} ±1.39	0.27^{b} ±0.43	0.68^{ab} ±1.07	0.20^{c} ±0.33	0.30^{c} ±0.43	0.49^{a} ±0.93	0.39^{c} ±0.73	0.49^{b} ±0.81
	9	0.16^{c} ±0.41	0.40^{cd} ±0.55	0.25^{b} ±0.52	0.39^{ab} ±0.58	0.66^{abc} ±0.93	0.45^{bc} ± 0.58	0.62^{a} ±0.85	0.72^{abc} ±0.91	0.61^{ab} ±0.93
	12	0.50^{abc} ±0.62	0.55^{bcd} ±0.87	0.36^{ab} ±0.56	0.47^{ab} ±0.52	0.60^{abc} ±0.82	0.50^{bc} ±0.68	0.67^{a} ±0.86	0.53^{bc} ±0.85	0.63^{ab} ±0.82
	15	0.45^{abc} ±0.55	0.30^{d} ±0.57	0.41^{ab} ±0.61	0.53^{ab} ±0.81	0.32^{bc} ± 0.61	0.70^{abc} ± 0.80	0.55^{a} ±0.76	0.56^{abc} ± 0.82	0.61^{ab} ±0.99

Mean ± standard deviations were evaluated by 10 trained panelists using nine category scales (0-8); 0: None, 2: Slightly difference, 4: Moderate, 6: Very difference, 8: Extremely difference.

NP: Non-pasteurized marinated shrimp in green curry paste. P = Pasteurized marinated shrimp in green curry paste at 70°C for 7 minutes. B: freshly prepared sample (non-pasteurized).

Blind sample: freshly prepared sample (non-pasteurized). Means with different superscripted letters in the same column are significantly different ($p \leq 0.05$).

CHAPTER V

CONCLUSION

5.1 Conclusion

As a conclusion, dipping frozen-thawed white shrimp in food additives was an important step for a good quality of final processed product. Treatments with non-phosphate additives were an alternative way to improve the quality of frozen white shrimp. Immersion white shrimp in 5% MTGase with 2% NaCl for 30 minutes increased the weight gain to 15.68%, moisture content to 84.00% and water holding capacity to 94.50%, and lowered cooking loss to 10.15%. The process also reduced total viable count for about 1.44 log CFU/g compared with control. The shear force value for this sample was not significantly different from control.

White shrimp was treated with 5% MTGase with 2% NaCl for 30 minutes and 0.5% citric acid for 5 minutes. For pasteurization parameter, treatment 5 (T5; 70°C for 7 minutes) with accumulated lethality of 2.17 minutes at 70°C was selected as the suitable pasteurization condition for marinated shrimp in green curry paste. The product pasteurized using condition T5 got the highest score for overall acceptability (5.75). The pH value of pasteurized marinated shrimp in green curry paste using treatment 5 was 7.55. The shear force and L*a* b* value for T5 was not significantly different from control ($p>0.05$).

After pasteurization, the safety level of marinated shrimp in green curry paste was increased, where the TVC was <10 CFU/g at the beginning of storage and 1.79 log CFU/g at the end of storage for 15 days. The TVC did not exceed 10^5 until the end of 15 days. *Listeria* spp., *E. coli,* yeast and mould were not detected in 25g sample.

The sensory evaluation result of cooked product (non-pasteurized and pasteurized marinated shrimp in green curry paste) was not significantly different from the blind sample (freshly prepared shrimp in green curry) for 9 attributes sensorial tested along the storage period of 15 days at 0-3°C.

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APPENDICES

APPENDIX A

A.1 Sensory evaluation form Quantitative Descriptive Analysis (QDA)

แบบทดสอบประเมินลักษณะทางประสาทสัมผัส

ชื่อ (Name)..... วันที่ (Date).....

ตัวอย่าง (Product): แกงเขียวหวานกุ้ง (marinated shrimp in green curry)

คำแนะนำ (Instruction):

1) กรุณาดื่มน้ำก่อนชิมตัวอย่างทุกครั้ง
2) กรุณาประเมินความเข้มคุณลักษณะของแต่ละตัวอย่างโดยใช้สเกลเชิงเส้น (0 = น้อยที่สุด และ 8 = มากที่สุด) กรุณาทำเครื่องหมาย | ลงบนเส้น ให้ตรงกับจุดที่ท่านมีต่อตัวอย่าง

1) Please rinse your mouth thoroughly before and after each sample.
2) Please mark the intensity for each attribute of the sample by the category scale (0 = weaker and 8 = strongest). Please mark (I) at the score you give.

รหัส (Sample Code): _____

1. **ลักษณะปรากฏ** (Appearance):

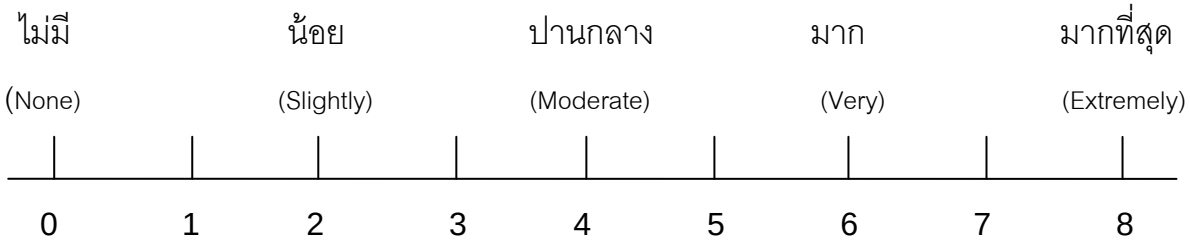
1.1 **สีของแกงเขียวหวาน** (Colour of the green curry): **ดูสีเขียวของน้ำ**

ไม่มี		น้อย		ปานกลาง		มาก		มากที่สุด
(None)		(Slightly)		(Moderate)		(Very)		(Extremely)
0	1	2	3	4	5	6	7	8

1.2 **สีของกุ้ง** (Colour of the shrimp): **ดูสีแดงของกุ้ง**

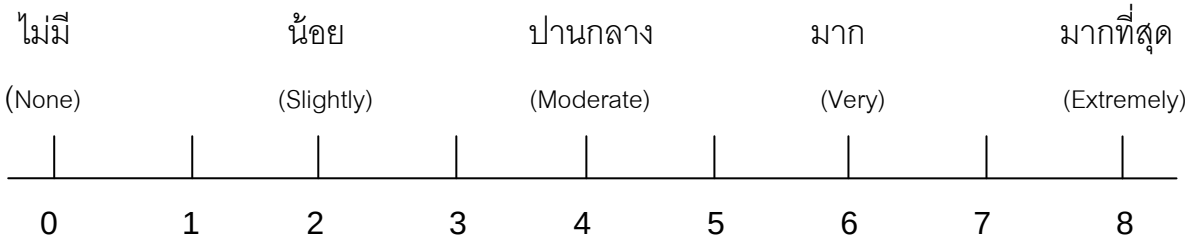
ไม่มี		น้อย		ปานกลาง		มาก		มากที่สุด
(None)		(Slightly)		(Moderate)		(Very)		(Extremely)
0	1	2	3	4	5	6	7	8

2. **กลิ่น** (Odour)

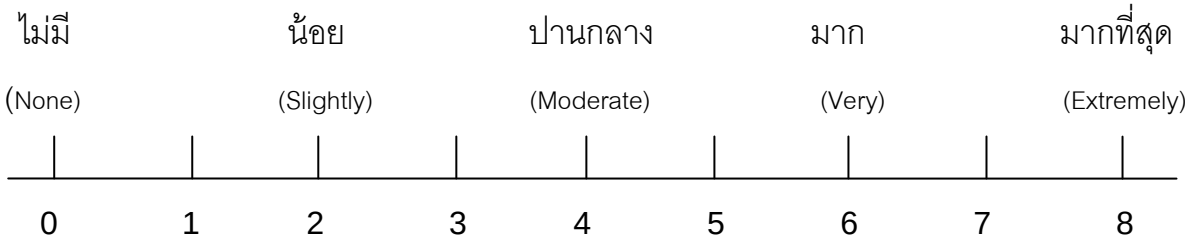


3. **กลิ่นรส** (Flavour)

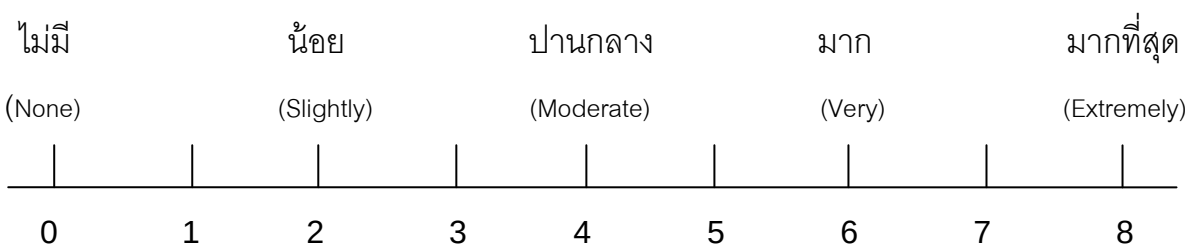
3.1 **รสหวาน** (Sweetness)



3.2 **รสเค็ม** (Saltiness)

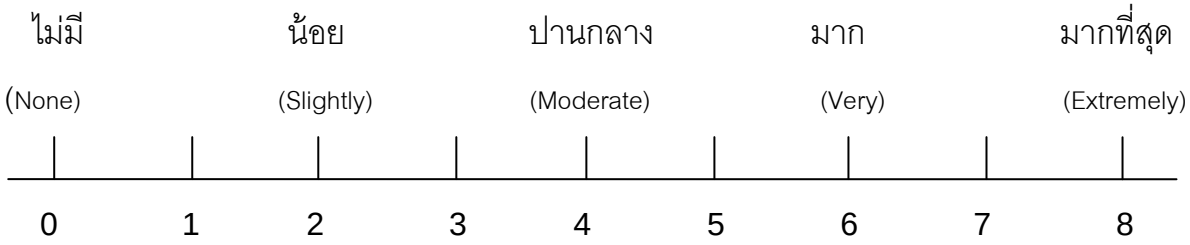


3.3 **กลิ่นรสแปลกปลอม** (Off flavour)**: เสีย หรือเหม็นหืน** (Rancidity)

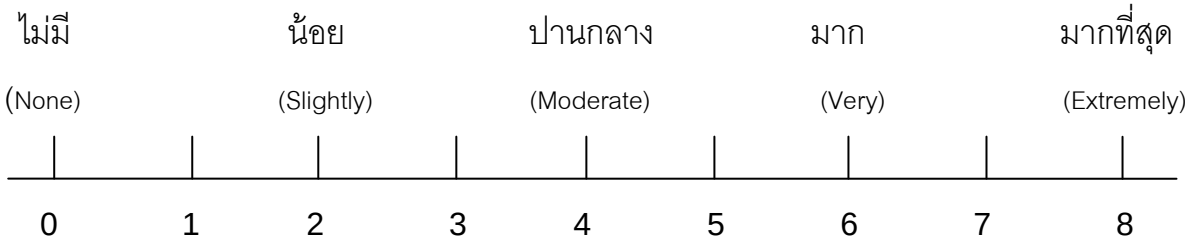


4. **<u>เนื้อสัมผัส</u>** <u>(Texture):</u>

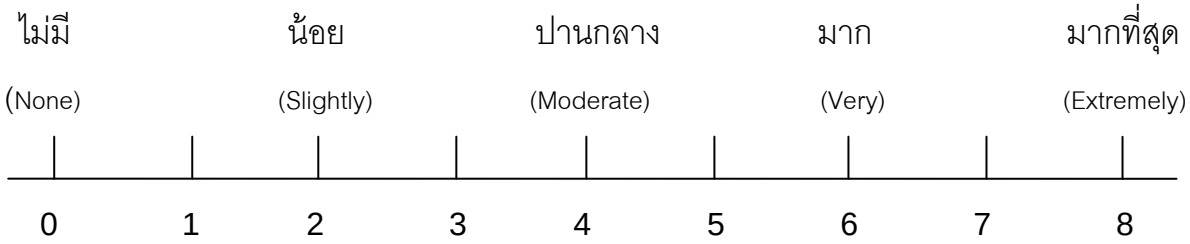
4.1 **ความฉ่ำน้ำ** (Juiciness): **ของกุ้ง** (shrimp)



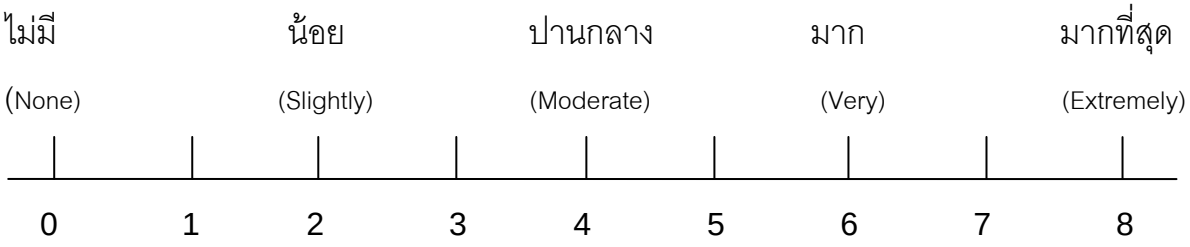
4.2 **ความแข็ง** (Toughness): **ของกุ้ง** (shrimp)



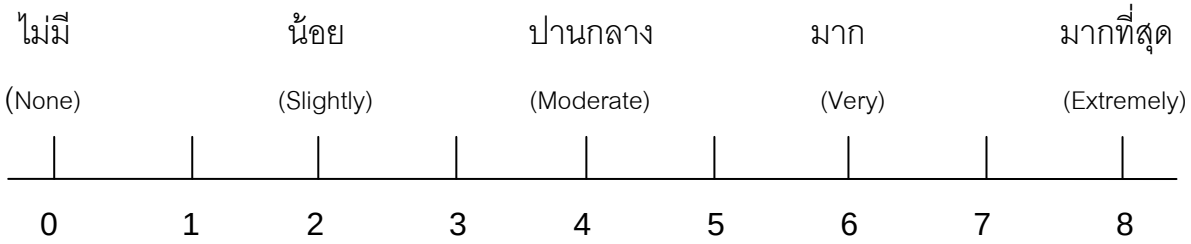
4.3 **ความนุ่ม** (Tenderness/ softness): **ของกุ้ง** (shrimp)



4.4 **ความแน่นเนื้อ** (Firmness): **ของกุ้ง** (shrimp)



5. **<u>การยอมรับโดยรวม</u>** <u>(Overall acceptability)</u>



ข้อเสนอแนะ (Comment)**:**

_____

_____

_____

Thank you

A.2 Sensory evaluation form Difference from control test

แบบทดสอบประเมินลักษณะทางประสาทสัมผัส

ชื่อ (Name)..... วันที่ (Date).....

ตัวอย่าง (Product): แกงเขียวหวานกุ้ง (Shrimp in green curry)

คำแนะนำ:

1) ท่านจะได้รับตัวอย่าง 2 ถ้วย โดยถ้วยแรกเป็นตัวอย่างควบคุมติดรหัส "R" และถ้วยสองเป็นตัวอย่างทดสอบติดรหัส "เลขสามหลัก"
2) กรุณาดื่มน้ำก่อนชิมตัวอย่างทุกครั้ง
3) กรุณาชิมตัวอย่างควบคุม (R) ก่อน
4) จากนั้นกรุณาชิมตัวอย่างทดสอบ (ที่มีรหัสเลขสามหลัก) และบอก ความแตกต่าง ของตัวอย่างทดสอบเปรียบเทียบกับตัวอย่างควบคุม (R) โดยทำเครื่องหมาย | ลงบนเส้นสเกลที่กำหนด

Instruction:

1) You have received two samples, a control sample labeled R and test sample labeled with three digit number.
2) Please rinse your mouth thoroughly before and after each sample.
3) Evaluate the control sample first (R)
4) Next evaluate the coded sample. Rate the degree of difference from the control on the category scale below by put the mark (I) at the number that you give.

Note: Remember that duplicate control (blind control; R) may be the test sample.

รหัส (Sample Code): _____

1. **ลักษณะปรากฏ** (Appearance):

1.1 **สีของแกงเขียวหวาน** (Colour of the green curry): **ดูสีเขียวของน้ำ**

ไม่แตกต่าง	แตกต่างน้อย	แตกต่างปานกลาง	แตกต่างมาก	แตกต่างมากที่สุด
(None)	(Slightly)	(Moderate)	(Very)	(Extremely)

0 1 2 3 4 5 6 7 8

1.2 **สีของกุ้ง** (Colour of the shrimp): **ดูสีแดงของกุ้ง**

ไม่แตกต่าง	แตกต่างน้อย	แตกต่างปานกลาง	แตกต่างมาก	แตกต่างมากที่สุด
(None)	(Slightly)	(Moderate)	(Very)	(Extremely)

0 1 2 3 4 5 6 7 8

2. **กลิ่น** (Odour)

ไม่แตกต่าง	แตกต่างน้อย	แตกต่างปานกลาง	แตกต่างมาก	แตกต่างมากที่สุด
(None)	(Slightly)	(Moderate)	(Very)	(Extremely)

0 1 2 3 4 5 6 7 8

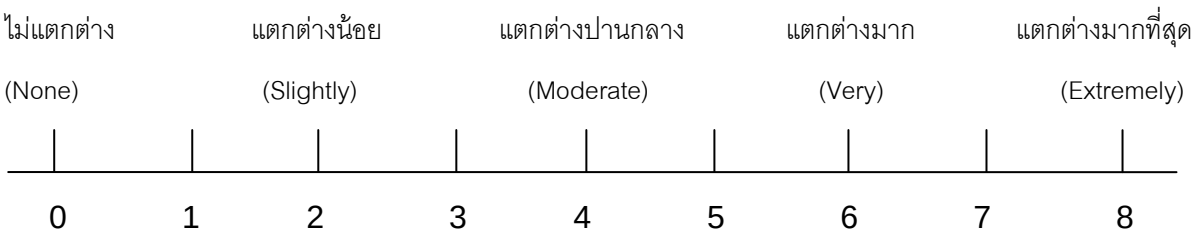
3. **กลิ่นรส** (Flavour)

ไม่แตกต่าง	แตกต่างน้อย	แตกต่างปานกลาง	แตกต่างมาก	แตกต่างมากที่สุด
(None)	(Slightly)	(Moderate)	(Very)	(Extremely)

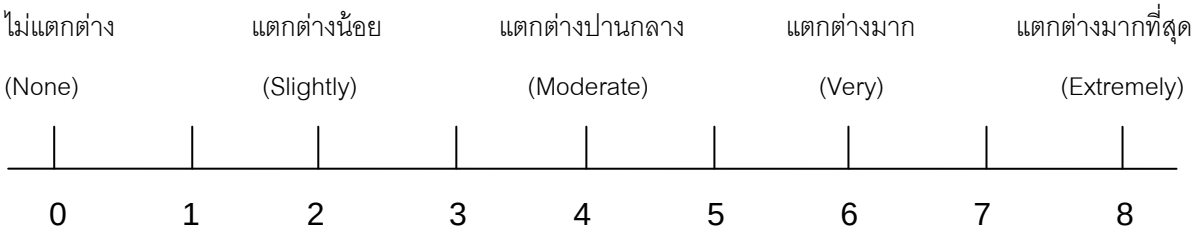
0 1 2 3 4 5 6 7 8

4. **เนื้อสัมผัส** (Texture):

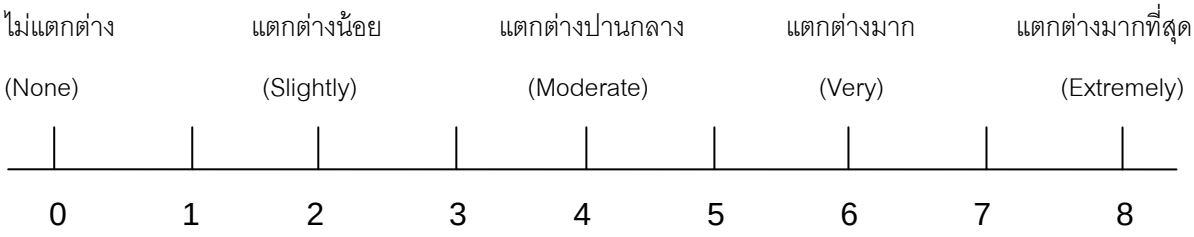
4.1 **ความฉ่ำน้ำ** (Juiciness): **ของกุ้ง** (shrimp)



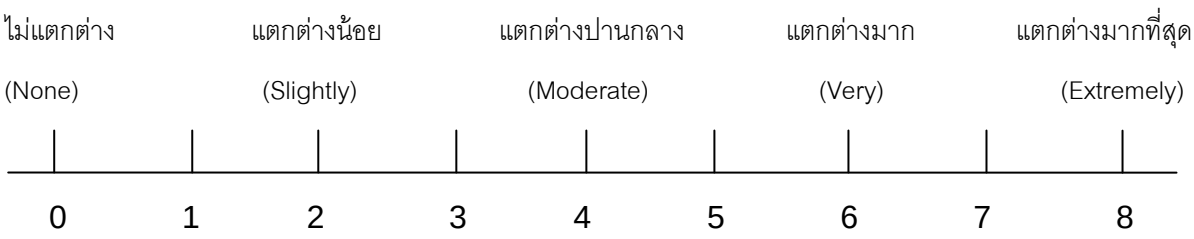
4.2 **ความแข็ง** (Toughness): **ของกุ้ง** (shrimp)



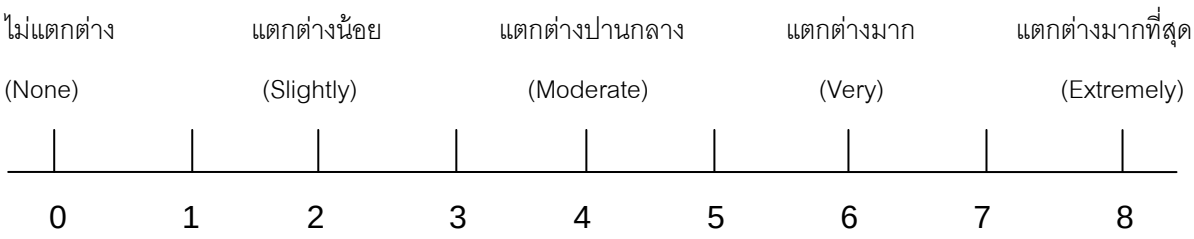
4.3 **ความนุ่ม** (Tenderness/ softness): **ของกุ้ง** (shrimp)



4.4 **ความแน่นเนื้อ** (Firmness): **ของกุ้ง** (shrimp)



5. **ความแตกต่างโดยรวม** (Overall difference)



ข้อเสนอแนะ (Comment)**:**

_____

_____

_____

Thank you

APPENDIX B

ANALYTICAL METHODS

B.1 Thiobarbituric acid (TBA) analysis

Thiobarbituric acid analysis (Vyncke, 1970).

(a) Reagent:

1. Trichloroacetic acid
2. Buthylated Hydroxy Toulene
3. 0.02 M 2-thiobarbituric acid
4. Ethylenediaminetetraacetic acid

(a) Extraction procedure:

1. Weight 20 g of shrimp and homogenized with 100ml of 7.5% trichloroacetic acid solution (TCA) for one minute in a Waring blender and filter using Whatman™ filter paper no.1, 150mm. Add an antioxidant 0.1% of Buthylated Hydroxy Toulene (BHT) and 0.1% EDTA Ethylenediaminetetraacetic acid (calculated on sample weight) in any case in order to avoid erroneously formed monaldehyde or other TBA reactive substances during blending and filtering the sample.
2. Add 5 ml of TBA reagent (0.02 M 2-thiobarbituric acid in distilled water) to 5 ml of filtrate in test tubes with screw caps; placed it in boiling water bath 100±2°C for 40 minutes.
3. Read absorbance (D) at 538 nm after cooling in tab water for 10 minutes, the original TCA extract was used as a blank. Blank values measured against pure TCA solution varied from 0.010 to 0.030 according to the species.

(b) Calculation:

TBA no. (as mg malonaldehyde per kg sample) = 7.8 D

Where D = the reading of the absorbance.

B.2 Total Volatile Base Nitrogen (TVB-N) analysis

Total Volatile Base Nitrogen (TVB-N) analysis (Pearson, 1981).

(a) Reagent:

1. Trichloroacetic acid
2. Sodium hydroxide
3. Hydrochloric acid
4. Methyl red indicator
5. Methyl blue indicator
6. Selenium mixture

(b) Procedure:

1. Weight 100±0.5 g of shrimp and homogenized with 300 ml of 5% m/v trichloroacetic acid for one minute in a Waring blender and filter using Whatman™ filter paper no.1, 150mm to obtain clear extract.
2. Transfer 5 ml of filtrate to a semi-micro distillation apparatus; add 5 ml of 2 M sodium hydroxide solution and 5 g selenium mixture before steam distillation.
3. In the receiving flask, prepare15 ml 0.01 M standard hydrochloric acid with the addition of 2 drop Tahiro's indicator solution (Tashiro's indicator; 0.08 g methyl red and 0.02 g methyl blue dissolved in 95% ethanol and make up to 100 ml with 95% ethanol).
4. Titrate the sample in the receiving flask with 0.01 M sodium hydroxide to a pale pink end point.

(c) Calculation:

$$\text{Total volatile base nitrogen (TVB-N)} = \frac{14(300+W) \times V}{500} \text{ mg per 100 g}$$

Where V ml = volume standard acid consumed in the titration.

W = water content of the sample mg/100g

B.3 Determination of moisture content

Moisture content analysis (Air oven method) AOAC 950.46 (AOAC, 2005, Tee et al., 1996)

(a) Definition

Moisture in this method refers to the amount of free water and volatile substances that are lost by drying the food under controlled temperature in an air oven. It is expressed in g per 100 g sample. The method is based on the drying of food sample under controlled temperature until constant weight is obtained. Moisture content is required to express the nutrient content per dry weight basis.

(b) Procedure

1. Dry the empty dish and lid in the oven at 105°C for 3 hours and transfer to dessicator to cool and weigh soon after it has attained room temperature.
2. Grind sample as finely as possible (homogenise in blender).
3. Weigh 10g of the homogenised sample into the aluminium dish. Spread the sample to the uniformity.
4. Place dish with sample, uncovered in the oven. Dry for overnight at 105°C.
5. Replace lid while dish is still in oven; remove dish from oven. Cool in desicator and weigh soon after attaining room temperature.
6. Repeat untill constant weight is obtained.

(c) Calculation

$$\text{Moisture (\%)} = \frac{(W2 - W3)}{(W2 - W1)} \times 100$$

Where: W1 = weight of container or empty dish (g)

W2 = weight of container + sample before drying (g)

W2- W1 = weight of sample (g)

W3 = weight of container + sample after drying (g)

W2– W3 = loss of weight of the sample (g)

B.4 Determination of protein

Protein was determined by the Kjeldahl Method AOAC 928.08 (AOAC, 2005; Tee *et al*., 1996)

(a) Reagents

1. Sulphuric acid (H_2SO_4), concentrated
2. 50% sodium hydoxide (NaOH)
3. 0.02 N Hydrochloric acid (HCl)
4. 4% Boric acid
5. Selenium mixture
6. Tashiro Indicator: Dissolve 80 mg methyl red and 20 mg methyl blue in 95% ethanol and make up to 100 ml with 95% ethanol

(b) Method

1. Weigh accurately 2g of the homogenised sample on Whatman filter paper no.1 and put in the digestion flask.
2. Add 5g selenium mixture and 20ml sulphuric acid.
3. Prepare blank containing 5g selenium mixture and 20ml sulphuric acid without sample.
4. Place flask in inclined position on an electric coil heating rack and heat gently until the liquid boils at moderate rate when the initial frothing had ceased. Boil briskly until solution clears (brown colour).
5. Cool with the fume exhaust unit in position. Add 60 ml of distilled water cautiously.
6. Turn on the water at the distillation unit. Connect digestion flask with distillation unit. In receiving Erlenmeyer flask add 50ml (4% boric acid) solution and 2 drop of mix indicator in the receiving flask.
7. 70 ml of 50% sodium hydroxide was automatically pumped by distillation unit in the distillation process. Distill for 6 minutes.

8. Remove receiver, wash tip of condenser by cleaning before and after use with distilled water 2/3 in 250 ml receiving flask.
9. Titrate the distillate with standard 0.02N HCl using semi automatic titrator.

(c) Calculation

Total protein (g) per 100 g of sample:

$$\text{Protein (\%)} = \frac{(A-B) \times M \times 1.4 \times 6.25}{\text{Weight in g of sample}}$$

Where A = Volume (ml) of 0.02N HCl used for sample titration

B = Volume (ml) of 0.02N HCl used for blank titration

1.4 = miliequivalent weight nitrogen x 100 (%)

6.25 = protein factor for fish and its by products

M = Normality 1ml 0.02N HCl

B.5 Determination of fat

Crude fat content of the sample was extracted using Solvent Extraction Method AOAC 991.36 (AOAC, 2005, Tee *et al*., 1996).

(a) Reagents

Petroleum ether (boiling point 40-60°C)

(b) Procedure

1. Place the round bottom flask in the oven at 105°C for 3 hours and transfer to dessicator to cool and weigh soon after it has attained room temperature (W2).
2. Weigh 2 g of homogenise sample (W1) in a filter paper (Whatman No.1) and wrap.
3. Put the sample in the extraction thimble and transfer into soxhlet.
4. Turn on the water 30 minutes before using the soxhlet system to make it cool. Preheat the furnace 10 minutes before used.
5. Fill the petroleum ether about 250 ml into the round bottom flask and put it on the heating mantle.
6. Heat the sample and extract for a minimum 8 hours to 12 hours.
7. Evaporate solvents (petroleum ether) completely on a water bath at 70-80°C.
8. Put the round bottom flask in oven at 100±5°C until constant weight is obtained.
9. Allow the flask to cool in a desiccator and weigh (W3).

(C) Calculation

Total Fat (g) per 100 g sample:

$$\text{Fat (\%)} = \frac{W3 - W2}{W1} \times 100$$

Where: W1 = Weight of sample in g

W2 = Weight of dried flask before extraction

W3 = Weight of dried flask + fat after extraction

B.6 Determination of ash

Ash content was determined according to AOAC 938.08 (AOAC, 2005).

(a) Procedure

1. Place the crucible and lid in the furnace at 550°C overnight to ensure that impurities on the surface of crucible are burn off.
2. Cool the crucible in the desiccator (30 minutes).
3. Weigh the crucible and lid to 3 decimal place (W1).
4. Weigh 5 g of homogenise sample into the crucible. Weight the crucible and lid with the sample (W2). Dry the sample in the dish in an oven at 130°C for one day.
5. Char the dried sample on a hot plate until it has ceased smoking.
6. Place the crucible in muffle furnace and heat at 550°C overnight. During heating do not cover the lid. Ash the sample until whitish or greyish ash was obtained.
7. Place the lid after heating to prevent loss of fluffy ash. Cool down in desiccator.
8. Weigh the ash with crucible and lid (W3) when the sample turns to grey. If not, return the crucible and lid to the furnace for further ashing.

(b) Calculation

The ash (g ash per 100g sample)

$$\text{Ash (\%)} = \frac{W3 - W2}{W1} \times 100$$

where: W1 = weight of crucible and lid

W2 = weight of crucible and lid + sample

W3 = weight of crucible and lid + ash

APPENDIX C

C.1 Example accumulated lethality calculation using general method

Time (minute)	Temperature (°C)	Lethal rate	Lethalithy	Accumulated Lethalithy
0.0	23.0	5.4117E-07		
0.5	51.0	0.003	0.001	0.001
1.0	57.9	0.024	0.007	0.008
1.5	60.0	0.046	0.018	0.025
2.0	63.5	0.136	0.046	0.071
2.5	64.5	0.185	0.080	0.151
3.0	66.0	0.293	0.119	0.270
3.5	66.5	0.341	0.159	0.429
4.0	66.8	0.374	0.179	0.608
4.5	67.0	0.398	0.193	0.801
5.0	67.7	0.494	0.223	1.024
5.5	68.0	0.541	0.259	1.283
6.0	68.2	0.575	0.279	1.562
6.5	68.4	0.612	0.297	1.859
7.0	68.6	0.651	0.316	2.174
7.5	68.8	0.692	0.336	2.510
8.0	69.0	0.736	0.357	2.867
8.5	69.2	0.782	0.379	3.246
9.0	69.3	0.807	0.397	3.643

Calculation lethal rate:

$$L = 10^{T-70/z}$$

Where T = Record temperature

70 =Reference temperature

Z = 7.5 for *Listeria monocytogenes*

APPENDIX D

ADDITIONAL DATA

D.1 ANOVA for pre-treatment analysis of white shrimp

Dependent Variable	Source	Sum of Squares	df	Mean Square	F	Sig
Cooking loss	Treatment	7871.29	27	291.53	466.53	0.00
pH	Treatment	60.97	27	2.26	9.39	0.00
Weight gain	Treatment	1017.28	27	37.68	132.70	0.00
Water holding capacity	Treatment	389.84	27	14.43	49.92	0.00
Total plate Count	Treatment	13.50	27	0.50	4.29	0.00
Moisture	Treatment	53.94	27	1.99	10.53	0.00
Shear force	Treatment	58.78	27	2.17	0.96	0.54
Colour L*	Treatment	272.30	27	10.08	4.28	0.00
Colour a*	Treatment	215.43	27	7.98	12.23	0.00
Colour b*	Treatment	326.38	27	12.09	10.96	0.00

Sig: Significance ($p \leq 0.05$)

D.2 ANOVA for physical analysis non-pasteurized (control) and five pasteurized marinated shrimp in green curry paste

Dependent Variable	Source	Sum of Squares	df	Mean Square	F	Sig
Shear Force	Treatment	13.59	5	2.72	20.87	0.00
Colour L*	Treatment	22.77	5	4.55	3.13	0.12
Colour a*	Treatment	1.09	5	0.22	3.87	0.08
Colour b*	Treatment	5.95	5	1.19	1.48	0.34
pH	Treatment	0.08	5	0.02	127.05	0.00

Sig: Significance ($p \leq 0.05$)

D.3 ANOVA for sensory evaluation of non-pasteurized (control) and five pasteurized marinated shrimp in green curry paste

Dependent Variable	Source	Sum of Squares	df	Mean Square	F	Sig
Colour of green curry	Treatment	1.57	5	0.31	0.93	0.45
Colour of shrimp	Treatment	0.82	5	0.16	1.17	0.34
Odour	Treatment	1.56	5	0.31	1.50	0.20
Sweetness	Treatment	0.32	5	0.06	0.46	0.80
Saltiness	Treatment	1.22	5	0.24	2.05	0.09
Off flavour	Treatment	0.00	5	0.00	0.00	0.00
Juiciness	Treatment	1.76	5	0.35	1.80	0.13
Toughness	Treatment	1.43	5	0.29	0.98	0.44
Tenderness	Treatment	1.88	5	0.37	0.54	0.20
Firmness	Treatment	2.61	5	0.52	0.97	0.10
Overall acceptability	Treatment	7.11	5	1.42	0.60	0.00

Sig: Significance ($p \leq 0.05$)

D.4 ANOVA for physical and chemical analysis of shelf-life study non-pasteurized and pasteurized marinated shrimp in green curry paste for 6 times sampling in 15 days

Dependent Variable	Source	Sum of Squares	df	Mean Square	F	Sig
pH	Treatment	0.28	11	0.03	100.25	0.00
TBA	Treatment	6.37	11	0.58	120.56	0.00
Shear Force	Treatment	27.32	11	2.48	3.75	0.02
Viscosity	Treatment	1.59	11	0.14	1388.98	0.00
Drain weight	Treatment	344.99	11	31.36	2260.97	0.00
Colour L*	Treatment	95.13	11	8.65	22.68	0.00
Colour a*	Treatment	2.74	11	0.25	10.84	0.00
Colour b*	Treatment	79.84	11	7.26	18.43	0.00

Sig: Significance ($p \leq 0.05$)

D.5 ANOVA for microbiology analysis of shelf-life study non-pasteurized and pasteurized marinated shrimp in green curry paste

Dependent Variable	Source	Sum of Squares	df	Mean Square	F	Sig
TVC	Treatment	67.01	10	6.70	182.05	0.00
Listeria. spp	Treatment	0.27	4	0.07	4.86	0.08
Coliform	Treatment	0.06	2	0.03	1.00	0.50
Yeast	Treatment	1.745	5	0.35	24.23	0.00

Sig: Significance ($p \leq 0.05$)

D.6 ANOVA for sensory evaluation of shelf-life study non-pasteurized and pasteurized marinated shrimp in green curry paste

Dependent Variable	Source	Sum of Squares	df	Mean Square	F	Sig
Colour of green curry	Treatment	3.15	14	0.22	1.77	0.05
Colour of shrimp	Treatment	10.04	14	0.72	1.88	0.03
Odour	Treatment	3.99	14	0.28	1.36	0.18
Flavour	Treatment	4.20	14	0.30	1.19	0.29
Juiciness	Treatment	7.85	14	0.56	2.05	0.02
Toughness	Treatment	7.29	14	0.52	1.89	0.03
Tenderness	Treatment	4.35	14	0.31	0.95	0.50
Firmness	Treatment	5.46	14	0.39	1.61	0.08
Overall different	Treatment	5.62	14	0.40	1.42	0.15

Sig: Significance ($p \leq 0.05$)

VITAE

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