Development of Reduced Sodium Shrimp Paste from Shrimp Head

Chanonkarn Rujirapong

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Functional Food and Nutrition

Prince of Songkla University

2022

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บทคัดย่อ

รายงานการลดลงของทรัพยากรธรรมชาติเกิดขึ้นทุกๆปี รวมไปถึงปัญหาการลดลงของ *Mesopodopsis* ซึ่งเป็นวัตถุดิบหลักในการผลิตกะปิที่เป็นส่วนประกอบหลักในอาหารไทยหลายชนิด แม้การลดลงของ *Mesopodopsis* เกิดขึ้นอย่างต่อเนื่อง ในขณะที่วัสดุเศษเหลือจากโรงงานแปรรูปกุ้งแช่แข็ง ตั้งแต่หัว เปลือก และหาง ยังไม่มีการนำไปใช้ประโยชน์อย่างเข้มข้นโดยเฉพาะหัวกุ้ง อย่างไรก็ตามหัวกุ้งมีไขมันไม่อิ่มตัวเป็นส่วนประกอบหลัก จึงง่ายแก่การเกิดออกซิเดชั่น นอกจากนี้การบริโภคอาหารที่มีปริมาณโซเดียมสูง เช่น กะปิ สามารถทำให้เกิดปัญหาทางสุขภาพได้หลายชนิด โดยเฉพาะความดันโลหิตสูง จากการได้รับโซเดียมเกินกว่าความต้องการในแต่ละวันประกอบกับโพแทสเซียมต่ำจะส่งเสริมการเกิดความดันโลหิตสูง ด้วยเหตุนี้ในการวิจัยนี้จึงออกแบบการทดลองเป็น 3 ประเด็นเป้าหมายหลักคือ (1) การผลิตกะปิลดโซเดียมจากหัวกุ้ง โดยนำหัวกุ้งผสมกับเกลือในอัตราส่วนหัวกุ้ง 12 14 และ 16 ต่อเกลือ 1 ส่วน หลังจากหมักเป็นระยะเวลา 90 วัน นำไปวัดและตรวจสอบคุณภาพทางเคมี กายภาพ จุลินทรีย์และประสาทสัมผัส พบว่าค่าน้ำอิสระลดลง (a_w) ในทุกชุดการทดลอง ปริมาณสารประกอบไนโตรเจนที่ระเหยได้ทั้งหมด (TVB-N) ไตรเมทิลเอมีน (TMA) ค่าการหืน (TBARS) และอัตราการย่อยสลาย (DH) มากที่สุดในชุดการทดลองที่มีอัตราส่วนหัวกุ้งมากที่สุด (16:1) อย่างไรก็ตามไม่มีการตรวจพบฮีสตามีน ในขณะที่อินโดลเพิ่มขึ้นอย่างเห็นได้ชัด ปริมาณจุลินทรีย์ทั้:งหมด (TVC) และปริมาณจุลินทรีย์ที่สร้างกรดแลคติก (LAB) ลดลงหลังจากหมักครบ 90 วันในทุกชุดการทดลอง และไม่พบจุลินทรีย์ก่อโรคทุกชนิด แม้ว่าในทุกชุดการทดลองมีคะแนนทางประสาทสัมผัสที่ประมาณ 6 คะแนน ซึ่งแตกต่างกันอย่างไม่มีนัยสำคัญ ชุดการทดลองที่มีหัวกุ้งในอัตราส่วน หัวกุ้ง 12 ต่อเกลือ 1 เป็นชุดที่มีคะแนนสูงกว่าชุดการทดลองอื่นๆ แต่ก็ยังต่ำกว่าชุดควบคุมจากกะปิในทางการค้าที่มีคะแนนประมาณ 7.5 จาก 9 (2) ประเด็นการป้องกันการเกิดออกซิเดชั่นของไขมันในกะปิจากหัวกุ้ง โดยใช้กระเทียมในขั้นตอนการหมักกะปิร้อยละ 0 3 และ 5 และทดสอบคุณภาพทางด้านต่างๆ เช่นเดียวกับตอนที่ 1 พบว่า ค่าน้ำอิสระลดลงในทุกชุดการทดลอง ในขณะที่

ค่ากรด-ด่างไม่เปลี่ยนแปลง นอกจากนี้ ปริมาณสารประกอบไนโตรเจนที่ระเหยได้ทั้งหมด (TVB-N) ไตรเมทิลเอมีน (TMA) ค่าการหืน (TBARS) และอัตราการย่อยสลาย (DH) เพิ่มขึ้นในทุกชุดการทดลอง แต่พบว่าการใช้กระเทียมร้อยละ 3 และ 5 สามารถยับยั้งการเกิดออกซิเดชั่นของไขมันได้ดีกว่าชุดการทดลองควบคุม (ไม่มีกระเทียม) ตรวจไม่พบฮีสตามีน ในขณะที่อินโดลเพิ่มขึ้นในทุกชุดการทดลอง แต่อยู่ในเกณฑ์ที่ยอมรับได้ ปริมาณจุลินทรีย์ทั้งหมด (TVC) และปริมาณจุลินทรีย์ที่สร้างกรดแลคติก (LAB) ลดลงเหลือประมาณ 2.54 log CFU/g และ 1 log CFU/g ตามลำดับหลังจากหมักครบ 90 วันในทุกชุดการทดลอง ตรวจไม่พบเชื้อก่อโรค แต่คะแนนการทดสอบทางประสาทสัมผัสในทุกชุดการทดลองอยู่ที่ประมาณ 6 ซึ่งน้อยกว่ากะปิทางการค้าที่ได้คะแนนประมาณ 7.5

สำหรับ (3) เป้าหมายสุดท้ายเป็นการลดปริมาณโซเดียม โดยทดแทนเกลือโซเดียมด้วยเกลือโพแทสเซียมที่ร้อยละ 0 , 30 และ 50 พบว่าในทุกชุดการทดลองค่าน้ำอิสระลดลงและค่ากรด-ด่างเพิ่มขึ้น นอกจากนี้ปริมาณสารประกอบไนโตรเจนที่ระเหยได้ทั้งหมด (TVB-N) ไตรเมทิลเอมีน (TMA) ค่าการหืน (TBARS) และอัตราการย่อยสลาย (DH) เพิ่มขึ้นในทุกชุดการทดลอง ปริมาณจุลินทรีย์ทั้งหมด (TVC) ลดลงจาก 5.90 log CFU/g เป็น 2.95 log CFU/g และปริมาณจุลินทรีย์ที่สร้างกรดแลคติก (LAB) ลดลงจาก 5.71 log CFU/g เป็น 1 log CFU/g ในทุกชุดการทดลอง หลังจากหมักครบระยะเวลา 90 วัน ไม่พบจุลินทรีย์ก่อโรคในทุกชุดการทดลอง เมื่อเปรียบเทียบคุณภาพทางเคมีและจุลินทรีย์กับมาตรฐานการผลิตกะปิพบว่ากะปิจากหัวกุ้งสามารถบริโภคได้อย่างปลอดภัย หัวกุ้งและกะปิที่หมักจากหัวกุ้ง ทั้งในชุดการทดลองควบคุม (NaCl 7.69%) และชุดการทดลองที่ใช้เกลือโพแทสเซียมทดแทนเกลือโซเดียม (NaCl 7.69%; S3 6.36% KCl 2.73% และ NaCl 4.55% KCl 4.55%) ที่ปริมาณ 1 มก./มล. สามารถในการยับยั้งเอนไซม์ ACE (Angiotensin-Converting Enzyme) ได้ 36.21±0.88% 53.47±2.81% 51.14±3.50% และ 45.80±3.45% ตามลำดับ ในขณะที่ captopril สารสังเคราะห์ที่ใช้ยับยั้งเอนไซม์ ACE ที่ความเข้มข้น 1.09 ng/ml (5 nM) 2.17 ng/ml (10 nM) และ 4.35 ng/ml (20 nM) สามารถยับยั้งเอนไซม์ได้ที่ 29.39±2.18% 36.00±4.34% และ 48.70±1.41%, ตามลำดับ ผู้บริโภคไม่สามารถบ่งบอกรสขมในชุดการทดลองที่ใส่เกลือโพแทสเซียมคลอไรด์ คะแนนการทดสอบทางประสาทสัมผัสในทุกชุดการทดลองยังอยู่ในช่วง 6 ซึ่งยังคงน้อยกว่ากะปิทางการค้าที่มีคะแนน 7.5

โดยสรุปสามารถกล่าวได้ว่าสามารถนำหัวกุ้งไปใช้ในการผลิตกะปิให้มีคุณภาพทางกายภาพ เคมี และจุลินทรีย์เทียบเท่ากับกะปิทางการค้า อย่างไรก็ตามยังจำเป็นต้องปรับปรุงคุณภาพทางประสาทสัมผัสเพิ่มเติม เช่น การใช้เครื่องมือหรือเอนไซม์เพื่อช่วยในการลดขนาดของหัวกุ้ง การเพิ่มน้ำตาลเพื่อช่วยปรับปรุงรสชาติ นอกจากนี้ยังพบว่าการผสมกระเทียมร้อยละ 3 และ 5 ในระหว่างการหมัก ไม่ทำให้คุณภาพด้านต่างๆของกะปิจากหัวกุ้งเปลี่ยนแปลงจากเดิมและช่วยลดการออกซิเดชั่นของไขมันได้ การทดแทนเกลือโซเดียมด้วยเกลือโพแทสเซียมที่ร้อยละ 50 ช่วยทำให้กะปิจากหัวกุ้งที่คุณภาพในด้านกายภาพ เคมี จุลินทรีย์ และประสาทสัมผัสที่เหมือนกับกะปิที่ใช้เกลือโซเดียมเพียงอย่างเดียว โดยไม่สามารถรับรู้รสขมได้อย่างชัดเจน นอกจากนี้การทดแทนเกลือโซเดียมด้วยเกลือโพแทสเซียมร้อยละ 50 สามารถยับยั้งการทำงานของเอนไซม์ ACE ได้ใกล้เคียงกับการใช้เกลือโซเดียมอย่างเดียว

Thesis Title Development of reduced sodium shrimp paste from shrimp head
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ABSTRACT

A significantly decreased of natural resources has been reported every year until now. This problem also including the decreasing of *Mesopodopsis*, a main raw material to produce tradition shrimp paste, Kapi which is a key ingredient in various Thai dishes. In contrast with decreasing of *Mesopodopsis*, most of by-product from frozen shrimp processing industry such as head, shell and tail were increased and still needed to utilize. However, shrimp head abundance with unsaturated fatty acid that susceptible to lipid oxidation. In addition, consumption of high sodium food as such Kapi can cause various health problems especially hypertension. Recently, there are many reports about hypertension due to high sodium consumption with low potassium intake. Therefore, this experiment was designed to meet 3 main objectives: The first aim was to produce the reduced sodium shrimp head paste. Shrimp head was mixed with salt as ratio shrimp head 12, 14 and 16 per 1 salt. After fermentation for 90 d, chemical, physical, microbiological and sensory quality of all treatments were determined. The result found that water activity (a_w) decreased in all treatments. The highest total volatile bases nitrogen (TVB-N), trimethylamine (TMA) thiobarbituric acid reactive substances (TBARS) and degree of hydrolysis (DH)were found in treatment with the highest shrimp head proportion (16:1). There was no histamine detected while indole was significantly increased. After fermentation for 90 d, significantly decreased of total viable count (TVC) and lactic acid bacteria (LAB) were found in all treatments with no pathogenic microorganisms detected. Although, no significantly difference in sensory score (approximately 6) was found, treatment with shrimp 12 per 1 salt seemed to have higher score in all attribute than other treatments which was lower than that of commercial shrimp paste with approximately 7.5 in 9. To prevent lipid oxidation in shrimp head paste, garlic was mixed at 0%, 3% and 5% and

determined the shrimp head paste quality as mentioned the first part. The resulted show that a_w reduced in all treatments while pH seemed to kept constant. In addition, TVB-N, TMA, TBARS and DH significantly increased in all treatments. When compared to control sample (without garlic), lipid oxidation of treatment with addition of garlic at 3% and 5% was retarded. There was no histamine detected while indole was significantly increased. TVC decreased to around 2.54 log CFU/g while LAB decreased to 1 log CFU/g in all treatments. Pathogenic microorganisms were not found in all treatments after fermentations for 90 days. Sensory score of all treatments were approximately 6 which was still lower than that of commercial shrimp paste with approximately 7.5 in 9.

The final aimed (3) was to reduce more sodium chloride by replacing with potassium chloride at 0%, 30% and 50%. After 90 d, reduction of a_w and increment of pH were found in all treatments. In addition, a significantly increased of TVB-N, TMA, TBARS and DH were found in all treatments. TVC decreased from 5.90 log CFU/g to around 2.95 log CFU/g while LAB decreased from 5.71 log CFU/g to 1 log CFU/g in all treatments. Pathogenic microorganisms were not found in all treatments after fermentations for 90 d. Based on production regulation on chemical and microbiological qualities of shrimp paste, shrimp head paste was considered as safe for consumption. Bitter taste was not detected in any treatment with added KCl. ACE Inhibition (%) of shrimp head (S1) and shrimp head paste (S2 NaCl 7.69%; S3 6.36% KCl 2.73%; S4 NaCl 4.55% KCl 4.55%) at 1 mg/ml were 36.21±0.88%, 53.47±2.81%, 51.14±3.50% and 45.80±3.45%, respectively while ACE Inhibition (%) of captopril at 5 nM, 10 nM and 20 nM were 29.39±2.18%, 36.00±4.34% and 48.70±1.41%, respectively. Sensory score of all treatments were approximately 6 which lower than that of commercial shrimp paste with approximately 7.5 in 9.

In summary indicated that shrimp head can be used to produce shrimp head paste with physical, chemical and microbiological qualities comparable to commercial shrimp paste. However, improvement of sensory quality by using properly machine or enzyme to reduce size of shrimp head and adding sugar to improve taste is required in further work. Addition of garlic at 3% and 5% seemed to retard lipid oxidation determined by TBARS assay and did not have any negative effect on other

qualities of shrimp paste. Based on physical, chemical, microbiological and sensory qualities, reduce sodium by replacement with KCl at 50% was comparable with 0% KCl shrimp head paste. In addition, no bitter taste was detected in shrimp head paste replacement NaCl with KCl up to 50%. Reduction of hypertension determined by ACE inhibition of shrimp head paste made from 50% KCl substitution was similar value to shrimp head paste made from 100% NaCl.

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LIST OF ABBREVATIONS

˚C	=	degree Celsius
µg	=	microgram
µl	=	microliter
µM	=	micromolar
ACE	=	angiotensin I-converting enzyme
a_w	=	water activity
cfu/g	=	colony forming unit per gram
d	=	day
g	=	gram
h	=	hour
L	=	liter
LAB	=	lactic acid bacteria
M	=	molarity
MUFA	=	monounsaturated fatty acid
m	=	month
mM	=	millimolar
mg	=	milligram
min	=	minute
ml	=	milliliter
N	=	normality
nm	=	nanometer
PUFA	=	polyunsaturated fatty acid
RDA	=	recommended dietary allowance
RDIs	=	reference daily intakes
SFA	=	saturated fatty acid
TBA	=	thiobarbituric acid
TBARS	=	thiobarbituric acid reactive substances
TCA	=	trichloroacetic acid
TMA	=	trimethylamine
TVB-N	=	total volatile bases nitrogen

TVC = total viable count

CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

1.1 Introduction

Thailand is top 5 global seafood producer and exporter particularly white shrimp. In 2020, Thailand produced around 257,262 tons of shrimp and 95.48% or 245,644 tons of the production was white shrimp (Fisheries Economics, 2020). Head, shell and tail are by-products, which may represent up to 55% of total shrimp weight (Hossain and Iqbal, 2014). In addition, shrimp head is a major by-product accounting for 37-45% of total shrimp weight (Ghorai, 2013). Recently, only 10% of total waste could be sold for 5 baht/kg, while other 90% is under-utilized and it could be sold at lower price as animal feed, fertilizer or disposed as waste leading to environment pollution.

In 2021, there is more than 7.7 billion people around the world with estimated increased to 8.5 billion in 2030 (United Nations, 2021). On the other hand, the more food consumption, the less natural resources supply leads to raw material and food supply shortage (Magdoff, 2013). Importantly, one of natural resource that must face with significantly decline in the near future is seafood (Stokstad, 2006) including krill (*Mesopodopsis oreintalis*) which is main ingredients used to produce shrimp paste (Pongsetkul *et al*., 2017b). Apart from krill, planktonic shrimp (*Acetes* species) also uses to produce shrimp paste. Shrimp paste, a natural salted fermented shrimp, is commonly used as condiment in various Asian countries including Cambodia, Indonesia, Malaysia, Myanmar, Philippines, Vietnam, and Thailand but may different in raw material, salt ratio and fermentation period (Ruddle and Ishige, 2010; Hajeb and Jinap, 2012; Pongsetkul *et al*., 2017b). However, similarity in the key production methods are drying, grinding, and fermentation (Hajeb and Jinap, 2012). Thai shrimp paste or Kapi is an essential raw material for cooking in various Thai dishes with special typical flavor and umami taste. Actually processing Kapi normally produced by taking krill or planktonic shrimp to mix with the salt as a ratio 3 to 5 per 1 (krill:salt) (Pongsetkul *et al.,* 2014) before taken to dry under sun light for a couple day and grind.

The mixture will be further fermented under restrict O_2 tension. From literature review, it revealed that white shrimp head composed of moisture 75.47 ± 0.43%, protein 60.13 ± 3.22% (dry basis), fat 4.48 ± 0.41% (dry basis), ash 17.73 ± 1.14% (dry basis) and carbohydrate 17.61% (dry basis) (Fernandes *et al.*, 2013). All mentioned compounds are quite close to composition of krill and planktonic shrimp which is a famous material used for shrimp paste (Kapi) processing. Planktonic shrimp (*Acetes vulgaris*) and krill (*Mesopodopsis*) normally contained moisture 86.74%, 86.25%; protein 87.93%, 66.97% (dry basis); fat 9.16%, 9.43% (dry basis); and ash 16.51%, 22.25% (dry basis), respectively (Kongpun and Kongrat, 2013). Although, fat content of shrimp head can be lower than krill and planktonic shrimp, among of unsaturated fatty acid (65.21%) in shrimp head especially eicosapentaenoic acid (EPA) (4.65 ± 0.01) and docosahexaenoic acid (DHA) (8.34 ± 0.02) were quite similar content even higher (Takeungwongtrakul, 2014). As well known that an unsaturated fatty acid is susceptible to lipid oxidation leading to unpleasant odor and unhealthy food (Domínguez *et al.* 2019). In addition, fermented shrimp paste contains high sodium content with salt not less than 36% (dry basis) and 12% (dry basis) based on regulation of Thailand Industrial Standards and Thai Community Product Standard, respectively (Ministry of Industry, 1992; Thai Industrial Standard Institute, 2018). As well known that consumption of high sodium food may cause some health problem including hypertension and other non-communicable diseases (NCDs).

Hypertension can increase risk for cardiovascular disease which cause of death and disability world-wide of approximately 9.4 million every year (Chen *et al.*, 2009; Rajati *et al*., 2019). In fact, the prevalence of hypertension is related to several factors such as high sodium and low potassium intake, consumption of food high in saturated and trans fats, consumption of alcohol and tobacco, obesity, diabetes, kidney disease as well as less physical activity and aging (Mayo Clinic, 2021; World Health Organization, 2021). World Health Organization Thailand (2017) reported that in 2015 at least 24% of Thai people with age equal or more than 18 years were hypertension. In addition, a number of registered patients who needed medications for hypertension increased from 3,946,902 people in 2013 to 5,584,007 people in 2017. Brouwer *et al*. (2015) reported that medication for hypertension can cost 916.54 bath (41.42 USD) per

patient per year. Many researchers reported that consuming healthy diet can help prevent or treat hypertension. Consumption food containing high potassium, calcium, magnesium, fiber etc. but low in sugar and fat, can lower blood pressure (Siervo *et al.*, 2015). In addition, consumption of low sodium soy sauce and miso can help lower diastolic blood pressure (DBP) in tester with aged 40 or older (Nakamura *et al*., 2003). Although, traditional fermented shrimp paste contains high sodium, however during fermentation, antihypertensive peptides obtaining from protein hydrolysis and Maillard reaction had been reported (Kleekayai *et al.,* 2015b). Therefore, there is possible that taking by-product from white shrimp industry for fermented shrimp paste production with proper process, using natural antioxidant to prevent lipid oxidation and replacement of potassium chloride for partial sodium chloride substitution might be essential for waste utilization, food sustainable and health benefit.

1.2 Review of Literature

1.2.1 Shrimp paste and its nutrition

Shrimp paste is a salted fermented shrimp or krill which commonly used as condiment in Asian. Table 1 shows the named list of shrimp paste in various countries. Thai traditional shrimp paste or Kapi is classified into two types: 1) Kapi Ta Deang is normally produced from planktonic shrimp in genus *Acetes* including *A. indicus*, *A. erythraeus* and *A. japonica* with a length between 10-40 mm (Wong *et al*., 2017) which is harvested from seas beds and 2) while Kapi Ta Dam is produced from krill (*Mesopodopsis orientalis*) which is normally harvested from mangrove canals (Kleekayai *et al*., 2015b). Production of shrimp paste can be affected by various factors similar to other fermented foods including freshness and proximate composition of raw material, temperature, oxygen concentration and salt content (Erkmen and Bozoglu, 2016). In general, Kapi is produced by mixing salt with shrimp or krill with ratio of 1:3 to 1:5 then the mixture will be taken to dry under sun light until moisture content of the paste reach 35-55%. Thereafter, finely ground to be a paste will be packed and fermented in the closed container at ambient temperature (25–35 °C) in restrict O_2 tension for 1-3 m (Department of Fisheries, 2013; Faithong and Benjakul, 2014; Pongsetkul *et al.*, 2014; Prapasuwannakul and Suwannahong, 2015). In addition, Thai Community Product issued that a good Kapi must have less fishy and ammonia smell,

not too salty, no bitter taste with several colors as pinkish, dark grayish brown and purplish gray (Thai Industrial Standard Institute, 2018).

Table 1. Named list of shrimp paste from Asian countries

Country	Name
Bangladesh	Nappi
Cambodia	Kapi
China	Shajiang
Indonesia	Terasi udang
Korea	Saewoojeot
Malaysia	Belacan
Myanmar	Ngapi seinsa
Philippines	Bagoong, Alamang, Dinailan, Guinamos Oyap
Thailand	Kapi
Vietnam	Mum ruoc, Mam tom

Source: Ruddle and Ishige, 2010

Proximate compositions, salt content, pH and a_w content in shrimp paste (Kapi) produced from identified shrimp species were presented in Table 2. According to notification of Ministry of Public Health (1998), shrimp paste can be considered as high protein food with protein content which accounted for 20% of Thai RDI (Thai RDI recommended as 50 g/d). Actually, carbohydrate of shrimp paste is quite low, however, it can be high due to addition of other carbohydrates from some processors including sugar, some tubers for texture, flavor and taste improving (Pongsetkul *et al*., 2014) as well as costing reduction. In addition, shrimp paste is classified as low-fat food (less than 3 g of total weight 50 g). However, shrimp paste is high ash (more than 20%) due to salt addition and minerals liberated from shrimp exoskeleton (Hossain and Iqbal, 2014; Pongsetkul *et al*., 2016). As a_w of shrimp paste is normally 0.62-0.77 which can be classified as intermediate moisture food (Pongsetkul *et al*., 2017b). pH of shrimp paste ranged between neutral to slightly basic pH mainly depends on degradation of

protein which is major macronutrient containing in shrimp paste (Daroonpunt *et al*., 2016).

Table 2. Proximate compositions of shrimp paste (Kapi).

Parameter	Kapi[A]	Kapi Ta-Dam[B]	Kapi Ta-Deang[B]	Kapi[C]
Moisture (%)	33.79-52.54	-		37.36-46.85
Carbohydrate (%)	4.9-32.48	18.4	16.5	4.27-17.96
Protein (%)	29.44-53.17	29.9±0.2	27.0±0.4	18.95-25.14
Fat (%)	1.41-3.67	2.1±0.0	2.9±0.0	0.69-2.05
Ash (%)	33.80-50.50	35.1±0.3	38.7±0.0	20.95-30.86
Salt (%)	22.77-35.47	13.1±0.1	14.7±0.2	19.78-22.96
a_w	0.669-0.774	0.62-0.63	0.62-0.63	0.70-0.74
pH	7.01-8.4	7.2-7.3	7.2-7.4	7.01-7.71

Source: Modified from Pongsetkul *et al*., 2014 (A); Kleekayai *et al*., 2015b (B); Prapasuwannakul and Suwannahong, 2015 (C)

Remark: - means no data.

Generally, Thai shrimp paste contained NaCl, moisture content, a_w, and pH as well as degree of hydrolysis between 22.77-35.47%, 33.79-52.54%, 0.695–0.774, 7.01-8.4 and 12.68-20.76%, respectively (Pongsetkul *et al.*, 2014). In addition, Pongsetkul *et al.* (2017b) reported that 30 d of fermented shrimp paste contained moisture content, a_w, pH and degree of hydrolysis approximately 35.07%, 0.694, 7.24 and 21.12%, respectively. In that experiment a_w of shrimp paste did not change during 30 d of fermentation, however, moisture increased in first 10 d then kept constant (Pongsetkul *et at*., 2017b). An increasing of moisture was due to moisture reabsorption from environment (Pongsetkul *et at*., 2017b). The initial pH of *Acetes vulgaris* was 7.21 before got in the fermentation process then it gradually decreased to approximately 6.9 after 10 d before slightly increased to 7.24 during fermentation as a result of proteolytic and lactic acid bacteria function (Pongsetkul *et at*., 2017b). As known that fermented food such as shrimp paste involved with enzymatic hydrolysis from both microbial and

indigenous proteolytic enzyme (Kleekayai *et al*., 2015b). The dominant bacteria in shrimp paste were reported as halophilic bacteria and proteolytic bacteria followed by lipolytic and lactic acid bacteria (Pongsetkul *et al.*, 2017b). Halophilic bacteria increased from approximately 4 log CFU/g (dry basis) to approximately 5 log CFU/g (dry basis) and proteolytic bacteria gradually increased from approximately 3.7 log CFU/g (dry basis) to approximately 4.6 log CFU/g (dry basis) throughout fermentation while lipolytic bacteria remained constant at approximately 3.6 log CFU/g (dry basis) (Pongsetkul *et al.*, 2017b). In contrast, lactic acid bacteria gradually increased before decreased after 10 d which correlated with increased pH (Pongsetkul *et al.*, 2017b). Although, high amount of halophilic, proteolytic, lipolytic and lactic acid bacteria was found in shrimp paste of Pongsetkul *et al.* (2017b), Kapi was considered as safe for consumption due to no spoilage and pathogenic microorganism detected (Majumdar *et al*., 2018). Standard regulation of Kapi includes total viable count (less than 5 log CFU/g), *Staphylococcus aureus* (must not detected in 0.1 g sample), coliform (less than 3 MPN/g), *Salmonella* (must not detected in 0.01 g sample), *Clostridium perfringens* (must not detected in 25 g sample), and yeast and mold (less than 1.7 log CFU/g) (Ministry of Industry, 1992). In addition, there was reported that all proteolytic, lipolytic, lactic acid and halophilic bacteria also contributed to unique taste, flavor and odor of shrimp paste from production of aldehyde, ketone, and ammonia etc. (Pongsetkul *et al.*, 2017b; Kleechaya *et al*., 2021).

Although, shrimp paste has salty taste due to high salt added during processing, Kapi also provides other tastes as a result of amino acid and Maillard reaction products. Actually, free amino acids can help improve 4 tastes in food such as sweetness, bitterness, sourness and umami taste (Table 3) (Kawai *et al*., 2012). Glycine, alanine, serine and threonine contribute to sweetness in food, while glutamic acid and asparagine mainly provide sour and umami taste. In addition, bitter taste could be derived from leucine, isoleucine, phenylalanine, tryptophan. However, the taste of amino acids also depends on types (L, D form) and difference of physicochemical properties such as hydrophobicity, size, isoelectric point and functional group of side chain (Kawai *et al*., 2012). Generally, major amino acids including essential amino acids in living organisms is L-form (Lopez and Mohiuddin, 2021). While D-form amino

acid mostly found in nonliving cells including soil, river, lake, sea, ocean and etc. (Naganuma *et al.*, 2018) or microbial metabolites (Bastings *et al*., 2019). However, recent research discovered that D-amino acids in human involves neural signaling (D-aspartate and D-serine) and innate immunity regulation as well as several diseases including amyotrophic lateral sclerosis (D-serine), schizophrenia (D-aspartate and D-serine) and cancer (D-leucine, D-lysine, D-valine and D-glutamic acid) in addition aging relates to disease including cataract and atherosclerosis (D-aspartate) were also reported (Bastings *et al*., 2019).

Table 3. Taste of amino acid (L and D form).

Amino acid	**Taste**	
	L-form	**D-form**
Glycine	Sweet or sweet and umami	-
Alanine	Sweet or sweet and umami	Sweet
Serine	Sweet or sweet, umami and sour	Sweet or sweet and sour
Threonine	Sweet or sweet and sour	Sweet
Glutamic acid	Sour or sour and umami	Sour
Asparagine	Sour or sour and umami	Sweet or sweet and sour
Leucine	Bitter	Sweet and bitter
Isoleucine	Bitter	Sweet and bitter
Phenylalanine	Bitter	Sweet and bitter
Tryptophan	Bitter	Sweet or sweet and bitter

Source: Modified from Kawai *et al*., 2012

Kleekayai *et al*. (2015b) reported that glutamic acid was the most abundant amino acid in both Kapi Ta-Dam and Kapi Ta-Deang followed by aspartic acid which was similar to finding of Pongsetkul *et al*. (2015) (Table 4). After fermentation for 6 m, most essential amino acids and non-essential amino acids in Kapi Ta-Dam slightly increased. In contrast, most essential amino acids and non-essential

amino acids in Kapi Ta-Deang slightly decreased compared with initial quality (0 d of fermentation).

Pongsetkul *et al*. (2017a) reported that *Acetes vulgaris* contained saturated fatty acid (SFA) as a major fatty acid (41.82%) followed by polyunsaturated fatty acid (PUFA) (30.41%) and monounsaturated fatty acid (MUFA) (25.65%). Palmitic acid and stearic acid were dominant in SFA while palmitoleic acid and oleic acid were mainly found in MUFA. For PUFA, arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA) were dominant compounds (Table 5). Decreasing of SFA and MUFA in shrimp paste had been found while PUFA content in shrimp paste was increased after fermentation for 1 m (Pongsetkul *et al.*, 2017a). In contrast, Peralta *et al*. (2008) reported that there was no change of PUFA in salt fermented shrimp paste after fermentation for 360 d.

Kim *et al*. (2014) reported that sodium, calcium and phosphorus were major mineral containing in shrimp paste (Table 6). According to Ministry of Public Health (1998) shrimp paste is classified as a high calcium and phosphorus diet which is higher than 20% of Thai RDI or higher than 160 mg/d. Calcium and phosphorus are essential minerals for good bone health (Lee *et al.*, 2014). Calcium also plays a role in contraction of muscle and vascular, nerve transmission and vasodilator (Institute of Medicine, 1997). While, phosphorus found in phospholipids, nucleotides and nucleic acids is commonly used to maintain pH, energy storage and phosphorylation (Institute of Medicine, 1997).

Table 4. Difference between amino acid profile of Kapi Ta-Dam, Kapi Ta-Deang and 11 local unidentified Kapi.

Amino acid (mg/100 g)	**Kp-B0[A]**	**Kp-B6[A]**	**Kp-R0[A]**	**Kp-R6[A]**	**Local kapi[B]**
Essential amino acid					
Histidine	0.37	0.26	0.46	0.26	0.36-0.98
Threonine	0.93	0.78	1.00	0.78	ND
Tyrosine	0.64	0.85	0.81	0.77	0.71-2.61
Valine	1.09	1.35	1.34	1.08	1.12-4.06
Lysine	1.76	2.19	2.05	1.86	1.69-6.23
Isoleucine	0.95	1.15	1.14	1.05	1.23-4.11
Leucine	1.69	1.92	1.99	1.84	1.79-6.42
Trytophan	-	-	-	-	0.00-0.05
Non-essential amino acid					
Aspartic acid	2.30	2.55	2.24	2.31	2.29-7.31
Serine	0.78	0.42	0.70	0.53	0.20-1.84
Glutamic acid	4.30	4.10	4.14	3.96	0.25-16.39
Glycine	1.12	1.62	1.78	1.32	1.33-4.48
Arginine	1.16	0.76	1.05	0.91	0.66-3.70
Alanine	1.48	1.96	1.99	1.77	1.69-6.65
Proline	0.89	1.02	1.08	0.99	1.90-5.80
Total	20.40	21.97	22.86	20.35	23.77-68.95

Source: Kleekayai *et al*., 2015b (A); Pongsetkul *et al.,* 2015 (B)

Remark: Kp-B6 means Kapi Ta-Dam after fermented for 6 m Kp-R6 means Kapi Ta-Deang after fermented for 6 m; local Kapi means 11 samples from Kapi produced and sold in Thailand; - means no data

Table 5. Fatty acid compositions in raw *Acetes vulgaris* and shrimp paste produced from *Acetes vulgaris*.

Fatty acid	*Acetes vulgaris*[A]	Thai shrimp paste[A]	Phillippine shrimp paste A[B]	Phillippine shrimp paste B[C]
Total SFA	41.82±2.08	38.39±2.13	30.8*	47.3±4.0
14:0	3.21±0.09	2.56±0.05	3.5±0.1	4.1±0.6
16:0	27.95±2.01	26.04±2.00	18.4±1.9	25.0±2.9
18:0	9.55±1.01	7.13±1.54	5.4±0.8	11.0±0.8
Total MUFA	25.65±1.96	24.05±1.21	20.3*	18.0±1.4
16:1(n-7)	11.11±0.99	11.42±1.00	10.0±0.6	6.6±0.7
18:1(n-9)	10.77±0.56	10.11±0.65	7.2±0.3	10.8±0.7
Total PUFA	30.41±1.14	35.54±0.95	43.5*	34.7±5.4
18:2(n-6)	1.99±0.07	4.25±0.22	2.0±0.1	2.9±0.2
20:4 (n-6) (AA)	8.06±0.09	8.03±0.83	5.4±0.2	5.8±0.6
20:5 (n-3) (EPA)	11.15±0.15	12.49±0.45	13.7±0.6	11.0±1.6
22:6 (n-3) (DHA)	7.88±0.97	7.02±0.23	16.7±0.7	11.5±3.3

Source: Modified from Pongsetkul *et al*., 2017a (A); Peralta *et al*., 2008 (B); Montaño *et al*. 2001 (C)

Remark: * means data calculated by substracting from sum of all fatty acid in that group in the same fatty acids type.

Table 6. Mineral content in shrimp paste (mg/100 g).

Minerals	A	B	C
Ca	1,457.82	931.07	3,598.05
Fe	2.09	2.26	28.90
K	345.98	548.87	751.12
Mg	193.33	438.80	254.65
Na	5,069.99	13,668.29	754.18
P	757.82	513.36	911.02
Zn	2.22	1.34	5.91

Source: Kim *et al.,* 2014 (A): Malaysian shrimp paste (B), Korean fermented and dried shrimp paste and Korean dried shrimp paste (C)

Besides being a source of protein and minerals particularly Ca and P of Kapi during fermentation protein hydrolysis and browning reaction generated others benefits compounds such as antioxidant (Prapasuwannakul and Suwannahong, 2015) and antihypertensive peptide. Faithong and Benjakul (2014) reported that increasing of antioxidant activity in shrimp paste had been noticed during first 8 m of fermentation before decreasing corresponding with decreasing of browning intensity.

In contrast to health benefits, shrimp paste has been addressed for two main problems. First, high sodium added into the shrimp for shrimp paste production may cause adverse effect to consumer health by increasing sodium in blood which related to blood pressure. Another problem is production of basic nitrogen containing compounds such as biogenic amines (BA), which are usually produced by microbial decarboxylation that carboxyl group is converted from amino acids. Spano *et al*. (2010) reported that the most important BA are histamine (histidine), tyramine (tyrosine), putrescine (ornithine), cadaverine (lysine) and β-phenylethylamine (β-phenylalanine). The symptom of BA toxicity is probably headache and migraine from tyramine or urticaria, hypotension, headache, flushing, and abdominal cramps from histamine as well as carcinogenic effect of putrescine and cadaverine (Calzada *et al*., 2013). Toxic

dose of others BA are as followed tyramine 100-800 mg/kg and phenylethylamine 30 mg/kg (Calzada *et al*., 2013). In addition, Prester (2011) reported that high quality crustacean contained no or low histamine and tyramine but can be high in putrescine and cadaverine during fermentation or spoilage. *Tetragenococcus*, dominant bacteria reported in Cambodia shrimp paste and also found in Thai shrimp paste (Kleekayai *et al*., 2015b; Pongsetkul *et al.*, 2017b), is major histamine-producing bacteria found in fishery products (Spano *et al*., 2010). Although, consumption BA can cause toxicity, only histamine has restriction (less than 100 mg/kg) (Satomi, 2016).

1.2.2 White shrimp (*Litopenaeus vannamei*)

Declining of krill, a natural material to produce shrimp paste, has been recorded (Stokstad, 2006; Pongsetkul *et al*., 2017b). However, many of by-products from seafood processing still has been left over especially *Litopenaeus vannamei* or white shrimp which is commonly used in Thailand seafood industry due to faster growing and more disease resistant compared with tiger prawn and others (Food Intelligence Center Thailand, 2015). In 2020, Thailand produced *vannamei* product approximately 257,262 tons (Fisheries Economics, 2020) or at least 125,000 tons of shrimp waste which is commonly sold in low price or dispose as waste. *Litopenaeus vannamei* in mature stage can be big as weight and length approximately 8.96±0.15 g and 11.82±0.07 cm respectively. Chemical compositions of *vannamei* and by-product (head and shell) are summarized in Table 7. Moreover, Takeungwongtrakul (2014) reported that lipid content of white shrimp head after iced storage 0 d and 6 d were 10.52±0.71 and 11.62±0.59, respectively (dry weight). In addition, Hossain and Iqbal (2014) reported that by product of shrimp contained lower protein but higher ash than shrimp meat. Head and shell of shrimp accounted approximately 45-55% of whole shrimp and composed of 30-50% (dry basis) calcium carbonate and calcium phosphate, 30-40% (dry basis) protein, and 20-30% (dry basis) chitin.

Table 7. Chemical compositions of shrimp meat, head and shell.

Shrimp portion	Proximate compositions (%)			
	Moisture	**Protein***	**Fat***	**Ash***
Head[A]	78.28±0.39	59.39±0.69	19.48±1.61	24.17±0.51
Shell[A]	77.07±0.59	57.48±0.39	14.78±2.53	21.50±0.61
Head[B]	75.47±0.43	60.13±3.22	4.48±0.41	17.73±1.14
Head[C]	78.60±0.95	58.08±3.04	4.11±0.33	0.79±5.23
Shrimp meat[D]	77.21±0.18	82.49±1.01	5.70±0.39	6.45±0.44

Source: Modified from Reerueangchai *et al*., 2014 (A); Fernandes *et al*., 2013 (B); Brasileiro *et al.*, 2012 (C); Sriket *et al*., 2007 (D)

Remark: Protein, fat, and ash based on dry basis (*).

Major amino acids found in white shrimp were arginine, proline, leucine, isoleucine, phenylalanine and glutamic acid (Table 8). Sriket *et al.* (2007) mentioned that seafood flavor in crustaceans was a result of high content of free arginine. Glycine, serine and threonine normally contributed to sweetness in shrimp meat, while arginine and proline work for both sweetness and bitterness (Sriket *et al*., 2007). In contrast to sweetness, leucine, isoleucine, phenylalanine play role for bitter taste (Sriket *et al*., 2007). However, Ghorai (2013) reported that shrimp by-product exhibited both sweetness and bitterness as a result of high lysine and methionine (Kawaii *et al*, 2012). In contrast to Ghorai (2013), Ibrahim *et al*. (1999) reported that glutamic acid, valine and arginine were dominant amino acid found in head of *Litopenaeus* spp.

Table 8. Amino acids compositions in white shrimp meat and head of *Litopenaeus* spp (mg/100 g sample).

Amino acid	Meat[A]	Head[B]
Histidine	666	3,500
Isoleucine	2,411	1,720
Leucine	3,153	3,540
Lysine	630	3,130
Methionine	1,298	2,760
Phenylalanine	1,967	2,370
Threonine	1,129	3,130
Valine	1,078	4,560
Aspartic acid + asparagine	1,704	2,360*
Hydroxyproline	215	-
Serine	1,027	2,570
Glutamic acid + glutamine	1,504	8,380*
Proline	3,862	2,000
Glycine	871	2,390
Alanine	1,601	2,590
Cysteine	547	2,230
Tyrosine	1,967	3,530
Arginine	3,494	3,550

Source: modified from Sriket *et al.*, 2007 (A); Ibrahim *et al*. 1999 (B) (dry basis)

Remark: - means no data. * means data in this research reported only aspartic acid or glutamic acid.

In general, shrimp contains fat mostly in hepatopancreas which located in shrimp cephalothorax. Cephalothorax and hepatopancreas of *vannamei* composed of PUFA with 39.30%, and 37.42% followed by SFA with 29.51% and 28.51% and MUFA with 25.91% and 29.95%, respectively (Takeungwongtrakul, 2014) which was similar to fatty acid profile found in Mungoong produced from the cephalothorax of *vannamei* as PUFA 40.86%, SFA 28.74% and MUFA 27.54%. The highest SFA in cephalothorax of *vannamei* was palmitic acid (16:0) while the most MUFA was oleic acid (18:1, ω-9). Among PUFA, linoleic (18:2, ω-6) was the most omega-6 fatty acid followed by docosahexaenoic acid (DHA; 22:6, ω-3) and eicosapentaenoic acid (EPA; 20:5, ω-3) which accounted for 8.34% and 4.65%, respectively (Table 9). PUFA containing in cephalothorax, hepatopancreas and mungoong was slightly lower than that of shrimp meats which composed of PUFA 42.2%, SFA 35.8% and MUFA 22%. However, as mentioned before that lipid composition in shrimp meat was four times lower than shrimp head. Ibrahim *et al*. (1999) and Reerueangchai *et al*. (2014) reported that shrimp head had a high ratio of unsaturated fatty acid to saturated fatty acid which supported to lipid oxidation susceptibility. Due to rancidity, therefore antioxidant addition particularly natural one to prevent or retard lipid oxidation is needed to consideration.

Table 9. Fatty acids compositions containing in white shrimp meats, head, hepotopancreas and its product (Mungoong) (g/100 g lipid).

Fatty acids	**Head[A]**	**Hepatopancreas[A]**	**Mungoong[B]**	**Meats[C]**
Total SFA	29.51±0.04	28.51±0.04	28.74	35.8
14:0	0.61±0.02	0.93±0.18	0.88	0.41
16:0	18.9±0.04	20.51±0.02	21.03	21.8
18:0	6.92±0.03	4.14±0.04	5.04	11.5
Total MUFA	25.91±0.05	29.95±0.02	27.54	22*

Source: Modified from Takeungwongtrakul, 2014 (A); Binsan *et al*., 2008 (B) (Mungoong produced from cephalothorax); Sriket *et al.*, 2007 (C)

Remark: ND means not detected.

Table 9. Fatty acids compositions containing in white shrimp meats, head, hepotopancreas and its product (Mungoong) (g/100 g lipid). (continued)

Fatty acids	Head[A]	Hepatopancreas[A]	Mungoong[B]	Meats[C]
16:1(n-7)	1.44±0.00	1.99±0.03	2.45	1.39
18:1 (n-7)	2.46±0.02	2.74±0.03	ND	2.41
18:1 (n-9)	18.66±0.03	21.76±0.01	21.89	11.4
Total PUFA	39.30±0.02	37.42±0.04	40.86	42.2
18:2 (n-6)	19.69±0.02	23.28±0.07	22.32	15.6
20:4 (n-6) (AA)	2.34±0.02	0.96±0.01	1.89	3.23
20:5 (n-3) (EPA)	4.65±0.01	2.15±0.00	4.31	9.46
22:6 (n-3) (DHA)	8.34±0.02	6.20±0.05	7.07	9.99

Source: Modified from Takeungwongtrakul, 2014 (A); Binsan *et al*., 2008 (B) (Mungoong produced from cephalothorax); Sriket *et al.*, 2007 (C)

Remark: ND means not detected.

In addition, shrimp meat and head were reported to contain various minerals as shown in Table 10 (Ibrahim *et al*., 1999; Sriket *et al.*, 2007).

1.2.3 Lipid oxidation and antioxidant

As mentioned above that shrimp head may prone to lipid oxidation due to high unsaturated fatty acid which leads to nutrition loss, rancidity and toxic component in food due to free radical, photo-oxidation, lypoxygenases, secondary oxidation product and transition metal. There are numerous factors such as type and amount of fatty acid, temperature, water activity (a_w), amount of oxygen etc. that affect lipid oxidation but generally polyunsaturated fatty acid, pro-oxidant and antioxidant are the most important. Ketones, aldehydes, and hydroxides are the products produced from lipid oxidation which relates to lowering in sensory quality of food (Sikorski and

Kolakowska, 2002). In addition, products from lipid oxidation including lipid peroxide and malondialdehyde can cause damage on cell of living organism, later lead to mutagenesis and carcinogenesis (Embuscado, 2015).

Table 10. Minerals content in white shrimp meat and head (mg/kg).

Mineral	Shrimp meat[A]	Shrimp head[B]
Iron (Fe)	12.2 ± 0.42	11.7
Copper (Cu)	4.07 ± 0.16	6.68
Manganese (Mn)	0.48 ± 0.00	13.0
Nickel (Ni)	0.36 ± 0.01	-
Zinc (Zn)	14.7 ± 0.56	71.9
Calcium (Ca)	247 ± 4.99	925
Magnesium (Mg)	361 ± 8.15	81.6
Potassium (K)	-	890
Sodium (Na)	-	1,400

Source: Modified from Sriket *et al.*, 2007 (A); Ibrahim *et al*., 1999 (B) (dry basis)
Remark: - means no data.

However, this problem can be retarded by adding antioxidant which can prevent lipid oxidation by donate electron or H^+ to free radical, chelate transition metal to prevent free radical formation, and/or propagation stage (lipid peroxidation chain reaction lead to production of hydroperoxides (ROOH) from peroxyl radical (ROO•)) (Nimse and Pal, 2015). In the past, there are several antioxidants used in food production, processing and storage. The high dose or long-term used of synthetic antioxidant such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and TBHQ exhibited many dangerous side effects. Kahl and Kappus (1993) reported that BHA and BHT are tumor promoter. BHA induced tumor in forestomach while BHT is inducer of liver cancer. Due to this problem, inhibition of lipid oxidation has shifted from synthetic to be natural antioxidant from particularly in plants which normally consist of three major groups such as polyphenols, carotenoids and sulfur

compounds (Abourashed, 2013). Carlsen *et al*. (2010) reported that many spices and herbs which are normally used to improve sensory quality and food preservation due to those plant are rich in phytochemical. Various spices and herbs also have high antioxidant value similar to vitamin and dietary supplements. In addition, Nugboon and Intarapichet (2015) who researched about antioxidant and antibacterial of Thai culinary herbs and spices reported that holy basil, Vietnamese coriander, turmeric and green peppercorn were great antioxidant sources among 22 herbs and spices. While, Nurwantoro *et al*. (2015) reported that garlic can slowdown increasing of TBARS value after applied garlic in beef fat.

Moreover, other natural antioxidants obtaining from Maillard reaction product (MRPS) are generated during food processing. Maillard reaction is non-enzymatic browning reaction between carbonyl group (carbohydrate and lipid) and amino group (protein, amino acids, ammonia) (Ames, 1992). Browning can cause many changes in color, odor and flavor of food. For instance, browning in grilled meat, baked, dehydrated foods and other food are related to Maillard reaction. Faithong, and Benjakul (2014) reported that antioxidant activity was significantly noticed as a result of Maillard reaction product during shrimp fermentation. Some of MRPs such as maltol, dihydroxyacetone, glyceraldehyde and reductones provided ability to act as antioxidant or precursor of antioxidant (Bailey and Um, 1992). Yilmaz and Toledo (2005) also reported that antioxidant activity found in MRPs was related to melanoidins and MRPs producing from histidine exhibited highest antioxidant activity. However, it was also reported that some of Maillard reaction products (MRPs) could be toxic substances (Ames, 1992). In addition, there are many researchers reported that Maillard reaction product (MRP) associated with many diseases such as diabetes, renal disease and cardiovascular disease. Adverse effects in human body from MRPs are associated with advanced glycation end-products (Nε-carboxymethyl-lysine) which related to cardiovascular disease and diabetes. In addition, Maillard reaction can cause loss of nutrition in food from changing of essential amino acid (lysine alanine, etc.) to others substance (glucosyllysine, lysinoalanine, etc.) (Tessier and Aragon, 2012).

1.2.4 Hypertension

A number of high blood pressure patients in Thailand is gradually increasing year by year especially in population age over than 40 years (Thai Hypertension Society, 2015). In 2019, Thailand World Health Organization reported that there were estimated 13.2 million of hypertension patients (Thailand World Health Organization, 2019). In addition, Bureau of Non- Communicable Diseases Thailand reported that during 2013-2015, death ratio of high blood pressure patients (per 100,000 healthy population) increased from 8.09 to 25.28 (more than 3 times) due to this problem can lead to various diseases such as stroke, heart failure, kidney disease and etc. (Chobanian *et al.*, 2003). Generally, optimal blood pressure of healthy human must be lower or equal 120 mmHg for systolic blood pressure (SBP) and/or 70 mmHg for diastolic blood pressure (DBP) (Table 11). However, when blood pressure is higher or equal 130 mmHg for SBP and/or 80 mmHg for DBP then high blood pressure or hypertension is identified (American Heart Association, 2018). Hypertension can be classified into two types: primary hypertension is high blood pressure with unidentified root cause (Hill, 2000) and secondary hypertension is caused by some diseases that can diagnose and cure such as glomerulonephritis (kidney inflammation), coarctation of the aorta (aorta narrowing) and aldosteronism (excessive secretion of aldosterone) (Puar *et al*., 2016).

It is known that when activity of angiotensin-converting enzyme (ACE) which is an enzyme in renin, angiotensin and aldosterone system (RAAS) (Figure 1), is inhibited or reduced then high blood pressure is declined or drop. Generally, low blood pressure was detected by baroreceptor which led to secretion of renin enzyme in kidney into blood (Kougias *et al.,* 2010; Chopra *et al*., 2011). Reaction between renin and angiotensinogen which primary secret by liver (Matsusaka *et al.,* 2012), cause rise in angiotensin I, a substrate for ACE to produce angiotensin II. In addition, ACE found in almost every cell but primary source is lung epithelial (Gallagher *et al.*, 2011), also degenerate bradykinin, a vasodilator, lead to vasoconstriction (Golias *et al.*, 2007; Chopra *et al*., 2011). Angiotensin II can act in various organ result in water and sodium retention in kidney by aldosterone, cortisol, adrenocorticotropic hormone (ACTH) and antidiuretic hormone (ADH or vasopressin) from adrenal cortex, anterior pituitary and

posterior pituitary (Patel *et al*., 2017) then increase thirst from hypothalamus (Patel *et al*., 2017) and vasoconstriction from prostaglandin (Chopra *et al*., 2011; Patel *et al*., 2017). For low blood pressure control mechanism was presented in Figure 1.

Table 11. Blood pressure level classified from systolic and diastolic blood pressure.

Stage of blood pressure	Systolic mm Hg		Diastolic mm Hg
Low	<80		<60
Normal	80-119	and	60-79
Elevated	120-129	and	60-79
High blood pressure (hypertension) stage 1	130-139	or	80-89
High blood pressure (hypertension) stage 2	140-180	or	90-120
Hypertension crisis	>180	and/or	>120

Source: Faniyan, 2016; American Heart Association, 2018

High blood pressure control mechanism composed of two ways (1) inhibition of RAAS and (2) kinin system (Figure 2). Inhibition of RAAS starts from high blood pressure detection by cardio pulmonary receptors which located in the atria and ventricles (Kougias *et al.*, 2010) lead to release of atrial natriuretic peptide (ANP) from atrial myocardium (Chopra *et al*., 2011). Reduction of renin secretion (Klabunde, 2016), inhibition of ADH (Chopra *et al*., 2011) and reduction of thirsty are involved with ANP function.

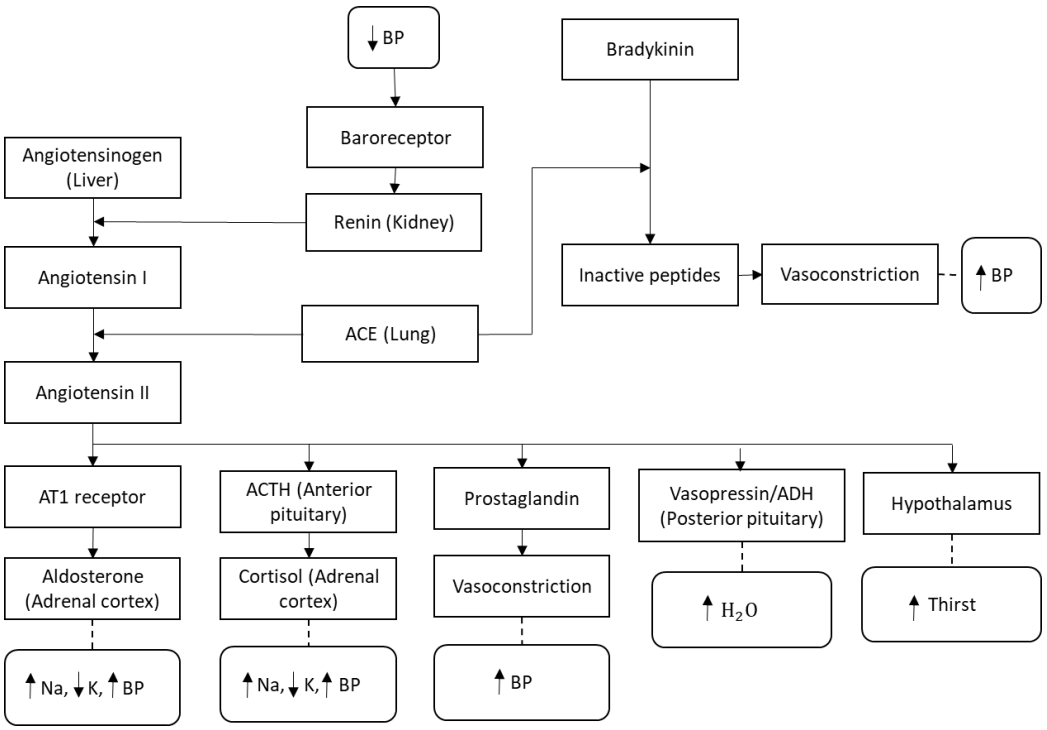

Figure 1. Low blood pressure control mechanism.

Source: Modified from Kougias *et al.*, 2010; Chopra *et al*., 2011; Patel *et al*., 2017

There are various factors affecting hypertension in human including consuming diet with high sodium and low potassium content, old age, high body mass index (BMI) and health problem such as obesity, diabetes, physical inactivity etc. (Sowers *et al*., 1991). Normally, high blood pressure can lower by two ways, medication and changing lifestyle. Medication involved antihypertensive drugs which are 15 different classes (total 69 drugs) such as ACE inhibitors and angiotensin receptor blockers (ARBs) to reduce blood pressure and organ injuring (Oparil and Schmieder, 2014). Changing life style to do more physical activity, reduce salt intake, consume more fruits and vegetables etc. can help control blood pressure even reduce need for medication by drugs which is quite costly (Yang *et al.*, 2017) and normally retains some side effects (dizziness, nausea, tiredness and etc.) (Carling *et al.*, 2010).

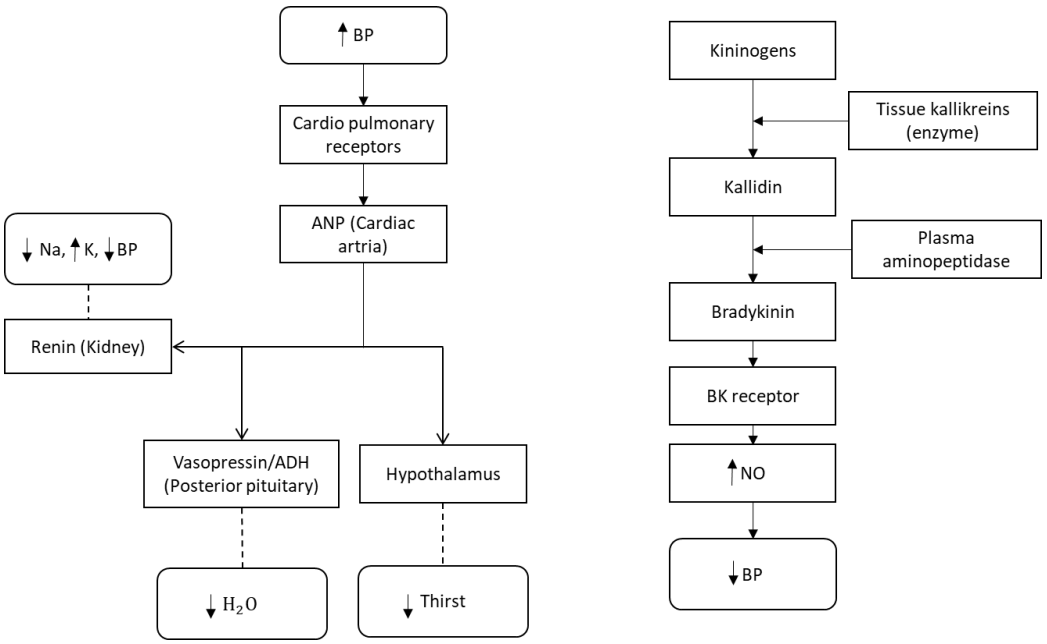

Figure 2. High blood pressure control mechanism.

Source: Modified from Golias *et al.*, 2007; Chopra *et al.*, 2011; Klabunde, 2016

1.2.5 Sodium and potassium intake

Although, numerous factors can cause hypertension, consuming diet with high sodium and low potassium content is a key role of high blood pressure (Staruschenko, 2018). In fact, sodium normally helps nerve and muscle function and involves in regulation of fluid balance of the body (Young *et al*., 2013; Ha, 2014), but when too much it can lead to other various chronic disease including cardiovascular disease, kidney disease, osteoporosis and cancer (Malta *et al*., 2018; Grillo *et al*., 2019). Generally, excessive sodium intake from processed food consumption is well related to high salt. A known that sodium chloride is the most salt used in food processing which naturally contains sodium (Na) 40% and chloride (Cl) 60% by weight (He *et al*., 2012). From this reason, the world health organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) reported that amount of table salt that people should consume daily must be lower than 5 g (Ha, 2014) which equal 2,000 mg of sodium. However, average table salt consumption of Thai population is high to 10.8 g (WHO Thailand, 2017) or equal 4000 mg of Na which exceeds limitation 2,000 mg/ d (Thailand Ministry of Public Health, 2018).

In fact, the purpose of using salt in food is to provide salty taste and prolonged shelf life of food (Smith and Van der Klaauw, 1995; Henney *et al*., 2010; Cepanec *et al.*, 2017). Preservation property of using salt is to lowering a_w in food which affects osmotic pressure of microbial intracellular leading to plasmolysis (microbial cell dry out) (Wijnker *et al.*, 2006). In addition, other mechanisms of salt for antimicrobial actions include limitation of oxygen solubility, inhibiting cellular enzymes, and forcing microorganism to use energy to secrete sodium ion until cell is exhausted (Henney *et al.*, 2010).

Hypertension that associated with high sodium intake also associated with low potassium intake because it will make higher sodium retention in kidney (Terker *et al*., 2015). In addition, low potassium intake also associated with glucose intolerance which related to diabetes. On the other hand, high potassium intake may prevent or slow progression of renal disease, lower urinary calcium excretion with help decrease risk of osteoporosis and management of hypercalciuria and kidney stones (He and MacGregor, 2008). Penton *et al*., (2015) also reported about various benefit from high potassium intake including vasodilation, reduce renin release and restore sodium balance by induced natriuresis (sodium excretion by kidney). Consumption more fruits and vegetables does not the only way to increase potassium intake but substitution sodium chloride with potassium chloride can help both problems: decrease sodium intake and increase potassium intake (Buren *et al*., 2016). However, Ministry of Public Health (1998) stated that Thai people should not take potassium over 3500 mg/d.

Potassium chloride is commonly used to substitute sodium chloride because chemical properties of both salt is almost similar. Characteristic of both compounds are white crystal, odorless and salty but potassium chloride also provides another bitter taste while salty of potassium chloride is lower than sodium chloride (Prinyawiwatkul and Sriwattana, 2013). Soglia *et al.* (2014) reported that potassium chloride has ability to preserve food but lower than sodium chloride. In addition, potassium chloride can be used as firming agent (strengthen structure of food) and used in treatment of patient that have hypokalemia or potassium in blood lower than 3.5 mmol/L while average between 3.6 and 5.2 mmol/L) to balancing potassium levels in blood (Terker *et al*., 2015).

As taste of potassium chloride is more bitter compared with sodium chloride leading to less sensory preference or even reject consumption. Generally, numerous researches reported that replacement sodium chloride with potassium chloride should not exceed 50% product to provide the attribute similar to control product (Ibañez *et al*., 1995). Jittrepotch *et al*. (2015) reported that sensory evaluation of Plaa-som, a Thai fermented fish which replaced sodium chloride with potassium chloride 25% and 50% exhibited sensory score similar to control (100% NaCl). In addition, Soglia *et al*. (2014) reported that replacement NaCl with KCl 30% in marinated rabbit meat obtained the sensory score similar to control (100% NaCl). However, it was found that using 50% KCl reduced saltiness and could not retard microbial load as much as control. Prinyawiwatkul and Sriwattana (2013) reported that the bitter taste reduction of reduced sodium Vienna sausages substituted with potassium chloride can be made by incorporation with glycine and L-arginine. Cepanec *et al*. (2017) reported that unwanted sensory in KCl replacement food can be modified by seven groups of taste improving ingredient: (1) mineral salts (dehydrate calcium chloride ($CaCl_2 \cdot 2H_2O$), magnesium chloride ($MgCl_2$) and potassium sulfate (K_2SO_4)) ; (2) food acids and amino acids and their nutritionally acceptable salt (citric acid,) potassium L-lactate and calcium gluconate);(3) simple carbohydrates, sugar alcohols and sugar substitutes (sucrose, sorbitol and aspartame); (4) food polymers (gluten, inulin and dextrin); (5) umami ingredients (monosodiumglutamate, monopotassium L-glutamate and calciumdi-L-glutamate); (6) spices, vegetables and flavors (oregano, garlic, meat flavor and cheese flavor); (7) miscellaneous taste improvers (taurine and betaine).

1.2.6 Garlic (*Allium sativum* Linn)

One of natural antioxidant which is popularly used in Asian diets to make food palatability is garlic. Garlic is a traditional herb or spice originates in central Asia (Naidu, 2000) with flat spear-like leaves which can growth up to 35 cm long and 2.5 cm wide. Normally, garlic bulb composes of 3 to 15 cloves which covers with white peel. In addition, it is used as natural medicine around the world because of many benefits such as antimicrobial, antiprotozoal, antimutagenic and antiplatelet etc.

(Rahman *et al*., 2012; Majewski, 2014). Generally, garlic can be stored for several months when dried (Benkeblia, 2004).

Proximate compositions of garlic were presented in Table 12. Recently, garlic is reported to contain various bioactive compounds however, main active compound is organosulfur compound in thiosulfinates group including alliin (S-allyl-L-cysteine sulfoxide) and allicin (diallylthiosulfinate) (Leelarungrayub *et al*., 2006). Allicin is produced from interaction between alliin and allinase when fresh garlic has been crushed. Both antioxidant and antibiotic effect of garlic were reported, for instance, allicin provided antibiotic activity equal to 15 IU or 0.009 mg of penicillin. However, disadvantage of allicin are bad odor, instability and hard to absorb (Benkeblia, 2004). Rahman *et al*. (2012) reported that stability of thiosulfinates can be improved by dilute and dissolve in water. Others thiosulfinates compounds beside allicin such as ajoene, *S*-allylcysteine, and saponin derived from allicin depend on environment and condition during processing were also reported (Majewski, 2014). Generally, fresh bulb and dry powder forms contained alliin as main compounds while in volatile oil contained mainly diallyl sulfide (DAS), diallyl disulfide (DADS) and diallyl trisulfide (DATS). In addition, E-ajoene, Z-ajoene, vinyl-1,3-dithiin and vinyl-1,2-dithiin were found mainly in oil macerate. Moreover, S-allylcystein (SAC), S-allylmercaptocysteine (SAMC) and saponins are water soluble compounds in garlic (Table 13.) (Majewski, 2014).

Table 12. Nutrition value of raw garlic (*Allium sativum* L.) (value/100 g wet basis).

Nutrients fact			
Energy	149 kcal	Lipid	0.50 g
Water	58.58 g	-SFA	0.089 g
Protein	6.36 g	-MUFA	0.011 g
Carbohydrate	33.06 g	-PUFA	0.249 g
-Sugars (total)	1.00 g	Vitamins	
-Fiber	2.10 g	-Vitamin C (ascorbic acid)	31.2 mg
Minerals		-Vitamin B_1 (Thiamin)	0.20 mg

Source: Sharifi-Rad *et al*., 2016

Table 12. Nutrition value of raw garlic (*Allium sativum* L.) (value/100 g wet basis). (continued)

Nutrients fact			
-Calcium (Ca)	181 mg	-Vitamin B_2 (rioboflavin)	0.11 mg
-Iron (Fe)	1.70 mg	-Vitamin B_3 (niacin)	0.70 mg
-Magnesium (Mg)	25 mg	-Vitamin B_6 (piridoxine)	1.23 mg
-Phosphorus (P)	153 mg	-Folate	3.00 µg
-Potassium (K)	401 mg	-Vitamin E (α-tocopherol)	0.05 mg
-Sodium (Na)	17 mg	-Vitamin K (phylloquinone)	1.70 µg
-Zinc (Zn)	1.16 mg		

Source: Sharifi-Rad *et al*., 2016

Table 13. Chemical compounds in various garlic forms.

Product	Compound[A]
Raw fresh garlic	Only alliin about 4-12 mg/g
Garlic essential oil	Only oil soluble sulfur compounds such as diallyl sulfide (DAS), diallyl disulfide (DADS)
Oil garlic extract (macerate)	Oil soluble sulfur compounds and alliin
Dried garlic and garlic powder (capsule)	1. Both alliin and alliinase 2. Low levels of oil soluble sulfur compounds
Aged garlic extract (AGE or water-alcohol extract)[B]	1. High amount of water-soluble compounds such as SAC, SAMC and saponins. 2. Small amounts of oil-soluble sulfur compounds (DADS)

Remark: A means no allicin can be detected in market products due to instability of allicin. B means safety usage reported only AGE

Source: Modified from Majewski, 2014

Majewski (2014) reported that garlic help reduce low density lipoprotein (LDL) cholesterol levels and triglycerides but increase high density lipoprotein (HDL) by inhibition enzyme involving in fatty acid synthase and pentose-phosphate metabolism. Garlic was also claimed as anti-atherosclerotic by reducing mRNA expression of inducible nitric oxide synthase (iNOS). Antimicrobial properties against various species of both Gram-negative and Gram-positive food bacteria such as *Escherichia, Salmonella, Staphylococcus, Streptococcus, Bacillus* and *Clostridium* etc. also found in garlic due to presence of the allyl group in allicin, DADS, DAD and DATS (Majewski, 2014).

Sallam *et al*. (2004) reported that various of garlic forms can reduce lipid oxidation in chicken sausage. Antioxidant activities with high to low was found in fresh garlic then followed by garlic powder, BHA, garlic oil. The lowest antioxidant activity of garlic oil might cause by changing of allicin to other sulfur compounds by heat during distillation (Sallam *et al*., 2004). Horita *et al*. (2016) reported that fresh garlic and garlic extract (pressurized liquid extraction) can reduce lipid oxidation (lower malondialdehyde) of Brazilian low-sodium frankfurters during 60 d of refrigerated storage.

Whereas, increasing of lipid oxidation after adding garlic also found in many research works. Park and Chin (2014) who studied about effect of garlic on lipid oxidation of pork patties reported that addition of garlic increased lipid oxidation in pork patties due to prooxidant property of garlic which was a result from iron in hemoglobin had been oxidized by allicin then change to be methemogoblin (Park and Chin, 2014).

Chen *et al*. (2009) reported that antihypertensive effect of garlic may be consequences of 4 mechanisms: (1) angiotensin-I-Coverting enzyme (ACE) inhibition, (2) vasoconstrictor prostanoids (thromboxane) reduction, (3) nitric oxide (NO) production and (4) peroxynitrite radical scavenger of S-allylcystein.

However, garlic has been reported for negative effect in diabetes patient due to interruption insulin therapy and hypoglycemia and other illness such as HIV,

pneumonia and kidney inflammation. Fresh garlic also causes problem in gastrointestinal tract such as abdominal pain, bloating and allergies (eczema, swollen, anaphylaxis etc.). Finally, children under 10 m, pregnant woman and nursing mother should avoid eating garlic and its derivative in large dose (Majewski, 2014). In addition, garlic also contains inulin (more than 75% dry weight), fructan and manganese (Swetwiwathana *et al*. 1998; Altuntaş and Korukluoglu, 2019; Gupta *et al.*, 2019) which was reported to enhance growth of lactic acid bacteria (Swetwiwathana *et al*., 1998).

1.3 Objectives

1.3.1 To utilize shrimp head waste as raw material for producing reduced sodium shrimp paste as value added product

1.3.2 To investigate the effect of garlic added on quality changes of shrimp head paste

1.3.3 To replace sodium with potassium into a reduced sodium shrimp paste product for lowering a risk of high blood pressure

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CHAPTER 2

PRODUCTION OF REDUCED SODIUM SHRIMP HEAD PASTE

2.1 Abstract

Thailand produced white shrimp (*Litopenaeus vannamei*) around 245,644 tons in 2020 which led to a large amount of low-cost white shrimp by-products especially head and shell. In controvert a decreased main raw material of shrimp paste production was found continuously. In addition, consumption of high sodium food as shrimp paste can lead to various health problems especially hypertension. Therefore, this study aimed to produce a reduced sodium shrimp head paste, using shrimp head as 12, 14 or 16 times to salt instead of 5-8 times to salt as conventional process. The salted mixture was dried at 60 °C after incubation for a night, then ground and fermented further for 30 d. The paste was dried again, until reached 40-45% moisture content, and fermented for another 90 d. The results showed that the total viable count and lactic acid bacteria significantly decreased in all treatments, and no pathogenic microorganisms were detected. After fermentation for 90 d, a_w decreased in all treatments. The highest shrimp head proportion (16:1) exhibited the highest total volatile bases nitrogen (TVB-N), trimethylamine (TMA) thiobarbituric acid reactive substances (TBARS) and degree of hydrolysis (DH) values among the treatments. After fermentation for 90 d, there was no histamine detected while indole was significantly increased. Although, no significantly difference between all treatments sensory score (approximately 6) was found, treatment with shrimp 12 per 1 salt seemed to have higher score in all attribute than other treatments which was lower than that of commercial shrimp paste with approximately 7.5 in 9. Therefore, to compare with the commercial shrimp paste, an improvement of sensory attribute of shrimp head paste is needed.

2.2 Introduction

Shrimp paste is commonly produced and consumed in Southeast Asian countries, including Burma, Cambodia, Indonesia, Malaysia, Myanmar, Philippines, Vietnam, and Thailand (Hajeb and Jinap, 2012). Although raw materials, salt ratios and fermentation times may vary by region and area, the key production methods involving drying, grinding, and fermentation are quite similar (Hajeb and Jinap, 2012). In Thailand, shrimp paste is commonly used as a condiment and is an important ingredient in some traditional main dishes. It is produced by mixing small shrimp, called krill, of *Acetes* and *Mesopodopsis* species with salt at 3-5:1 ratio of shrimp to salt, then dried, ground and fermented for at least 1 m (Pongsetkul *et al*., 2014). However, decreasing marine populations of krill have been noticed over the recent years (World wildlife fund, 2015) including also *Mesopodopsis* raw material of shrimp paste (Pongsetkul *et al*., 2017). In contrast to the scarcity of these raw materials, by-products from shrimp processing industry are increasingly available. This includes head, shell and tail, especially shrimp head that represents up to 45% of the total shrimp weight (Ghorai, 2013). In 2020, Thailand produced around 245,644 tons of shrimp and exported 149,490 tons (Fisheries Economics, 2020) and about 90% of the by-products from shrimp processing have not yet been properly and optimum utilized. In general, solid by-products may be used as animal feed or fertilizer with a low value/price. Recently, shrimp head cost about 5 baht/kg (0.15 USD), which is much less than the 40 Baht/kg (1.23 USD) of *Acetes vulgaris*.

Shrimp head is composed of moisture 78.28 ± 0.39%, protein 12.90 ± 0.15%, fat 4.23 ± 0.35% and ash 5.25 ± 0.11% (Reerueangchai *et al*., 2014), while planktonic shrimp (*Acetes vulgaris*) and krill (*Mesopodopsis*) are composed of moisture 86.74%, 86.25%; protein 8.88%, 12.09%; fat 1.25%, 1.26%; ash 2.95%, 2.27%; and salt 1.26%, 1.16%, respectively (Kongpun, and Kongrat, 2013). Therefore, it is hypothesized that shrimp paste could be produced from shrimp head with proper process.

Generally, a good Kapi (Thai shrimp paste) must have low fishy and ammonia smells, not too salty, without a bitter taste, with various colors such as pinkish, dark grayish brown and purplish gray. It must contain over 36% table salt (dry basis) (Ministry of Industry Thailand, 1992) or 14,400 mg sodium in 100 g of shrimp paste. However, one serving size of shrimp paste (15 g) contains 2,160 mg sodium and is therefore considered high sodium food (any food containing more than 20% of daily intake per serving or 460 mg sodium is considered high sodium food) (The United States Food and Drug Administration, 2018). It is well known that the consumption of high sodium food may increase the risk of hypertension, a major cause of death and various disability worldwide. Excessive sodium intake not only causes hypertension but also can lead to stroke, calcium loss, osteoporosis, cardiovascular disease, and kidney disease (Malta *et al*., 2018; Grillo *et al*., 2019). Therefore, this study aimed to produce a reduced sodium shrimp head paste with reduced risk of hypertension from its consumption.

2.3 Materials and methods

2.3.1 Shrimp paste production

Shrimp head containing pereiopods along with internal organs was received from a frozen food manufacturer in Songkhla province, and was used in this experiment (Figure 3). Since, this work aimed to reduce salt content, therefore, an increased ratio of shrimp to salt was performed from normal or traditional used. These treatments were decided based on preliminary experiments, in which the highest shrimp to salt ratio was up to 8. Shrimp head and NaCl were well mixed together at shrimp to salt ratio 12, 14 or 16 as showed in Table 14. The mixture was incubated indoors for 14-16 hr at room temperature (30-33˚C). After that, the mixture was dried in a hot air oven at 60 °C for 6 h, or until reached 60% moisture content, and was then ground to make the solids finer with grinder machine. After grinding, earthen jar was used to ferment the paste for 30 d on the roof deck at 35-39 ˚C with humidity 77-81%. The paste was again dried at 60 °C for 5 h until reached 40-45% moisture content, following the standard of shrimp paste production (Ministry of Industry Thailand, 1992). The paste was ground and further fermented for 90 d.

Figure 3. Shrimp head used in this experiment

Source: Photographed by author

Table 14. Ratio of shrimp head to salt used in this experiment.

Treatment	Ingredients	
	Shrimp head	**Salt (NaCl)**
Shrimp head 12: salt 1	92.31%	7.69%
Shrimp head 14: salt 1	93.33%	6.67%
Shrimp head 16: salt 1	94.12%	5.88%

2.3.2 Chemical and physical quality analyses

Physical quality indicators including color and a_w, and chemical quality parameters for instance proximate composition, pH, total volatile basic nitrogen (TVB-N), trimethylamine (TMA), thiobarbituric acid reactive substances (TBARS), degree of hydrolysis (DH), histamine and indole were determined for the shrimp head (raw material) and the finished products.

2.3.2.1 Proximate analysis (moisture, crude protein, fat, ash, carbohydrate)

-Moisture (AOAC, 2000)

Moisture content was determined by weighing (Sartorius-Sartorius AG, BSA 224 S-CW, Goettingen, Germany) 3 g of sample and drying it in an oven at 105℃ for 3 h. The sample was reweighed and redried until obtained constant weight. After that, the moisture was calculated as Equation 1.

$$\text{Moisture (\%)} = \frac{W_1 - W_2}{W_1} \times 100 \quad (1)$$

where W_1 = weight of a sample before drying (g)

W_2 = weight of a sample after drying (g)

-Crude protein (AOAC, 2000)

Crude protein content in the sample was determined by adding 0.5-1 g sample to a test tube. Then Kjedahl catalyst was added (mix of K_2SO_4 with $CuSO_4$ in 9:1 ratio) for 5 g along with 200 ml of concentrated H_2SO_4. Blank was prepared by adding Kjedahl catalyst and concentrated H_2SO_4 without a sample to a test tube. Then mixture was boiled until it was clear and then cooled down. After that, 60 ml distilled water was added to the mixture.

The flask was connected with a condenser and 40% NaOH was added, while another flask that contained H_3BO_3 and indicator solution was served as the receiver. The solution was heated until all NH_3 had been distilled. The distilled solution was removed from receiver and titrated with 0.02 N HCl until it turned to colorless. The process was repeated again using the blank. Crude protein content was calculated as Equation 2.

$$\text{Protein (\%)}=(\text{A-B})\times\frac{(\text{A-B})\times\text{N}\times14.007\times5.6}{\text{W}} \quad (2)$$

where A = volume of HCl used to titrate sample (ml)

B = volume of HCl used to titrate blank (ml)

N = Normality of HCl

W = weight of sample (g)

14.007 = atomic weight of nitrogen

5.6 = the protein-nitrogen conversation factor

-Fat content (AOAC, 2000)

Filter paper was used to wrap 3-5 g of sample and weighed. Then the sample was transferred to Soxhlet device. Bottle on the heating mantle was filled with petroleum ether (250 ml) and heated for 14 h at the rate of 150 drops/min until there was no more drip of solvent. The bottle was allowed to cool down and was taken to evaporate the solvent until completely dry, and the dried film was weighed. Fat content (%) was calculated as Equation 3.

$$\text{Fat (\%)}=\frac{\text{Weight of fat}}{\text{Weight of sample}}\times100 \quad (3)$$

-Ash content (AOAC, 2000)

The crucible with 5 g sample was weighed before heating with a Bunsen burner until it no longer produced fumes, and then a furnace was used to heat the sample overnight. After cooling down, ash of the sample was weighed. The ash content was calculated as Equation 4.

$$\text{Ash (\%)}=\frac{\text{Weight of ash}}{\text{Weight of sample}}\times100 \quad (4)$$

-Carbohydrate content

Total carbohydrate content was calculated by subtracting as Equation 5.

$$\text{Carbohydrate (\%)} = 100 - (\text{moisture} + \text{crude protein} + \text{total fat} + \text{ash}) \quad (5)$$

2.3.2.2 Determination of salt content (A.O.A.C, 2000)

The sample and 20 ml 0.1 N $AgNO_3$ were mixed together. After that, 10 ml of concentrated HNO_3 was added. Then the solution was boiled on a hot plate until all solid except for $AgNO_3$ was completely dissolved. Tap water was used to cool down the solution. Ferric alum indicator (ammonium iron sulfate) was added to the solution. After that, the solution was titrated with 0.1 N KSCN until it became light brown. Salt content of the sample was calculated as Equation 6.

$$\text{NaCl(\%)} = \frac{(\text{vol. } AgNO_3 \times \text{Conc. of } AgNO_3) - (\text{vol. of KSCN} \times \text{Conc. of KSCN})}{\text{weight of sample (g)}} \quad (6)$$

2.3.2.3 Water activity (a_w)

Water activity was measured using a water activity analyzer (METER-AQUALAB PRE, Decagon Devices Inc., Washington, USA).

2.3.2.4 pH determination

The sample was homogenized in distilled water at 1:5 ratio and the pH of the mixture was determined by using a pH meter (Sartorius-Sartorius AG, Docu-pH+ Meter, Goettingen, Germany).

2.3.2.5 Total volatile basic nitrogen (TVB-N) and trimethylamine-nitrogen (TMA)

Conway's micro-diffusion assay was used to determine TVB-N and TMA according to method of Junsi (2012). 4% trichloroacetic acid (TCA, 2 ml) was used to extract an 8 g sample. Then the extracted mixture was filtered through Whatman No. 41 and 4% TCA was added until the solution reached 10 ml. After that, the filtrate solution was added into the outer ring of a Conway unit, while boric acid with indicator

was added into the inner ring. Saturated K_2CO_3 was added in the outer ring opposite to the sample. The Conway unit was rotated to mix K_2CO_3 with the sample solution. The solution was incubated at ambient temperature for 3 h. After incubation, 0.02 N HCl was used to titrate the indicator in inner ring until it turned to the initial inner ring color. The process was repeated for the blank without a sample, using TCA solution instead. For determination of TMA, 1 ml of 10% formaldehyde solution was added to the sample solution before saturated K_2CO_3. TVB-N and TMA of sample were calculated as Equation 7.

$$\text{TVB-N or TMA (mg.nitrogen/100g of sample)} = \frac{(N)(14)(A-B)(V)(100)}{\text{weight of sample}} \quad (7)$$

where N = normality of HCl

A = ml of HCl used to titrate sample mixture

B = ml of HCl used to titrate blank

V = total volume of sample and TCA in sample preparation

2.3.2.6 Determination of thiobarbituric acid reactive substances (TBARS)

Determination of thiobarbituric acid reactive substances was determined according to method of Buege and Aust, 1978 with slight modification by using 5000 x g for 25 min instead of 3,600 x g form 20 min. Sample and thiobarbituric acid (TBA) solution were mixed and heated for 10 min at 95 ˚C, after which the mixture was centrifuged at 5000 x g for 25 min. The supernatant was testes for absorbance at 532 nm. Malondialdehyde (MDA) was used as the standard and TBARS was calculated to mg MDA/kg sample.

2.3.2.7 Degree of hydrolysis (DH)

Degree of hydrolysis was determined according to the method of Qi *et al.*, 1997 with slight modification. The sample was mixed with 20% TCA solution at 1:1 proportion. The mixture was left for 30 min, then centrifuged at 10,000 x g. After that, the supernatant was titrated with 0.02 N HCl. The DH was calculated as Equation 8.

$$\text{DH \%} = \frac{(\text{amount N soluble in 10\% TCA})(100)}{\text{Total N in sample}} \quad (8)$$

2.3.2.8 Histamine and Indole

Histamine analysis was sent out to investigate at ALS Laboratory Group (Thailand) Co., Ltd. while indole was analysed at Central laboratory (Thailand) Co., Ltd. Both laboratories are ISO/IEC 17025:2017 certified.

2.3.3 Microbiological quality analyses

Microbiological quality measurement, including TVC, coliforms, *Escherichia coli*, *Clostridium perfringens*, *Salmonella*, *Staphylococcus aureus*, yeast and mold, and lactic acid bacteria (LAB), were determined for the shrimp head (raw material) and the finished products.

2.3.3.1 Total viable count or total mesophilic count (TVC or TMC)

TVC was analyzed according to the method of The United States Food and Drug Administration (2001a) with some modifications (using 0.85% NaCl solution instead of 0.1% peptone water). Briefly, sample and 0.85% NaCl in distilled water were mixed together. The mixture was added to blender jar and blended to 10^{-1} dilution. Then appropriate dilutions were made. One ml of each dilution was transferred to a plate. Then 15 ml of plate count agar was added to the plate. The plate was rotated to spread agar, and the agar was allowed to solidify, then it was inverted and incubated at 35 °C for 48 h. Plates that contained 30-300 colonies were counted and recorded in colony forming units per gram (log CFU/g).

2.3.3.2 Coliform bacteria

Coliform bacteria were analyzed according to the method of The United States Food and Drug Administration (2020) with some modifications (using 0.85% NaCl solution instead of 0.1% peptone water). The sample was prepared as if it was for TVC. Each dilution was transferred to 3 lactose broth tubes. All tubes were incubated at 35°C for 24 h. The tubes containing gas were recorded and further incubated for another 24 h to record the gas production. One loopful from lactose broth tubes was transferred to brilliant green lactose bile (BGLB) broth tube. The tubes were incubated at 35 °C for 48 h. All tubes containing active gas were recorded to calculate the most probable number (MPN).

2.3.3.3 *Staphylococcus aureus*

Staphylococcus aureus was analyzed according to the method of The United States Food and Drug Administration (2016) with some modifications (using 0.85% NaCl solution instead of 0.1% peptone water). Sample was prepared similarly as for TVC. One ml of each dilution was divided to 0.1 ml aliquots and transferred to plates with Baird-Parker (BP) agar. The plates were incubated. Plates that contained 20-200 colonies were selected and counted before reporting “detected” or “not detected” per 0.1 g.

2.3.3.4 *Salmonella*

Salmonella was analyzed according to the method of The United States Food and Drug Administration (2021) with some modifications (using 0.85% NaCl solution instead of 0.1% peptone water). Sample and sterile lactose broth were mixed together and added into sterile blending container. The mixture was blended and transferred to container and incubated at room temperature for 1 h. After incubation, the mixture was transferred to Rappaport-Vassiliadis (RV) medium and Tetrathionate broth (TT). Then RV medium and TT broth were incubated in water bath at 42 ± 0.2°C and 43 ± 0.2°C for 24 ± 2 h respectively. Thereafter, the mixture was shaken. One loopful of incubated sample in TT broth and RV medium were streaked on bismuth sulfite (BS) agar, xylose lysine desoxycholate (XLD) agar, and Hektoen enteric (HE) agar. Plates containing BS, XLD, and HE agar were incubated at 35 °C for 24 h. Any

plate that had more than one colony of *Salmonella* was selected and sample from the selected plate was streaked on triple sugar iron agar (TSI) and lysine iron agar (LIA) and incubated.

2.3.3.5 *Clostridium perfringens*

Clostridium perfringens was sent out to perform according to Bam 2001 chapter 16 at ALS Laboratory Group (Thailand) Co., Ltd. with ISO/IEC 17025:2017 certified.

2.3.3.6 Yeast and mold

Yeast and mold were analyzed according to the method of The United States Food and Drug Administration (2001c) with some modifications (using 0.85% NaCl solution instead of 0.1% peptone water). Sample and 0.85% NaCl in distilled water were mixed together to make 10^{-1} dilution. The mixture was homogenized. Appropriate dilutions were made. One ml of each sample dilution was transferred into plates. After that dichloran 18% glyceral (DG18) agar was added. Plates were mixed by swirling clockwise and counterclockwise then incubated in the dark at 25 °C. The incubated plates containing 10-150 colonies were selected for counting. Number of microbial colonies was recorded in log CFU/g.

2.3.3.7 Lactic acid bacteria (LAB)

LAB was determined according to Pongsetkul *et al.* (2017a) with some modifications (using normal MRS instead of MRS agar plus 10% NaCl) as the sample was prepared similarly as for TVC, and 1 ml of each dilution was transferred to plate. Then De Man, Rogosa, and Sharpe (MRS) agar with 0.0005% bromocresol purple with pH 7.5 was incubated at 30 °C for 3 d.

2.3.4 Sensory evaluation

All treatments (shrimp head paste after fermentation for 90 d) were evaluated by a 9- point Hedonic scale test with 9 (like extremely) to 1 (dislike extremely). Fifty untrained panelist who were familiarized with shrimp paste would be asked used to determine 6 attributes based on appearance, color, odor, taste, texture,

and overall acceptability and this evaluation was performed in laboratory. During evaluation, water, cucumber and raisin were used to refreshing panelist between samples.

2.3.5 Statistical analysis

The experiment was conducted in three replicates and data from each experiment were analyzed by one-way analysis of variance and presented as mean ($\bar{x}$) ± standard deviation (S.D.). Means comparisons were evaluated by Tukey's multiple range test. Completely randomize design (CRD) was used in testing chemical, physical, and microbiological properties while randomized completely block design (RCBD) was used in sensory evaluation.

2.4 Results and discussion

2.4.1 Chemical and physical qualities

2.4.1.1 Proximate compositions

Initial quality terms as, moisture, carbohydrate, protein, fat and ash of shrimp head were 75.38±0.51%, 7.95±0.62%, 11.22±0.21%, 0.95±0.07% and 4.51±0.08%, respectively. Proximate composition of the shrimp head used in this experiment was similar to the proximate composition of Brazilian white shrimp (*Litopenaeus vannamei*) head, containing above mentioned components at 75.47±0.43, 4.33, 14.75±0.79, 1.10±0.10 and 4.35±0.28 in the same order (Fernandes *et al*., 2013), and also close to those of *Acetes vulgaris* and *Mesopodopsis* raw materials used to produce Kapi, which contained moisture at 86.74%, 86.25%; protein 8.88%, 12.09%; fat 1.25%, 1.26% and ash 2.95%, 2.27, respectively (Kongpun and Kongrat, 2013). At the end of experiment (90 d), it was found that moisture content decreased from 75.38±0.51% down to 42.5%. Similar decreasing trend was found for carbohydrate content, which decreased from 7.95±0.62 to 4.14-5.29% (wet basis). However, protein content increased from 11.22% to 22.70-26.1% (wet basis) due to both fermentation and drying. Expectedly, the ash increased from 4.51±0.08% to 20.60-24.70% (wet basis) as a result of the salt added. When more salt was added the ash content increased. Comparison of the experimental shrimp head paste with a commercial one indicated a

large difference in carbohydrate, maybe due to starch and/or sugar that are usually added to improve texture, flavor and taste attributes, as well as to reduce the price. The proximate composition of commercial Kapi in Thailand contained moisture 33.79-52.54% (wet basis), carbohydrate 2.91-19.41% (wet basis), protein 17.59-29.77% (wet basis), fat 0.75-1.76% (wet basis) and ash 17.18-29.68% (wet basis) (Pongsetkul *et al*., 2014).

2.4.1.2 Salt content

Salt content of the initial white shrimp head was 1.22±0.08% (4.96±0.32% dry basis), which was similar to the salt contents 1.26% (8.44% dry basis) and 1.16% (9.50% dry basis) reported for *Acetes vulgaris* and *Mesopodopsis*, respectively (Kongpun and Kongrat, 2013). At the end of experiment (90 d), salt contents of shrimp pastes made at ratios 12:1, 14:1 and 16:1 were 13.95±0.62% (24.26±1.04% dry basis), 10.34± 1.26% (20.68±2.52% dry basis) and 9.37±0.57% (16.76±1.02% dry basis) which were much lower than the 14.72-22.93% (22.77-35.47% dry basis) of commercial Kapi (Pongsetkul *et al.*, 2014). When compared to a traditional Kapi, it pointed out that the experiment shrimp head paste could be reduced salt content by at least 25% in shrimp paste made with ratio 14:1 and 16:1.

2.4.1.3 Water activity (a_w)

After salting the a_w before first drying in all treatments decreased but still exceeded 0.91 (Figure 4), which normally cannot retard the growth of spoilage microorganisms (Majumdar *et al*., 2018). However, the a_w decreased further. Therefore, this may explain the reduction of TVC and LAB in some treatments. Clearly, a_w in all treatments decreased until it reached approximately 0.80. During fermentation, after second drying step, the a_w in all treatments was about 0.78. The higher the salt ratio (12:1) the lower a_w was noticed, such that supports halophilic growth mainly (Resnik and Chirife, 1988).

Comparison of a_w with TVC and LAB indicated that a lesser a_w lowered both microbial values. Both salt addition and drying process reduced the growth of general microorganisms (Pittia and Paparella, 2016).

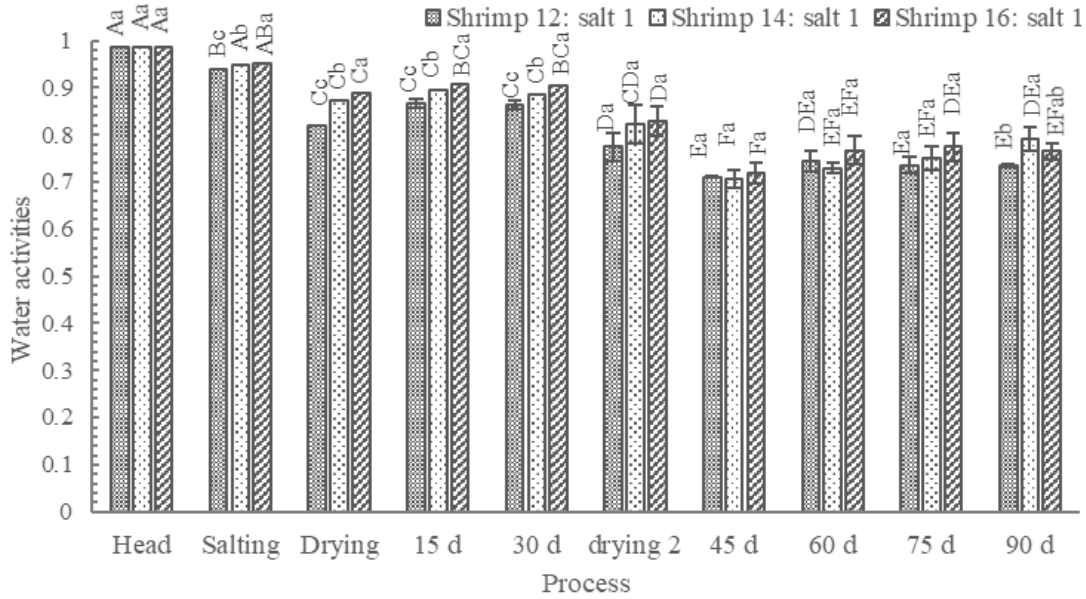

Figure 4. Water activity (a_w) of shrimp head (*Litopenaeus vannamei*) and of shrimp paste with shrimp head to salt ratios 12, 14 or 16. Different uppercase letters indicate significant differences within a salt ratio. Different lowercase letters indicate significant difference within a process ($p < 0.05$). Head means shrimp head; Salting is after salting step 14-16 h; Drying is after primary drying step; 15d is after fermentation for 15 d; 30d is after fermentation for 30 d; Drying2 is after secondary drying; 45d is after fermentation for 45 d; 60d is after fermentation for 60 d; 75d is after fermentation for 75 d; 90d is after fermentation for 90 d (end of fermentation).

2.4.1.4 pH

After salting the pH in all treatments increased, matching the increased TVB-N and TMA as functions of spoilage microorganisms and endogenous enzymes (Etienne, 2005) (Figure 5). Then the pH decreased after drying from evaporation of TVB-N and TMA (O'Neil, 2013). It is interesting that pH of the 16:1 treatment decreased after 15 d, and then increased to equal the other ratios after 30 d. After secondary drying, pH in all treatments decreased again. However, decreasing pH was observed after 45 d except for the ratio 16:1. After that pH in all treatments increased and then stayed constant until 90 d. In addition, there were no differences in pH between the ratios, except for the ratio 16:1 in some process stages. This might be because of

the highest water activities for ratio 16:1 lead to increasing of alkaline compounds such as ammonia, trimethylamine and ketone from protein degradation by spoilage microorganisms.

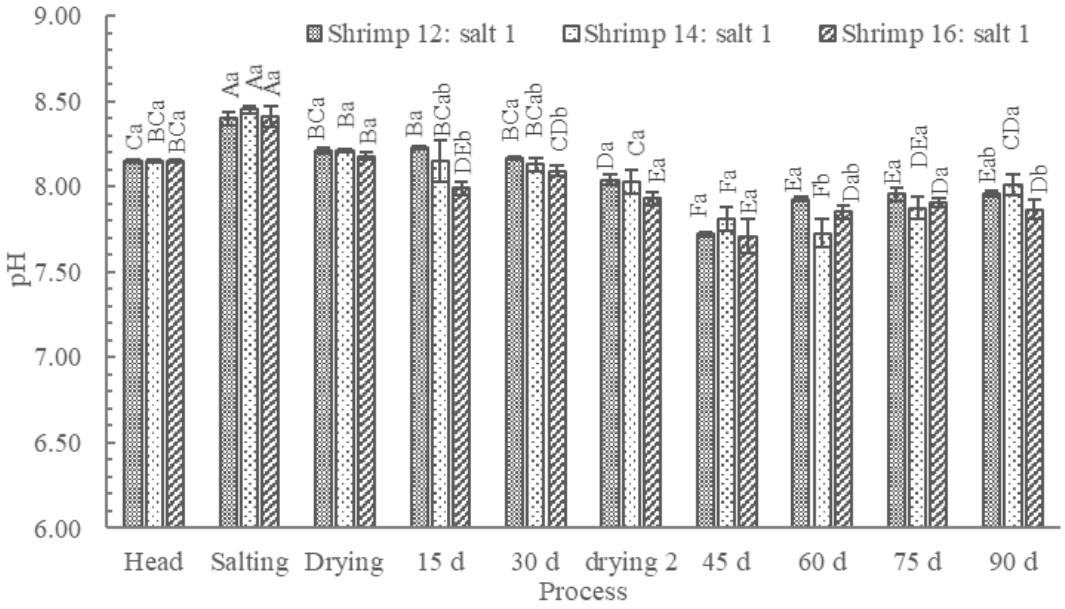

Figure 5. pH of shrimp head (*Litopenaeus vannamei*) and of shrimp paste prepared with shrimp head to salt ratios 12, 14 or 16. Different uppercase letters indicate significant difference within a salt ratio. Different lowercase letters indicate significant difference within a process ($p < 0.05$). Head means shrimp head; Salting is after salting step 14-16 h; Drying is after primary drying step; 15d is after fermentation for 15 d; 30d is after fermentation for 30 d; Drying2 is after secondary drying; 45d is after fermentation for 45 d; 60d is after fermentation for 60 d; 75d is after fermentation for 75 d; 90d is after fermentation for 90 d (end of fermentation).

In general, pH of raw shrimp head was about 8, possibly increased by enzymatic hydrolysis after death of the shrimp (Guo *et al.*, 2019). The pH results may indicate that the raw material had a high content of alkaline compounds. In addition, pH in all treatments kept increasing after salting. This suggests that there were some chemical reactions taking place. It is known that both TVB-N, TMA and other amine compounds are alkaline. These compounds actually can be derived from endogenous (autolysis) and exogenous enzymes (microorganism enzymes) as time passes (Etienne,

2005). In fact, high activities of digestive enzymes such as protease, lipase and amylase are found in the shrimp head (Kanduri and Eckhardt, 2009). In addition, the digestive tract also carries gut microflora dependent on the food and environment that the shrimp was cultured in (Li *et al*., 2019). For these reasons, spoilage signs definitely emerge first at the head or a shrimp. It is pointed out that while the shrimp head can be used for a fermented product, some quality parameters such as pH and TVB-N as well as TMA may not be on desired level.

2.4.1.5 Total volatile basic nitrogen and trimethylamine (TVB-N and TMA)

As shown in Figure 6 and Figure 7, both TVB-N and TMA significantly increased after salting. Particularly the TVB-N increased along the fermentation process. However, every drying step reduced both TVB-N and TMA. This might be because the volatile compounds are sensitive to temperature. However, a different change pattern was found for TMA that seemed to increase with fermentation time. Actually, TMA is used to indicate fishy odor, which also is associated with spoilage or interior quality of a raw seafood product (Herath *et al*., 2019). However, TMA is also an essential compound for proper smell in some fermented fish products, particularly in fish sauce (Yimdee and Wang, 2016). Generally, changes of both TVB-N and TMA during fermentation were similar to a wave going up and down or not consistently increasing with fermenting time as might be expected. This may be due to some chemical reaction like the browning reaction, which can consume ammonia as amine compound to react with carboxylic group of sugar or carbohydrate compound (Izzo and Ho, 1992). The volatile compounds could also change as a result of chemical reactions from both microbial action and its metabolites, as well as from autolysis from indigenous enzyme (Etienne, 2005).

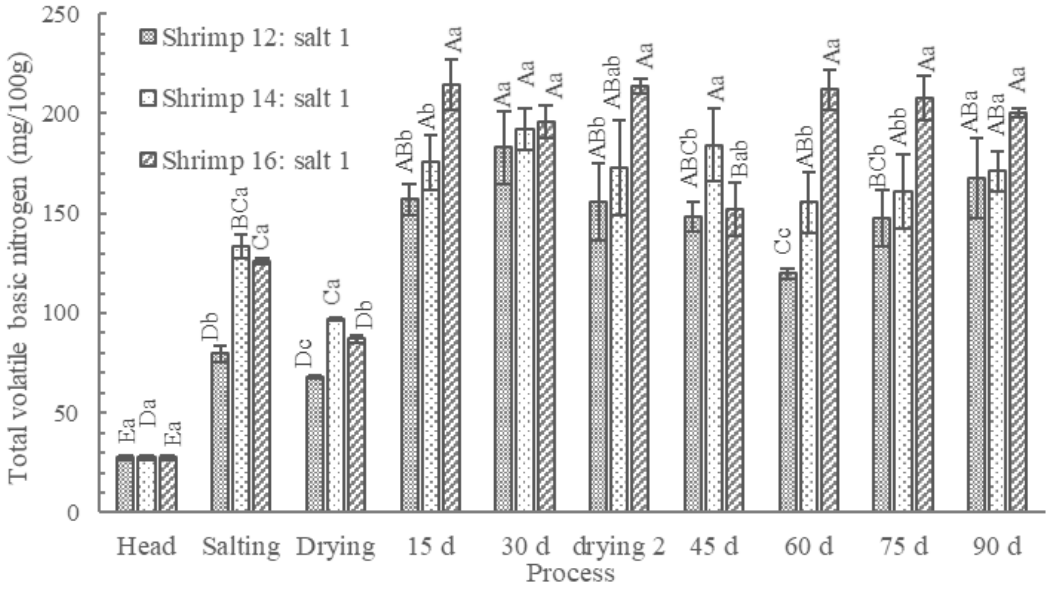

Figure 6. Total volatile basic nitrogen (TVB-N, mg/ 100 g) of shrimp head (*Litopenaeus vannamei*) and shrimp pastes prepared with shrimp head to salt ratio 12, 14 or 16. Different uppercase letters indicate significant difference within a salt ratio. Different lowercase letters indicate significant difference within a process ($p < 0.05$). Head means shrimp head; Salting is after salting step 14-16 h; Drying is after primary drying step; 15d is after fermentation for 15 d; 30d is after fermentation for 30 d; Drying2 is after secondary drying; 45d is after fermentation for 45 d; 60d is after fermentation for 60 d; 75d is after fermentation for 75 d; 90d is after fermentation for 90 d (end of fermentation).

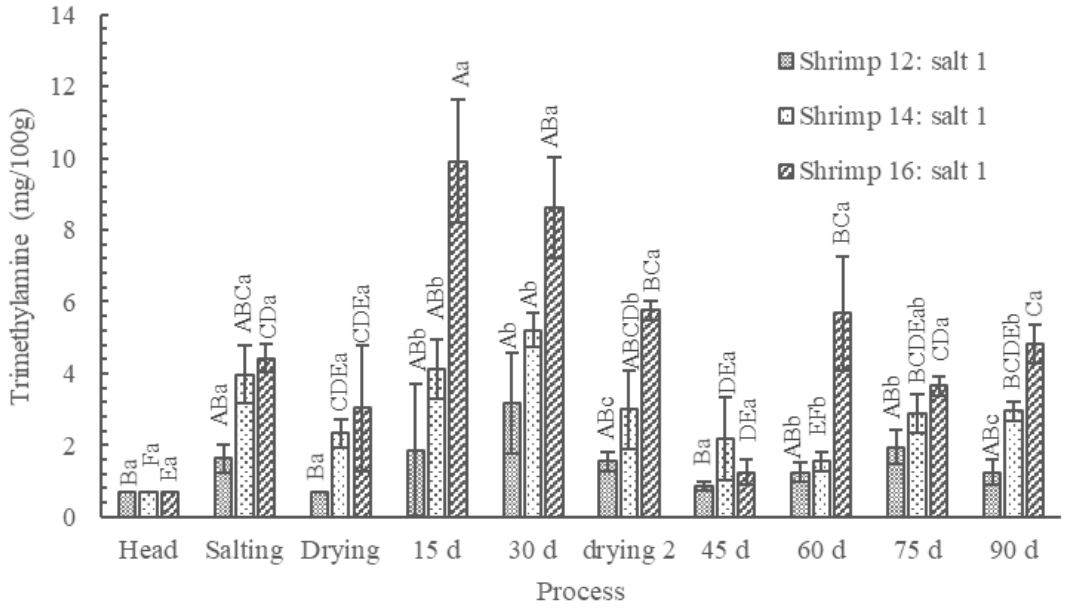

Figure 7. Trimethylamine (TMA, mg/ 100 g) of shrimp head (*Litopenaeus vannamei*) and shrimp paste prepared with shrimp head to salt ratio 12, 14 or 16. Different uppercase letters indicate significant difference within a salt ratio. Different lowercase letters indicate significant difference within a process ($p < 0.05$). Head means shrimp head; Salting is after salting step 14-16 h; Drying is after primary drying step; 15d is after fermentation for 15 d; 30d is after fermentation for 30 d; Drying2 is after secondary drying; 45d is after fermentation for 45 d; 60d is after fermentation for 60 d; 75d is after fermentation 75 d; 90d is after fermentation for 90 d (end of fermentation).

In addition, it was found that at a higher salt content (12:1), TVB and TMA were lower than in other treatments. This may due to the spoilage microorganisms normally not being halophilic bacteria or halo tolerant bacteria (Larsen, 1986) could not grow. It is known that TVB-N consists of ammonia, trimethylamine and dimethylamine, which are all volatile alkaline compounds. Therefore, TVB-N should positively correlate with the pH. However, changes in pH of the system actually relate to the amounts of bases, acids and the buffering capacity. An increased TVB-N and TMA may not increase pH, because also acids are generated, or because of the buffering capacity including protein and amino acids of the system (Bhagavan and Ha, 2015; Pongsetkul *et al.*, 2017b).

2.4.1.6 Determination of thiobarbituric acid reactive substances (TBARS)

TBARS in this experiment was shown in Table 15. TBARS of shrimp head in this experiment was 4.67±0.13 which closed to preliminary experiment (4.47±0.28 mg MDA/kg) and considered as rancidity (more than 2.5 mg MDA/kg) (Domínguez *et al*., 2019). This may due to high unsaturated fatty acid containing in shrimp head. However, TBARS of *Acetes vulgaris* (raw material to produce commercial shrimp paste) which was also contained high unsaturated fatty acid (56.06%), was lower (around 0.6 mg MDA/kg) than shrimp head in this experiment (Pongsetkul *et al*., 2017). As mentioned before that shrimp head was susceptible to lipid oxidation, therefore, rancidity in shrimp head may start since harvesting period and processing before obtaining to run experiment. In addition, by-product has not had well manage, thereafter deteriorate process was easy to undergo especially shrimp head which contained high both substrates, enzymes and microorganisms.

Table 15. TBARS in shrimp head (*Litopenaeus vannamei)* and shrimp paste with shrimp head 12, 14 and 16 per 1 salt after fermentation for 90 d.

Process	TBARS value (mg/kg)		
	12:1	**14:1**	**16:1**
Shrimp head	4.67±0.13^{d}	4.67±0.13^{d}	4.67±0.13^{d}
90 d fermentations	9.80±0.42^{b}	6.87±0.57^{c}	11.09±0.78^{a}

Remark: Different letters indicate significant differences ($p < 0.05$).

2.4.1.7 Degree of hydrolysis (DH)

In this experiment, DH was showed in Table 16. DH of shrimp head in this experiment was around 2.4% which was in the range of previous study. This indicated that freshness of shrimp head used in this experiment was comparable to preliminary test. In addition, DH in this experiment significantly increased after fermentation for 90 d. As expected, DH decreased with increasing of salt ratio. This could be explained by reduction of water activity and microbial activity as well as proteolysis enzyme activity. In addition, DH of shrimp head paste after fermentation

for 90 d was similar to commercial shrimp paste and another experiment which ranged 12.68-20.67% and 10.72±0.61%, respectively (Pongsetkul *et al*., 2014).

Table 16. Degree of protein hydrolysis of shrimp head (*Litopenaeus vannamei)* and shrimp paste with shrimp head 12, 14 and 16 per 1 salt after fermentation for 90 d.

Sample	Degree of protein hydrolysis (%)		
	12:1	**14:1**	**16:1**
Shrimp head	2.12±0.20^{d}	2.66±0.21^{d}	2.81±0.25^{d}
90 days fermentations	10.72±0.61^{c}	14.28±0.30^{b}	16.03±1.10^{a}

Remark: Different letters indicate significant differences ($p < 0.05$).

2.4.1.8 Histamine and indole

As shown in Table 17, an increasing of histamine in all treatments were found after secondary drying. However, after fermentation for 90 d, histamine decreased until not detected while indole was found in all treatments. A decreasing of histamine might be caused by some microorganism presence in the product. Kung *et al*. (2017) reported that *Lactobacillus plantarum* isolated from Miso products can degrade histamine. In addition, eight histamine-degrading bacteria including *Rummeliibacillus stabekisii*, *Agrobacterium tumefaciens*, *Bacillus cereus*, *Bacillus polymyxa*, *Bacillus licheniformis*, *Bacillus amyloliquefaciens*, and *Bacillus subtilis* were found in salted fish product (Lee *et al*., 2015).

Generally, during production of fermented food particularly protein based, toxic biogenic amine including histamine and indole is producing (Sang *et al.*, 2021). Histamine which is often used to measure the decomposition produce from histidine by histamine producing bacteria including *Vibrio parahaemolyticus*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Proteus mirabilis* etc. Histamine can be found often in Scombroid fish, fermented fish products, wine, cheese, meat products and etc. (Sadayan and Thangaradjou, 2007; Satomi, 2016). Consumption of food containing histamine may cause various symptom including hypertension, hypotension, headache, urticaria, nausea, and vomiting. CODEX Alimentarius stated that fish and fish products

must contain histamine lower than 100 mg/kg and lower than 400 mg/kg for fish sauce for being consumer safe. Furthermore, the European Union (EU) also stated that histamine in fish and fish products must lower than 100 mg/kg while fermented product not higher than 200 mg/kg (Satomi, 2016).

Universal spoilage indicator of shrimp is indole content which is produced from break down of trytophan by spoilage bacteria such as *Bacillus alvei*, *E. coli*, *Shigella* sp., *Enterococcus faecalis*, and *V. cholerae* (Lee and Lee, 2010; Pongsetkul, 2018). In addition, Pongsetkul (2018) reported that indole of commercial shrimp pastes varied from ND to 1852 at peak area and the shrimp paste producing from *Acetes vulgaris* after fermentation for 30 dcontained indole as 180.45 at peek area. Based on US FDA guideline, shrimp product was considered as spoilage when indole presence more than 25 µg/100 g (National Research Council (US) Subcommittee on Microbiological Criteria, 1985). Based on histamine and indole value it confirmed that both raw material and products in this experiment after fermentation for 90 d could be consumption safely.

Table 17. Histamine and indole of shrimp head (*Litopenaeus vannamei)* and shrimp paste with shrimp head 12, 14 and 16 per 1 salt after fermentation for 90 d. (mg/ kg).

Sample	**12:1**		**14:1**		**16:1**	
	Histamine	**Indole**	**Histamine**	**Indole**	**Histamine**	**Indole**
Shrimp head	5.57	ND	5.57	ND	5.57	ND
After 2nd time drying	20.5	-	19.6	-	28.2	-
90 d	ND	0.14	ND	0.20	ND	0.13

Remark: ND mean not detected. – mean measure

2.4.2 Microbiological qualities

2.4.2.1 Total viable count (TVC)

Interestingly, TVC in all treatments significantly increased after salting and did not decrease after drying as would be expected (Figure 8). This may be due to not having high enough salt content, as indicated by the high a_w (0.910). In addition, temperature of sample during first time drying was around 41℃, which is in the optimum temperature range of most mesophilic microorganisms (20-45℃) (Keenleyside, 2019). A high a_w with not too high temperature (< 50 °C) supports microbial growth, particularly microflora in mesophilic and thermophilic groups. Remarkably, the TVC of ratio 12:1 treatment seemed to be higher than for the other treatments, even the highest in some fermentation steps. Based on the percentage of salt there should have been more antimicrobial activity, and TVC of 12:1 treatment should be the lowest among the ratios tested. This may be due to two reasons: (1) microbial profile of the raw material could be halophilic bacteria in brackish water shrimp; and (2) amount of salt used was suitable, supporting bacterial growth as a result of high a_w (Pakdeeto, 2016**)**. Additionally, it was found that TVC in all treatments decreased with fermentation time after the secondary drying step. A decrease of TVC during fermentation may be due to a reduction in a_w mainly from drying. Based on microbiological standards, this indicates that the shrimp paste produced from shrimp head at ratios 12:1, 14:1 and 16:1 to salt was safe to consume. However, it was noted that the smell of treatments with 14:1 and 16:1 ratios was quite foul, similar to a dead rat, after the secondary drying, although that smell was not as strong as before drying. This indicates that a too low salt content may allow growth of some spoilage bacteria. Therefore, TVC may not be an appropriate indicator of quality, instead spoilage bacteria should be determined in a further study.

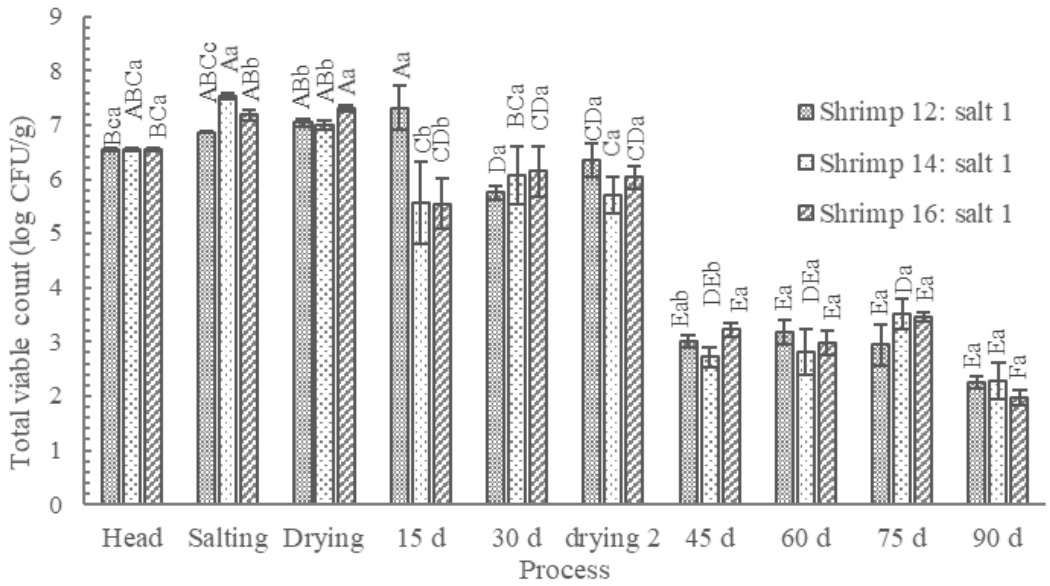

Figure 8. Total viable counts in shrimp head (*Litopenaeus vannamei*) and in shrimp paste prepared with shrimp head to salt ratio 12, 14 or 16. Different uppercase letters indicate significant difference within a salt ratio. Different lowercase letters indicate significant difference within a process ($p < 0.05$). Head means shrimp head; Salting is after salting step 14-16 h; Drying is after primary drying step; 15d is after fermentation for 15 d; 30d is after fermentation for 30 d; Drying2 is after secondary drying; 45d is after fermentation for 45 d; 60d is after fermentation for 60 d; 75d is after fermentation 75 d; 90d is after fermentation for 90 d (end of fermentation).

2.4.2.2 Lactic acid bacteria (LAB)

Changes in amount of LAB in shrimp paste had trends similar to TVC, with a sharp decrease after the secondary drying (Figure 9). Decrease of LAB after secondary drying may be from decreased water activity (Battcock and Azam-Ali, 2001). An increase in LAB after fermentation for 90 d was noted but it did not exceed 3 log CFU/g. This indicates that LAB population might recover. This was similar to the other fermented products, including fish sauce and soy sauce, because of the natural changes in a live population.

As is known, LAB growth can provide antimicrobial substances such as lactic acid, bacteriocin, hydrogen peroxide and so on (Sudalayandi, 2011), which can reduce or retard the growth of other microorganisms and their metabolites (Saranraj *et al.*, 2013). Therefore, a higher LAB count may inhibit volatile compounds accumulation, which is associated with the pH and TVB-N values.

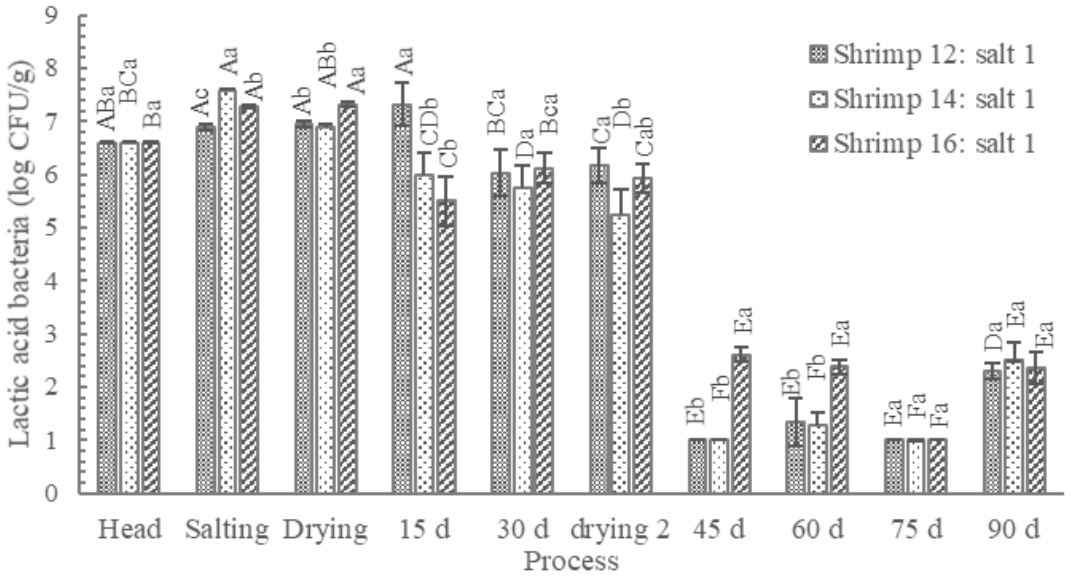

Figure 9. Amount of lactic acid bacteria in shrimp head (*Litopenaeus vannamei*) and shrimp paste prepared with shrimp head to salt ratio 12, 14 or 16. Different uppercase letters indicate significant difference within a salt ratio. Different lowercase letters indicate significant difference within a process ($p < 0.05$). Head means shrimp head; Salting is after salting step 14-16 h; Drying is after primary drying step; 15d is after fermentation for 15 d; 30d is after fermentation for 30 d; Drying2 is after secondary drying; 45d is after fermentation for 45 d; 60d is after fermentation for 60 d; 75d is after fermentation for 75 d; 90d is after fermentation for 90 d (end of fermentation).

2.4.2.3 Pathogenic microorganisms

Staphylococcus aureus, Clostridium perfringens and *Salmonella* that are pathogenic bacteria were not found. Coliform and yeast and mold were lower than 3 MPN/g and 2.15 log CFU/g, respectively. The results confirmed that initial raw material used in this experiment was of quite high quality in terms of microbiological standards, maybe due to both good agricultural practices (GAP) and good manufacturing practices (GMP). It also proved that the raw material used in this experiment was safe to produce and to consume. Yeast and mold decreased and disappeared after the first drying step, maybe due to sanitation and fermentation processes.

2.4.3 Sensory evaluation

Sensory score of commercial shrimp paste, shrimp head paste shrimp head 12, 14 and 16 per 1 salt were significant difference (Table 18). Difference score might mainly cause by different between raw material used including species (commercial one used krill) and freshness. However, commercial shrimp paste usually added sugar, monosodium glutamate and sweet potatoes to improve the quantity and quality (marketing survey and personal data during plant visiting). Low score in appearance and texture attribute was a result of coarse and rough texture and appearance which caused by hardness of shrimp head. High calcium (3,394 mg/100g) was found in shrimp while krill only contained half (1,565 mg/100g) (Benjakul *et al*., 2011). High chitin content (27.00 ± 0.20 % dry basis) might also contribute to hardness of shrimp head (Diaz-Rojas *et al*., 2006). This problem may solve in the future by using of chitinase enzyme to breakdown chitin. Another problem was odor which was a bit far from commercial one. Although, no significantly difference between all treatments was found, treatment with shrimp 12 per 1 salt seemed to have higher score in all attribute than other treatments. This indicated that shrimp head needed proper salt content to control autolysis and bacterial growth.

Table 18 Sensory score shrimp paste with shrimp head 12, 14 and 16 per 1 salt after fermentation for 90 d and commercial shrimp paste.

Sample	Treatment					
	Appearance	**Color**	**Texture**	**Odor**	**Taste**	**Overall**
12:1	5.70± 1.53^{b}	6.02± 1.25^{b}	5.50± 1.62^{b}	5.92± 1.70^{b}	6.10± 1.39^{b}	6.08± 1.32^{b}
14:1	5.46± 1.57^{b}	5.78± 1.47^{b}	5.32± 1.50^{b}	5.58± 1.69^{b}	5.66± 1.47^{b}	5.64± 1.29^{b}
16:1	5.42± 1.47^{b}	5.68± 1.19^{b}	5.26± 1.66^{b}	5.68± 1.80^{b}	5.68± 1.67^{b}	5.70± 1.39^{b}
Commercial shrimp paste	7.68± 1.00^{a}	7.56± 1.13^{a}	7.48± 1.30^{a}	7.64± 1.10^{a}	7.62± 1.05^{a}	7.88± 0.77^{a}

Remark: Different uppercase letters indicate significant differences within column ($p < 0.05$).

2.5 Conclusion

Microbiological qualities and pH did not much differ between the salt ratios tested. However, a higher salt ratio led to a_w, TVB-N, TMA and DH reduction. Moreover, the drying step seemed to have a larger sized effect on a_w than the salt ratio range tested. Shrimp head can be a great alternative source for making shrimp paste (actually an equivalent closely similar product). Based on histamine and indole content, fermented shrimp head at ratio shrimp 12, 14 and 16 per 1 salt was safe for consumption.

Although, some quality including degree of hydrolysis in all treatments after fermentation for 90 d was close to commercial shrimp paste, sensory quality of all treatments in this experiment was lower than the commercial shrimp paste. Using of enzyme to improve chitin hydrolysis or added sugar may necessary to solve the texture and odor problem and improve sensory quality in the further.

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CHAPTER 3

PRODUCTION OF SHRIMP HEAD PASTE ADDED WITH GARLIC

3.1 Abstract

Head of *Litopenaeus vannamei* is a low-cost by-product that was used to make fermented shrimp paste instead of the traditional raw material, which is becoming a scarce resource. However, internal organ in shrimp head mainly consisted of unsaturated fatty acid which is susceptible to lipid oxidation. To retard lipid oxidation, therefore garlic providing some biological properties was added at 0%, 3% and 5% in shrimp head paste production. Chemical, physical, microbiological and sensory quality during fermentation for 90 d were determined. Moisture, protein and ash were main compositions of shrimp head paste added with garlic 0%, 3% and 5%. The reduction of water activity (a_w) was found in all treatments while pH seemed to kept constant during fermentation. In addition, total volatile bases nitrogen (TVB-N), trimethylamine (TMA), thiobarbituric acid reactive substances (TBARS) and degree of hydrolysis (DH) significantly increased in all treatments. Addition of garlic at 3% and 5% seemed to retard of lipid oxidation compared to control sample without added garlic. After fermentation for 90 d, while indole was significantly noticed, there was no histamine detected. Total viable count (TVC) decreased to around 2.54 log CFU/g while lactic acid bacteria (LAB) decreased to reach 1 log CFU/g in all treatments. Pathogenic microorganisms were not found in all treatments after fermentations for 90 d. Sensory score of all treatments were approximately 6 which lower than that of commercial shrimp paste with approximately 7.5 in 9. Improvement of sensory is needed in the future work.

3.2 Introduction

Thailand is among the top 5 global seafood exporters, particularly with the white shrimp production. Shrimp head accounts for about 40% of the total body weight and is judged as by-product having low price and energy consumption problem. Recently, only about 5% of the shrimp heads are utilized, with low profit. All frozen shrimp industries attempt to develop or convert shrimp heads to be higher value items

or demanded products. Shrimp paste or Kapi is an essential ingredient in various Thai dishes. Generally, main raw material to produce Kapi are *Mesopodopsis* spp. and *Acetes vulgaris* (Kleechaya *et al.*, 2021) which undergoes to decline continuously. Therefore, using shrimp head to produce shrimp paste would be an attractive alternative way to prevent essential condiment collapse.

According to preliminary data dealing on nutritional values, shrimp head contained moisture, carbohydrate, protein, fat and ash at 75.38±0.51%, 32.28±2.52% (dry basis), 45.55±0.85% (dry basis), 3.86± 0.28% (dry basis) and 18.31±0.32% (dry basis), respectively. In addition, shrimp heads are source of unsaturated fatty acids (Reerueangchai *et al*. 2014) which composed of monounsaturated fatty acid (MUFA) as 25.91±0.05% and polyunsaturated fatty acids (PUFA) 39.30±0.02% of total lipid (Takeungwongtrakul, 2014). Among PUFA, linoleic acid (LA) (19.69±0.02%) was the most omega-6 fatty acid followed by docosahexaenoic acid (DHA) (8.34±0.02%) and eicosapentaenoic acid (EPA) (4.65±0.01%). As well known that LA, DHA and EPA are essential fatty acids having various health benefits including prevention of cardiovascular diseases, myocardial infarction, several type cancers and etc. (Whelan and Fritsche, 2013; Kaur *et al*. 2014; Taha, 2020). However, unsaturated fatty acids are prone to oxidation forming lipid alcohols, ketones, epoxides, aldehydes and hydrocarbons leading to an unpleasant odor and several diseased such as atherosclerosis, cancer, inflammation and etc. (Domínguez *et al*. 2019). Natural antioxidants have been investigated to prevent or retard such lipid oxidation. Garlic is used worldwide as a spice and used in various Thai curry pastes such as green curry and Massaman curry and dishes particularly side dip as spicy shrimp paste dip (Nam Phrik Ka Pi) (Khanthapok and Sukrong, 2019). There are many antioxidant compounds found in garlic including quercetin and organosulfur compound (allicin, diallyl disulfide and ajoene) have been addressed (Khanthapok and Sukrong, 2019: Shang *et al*. 2019). In addition, garlic is used for masking and modifying property of unpleasant smell flavor and taste in many cooking recipes (Li *et al.*, 2016). Therefore, this work aimed to investigate the effects of garlic on shrimp head fermentation to make shrimp paste.

3.3 Materials and methods

3.3.1 Making shrimp paste with garlic

Shrimp head containing pereiopods and internal organs from frozen food industry in the Songkhla province of Thailand was used in this study. Shrimp head and NaCl were well mixed together at a fresh weight ratio of 12 shrimp to 1 salt. The mixture was then incubated for 1 night at room temperature. After that, the mixture was dried in a hot air oven at 60 °C for 6 h, or until the moisture content reached 60%, and was then ground to reduce particle sizes. After grinding, garlic was added at 0%, 3% or 5% based on preliminary data thereafter the paste was fermented for 30 d in an earthen jar. The paste was dried again at 60 °C for 5 h until the moisture content reached 40-45%, following a convention in shrimp paste production. Later, the paste was ground and fermented further for 90 days.

3.3.2 Chemical and physical quality analyses

Physical quality indicators including color and $a_{w,}$ and chemical quality parameters including proximate composition, pH, total volatile basic nitrogen (TVB-N), trimethylamine (TMA), thiobarbituric acid reactive substances (TBARS), degree of hydrolysis (DH), histamine and indole were determined for the shrimp head (raw material) and the finished products.

3.3.2.1 Proximate analysis (moisture, crude protein, fat, ash, carbohydrate)

-Moisture (AOAC, 2000)

Moisture content was determined by weighing (Sartorius-Sartorius AG, BSA 224 S-CW, Goettingen, Germany) 3 g of sample then brought to dry it in an oven at 105℃ for 3 h. The sample was reweighed and redry until constant weight. After that, the moisture was calculated as Equation 9.

$$\text{Moisture (\%)} = \frac{W_1 - W_2}{W_1} \times 100 \quad (9)$$

where W_1 = weight of a sample before drying (g)

W_2 = weight of a sample after drying (g)

-Crude protein (AOAC, 2000)

Crude protein content in the sample was determined by adding 0.5-1 g sample to a test tube. Then Kjedahl catalyst was added (mix use of K_2SO_4 with $CuSO_4$ in 9:1 ratio) for 5 g along with 200 ml of concentrated H_2SO_4. Blank was prepared by adding Kjedahl catalyst and concentrated H_2SO_4 without a sample to a test tube. Then mixture was boiled until it was clear and then cooled down. After that, 60 ml distilled water was added to the mixture.

The flask was connected with a condenser and 40% NaOH was added, while another flask that contained H_3BO_3 and indicator solution was served as the receiver. The solution was heated until all NH_3 had been distilled. The distilled solution was removed from receiver and titrated with 0.02 N HCl until it turned colorless. The process was repeated again using the blank. Crude protein content was calculated as Equation 10.

$$\text{Protein (\%)} = (A-B) \times \frac{(A-B) \times N \times 14.007 \times 5.6}{W} \quad (10)$$

where A = volume of HCl used to titrate sample (ml)

B = volume of HCl used to titrate blank (ml)

N = Normality of HCl

W = weight of sample (g)

14.007 = atomic weight of nitrogen

5.6 = the protein-nitrogen conversation factor

-Fat content (AOAC, 2000)

Filter paper was used to wrap 3-5 g of sample and weighed. Then the sample was transferred to Soxhlet device. Bottle on the heating mantle was filled with petroleum ether (250 ml) and heated for 14 h at the rate of 150 drops/min until there was no more drip of solvent. The bottle was allowed to cool down and was taken to evaporate the solvent until completely dry, and the dried film was weighed. Fat content (%) was calculated as Equation 11.

$$\text{Fat (\%)} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100 \quad (11)$$

-Ash content (AOAC, 2000)

The crucible with 5 g sample was weighed before heating with a Bunsen burner until it no longer produced fumes, and then a furnace was used to heat the sample overnight. After cooling down, ash of the sample was weighed. The ash content was calculated as Equation 12.

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100 \quad (12)$$

-Carbohydrate content

Total carbohydrate content was calculated by subtracting as Equation 13.

$$\text{Carbohydrate (\%)} = 100 - (\text{moisture} + \text{crude protein} + \text{total fat} + \text{ash}) \quad (13)$$

3.3.2.2 Determination of salt content (AOAC, 2000)

The sample and 20 ml 0.1 N $AgNO_3$ were mixed together. After that, 10 ml of concentrated HNO_3 was added. Then the solution was boiled on a hot plate until all solid except for $AgNO_3$ was completely dissolved. Tap water was used to cool down the solution. Ferric alum indicator (ammonium iron sulfate) was added to the solution. After that, the solution was titrated with 0.1 N KSCN until it became light brown. Salt content of the sample was calculated as Equation 14.

$$\text{NaCl}(\%) = \frac{(\text{vol. } AgNO_3 \times \text{Conc. of } AgNO_3) - (\text{vol. of KSCN} \times \text{Conc. of KSCN})}{\text{weight of sample (g)}} \quad (14)$$

3.3.2.3 Water activity (a_w)

Water activity was measured using a water activity analyzer (METER-AQUALAB PRE, Decagon Devices Inc., Washington, USA).

3.3.2.4 pH determination

The sample was homogenized in distilled water at 1:5 ratio and the pH of the mixture was determined by using a pH meter (Sartorius-Sartorius AG, Docu-pH+ Meter, Goettingen, Germany).

3.3.2.5 Total volatile basic nitrogen (TVB-N) and trimethylamine-nitrogen (TMA)

Conway's micro-diffusion assay was used to determine TVB-N and TMA according to method of Junsi (2012). 4% trichloroacetic acid (TCA, 2 ml) was used to extract an 8 g sample. Then the extracted mixture was filtered through Whatman No. 41 and 4% TCA was added until the solution reached 10 ml. After that, the filtrate solution was added into the outer ring of a Conway unit, while boric acid with indicator was added into the inner ring. Saturated K_2CO_3 was added in the outer ring opposite to the sample. The Conway unit was rotated to mix K_2CO_3 with the sample solution. The solution was incubated at ambient temperature for 3 h. After incubation, 0.02 N HCl was used to titrate the indicator in inner ring until it turned to the initial inner ring color. The process was repeated for the blank without a sample, using TCA solution instead. For determination of TMA, 1 ml of 10% formaldehyde solution was added to the sample solution before saturated K_2CO_3 was added. TVB-N and TMA of sample were calculated as Equation 15.

$$\text{TVB-N or TMA (mg.nitrogen/100g of sample)} = \frac{(N)(14)(A\text{-}B)(V)(100)}{\text{weight of sample}} \qquad (15)$$

where N = normality of HCl

A = ml of HCl used to titrate sample mixture

B = ml of HCl used to titrate blank

V = total volume of sample and TCA in sample preparation

3.3.2.6 Determination of thiobarbituric acid reactive substances (TBARS)

Determination of thiobarbituric acid reactive substances was determined according to method of Buege and Aust, 1978 with slight modification by using 5000 x g for 25 min instead of 3,600 x g form 20 min. Sample and thiobarbituric acid (TBA) solution were mixed and heated for 10 min at 95 ˚C, after that the mixture was centrifuged at 5000 x g for 25 min. The supernatant was tested for absorbance at 532 nm. Malondialdehyde (MDA) was used as the standard and TBARS was calculated to mg MDA/kg sample

3.2.2.7 Degree of hydrolysis (DH)

Degree of hydrolysis was determined according to the method of Qi *et al.*, 1997 with slight modification. The sample was mixed with 20% TCA solution at 1:1 proportion. The mixture was left for 30 min, then centrifuged at 10,000 x g. After that, the supernatant was titrated with 0.02 N HCl. The DH was calculated as Equation 16.

$$\text{DH \%} = \frac{(\text{amount N soluble in 10\% TCA})(100)}{\text{Total N in sample}} \qquad (16)$$

3.2.2.8 Histamine and Indole

Histamine analysis was sent out to perform at ALS Laboratory Group (Thailand) Co., Ltd. while indole was done at Central laboratory (Thailand) Co., Ltd. Both laboratories are ISO/IEC 17025:2017 certified.

3.3.3 Microbiological quality analyses

Microbiological quality measures, including TVC, coliforms, *Escherichia coli*, *Clostridium perfringens*, *Salmonella*, *Staphylococcus aureus*, yeast and mold, and lactic acid bacteria (LAB), were determined for the shrimp head (raw material) and the finished products.

3.3.3.1 Total viable count or total mesophilic count (TVC or TMC)

TVC was analyzed according to the method of The United States Food and Drug Administration (2001a) with some modifications due to using 0.85% NaCl solution instead of 0.1% peptone water. Briefly, sample and 0.85% NaCl in distilled water were mixed together. The mixture was added to blender jar and blended to 10^{-1} dilution. Then appropriate dilutions were made. One ml of each dilution was transferred to a plate. Then 15 ml of plate count agar was added to the plate. The plate was rotated to spread agar, and the agar was allowed to solidify, then it was inverted and incubated at 35 °C for 48 h. Plates that contained 30-300 colonies were counted and recorded in colony forming units/gram (log CFU/g).

3.3.3.2 Coliform bacteria

Coliform bacteria were analyzed according to the method of The United States Food and Drug Administration (2020) with some modifications by using 0.85% NaCl solution instead of 0.1% peptone water. The sample was prepared as if it was for TVC. Each dilution was transferred to 3 lactose broth tubes. All tubes were incubated at 35°C for 24 h. The tubes containing gas were recorded and further incubated for another 24 h to record the gas production. One loopful from lactose broth tubes was transferred to brilliant green lactose bile (BGLB) broth tube. The tubes were incubated at 35 °C for 48 h. All tubes containing active gas were recorded to calculate the most probable number (MPN).

3.3.3.3 *Staphylococcus aureus*

Staphylococcus aureus was analyzed according to the method of The United States Food and Drug Administration (2016) with some modifications by using 0.85% NaCl solution instead of 0.1% peptone water). Sample was prepared similarly as for TVC. One ml of each dilution was divided to 0.1 ml aliquots and transferred to plates with Baird-Parker (BP) agar. The plates were incubated. Plates that contained 20-200 colonies were selected and counted before reporting "detected" or "not detected" per 0.1 g.

3.3.3.4 *Salmonella*

Salmonella was analyzed according to the method of The United States Food and Drug Administration (2021) with some modifications by using 0.85% NaCl solution instead of 0.1% peptone water. Sample and sterile lactose broth were mixed together and added into sterile blending container. The mixture was blended and transferred to container and incubated at room temperature for 1 h. After incubation, the mixture was transferred to Rappaport-Vassiliadis (RV) medium and Tetrathionate broth (TT). Then RV medium and TT broth were incubated in water bath at 42 ± 0.2°C and 43 ± 0.2°C for 24 ± 2 h respectively. Thereafter, the mixture was shaken. One loopful of incubated sample in TT broth and RV medium were streaked on bismuth sulfite (BS) agar, xylose lysine desoxycholate (XLD) agar, and Hektoen enteric (HE) agar. Plates containing BS, XLD, and HE agar were incubated at 35 °C for 24 h. Any plate that had more than one colony of *Salmonella* was selected and sample from the selected plate was streaked on triple sugar iron agar (TSI) and lysine iron agar (LIA) and incubated.

3.3.3.5 *Clostridium perfringens*

Clostridium perfringens was sent out to perform according to Bam 2001 chapter 16 at ALS Laboratory Group (Thailand) Co., Ltd. with ISO/IEC 17025:2017 certified.

3.3.3.6 Yeast and mold

Yeast and mold were analyzed according to the method of The United States Food and Drug Administration (2001c) with some modifications by using 0.85% NaCl solution instead of 0.1% peptone water). Sample and 0.85% NaCl in distilled water were mixed together to make 10^{-1} dilution. The mixture was homogenized. Appropriate dilutions were made. One ml of each sample dilution was transferred into plates. After that dichloran 18% glyceral (DG18) agar was added. Plates were mixed by swirling clockwise and counterclockwise then incubated in the dark at 25 °C. The incubated plates containing 10-150 colonies were selected for counting. Number of microbial colonies was recorded in log CFU/g.

3.3.3.7 Lactic acid bacteria (LAB)

LAB was determined according to Pongsetkul *et al*. (2017a) with some modifications (using normal MRS instead of MRS agar plus 10% NaCl) as the sample was prepared similarly as for TVC, and 1 ml of each dilution was transferred to plate. Then De Man, Rogosa, and Sharpe (MRS) agar with 0.0005% bromocresol purple with pH 7.5 was incubated at 30 °C for 3 d.

3.3.4 Sensory evaluation

All shrimp head paste after fermentation for 90 d were evaluated by a 9-point Hedonic scale test with 9 (like extremely) to 1 (dislike extremely). Fifty untrained panelist who were familiarity with shrimp paste were used to determine for 6 attributes based on appearance, color, odor, taste, texture, and overall acceptability and this evaluation was performed in laboratory. During evaluation, water, cucumber and raisin were used to refreshing panelist between samples.

3.3.5 Statistical analysis

The experiment was conducted in three replicates and data from experiment were analyzed by one-way analysis of variance and are shown as mean ($\overline{x}$) ± standard deviation (S.D.). Means comparisons were evaluated by Tukey's multiple range test. Completely randomize design (CRD) was used in testing chemical, physical,

and microbiological properties while randomized completely block design (RCBD) was used to determine sensory acceptability.

3.4 Results and discussion

3.4.1 Chemical and physical qualities

3.4.1.1 Proximate compositions and salt content

Proximate compositions of initial shrimp head were presented in Table 19. Shrimp head in this experiment contained slightly less protein while fat was higher than in preliminary test with moisture, carbohydrate, protein, fat and ash levels of shrimp head as 75.38±0.51%, 32.28±2.52% (dry basis), 45.55±0.85% (dry basis), 3.86± 0.28% (dry basis) and 18.31±0.32% (dry basis), respectively. However, protein and ash in this experiment were lower than in white shrimp head reported by Fernandes *et al.* (2013), which had moisture at 75.47±0.43% (dry basis), protein 60.13±3.22% (dry basis), fat 4.48±0.40% (dry basis), ash 17.73±1.14% (dry basis) and carbohydrate approximately 4.33% (dry basis), while fat and carbohydrate in this experiment were higher. Difference between proximate compositions of shrimp head in this experiment and Fernandes *et al.* (2013) might be explained by different rearing conditions, including environment, food and salt content in culture water. Higher carbohydrate contents in this white shrimp head may be due to high chitin content at 27.00±0.20% (dry basis) as explained by Diaz-Rojas *et al*. (2006).

After fermentation for 90 d (Table 19), moisture and ash were close to the proximate contents in preliminary experiment, which had moisture 42.5%, carbohydrate 9.20% (dry basis), protein 42.78% (dry basis), fat 5.06% (dry basis), and ash 42.96% (dry basis). All treatments in this experiment contained more carbohydrate than in the preliminary experiment, but these values were similar to commercial shrimp paste with moisture at 33.79-52.54%, carbohydrate 4.90-32.48%, protein 29.44-53.17%, fat 41-3.67% and ash 33.80-50.50% (Pongsetkul *et al*., 2014). Since this experiment used only head, the fat content was higher than in commercial shrimp paste that used whole small shrimp or krill. Generally, shrimp head with internal organs has less or no meat, but more fat than whole *Acetes vulgaris* which contained approximately 9.39±0.15% (dry basis) (Kongpun, and Kongrat, 2013).

Initial salt content of white shrimp head in this experiment was much higher (38.68±0.41) (dry basis) than in preliminary experiment, which had 4.96±0.32 (dry basis) (Table 19). In general, before harvesting, microbiological quality (TVC of shrimp) would be checked and reported to frozen plant to set up concentration of chloride solution for reduction of microbial load. The more TVC, the higher chlorine concentration was prepared for washing step. This might be due to high concentration of chlorine during washing, so that chlorine residue was retained. It is known that chlorine determination also uses silver nitrate (United States Environmental Protection Agency, 1994) similar to the salt determination in this experiment. However, salt contents in shrimp pastes with garlic at 0%, 3% and 5% after 90 d of fermentation were close to 28.05-42.48% (dry basis) of commercial Kapi (Kongpun and Kongrat 2013) but lower than in a previous study in which salt content was 24.26±1.04%. Addition of garlic did not change protein, fat, ash, carbohydrate or salt, as expected. However, there was some loss of protein, fat and carbohydrate in shrimp head paste compared to raw shrimp head. The main causes for such loss of protein and fat maybe solubilization of internal organs containing various enzymes, proteins and fats, after salting

Table 19. Proximate compositions of shrimp head (SH) (*Litopenaeus vannamei)* and shrimp paste made with garlic at 0% (SP-0G), 3% (SP-3G), and 5% (SP-5G), after fermentation for 90 d.

Sample	Moisture	Protein	Fat	Ash	Carbohydrate	Salt
SH	78.26± 4.48^{a}	42.56± 1.38^{a}	12.14± 0.35^{a}	14.94± 1.09^{b}	30.36± 1.48^{a}	38.68± 0.41^{a}
SP-0G	48.69± 3.49^{b}	25.36± 0.51^{b}	8.54± 1.55^{bc}	39.15± 3.87^{a}	26.96± 2.19^{ab}	29.96± 2.10^{b}
SP-3G	44.35± 7.76^{b}	27.86± 1.82^{b}	5.80± 1.64^{c}	39.70± 0.67^{a}	26.63± 1.04^{ab}	29.09± 2.93^{b}
SP-5G	44.82± 2.38^{b}	27.52± 0.48^{b}	9.75± 0.86^{ab}	39.47± 0.40^{a}	23.26± 0.95^{b}	29.61± 0.98^{b}

Remark: Protein, fat, ash, carbohydrate and salt on dry basis. Different superscripts indicate significant differences within a column ($p < 0.05$).

3.4.1.2 Water activities (a_w)

Not surprisingly, a_w decreased after salting and drying (Figure 10). After salting, a_w still exceeded 0.91, which provides an opportunity for spoilage microorganisms to grow (Majumdar *et al.*, 2018). a_w reduced after primary drying as expected and remained constantly until 30 d of fermentation, and then decreased below 0.75 in all treatments after secondary drying. After secondary drying, a_w in all treatments kept constant until the end of fermentation. Addition of garlic at 3 to 5% did not affect a_w. Therefore, it is pointed out that the garlic addition up to 5% were not large enough to alter the a_w.

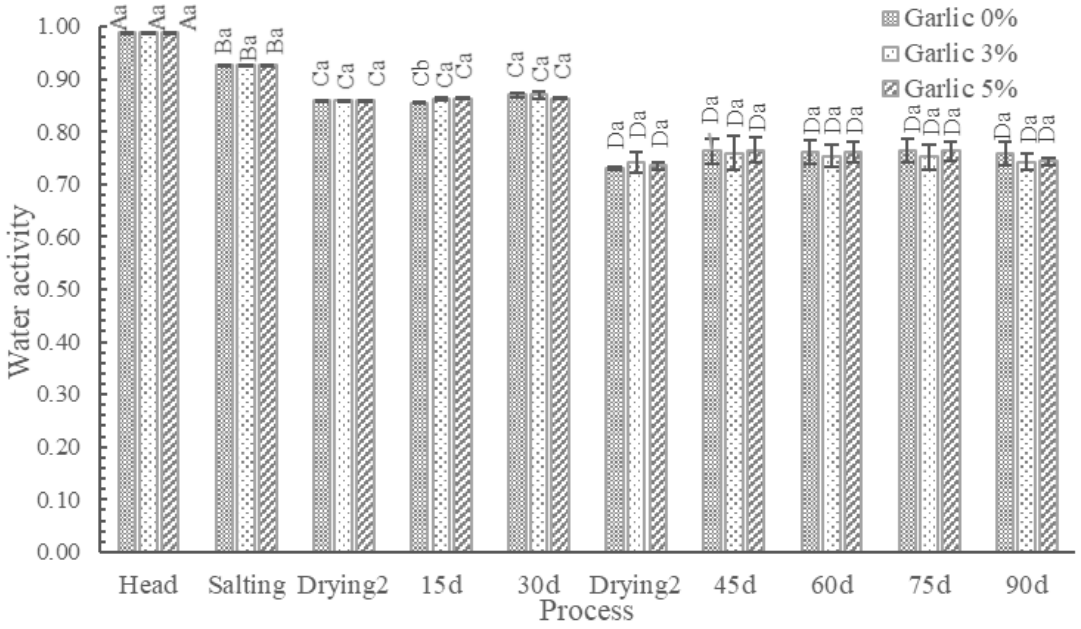

Figure 10. Water activity (a_w) of shrimp head (*Litopenaeus vannamei*) and shrimp paste with garlic at 0%, 3% and 5% after fermentation for 90 d. Different uppercase letters indicate significant differences within a salt ratio. Different uppercase letters indicate significant difference within a salt ratio. Different lowercase letters indicate significant difference within a process ($p < 0.05$). Head means shrimp head; Salting is after salting step 14-16 h; Drying is after primary drying step; 15d is after fermentation for 15 d; 30d is after fermentation for 30 d; Drying2 is after secondary drying; 45d is after fermentation for 45 d; 60d is after fermentation for 60 d; 75d is after fermentation for 75 d; 90d is after fermentation for 90 d (end of fermentation).

3.4.1.3 pH

Initial pH in all cases was around 8 (Figure 11). During making shrimp paste, pH in all cases seemed to slightly decrease to around 7.6-7.8. This agrees with Guo *et al.* (2019) who reported that the initial pH of shrimp head is around 8. After secondary drying, pH of case with garlic at 5% was lower and stayed constant until the end of fermentation. With garlic at 3% it seemed to decrease with fermentation time, but no significant difference was detected. In addition, there was no significant difference of pH by case during production. It is known that changes in pH relate to alkaline compounds from amine or ammonia production, and acidic compounds from lactic acid and acetic acid contents during fermentation (Pongsetkul *et al.* 2017b). Generally, a slight decrease in pH was found in all cases. This may be because of enzyme production by lactic acid bacteria that mainly produce organic acids such as lactic acid, acetic acid and other weak acids. While also volatile bases were produced, those can easily evaporate. That pH was decreased by the fermentation of shrimp paste was also found in Kapi Ta Dam (produced from *Mesopdopsis orienttalis*) and Kapi Ta Deang (produced from *Acetes* sp.) of which Kapi Ta Dam decreased from 7.9-8.0 to 7.2-7.3 and Kapi Ta Deang decreased from 7.8-8.0 to 7.2-7.4 (Kleekayai *et al*. 2015a). However, the pH differences in each experiment may due to many factors, including different raw materials, degree of freshness, salt ratio, and bacterial load and type. It is pointed out that the change of pH might depend on the amounts of acidic and alkaline compounds, as well as on buffering capacity of the product.

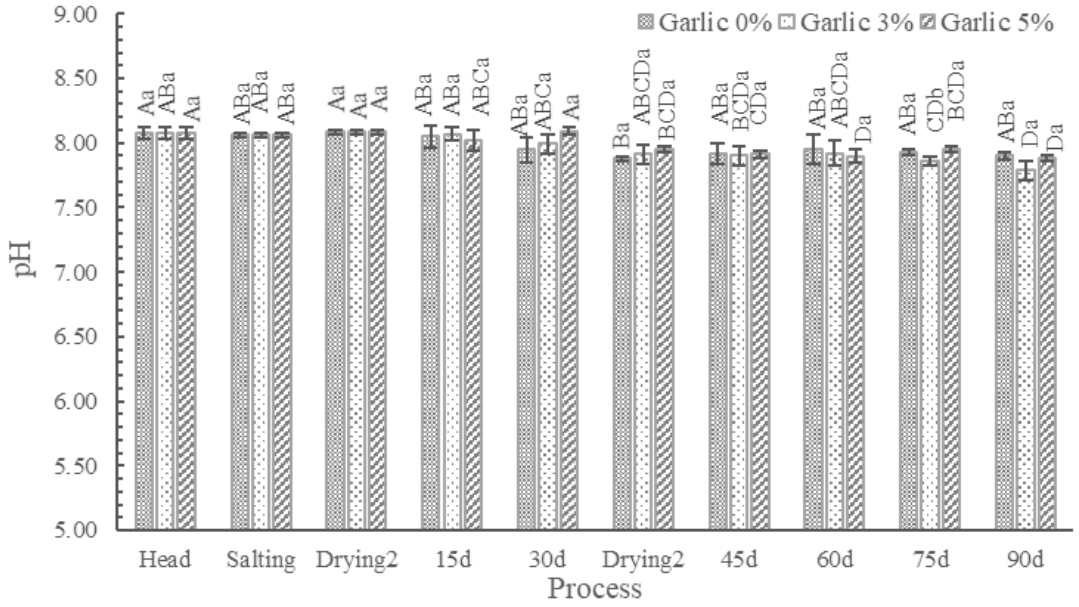

Figure 11. pH of shrimp head (*Litopenaeus vannamei*) and shrimp paste with garlic at 0%, 3% and 5% after fermentation for 90 d. Different lowercase letters indicate significant difference within a process ($p < 0.05$). Head means shrimp head; Salting is after salting step 14-16 h; Drying is after primary drying step; 15d is after fermentation for 15 d; 30d is after fermentation for 30 d; Drying2 is after secondary drying; 45d is after fermentation for 45 d; 60d is after fermentation for 60 d; 75d is after fermentation for 75 d; 90d is after fermentation for 90 d (end of fermentation).

3.4.1.4 Total volatile basic nitrogen and trimethylamine (TVB-N and TMA)

TVB-N of shrimp head in this experiment was higher than in another study which reported as 9.00±1.44 (Reerueangchai *et al*., 2014). Figures 12 and 13 showed that TVB-N in all treatments sharply increased after salting, while TMA seemed to slightly increase. Initial TVB-N of shrimp head was higher than 12 mg/100 g but still lower than 20 mg/100 g, so it was considered acceptable for consumption (Sringarm *et al.* 2020). Actually, TVB-N and TMA are indicators of freshness of seafood (Summers *et al*., 2016). Raw seafood with TVB-N higher than 25 mg/100 g is considered spoiled, but this threshold is not suitable for fermented sea food (Sringarm *et al.*, 2020). After fermentation for 15 d both TVB-N and TMA increased, but

decreased after secondary drying. As expected, increasing TVB-N and TMA during salting and fermentation for 15 d were found. TVB-N compounds, including ammonia, TMA and dimethylamine as well as other amines, were produced via hydrolysis of protein by spoilage microorganisms and endogenous enzymes (Summers *et al*., 2016). In fact, TMA is also produced from the degradation of trimethylamine oxide (TMA-O) by both endogenous enzymes and bacterial growth (*Pseudomonas* sp., *Photobacterium phosphoreum* and *Shewanella putrefacien*) (Summers *et al*., 2016; Zhao *et al*., 2018). Khairina *et al*. (2017) reported that TVB-N of Ronto (Indonesian traditional shrimp paste) after 18 d of fermentation was around 150 mg/100 g which is close to all treatments in this experiment after fermentation for 15 d. Decreases in TVB-N and TMA after drying were caused by evaporation of volatile compounds when heat was applied. Thereafter, TVB-N and TMA of all treatments slightly increased and then kept constant until the end of fermentation. There was no significant difference of TVB-N and TMA among the all fermented shrimp paste product. Throughout the experiment, TMA was below 5 mg/100 g, which is also considered safe for consumption (Siripongvutikorn *et al*., 2008). Although, TMA also provides a fishy odor with not fresh characteristic of food (Herath *et al*., 2019), this odor actually is essential and indicated for completely typical fermented seafood products such as the fish sauce, an important condiment in many Thai foods. The Ministry of Industry (1992) stated that fishy odor must not be detectable in shrimp paste. Burdock (2009) addressed that 5 mg/100g was used an odor threshold of TMA acceptability, therefore, it confirmed that there was no detectable fishy odor in the shrimp paste made from white shrimp head after fermentation for 90 d.

It is well known that TVB-N comprising ammonia, TMA and dimethylamine, are volatile alkaline compounds. However, the increased TVB-N and TMA in this experiment did not change pH much. Changes in pH of a food system actually relate to acids, bases and buffering capacity. It was hypothesized that there was some lactic acid content derived from lactic acid bacteria, which would be discussed later. In addition, buffering capacity in food is common with high protein and amino acid foods including histidine, cysteine, aspartic acid and glutamic acid (Bhagavan and Ha, 2015; Govela *et al*. 2019).

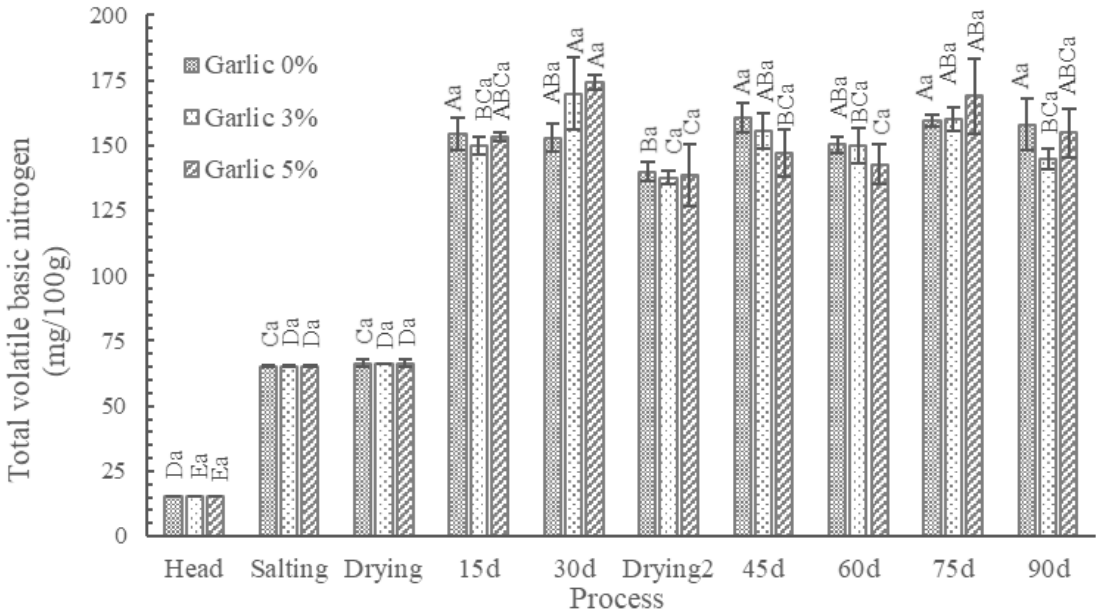

Figure 12. Total volatile basic nitrogen (TVB-N) of shrimp head (*Litopenaeus vannamei*) and shrimp paste with garlic at 0%, 3% and 5% after fermentation for 90 d (mg/ 100 g). Different uppercase letters indicate significant differences within a salt ratio. Different lowercase letters indicate significant difference within a process ($p < 0.05$). Head means shrimp head; Salting is after salting step 14-16 h; Drying is after primary drying step; 15d is after fermentation for 15 d; 30d is after fermentation for 30 d; Drying2 is after secondary drying; 45d is after fermentation for 45 d; 60d is after fermentation for 60 d; 75d is after fermentation for 75 d; 90d is after fermentation for 90 d (end of fermentation).

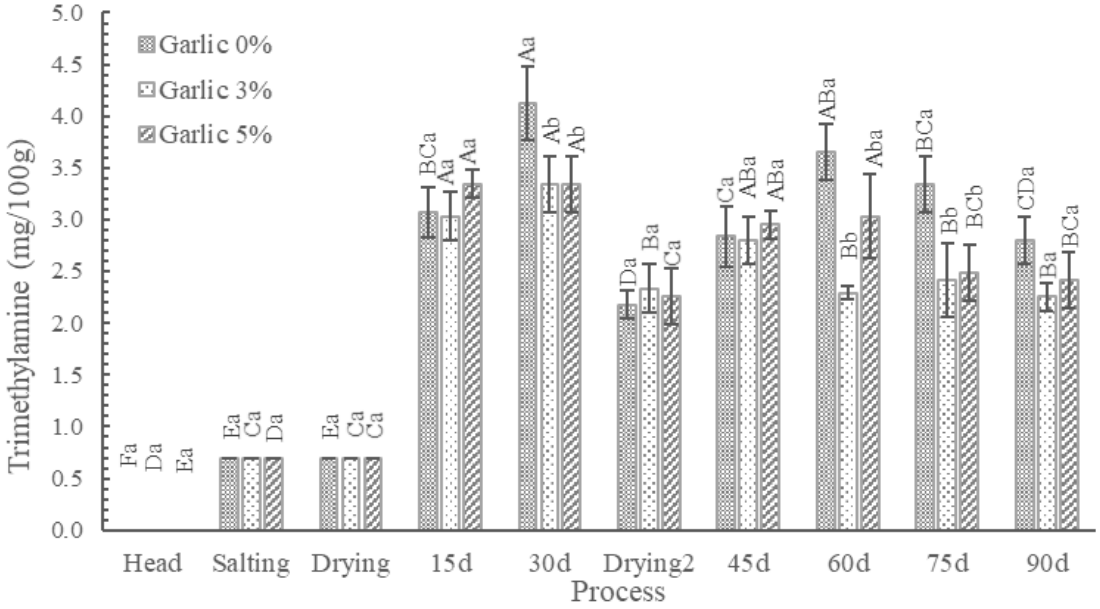

Figure 13. Trimethylamine (TMA) of shrimp head (*Litopenaeus vannamei*) and shrimp paste with garlic at 0%, 3% and 5% after fermentation for 90 d (mg/ 100 g). Different lowercase letters indicate significant difference within a process ($p < 0.05$). Head means shrimp head; Salting is after salting step 14-16 h; Drying is after primary drying step; 15d is after fermentation for 15 d; 30d is after fermentation for 30 d; Drying2 is after secondary drying; 45d is after fermentation for 45 d; 60d is after fermentation for 60 d; 75d is after fermentation for 75 d; 90d is after fermentation for 90 d (end of fermentation).

3.3.1.5 Determination of thiobarbituric acid reactive substances (TBARS)

TBARS in this experiment is shown in Table 20. The determination of TBARS was done by measurement of malondialdehyde (MDA) production from oxidation of unsaturated fatty acids (Kumar *et al.*, 2018). Generally, high TBARS indicates rancidity of a product. Domínguez *et al*. (2019) reported that meat products with TBARS below 2.5 mg MDA/kg are considered not rancid. However, high TBARS in shrimp head used in this experiment indicated rancidity before start of the experiment, due to the high unsaturated fatty acid content in shrimp head, prone to lipid oxidation. Takeungwongtrakul (2014) reported that shrimp head had high unsaturated

fatty acid at 65.21%, which supports lipid oxidation. Pongsetkul *et al*. (2017a) reported that *Acetes vulgaris* contained unsaturated fatty acid at 56.06% and exhibited TBARS around 0.6 mg MDA/kg (Pongsetkul *et al*., 2016). A higher TBARS value found in white shrimp head used in this experiment indicated the loss of freshness and careless handling, as this by-product raw material is of not much value. Generally, after shrimp has been beheaded, the head is discarded without care or proper icing. In addition, the head of shrimp with internal organs has high enzyme contents and microorganisms that contribute to its faster deterioration.

TBARS in all treatments was increased after 90 d of fermentation (Table 20). In addition, previous research also reported that TBARS in shrimp paste produced from *Acetes vulgaris* was around 18 mg MDA/kg (Chaijan and Panpipat, 2012), which is close to TBARS of the experimental shrimp paste after fermentation for 90 d. Adding garlic reduced TBARS in shrimp paste, as expected. As mentioned above, garlic has antioxidant compounds including allicin and ajoene (Shang *et al*. 2019). Interesting, TBARS value of shrimp paste with garlic at 3% was the lowest, followed by shrimp paste with garlic at 5%. Park and Chin (2014) reported that allicin can be a prooxidant when reacted with copper, which is a central component in the hemocyanin of shrimp blood (Kong *et al*. 2016), potentially contributing to TBARS. It pointed out that higher garlic addition in fermented shrimp head may lead to be prooxidant instead of antioxidant function.

Table 20. TBARS in shrimp head (*Litopenaeus vannamei)* and shrimp paste with garlic at 0%, 3% and 5% after fermentation for 90 d (mg MDA/kg).

Sample	**Treatment**		
	Garlic 0%	**Garlic 3%**	**Garlic 5%**
Shrimp head	2.88 ± 0.47^{d}	2.88 ± 0.47^{d}	2.88 ± 0.47^{d}
90 d fermentations	15.96 ± 1.58^{a}	10.03 ± 1.00^{c}	13.18 ± 0.71^{b}

Remark: Different superscripts indicate significant differences ($p < 0.05$).

3.4.1.6 Degree of hydrolysis (DH)

As expected, DH in this experiment was increased by fermentation for 90 d (Table 21). In addition, it was found that initial of DH of shrimp head used in this experiment was higher than in the preliminary experiment, which was 2.12±0.20%. DH measures protein hydrolysis by the cleavage of peptide bonds (Pongsetkul *et al.*, 2014) therefore, the more DH the more digested protein turned to small peptides and amino acids indicating of less freshness quality. In addition, freshness of any food or products also depends on time, salt ratio or a_w, and activity of enzymes in the raw material and microbial activity (Sbroggio *et al.*, 2016). The shrimp head used in this experiment was initially less fresh compared with the previous one (2.12±0.20%), but DH of all treatments after fermentation for 90 d was similar value to commercial shrimp paste that exhibited a DH at 12.68-20.67% (Pongsetkul *et al*., 2014).

Interesting, using 3% garlic retarded fermentation when determined by DH, while using 5% garlic seemed to not inhibit DH compared with control. Although a_w, TVC and LAB in all treatments did not significantly differ, DH of treatments with 0% and 5% garlic was higher than when using 3% garlic. DH of treatment with added 3% garlic was lower may cause by antimicrobial properties of allicin, ajoenes, and allyl sulfides from garlic (Bhatwalkar *et al*., 2021). While, the 5% garlic seemed to induce a higher DH, possibly because of prebiotic compounds such as fructan and fructooligosaccharide facilitating bacterial growth (Altuntas and Korukluoglu, 2019; Sunu *et al.*, 2019). It pointed out that everything has naturally both pro and con therefore, optimization needs to reach and manage to obtain more positive effect or get proper result.

Table 21. Degree of hydrolysis of shrimp head (*Litopenaeus vannamei)* and shrimp paste with garlic at 0%, 3% and 5% after fermentation for 90 d (%).

Sample	Treatment		
	Garlic 0%	**Garlic 3%**	**Garlic 5%**
Shrimp head	4.61 ± 0.02^{c}	4.61 ± 0.02^{c}	4.61 ± 0.02^{c}
90 d fermentation	18.28 ± 0.76^{a}	14.13 ± 0.96^{b}	17.09 ± 0.36^{a}

Remark: Different superscripts indicate significant differences ($p < 0.05$).

3.4.1.8 Histamine and indole

Histamine and indole of shrimp head used in this experiment are shown in Table 22. Freshness of seafood can be evaluated by histamine and indole contents, depending on the seafood species. CODEX Alimentarius and the European Union (EU) states that fish and fish products, particularly scombroid fish, must contain less than 100 mg/kg histamine for human consumption with safe (Satomi, 2016), while the U.S. FDA guideline states that fresh shrimp must have indole content less than 25 µg/100 g (National Research Council (US) Subcommittee on Microbiological Criteria, 1985). Therefore, shrimp head used in this experiment can be considered fresh due to its histamine content below 100 mg/kg and no indole detected. However, based on histamine content, shrimp head in this experiment had better quality (more freshness) than shrimp head in the preliminary experiment with 5.57 mg/kg. An increase in histamine in all treatments was found after primary drying, which indicated microbial growth. However, histamine was not detected after the secondary drying, while indole increased to more than 0.25 mg/kg, which was significantly higher than indole in the preliminary experiment (around 0.2 mg/kg). Decreased histamine may indicate degradation of histamine by lactic acid bacteria that happens in various fermented foods (Lee *et al.,* 2015; Kung *et al*., 2017). After fermentation for 90 d, histamine was still not detected in while increased indole was detected later. In addition, decreased indole with more garlic was detected after the secondary drying and after fermentation for 90 d. It is concluded that garlic can reduce indole formation by its antimicrobial properties (Leontiev *et al.,* 2018; Nakamoto *et al*., 2019; Bhatwalkar *et al*., 2021). Odor threshold of indole having a feces odor was reported around 0.1 mg/kg (National Research Council, 1996). Therefore, a high amount of indole found in all treatments might responsible for the poor odor scores in this experiment (Table 24). In addition, histamine content in commercial shrimp paste is in the range from 20 to 53.85 mg/kg, while indole is not detected (ND) to 1852×10^5 MAU of peak area (abundance) based on GC-MS method (Kleekayai *et al*., 2016; Pongsetkul, 2018). Therefore, in terms of histamine and indole in raw materials and products after fermentation for 90 d would have good quality and safety for consumption.

Generally, histamine and indole are used to indicate decomposition in raw seafood, but indole is a specific parameter used to indicate the spoilage of shrimp. Histidine and tryptophan are degraded by spoilage microorganisms. *Vibrio parahaemolyticus*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Proteus mirabilis* etc. are considered responsible for histamine production, and *Bacillus alvei*, *E. coli*, *Shigella* sp., *Enterococcus faecalis*, and *V. cholerae etc.* are bacteria contributing indole (Lee and Lee, 2010; Pongsetkul, 2018). However, it has been reported that consumption of food containing histamine may cause various symptoms alike allergic reactions, including hypertension, hypotension, headache, urticaria, nausea, asthma, diarrhea, flushing, arrhythmia, and vomiting (Maintz and Novak, 2007; Satomi, 2016).

Table 22. Histamine and indole content of shrimp head (*Litopenaeus vannamei)* and shrimp paste with added garlic at 0%, 3% and 5% after fermentation for 90 d (mg/kg).

Sample	Garlic 0%		Garlic 3%		Garlic 5%	
	Histamine	Indole	Histamine	Indole	Histamine	Indole
Shrimp head	ND	ND	ND	ND	ND	ND
After 1st drying	24.5	-	24.5	-	24.5	-
After 2nd drying	ND	1.09	ND	0.77	ND	0.67
After 90 d	ND	2.72	ND	2.52	ND	2.35

Remark: ND mean not detected. - mean not measured.

3.4.2 Microbiological qualities

3.4.2.1 Total viable count (TVC) and lactic acid bacteria (LAB)

TVC and LAB are shown in Figures 14 and 15, respectively. Both TVC and LAB in all treatments significantly increased after salting and continuously kept raising during fermentation up to 15 d. Increasing TVC and LAB after salting may be due to high a_w (>0.95) and proper conditions (temperature < 45 ˚C) for the growth of mesophilic microorganisms (Keenleyside, 2019). In addition, temperature of sample during first time drying was lower than 50 ℃. TVC without garlic addition seemed to reach its peak at 15 d, while adding garlic extended the TVC peak to 30 d. An increased TVC may relate to the degree of hydrolysis. The more TVC or longer time of

fermentation, the higher DH was found. The higher number of TVC in the treatments with added garlic may be due to some compounds such as fructan, inulin, and manganese in garlic, enhancing microbial growth (Swetwiwathana *et al*. 1998; Altuntaş and Korukluoglu, 2019; Gupta *et al.,* 2019) particularly of heterofermentative lactic acid bacteria (Swetwiwathana *et al*., 1998; Siripongvutikorn, 2004; Siripongvutikorn *et al*., 2008). However, after 90 d, both TVC and lactic acid bacteria reduced to about 3 log CFU and 1 log CFU, respectively may due to low a_w and toxicity compounds productions from each microbial type as well as cell life cycle as death phase phenomenon (Majumdar *et al.*, 2018; Osborne *et al.*, 2021). According to plating method and recorded colony forming units, the shrimp paste was quite safe for consumption even without any further heat treatment. Reduction of TVC and lactic acid bacteria in fermented products such as shrimp paste could be explained by NaCl content and drying to lower a_w, and maybe by some secondary metabolites produced, such as niasin and betaine (Lindstedt *et al.* 1990; Murray, 2003). As mentioned above regarding the increased TVB-N and TMA while pH did not much change during 30 d of fermentation, during fermentation lactic acid, acetic acid and other weak acids were provided by the lactic acid bacteria, particularly during 30 d of fermentation. Interesting, there was no significant difference between TVC and lactic acid bacterial count in any treatments as expected. This may be due to the relatively low garlic contents and/or quality of raw materials (both shrimp head and garlic bulb) used not fresh and some microbial type contamination, leading to no effects from the active compounds such as allicin, ajoene and other organosulfurs that are antimicrobial and antioxidants. However, Siripongvutikorn (2004) reported that seabass flesh marinated with Tom-Yam containing garlic supported lactic acid bacterial growth due to prebiotics in fresh garlic.

Comparison of TVC to lactic acid bacterial count seemed to indicate that this raw material had lots of lactic acid bacteria, which may explain why the pH was not much reduced as expectation. As known that shrimp head contains various enzymes, proteins and peptides as well as free amino acid, which all have buffering capacity (Govela *et al*., 2019), therefore the small changes in pH found in this experiment may also be due to the high buffering capacity in the Kapi product.

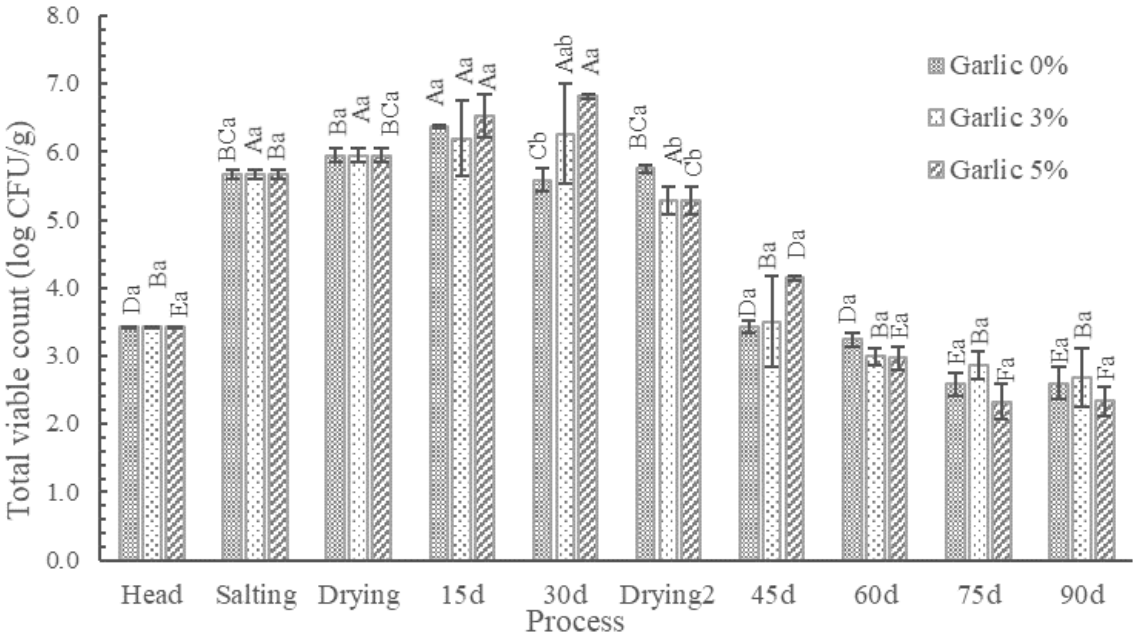

Figure 14. Total viable counts in shrimp head (*Litopenaeus vannamei*) and shrimp paste with garlic at 0%, 3% and 5% after fermentation for 90 d. Different lowercase letters indicate significant difference within a process ($p < 0.05$). Head means shrimp head; Salting is after salting step 14-16 h; Drying is after primary drying step; 15d is after fermentation for 15 d; 30d is after fermentation for 30 d; Drying2 is after secondary drying; 45d is after fermentation for 45 d; 60d is after fermentation for 60 d; 75d is after fermentation for 75 d; 90d is after fermentation for 90 d (end of fermentation).

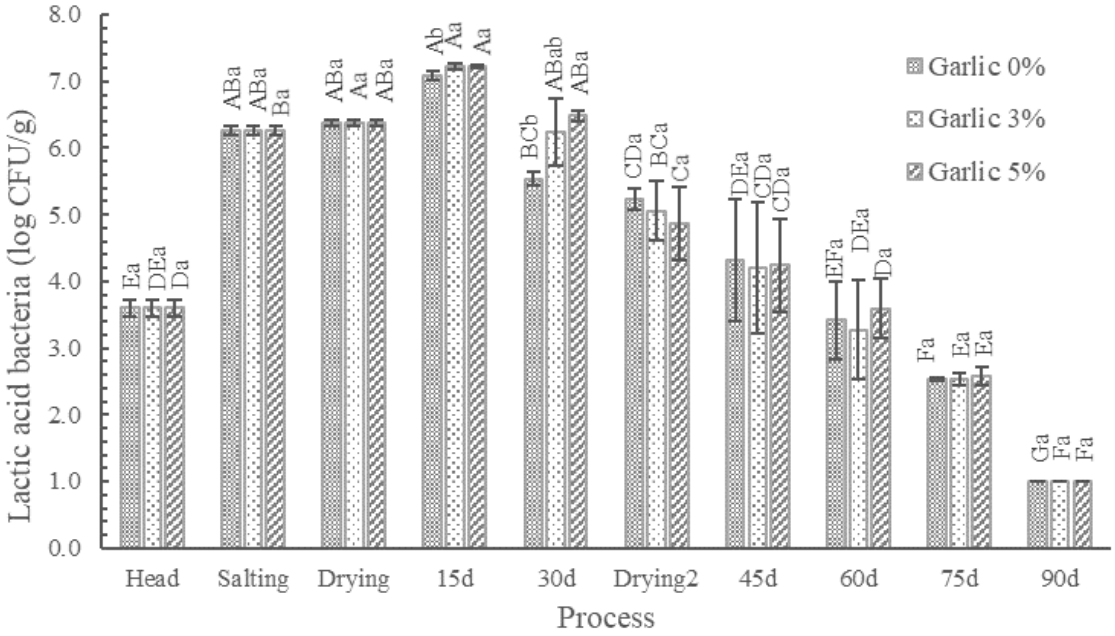

Figure 15. Amount of lactic acid bacteria in shrimp head (*Litopenaeus vannamei*) and shrimp paste with garlic at 0%, 3% and 5% after fermentation for 90 d. Different lowercase letters indicate significant difference within a process ($p < 0.05$). Head means shrimp head; Salting is after salting step 14-16 h; Drying is after primary drying step; 15d is after fermentation for 15 d; 30d is after fermentation for 30 d; Drying2 is after secondary drying; 45 d is after fermentation for 45 d; 60d is after fermentation for 60 d; 75d is after fermentation for 75 d; 90 d is after fermentation for 90 d (end of fermentation).

3.4.2.2 Pathogenic microorganisms

Initial pathogenic microorganisms in shrimp head are shown in Table 23. Unexpectedly, *Clostridium perfringens* was found in shrimp head which might indicate not enough hygiene of cultured pond, transportation process and icing etc., while coliforms and yeast and mold in shrimp head were at 430 MPN/g and 2.91 log CFU/g, respectively. *S. aureus* appeared after the first drying, which might indicate some contamination from garlic bulb during salting and drying. However, after secondary drying and after 90 d of fermentation, no pathogenic microorganisms were found. This may due to a reduction of a_w and antimicrobial effects of salt (Wijnker *et al*. 2006) and drying process. Based on the standard regulations for a good shrimp paste

(Table 23: Ministry of Industry, 1992), it was confirmed that white shrimp head paste was safe for consumption. However, it is pointed out that sanitation and freshness of raw materials must be properly controlled.

Table 23. Pathogenic microorganisms of shrimp head (*Litopenaeus vannamei*) and of shrimp paste after fermentation for 90 d with garlic at 0%, 3% and 5% after fermentation for 90 d.

Sample	**Pathogenic microorganism**				
	Coliform (MPN/g)	***Staphylococcus aureus* (per 0.1 g)**	***Clostridium perfringens* (per 0.01 g)**	***Salmonella* (per 25 g)**	**Yeast mold (log CFU/g)**
Shrimp paste production standard regulation[A]	<3	ND	ND	ND	<1.7
Shrimp head	430	ND	D	ND	2.91
Garlic 0% after 1st time drying	<3	D	ND	ND	1.48
Garlic 3% after 1st time drying	<3	D	ND	ND	1.48
garlic 5% after 1st time drying	<3	D	ND	ND	1.48
Garlic 0% after 2nd time drying	<3	ND	ND	ND	<1

Remark: D means detected. ND means non-detected. A means data are from the Ministry of Industry, 1992.

Table 23. Pathogenic microorganisms of shrimp head (*Litopenaeus vannamei*) and of shrimp paste after fermentation for 90 d with garlic at 0%, 3% and 5% after fermentation for 90 d. (continued)

Sample	Pathogenic microorganism				
	Coliform (MPN/g)	***Staphylococcus aureus* (per 0.1 g)**	***Clostridium perfringens* (per 0.01 g)**	***Salmonella* (per 25 g)**	**Yeast mold (log CFU/g)**
Garlic 3% after 2nd time drying	<3	ND	ND	ND	<1
Garlic 5% after 2nd time drying	<3	ND	ND	ND	<1
Garlic 0 % after 90 d	<3	ND	ND	ND	<1
Garlic 3 % after 90 d	<3	ND	ND	ND	<1
Garlic 5 % after 90 d	<3	ND	ND	ND	<1

Remark: D means detected. ND means non-detected. A means data are from the Ministry of Industry, 1992.

3.4.3 Sensory evaluation

In general, addition of garlic did not change organoleptic attributes. Likeness scores of commercial shrimp paste based on 9-point Hedonic scale determination was significantly different from shrimp head paste with added garlic at 0%, 3% and 5% (Table 24). Panelists gave a higher sensory score of commercial one compared with treatments. Sensory score of treatment was lower than commercial may due to different raw materials used as such krill (commercial) and shrimp head (by-product from frozen plant) and their freshness, in addition, a commercial product is always improved sensory quality and reducing cost by using sugar and monosodium glutamate, possibly with also sweet potato added (data from marketing survey and

personal observations during plant visits). In addition, it needs to be accepted that the shell of shrimp head in this experiment was quite thick and tough giving a coarse texture, which was a big problem affecting to texture attribute and leading to lower scores. From preliminary testing, it was also found that shrimp paste made from shrimp head contained calcium at 3,394 mg/100 g which was more than twice of the calcium content in commercial shrimp paste at about 1,565 mg/100 g (Benjakul *et al*., 2011). Generally, odor of shrimp paste in this experiment was poor and far from commercial product due to highly autolysis as nature of internal organ. Therefore, using the salt ratio 12:1 for shrimp heads may be needed to better control of spoilage bacterial or otherwise really fresh shrimp head must be controlled and used as premium grade not as by-product as usual.

Table 24. Sensory scores of shrimp paste with garlic at 0%, 3% and 5% after fermentation for 90 d and of commercial shrimp paste.

Sample	Treatment					
	Appearance	**Color**	**Texture**	**Odor**	**Taste**	**Overall**
Commercial shrimp paste	7.78± 1.01[a]	7.62± 0.93[a]	7.58± 0.87[a]	7.52± 1.12[a]	7.46± 1.28[a]	7.64± 0.99[a]
Garlic 0%	5.44± 1.72[b]	5.66± 1.57[b]	5.38± 1.71[b]	5.50± 1.85[b]	5.70± 1.79[b]	5.66± 1.67[b]
Garlic 3%	5.78± 1.40[b]	5.96± 1.37[b]	5.66± 1.42[b]	6.06± 1.55[b]	5.90± 1.56[b]	6.00± 1.44[b]
Garlic 5%	5.88± 1.39[b]	5.82± 1.43[b]	5.68± 1.52[b]	6.04± 1.70[b]	5.84± 1.75[b]	5.84± 1.66[b]

Remark: Different superscripts indicate significant differences in one column between the rows ($p < 0.05$).

3.5 Conclusion

Addition of garlic at 3% and 5% did not significantly reduce any good quality parameter of shrimp paste. However, addition of 3% garlic was a proper condition to retard lipid oxidation based on TBARS assay. Shrimp head can be used as alternative raw material for producing Kapi. However, texture improvement either by

size reduction using proper machines or enzymes for chitin hydrolysis must be further investigated. Furthermore, sugar might improve the sensory scores, and could be addressed in further studies to obtain higher likeness score.

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CHAPTER 4

PRODUCTION OF REDUCED SODIUM SHRIMP HEAD PASTE BY REPLACING SOME SODIUM CHLORIDE WITH POTASSIUM CHLORIDE

4.1 Abstract

A significant decrease in main raw materials of shrimp paste production is a big problem, while most by-products of white shrimp (*Litopenaeus vannamei*) processing – especially head – are still underutilized. Therefore, producing shrimp head paste is well motivated. However, traditional shrimp paste is claimed as a high salt product that causes a big concern regarding health impacts via hypertension. To dissolve this problem, KCl at 0%, 30% and 50% was used to replace NaCl. Chemical, physical, microbiological and sensory quality changes during fermentation for 90 d were determined. The result showed that after fermentation for 90 d, moisture, protein and ash were main compositions of shrimp head paste. The reduction of a_w and increment of pH were found in all treatments. In addition, significantly increased of total volatile bases nitrogen (TVB-N), trimethylamine (TMA), thiobarbituric acid reactive substances (TBARS) and degree of hydrolysis (DH) were found in all treatments. Total viable count (TVC) decreased from 5.90 log CFU/g to around 2.95 log CFU/g while lactic acid bacteria (LAB) decreased from 5.71 log CFU/g to 1 log CFU/g in all treatments. Pathogenic microorganisms were not found in all treatments after fermentations for 90 d. Based on production regulation on chemical and microbiological qualities of shrimp paste, shrimp head paste was considered as safe for consumption. ACE Inhibition (%) of shrimp head (S1) and shrimp head paste (S2 NaCl 7.69%; S3 6.36% KCl 2.73%; S4 NaCl 4.55% KCl 4.55%) at 1 mg/ml were 36.21±0.88%, 53.47±2.81%, 51.14±3.50% and 45.80±3.45%, respectively while ACE Inhibition (%) of captopril at 1.09 ng/ml, 2.17 ng/ml and 4.35 ng/ml were 29.39±2.18%, 36.00±4.34% and 48.70±1.41%, respectively. Sensory score of all treatments were approximately 6 which lower than that of commercial shrimp paste with approximately 7.5 in 9. Bitter taste was not detected in any treatment with added KCl, however, the

texture and odor qualities of shrimp head paste still needed to improve comparing with commercial shrimp paste.

4.2 Introduction

Various of Thai dishes could not lack of shrimp paste or Kapi having special typical flavor and umami taste due to high free amino acid contents. Generally, shrimp paste contains many healthy compounds such as high unsaturated fatty acid including docosahexanoic acid (DHA) and eicosapentaenoic acid (EPA) (Pongsetkul *et al*., 2017a), astaxanthin and antihypertensive peptides (Pongsetkul *et al*., 2014; Kleekayai *et al*., 2015b; Prapasuwannakul and Suwannahong, 2015). Due to raw material including *Mesopodopsis* and *Acetes vulgaris* for producing shrimp paste have been decreasing further, however, a large-scale of by-product especially shrimp head from shrimp processing industry has not been properly utilized. Furthermore, a standard of shrimp paste based on regulation of (1) Thailand Industrial Standards and (2) Thai Community Product Standard contains high sodium content with salt not less than 36% (dry basis) and 12% (dry basis), respectively (Ministry of Industry, 1992; Thai Industrial Standard Institute, 2018). As well known that consumption of high sodium diet may cause some health problems particularly hypertension which is one of a risk factors for cardiovascular disease leading to approximately 9.4 million deaths every year (Rajati *et al*., 2019). However, hypertension is not caused only by high sodium intake but also by low potassium intake (Staruschenko, 2018). Therefore, using KCl replacement of high Na content to reduce hypertension affect is more interesting and using in many foods or drink product.

4.3 Materials and methods

4.3.1 Shrimp paste replace NaCl with KCl production

Shrimp head containing pereiopods and internal organs from a frozen food manufacturer in Songkhla province were used in this experiment. Shrimp head was divided to three treatments based on a_w content controlling as 0.916±0.02. The first group was mixed with salt at ratio 12:1 (NaCl 7.69%). The second and third were mixed with salt at ratio 10:1 with replacement of KCl 30% (NaCl 6.36%, KCl 2.73%) and 50% (NaCl 4.55%, KCl 4.55%), respectively to get a similar a_w level for all treatments.

Each mixture was incubated for 1 night at room temperature, dried in a hot air oven at 60 °C for 6 h or until reached 60% moisture content. The sample was then ground to make the finer solid. After grinding, earthen jar was used to ferment the paste for 30 d. The paste was again dried at 60 °C for 5 h until obtaining 40-45% moisture content, following the standard of shrimp paste production (Ministry of Industry, 1992). The paste was ground and further fermented for 90 d.

4.3.2 Chemical and physical quality analyses

Physical quality indicators including color and $a_{w,}$ and chemical quality parameters including proximate composition, pH, total volatile basic nitrogen (TVB-N), trimethylamine (TMA), thiobarbituric acid reactive substances (TBARS) and degree of hydrolysis (DH) were determined for the shrimp head (raw material) and the finished products.

4.3.2.1 Proximate analysis (moisture, crude protein, fat, ash, carbohydrate)

- Moisture (AOAC, 2000)

Moisture content was determined by weighing (Sartorius-Sartorius AG, BSA 224 S-CW, Goettingen, Germany) 3 g of sample then brought to dry in an oven at 105℃ for 3 h. The sample was reweighed and redry until constant weight. After that, the moisture was calculated as Equation 17.

$$\text{Moisture (\%)} = \frac{W_1 - W_2}{W_1} \times 100 \quad (17)$$

where W_1 = weight of a sample before drying (g)

W_2 = weight of a sample after drying (g)

-Crude protein (AOAC, 2000)

Crude protein content in the sample was determined by adding 0.5-1 g sample to a test tube. Then Kjedahl catalyst was added (mix use of K_2SO_4 with $CuSO_4$ in 9:1 ratio) for 5 g along with 200 ml of concentrated H_2SO_4. Blank was prepared by adding Kjedahl catalyst and concentrated H_2SO_4 without a sample to a test tube. Then mixture was boiled until it was clear and then cooled down. After that, 60 ml distilled water was added to the mixture.

The flask was connected with a condenser and 40% NaOH was added, while another flask that contained H_3BO_3 and indicator solution was served as the receiver. The solution was heated until all NH_3 had been distilled. The distilled solution was removed from receiver and titrated with 0.02 N HCl until it turned colorless. The process was repeated again using the blank. Crude protein content was calculated as Equation 18.

$$\text{Protein (\%)} = (A-B)\times\frac{(A-B)\times N\times 14.007\times 5.6}{W} \quad (18)$$

where A = volume of HCl used to titrate sample (ml)

B = volume of HCl used to titrate blank (ml)

N = Normality of HCl

W = weight of sample (g)

14.007 = atomic weight of nitrogen

5.6 = the protein-nitrogen conversation factor

-Fat content (AOAC, 2000)

Filter paper was used to wrap 3-5 g of sample and weighed. Then the sample was transferred to Soxhlet device. Bottle on the heating mantle was filled with petroleum ether (250 ml) and heated for 14 h at the rate of 150 drops/min until there was no more drip of solvent. The bottle was allowed to cool down and was taken to

evaporate the solvent until completely dry, and the dried film was weighed. Fat content (%) was calculated as Equation 19.

$$\text{Fat (\%)} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100 \quad (19)$$

-Ash content (AOAC, 2000)

The crucible with 5 g sample was weighed before heating with a Bunsen burner until it no longer produced fumes, and then a furnace was used to heat the sample overnight. After cooling down, ash of the sample was weighed. The ash content was calculated as Equation 20.

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100 \quad (20)$$

-Carbohydrate content

Total carbohydrate content was calculated by subtracting as Equation 21.

$$\text{Carbohydrate (\%)} = 100 - (\text{moisture} + \text{crude protein} + \text{total fat} + \text{ash}) \quad (21)$$

4.3.2.2 Determination of salt content (AOAC, 2000)

The sample and 20 ml 0.1 N $AgNO_3$ were mixed together. After that, 10 ml of concentrated HNO_3 was added. Then the solution was boiled on a hot plate until all solid except for $AgNO_3$ was completely dissolved. Tap water was used to cool down the solution. Ferric alum indicator (ammonium iron sulfate) was added to the solution. After that, the solution was titrated with 0.1 N KSCN until it became light brown. Salt content of the sample was calculated as Equation 22.

$$\text{NaCl(\%)} = \frac{(\text{vol. } AgNO_3 \times \text{Conc. of } AgNO_3) - (\text{vol. of KSCN} \times \text{Conc. of KSCN})}{\text{weight of sample (g)}} \quad (22)$$

4.3.2.3 Water activity (a_w)

Water activity was measured using a water activity analyzer (METER-AQUALAB PRE, Decagon Devices Inc., Washington, USA).

4.3.2.4 pH determination

The sample was homogenized in distilled water at 1:5 ratio and the pH of the mixture was determined by using a pH meter (Sartorius-Sartorius AG, Docu-pH+ Meter, Goettingen, Germany).

4.3.2.5 Total volatile basic nitrogen (TVB-N) and trimethylamine-nitrogen (TMA)

Conway's micro-diffusion assay was used to determine TVB-N and TMA according to method of Junsi (2012). 4% trichloroacetic acid (TCA, 2 ml) was used to extract an 8 g sample. Then the extracted mixture was filtered through Whatman No. 41 and 4% TCA was added until the solution reached 10 ml. After that, the filtrate solution was added into the outer ring of a Conway unit, while boric acid with indicator was added into the inner ring. Saturated K_2CO_3 was added in the outer ring opposite to the sample. The Conway unit was rotated to mix K_2CO_3 with the sample solution. The solution was incubated at ambient temperature for 3 h. After incubation, 0.02 N HCl was used to titrate the indicator in inner ring until it turned to the initial inner ring color. The process was repeated for the blank without a sample, using TCA solution instead. For determination of TMA, 1 ml of 10% formaldehyde solution was added to the sample solution before saturated K_2CO_3 was added. TVB-N and TMA of sample were calculated as Equation 23.

$$\text{TVB-N or TMA (mg.nitrogen/100g of sample)} = \frac{(N)(14)(A-B)(V)(100)}{\text{weight of sample}} \qquad (23)$$

where N = normality of HCl

A = ml of HCl used to titrate sample mixture

B = ml of HCl used to titrate blank

V = total volume of sample and TCA in sample preparation

4.3.2.6 Determination of thiobarbituric acid reactive substances (TBARS)

Determination of thiobarbituric acid reactive substances was determined according to method of Buege and Aust, 1978 with slight modification by using 5000 x g for 25 min instead of 3,600 x g form 20 min. Sample and thiobarbituric acid (TBA) solution were mixed and heated for 10 min at 95 ˚C, after that the mixture was centrifuged at 5000 x g for 25 min. The supernatant was tested for absorbance at 532 nm. Malondialdehyde (MDA) was used as the standard and TBARS was calculated to mg MDA/kg sample.

4.3.2.7 Degree of hydrolysis (DH)

Degree of hydrolysis was determined according to the method of Qi *et al.*, 1997 with slight modification. The sample was mixed with 20% TCA solution at 1:1 proportion. The mixture was left for 30 min, then centrifuged at 10,000 x g. After that, the supernatant was titrated with 0.02 N HCl. The DH was calculated as Equation 24.

$$\text{DH \%} = \frac{(\text{amount N soluble in 10\% TCA})(100)}{\text{Total N in sample}} \quad (24)$$

4.3.3 Microbiological quality analyses

Microbiological quality measures, including TVC, coliforms, *Escherichia coli*, *Clostridium perfringens*, *Salmonella*, *Staphylococcus aureus*, yeast and mold, and lactic acid bacteria (LAB), were determined for the shrimp head (raw material) and the finished products.

4.3.3.1 Total viable count or total mesophilic count (TVC or TMC)

TVC was analyzed according to the method of The United States Food and Drug Administration (2001a) with some modifications due to using 0.85% NaCl solution instead of 0.1% peptone water. Briefly, sample and 0.85% NaCl in distilled water were mixed together. The mixture was added to blender jar and blended to 10^{-1} dilution. Then appropriate dilutions were made. One ml of each dilution was transferred to a plate. Then 15 ml of plate count agar was added to the plate. The plate was rotated to spread agar, and the agar was allowed to solidify, then it was inverted and incubated at 35 °C for 48 h. Plates that contained 30-300 colonies were counted and recorded in colony forming units/gram (log CFU/g).

4.3.3.2 Coliform bacteria

Coliform bacteria were analyzed according to the method of The United States Food and Drug Administration (2020) with some modifications by using 0.85% NaCl solution instead of 0.1% peptone water. The sample was prepared as if it was for TVC. Each dilution was transferred to 3 lactose broth tubes. All tubes were incubated at 35°C for 24 h. The tubes containing gas were recorded and further incubated for another 24 h to record the gas production. One loopful from lactose broth tubes was transferred to brilliant green lactose bile (BGLB) broth tube. The tubes were incubated at 35 °C for 48 h. All tubes containing active gas were recorded to calculate the most probable number (MPN).

4.3.3.3 *Staphylococcus aureus*

Staphylococcus aureus was analyzed according to the method of The United States Food and Drug Administration (2016) with some modifications by using 0.85% NaCl solution instead of 0.1% peptone water). Sample was prepared similarly as for TVC. One ml of each dilution was divided to 0.1 ml aliquots and transferred to plates with Baird-Parker (BP) agar. The plates were incubated. Plates that contained 20-200 colonies were selected and counted before reporting “detected” or “not detected” per 0.1 g.

4.3.3.4 *Salmonella*

Salmonella was analyzed according to the method of The United States Food and Drug Administration (2021) with some modifications by using 0.85% NaCl solution instead of 0.1% peptone water. Sample and sterile lactose broth were mixed together and added into sterile blending container. The mixture was blended and transferred to container and incubated at room temperature for 1 h. After incubation, the mixture was transferred to Rappaport-Vassiliadis (RV) medium and Tetrathionate broth (TT). Then RV medium and TT broth were incubated in water bath at 42 ± 0.2°C and 43 ± 0.2°C for 24 ± 2 h respectively. Thereafter, the mixture was shaken. One loopful of incubated sample in TT broth and RV medium were streaked on bismuth sulfite (BS) agar, xylose lysine desoxycholate (XLD) agar, and Hektoen enteric (HE) agar. Plates containing BS, XLD, and HE agar were incubated at 35 °C for 24 h. Any plate that had more than one colony of *Salmonella* was selected and sample from the selected plate was streaked on triple sugar iron agar (TSI) and lysine iron agar (LIA) and incubated.

4.3.3.5 *Clostridium perfringens*

Clostridium perfringens was sent out to perform according to Bam 2001 chapter 16 at ALS Laboratory Group (Thailand) Co., Ltd. with ISO/IEC 17025:2017 certified.

4.3.3.6 Yeast and mold

Yeast and mold were analyzed according to the method of The United States Food and Drug Administration (2001c) with some modifications by using 0.85% NaCl solution instead of 0.1% peptone water). Sample and 0.85% NaCl in distilled water were mixed together to make 10^{-1} dilution. The mixture was homogenized. Appropriate dilutions were made. One ml of each sample dilution was transferred into plates. After that dichloran 18% glyceral (DG18) agar was added. Plates were mixed by swirling clockwise and counterclockwise then incubated in the dark at 25 °C. The incubated plates containing 10-150 colonies were selected for counting. Number of microbial colonies was recorded in log CFU/g.

4.3.3.7 Lactic acid bacteria (LAB)

LAB was determined according to Pongsetkul *et al*. (2017a) with some modifications (using normal MRS instead of MRS agar plus 10% NaCl) as the sample was prepared similarly as for TVC, and 1 ml of each dilution was transferred to plate. Then De Man, Rogosa, and Sharpe (MRS) agar with 0.0005% bromocresol purple with pH 7.5 was incubated at 30 °C for 3 d.

4.3.4 Angiotensin converting enzyme (ACE) inhibition assay

Sample solution was prepared according to Kleekayai *et al.* (2015a) with slight modification by using 50 mM tris-HCl buffer (pH 7.0) instead of distill water. The dried samples (dried with hot air oven at 50 ˚C) was mixed with 50 mM tris-HCl buffer (pH 7.0) in the ratio of 1:5 (w/v). The mixtures were homogenized and shaken at 150 rpm on an orbital shaker for 1 h at 30 °C. The mixtures were then centrifuged at 8,400 × g for 10 min at 4 °C to remove undesired debris. The supernatants were collected and adjusted to pH 7.0 using 1 M HCl.

ACE inhibition assay was determined according to Onuh *et al*. (2015) with slight modification. 1 ml of N-[3-(2-Furyl)acryloyl]-L-phenylalanyl-glycyl-glycine (FAPGG) 0.35 mM dissolving in 50 mM Tris-HCl buffer containing 0.3 M NaCl, pH 7.5) was mixed 20 µl of ACE 20 mU and 200 µl of sample mixture (0.7 mg/ml) dissolving in the 50 mM Tris–HCl buffer. The absorbance were read by microplate reader at 340 nm for 30 min at 37 ˚C. four controls was prepared by replace sample mixture with tris-HCL buffer, tris-HCl buffer with 0.3 M NaCl and tris-HCL buffer containing NaCl and/or KCl same as concentration of NaCl and/or KCl in shrimp paste while positive controls were prepared by using captopril (20 nM, 10 nM and 5 nM) instead of sample solution. ACE inhibition activity was calculated as Equation 25.

$$\text{ACE inhibition } (\%)=\left[\frac{\Delta\text{Amin}^{-1}(\text{blank})-\Delta\text{Amin}^{-1}(\text{sample})}{\Delta\text{Amin}^{-1}\ (\text{blank})}\right]\times 100 \qquad (25)$$

When ΔAmin^{-1} (blank) = ACE activity without sample

ΔAmin^{-1} (sample) = ACE activity with sample

4.3.5 Sensory evaluation

All shrimp head paste after fermentation for 90 d were evaluated by a 9-point Hedonic scale test with 9 (like extremely) to 1 (dislike extremely). Fifty untrained panelist who were familiarity with shrimp paste were used to determine for 6 attributes based on appearance, color, odor, taste, texture, and overall acceptability and this evaluation was performed in laboratory. During evaluation, water, cucumber and raisin were used to refreshing panelist between samples.

4.3.6 Statistical analysis

The experiment was conducted in three replicates and data from experiment were analyzed by one-way analysis of variance and are shown as mean ($\overline{x}$) ± standard deviation (S.D.). Means comparisons were evaluated by Tukey's multiple range test. Completely randomize design (CRD) was used in testing chemical, physical, and microbiological properties while randomized completely block design (RCBD) was used to determine sensory acceptability.

4.4 Result and discussion

4.4.1Chemical and physical qualities

4.4.1.1 Proximate compositions and salt content

Proximate compositions of shrimp head were shown in Table 25. These results were close to shrimp head in preliminary experiment with moisture as 75.38±0.51%, protein as 45.55±0.85% (dry basis), fat as 3.86± 0.28%, ash as 18.31±0.32% (dry basis) and carbohydrate as 32.28±2.52%. However, shrimp head in this experiment contained higher protein and lower carbohydrate compared with *Litopenaeus vannamei* head in experiment of Fernandes *et al*. (2013) with containing moisture as 75.47±0.43% (dry basis), protein as 60.13±3.22% (dry basis), fat as 4.48±0.40% (dry basis), ash as 17.73±1.14% (dry basis) and carbohydrate as approximately 4.33% (dry basis). Difference between proximate compositions in shrimp head of each lot or experiment may indicate the natural phenomenon of raw materials and cultivation and aquaculture condition particularly when process steps were not well controlled. Since shrimp head used in this experiment was a by-product

of frozen shrimp, therefore, taking care of the by-product may not high leading to faster deterioration which was confirmed with TVB-N of shrimp head in this experiment as approximately 16 mg/100g is much higher when compared with TVB-N approximately 1 mg/100g of white shrimp head reported in Liu *et al.* (2021).

After fermentation for 90 d, moisture in all treatments decreased due to salting and drying process (Table 25). Moisture content of treatment with added NaCl 6.36% and KCl 2.73% (treatment 2) was the lowest while treatment with added NaCl 4.55% and KCl 4.55% (treatment 3) was the highest. This might be caused by K^+ inhibit Na^+ penetration in the muscle by react with muscle surface protein led to slow drying rate in treatment with higher KCl replacement (Chen *et al.*, 2019). No surprisingly, ash content of all treatments also increased compared with shrimp head because of the added salt during shrimp paste production. In addition, treatment (2) NaCl 6.36%, KCl 2.73% and (3) NaCl 4.55%, KCl 4.55% contained higher ash content due to higher salt added compared with treatment 1 (control treatment). Decreasing of fat and protein in all treatments after fermentation for 90 d may cause by leaching and melting of internal organ containing a pool of enzymes, protein and fat content during salting and fermenting. Penetration of salt during salting caused water loss in raw material (Pongsetkul *et al.,* 2017b). Moisture, fat and ash of all treatments in this experiment were similar to preliminary experiment which moisture was 42.5%, carbohydrate was 9.20% (dry basis), protein was 42.78% (dry basis), fat was 5.06% (dry basis), and ash was 42.96% (dry basis). All treatments in this experiment contained carbohydrate higher than preliminary experiment while protein was lower may due to different of raw material indicating the uncertainty of natural phenomenon. However, moisture, carbohydrate, protein and ash in this experiment after fermentation for 90 d was close to proximate compositions of commercial shrimp paste containing moisture as 33.79-52.54%, carbohydrate as 4.90-32.48% (dry basis), protein as 29.44-53.17% (dry basis), fat as 1.41-3.67% (dry basis) and ash as 33.80-50.50% (dry basis) (Pongsetkul *et al*., 2014).

Interesting, salt content in shrimp head using in this experiment was much higher (14.32±0.55) than preliminary experiment which was 4.96±1.09 (dry basis) (Table 25). This might be due to some residue of chlorine solution during

washing step still remained, since silver nitrate was a main chemical used in the determination of chlorine and salt (United States Environmental Protection Agency, 1994). Generally, fresh shrimp in frozen plant would be washed with different chlorine concentration based on microbiological quality recorded before purchasing and harvesting process. The more microorganism count the higher chlorine content would be applied.

Treatment 2 containing NaCl 6.36% and KCl 2.73% seemed to lower moisture content better than other treatments. This may due to proper replacement of NaCl with KCl to not high until can not reduce a_w or reabsorbed moisture back during drying and fermentation. As known that NaCl has a high potency to trap or absorb water in the dried sample stronger than KCl (Song *et al.*, 2020) therefore, it caused a harder water removal during drying (Fijal-kirejczyk *et al.*, 2013). After fermentation for 90 d, an increasing of salt content was found due to mainly drying. Although, significant different between treatment 2 and 3 was found, salt content in both treatments still not exceeded 30% which is maximum limit that extreme halophiles can grow (Pakdeeto, 2016). However, salt content in all treatments was in the range close to commercial Kapi containing salt as 22.77-42.48% (dry basis) (Kongpun and Kongrat, 2013; Pongsetkul *et al.*, 2014). Although, initial salt content of shrimp head using in this experiment was higher with about 14%, the final product was in the same range of other experiments or commercial one. This result indicated that salt content determination in the shrimp head should not be real salt but it should be chlorine residue as washing process which was explained earlier. Therefore, using $AgNO_3$ method for salt determination may give fault positive when the sample was contaminated with chlorine compounds that producer should take note. Although, all shrimp head paste in this experiment contained sodium lower than production standard of shrimp paste (Table 26), only shrimp head paste added with NaCl 6.36% and KCl 2.73% and shrimp head paste added with NaCl 4.55% and KCl 4.55 can categorized to be reduced sodium shrimp paste (sodium lower than 25% from standard) (Thai Food and Drug Administration, 2018). In addition, both mentioned shrimp head pastes were claimed as excellent source of potassium due to potassium content was more than 30% or 1,050

mg/100g of Thai daily potassium intake which is recommended at 3,500 mg/d (Thai Food and Drug Administration, 2018).

Table 25. Proximate compositions of shrimp head (*Litopenaeus vannamei*) and shrimp paste added with (1) NaCl 7.69%, (2) NaCl 6.36%, KCl 2.73% and (3) NaCl 4.55%, KCl 4.55% after 90 d of fermentation.

Sample	Proximate compositions (%) (dry basis except moisture)					
	Moisture	**Protein**	**Fat**	**Ash**	**Carbohydrate**	**Salt**
Head	76.54± 1.40^{a}	54.39± 0.38^{a}	6.35± 0.05^{a}	18.97± 0.78^{d}	20.29± 0.70^{b}	14.32± 0.55^{c}
1	42.72± 0.76^{bc}	33.25± 1.12^{b}	5.39± 0.08^{b}	36.80± 0.28^{c}	24.56± 0.77^{a}	27.78± 0.24^{ab}
2	40.82± 0.93^{c}	34.03± 1.51^{b}	3.38± 0.26^{c}	39.51± 0.76^{b}	23.08± 1.63^{ab}	27.03± 0.58^{b}
3	43.30± 0.25^{b}	32.05± 1.02^{b}	3.42± 0.07^{c}	41.33± 0.35^{a}	23.20± 1.32^{ab}	28.28± 0.29^{a}

Remark: Protein, fat, ash, carbohydrate and salt based on dry basis. Different letters indicate significant differences within value ($p < 0.05$).

Table 26. Sodium and potassium content in standard shrimp paste and shrimp head paste added with (1) NaCl 7.69%, (2) NaCl 6.36%, KCl 2.73% and (3) NaCl 4.55%, KCl 4.55% after 90 d of fermentation.

Sample	Sodium	Potassium
Standard	> 14.4%	-
1	11.11%	-
2	7.57%	4.25%
3	5.66%	7.42%

Remark: - means no potassium addition or regulation

4.4.1.2 Water activity (a_w)

After salting, a_w of treatments added with NaCl 6.36%, KCl 2.73% (treatment 2) and NaCl 4.55%, KCl 4.55% (treatment 3) reduced from 0.984 to 0.912 and 0.938, respectively (Figure 16). In initial process, only treatment added with NaCl 7.69% (treatment 1) provided a_w lower than 0.91 which can retard growth of microbial. As known that NaCl can bind to water (water holding capacity) higher than KCl at the same molarity, therefore a_w increased with higher KCl replacement. However, after drying, a_w of all treatments decreased to lower than 0.91 and kept constant until fermentation for 30 d. In fact, a_w lower than 0.91 but far to 0.7 still allows halophilic bacteria growth but inhibits spoilage microorganism (Majumdar *et al*. 2018). After secondary drying, a_w of all treatments sharply decreased to lower than 0.75, which can inhibit most microorganism growth including halophilic bacteria (Majumdar *et al.*, 2018). Furthermore, a_w kept constant until the end of fermentation. Most commercial shrimp pastes have a_w in the range of 0.669 – 0.774 which was in agreement of experiment of Pongsetkul *et al*., 2014. In addition, a_w of shrimp paste in this experiment and commercial was less than 0.85 which was under a regulation of recently shrimp paste production standard (Thai Industrial Standard Institute, 2018). However, it was found that replacing NaCl with KCl as 30% which was NaCl 6.36% and KCl 2.73% (treatment 2) exhibited the a_w lowest even though salt content was not highest (Table 25). Although, ionic strength of KCl was lower than NaCl in the same molarity, higher salt ratio (shrimp head 10: salt 1) in NaCl 6.36%, KCl 2.73% (treatment 2) and NaCl 4.55%, KCl 4.55% (treatment 3) may help to reduce water activity similarly to treatment containing 7.69% NaCl (treatment 1). Richardson and Jones (2007) reported that NaCl can significantly reduce a_w in raw material, however, during drying NaCl can caused slower drying rate due to its high water holding capacity (Chen *et al*., 2019).

Taken moisture content to compare with a_w each treatment found that there was a good relationship with R^2 between 0.7747 to 0.9774 was found in each treatment. It was interesting that taking 30% KCl to replace NaCl could control moisture content and a_w better than others particularly after drying and fermenting process.

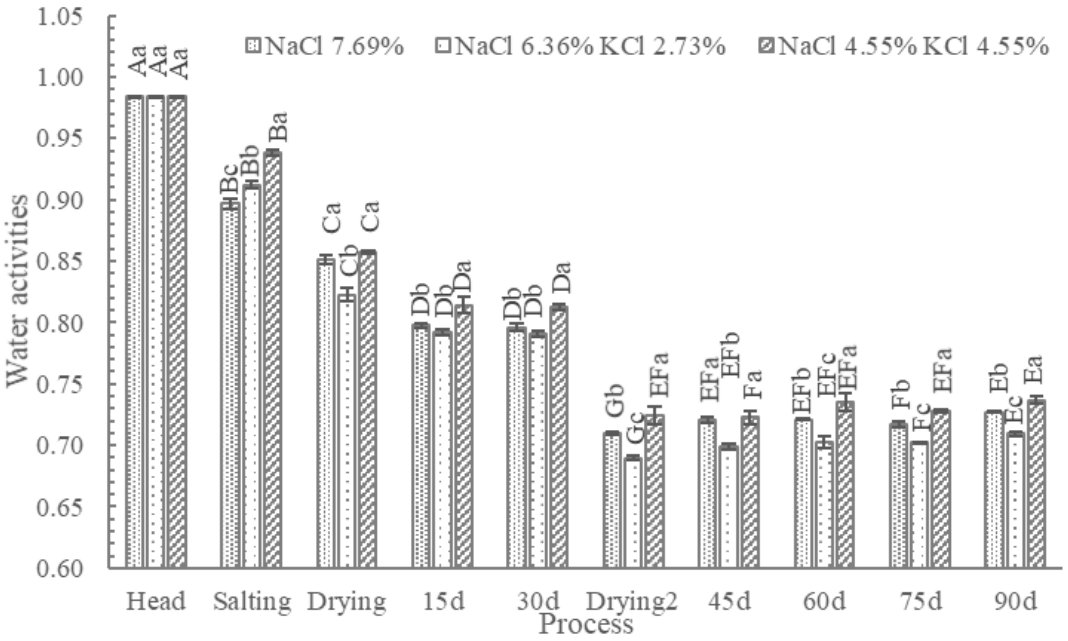

Figure 16. Water activity (a_w) of shrimp head (*Litopenaeus vannamei*) and shrimp paste added with (1) NaCl 7.69%, (2) NaCl 6.36%, KCl 2.73% and (3) NaCl 4.55%, KCl 4.55% after 90 d of fermentation. Different lowercase letters indicate significant difference within a process ($p < 0.05$). Head means shrimp head; Salting is after salting step 14-16 h; Drying is after primary drying step; 15d is after fermentation for 15 d; 30d is after fermentation for 30 d; Drying2 is after secondary drying; 45d is after fermentation for 45 d; 60d is after fermentation for 60 d; 75d is after fermentation for 75 d; 90d is after fermentation for 90 d (end of fermentation).

4.4.1.3 pH

After salting, pH increased in all treatments (Figure 17). Increasing of pH after salting indicated activity of endogenous enzyme and spoilage microorganism as a result of liberating of amine compound as a fermentation of protein and peptides hydrolysis of meat product (Etienne, 2005). This result was in agreement with increasing of volatile base nitrogen including TVB-N and TMA. However, pH in all treatments did not increase with increasing of TVB-N which the highest was found in treatment 3 and the lowest was treatment 1. Changes of pH in all treatments after salting might be affected by acid production which confirmed by increasing of LAB after

salting. After drying, pH in all treatments decreased due to heat treatment and remained constant until the end of fermentation. Volatile base compounds are known to be responsible for the increase of pH, however, generated heat during drying also accelerated evaporation of volatile base nitrogen, causing drop down of pH in the sample. The final pH of the product was approximately 8.07, which was close to commercial shrimp as 7.01-8.4 (Pongsetkul *et al*., 2014). According to production standard shrimp paste, pH generally range 6.5-7.8 (Ministry of industry). However, pH regulation was take out in the recently shrimp paste production standard (Thai Industrial Standard Institute, 2018). Higher pH of shrimp paste in this experiment may explain by nature of shrimp head containing internal organ which was easily digested by enzymes and microorganism as well as not proper management due to low price margin of by product. However, pH of shrimp head in this experiment was closely bound to pH 7.7 which was similar to the reported of Nargis *et al*. (2007).

Changes of pH involved with base and acid content as a function of microorganism and enzyme during fermentation. The unclearly change of pH after first time drying until end of fermentation may due to stabilization of production of base, acid, and vaporize of volatile acid and base as well as buffering capacity. Although, microorganism could not grow or even rapidly dead after secondary drying because low a_w, basic and acid compounds derived from enzyme of the dead microorganisms still provided (Tan and Qian, 1996). In addition, food containing high protein and amino acid such as histidine, cysteine, aspartic acid and glutamic acid were reported as high buffering capacity (Bhagavan and Ha, 2015; Govela *et al*. 2019). Amino acids mentioned above also found in shrimp head especially high glutamic acid (Ibrahim *et al*., 1999) indicating buffering capacity in shrimp paste leading to unchanged pH.

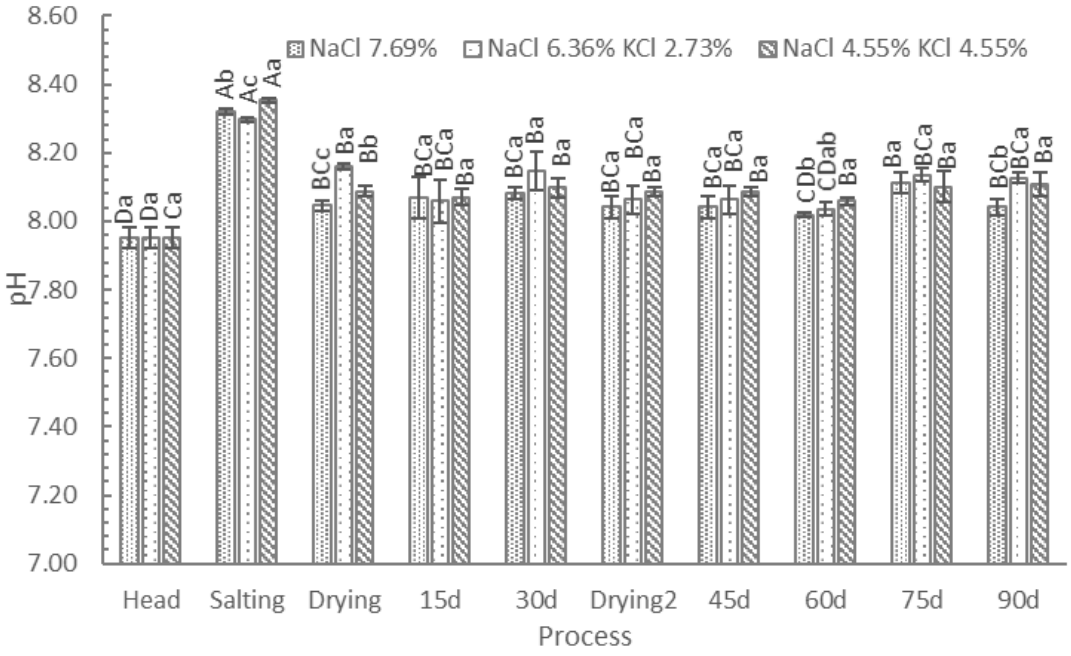

Figure 17. pH of shrimp head (*Litopenaeus vannamei*) and shrimp paste added with (1) NaCl 7.69%, (2) NaCl 6.36%, KCl 2.73% and (3) NaCl 4.55%, KCl 4.55% after 90 d of fermentation. Different lowercase letters indicate significant difference within a process ($p < 0.05$). Head means shrimp head; Salting is after salting step 14-16 h; Drying is after primary drying step; 15 d is after fermentation for 15 d; 30 d is after fermentation for 30 d; Drying2 is after secondary drying; 45 d is after fermentation for 45 d; 60 d is after fermentation for 60 d; 75 d is after fermentation for 75 d; 90 d is after fermentation for 90 d (end of fermentation).

4.4.1.4 Total volatile base nitrogen (TVB-N) and trimethylamine (TMA)

TVB-N and TMA of all treatments were showed in Figure 18 and Figure 19. TVB-N of shrimp head used in this experiment was lower than 25 mg/100 g which was considered as not spoilage but also not fresh. In addition, TVB-N of *Acetes vulgaris* (34.08 mg/100 g) using in the production of Indonesian shrimp paste was higher than TVB-N of the shrimp head in this experiment (Khairina *et al*., 2017). However, TVB-N of raw material used in this experiment was lower than Khairina *et al*. (2017). The different of TVB-N of raw material might due to difference freshness obtaining each

lot. TVB-N and TMA of all treatments increased after salting as expected corresponding with the increasing of TVC. Salting for 1 night can significantly increase TVB-N and TMA particularly treatment with replaced 50% KCl (treatment 3). It indicating that there was high protein hydrolysis process leading to volatile compound. However, after drying TVB-N and TMA of treatments with added NaCl 4.55%, KCl 4.55% (treatment 3) reduced to around 60 mg/ 100 g. TVB-N of all treatments later significantly increased then seemed to reduce after secondary drying. After that TVB-N of all treatments seemed to increase then kept constant until the end of fermentation. The tendency of TVB-N changes was mainly drying dependent nature. TMA seemed to increase during 30 d of the fermentation and decreased after secondary drying. TMA in all treatments seemed to increase with fermentation time increased, however, no significantly difference were found until the end of fermentation. It was noticed that drying step having both temperature and time can reduce both TVB-N and TMA value due to the nature of volatile phenomenon reduction. As affected of low a_w due to drying process and salting led to microorganism reduction, however, TVB-N and TMA kept constant as a result of their accumulation during fermentation process. Moreover, throughout this experiment TVB-N of all treatments seemed to lower than TVB-N of Indonesian shrimp paste after fermentation for 20 d with 150 mg/100 g (Khairina *et al*., 2017). The higher of TVB-N found in Indonesian shrimp paste even using higher salt content (12.5%) and shorter fermentation time may due to no drying process. In addition, Taiwan shrimp paste also contained TVB-N and TMA as 73 to 275 mg/100 g (158±45) and 10.3 to 44.9 mg/100 g (26.7±10.2), respectively (Tsai *et al.,* 2006). It pointed out the nature of fermented protein source yielded a big value of volatile base.

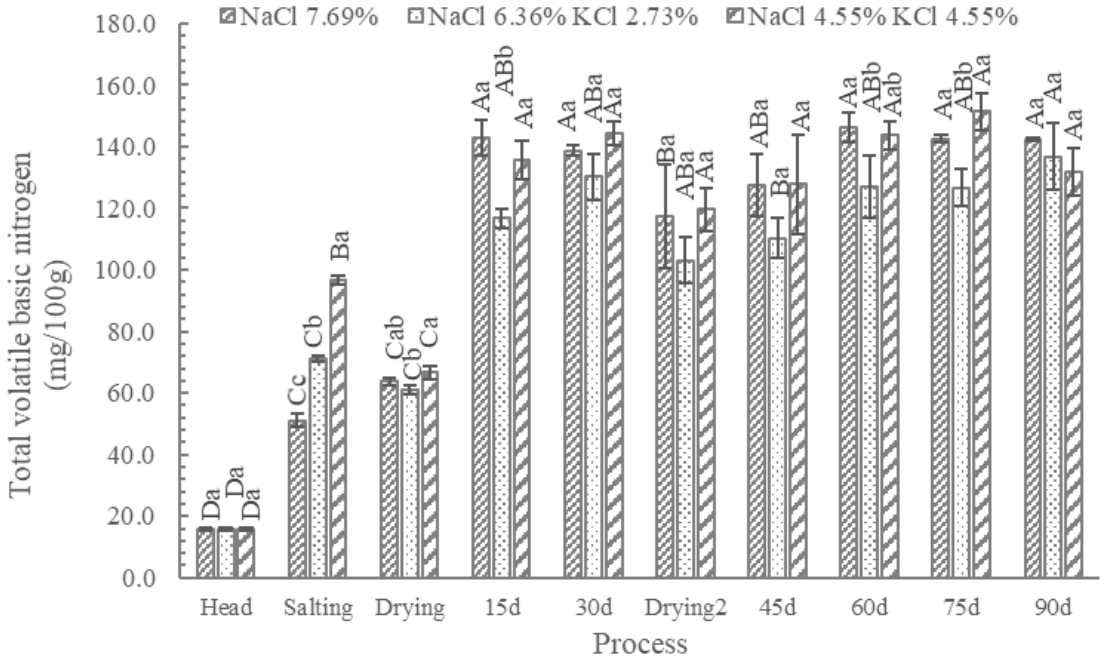

Figure 18. TVB-N of shrimp head (*Litopenaeus vannamei*) and shrimp paste added with (1) NaCl 7.69%, (2) NaCl 6.36%, KCl 2.73% and (3) NaCl 4.55%, KCl 4.55% after 90 d of fermentation. Different lowercase letters indicate significant difference within a process ($p < 0.05$). Head means shrimp head; Salting is after salting step 14-16 h; Drying is after primary drying step; 15d is after fermentation for 15 d; 30d is after fermentation for 30 d; Drying2 is after secondary drying; 45d is after fermentation for 45 d; 60d is after fermentation for 60 d; 75d is after fermentation for 75 d; 90d is after fermentation for 90 d (end of fermentation).

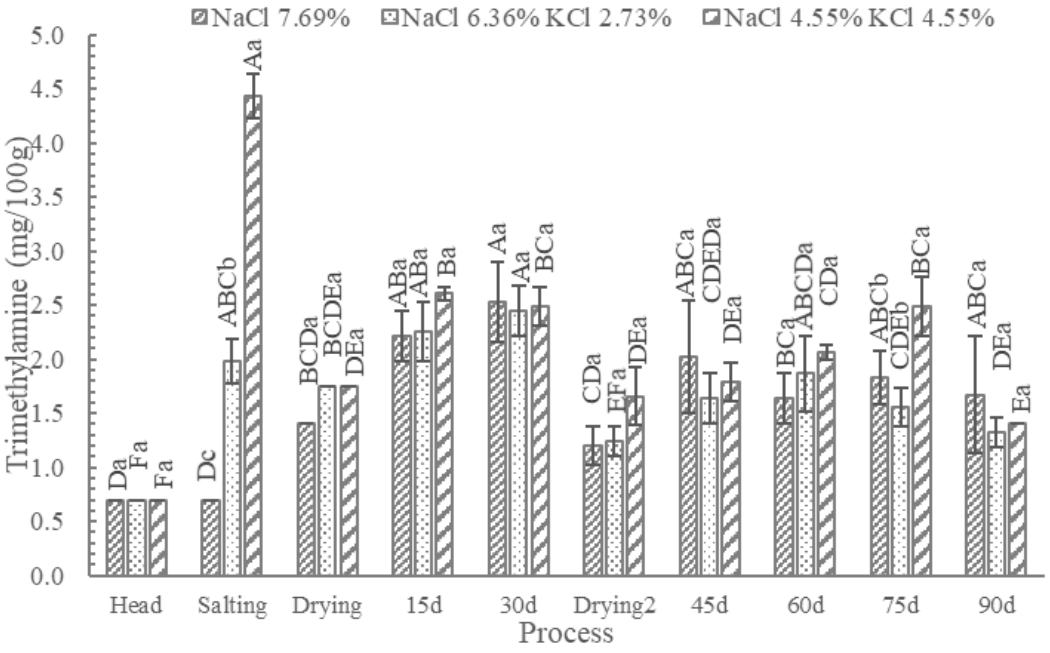

Figure 19. TMA of shrimp head (*Litopenaeus vannamei*) and shrimp paste added with with (1) NaCl 7.69%, (2) NaCl 6.36%, KCl 2.73% and (3) NaCl 4.55%, KCl 4.55% after 90 d of fermentation. Different uppercase letters indicate significant difference within a salt ratio. Different lowercase letters indicate significant difference within a process ($p < 0.05$). Head means shrimp head; Salting is after salting step 14-16 h; Drying is after primary drying step; 15d is after fermentation for 15 d; 30d is after fermentation for 30 d; Drying2 is after secondary drying; 45d is after fermentation for 45 d; 60d is after fermentation for 60 d; 75d is after fermentation for 75 d; 90d is after fermentation for 90 d (end of fermentation).

Volatile alkaline compounds particularly TVB-N consist of ammonia, TMA and dimethylamine. Generally, TVB-N compounds were produced from hydrolysis of protein by spoilage microorganism and endogenous enzyme, in addition, TMA which was responsible for fishy odor (Herath *et al*., 2019) was produced from degradation of trimethylamine oxide (TMA-O) by both endogenous enzymatic and bacterial action (Summers *et al.*, 2016). As well known that TVB-N and TMA also are used to indicate freshness and spoilage of food especially seafood (Summers *et al*., 2016). However, in the case of fermented food, increasing of TVB-N from protein degradation turned to indicate a signal of a typical fermentation process. It pointed out

that without an increase of TVB-N may indicate unfermented process particularly protein source material. Based on the standard of shrimp paste product, ammonia containing in shrimp paste must lower than 700 mg/100 g and must not detected fishy odor in shrimp paste (Ministry of Industry, 1992). However, no ammonia regulates in recently shrimp paste production standard (Thai Industrial Standard Institute, 2018). Burdock (2009) also reported that odor threshold of TMA was 5 mg/100g. Throughout this experiment, TVB-N and TMA in this experiment was lower than 700 mg/100g and 5 mg/100 g, respectively which was also considered safe for consumption (Ministry of Industry, 1992; Siripongvutikorn *et al*., 2008; Khairina *et al*. 2017). In general, TVB-N and TMA significantly reduced after drying was applied. In addition, treatment 2 seemed to exhibit the both values lower than others which related to the lower a_w as explained earlier. It was quite confirmed that using KCl for NaCl may not good only health benefit as hypertension but also help control fermentation process into the proper way.

4.4.1.5 Determination of thiobarbituric acid reactive substances (TBARS)

TBARS in this experiment was shown in Table 26. After fermentation for 90 d TBARS of all treatments increased. However, there was no difference between all treatments. It pointed out that KCl replacement in the sample 30 to 50% did not affect to lipid oxidation. This experiment was in agreement with the finding of Santos *et al.* (2017) who addressed that the replacement of NaCl with KCl 50% in dry fermented sausages did not modify lipid oxidation. As well known that lipid oxidation was affected by type of lipid, amount of unsaturated fatty acid, light, heat, oxygen, pro-oxidant and antioxidant (Amaral *et al.*, 2018). High lipid oxidation found in shrimp paste after fermentation for 90 d must cause by high unsaturated fatty acid as 65.21%, high a_w content in shrimp head, heat applied during dry and salt added which can act as pro-oxidant during fermentation. In addition, Vilgis (2015) also reported that lipid oxidation still occurred at a_w 0.70. It pointed out lipid oxidation in this experiment still existed throughout the fermentation process.

As know that oxidation of unsaturated fatty acids relate to rancidity of product which normally was determined through malondialdehyde (MDA) by using TBARS method (Kumar *et al*., 2018). Domínguez *et al*. (2019) stated that TBARS with more than 2.5 mg MDA/kg in meat product indicated rancidity, therefore, shrimp head using in this experiment exhibited definitely lipid oxidation before taking to experiment. High unsaturated fatty acid containing in shrimp head was susceptible to lipid oxidation since the beginning of post-harvest particularly when did not proper pay attention as by-product nature. Unsaturated fatty acid content in this shrimp head (65.21%) was similar to the finding of Takeungwongtrakul (2014). However, lipid oxidation found in whole *Acetes vulgaris* containing high unsaturated fatty acid (56.06%) was only around 0.6 mg MDA/kg (Pongsetkul *et al*., 2016; Pongsetkul *et al*., 2017a). The poor management included unchilled, unclean and hard handling of shrimp head containing high enzymes and microorganism as well as vulnerable structure which all supported rapidly deterioration and lipid oxidation further. It pointed out that quantity of unsaturated fatty acid may and may not well indicate potency of lipid oxidation if post-harvest handling did not specify.

Table 27. TBARS in shrimp head (*Litopenaeus vannamei)* and shrimp paste added with (1) NaCl 7.69%, (2) NaCl 6.36%, KCl 2.73% and (3) NaCl 4.55%, KCl 4.55% after 90 d of fermentation (mg/kg).

Process	**TBARS value (mg/kg)**		
	1	**2**	**3**
Shrimp head		4.47 ± 0.28^{b}	
90 d fermentations	13.43 ± 0.69^{a}	14.01 ± 1.00^{a}	13.08 ± 0.37^{a}

Remark: Different letters indicate significant differences ($p < 0.05$).

4.4.1.6 Degree of hydrolysis (DH)

Degree of hydrolysis (DH) in this experiment was showed in Table 27. The value in this experiment closed with preliminary experiment which was around 2.4%. This indicated that freshness of shrimp head used in this experiment was still comparable to preliminary test. Degree of hydrolysis in this experiment increased after fermentation for 90 d. In addition, the result in this experiment was similar to

commercial shrimp paste (12.68-20.67%) and preliminary experiment (10.72±0.61%) (Pongsetkul *et al.*, 2014).

Comparing the degree of hydrolysis with a_w indicated that the degree of hydrolysis well related to a_w with R^2 between 0.7747 to 0.9774. The lower a_w the lesser DH as showed in Figure 16 and Table 27. It pointed out that treatment 2 (added with NaCl 6.36% and KCl 2.73%) having the lowest a_w provided the lowest DH. This may due to reduction of enzymatic reaction from both microbial and endogenous enzymes (Barbosa-Cánovas *et al.*, 2003). Replacement KCl at 30% led to a_w and degree of protein hydrolysis reduction during fermentation which drying step was applied.

Table 28. Degree of protein hydrolysis of shrimp head (*Litopenaeus vannamei)* and shrimp paste added with (1) NaCl 7.69%, (2) NaCl 6.36%, KCl 2.73% and (3) NaCl 4.55%, KCl 4.55% after 90 d of fermentation.

Sample	Degree of protein hydrolysis (%)		
	1	**2**	**3**
Shrimp head	1.81 ± 0.02^{c}	1.80 ± 0.62^{c}	1.88 ± 0.63^{c}
90 d fermentations	11.71 ± 0.06^{ab}	10.74 ± 0.42^{b}	12.38 ± 0.24^{a}

Remark: Different letters indicate significant differences ($p < 0.05$).

4.4.2 Microbiological qualities

4.4.2.1 Total viable count (TVC) and Lactic acid bacteria (LAB)

TVC and LAB of all treatments increased after salting and fermentation for 1 night (Figure 20 and Figure 21). An increase of TVC and LAB in all treatments may explain the rule of high a_w and microbial selection (Majumdar *et al.*, 2018). TVC and LAB of all treatments sharply decreased after 45 d of fermentation and remained constant until the end of fermentation. A reduction of TVC and LAB after secondary drying may indicate the low a_w during fermentation process (drying and salting) and production of secondary metabolites of lactic acid bacteria such as antimicrobial agents as niasin and betaine (Murray, 2003; Lindstedt *et al.*, 2021) as well as nature of living cell life cycle (log stationary and death phase).

An increasing of TVC during salting and primary drying as well as further fermentation strongly related to higher degree of hydrolysis which indicated by the amount of amine and acid content. As known that LAB mainly produced organic acids especially lactic acid. while, proteolytic bacteria which normally are spoilage bacteria involve with amine production. The more amine production the higher pH particularly when the acid product is less. Therefore, constant of pH may indicate the basic and acid production balancing via degree of hydrolysis and LAB growth as well as buffering capacity. TVC and LAB values in this experiment were quite closed in number indicating that the predominant microorganism in shrimp head paste should be lactic acid bacteria. After fermentation for 90 d, TVC of all treatments were similar to TVC of commercial shrimp paste which varied from 1 to 6 log CFU/g (Kongpun and Kongrat, 2013). Based on production standard of shrimp paste, shrimp paste in this experiment after fermentation for 90 d which contained TVC, lower than log 5 CFU/g, was safe for consumption (Ministry of Industry, 1992). However, recently no regulation on TVC in standard of shrimp paste (Thai Industrial Standard Institute, 2018). Surprisingly, TVC and LAB of all treatments did not decrease after drying both first and second one as expected. It was possible that drying step did not provide high heat enough to suddenly destroy or kill the bacterial cell and spore. However, drying step may help to injure cells those would be death later. Therefore, reduction of TVC and LAB were found after drying instead. In addition, drying with hot air oven at 60-65 ˚C may facilitate some mesophile and thermophile as well as sporadic bacteria and mold to grow. This changing was under natural rule of number living cell which is dying and growing as growth cycle.

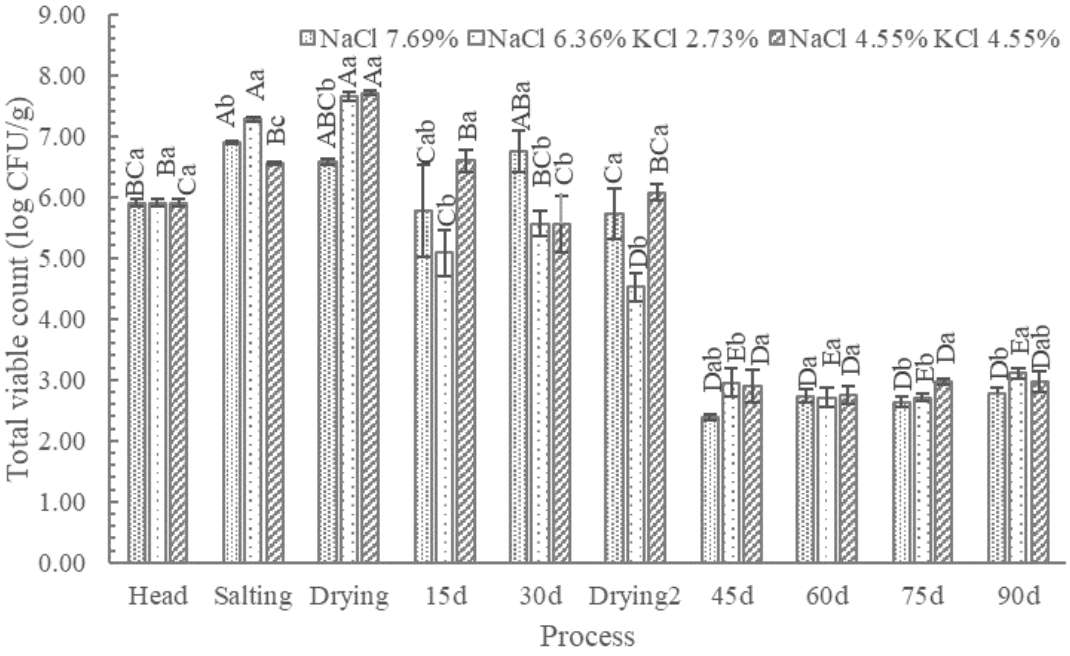

Figure 20. TVC of shrimp head (*Litopenaeus vannamei*) and of shrimp paste added with (1) NaCl 7.69%, (2) NaCl 6.36%, KCl 2.73% and (3) NaCl 4.55%, KCl 4.55% after 90 d of fermentation. Different lowercase letters indicate significant difference within a process ($p < 0.05$). Head means shrimp head; Salting is after salting step 14-16 h; Drying is after primary drying step; 15d is after fermentation for 15 d; 30d is after fermentation for 30 d; Drying2 is after secondary drying; 45d is after fermentation for 45 d; 60d is after fermentation for 60 d; 75d is after fermentation for 75 d; 90d is after fermentation for 90 d (end of fermentation).

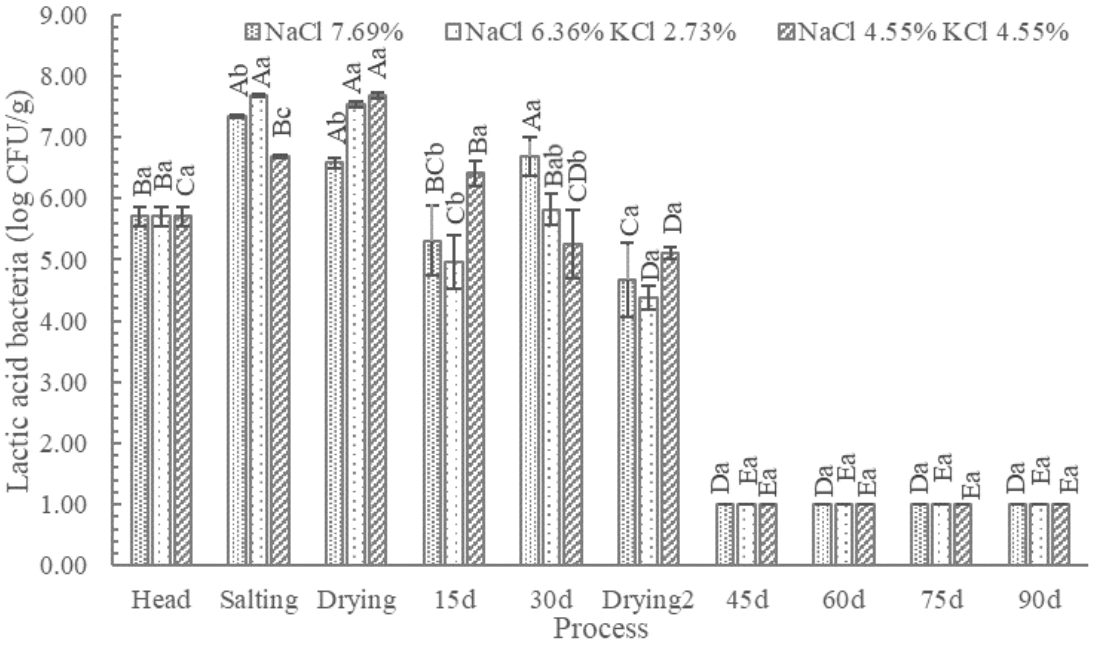

Figure 21. LAB of shrimp head (*Litopenaeus vannamei*) and of shrimp paste added with (1) NaCl 7.69%, (2) NaCl 6.36%, KCl 2.73% and (3) NaCl 4.55%, KCl 4.55% after 90 d of fermentation. Different lowercase letters indicate significant difference within a process ($p < 0.05$). Head means shrimp head; Salting is after salting step 14-16 h; Drying is after primary drying step; 15d is after fermentation for 15 d; 30d is after fermentation for 30 d; Drying2 is after secondary drying; 45d is after fermentation for 45 d; 60d is after fermentation for 60 d; 75d is after fermentation for 75 d; 90d is after fermentation for 90 d (end of fermentation).

4.4.2.2 Pathogenic microorganisms

Pathogenic microorganism in shrimp head used in this experiment was showed in Table 28. Based on safety requirements for agricultural commodity and food such as frozen and chilled shrimp, TVC, *E.coli* and *S. aureus* must contain lower than 6.70 log CFU, 3 MPN/g, 1000 MPN/g and no detected *Salmonella* in sample 25 g (National Bureau of Agricultural Commodity and Food Standards, 2008). Therefore, it indicated that shrimp heads used in this experiment were considered as safe. Yeast and mold gradually reduced until lower than 1 log CFU/g in all treatments after secondary drying. Reduction of a_w and antimicrobial effect of salt must be a great explanation of

yeast and mold reduction (Wijnker *et al*. 2006). Generally, all treatments after fermentation for 90 d were safe for consumption based on standard of microbiological quality. Therefore, replacement of NaCl with KCl at 30% and 50% did not provided any negative affect to pathogenic microorganism quality of shrimp head paste. In addition, using KCl to substitute NaCl would not cause any microbiological quality problem. And may help to reduce NaCl consumption which relates to hypertension as well. It pointed out using KCl substitution for shrimp head paste may be possible to carry on without any negative result.

Table 29. Pathogenic microorganism of shrimp head (*Litopenaeus vannamei*) and of shrimp paste after fermentation for 90 dd with (1) NaCl 7.69%, (2) NaCl 6.36%, KCl 2.73% and (3) NaCl 4.55%, KCl 4.55%.

Sample	**Pathogenic microorganism**				
	Coliform (MPN/g)	***Staphylococcus aureus* (per 0.1 g)**	***Clostridium perfringens* (per 0.01 g)**	**Salmonella (per 25 g)**	**Yeast mold (log CFU/g)**
Shrimp paste production standard[A]	<3	ND	ND	ND	<1.7
Shrimp head	<3	ND	ND	ND	2.97
(1) after 1st time drying	<3	ND	ND	ND	1.56
(2) after 1st time drying	<3	ND	ND	ND	1.50
(3) after 1st time drying	<3	ND	ND	ND	1.16
(1) after 2nd time drying	<3	ND	ND	ND	<1

Remark: ND means non-detected. A means data are from the Ministry of Industry, 1992.

Table 29. Pathogenic microorganism of shrimp head (*Litopenaeus vannamei*) and of shrimp paste after fermentation for 90 dd with (1) NaCl 7.69%, (2) NaCl 6.36%, KCl 2.73% and (3) NaCl 4.55%, KCl 4.55%. (continued)

Sample	Pathogenic microorganism				
	Coliform (MPN/g)	***Staphylococcus aureus* (per 0.1 g)**	***Clostridium perfringens* (per 0.01 g)**	**Salmonella (per 25 g)**	**Yeast mold (log CFU/g)**
(2) after 2nd time drying	<3	ND	ND	ND	<1
(3) after 2nd time drying	<3	ND	ND	ND	<1
(1) after 2nd time after 90 d	<3	ND	ND	ND	<1
(2) after 2nd time after 90 d	<3	ND	ND	ND	<1
(3) after 2nd time after 90 d	<3	ND	ND	ND	<1

Remark: ND means non-detected. A means data are from the Ministry of Industry, 1992.

4.4.3 ACE inhibition (%)

ACE inhibition of all treatments in this experiment were showed in Figure 22. ACE which its role is to convert angiotensin I to a vasoconstrictor angiotensin II and also promote the degradation of the vasodilator bradykinin leading to increase of blood pressure (Paiva *et al*., 2017). Therefore, the more ACE inhibition, the less blood pressure detected. No significantly difference between ACE inhibition of both control and blank sample with indicated that NaCl and KCl as well as NaCl plus KCl at set up in fermentation did not change activity of ACE. Increasing of ACE inhibition of positive control (captopril) with increasing of captopril concentration was

as expectation. Generally, captopril is used in medical treatment for hypertension, congestive heart failure and myocardial infarction at minimum dose 25 mg daily to maximum 150 mg (The Electronic Medicines Compendium, 2017). From the result of ACE inhibition, IC_{50} of captopril in this experiment was approximately 4.56 ng/ml (21 nM) which was higher than IC_{50} of captopril 0.39-3.28 ng/ml (1.79-15.1 nM) reported in Henda *et al.* (2013). Shrimp head used in this experiment containing ACE inhibition activity comparable with captopril at 2.17 ng/ml (10 nM) but significantly lower than captopril at 4.35 ng/ml (20 nM) and shrimp head pastes with or without KCl (S2, S3 and S4) in this experiment. In addition, ACE inhibition of shrimp head pastes with or without KCl in this experiment was comparable to captopril at 4.35 ng/ml (20 nM). ACE inhibition of shrimp head paste was higher than fresh shrimp head indicated that more antihypertensive peptides was generated during fermentation which also reported in Kleekayai *et al*. (2015b). In addition, Kleekayai *et al.* (2015a) also reported that two antihypertensive dipeptides were found in Kapi with IC_{50} 60.68±1.06 μM (Ser-Val) and 70.03±1.45 μM (Ile-Phe), respectively. Paiva *et al*. (2017) also confirmed that ACE-inhibitory peptides were usually found between peptides with 2 and 30 amino acids. Although, no significantly difference between all shrimp head paste (S1, S2 and S3) were not found, ACE inhibition seemed to reduce with amount of KCl in treatments increased. However, no significantly effect of KCl substitution on ACE inhibition was also reported in Ayyash *et al.* (2012) who studied cheese substitution with KCl up to 75%.

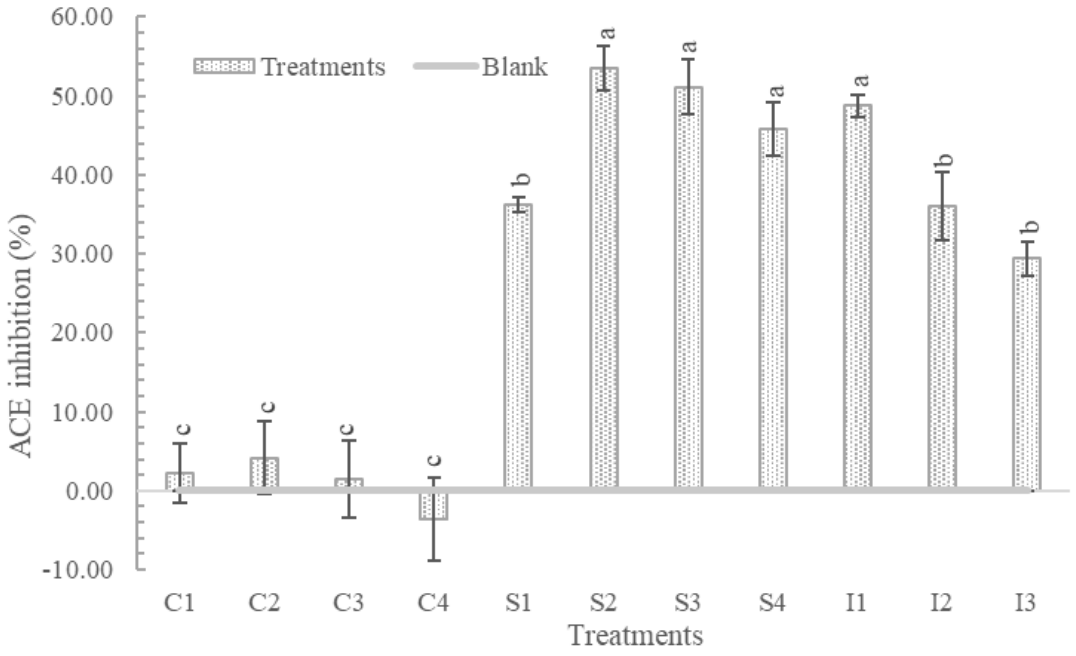

Figure 22. ACE inhibition (%) of shrimp head (*Litopenaeus vannamei*) and of shrimp head paste after 90 d of fermentation. Blank is tris-HCl; C1 is tris-HCl with 1.75% NaCl (0.3 M NaCl); C2 is tris-HCl with NaCl 7.69%; C3 is tris-HCl with NaCl 6.36%, KCl 2.73%; C4 is tris-HCl with NaCl 4.55%, KCl 4.55%; S1 is shrimp head; S2 shrimp head paste added with NaCl 7.69%, S3 is shrimp head paste added with NaCl 6.36%, KCl 2.73%; S4 is shrimp head paste added with NaCl 4.55%, KCl 4.55%; I1 is captopril 20 nM; I2 is captopril 10 nM; I3 is captopril 5 nM. Different letters indicate significant difference within a process ($p < 0.05$).

Therefore, in this experiment it can be concluded that shrimp head also contained some natural antihypertensive activity while fermentation did more increasing and replacement of sodium chloride with potassium chloride did not reduce ACE activity. However, blood pressure regulation did not control by only ACE, it involves various organ and mechanisms including renin angiotensin aldosterone system (RAAS), Kinin–kallikrein system (KKS) (Penton *et al*., 2015; Bekassy *et al.*, 2021). In addition, determination of antihypertension compound or compound with vasodilation properties can be done with various methods such as nitric oxide (NO) production (induce vasodilation in blood vessel), renin inhibition (inhibit angiotensin I production), gene eNOS (endothelial nitric oxide synthase) expression (increase of nitric oxide

synthase) (Onuh *et al*., 2015, Takashima *et al.*, 2017). While, Penton *et al*. (2015) also confirmed that the effect of high intake potassium on blood pressure reduction was not ACE inhibition but via stimulate sodium excretion while Takashima *et al*. (2017) reported that L-arginine of garlic induced NO production from nitric oxide synthase (NOS). In addition, Penton *et al*. (2015) reported that high potassium intake altered plasma K^+ levels led to reduce stiffness of endothelial cell, vasodilation by increasing nitic oxide (NO). K^+ also reduce excessive water by increasing sodium excretion, reducing sympathetic nervous system activity, hyperpolarization of endothelial and vascular smooth muscle cell lead to vasodilation, an altered response of arterial baroreceptors and reduced renal renin. Vio *et al.* (2020) also detected decreasing of renal renin, angiotensin I converting enzyme (ACE) and angiotensin converting enzyme II in rat received high potassium diet (3%) when compared to rat received normal potassium (0.9%). Increasing of potassium in blood was detected by inwardly rectifying potassium channel 4.1 and 5.1 (Kir 4.1 and Kir 5.1) in basolateral membrane which led to inhibition of chlorine channel. Accumulation of chlorine in intracellular therefore led to inhibition of apical sodium chlorine cotransporter (NCC) and decreasing of sodium reabsorption (Vio *et al*. 2020).

4.4.4 Sensory evaluation

Sensory score of shrimp head paste and commercial shrimp paste were significant difference (Table 30). Difference sensory acceptability between shrimp head paste and commercial shrimp paste may reflect many causative factors including freshness of raw material, different type of used raw material (whole krill for commercial one), and process improvement of sensory attributes (sugar, monosodium glutamate and other sweet potatoes) (marketing survey and personal data during shrimp paste plant visiting). However, one of a big problem that caused inferior quality in appearance and texture attribute of this shrimp head paste compared to commercial one was coarse appearance and rough texture. This problem was generated by thickness and toughness of shrimp head due to high content of calcium 3394 mg/100 g which was 2-fold of calcium value which was higher than in commercial shrimp paste (1565 mg/100 g) (Benjakul *et al*., 2011). Odor score of shrimp head paste was also inferior compared with commercial one may due to high deterioration rate of internal organ, however, this

problem could be resolved by increasing salt ratio to control both autolysis enzymes and microbial growth and increasing drying time of primary drying for more moisture decreasing. Although, sensory score of shrimp head paste in this experiment were lower than commercial shrimp paste, substitution of KCl for 30 and 50% did not showed any significant difference even bitter taste. It confirmed that using KCl to replace NaCl within 50% did not cause bitter taste and any negative effect on sensory evaluation.

Table 30. Sensory score of shrimp head (*Litopenaeus vannamei*) paste added with (1) NaCl 7.69%, (2) NaCl 6.36%, KCl 2.73% and (3) NaCl 4.55%, KCl 4.55% after 90 d of fermentation and commercial one.

Sample	Treatment					
	Appearance	**Color**	**Texture**	**Odor**	**Taste**	**Overall**
1	6.02± 1.02^{b}	6.13± 1.18^{b}	5.87± 1.12^{b}	6.25± 1.13^{b}	6.48± 1.12^{b}	6.18± 1.05^{b}
2	6.07± 0.95^{b}	6.18± 1.01^{b}	5.71± 1.14^{b}	5.83± 1.11^{b}	5.85± 1.18^{b}	5.79± 1.15^{b}
3	5.86± 1.28^{b}	6.13± 1.14^{b}	5.76± 1.22^{b}	5.81± 1.28^{b}	6.21± 1.32^{b}	6.08± 1.31^{b}
Commercial shrimp paste	7.60± 0.96^{a}	7.67± 0.88^{a}	7.50± 0.86^{a}	7.58± 1.11^{a}	7.55± 1.12^{a}	7.64± 1.09^{a}

Remark: Different letters indicate significant differences ($p < 0.05$).

4.5 Conclusion

Generally, there was no difference in physical, chemical, and microbiological quality in any treatment substituted with KCl up to 50%. Only reduction of moisture, a_w and degree of hydrolysis were noticed with the increase of KCl. Inhibition of ACE could be found in shrimp head even lower than shrimp head paste and, there was no difference in shrimp head paste among the treatment of using with 0% KCl and substituted with KCl up to 50%. In addition, bitter taste was not detected in any treatment with added KCl, however, further improvement of sensory qualities is still required to solve the poor texture and odor problem.

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CHAPTER 5

CONCLUSIONS AND SUGGESTIONS

5.1 Conclusion

5.1.1 The production of reduced sodium chloride shrimp head by using ratio of shrimp head to salt (12:1, 14:1 and 16:1). It was indicated that shrimp head can be used to produce shrimp head paste with physical, chemical and microbiological qualities comparable to commercial shrimp paste, however, Sensory quality need to improve. Based on by-product, it pointed out that product cost can be reduced at least 4 times due to raw material price of the shrimp head and commercial one was around 5 and 40 baht, respectively. Since, salt added in this experiment was lower than commercial one around 25%, then yield of this experiment may be a bit lower compared with traditional. Although, physical, chemical and microbiological of shrimp head paste was comparable to commercial shrimp paste, lipid oxidation of high unsaturated fatty acid containing in shrimp head and higher browning from Maillard reaction may lead to not long shelf-life as traditional that should be further studied.

5.1.2 The prevention of lipid oxidation in shrimp head paste using garlic showed that quality of shrimp paste were not significantly affect by addition of garlic at 3% and 5%, however, addition of garlic at 3% and 5% seemed to retard lipid oxidation determined by TBARS assay.

5.1.3 Based on physical, chemical, microbiological and sensory qualities, reduce sodium by replacement with KCl at 50% was comparable with 0% KCl shrimp head paste. Shrimp head paste can reduce hypertension by inhibition of ACE with shrimp head paste replacement with KCl at 50% was comparable with 0% KCl shrimp head paste. In addition, no bitter taste was detected in shrimp head paste replacement NaCl with KCl up to 50%. Shrimp paste substituted some NaCl with KCl may good for healthy person and hypertension patient, however, it may have adverse health effect on patient with kidney, diabetes and cardiovascular disease. According to cost of potassium chloride which is higher than 5 times of sodium chloride, but product cost of

replacement with KCl at 50% does increase the price only 7 baht/kg product therefore, it is still lower than 4 times of commercial shrimp paste.

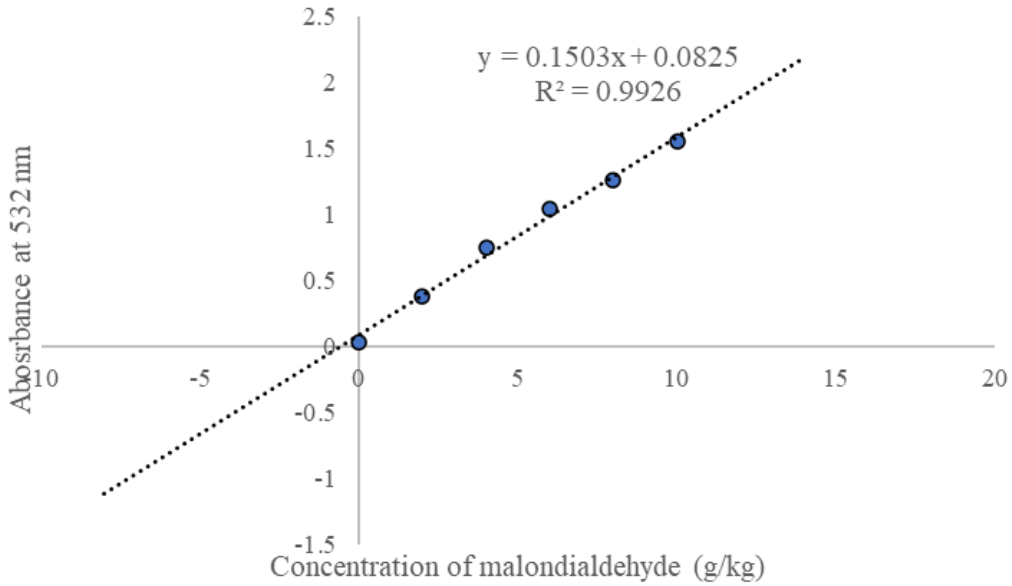
5.2 Suggestion for the future work

Since the sensory quality of reduce sodium chloride shrimp head pastes were lower comparing to ordinary shrimp paste, the ratio of shrimp head to salt may need to increase from 12:1. The used of shrimp head paste added with garlic may limit to only dishes that normally used shrimp paste with garlic such as green curry, Massaman curry and spicy shrimp paste dip (Nam Phrik Ka Pi). The replacement of KCl at 50% of NaCl may be alternative way to reduce NaCl in shrimp head paste. Using properly machine or enzyme to reduce size of shrimp head to improve the textural properties and adding sugar to improve taste were needed.

Appendices

APPENDIX A

STANDARD GRAPH OF THE MALONDIALDEHYDE



APPENDIX B

PRODUCTION STANDARD OF SHRIMP PASTE

1.General qualities

Shrimp paste must finely ground not too dried or wet with less fishy and ammonia smell, not too salty, no bitter taste and can be vary in color as pinkish, dark grayish brown and purplish gray

2. Filt and physical contamination

No filt and physical contamination such as cassava, flour, sand, pebble, part of insect or feces of animal

3. Chemical qualities

Table 31. Regulation standard for chemical qualities standard of shrimp paste

Chemical qualities	amount
Total nitrogen (dry basis)	≥58 g/kg or 5.8%
Salt content (dry basis)	≥36%
Ash (acid insoluble) (dry basis)	<0.5%
pH	6.5-7.8
Nitrogen from amino acid	≥50 g/kg or 5%
Ammonia nitrogen	<7 g/kg or 0.7%
Moisture	<45%

Source: Ministry of Industry, 1992

4. Food additives

Shrimp paste must not contain any preservative except for sulfur dioxide in raw material and fermentation must lower than 20 mg/kg with no food coloring and sugar substitutes.

5. Contaminants

Table 32. Regulation standard for heavy metals of shrimp paste (mg/kg).

Heavy metal	amount
Mercury	<0.5
Lead	<1.0
Cadmium	<1.0

Source: Ministry of Industry, 1992

6. Microbiological qualities

Table 33. Regulation standard for microbiological qualities of shrimp paste.

Microbiological qualities	Amount
Total viable count	<log 5 CFU/g
Coliform	<3 MPN/g
Staphylococcus aureus	Not detected in sample 0.1 g
Salmonella	Not detected in sample 25 g
Clostridium perfringens	Not detected in sample 0.01 g
Yeast and mold	<log 1.7 CFU/g

Source: Ministry of Industry, 1992

VITAE

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List of Publications and Proceedings

Rujirapong, C., Siripongvutikorn, S., Usawakesmanee, W. & Wanikorn, B. (2020). Quality of reduced sodium shrimp paste from shrimp head as alternative source. Food Science and Technology. https://doi.org/10.1590/fst.36921