CHANGES OF BIOCHEMICAL, PROTEIN CONFORMATION AND ODOR ACTIVE COMPOUNDS OF SILVER CARP WASHED MINCE AS AFFECTED BY ICE STORAGE OF RAW MATERIAL

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Food Technology

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การเปลี่ยนแปลงสมบัติทางชีวเคมี โครงร่างโปรตีนและสารให้กลิ่นสำคัญในเนื้อปลาเกล็ดเงินบดล้างน้ำที่ผลิตจากปลาที่เก็บรักษาในน้ำแข็ง

นางสาวศศิณี กันยาบุญ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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ศศิณี กันยาบุญ : การเปลี่ยนแปลงสมบัติทางชีวเคมี โครงร่างโปรตีนและสารให้กลิ่นสำคัญในเนื้อปลาเกล็ดเงินบดล้างน้ำที่ผลิตจากปลาที่เก็บรักษาในน้ำแข็ง (CHANGES OF BIOCHEMICAL, PROTEIN CONFORMATION AND ODOR ACTIVE COMPONDS OF SILVER CARP WASHED MINCE AS AFFECTED BY ICE STORAGE OF RAW MATERIAL) อาจารย์ที่ปรึกษา : รองศาสตราจารย์ ดร. จิรวัฒน์ ยงสวัสดิกุล, 129 หน้า.

กิจกรรมการย่อยสลายตัวเองและค่าเค ในปลาเกล็ดเงินเพิ่มขึ้นตามระยะเวลาในการเก็บรักษาในน้ำแข็ง ปริมาณพื้นผิวไฮโดรโฟบิกและหมู่ซัลฟ์ไฮดริลทั้งหมดที่เพิ่มขึ้นอย่างช้าๆ เมื่อระยะเวลาในการเก็บเพิ่มขึ้น บ่งชี้ให้เห็นว่าโปรตีนกล้ามเนื้อปลามีการเปลี่ยนแปลงโครงร่างหลังจากเก็บรักษาปลาเกล็ดเงินในน้ำแข็งเป็นเวลา 14 วัน พบว่าปริมาณผลผลิตของเนื้อปลาบดล้างน้ำที่ได้มีค่าลดลงถึง 13.27% ผลของฟลูเรียร์ทรานส์ฟอร์ม อินฟราเรดสเปคทรา พบว่าระยะเวลาในการเก็บปลาที่เพิ่มขึ้น โครงสร้างเกลียวแอลฟาในเนื้อปลาเกล็ดเงินลดลงเล็กน้อย ในขณะที่โครงสร้างเบตาชีทเพิ่มมากขึ้น ผลรามานสเปกทราแสดงให้เห็นว่าระยะเวลาในการเก็บนานขึ้นโปรตีนในเนื้อปลาบดและเนื้อปลาบดล้างน้ำมีการคลายตัวและเสียสภาพเพิ่มขึ้น บ่งชี้จากผลรวมของกรดอะมิโนอะโรมาติกที่เพิ่มขึ้น และการเกิดแลกเปลี่ยนกันของหมู่ไดซัลไฟด์ในเนื้อปลาเกล็ดเงินและปลาเกล็ดเงินบดล้างน้ำที่เพิ่มขึ้น

การเก็บรักษาในน้ำแข็งส่งผลต่อการเกิดออกซิเดชันของไขมันในอวัยวะต่างๆ ของปลาที่แตกต่างกัน พุงปลามีค่ากรดไทโอบาร์บิวทูริก (TBARS) สูงที่สุด ตามด้วยเนื้อปลาบดล้างน้ำ หนังปลา และเนื้อปลาบด ฟอสโฟลิปิดในเนื้อปลาบดล้างน้ำมีปริมาณสูงกว่าเนื้อปลาบด การเพิ่มระยะเวลาในการเก็บส่งผลให้ปริมาณไขมัน ฟอสโฟลิปิด กรดอะมิโนชนิดไม่อิ่มตัว และกรดอะมิโนชนิดอิ่มตัวในหนังปลา พุงปลา เนื้อปลาบด และเนื้อปลาบดล้างน้ำลดลง การเก็บรักษาในน้ำแข็งส่งผลให้เกิดผลิตภัณฑ์ที่สามารถระเหยได้จากปฏิกริยาออกซิเดชัน ซึ่งได้แก่ สารระเหยจำพวกแอลกอฮอล์ แอลดีไฮด์ คีโตน และไฮโดรคาร์บอน ผลของฟลูเรียร์ทรานส์ฟอร์ม อินฟราเรด *สเปค*ทราและผลรามานสเปกทราแสดงให้เห็นว่า ระยะเวลาการเก็บเพิ่มขึ้นส่งผลต่อการเกิดออกซิเดชันของไขมัน บ่งชี้จากการลดลงของพันธะคู่ที่มีโครงสร้างแบบซีส การลดลงของกลุ่มเมทิลีนและกลุ่มฟอสเฟส ในขณะที่พันธะเอสเทอร์เพิ่มสูงขึ้น และกรดไขมันเปลี่ยนโครงสร้างจากซีสให้อยู่ในรูปทรานส์

การแช่เยือกแข็ง-การละลายน้ำแข็งซ้ำ และความร้อน ส่งผลให้เนื้อปลาบดล้างน้ำเกิดออกซิเดชันของไขมันเพิ่มขึ้น โดยบ่งชี้จากการเพิ่มขึ้นของค่ากรดไทโอบาร์บิวทูริก (TBARS) และการเกิดการสารระเหยที่เป็นผลผลิตจากปฏิกริยาออกซิเดชันซึ่งได้จากการวิเคราะห์ด้วยแก๊สโครมาโทกราฟี-ออลแฟกโตรเมตรี (GC-O) และแก๊สโครมาโทกราฟี-แมสสเปกโตรเมตรี (GC-MS) ใน

จำนวนของสารระเหยให้กลิ่นที่เพิ่มขึ้นนี้ ผลที่ได้จากการคำนวณค่า odor activity value (OAV) พบว่า Z-1,5-octadien-3-one, (E)-2-nonenal และ 1-octen-3-ol เป็นสารให้กลิ่นสำคัญในเนื้อปลาปลาบดล้างน้ำ การวิเคราะห์หาปริมาณสารให้กลิ่นแสดงให้เห็นว่า hexanal และ 1-octen-3-ol สามารถนำมาใช้ติดตามการเกิดออกซิเดชันของไขมันในเนื้อปลาบดได้

สาขาวิชาเทคโนโลยีอาหาร ลายมือชื่อนักศึกษา_____

ปีการศึกษา 2559 ลายมือชื่ออาจารย์ที่ปรึกษา_____

SASINEE KUNYABOON : CHANGES OF BIOCHEMICAL, PROTEIN CONFORMATION AND ODOR ACTIVE COMPOUNDS OF SILVER CARP WASHED MINCE AS AFFECTED BY ICE STORAGE OF RAW MATERIAL. THESIS ADVISOR : ASSOC. PROF. JIRAWAT YONGSAWATDIGUL, Ph. D., 129 PP.

SILVER CARP/GAS CHROMATOGRAPHY-OLFACTOMETRY (GC-O)/ CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)/FOURIER TRANSFORM INFRARED (FTIR)/FOURIER TRANSFORM RAMAN (FT-RAMAN)/ODORANT VOLATILE COMPOUNDS/ODOR ACTIVE VALUES (OAVS)

Autolytic activity and K-value of silver carp increased with ice storage. Surface hydrophobicity and total sulfhydryl content also increased with storage time, implying conformational changes of muscle proteins. Protein yield of washed mince decreased by 13.27% after 14 days in ice storage. Fourier transform infrared (FTIR) spectra revealed that α-helix content of mince slightly decreased in concomitant with an increase in β-sheet with extended ice storage. FT-Raman spectra demonstrated that prolonged ice storage induced protein unfolding and protein denaturation of mince and their respective washed mince as indicated by an increase in aromatic amino acid residues of mince and the formation of a disulfide interchange of mince and their respective washed mince, respectively.

The degree of lipid oxidation in various fish tissues were affected by ice storage. Fish belly exhibited the highest thiobarbituric acid reactive substances

(TBARS) value, followed by washed mince, skin and mince. Phospholipid content of washed mince was higher than that of mince. Extended ice storage led to the reduction of total lipid, phospholipid, polyunsaturated fatty acid (PUFA) and monounsaturated fatty acid (MUFA) contents of skin, belly, mince and their respective washed mince. Alcohols, aldehydes, ketones and hydrocarbon were the oxidative volatile products accumulated during ice storage. FTIR and FT-Raman revealed that prolonged ice storage induced lipid oxidation as indicated by a decrease of *cis* double bonds, methylene groups and phosphate groups whereas ester bonds were increased, and conversion of *cis* to *trans* isomerize fatty acid.

Freeze-thaw and the thermal process induced lipid oxidation in washed mince as indicated by an increase in the TBARS value and a formation of oxidative volatile compounds identified by gas chromatography-olfactometry (GC-O) and gas chromatography-mass-spectrometry (GC-MS). Among odor active compounds identified, Z-1,5-octadien-3-one, (E) -2-nonenal and 1-octen-3-ol were considered important odorants in washed silver carp mince. Quantitative measurements suggested that hexanal and 1-octen-3-ol could be used as markers of lipid oxidation in silver carp washed mince.

School of Food Technology Student's Signature_____

Academic Year 2016 Advisor's Signature_____

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LIST OF ABBREVIATIONS

MT	=	Metric ton
IUU	=	Illegal, unreported and unregulated
PV	=	Peroxide value
TBARS	=	Thiobarbituric acid reactive substances
FTIR	=	Fourier transform infrared spectroscopy
FT-Raman	=	Fourier transform Raman spectroscopy
GC	=	Gas chromatography
GC-MS	=	Gas chromatography-mass- spectrometry
GC-O	=	Gas chromatography-olfactometry
SIDA	=	Stable isotope dilution assay
°C	=	Degree Celsius
ATP	=	Adenosine triphosphate
ADP	=	Adenosine diphosphate
AMP	=	Adenosine monophosphate
IMP	=	Inosine monophosphate
Ino	=	Inosine
Hx	=	Hypoxanthine
PUFA	=	Polyunsaturated fatty acids
PL	=	Phospholipids
pH	=	Potential of hydrogen ion
NaCl	=	Sodium chloride

LIST OF ABBREVIATIONS (Continued)

M	=	Molar
AnV	=	Anisidine value
OAV	=	Odor activity value
BSA	=	Bovine serum albumin
TCA	=	Trichloroacetic acid
AOAC	=	The Association of Official Analytical Chemists
T-SH	=	Total sulfhydryl content
R-SH	=	Reactive sulfhydryl content
mM	=	Millimolar
FI	=	Fluorescence intensity
ATR	=	Attenuated total reflectance
MHC	=	Myosin heavy chain
AC	=	Actin
TM	=	Tropomyosin
FAME	=	Fatty acid methyl esters
FID	=	Flame ionization detector
TEP	=	Tetraethyloxypropane
SPME	=	Solid phase microextraction
CAR/DVB/PDMS	=	Carboxen–divinylbenzene–olydim-ethyl–siloxane
PTFE	=	Polytetrafluoroethylene
SFA	=	Saturated fatty acid
MUFA	=	Monounsaturated fatty acid

LIST OF ABBREVIATIONS (Continued)

PUFA	=	Polyunsaturated fatty acid

CHAPTER I

INTRODUCTION

1.1 Introduction

Demand of surimi or washed mince as an intermediated raw material to produce variety of products, such as crabstick, fish ball, fish tofu, and etc., has reached 830,000 T in 2016 (Wietecha, 2017) . Thailand is the one of the largest surimi producers in Southeast Asia with the production was estimated at 90,000 MT in 2012 (Guenneugues and Lanelli, 2013). Tropical fish species used for surimi production in Thailand include threadfin bream, lizardfish,, big eye snapper and goat fish. However, surimi industry in Thailand has encountered a problem with overexploitation of fish stock in the Gulf of Thailand and enforcing the of illegal, unreported and unregulated (IUU) fishing since 2015 (Ministry of Foreign Affairs, 2017) . There has been an attempt to find new resources for surimi industry.

Surimi can be produced from both marine and freshwater fish. Functional and compositional properties vary depending on species used. The use of alternative species in order to obtain good gel-forming ability of surimi is one of the aims of the industry. Silver carp (*Hypophthalmichthys molitrix*) is one of important freshwater fish. World production of silver carp in 2015 reached to 5.13 MT which was ranked as the second largest quantity in the world (FAO, 2017). The problems in utilization of freshwater species include soft texture, pin bones and muddy flavor. Utilization and distribution as high value fish is very limited. The use of alternative species in order to

obtain good gel-forming ability of surimi is one of the aims of the industry (Nopianti, Huda, and Ismail, 2010). Thus, a means to fully utilize and increase value of silver carp is needed.

Ice storage is required for surimi production in Thailand as fish must be delivered from farm to plant. The rate of deterioration during ice storage is species-specific and depends on post-harvest handlings (Pacheco-Aguilar, Lugo-Sánchez, and Robles-Burgueño, 2000). Study on biochemical changes of during ice storage of silver carp is limited.

Lipid oxidation is one of major causes affecting quality of surimi. Triacylglycerides and phospholipids are generally thought to be major lipid fraction responsible for oxidative deterioration. It produces unstable intermediate compounds, such as free radicals and hydroperoxides and then decompose to form secondary oxidative products leading to formation of off-flavor volatile compounds (Eymard, et al., 2005). During washing, triacylglycerides are largely removed whereas membrane phospholipids mostly remain (Raghavan and Kristinsson, 2008). However, a small percentage of phospholipids are highly unsaturated and often in contact with muscle heme iron and are, therefore, sensitive to oxidation (Lanier, 2000).

Evaluation of lipid oxidation of flesh fish and surimi is normally based on analytical methods like peroxide value (PV) and thiobarbituric acid reactive substances (TBARS). However, such methods provide only partial information on the oxidative process. In the case of TBARS, malondialdehyde is only one of many possible secondary oxidation products formed and other compounds not resulting from the oxidation process, can contribute to TBARS values (Gimenez, Gomez-Guillen, Pérez-Mateos, Montero, and Márquez-Ruiz, 2011). Recently, the evaluation of volatile compounds has become an additional indicator of lipid oxidation in fish

and surimi samples (Iglesias and Medina, 2008). In addition, vibrational spectroscopy including Fourier transform infrared spectroscopy (FTIR) and Fourier transform (FT)-Raman techniques, is a useful analytical technique to characterize lipids and monitor oxidative changes due to non-destructive, fast and high information content of spectra.. However, there are only few reports dealing with oxidative changes of fish and surimi by vibrational spectroscopic techniques.

Odors are volatile compounds perceived by human nose. It is an importance factor influencing consumer acceptance. Intensity of off-odor of surimi is typically less than of mince counterpart as repeating washing process could remove significant amount of odorous compounds. Changes of odor profile of surimi could take place, particularly during frozen storage. Lipid oxidation during frozen storage can be a prime reason of off-odor in raw surimi. In addition, odor characteristics of raw surimi would be different from surimi gel which is subjected to heat. To understand volatile compounds responsible for odor of raw surimi and cooked surimi gel will help to explain the odorants that contributed to the overall aroma in sample. Moreover, lipid oxidation marker in raw surimi and cooked surimi gel will be obtained.

1.2 Research objectives

The objectives of this study were:

1. To determine biochemical changes at postharvest silver carp stored in ice and their respective washed mince

2. To evaluate the effect of ice storage on lipid oxidation of various tissue of silver carp and its washed mince

3. To identify and quantify the odor active volatile compounds of silver carp washed mince under freeze-thaw cycles and cooked gel.

1.3 Research hypotheses

Iced storage induces biochemical and conformational changes of proteins of silver carp and their respective washed mince, which eventually affect yield and textural properties of washed mince Lipid oxidation of silver carp during ice storage varies with parts of tissue. In addition, extensive temperature abuse during freezing and thermal process induces lipid oxidation, leading to various volatile compounds with distinct notes.

1.4 Scope of the study

Biochemical changes of silver carp stored in ice for 0 7 and 14 days and their respective washed mince were investigated. Proximate analyses, muscle composition, total lipid content were evaluated. Freshness quality of fish was assessed by K-value. Yield and textural properties of its washed mince was investigated. Protein conformational changes of minces and respective washed minces were monitored by FT-Raman and FT-IR.

Lipid oxidation of fish at various parts, namely fish flesh, skin and belly, was followed during ice storage of 0 7 and 14 days. Lipid oxidation of washed mince prepared from fish at various storage time was also evaluated.. Lipid oxidation products were measured by TBARS. Volatile compounds were analyzed by GC-MS. Total lipid content, phospholipid content, fatty acid profile were investigated. Changes of lipid structure were monitored by FT-Raman and FTIR.

Silver carp washed mince was prepared and subjected to 10 freeze-thaw cycles and analyzed for TBARS. Changes of odorants were monitored by gas chromatography-olfactometry-mass spectrometry (GC-O-MS). A stable isotope dilution assay (SIDA) was used for quantitation of selected compounds.

1.5 References

Eymard, S., Carcouët, E., Rochet, M. J., Dumay, J., Chopin, C., and Genot, C. (2005). Development of lipid oxidation during manufacturing of horse mackerel surimi. **Journal of the Science of Food and Agriculture**, 85(10): 1750-1756.

FAO. (2017). FAO yearbook. Fishery and Aquaculture Statistics. 2015/FAO annuaire. Statistiques despêches et de l'aquaculture. 2015/FAO anuario. Estadísticas de pesca y acuicultura. 2015. Rome/Roma, Italy/Italie/Italia.

Gimenez, B., Gomez-Guillen, M., Pérez-Mateos, M., Montero, P., and Márquez-Ruiz, G. (2011. Evaluation of lipid oxidation in horse mackerel patties covered with borage-containing film during frozen storage. **Food Chemistry**, 124(4): 1393-1403.

Guenneugues, P., and Lanelli, J. (2013). Surimi resources and market. **Surimi and surimi seafood**: 25.

Iglesias, J., and Medina, I. (2008). Solid-phase microextraction method for the determination of volatile compounds associated to oxidation of fish muscle. **Journal of Chromatography A**, 1192(1): 9-16.

Lanier, T. C. (2000). *Surimi gelation chemistry. In J. W. Park (Ed.),Surimi and surimi seafood*: New York, USA: Marcel Dekker.

Ministry of Foreign Affairs, D. o. E. A., Thailand,. (2017). Hightlights of progress: Thailands's commitment to combating IUU fishing. Available at: http://www.thaiembassy.org/bucharest/contents/files/news-20170125-163408-373852.pdf(Accessed on Febuary 21, 2018).

Nopianti, R., Huda, N., and Ismail, N. (2010). Loss of functional properties of proteins during frozen storage and improvement of gel-forming properties of surimi. **Asian Journal of Food and Agro-Industry**, 3(6): 535-547.

Pacheco-Aguilar, R., Lugo-Sánchez, M., and Robles-Burgueño, M. (2000). Postmortem biochemical and functional characteristic of Monterey sardine muscle stored at 0 C. **Journal of Food Science**, 65(1): 40-47.

Raghavan, S., and Kristinsson, H. G. (2008). Antioxidative efficacy of alkali-treated tilapia protein hydrolysates: a comparative study of five enzymes. **Journal of agricultural and food chemistry**, 56(4): 1434-1441.

Wietecha, O. (2017). Global 2017 surimi supply expected to remain stable. Available at: https://www.undercurrentnews.com/2017/05/19/global-2017-surimi-supply-expected-to-remain-stable/, Accessed Febuary 19, 2018.

CHAPTER II

LITERATURE REVIEWS

2.1 Silver carp

Silver carp (*Hypophthalmichthys molitrix*) is a freshwater species living in temperate conditions (6-28°C) and its natural distribution is in Asia. This species requires static or slow-flowing water, as found in impoundments or the backwaters of large rivers. In its natural range, it is potamodromous, migrating upstream to breed; eggs and larvae float downstream to floodplain zones. While it is fundamentally benthopelagic, as an active species it swims just below the water surface and is well known for its habit of leaping clear of the water when disturbed. Silver carp are typical planktivores, the gillrakers being the main means of filtration. Silver carp consume diatoms, dinoflagellates, chrysophytes, xanthophytes, some green algae and cyanobacteria ('blue green algae'). Silver carp is the most commonly aquacultured carp species. FAO reported that silver carp is also low value (FAO, 2007). After they are harvested, their distribution sphere and storage period are very limited. Subsequently, although there is high production, the commercial value of their fresh fish flesh is low. Silver carp is well accepted for human consumption due to its attractive white color. It has been processed in many forms including fillet and surimi products. The fillet production can cause a waste of meat because the edible portion has a high amount of fish-bone (Ramirez et. al., 2000). In contrast, surimi products have been considered to be promising due to its more efficiently utilization of silver carp resources. However,

silver carp surimi exhibited considerable gel softening (modori) in the course of heating, resulting in a decrease in textural quality, eventually having a negative effect on acceptance and price of the surimi products (Luo et al., 2001).

2.2 Overview of surimi

Surimi is a washed fish mince or concentrated myofibrillar protein obtained after repeatedly cold washing process to remove sarcoplasmic protein. Surimi is introduced to Thailand since 1978-1979. Tropical marine fish species such as threadfin bream, lizardfish, bigeye snaper and goat fish is important raw material. In 2012, there were 15 surimi manufacturers produced surimi and surimi based products (Park, Nozaki, Suzuki, and Beliveau, 2013).

Washing process is a very important step in surimi processing. Washing is necessary to remove water-soluble substances, mainly sarcoplasmic proteins, fat and other undesirable materials like pigments. The removal of sarcoplasmic proteins concentrates myofibrillar proteins which are the primary component in the formation of three-dimensional gel structure (Hall and Ahmad, 1997). The number of washing cycles and the volume of water varies with fish species, freshness quality, type of washing unit and the desired quality of the surimi (Hall and Ahmad, 1997). In general, a five minute agitation in each of two washing cycles using a 3:1 (v/w; water to mince) ratio has been considered to be adequate for raw fish surimi production (Lee, 1986). Pacheco-Aguilar, Crawford and Lampila (1989) investigated the effect of washing in Pacific whiting surimi production and reported that removal of lipids was not efficiently achieved using a single wash with a 3:1 ratio (v/w; water to mince) but resulted in high solids and protein recoveries which are often low in multiple washing cycles. Lin and Park (1996)

reported that an effective washing process can now be accomplished with two washing cycles at water to meat ratio of less than 2:1 with typical wash ratio of 0.9-1.2 parts water in the first wash and 0.7-0.8 in a second wash. An insufficient washing process could result in substantial loss of gel quality during frozen storage. On the other hand, over- washing could cause substantial loss of fine particles and excessive moisture content, resulting in difficulty of refining and dewatering in the screw press or decanter. Excessive washing also enhances myofibrillar protein solubilization as mechanical agitation increases.

Neutral lipids were more easily removed during washing than membrane phospholipids (Raghavan and Kristinsson, 2008). Phospholipid content ofsurimi is higher than in mince.Membrane polar lipids, interacting with proteins and consequently being less easily removed than neutral lipids during washing process (Eymard, et al., 2005). Tongnuanchan et al., (2011) reported that lipid content of red tilapia washed mince was decreased by 14.4%, in comparison with that found in mince. Eymard et al., (2005) reported that most of lipids in horse mackerels mince were removed during washing stage and neutral lipids were lost in higher proportion than polar lipids. Dawson et al., (1990) found that washing removed more neutral lipids than phospholipids from mechanically separated chicken meat.

Srinivasan, Xiong, and Decker (1996) reported that mince sardine washed with sodium bicarbonate (0.5%) showed high reductions in lipid components. Hrynets et al., (2011) reported that citric acid significantly influenced protein yield and lipid removal during extraction of proteins from mechanically separated turkey meat (MSTM). The optimal concentration of citric acid for the maximum protein yield was 6 mM. However, 2 mM of citric acid was the most efficient for removing phospholipids. This resulted in

greater stability of isolated proteins against lipid oxidation compared with raw MSTM. Rhee et al., (1998) reported that washing ground lamb 4 times with high-pH (8.2) tap water, sharply lowered total fat content (primarily by removing neutral lipids), and decreased lipid oxidation during 4^{o}C storage.

Myofibrillar protein is a dominant functional component in surimi. Ice storage is a widely used method for short-term storage of surimi products. During ice storage, surimi may lose its functional properties as a result of myofibrillar protein denaturation, such as a decrease of water-holding capacity, protein solubility and gel-forming ability (Xiong, et al., 2009). Jasra, Jasra, and Talesara (2001) reported that after muscle fillets of rohu carp (*Labeo rohita*) were kept either at 2°C for up to 15 days or frozen at -8°C or -20°C for up to 6 months, myofibrillar protein fractions, myosin light chains and α-actinin, degraded during such frozen storage conditions. The importance of the post-mortem storage temperature for controlling the degradation of the myofibrillar proteins was emphasized.

2.3 Postharvest changes during ice storage

Several postmortem changes in fish starts immediately after death. Freshness continuously decreases postmortem. K-value is a useful method to determine changes of freshness of many fish species. It was firstly introduced by Saito, Araki, and Matsuyoshi (1959) which calculated on the basis of the results of the adenosine triphosphate (ATP) content in muscle tissue as well as its nucleotide breakdown products, namely adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), inosine (Ino), and hypoxanthine (Hx). Lin and Morrissey (1994) suggested that value lower than 20% is considered a very good quality

fish, a value of 60% is being the rejection point and more than 80% is inadequate for consumption. Degradation of ATP-related compounds during ice storage was varies with fish species depending on the species. Puchała, Białowąs, and Pilarczyk (2005) reported that K-value of carp (*Cyprinus carpio*) stored on ice increased from 3.8% to 52.6% after seven days of storage.. Ocaño-Higuera, et al. (2009) found that K-value of ice stored cazon fish (*Mustelus lunulatus*) reached to 58.9% at day 18. They classified that less than 6-days ice stored cazon fish was very fresh and moderately fresh up to day 16 followed by a minimum freshness until the end of storage. Lakshmanan, Antony, and Gopakumar (1996) reported that 4 and 8 ice stored of mullet (*Liza corsula*) and pearlspot (*Etroplus suratensis*) with K-value of 29.8% and 23.5%, respectively, were considered as fresh fish, remained a good quality after 8 and 13 storage with K-value of 29.8%and 23.5%, respectively and rejected after 12 and 14 day in ice with K-value of 70.59% and 54.94%, respectively. Özoğul, Boğa, Tokur, and Özoğul (2011) reported that K-value of wild common sole (*Solea solea*) was more than 80% after 16-18 days ice storage suggesting fair quality.

2.4 Lipid component in fish muscle

Fish lipids are well known to have a high content of n-3 and n-6 polyunsaturated fatty acids (PUFA) (Lei, et al., 2012). Although freshwater fish in general contain higher proportions of n-6 PUFA (2.42-21.92%) than marine species (0.43-14.2%), they also have excellent levels of n-3 PUFA, such as 20:5n-3 (1.15-13.8%) and 22:6n-3 (0.94-24.8%), with a ratio of (n−6)/(n−3) PUFA varying between 0.04 and 4.55 (Özogul, Özogul and Alagoz, 2007). The lipid content and fatty acid profile of fish vary with species even in dark and white muscle, which are affected by many factors such as the

temperature, salinity, season, size, age, species habitat, life stage, and the type and abundance of food, especially whether a species is herbivorous, omnivorous or carnivorous (Hossain, 2011; Saito et al., 1999; Sargent et al., 1995). The seasonal changes in the contents of fatty acids were previously recorded in gilthead sea bream (*Sparus aurata*) (Grigorakis et al., 2002), in baltic herring (*Clupea harengus membras*) (Aro, et al., 2000), and some other fish species (Luzia et al., 2003; Tanakol et al., 1999). Although fatty acid composition of triglycerides closely resembles that of the fish diet and has been used to identify the production method (that is, farmed versus wild), the positional distribution of the fatty acids in the carbons of the glycerol has been used to identify species and adulterations in oil and oil mixtures (Rehbein and Oehlenschlager, 2009).

Lipids in fish can be divided into two main classes, neutral lipids (NL-triacylglycerols, TAG) and phospholipids (PL) (Rehbein and Oehlenschlager, 2009). Phospholipids are made up of four components: fatty acids, a glycerol backbone, a negatively charged phosphate group and a headgroup of nitrogen-containing alcohol. Triglycerides are highly hydrophobic, whereas phospholipids are hydrophilic due to the polar headgroup (Burri et al., 2012). Phospholipids are known to interact with proteins and being difficult to remove by washing (Lanier, Carvajal, and Yongsawatdigul, 2000).

2.5 Lipid oxidation in surimi

Lipid oxidation has been recognized as a leading cause of quality deterioration in muscle foods and is often used to determine shelf-life of food products (Frankel, 1993). Lipid oxidation is a complex process whereby unsaturated fatty acids react with molecular oxygen via a free radical mechanism (Asghar et al., 1988). The basic

mechanisms of lipid oxidative reactions can be characterized by three distinctive steps: initiation, propagation and termination reactions (Nawar, 1996). The major primary products of this reaction, are hydroperoxides which are relatively unstable and essentially odorless, and decompose into a wide range of secondary compounds, including alkanes, alkenes, aldehydes, ketones, alcohols, esters, acids, and hydrocarbons. Of these compounds, aldehydes are considered the most important breakdown products because they possess low threshold values and are the major contributors to the development of rancid off-flavors and odors (Ladikos and Lougovois, 1990; Ross and Smith, 2006). Other problems associated with lipid oxidation include deterioration in flavor, color, and texture (Kanner, 1994). Among different lipid classes of fish muscle, phospholipids (membrane lipids) which usually represent ~1% of the total lipids are believed to be the primary subtrates of lipid oxidation leading to rancidity (Gandemer and Meynier, 1995). This is because of their high degree of unsaturation, large surface area and the proximity to oxidation catalysts located in the aqueous cell phases (Medina, et al., 2012).

In surimi manufacture, triacyglycerides are largely removed by flotation, aided by mechanical action and possible melting/softening. Most of the depot fat is removed when fish are headed, gutted, and skinned because fish generally deposit most of their fat in these region. However, a small percentage of membrane phospholipids are present in fish muscle, which are difficult to remove by washing. These phospholipids are highly unsaturated and often in contact with muscle heme iron and therefore sensitive to oxidation. Such oxidation causes off-flavors and may hasten denaturation of myofibrillar proteins. Lipids in surimi are even more problematic if pro-oxidants, such as iron (from water pipes, machinery, or residual heme proteins), are present. The

mincing and washing procedures generally incorporate a large amount of oxygen into the surimi, resulting in lipid oxidation (Park, 2000). This leads to the formation of free radicals and lipid hydroperoxides, primary products of oxidation which break down to secondary lipid oxidation compounds, such as alcohols, aldehydes, and ketones. Among the consequences of these reactions are the development of unpleasant odor, rancid taste and discoloration (E. N. Frankel, 2005). Saeed and Howell (2002) reported that the primary and secondary lipid oxidation products in muscle proteins of Atlantic mackerel was increased with storage time and at a higher storage temperature of -20°C compared with samples stored at -30°C.

Surimi and washed mince progressively lose its natural protection and become very sensitive to oxidation (Eymard, et al., 2005). Eymard, Baron and Jacobsen (2009) found that horse mackerel mince washed in cold distilled water creates an imbalance in the initial prooxidant-antioxidant equilibrium in the muscle tissue. Washing process promotes lipid oxidation, and the oxidative ranking was as follows: mince < one washing step < two washing step ≤ three washing step. Srinivasan, et al. (1996) reported that washing beef heart surimi-like material two times in 25 mM phosphate buffer at pH 7, and the presence of salt (0.1 M NaCl) inhibited lipid oxidation during storage. This result suggested that Na^+ interfered with the ability of H^+ to catalyze redox reactions of myoglobin or that washing in 0.1 M NaCl may have removed some factors or cofactors that are capable of catalyzing lipid or pigment oxidation in beef heart muscle. Wimmer, Sebranek and McKeith (1993) reported that washing pork mince with 1.5% NaCl solution at pH 5.5 and 6.5, resulted in fat content less than 1% and lipid oxidation decreased during storage.

2.6 Vibrational spectroscopy

Vibrational spectra can be obtained either by infrared absorption (IR) or Raman scattering spectroscopy. Two techniques provide complementary information. IR results from the absorption of energy by vibrating chemical bonds (primarily stretching and bending motions). Raman scattering results from the same types of transitions, but the selection rules are somewhat different so that weak bands in the IR may be strong in the Raman and vice versa (Li-Chan, Griffiths and Chalmers, 2010). The amide band I (C=O stretch, near 1650 cm^{-1}), II (N–H bend and C–N stretch, near 1550 cm^{-1}), and III (C–N stretch, N–H bend, near 1300 cm^{-1}) are generally employed to study protein structure (Pelton and McLean, 2000).

2.6.1 Raman spectroscopy

Raman spectroscopic analysis is based on the inelastic scattering of photons resulting from vibrational transitions of the functional groups of molecules. Both frequency and intensity of molecular vibrations are sensitive to chemical changes and microenvironment of the functional groups, which are reflected by changes in the Raman spectrum (Careche and Li-Chan, 1997). Raman spectroscopy is a non-invasive method, which does not require sample pre-treatments, moreover only small portions of sample are needed for analysis. Many studies using Raman spectroscopy have been reported on proteins, including protein-protein interactions, which occur during processing and storage of many food products (Marquardt and Wold, 2004). Some studies have also been conducted to elucidate protein-lipid interactions in emulsions (Howell, Herman and Li-Chan, 2001). Since water has a very weak Raman signal, this technique is applicable to study biological systems such as foods that contains significant amounts of water (Careche and Li-Chan, 1997).

2.6.1.1 Proteins structural changes by Raman spectroscopy

Spectral assignments of proteins Raman bands are usually based on model compounds such as amino acids or short peptides. Raman spectroscopy can provide information on the secondary and tertiary structure of proteins in solid samples (Li-Chan, Nakai and Hirotsuka, 1994) like muscle foods. The most useful Raman bands for determining the secondary structure of proteins are amide I (1645–1685 cm^{-1}) and amide III bands (1200-1350 cm^{-1}) since they are contributions from α-helix, β-sheet, turns and random coil structures. Information about tertiary structure is provided by local environments such as tryptophan (544, 577,760, 879, 1014, 1340, 1363, 1553 and 1582 cm^{-1}), tyrosil doublet (855 and 828 cm^{-1}), and aliphatic hydrophobic residues assigned at 1450 cm^{-1} (CH_2 and CH_3 bending vibrations) and at 2930 cm^{-1} (C-H stretching) (Li-Chan, 1996).

Raman spectroscopy has been shown to be a useful tool to study structural changes in solid food systems such as fish and meat, and to determine structural changes that occur during processing and storage of fish and seafood products including surimi (Herrero, Carmona and Careche, 2004). It can also be used to investigate *in situ* structural changes in proteins during denaturation and aggregation (Li-Chan, Nakai and Hirotsuka, 1994).

The study of Fourier transform Raman (FT-Raman) by Careche et al. (1999) on hake (*Merluccius merluccius* L.) during frozen storage for 10 months at -10°C or -30°C revealed that secondary structure of proteins changed during storage temperature. These structural transitions mainly involved an increase in β-sheets structures at the expense of α-helices and accompanied by changes in apparent viscosity and shear resistance, indicating the loss of quality of the muscle proteins.

Leelapongwattana (2008) revealed Raman spectra of natural actomyosin from haddock during refrigerated (4°C) and frozen (-10°C) storage. The amide I and amide III were affected by storage temperature. These changes in both α- helix and β- sheet conformation could be due to an unfolding of helical structures, followed by the formation of β- sheet structures possibly through intermolecular interactions between exposed hydrophobic residues. Herrero, Carmona and Careche (2004) revealed some structural changes in hake muscle proteins during frozen storage involving secondary and tertiary protein structures. Changes in secondary structure showed an increase of β-sheet at the expense of α-helix structure. The νC-H stretching band near 2935 cm^{-1} increased in intensity, indicating denaturation of muscle proteins through the exposure of aliphatic hydrophobic groups to the solvent. Herrero et al. (2005) used Raman spectroscopy to study structural changes in hake muscle during frozen storage, the results showed changes in spaces between myofibrils could be related to modifications of shear resistance. Strong 160 cm^{-1} band could be related to conformational transitions of muscle proteins, and/or to alterations in protein-water interactions. Results also showed that there were intensity changes in the ν(O-H) band that may be attributable to transfer of water to larger spatial domains during frozen storage. Sultanbawa and Li- Chan (2001) revealed the effects of frozen storage for natural actomyosin (NAM) from ling cod stored at -10°C and for surimi after freeze-thaw cycles. Raman analysis showed an increased in the percentage of α-helical content, suggesting that the secondary structural changes were induced by freezing and frozen storage. Sun et al. (2011) revealed myofibrillar proteins structural changes during Cantonese sausage processing based on Raman spectroscopy. The results from amide I, amide III, and C-C stretching vibrations

in 890-1060 cm^{-1} showed that α-helix decreased accompanied by an increase in β-sheet structure during the first 18 h.

2.6.1.2 Lipid and lipid oxidation by Raman spectroscopy

Raman spectroscopy has also been used to investigate the structure of lipids and changes resulting from storage conditions or lipid oxidation (Howell et al., 2001). Lipid structural changes in relation to chain order-disorder can be visualized through the frequency positions of the ν(CH) infrared bands located in the 2800-3000 cm^{-1} region (Li-Chan, 1996), and lipid hydrolysis leads to a decrease intensity of ester bands (e.g., ν(C=O) band near 1745 cm^{-1}) and a concomitant increase in intensity of some bands generated by carboxylic acids located near 1710 (ν(C=O)) and 920 cm^{-1} (γ(OH)) (Carmona, Sánchez-Alonso and Careche, 2006; Li-Chan, 1996).

Study of lipid oxidation can be carried out through different ways. One of them is the use of the ν(OH) band of hydroperoxides and/or oxidation-derivative hydroxyl compounds located in the 3500-3000 cm^{-1} region. Although this procedure is reliable for dried sample, this band is masked by the analogous band of water when studying aqueous samples. Another alternative is to measure the intensities of ν(CH) bands resulting from unsaturated carbon-carbon double bonds that absorb between 3060 and 3000 cm^{-1}. However, care should be taken in the sense that the intensity measurement of these bands can be influenced by waterto some extent. Therefore, deuteration of the aqueous sample in question should be carried out. Finally, the peroxide O-O stretching mode appeared in the 900-800 cm^{-1} in both the infrared and Raman spectra should be evaluated, although its weak intensity makes this band not very useful for vibrational spectroscopic analyses during the initial phase of oxidation (Li-Chan, Griffiths and Chalmers, 2010).

Raman spectroscopy has been used to characterize the degree of fatty acid unsaturation (iodine value) of salmon (*Salmo salar*) muscle, ground salmon and extracted salmon oil (Afseth, Wold and Segtnan, 2006). Sarkardei and Howell (2007) investigated the structural changes of extracted lipids from freeze-dried mackerel and horse mackerel. Raman spectral analysis revealed significant reductions in the intensity of bands associated with CH_2 stretches and C=O ester stretches. This was also confirmed by an increase in the intensity of the bands at 3011 cm^{-1} and 2960–2850 cm^{-1}, suggesting alterations in lipid structure involving CH groups. An initial rise followed by a decline of peroxide value of the oil extracted from freeze-dried mackerel and horse mackerel stored at 22°C for 12 weeks confirmed the occurrence of lipid oxidation. Sánchez-Alonso, Carmona and Careche (2012) monitored lipids extracted from hake fillets during frozen storage at -10°C by FT-Raman. Changes in Raman ν(C=C) stretching region (1658 cm^{-1} band) partially attributed to conjugated dienes development.

2.6.2 Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR) is an analytical technique, which measures the infrared intensity versus wavenumber of light. The resulting spectrum is characteristic of the organic molecules, which absorb infrared energy at specific frequencies so that the basic structure of compounds can be determined by the spectral locations of their IR absorptions (Klaypradit, Kerdpiboon and Singh, 2011).

2.6.2.1 Proteins structural changes by Fourier transform infrared spectroscopy (FTIR)

FTIR spectroscopy is a rapid, non-destructive and reproducible

technique. FTIR spectroscopy provides information about the secondary structure content of proteins. Characteristic bands found in the infrared spectra of proteins and polypeptides include Amide I and Amide II. The absorption associated with the Amide I band (1700-1600 cm^{-1}) leads to stretching vibrations of the C=O bond of the peptide linkages, absorption associated with Amide II band leads primarily to bending vibrations of the N-H bond. Because both the C=O and the N-H bonds are involved in the hydrogen bonding that takes place between different elements of secondary structure, the locations of both Amide I and Amide II bands are sensitive to the secondary structure content of a protein (Kong and Yu, 2007).

FTIR spectra of Alaska pollock washed minced revealed that amide I band revealed that α-helix content increased with increased number of washing cycles and contained more unordered or random coil structure (1641- 1645 cm^{-1} (Moosavi-Nasab (2003)). Alaska pollock surimi showed more α- helix content than minced fish since the washing process removed compounds containing β- sheet structure. The loss of α- helical content was more significant in slowly frozen Alaska pollock surimi compared with rapidly- frozen counterparts (Moosavi-Nasab, Alli, Ismail, and Ngadi (2005). Rapid freezing is effective in maintaining the secondary structure of proteins during long- term frozen storage. . FTIR spectra of Cantonese sausage showed nine major bands associated with conformation of proteins, particularly amide I region (Sun, Zhou, Zhao, Yang, and Cui (2011) These bands, mainly attributed to the C=O stretching vibration and to a small extent to C-N stretching vibration of the peptide bonds, have been widely used to reflect the secondary structure of proteins.

2.6.2.2 Lipids and lipid oxidation monitored by FTIR

FTIR can be used to investigate lipid oxidation of oil and many other applications. Sinelli et al. (2007) demonstrated that FTIR spectroscopy has the ability to classify fresh and oxidized virgin olive oil samples. They found the variation in the absorption bands, related to a higher concentration of aldehydes, ketones, epoxides in the stored oil samples than in the fresh oils: 1743 cm^{-1}, corresponds to C=O stretching vibration of aldehydes and ketones; 1363 cm^{-1} corresponds to CH_3 scissoring vibration of ethers; 1218 cm^{-1} corresponds to C-O stretching vibration of epoxides. In addition, FTIR spectra results are in agreement with oxidation measured by classical methods. Klaypradit, Kerdpiboon and Singh (2011) revealed FTIR spectra of Menhaden fish oil stored under light at room temperature to monitor oxidative quality. It was found that changes in the region between 3,500 and 1,700 cm^{-1} and the absorbance were related to peroxide value (PV) and anisidine value (AnV) of the chemical analysis. The FTIR spectral showed an increase in the absorbance band at 3600-3200 cm^{-1} and increased peroxide values were observed in sardine (*Sardinella gibbosa*) muscle during the first 9 days of iced storage, indicating the formation of hydroperoxide in the sample (Chaijan et al. 2006). Thereafter, the absorbance in this region was not detectable up to 15 days of storage, suggesting the decomposition of hydroperoxides to secondary lipid oxidation products. Sánchez-Alonso, Carmona and Careche (2012) reported that FTIR can be used for monitoring lipid hydrolysis of lipid extracted from hake fillets during frozen storage at -10°C. FTIR spectra revealed significant changes in the carbonyl region (1800-1600 cm^{-1}) during the whole storage period. The main features are differences in absorbance intensities of bands near 1742 and 1712 cm^{-1}. Conformational changes of proteins and lipids of silver carp during ice storage and their respective

washed mince during frozen can be monitored using vibrational spectroscopic technique.

2.7 Overview of odorant volatile compounds in fish

An odor volatile compound is one of important fish quality related to consumer acceptance. Olafsdottir, et al. (1997) classified fish odor as species-specific fish odor, microbial spoilage odor, oxidized odor, processing-induced odor and environmentally derived odor (**Table 1**). Action of microorganisms is negligible at frozen temperatures, volatiles formed by this action should be minimal in fresh/thawed fish and fish products. However, progress of lipid oxidation leads to the formation of several volatiles resulting from degradation of polyunsaturated fatty acids (PUFA). Consequently, lipid oxidation has long been recognized as a leading cause of quality deterioration in fish muscle foods and is considered to be a critical parameter in determining their shelf-life (E. Frankel, 1993).

Volatile compounds of fish have been widely studied. Although numerous volatiles have been identified, but not all contribute to aroma of these products. Odor activity value (OAV; also call aroma value, flavor unit, or odor unit) has been proposed to estimate the influence of specific odorants to the overall aroma of analyzed food (REF). OAV is calculated from the ratio of concentration of odorant in foods to its odor detection threshold in food matrix. It is, however, difficult to determine the odor thresholds of all volatiles present in food in published literatures. For this reason, GC-olfactometry (GCO) was developed to determine the aroma-active compounds (odorants) in a complex mixture comprised of odorants and non-odorants (volatiles have no or just minimal odors). GCO is the technique which uses the human nose as a GC

detector. There are three categories of GCO techniques, including dilution analysis, detection frequency analysis, and perceived intensity analysis. Odorant with an OAV greater than one is considered an aroma-active compound (Grosch, 2001).

Changes in odorants of frozen food have been reported. Lipid oxidation has been recognized as a problem occurring during storage. Shenouda (1980) reported that off-odor in fish is due to the formation of low-molecular weight compounds from lipid oxidation or protein degradation during storage. Milo and Grosch (1993) reported that (Z)-1,5-octadien-3-one, (E,Z)-2,6-nonadienal and methional were the most potent odorants of freshly prepared boiled trout (*Salmo fario*) homogenates. After storage of raw material at -13^{o}C for 14 weeks, homogenates boiled trout exhibited fatty, fishy off-odor in which (Z)-3-hexenal and (Z,Z)-3,6-nonadienal showed the highest OAVs (Table 2). They suggested that (Z)-3-hexenal is more stable than (Z,Z)-3,6-nonadienal and is proposed as an indicator for objective determination of off-odor of boiled trout.

Table 2.1 Classes of fish odors and compounds contributing to the odor.

Fish odor	Class of chemical species	Examples of compounds	Aroma description	Odor threshold in water
Species related fresh fish odor	C6-C9 alcohols and carbonyls	Hexanal / t-2-hexenal, 1-octen-3-ol, /1-octen-3-one 1,5-octadiene-3-ol 1,5-octadiene-3-one 2,6-nonadienal 3,6-nonadienol	Green, aldehyde-like Mushroom Heavy earthy, mushrooms Geranium Cucumber Cucumber, melon-like	4.5ppb / 17 ppb[1] 10 ppb/ 0,009 ppb[1] 10 ppb[1] 0.001 ppb[1] 0.001 ppb[1] 10 ppb[1]
	Bromophenols	2,6-dibromophenol 2,4,6-tribromophenol 2-bromophenol	iodine- and shrimp-like saltwater fish, brine-like. Sea, marine-like flavour	0.0005 µg/kg[2] 0.6 µg/kg
	N-cyclic compounds	Pyrrolidine Piperidine	Earthy	
Microbial spoilage odor	Short chain alcohols	ethanol, propanol, butanol, 3-methyl-1-butanol	solvent like	1-100 ppm[3]
	Short chain carbonyls	acetone, butanone ethanal, propanal 3-methylbutanal 2-methylbutana	solvent like malty malty	 0.06 ppm[4] 0.04 ppm[4]
	Amines	ammonia, TMA DMA histamine, putrecine, cadeverine	ammoniacal fishy, ammoniacal putrid, rotten	110 ppm[3] 30 ppm[3] 0.6 ppm[3]

[1] Josephson (1991); [2] Whitfield et al. (1988); [3] Kawai (1996); [4] Sheldon et al. (1971); [5] Fazzalari (1978); [6] Whitfield and Tindale (1984); [7] Buttery et al. (1976); [8] McGill et al. (1974) **Modified form:** Olafsdottir G and J. (1998)

Table 2.1 Classes of fish odors and compounds contributing to the odor (continued).

Fish odor	Class of chemical species	Examples of compounds	Aroma description	Odor threshold in water
	Sulphur compounds	hydrogen sulfide methylmercaptan methyl sulfide dimethyl disulfide dimethyl trisulfide bis-methylthio methane thioesters	sulphury, boiled eggs rotten, cabbage cabbage-like putrid, onion-like putrid, cabbage and onion garlic like	5-40 ppb[5] 0.05 ppb[5] 0.05 ppb[5] 0.9 µg/kg[6] 12 ppb[7] 0.01 ppb[7] 0.3 µg/kg[6]
	Aromatics	phenethyl alcohol phenol, p-cresol	old roses phenolic, pigpen-odours , horse manure moth ball or faecal like	 2 ppm 300 µg/kg[6]
	N-cyclic compounds	Skatole acetic acid,		34.2 ppm[3]
Oxidized odor	Unsaturated aldehydes	hexanal c4-heptenal 2,4-heptadienal, 2,4,7-decatrienal,	green, planty cardboard-like, potato-like fishy oxidised flavour burnt, fishy, cod-liver oil like	4.5 ppb[6] 0.04 ppb[8]
Processing odor		2,4-heptadienal and 3,5-octadien-2-one methional 2-methyl-3-furanthiol	ripened anchovies boiled potato - like odour meaty odour in canned tuna	
Environmental odor	methyl sulfite geosmin 2-methyl-iso-borneol	methyl sulfite geosmin 2-methyl-iso-borneol	petroleum odours earthy, muddy odours	

[1] Josephson (1991); [2] Whitfield et al. (1988); [3] Kawai (1996); [4] Sheldon et al. (1971); [5] Fazzalari (1978); [6] Whitfield and Tindale (1984); [7] Buttery et al. (1976); [8] McGill et al. (1974) **Modified from:** (Olafsdottir G and J. (1998)

Table 2.2 Concentration and OAVs of potent odorants of boiled trout homogenates before and after storage of the raw material.

No.	Compound	Freshly boiled[a]		Boiled after storage[b]	
		(ug/kg)	OAV	(ug/kg)	OAV
1	2,3-Butanedione	255	51	297	59
2	2,3-Pentanedione	17	3	59	12
3	**(Z)-3-Hexenal**	**1.4**	**47**	**24**	**800**
4	Hexanal	14	1	33	3
5	(Z)-Heptanal	1.1	18	6.0	100
6	Methional	4.0	100	5.2	130
7	1-Octen-3-one	0.16	16	0.16	16
8	(Z)-1,5-Octadien-3-one	0.17	400	0.16	400
9	**(Z,Z)-3,6-Nonadienal**	**1.2**	**24**	**22**	**440**
10	(E)-2-Nonenal	1.1	14	2.1	26
11	(E,Z)-2,6-Nonadienal	2.1	105	4.8	240
12	(E,E)-2,4-Nonadienal	2.7	45	2.6	43

[a] The homogenate was boiled immediately after preparation and [b] after storage for 14 weeks at -13ºC.

Modified from: (Milo and Grosch (1993)

Milo and Grosch (1996) reported that frozen storage of salmon (*Salmo salar*) and cod (*Gadus morhua*) at -60ºC and -13ºC for 26 weeks exhibited an off-odor in which mainly caused by (E,Z)-2,6-nonadienal, (Z)-3-hexenal, and (Z,Z)-3,6-nonadienal and 3-methylbutanal. OAVs revealed (Z)-1,5-octadien-3-one (I), (E,Z)-2,6-nonadienal (II), propionaldehyde (III), acetaldehyde (IV), and methional (V) as the character impact odorants of -60ºC stored salmon as well as I, II, IV, V, and (E,E)-2,4-decadienal as those of -60ºC stored cod. After boiling, the -60ºC stored salmon and cod exhibited mild flavor of the fresh fish, whereas -13ºC stored salmon smelled fatty and train-oily and the -13ºC stored cod showed a malty odor defect. Selli, Rannou, Prost, Robin, and Serot (2006) reported that numerous odorant compounds were perceived on cooked rainbow trout (*Oncorhynchus mykiss*). Among these, (*E*)-2-nonenal, 2-ethyl-1-hexanol, 2- methylisoborneol, geosmin, 2- methylnaphthalene, and 8- heptadecene were

considered as off-odor compounds. The most powerful off-odor compounds identified by OAVs were 2-methylisoborneol and geosmin.

2.8 References

Aro, T., Tahvonen, R., Mattila, T., Nurmi, J., Sivonen, T., and Kallio, H. (2000). Effects of Season and Processing on Oil Content and Fatty Acids of Baltic Herring (Clupea h arengus m embras). **Journal of agricultural and food chemistry**, 48(12): 6085-6093.

Eymard, S., Carcouët, E., Rochet, M. J., Dumay, J., Chopin, C., and Genot, C. (2005). Development of lipid oxidation during manufacturing of horse mackerel surimi. **Journal of the Science of Food and Agriculture**, 85(10): 1750-1756.

Frankel, E. (1993). In search of better methods to evaluate natural antioxidants and oxidative stability in food lipids. **Trends in Food Science & Technology**, 4(7): 220-225.

Frankel, E. N. (2005). *Lipid oxidation*: The Oily Press. Bridgewater, England: P.J. Barnes and Associates.

Grosch, W. (2001). Evaluation of the key odorants of foods by dilution experiments, aroma models and omission. **Chemical senses**, 26(5): 533-545.

Jasra, S. K., Jasra, P. K., and Talesara, C. L. (2001). Myofibrillar protein degradation of carp (Labeo rohita (Hamilton)) muscle after post-mortem unfrozen and frozen storage. **Journal of the Science of Food and Agriculture**, 81(5): 519-524. doi: 10.1002/jsfa.841.

Kanner, J. (1994). Oxidative processes in meat and meat products: quality implications. **Meat Science**, 36(1): 169-189.

Lakshmanan, P., Antony, P., and Gopakumar, K. (1996). Nucleotide degradation and quality changes in mullet (Liza corsula) and pearlspot (Etroplus suratensis) in ice and at ambient temperatures. **Food Control**, 7(6): 277-283.

Lanier, T. C., Carvajal, P., and Yongsawatdigul, J. (2000). Surimi gelation chemistry. **Surimi and surimi seafood**, 2.

Lee, C. M. (1986). Surimi manufacturing and fabrication of surimi-based products. **Food Technol**, 40(3): 115-124.

Lei, L., Li, J., Li, G.-Y., Hu, J.-N., Tang, L., Liu, R., . . . Deng, Z.-Y. (2012). Stereospecific analysis of triacylglycerol and phospholipid fractions of five wild freshwater fish from Poyang Lake. **Journal of agricultural and food chemistry**, 60(7): 1857-1864.

Lin, D., and Morrissey, M. T. (1994). Iced storage characteristics of Northern squawfish (Ptychocheilus oregonensis). **Journal of aquatic food product technology**, 3(2): 25-43.

Medina, I., Undeland, I., Larsson, K., Storrø, I., Rustad, T., Jacobsen, C., . . . Gallardo, J. M. (2012). Activity of caffeic acid in different fish lipid matrices: A review. **Food Chemistry**, 131(3): 730-740.

Milo, C., and Grosch, W. (1993). Changes in the odorants of boiled trout (Salmo fario) as affected by the storage of the raw material. **Journal of Agricultural and Food Chemistry**, 41(11): 2076-2081.

Milo, C., and Grosch, W. (1996). Changes in the odorants of boiled salmon and cod as affected by the storage of the raw material. **Journal of Agricultural and Food Chemistry**, 44(8): 2366-2371.

Moosavi-Nasab, M. (2003). *Protein Structural Changes During Preparation and Storage of Surimi* (Graduate and Post-Doctoral), McGill University, Montréal,

Canada.

Moosavi-Nasab, M., Alli, I., Ismail, A. A., and Ngadi, M. O. (2005). Protein Structural Changes During Preparation and Storage of Surimi. **Journal of Food Science**, 70(7): c448-c453. doi: 10.1111/j.1365-2621.2005.tb11467.x.

Nawar, W. W. (1996). Lipids. In: Fennema OR, editor. Food chemistry, 3rd ed. New York: Marcel Dekker. 225-319.

Ocaño-Higuera, V., Marquez-Ríos, E., Canizales-Dávila, M., Castillo-Yáñez, F., Pacheco-Aguilar, R., Lugo-Sánchez, M., . . . Graciano-Verdugo, A. (2009). Postmortem changes in cazon fish muscle stored on ice. **Food Chemistry**, 116(4): 933-938.

Olafsdottir G, and J., F. (1998). Evaluation of fish freshness using volatile compounds-classification of volatile compounds in fish. **In: Olafsdottir G, Luten J, Dalgaard P, Careche M, Verrez-Bagnis V, Martinsdóttir E, Heia K, editors. Methods to Determine the Freshness of Fish in Research and Industry. Proceedings of the final meeting of the concerted action "Evaluation of Fish Freshness" AIR3 CT94 2283. Paris: International Institute of Refrigeration**: 55-69.

Olafsdottir, G., Martinsdóttir, E., Oehlenschläger, J., Dalgaard, P., Jensen, B., Undeland, I., . . . Nilsen, H. (1997). Methods to evaluate fish freshness in research and industry. **Trends in Food Science & Technology**, 8(8): 258-265.

Özoğul, Y., Boğa, E. K., Tokur, B., and Özoğul, F. (2011). Changes in biochemical, sensory and microbiological quality indices of Common Sole (Solea solea) from the Mediterranean sea, during ice storage. **Turkish Journal of Fisheries and Aquatic Sciences**, 11(2).

Park, J. W., Nozaki, H., Suzuki, T., and Beliveau, J.-L. (2013). Historical review of

Surimi technology and market developments. **Surimi and Surimi seafood**, 1(1): 1.

Puchała, R., Białowąs, H., and Pilarczyk, M. (2005). Influence of cold and frozen storage on carp (cyprinus carpio) flesh quality. **Polish journal of food and nutrition sciences**, 14(55): S1.

Saeed, S., and Howell, N. K. (2002). Effect of lipid oxidation and frozen storage on muscle proteins of Atlantic mackerel (Scomber scombrus). **Journal of the Science of Food and Agriculture**, 82(5): 579-586.

Saito, T., Araki, K., and Matsuyoshi, M. (1959). A new method for the estimating the freshness of fish. **Bulletin of the Japanese Society of Scientific Fisheries**, 24: 749-750.

Sarkardei, S., and Howell, N. K. (2007). The effects of freeze-drying and storage on the FT-Raman spectra of Atlantic mackerel (< i> Scomber scombrus</i>) and horse mackerel (< i> Trachurus trachurus</i>). **Food chemistry**, 103(1): 62-70.

Selli, S., Rannou, C., Prost, C., Robin, J., and Serot, T. (2006). Characterization of aroma-active compounds in rainbow trout (Oncorhynchus mykiss) eliciting an off-odor. **Journal of agricultural and food chemistry**, 54(25): 9496-9502.

Shenouda, S. Y. (1980). Theories of protein denaturation during frozen storage of fish flesh *Advances in food research* (Vol. 26, pp. 275-311): Elsevier

Srinivasan, S., Xiong, Y. L., and Decker, E. A. (1996). Inhibition of protein and lipid oxidation in beef heart surimi-like material by antioxidants and combinations of pH, NaCl, and buffer type in the washing media. **Journal of agricultural and food chemistry**, 44(1): 119-125.

Sultanbawa, Y., and Li-Chan, E. C. (2001). Structural changes in natural actomyosin

and surimi from ling cod (Ophiodon elongatus) during frozen storage in the absence or presence of cryoprotectants. **Journal of agricultural and food chemistry**, 49(10): 4716-4725.

Sun, W., Zhou, F., Zhao, M., Yang, B., and Cui, C. (2011). Physicochemical changes of myofibrillar proteins during processing of Cantonese sausage in relation to their aggregation behaviour and< i> in vitro</i> digestibility. **Food Chemistry**, 129(2): 472-478.

Xiong, G., Cheng, W., Ye, L., Du, X., Zhou, M., Lin, R., . . . Cai, Y.-Z. (2009). Effects of konjac glucomannan on physicochemical properties of myofibrillar protein and surimi gels from grass carp (Ctenopharyngodon idella). **Food Chemistry**, 116(2): 413-418. doi: http://dx.doi.org/10.1016/j.foodchem. 2009.

CHAPTER III

VIBRATIONAL SPECTROSCOPY AND BIOCHEMICAL CHANGES IN SILVER CARP AS RELATED TO QUALITY OF WASHED MINCE

3.1 Abstract

Biochemical and protein conformational changes of silver carp (*Hypophthalmichthys molitrix*) stored in ice for up to 14 days and their respective washed mince were investigated. The autolytic activity of silver carp gradually increased with storage time. The K value increased from 15.8% to 85.0% after 14 days on ice. The surface hydrophobicity of silver carp muscle proteins increased during ice storage, and its thrice-washed mince showed the same trend. The yield and textural properties of washed mince continually decreased as the storage time was extended. Fourier-transform infrared spectroscopy revealed that, as storage time increased, the α-helix content of mince decreased, while the β-sheet content increased. Prolonged ice storage led to the exposure of buried aromatic amino acid residues and an increase in disulfide interchanges in mince and washed mince. Changes in the Σβ-sheet structure and Raman intensity at 828 cm^{-1} observed in mince correlated well with the K value. The α-helix content and Raman intensity of raw washed mince at 621 and 828 cm^{-1} showed a strong correlation with its textural properties.

Keywords: Silver carp, washed mince, FT-IR and FT-Raman, ice storage

3.2 Introduction

High-quality surimi is typically obtained from very fresh fish (Lin and Morrissey, 1995). Functional properties of surimi gel deteriorate rapidly during the ice storage of raw materials (Hossain et al., 2005; Mehta et al., 2014; Yathavamoorthi, Sankar, and Ravishankar, 2010; Yongswawatdigul and Park, 2002). Yongswawatdigul and Park (2002) reported that threadfin bream (*Nemipterus hexodon*) should not be kept on ice for more than 3 days to obtain high-quality surimi. Hossain et al. (2005) found a rapid decrease in the gel forming ability of surimi when queen fish (*Chrorinemus lysan*) was used after 10 days of ice storage. Yathavamoorthi et al. (2010) reported that surimi prepared from black rohu (*Labeo calbasu*) stored on ice for 2 days exhibited a significant decrease in gel properties. Mehta et al. (2014) also reported that the gel quality of surimi prepared from three species of Indian major carps, namely, catla (*Catla catla*), mrigala (*Cirrhinus mrigala*), and rohu (*Labeo rohita*) decreased as the storage time on ice was extended. Catla and rohu exhibited the best surimi gel properties when processed within 3 days, whereas mrigala stored on ice for up to 6 days still yielded a good surimi gel. The optimal ice storage of raw material for surimi production appeared to vary with species.

Important tropical marine fish for surimi include threadfin bream, lizardfish, bigeye snapper, and goatfish (Park et al., 2013). The depletion of the tropical marine fishery stock has meant that freshwater fish has become an important raw material with greater sustain ability. Silver carp (*Hypophthalmichthys molitrix*) is one important freshwater species with a global production of approximately 5.3 million MT, ranking as the second largest aquacultured fish after bighead carp (FAO, 2017). Postharvest changes in silver carp during ice storage have not been clearly elucidated, particularly

in relation to surimi quality, despite the relatively large volume of production of this species. An understanding of postharvest changes in silver carp is critical in establishing control measures for its surimi production. Several indicators, including sensory assessment, microbial, physical, and biochemical parameters, have been used to evaluate fish freshness during ice storage (Cheng et al., 2015; Soncin et al., 2009). Changes in the protein conformation monitored during ice storage include protein surface hydrophobicity, sulfhydryl group content, Ca^{2+}-ATPase activity, and circular dichroism (CD). These assays only illustrate the properties of extractable soluble proteins, rather than overall muscle proteins. Typically, the quality of surimi is mainly judged by its gel quality, which is a time-consuming and rather skill-dependent measurement. A technique that can rapidly and accurately evaluate the freshness quality of fish flesh and can assess the quality of surimi would be ideal for the surimi industry.

Vibrational spectroscopy, including Fourier transform infrared (FTIR) and Fourier transform Raman (FT-Raman) spectroscopy, have been applied for food-quality assessment. Fourier trans form infrared spectroscopy is based on infrared (IR) absorption of the electrical dipole moment of a compound, whereas FT-Raman detects inelastic scattering vibrations, resulting from electrical polarizability changes (Li-Chan, 2006). Fourier transform infrared spectroscopy can provide important information on secondary structure at wavenumbers of 1600-1700 cm^{-1}, designated for amide-I, while the Raman spectrum can provide information on the tertiary structure and the environment of protein side chains, such as tyrosine, tryptophan, and local conformations of disulfide bonds (Li-Chan, 1996). These two techniques therefore complement each other for elucidating protein conformation in situ. Vibration spectroscopy combined with chemometric analysis has also been applied to elucidate

various properties of food products (Careche et al., 2002; Careche et al., 1999; Hernández-Martínez et al., 2014; Herrero, Carmona, and Careche, 2004; Leelapongwattana et al., 2008; Moosavi-Nasab et al., 2005; Uysal, Mentes Yilmaz, and Boyaci, 2019). However, a relationship between freshness quality of fish and vibrational spectra has not been clearly established. The objective of this study was to investigate biochemical and conformational changes in silver carp and its washed mince during ice storage. Fourier transform infrared spectroscopy and FT-Raman spectra of mince and washed mince prepared from carp with varied freshness were measured, and the correlation between spectra and biochemical / textural properties was evaluated.

3.3 Materials and methods

3.3.1 Chemicals and Reagents

Bovine serum albumin (BSA) and L-tyrosine were purchased from Fluka (Buchs, Switzerland). Trichloroacetic acid (TCA) was purchased from BDH Chemicals Ltd (Poole, UK). 1 Anilino-8-napthalenesulfonate (ANS) and 5, 5'- dinitrobis (2-nitrobenzoic acid) were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). A broad-range molecular weight standard was obtained from Bio-Rad (Hercules, Calif., U.S.A.). Other chemicals and reagents used were of analytical grade.

3.3.2 Preparation of silver carp surimi samples

Silver carp (*Hypophthalmichthys molitrix*) with a weight of 1.0-1.5 kg were obtained from the Khon Kaen Inland Fisheries Research and Development Center (Khon Kaen, Thailand). Fish were transported alive to a laboratory at Suranaree University of Technology within 3 h. Upon arrival, fish were stunned by an accurate blow to the head in the most humane way possible and immediately packed in

polystyrene foam boxes filled with ice, with a fish-to-ice ratio of approximately 1:2. Polystyrene containers were kept in a cold room (4 ^{o}C) throughout the experiment. The draining and addition of ice was conducted every 2 days during the 14 days of storage. At 0, 7, and 14 days of storage, fish were taken from the ice, beheaded, eviscerated, washed, deskinned, filleted and minced using a grinder equipped with a 5 mm diameter perforated plate. The mince was washed twice with portable reverse osmosis water at a mince : water ratio of 1:3 for 5 min. The temperature of the water was below 5 ^{o}C. The third washing cycle was carried out in the same manner using 0.3% NaCl solution. Centrifugation was carried out at 4000×g at 4 ^{o}C for 15 min at each washing step. The supernatant was referred to as washed water and collected for analysis. Mince and its respective washed mince were vacuum packed in a polyethylene bag, kept at -80 ^{o}C, and used within 10 days with the assumption of no significant protein denaturation. Proximate compositions (moisture, ash and crude protein) of all samples were analyzed according to the AOAC official method (AOAC, 2005). Total lipid content was determined according to the Folch method (Folch, Lee and Stanley, 1957). Total solid recovery was also calculated.

3.3.3 Determination of muscle compositions

The muscle composition of mince and its respective washed mince samples was analyzed according to the method of Hashimoto et al. (1979) with slight modifications. Ten grams of sample was homogenized with 100 mL of phosphate buffer (15.6 m mol L^{-1} Na_2HPO_4, 3.5 m mol L^{-1} KH_2PO_4), with pH 7.5, using a homogenizer (IKA T25 Digital Ultra-Turrax, Model T 25 D, Staufen, Germany) at 10000 rpm for 30 s. The homogenate was centrifuged at 5000×g for 15 min at 4 ^{o}C, and the supernatant was collected for the sarcoplasmic protein fraction. Precipitates were collected and

homogenized with 100 mL of phosphate buffer (15.6 m mol L^{-1} Na_2HPO_4, 3.5 m mol L^{-1} KH2PO4) containing 0.45 mol L^{-1} KCl with pH 7.5. The homogenate was centrifuged at 5000×*g* for 15 min at 4 °C. The residue was again extracted with the same buffer, and all combined supernatants were referred to as the myofibrillar protein fraction or salt soluble protein (SSP). The pellets were then mixed with a solution of five times their volume of 0.1 N NaOH and stirred at 4 °C for 12 h. The mixture was centrifuged at 5000×*g* at 4 °C for 15 min and referred to as the alkali-extractable protein fraction. The final residues were considered as stroma proteins. The protein content of each fraction was determined using the Kjeldahl method with a protein conversion factor of 6.25 (AOAC, 2005).

3.3.4 K value

The K value was obtained using a high-performance liquid chromatography (HPLC) procedure according to Yongswawatdigul and Park (2002). Five grams of mince were homogenized with 40 mL of cold 7.5% perchloric acid at 10000 rpm for 30 s. The homogenates were centrifuged at 990×g at 4 °C for 5 min. The supernatant was collected and adjusted to a volume of 50 mL with cold 7.5% perchloric acid and then kept at -20 °C. For HPLC analysis, the extracts were thawed, and 0.6 mol L^{-1} potassium phosphate buffer (pH 7.6) was added at an equal volume. The mixture was allowed to stand at 4 °C for 30 min for potassium perchlorate precipitation. The supernatant was filtered through a 0.45 μm nylon filter membrane before HPLC injection.

Adenosine triphosphate (ATP) and its breakdown products, namely, adenosine diphosphate (ADP), adenosine monophos phate (AMP), inosine monophosphate (IMP), inosine (HxR), and hypoxanthine (Hx) were separated on a

reverse phase C18 column, Hypersil ODS C18 column (3 μm, 150 × 4.6 mm) (Thermo Scientific, Waltham, MA, USA). Mobile phase A consisted of 150 m mol L^{-1} KH_2PO_4 and 150 m mol L^{-1} KCl (adjusted to a pH of 6.0 with 50% KOH). Mobile phase B consisted of 20% acetonitrile in mobile phase A. The flow rate was set at 2.0 mL min^{-1} with a linear gradient of 0 to 100% mobile phase B in 25 min. The K value was calculated as follows:

$$\text{K} - \text{value}\ (\%) = \frac{\text{HxR} + \text{Hx}}{\text{ATP} + \text{ADP} + \text{AMP} + \text{IMP} + \text{HxR} + \text{Hx}} \text{ x } 100$$

3.3.5 Autolytic activity

The autolytic activity of muscle tissue during ice storage was determined using trichloroacetic acid (TCA)-soluble oligopeptide contents (Yongsawatdigul and Piyadhammaviboon, 2004). Three grams of mince was incubated at the optimum temperature of autolytic activity of silver carp mince, 65 °C, for 1 h. To stop the reaction, 27 mL of 5% cold TCA was added. The mixture was homogenized and then centrifuged at 10000×g at 4 °C for 15 min. Oligopeptide content was analyzed by the Lowry et al.'s method (Lowry et al., 1951) using tyrosine as a standard. The sample without incubation was used as a control. Autolytic activity was expressed as μmole tyrosine g^{-1}sampleh^{-1}.

3.3.6 Protein conformational changes

To monitor conformational changes in mince at various storage times in ice, as well as its washed mince, the total sulfhydryl content (T-SH), reactive sulfhydryl content (R-SH), and surface hydrophobicity (S_0-ANS) were evaluated. The salt-soluble protein of mince and its extracted washed mince were used for analyses.

Determination of T-SH groups was carried out using Ellman's reagent, as described by Yongsawatdigul and Park (2003). To 1 mL of SSP (4 mg mL^{-1}), 9 mL of cold buffer solution (50 m mol L^{-1} sodium phosphate buffer, 10 m mol L^{-1}

ethylenediaminetetraacetic acid, 0.6 mol L^{-1} NaCI and 8 mol L^{-1} urea; pH 7.0) was added. The mixture (4 mL) was then mixed with 0.4 mL of 0.1% 5,5′-dinitrobis (2-nitrobenzoic acid) and subsequently incubated at 40 ^{o}C for 25 min. The mixture was measured at an absorbance of 412 nm, and T-SH groups were calculated using an extinction coefficient of 13600 mol L^{-1} cm^{-1}. The R-SH groups were determined by incubating SSP at 4 ^{o}C for 1 h in the absence of urea. The T-SH and R-SH groups were expressed as moles 105 g^{-1} protein.

Changes in the S_0 were measured using 8-anilino-1-naphthalene sulfonate (ANS) following Hayakawa and Nakai (1985). One milliliter of SSP (1 mg mL^{-1}) was added to 10 μL of 8 mol L^{-1} ANS solution in 0.01 mol L^{-1} phosphate buffer (pH 7.0) and mixed well. The reaction was incubated in the dark for 10 min. Subsequently, the fluorescence intensity (FI) of the mixture was monitored at excitation and emission wavelengths of 374 nm and 485 nm, respectively, using a spectrofluorometer (FP-8300, Jasco, Tokyo, Japan). Surface hydrophobicity (S_0-ANS) was calculated from the slope of the relative fluorescence intensity against the protein concentration (% w/v) plot.

3.3.7 Vibrational spectroscopy

For secondary structure analysis, FTIR spectroscopy was per formed using attenuated total reflectance (ATR)-FTIR spectroscopy with a single reflection ATR sampling module, coupled with an MCT detector, cooled with liquid nitrogen over a measurement range from 4000 to 600 cm^{-1}. The measurement was performed with a spectral resolution of 4 cm^{-1} with 64 scans using a Bruker Tensor 27 (Bruker Optics Ltd, Ettlingen, Germany). Lyophilized mince and washed mince samples were placed in contact with a horizontal ATR plate. At least 30 spectra were collected from each sample and they were analyzed with the OPUS 7.2 program. Spectra were taken from three

replications and then averaged. Curve fitting was carried out on the baseline corrected amide I (1730-1587 cm^{-1}) region. The results were expressed as the relative integrated intensity of each component (parallel β-sheet, 1613-1636 cm^{-1}; α-helix, 1647-1669 cm^{-1}; β-turn, 1680 cm^{-1}; and antiparallel β-sheet, 1691 cm^{-1}).

Fourier transform Raman spectra were recorded on a Bruker RAM II FT Raman module coupled to a Bruker Vertex 70v interferometer (Bruker Co., Ettlingen, Germany). The excitation source was an Nd:YAG laser, emitting at a wavelength of 1064 nm. The scattered radiation was collected from 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1}. Mince and washed mince samples were packed in a tablet, placed in a sample holder, and monitored via video camera. The sample was measured at a set laser power of 300 mW and 300 scans. At least nine Raman spectra per sample were collected, averaged, and normalized using the OPUS program, version 7.2. The integrated intensity of the second derivative (nine-point smoothing) was then performed to distinguish the overlapped peaks, and was expressed as the relative integrated intensity (Dong, Huang, and Caughey, 1990).

3.3.8 SDS-PAGE

Protein patterns of mince, its washed mince, and washed water were performed according to Laemmli's method (Laemmli, 1970). Mince and its respective washed mince samples (2 g) were homogenized with 18 mL 5% SDS. Washed water samples (2 mL) were solubilized with 18 mL 10% SDS. All mixtures were subsequently heated at 90 °C for 30 min and centrifuged at 5000×*g* for 15 min at 4 °C. Protein content was determined using the Lowry method (Lowry et al., 1951). Gel electrophoresis was determined using a 4% stacking gel and 10% acrylamide gel with applied protein of 20 μg.

3.3.9 Gel quality assessment

Washed mince samples containing 6% sucrose and 0.3% sodium tripolyphosphate were mixed with ice and NaCl to obtain the final content of 80% moisture and 2% NaCl. Samples were ground manually using a mortar and pestle for 7 min. Surimi paste was then injected into a microplate well with a 5 mm diameter and 10 mm depth. The filled microplates were individually packed in a polyethylene bag and vacuum-sealed. The packed samples were pre-incubated either at 40 ^{o}C or 60 ^{o}C for 30 min prior to heating at 90 ^{o}C for 30 min. Direct heating treatment (90 ^{o}C for 30 min) was also conducted. Samples were immediately cooled in ice water for 20 min and kept at 4 ^{o}C overnight. Prior to texture analysis, samples were equilibrated at room temperature for 2 h. The breaking force (g) and penetration distance (mm) were evaluated using a texture analyzer (XTPlus, Stable Micro System, Surrey, UK) equipped with a 2 mm cylindrical probe at a test speed of 1 mm s^{-1}. Breaking force and distance values of at least 8 gel samples were recorded for each treatment.

3.3.10 Statistical analyses

Results were statistically evaluated by one-way analysis of variance (ANOVA). Duncan's multiple range tests were carried out to determine significant differences within the 95% confidence interval using SPSS 17.0 software (SPSS Inc, Chicago, IL, USA). All values were expressed as the mean ± SD. The significance level was $P < 0.05$. A principal component analysis (PCA) of all measured parameters at various storage times was also performed using the XLSTAT software, version 2018.5. A Pearson correlation analysis was carried out to evaluate the relationship between pairs of measured variables.

3.4 Results and Discussion

3.4.1 Freshness quality

Initially, ATP in silver carp was not detected, while the ADP and AMP content were relatively low (5.63 and 4.85 μg g^{-1}, respectively). In contrast, high IMP content, 77.39 μg g^{-1}, was observed (**Figure 3.1a**). Rapid degradation of ATP in fish muscle to IMP by endogenous enzymes, namely ATPase, myokinase, and AMP deaminohydrolase, has been reported (Hattula, 1997). Struggle during capture and stress before death greatly affect ATP degradation (Alasalvar, Taylor, and Shahidi, 2002; Tejada, 2009). The IMP decreased concomitantly with an increase in HxR with prolonged ice storage. It is widely accepted that a low level of IMP is related to a loss of freshness quality. Accumulation of Hx is due to both autolytic degradation and bacterial spoilage (Woyewoda et al., 1986).

The K value continually increased to 63.6% at day 7 and reached 85.0% at day 14 (**Figure 3.1a**). After 7 days of ice storage, moderate freshness was observed with a slightly soft texture and slightly opaque eye. Spoilage was noticed at 14 days of ice storage, as evidenced by a softer texture, slightly abnormal odor, and opaque and cloudy eyes. Similar results were found in rainbow trout (*Oncorhynchus mykiss*), which showed unacceptable sensory qualities at 12 days of ice storage, with a K value greater than 73% (Özogul and Özogul, 2001). Özogul et al. (2006) reported that wild turbot (*Scophthalmus maximus*) was still acceptable after 15 days of ice storage, with a K value of 75–85%. Rohu kept on ice for 21 days, with acceptable quality, showed a K value of 56% (Mohan, Ramachandran, and Sankar, 2006). Based on the K value and sensory characteristics, silver carp could be stored in ice for up to 7 days, and the K value of silver carp with acceptable freshness quality should not be over 65%.

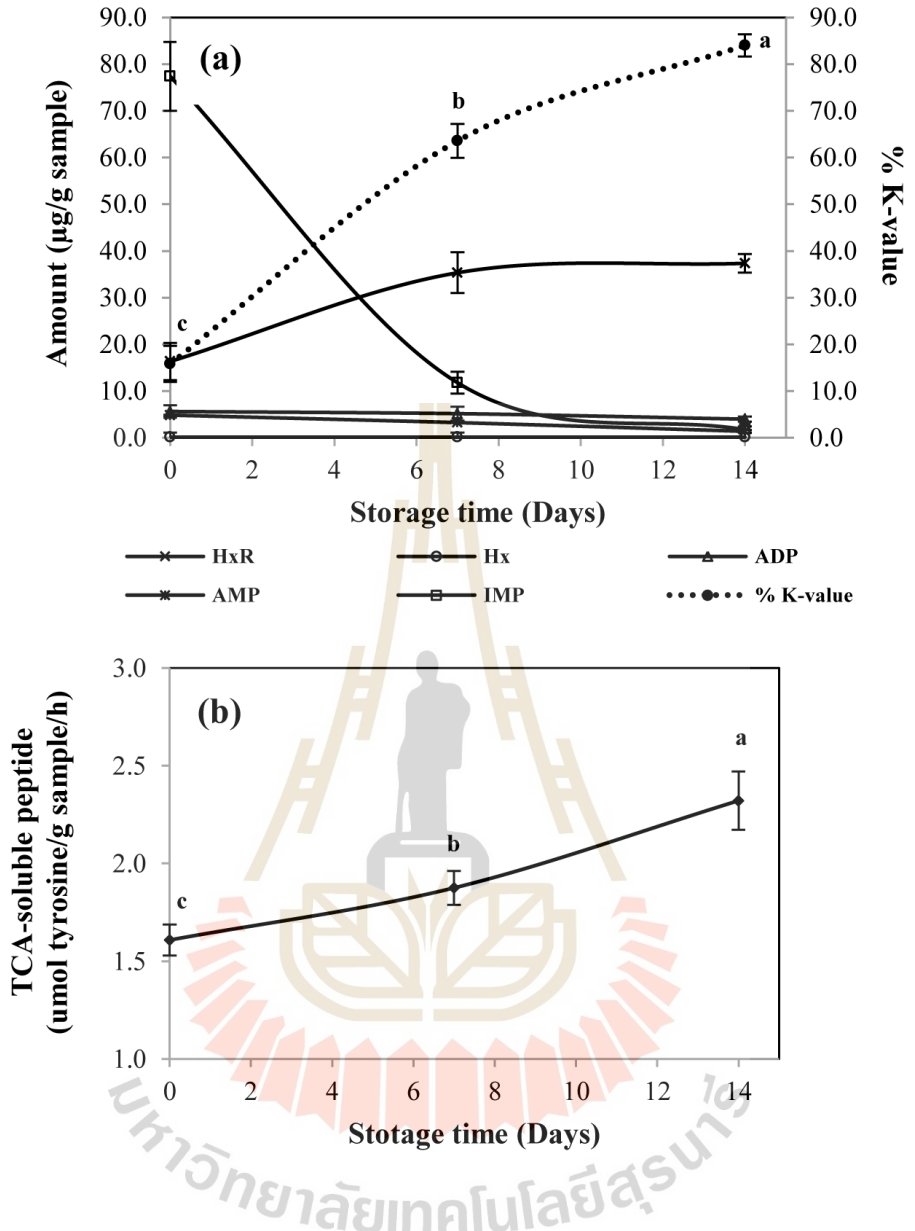


Figure 3.1 Changes in concentrations of nucleotide degradation products and the K value (A) and TCA-soluble peptide contents (B) of silver carp during ice storage for 14 days.

3.4.2 Chemical composition

Changes in the moisture content of silver carp during ice storage were subtle ($P > 0.05$, **Table 3.1**). The ash content of fish decreased with prolonged ice storage. This could be because ions in fish muscle leached out during ice storage. The washing process also removed ions from the muscle, resulting in a lower ash content in washed mince samples.

The protein content of fish decreased as storage time increased concomitantly with lower proteins of washed mince prepared from fish with extended storage time. Proteolysis from endogenous and microbial proteases during ice storage could be responsible for protein degradation, leading to a decrease in protein content with extended ice storage. This was confirmed by a continual increase in TCA-soluble oligopeptide content of fish during ice storage (**Figure 3.1b**). Muscle degradation also led to more protein leaching out in wash water, resulting in lower protein content of washed mince prepared from aged fish.

Changes in the total lipids showed a similar trend to those in proteins. Lipolysis and lipid oxidation during ice storage could be the main causes of such changes (Wongwichian et al., 2015). Washing reduced the total lipid content by 60-70% but approximately 5.8-6.5% of lipids remained. Myofibrillar and alkali-extractable proteins of fish and its respective washed mince decreased with the storage time of raw material ($P < 0.05$, **Table 3.1**). Alkali-extractable proteins are derived from the aggregation of denatured myofibrillar proteins (Pacheco-Aguilar, Lugo-Sánchez, and Robles-Burgueño, 2000). Thus, these two fractions indicated the overall myofibrillar content of samples. Changes in the sarcoplasmic and stroma proteins both of fish and washed mince were subtle during ice storage ($P > 0.05$). This finding was in agreement

with Pacheco-Aguilar, Lugo-Sánchez, and Robles-Burgueño (2000) who found that the content of sarcoplasmic proteins of the Monterey sardine did not change during 15 days of ice storage. Stroma proteins of silver carp tended to decrease during ice storage, which could have resulted from collagenases (Delbarre-Ladrat et al., 2006). Our results indicated that extended ice storage of whole silver carp led to a loss of myofibrillar proteins. The washing process resulted in a reduction in sarcoplasmic proteins and an increase in myofibrillar and alkali-extractable proteins (**Table 3.1**). The proportion of stroma proteins also increased after the washing process. Connective tissues are not water soluble. Thus, they remained after the three-cycle washing. In surimi production, stroma proteins are eliminated in both the deboning / mincing and the refining process. Washed mince prepared from aged fish contained less myofibrillar proteins than that prepared from freshly caught fish ($P < 0.05$, **Table 3.1**), indicating a loss of myofibrillar proteins through possible degradation during ice storage.

Table 3.1 Chemical composition changes of silver carp during iced storage for 14 days and their respective trice-washed mince.

Composition	Mince			Washed mince		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Moisture	79.32±0.66	78.84±0.61	78.72±1.04	87.43±0.71^{A}	86.75±0.20^{B}	86.60±0.38^{B}
Ash (g kg^{-1} dry weight)	0.58±0.01^{a}	0.49±0.02^{b}	0.47±0.07^{b}	0.24±0.01^{A}	0.25±0.03^{A}	0.25±0.03^{A}
Protein (g kg^{-1} dry weight)	7.88±0.19^{a}	7.25±0.11^{b}	7.15±0.17^{b}	8.36±0.17^{A}	7.65±0.20^{B}	7.10±0.20^{C}
Lipid (g kg^{-1} dry weight)	1.14±0.15^{a}	1.07±0.33^{b}	0.90±0.14^{c}	0.65±0.35^{A}	0.62±0.28^{AB}	0.58±0.27^{B}
Muscle composition (g kg^{-1} dry weight)						
Sarcoplasmic	2.92±0.19	2.90±0.09	2.83±0.17	1.26±0.10	1.29±0.10	1.26±0.09
Myofibrillar	3.41±0.06^{a}	3.25±0.08^{ab}	3.16±0.17^{b}	4.24±0.18^{A}	4.01±0.07^{B}	3.91±0.11^{B}
Alkali-extractable	1.08±0.11^{a}	0.55±0.05^{b}	0.55±0.07^{b}	2.08±0.17^{A}	1.21±0.12^{B}	0.78±0.05^{C}
Stroma	0.35±0.05	0.30±0.11	0.29±0.10	0.84±0.06^{A}	0.80±0.07^{A}	0.57±0.10^{B}

Different lowercase letters (a–c) indicate differences in ice storage time of raw material ($P < 0.05$). Different uppercase letters (A–B) indicate significant differences among washed mince prepared from fish stored in ice at various times ($P < 0.05$).

3.4.3 Protein loss and yield

The autolytic activity of silver carp muscle increased with storage time (**Figure 3.1b**). During ice storage, fish protein degradation was influenced by the activity of endogenous and microbial proteases. Proteolysis in silver carp muscle has been contributed from the action of cathepsin L and B (Liu et al., 2006, 2008).

Although the autolytic activity of silver carp and loss of myofibrillar proteins increased during ice storage, degradation of myosin heavy chain (MHC), actin (AC), and tropomyosin (TM) was not visibly noticed (**Figure 3.2**). However, the intensity of low molecular weight proteins (< 29 kDa) slightly increased at days 7 and 14 of ice storage, which was likely caused by proteolysis of muscle proteins. Washed water of the third cycle of fish stored for 14 days exhibited the highest intensity of myofibrillar proteins (**Figure 3.2**). Surimi and washed mince should therefore be processed within 7 days of ice storage to minimize myofibrillar protein loss during washing.

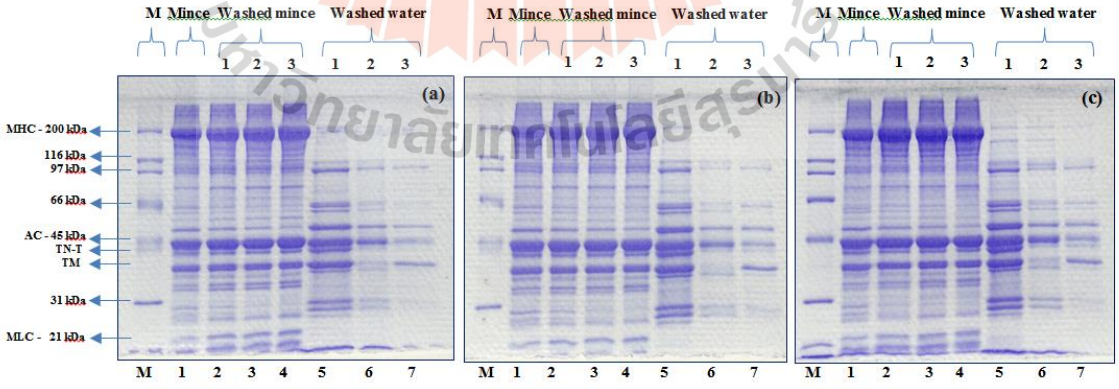


Figure 3.2 Protein patterns of silver carp mince, its respective washed mince and washed water during stored in ice for 0 (a), 7 (b) and 14 (c) days determined by SDS-PAGE using 4% stacking gel and 10% separating gel with the

applied protein of 20 µg. M: molecular weight standard; lane 1: mince; lane 2-4: the 1st, 2nd, 3rd washing cycle of washed mince; lane 5-7: the 1st, 2nd, 3rd washing cycle of washed water.

The total solid recovery or yield of thrice-washed mince prepared from freshly caught silver carp was 70.53%, which was similar to that in previous studies of 74–76.8% (Li-Chan, Nakai, and Hirotsuka, 1994; Liu et al., 2008). A significant decrease in yield was observed as the storage time of raw material was extended ($P < 0.05$, **Figure 3.3**). The loss of myofibrillar proteins induced by proteolysis would be a prime factor for a decreased yield.

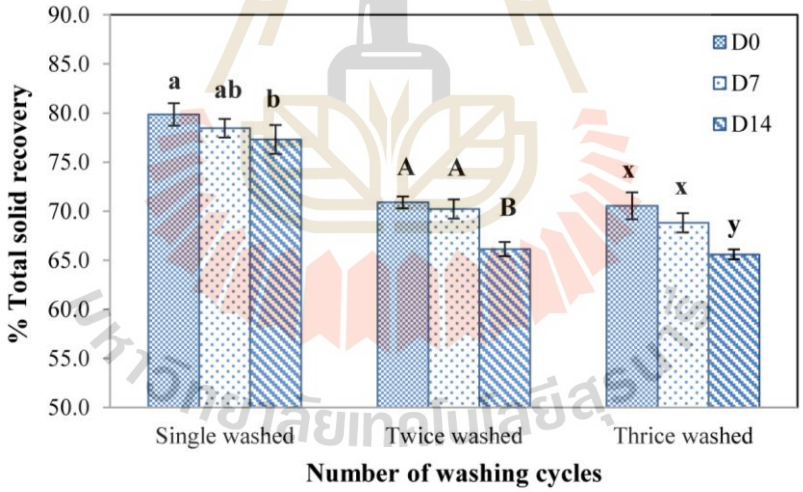


Figure 3.3 Total solid recovery of silver carp affected by various storage time in ice and the washing cycle. Different letters in each washing cycles indicate a significant difference ($P < 0.05$).

3.4.4 Protein conformational changes

Changes in the T-SH of both mince and washed mince were subtle during ice storage ($P > 0.05$, **Figure 3.4a**), suggesting that oxidation of sulfhydryl groups to disulfide bonds was insignificant. In contrast, the R-SH content continually decreased with storage time ($P < 0.05$, **Figure 3.4b**). Ice storage induced exposure of the R-SH groups of myofibrillar proteins. The S0-ANS of myofibrillar proteins extracted from mince and washed mince also increased during ice storage ($P < 0.05$, **Figure 3.4c**), confirming the unfolding of myofibrillar proteins during ice storage. The extent of unfolding drastically increased in washed mince prepared from fish after 14 days of ice storage. Washing also appeared to induce more unfolding, as the surface hydrophobicity of washed mince was higher than that of mince. Our results indicated that washed mince produced from fish stored in ice for an extended period exhibited more extensive conformational changes than that produced from fresh fish.

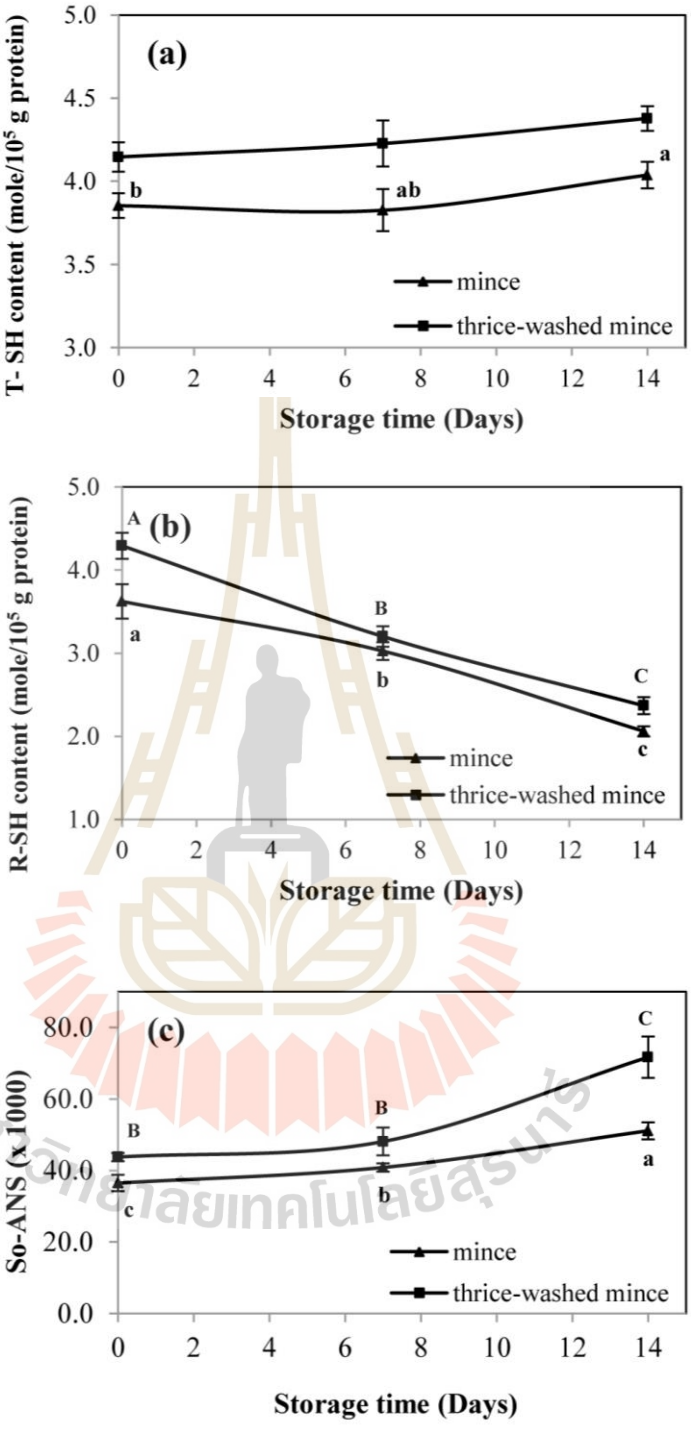


Figure 3.4 Effect of iced storage and washing process on the total SH (a) and reactive SH content (b) and surface hydrophobicity (c)

3.4.5 Vibrational spectroscopy

The major secondary structures of mince and washed mince were the α-helix and β-sheet estimated from the amide I region obtained from FTIR (**Table 3.2**). In mince, the α-helical and β-turn structures decreased, while the β-sheet increased, with an extended period of ice storage. These results were in agreement with an increase in the S_0-ANS of myofibrillar proteins extracted from fish stored in ice for an extended period (**Figure 3.4c**). Washed mince contained a higher proportion of the α-helix structure than its mince counterparts (**Table 3.2**). Changes in the secondary structure of washed mince with respect to the freshness quality of their raw materials was not obvious (**Table 3.2**). Our results indicated that ice storage induced structural changes in muscle proteins in mince to a greater extent than in its respective washed mince.

Raman spectra of mince samples obtained from fish stored in ice for varied durations and its respective washed mince were different at the 3200-2800 cm^{-1} wavenumbers (**Figure 3.5a**) and 1800-800 cm^{-1} (**Figure 3.5b**), respectively. Changes in total aliphatic residues were subtle during ice storage of raw material (**Table 3.3**). Total aromatic residues, namely, tryptophan, tyrosine, and phenylalanine in mince and washed mince increased during 14 days of ice storage of raw material ($P < 0.05$). The tyrosine doublet near 830 and 850 cm^{-1} has been used to monitor the microenvironment around tyrosine residues and is associated with the vibration of the para-substituted benzene ring (Li-Chan et al., 1994). The intensity value of I850/830 has been used to indicate changes in tyrosine residues on the protein surface to a more polar environment. If the ratio is more than 1, tyrosine residues are exposed to a more polar environment and interact with water molecules through hydrogen bonds. However, if it is less than 1, tyrosine residues are expected to be buried (Shao et al., 2011; Soncin et al., 2009). In this study, the ratio of the relative integrated intensity of the doublet bands at 854 cm^{-1} and 828 cm^{-1}

(854/828) of mince increased with storage time, implying the unfolding of muscle proteins during ice storage. These values of washed mince were also higher than those of mince, suggesting that the washing process caused exposure of tyrosine residues and induced the unfolding of muscle proteins. Raman bands at 536, 525, and 507 cm^{-1} have been assigned to S-S stretching vibrations of disulfide bonds in the trans-gauche-trans, gauche-gauche-trans, and gauche-gauche-gauche conformations, respectively (Bouraoui, Nakai, and Li-Chan, 1997). The formation of disulfide bonds appeared to increase in silver carp muscle during ice storage and its respective washed mince ($P < 0.05$, **Table 3.3**). These results suggested that ice storage induced oxidation of the sulfhydryl groups of muscle proteins. An increase in disulfide bonds was also promoted by three-cycle washing. Thus, the use of silver carp with lower freshness quality not only resulted in a lower yield of washed mince but also rendered the greater extent of conformational changes in myofibrillar proteins, namely, the exposure of hydrophobic amino acid residues and oxidation of sulfhydryl groups to disulfide linkages.

Table 3.2 Changes in secondary structures estimated from the amide I band profiles

Secondary structure	Mince			Washed mince		
	Day 0	**Day 7**	**Day 14**	**Day 0**	**Day 7**	**Day 14**
α-helix	51.78±0.70^{a}	50.83±0.71^{ab}	49.75±1.11^{b}	55.32±1.24	55.66±0.86	53.90±0.65
β-turn	13.18±0.78^{a}	8.67±0.22^{b}	9.15±0.66^{b}	10.25±1.52	11.17±2.36	13.00±0.86
pararell β-sheet	33.16±0.62^{a}	32.07±0.99^{ab}	31.09±0.74^{b}	30.33±0.72^{A}	28.61±0.73^{B}	28.82±0.57^{B}
anti pararell β-sheet	4.21±0.37^{c}	7.43±1.00^{b}	10.33±0.88^{a}	4.43±1.51	4.56±1.07	3.95±0.48
∑β-sheet (pararell and anti pararell β-sheet	37.37±1.73^{b}	39.50±1.13^{ab}	41.41±0.23^{a}	34.77±1.81	33.16±1.51	32.70±0.90

Different lowercase letters (a–c) within each row indicate difference of ice storage time of raw material ($P < 0.05$). Different uppercase letters (A–B) within each row indicate difference among washed mince prepared from fish stored in ice at various times ($P < 0.05$).

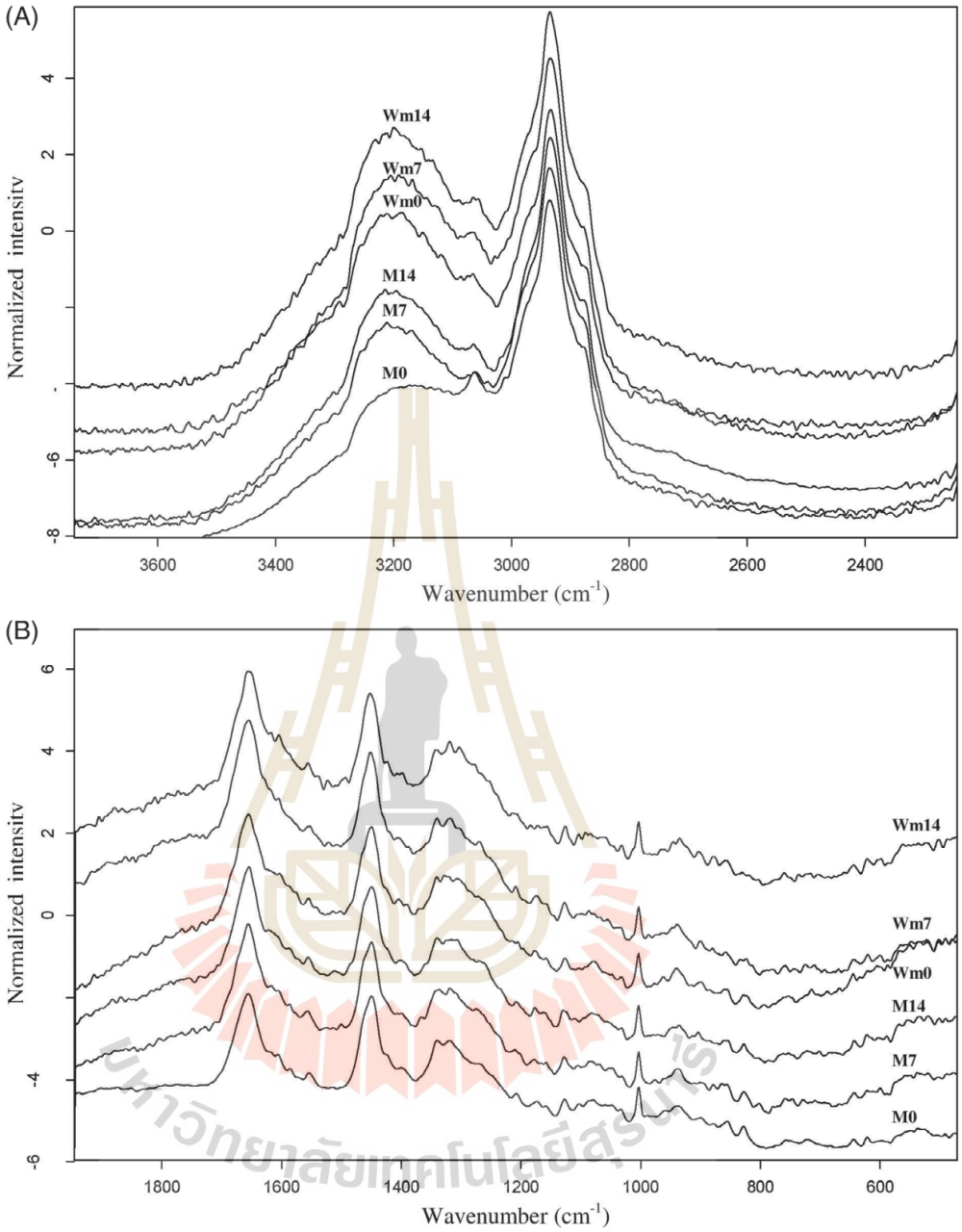


Figure 3.5 The average FT-Raman spectra from the (A) 3700-2400 cm^{-1} and (B) 1800-500 cm^{-1} regions of silver carp mince during storage in ice for 0 (M0), 7 (M7) and 14 (M14) days and its respective washed mince (Wm0, Wm7, Wm14)

Table 3.3 Percentage of selected relative area of Raman bands of silver carp mince and their respective thrice-washed mince.

Wavenumber (cm^{-1})	Band assignment	mince			thrice-washed mince		
		0	7	14	0	7	14
∑ aliphatic amino acid residues		**43.525±1.64[a]**	**42.640±2.32[a]**	**44.907±2.38[a]**	**45.184±1.73**	**45.336±0.67**	**45.066±0.70**
2980	ν_{as}(CH3)	1.338	1.589	1.504	2.696	3.263	2.435
2937	ν_{as}(CH2), ν_{s}(CH3)	19.877	19.582	20.603	19.110	17.405	21.367
2872	ν_{s}(CH2)	6.969	6.471	8.182	8.602	5.647	5.823
1450	δ_{s}CH2, δ_{as}CH3	10.616	10.561	10.128	7.626	10.587	9.320
1341	δ(CH)	3.160	2.202	3.818	4.191	3.912	2.115
1319	δ(CH)	1.079	2.236	0.000	2.503	3.260	2.565
720	rCH2	0.486	0.000	0.672	0.456	1.262	1.441
∑ aromatic amino acid residues		**32.698±1.29[c]**	**35.794±1.57[b]**	**38.983±1.12[a]**	**36.441±1.67[B]**	**37.131±1.43[B]**	**40.095±1.25[A]**
3064	Aromatic ν(CH)	3.436	3.847	3.648	3.907	2.268	3.064
1618	Aromatic ν-ring (Trp, Phe, Tyr ν-ring)	0.530	0.760	0.864	0.501	1.060	1.227
1605	Aromatic ν-ring (Trp, Phe, Tyr ν-ring)	2.027	2.157	2.642	3.077	2.342	2.897
1584	ν(Trp-ring)	0.576	0.702	0.832	0.271	0.332	0.662
1554	ν(Trp-ring)	1.680	2.199	2.331	1.414	1.680	1.676
1207	Tyr, Phe	1.460	1.153	1.453	0.963	1.573	1.620
1175	Tyr	1.077	1.256	1.697	2.911	2.564	1.366

Abbreviation s, symmetric; vs, asymmetric; ν, stretch; δ, deformation; r, rock

Table 3.3 Percentage of selected relative area of Raman bands of silver carp mince and their respective thrice-washed mince (Continued).

Wavenumber (cm^{-1})	Band assignment	mince			thrice-washed mince		
		0	7	14	0	7	14
1033	δ(Phe-ring)	1.577	2.086	2.984	2.418	2.382	3.375
1003	ν(Phe-ring)	9.935	9.821	10.085	9.345	9.660	8.203
877	tryptophan	0.412	0.380	0.566	0.052	0.584	1.254
854	ν(Tyr-ring)	1.808	2.159	2.594	2.383	3.581	4.444
828	ν(Tyr-ring)	2.567	2.641	2.658	0.880	0.380	0.101
758	Trp	1.267	1.717	1.274	0.572	1.661	1.619
645	Tyr	2.302	2.848	2.762	3.343	2.445	4.425
621	Phe	2.044	2.067	2.592	4.405	4.620	4.161
∑ disulfide bonds		**0.373 ± 0.02^{b}**	**0.573 ± 0.05^{a}**	**0.612 ± 0.04^{a}**	**0.796 ± 0.08^{B}**	**0.900 ± 0.05^{AB}**	**1.067 ± 0.10^{A}**
536	S-S (tgt)	0.137	0.234	0.326	0.150	0.304	0.333
525	S-S (ggt)	0.097	0.127	0.156	0.432	0.293	0.405
507	S-S (ggg)	0.139	0.212	0.131	0.214	0.303	0.329

Abbreviation s, symmetric; vs, asymmetric; ν, stretch; δ, deformation; r, rock. Different lowercase letters (a–c) within a row indicate differences in ice-storage time of raw material ($P < 0.05$. Different uppercase letters (A–B) within a row indicate differences in washed mince prepared from fish stored in ice at various times ($P < 0.05$)

3.4.6 Gel quality assessment

The textural properties of gels made from washed mince decreased with the storage time of fish (**Figure 3.6**). This finding was probably associated with the loss of myofibrillar proteins and conformational changes in proteins during ice storage. Extended ice storage induced conformational changes in myofibrillar proteins, exposing more hydrophobic residues, and oxidation of sulfhydryl groups/disulfide interchanges. All these factors would have led to an inferior gel-forming ability of washed silver carp mince.

Ice storage also induced proteolysis, resulting in poor gel network formation. A reduction in gel strength of calbasu surimi by 19.12% and 25% was reported in fish stored in ice for 24 and 48 h, respectively (Mehta et al., 2014). Phatcharat, Benjakul, and Visessanguan (2006) also reported a continuous decrease in the breaking force of bigeye snapper surimi when fish were stored in ice for 14 days. This study revealed that the gel-forming ability of silver carp washed mince decreased as the storage time of fish in ice was prolonged.

Textural properties of gels made from washed mince were affected by the thermal process applied ($P < 0.05$). Silver carp gels set at 40 ^{o}C for 30 min showed the highest breaking force and distance, and the lowest textural properties were found in gels preincubated at 60 ^{o}C for 30 min. This finding was in agreement with that of (Luo et al., 2008). The inferior gel quality of washed silver carp mince at 60 ^{o}C was likely due to endogenous proteolysis, which has its optimal activity between 60 and 70 ^{o}C (An et al., 1994). Textural improvement at 40 ^{o}C was probably due to the activity of residual transglutaminase, which catalyzed protein cross-linking between the γ-carboxamide group of glutamine and ε-amino group of lysine. Ramírez et al. (2000) found the

optimum setting temperature of silver carp at 39.6 ^{o}C for 1 h. Gel enhancement mediated by setting was reduced as the freshness of fish decreased (**Figure 3.6**). Changes in protein conformation, namely the loss of the α-helical structure at wavenumbers of 1647-1669 cm^{-1} (amide I) and exposure of aromatic residues occurring during ice storage, could partly hamper proper gel network formation induced by setting.

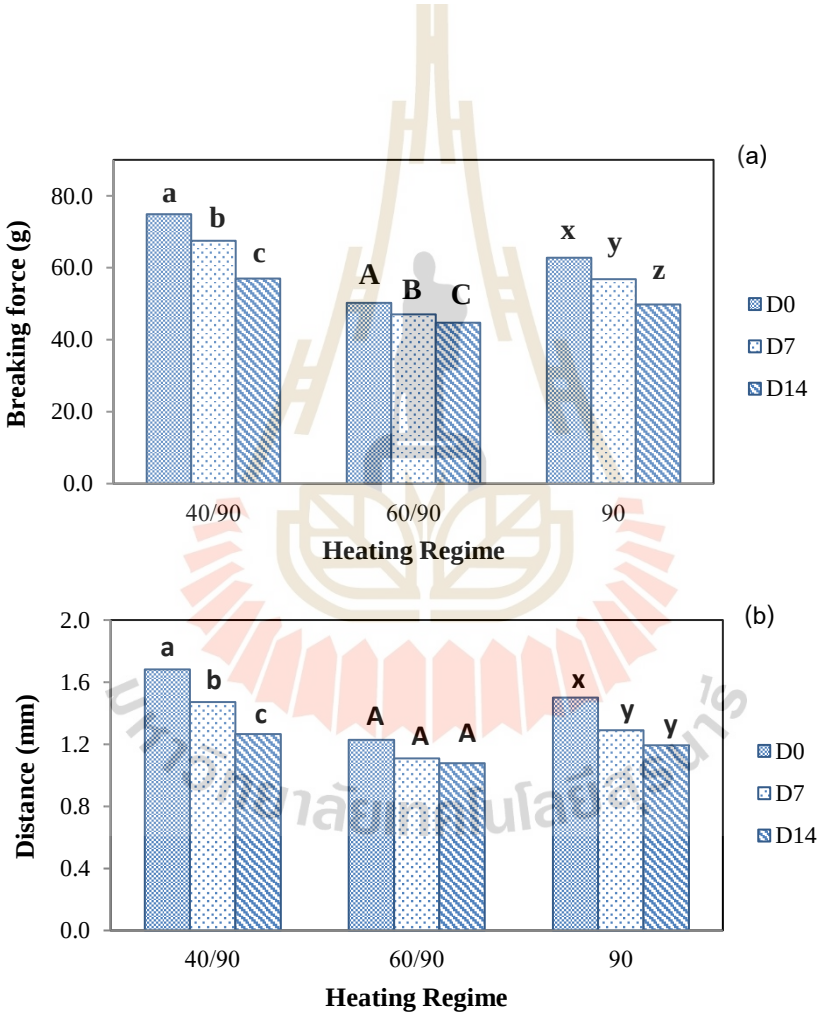


Figure 3.6 Breaking force (a) and distance (b) of silver carp washed mince gel prepared from fish stored in iced for various time. Different letters in each heating regime indicate a significant difference ($P < 0.05$).

3.4.7 Correlations between vibrational spectra and physico-chemical properties

Two components, F1 and F2, represented a data variance of 83.97% based on PCA (**Figure 3.7**). Clear discrimination between mince and washed mince was observed in F1. The storage time of raw material not only affected measured parameters of mince but also those of washed mince. Aged fish (7 days and 14 days of storage, M7 and M14) were located in the negative F2 quadrants, corresponding to an increase in autolytic activity and K value and a decrease in lipid and ash content with a loading factor of 0.974, 0.803, 0.947, and 0.976, respectively (**Figure 3.7**). Our results also indicated that IR and Raman spectra showed the potential to differentiate aged fish from fresh counterparts based on the Σβ-sheet and relative integrated intensity at 828 cm^{-1} ν(Tyr-ring) with loading factors of 0.916 and 0.985, respectively. Pearson's correlations also confirmed a strong positive correlation between the K value and Σβ-sheet ($P < 0.01$, **Table 3.4**). Infrared and Raman spectroscopy based on information from the Σβ-sheet and 828 cm^{-1} *ν*(Tyr-ring), respectively, could be developed as indices of freshness quality for ice-stored silver carp.

Mince and its respective washed mince were clearly separated into two clusters (**Figure 3.7**). Silver carp mince samples were characterized by a higher content of ash, lipid, and sarcoplasmic protein as well as Σβ-sheet structure and 828 ν(Tyr-ring) than those of washed mince. A high content of myofibrillar protein and α-helix structures, intensity of 621 (Phe), 525 (S-S, ggt), and 507 (S-S, ggg) cm^{-1}, and Σdisulfide bonds were characteristics of washed mince (**Figure 3.7**).

Extended ice storage of silver carp affected not only the quality of mince but also washed mince, which was clearly separated by F2. Washed mince prepared

from raw material with a lower freshness quality tended to have a lower content of R-SH and higher relative integrated intensity at 536 cm^{-1} (S-S, tgt) with loading factors of 0.958 and 0.912, respectively. Changes in Raman 525 cm^{-1} were an indicator used to trace the freshness quality of fish used for washed mince. Pearson's correlations showed that the textural properties of both breaking force and distance were positively correlated with the α-helix ($P < 0.01$), relative integrated intensity at wavenumbers of 621 (Phe, $P < 0.01$) and 525 (S-S, ggt, $P < 0.01$) cm-1, and Σdisulfide bonds ($P < 0.05$). Textural properties of gels made from washed mince also negatively correlated with the parallel β-sheet ($P < 0.05$), β-sheet ($P < 0.05$), and intensity at a wavenumber of 828 (Tyr-ring, $P < 0.01$). Thus, these vibrational spectra could have potential to be used for monitoring the gel quality of washed mince / surimi and for tracing the freshness quality of raw materials used.

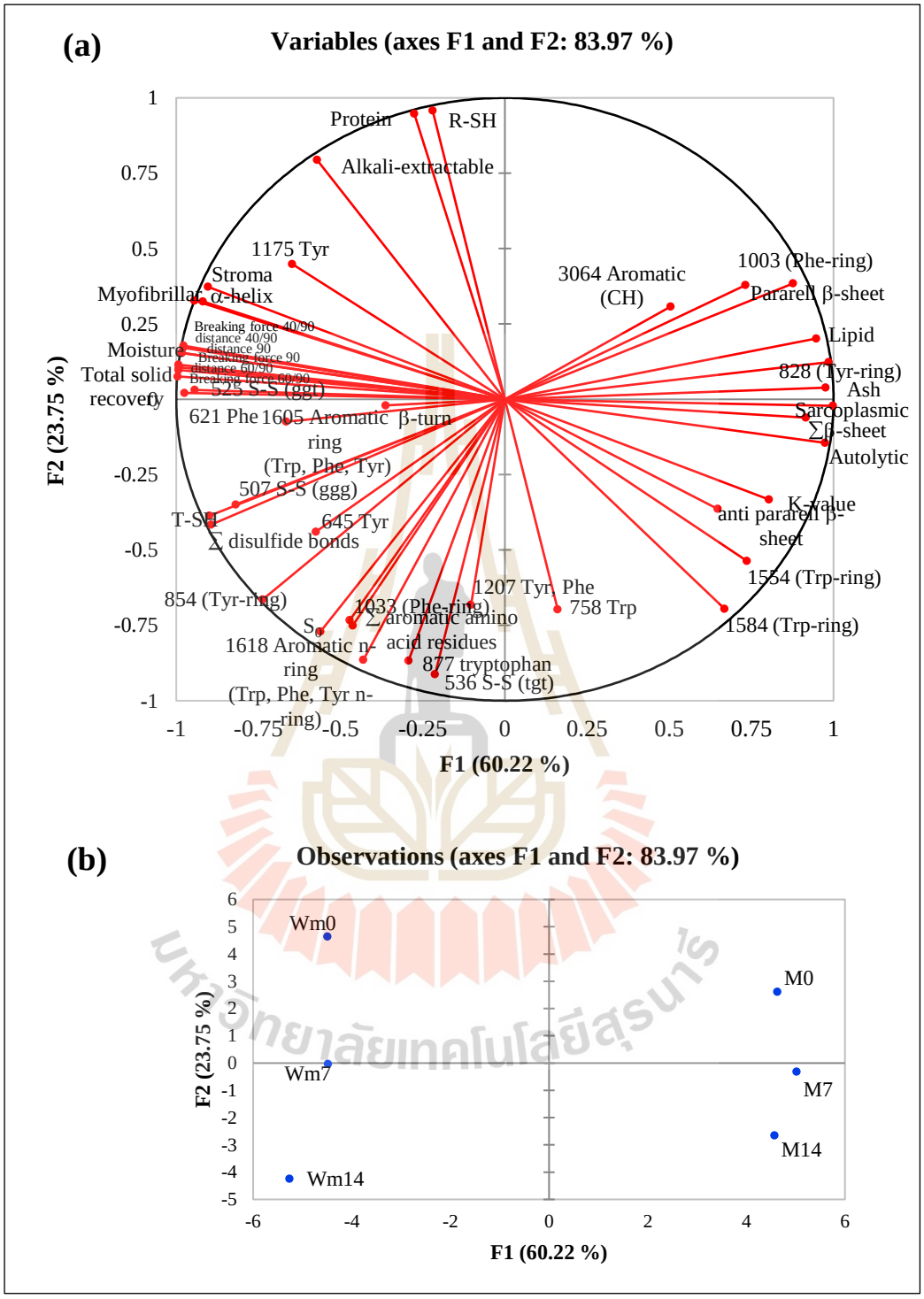


Figure 3.7 Principal component analysis determined by principal components 1(PC1) and 2 (PC2) of iced storage period of mince (M) and its respective washed

mince (Wm). Plots (a) and (b) represent variables and obsevations, respectively.

3.5 Conclusions

Prolonged ice storage of silver carp induced conformational changes in proteins not only in mince but also in washed mince, as indicated by an increase in the surface hydrophobicity and a decrease in the reactive sulfhydryl content. Proteolysis also progressed during ice storage, leading to a greater loss of myofibrillar proteins during washing and a subsequent lower yield and gel-forming ability. To obtain the optimal textural properties of washed mince, silver carp should not be kept in ice for longer than 7 days before being processed. Vibrational spectroscopy can effectively differentiate between mince and washed mince based on the contents of the α-helix and Σβ-sheet structures and Σdisulfide bonds. Changes in the secondary structure determined from the FTIR and Raman wavenumber at 828 cm^{-1}, indicating changes in the tryptophan ring, correlated well with the K value of silver carp. The content of the parallel β-sheet and the relative integrated intensity of the Raman wavenumber at 828 were negatively correlated with the textural properties of washed mince, while the vibration of phenylalanine and disulfide at 621 and 525 cm^{-1}, respectively, increased with the breaking force value. Fourier transform infrared and FT-Raman spectroscopy showed potential as rapid techniques to monitor the freshness of raw material and to assess the gel quality of washed mince.

3.6 References

Alasalvar, C., Taylor, K. A., & Shahidi, F. (2002). Comparative quality assessment of cultured and wild sea bream (Sparus aurata) stored in ice**. Journal of Agricultural and Food Chemistry,** 50(7), 2039-2045.

An, H., Weerasinghe, V., Seymour, T. A., & Morrissey, M. T. (1994). Cathepsin degradation of Pacific whiting surimi proteins. **Journal of food science,** 59(5), 1013-1017.

AOAC (2005) Official method of Analysis. 18th Edition, Assocoation of Official Analytical Chemists International, Maryland, Washington DC, Method 935.14 and 992.24.

Bouraoui, M., Nakai, S., & Li-Chan, E. (1997). In situ investigation of protein structure in Pacific whiting surimi and gels using Raman spectroscopy. **Food Research International,** 30(1), 65-72.

Careche, M., García, M., Herrero, A., Solas, M. T., & Carmona, P. (2002). Structural properties of aggregates from frozen stored hake muscle proteins. **Journal of food science,** 67(8), 2827-2832.

Careche, M., Herrero, A. M., Rodríguez-Casado, A., Del Mazo, M., & Carmona, P. (1999). Structural changes of hake (Merluccius merluccius L.) fillets: effects of freezing and frozen storage. **Journal of Agricultural and Food Chemistry,** 47(3), 952-959.

Cheng, J.-H., Sun, D.-W., Zeng, X.-A., & Liu, D. (2015). Recent advances in methods and techniques for freshness quality determination and evaluation of fish and fish fillets: A review. **Critical reviews in food science and nutrition,** 55(7), 1012-1225.

Delbarre-Ladrat, C., Chéret, R., Taylor, R., & Verrez-Bagnis, V. (2006). Trends in postmortem aging in fish: understanding of proteolysis and disorganization of the myofibrillar structure. **Critical reviews in food science and nutrition,** 46(5), 409-421.

Dong, A., Huang, P., & Caughey, W. S. (1990). Protein secondary structures in water from second-derivative amide I infrared spectra. **Biochemistry,** 29(13), 3303-3308.

FAO. (2017). FAO year book. Fishery and Aquaculture Statistics. Annuaire. Statistiques despêches et de l'aquaculture. 2015/FAO Anuario. Estadísticas de pesca y acuicultura. 2015., Rome/Roma.Italy/Italie/Italia.

Folch, J., Lees, M., & Stanley, G. S. (1957). A simple method for the isolation and purification of total lipides from animal tissues. **Journal of biological chemistry,** 226(1), 497-509.

Hashimoto, K., Watabe, S., Kono, M., & Shiro, M. (1979). Muscle protein composition of sardine and mackerel. **Bulletin of the Japanese Society of Scientific Fisheries.**

Hayakawa, S., & Nakai, S. (1985). Relationships of hydrophobicity and net charge to the solubility of milk and soy proteins. **Journal of food science,** 50(2), 486-491.

Hattula, T. (1997). Adenosine triphosphate breakdown products as a freshness indicator of some fish species and fish products: **Technical Research Centre of Finland.**

Hernández-Martínez, M., Gallardo-Velázquez, T., Osorio-Revilla, G., Almaraz-Abarca, N., & Castañeda-Pérez, E. (2014). Application of MIR-FTIR spectroscopy and chemometrics to the rapid prediction of fish fillet quality. **CyTA-Journal of Food,** 12(4), 369-377.

Herrero, A. M., Carmona, P., & Careche, M. (2004). Raman spectroscopic study of structural changes in hake (Merluccius merluccius L.) muscle proteins during frozen storage. **Journal of Agricultural and Food Chemistry,** 52(8), 2147-2153.

Hossain, M., Kamal, M., Sakib, M., Shikha, F., Neazuddin, M., & Islam, M. (2005). Influence of Ice Storage on the Gel Forming Ability, Myofibrillar Protein Solubility and Ca"-ATPase Activity of Queen Fish (Chorinemus lysan). **Journal of Biological Sciences,** 5(4), 519-524.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. **Nature,** 227(5259), 680-685.

Leelapongwattana, K., Benjakul, S., Visessanguan, W., & Howell, N. K. (2008). Raman spectroscopic analysis and rheological measurements on natural actomyosin from haddock (*Melanogrammus aeglefinus*) during refrigerated (4° C) and frozen (− 10° C) storage in the presence of trimethylamine-N-oxide demethylase from kidney of lizardfish (Saurida tumbil). **Food chemistry,** 106(3), 1253-1263.

Li-Chan, E., Nakai, S., & Hirotsuka, M. (1994). Raman spectroscopy as a probe of protein structure in food systems. In Protein structure-function relationships in foods (pp. 163-197): Springer.

Li-Chan, E. C. Y. (1996). The applications of Raman spectroscopy in food science. **Trends in Food Science & Technology,** 7(11), 361-370.

Li-Chan, E. C. (2006). Introduction to vibrational spectroscopy in food science. Handbook of Vibrational Spectroscopy.

Lin, D., & Morrissey, M. (1995). Northern squawfish (PtychocheHus oregonensis) for Surimi production. **Journal of food science,** 60(6), 1245-1247.

Liu, H., Yin, L., Zhang, N., Li, S., & Ma, C. (2006). Purification and characterization of cathepsin L from the muscle of silver carp (*Hypophthalmichthys molitrix*). **Journal of Agricultural and Food Chemistry,** 54(25), 9584-9591.

Liu, H., Yin, L., Zhang, N., Li, S., & Ma, C. (2008). Isolation of cathepsin B from the muscle of silver carp (*Hypophthalmichthys molitrix*) and comparison of cathepsins B and L actions on surimi gel softening. **Food chemistry,** 110(2), 310-318.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. **Journal of biological chemistry,** 193, 265-275.

Luo, Y., Shen, H., Pan, D., & Bu, G. (2008). Gel properties of surimi from silver carp (Hypophthalmichthys molitrix) as affected by heat treatment and soy protein isolate. **Food Hydrocolloids,** 22(8), 1513-1519.

Mehta, N. K., Elavarasan, K., Reddy, A. M., & Shamasundar, B. (2014). Effect of ice storage on the functional properties of proteins from a few species of fresh water fish (Indian major carps) with special emphasis on gel forming ability. **Journal of food science and technology,** 51(4), 655-663.

Mohan, M., Ramachandran, D., & Sankar, T. (2006). Functional properties of Rohu (Labeo rohita) proteins during iced storage. **Food Research International,** 39(8), 847-854.

Moosavi-Nasab, M., Alli, I., Ismail, A. A., & Ngadi, M. O. (2005). Protein structural changes during preparation and storage of surimi. **Journal of food science,** 70(7), c448-c453.

Özogul, Y., & Özogul, F. (2001). Degradation products of adenine nucleotide in rainbow trout (Oncorhynchus mykiss) stored in ice and in modified atmosphere packaging. **Turkish Journal of Zoology,** 26(1), 127-130.

Özogul, Y., Özogul, F., Kuley, E., Özkutuk, A. S., Gökbulut, C., & Köse, S. (2006). Biochemical, sensory and microbiological attributes of wild turbot (Scophthalmus maximus), from the Black Sea, during chilled storage. **Food chemistry,** 99(4), 752-758.

Pacheco-Aguilar, R., Lugo-Sánchez, M., & Robles-Burgueño, M. (2000). Postmortem biochemical and functional characteristic of Monterey sardine muscle stored at 0 C. **Journal of food science,** 65(1), 40-47.

Park, J. W., Nozaki, H., Suzuki, T., & Beliveau, J.-L. (2013). Historical review of surimi technology and market developments. Surimi and Surimi seafood, 1(1), 1.

Phatcharat, S., Benjakul, S., & Visessanguan, W. (2006). Effects of washing with oxidising agents on the gel-forming ability and physicochemical properties of surimi produced from bigeye snapper (Priacanthus tayenus). **Food chemistry,** 98(3), 431-439.

Ramírez, J., Santos, I., Morales, O., Morrissey, M., & Vázquez, M. (2000). Application of microbial transglutaminase to improve mechanical properties of surimi from silver carp utilizaciónde transglutaminasa microbiana paramellora-laspropiedades mecánicas de surimide carpaplateada utilizaciónde transglutaminasamicrobiana paramejorarlaspropiedades mecánicas de surimide carpaplateada. **CyTA-Journal of Food,** 3(1), 21-28.

Shao, J.-H., Zou, Y.-F., Xu, X.-L., Wu, J.-Q., & Zhou, G.-H. (2011). Evaluation of structural changes in raw and heated meat batters prepared with different lipids using Raman spectroscopy. **Food Research International,** 44(9), 2955-2961.

Soncin, S., Chiesa, L. M., Panseri, S., Biondi, P., & Cantoni, C. (2009). Determination of volatile compounds of precooked prawn (Penaeus vannamei) and cultured gilthead sea bream (*Sparus aurata*) stored in ice as possible spoilage markers using solid phase microextraction and gas chromatography/mass spectrometry. **Journal of the Science of Food and Agriculture,** 89(3), 436-442.

Tejada, M. (2009). ATP-derived products and K-value determination. In (pp. 68-88): Wiley Online Library.

Uysal, R. S., Mentes Yilmaz, O., & Boyaci, I. H. (2019). Determination of liquid egg composition using attenuated total reflectance Fourier transform infrared spectroscopy and chemometrics. **Journal of the Science of Food and Agriculture,** 99(7), 3572-3577.

Wongwichian, C., Klomklao, S., Panpipat, W., Benjakul, S., & Chaijan, M. (2015). Interrelationship between myoglobin and lipid oxidations in oxeye scad (Selar boops) muscle during iced storage. **Food chemistry,** 174, 279-285.

Woyewoda, A., Shaw, S., Ke, P., & Burns, B. (1986). Recommended laboratory methods for assessment of fish quality.

Yathavamoorthi, R., Sankar, T., & Ravishankar, C. (2010). Effect of ice storage and washing on the protein constituents and textural properties of surimi from Labeo calbasu (Hamilton, 1822).

Yongsawatdigul, J., & Piyadhammaviboon, P. (2004). Inhibition of autolytic activity of lizardfish surimi by proteinase inhibitors. **Food chemistry,** 87(3), 447-455.

Yongswawatdigul, J., & Park, J. W. (2002). Biochemical and conformation changes of actomyosin from threadfin bream stored in ice. **Journal of food science,** 67(3), 985-990.

CHAPTER IV

EVALUATION OF LIPID OXIDATION, VOLATILE COMPOUNDS AND VIBRATIONAL SPECTROSCOPY OF SILVER CARP (*Hypophthalmichthys molitrix*) DURING ICE STORAGE AS RELATED TO THE QUALITY OF ITS WASHED MINCE

4.1 Abstract

Changes in the lipid oxidation of silver carp (*Hypophthalmichthys molitrix*) stored in ice for 14 days and that of its respective washed mince were evaluated. Total lipid, phospholipid, polyunsaturated fatty acid (PUFA) and monounsaturated fatty acid (MUFA) contents of the skin, belly flap and mince decreased as the storage time in ice increased. The washing process decreased the lipid contents but concentrated their phospholipid counterparts. The fish belly flap exhibited the highest thiobarbituric acid reactive substances (TBARS) value, while the mince had the lowest. 1-Hexanol, 1-octen-3-ol, and 1-hexanal were key volatile compounds detected in the belly flaps of fish stored for 7-14 days. Hexanal was the only major volatile compound found in washed mince prepared from fish stored for an extended period in ice, but in a much lower amount compared with that in the belly flap. FTIR (Fourier transform infrared) spectra revealed a decrease in the number of *cis* double bonds, methylene groups and phosphate groups in lipids extracted from fish stored in ice for 7-14 days as compared

with those extracted from fresh fish. Principle component analysis (PCA) revealed that the FT-Raman band at 1747 cm^{-1} could be a potential marker for tracking the degree of lipid oxidation in the belly flap of silver carp stored in ice. In addition, IR bands indicating phosphate group (925, 825 cm^{-1}) in oil extracted from washed mince were correlated with the extent of the lipid oxidation of the raw material.

Keywords: Silver carp, washed mince, FTIR, FT-Raman, lipid oxidation, volatile compounds

4.2 Introduction

Silver carp (*Hypophthalmichthys molitrix*) is an important freshwater fish species with global production of 4,704,673 tons in 2017 (FAO, 2017). At the industry level, silver carp has proven useful as a potential raw material for surimi production, providing good gel-forming ability with exceptional white color (Park et al., 2013; Weng and Zheng, 2015). In tropical surimi production, whole fish are typically kept in ice before being processed**.** It is well recognized that various parts of the fish body contain different lipid contents, leading to varied degree of lipid oxidation during ice storage. Lipid oxidation is known to produce undesirable flavors and oxidized products that induce protein oxidation. This ultimately leads to a deterioration in the texture and sensory characteristics of surimi. Fish freshness quality has been extensively studied with regard to nucleotide degradation and changes in protein conformation. However, changes in the lipid oxidation of silver carp during ice storage have not been well characterized.

Many methods have been applied to evaluate lipid oxidation in fish and surimi, including (1) measuring changes in oxidative substrates, such as fatty acids, total lipid

and phospholipid contents; and (2) assessing the quantity of primary and secondary products of oxidation. The thiobarbituric acid reactive substances (TBARS) level, which is a widely used lipid oxidation indicator, has some limitations. Malondialdehyde (MDA) is only one of many possible secondary oxidation products formed (Reitznerová et al., 2017). In addition, other compounds that do not result from the oxidation process can contribute to TBARS levels (Giménez et al., 2011). Recently, the evaluation of volatile compounds has become an additional indicator of lipid oxidation in fish and surimi samples (Iglesias and Medina, 2008). In addition, Fourier transform infrared (FT-IR) and FT-Raman spectroscopy are techniques that can be used to monitor oxidative changes. FT-Raman spectroscopy was used to monitor lipid oxidation in hake fillets during frozen storage (Sánchez-Alonso, Carmona, and Careche, 2012) and in beef during repeated freeze-thaw cycles (Chen et al., 2018). Vibrational spectroscopy can provide additional insightful information at the molecular level. Therefore, the objective of this study was to evaluate the lipid oxidation of various parts of silver carp stored in ice for 14 days. In addition, the lipid oxidation of washed mince prepared from fish stored in ice for various periods was analyzed. Changes in volatile compounds were also investigated. Vibrational spectroscopic techniques, namely FTIR and FT-Raman, were also applied to monitor the lipid oxidation of oil extracted from raw materials stored in ice for up to 14 days.

4.3 Materials and methods

4.3.1 Sample preparation

Live silver carp (*Hypophthalmichthys molitrix*) weighing 1.0–1.5 kg were transported from the Khon Kaen Inland Fisheries Research and Development Center to

a laboratory at Suranaree University of Technology within 4 h. Upon arrival, fish were stunned by an accurate blow to the head, with regard to animal welfare law, and immediately packed in polystyrene foam boxes filled with ice, with a fish-to-ice ratio of approximately 1:2. The polystyrene foam boxes were kept in a cold room (4 °C) for 14 days. Ice was added every 2 days. At 0, 7 and 14 days of storage, fish were randomly selected and washed with tap water (27 °C). Fish skin and belly flap were manually separated and collected. Fish flesh was minced using a grinder with a 5-mm perforation plate. Fish mince was washed three times with potable water (<5 °C) at a mince/water ratio of 1:3. The third washing cycles were carried out using the same volume of 0.3% NaCl solution. Centrifugation was carried out at 5000× *g* for 15 min at 4 °C at each washing step. Any floating matter, including muscle tissue and fats, was manually removed after centrifugation. Fish skin, belly flap and unwashed and washed mince in a total of 36 samples were vacuum packed and kept at -80 °C and used within 1 week.

4.3.2 Analysis of Lipid and Fatty acids

Total lipid content was analyzed according to the Folch method (Folch, Lees, and Stanley, 1957) with slight modifications. Each ground sample (30 g) was homogenized with 180 mL of a chloroform and methanol solution (2:1) for 1 min and centrifuged at 2000× *g*, 4 °C, for 10 min. The solution was then filtered through Whatman No. 1 filter paper into a separatory funnel. Chloroform (60 mL), deionized water (60 mL) and 0.58% NaCl (10 mL) were then added and thoroughly mixed. After phase separation, the lower layer of the chloroform phase was collected, and anhydrous sodium sulfate was added to remove water. The chloroform phase was then filtered through Whatman No.1 filter paper. The chloroform was evaporated under nitrogen. The extracted lipid was determined gravimetrically and total lipid was expressed as

g/100 g dry weight. Some portions of the extracted oil were kept at -80 °C before further analysis of phospholipid, fatty acid profile and FTIR and FT-Raman spectra.

Phospholipid content was estimated as described by Eymard et al. (2005). Extracted lipid was dissolved in chloroform (0.25 mg/mL). Then, 2 mL of solution was mixed with 1 mL of thiocyanate reagent (0.10 M ferric chloride hexahydrate and 0.40 M ammonium thiocyanate). The mixture was centrifuged at 750× *g*, 4 °C, for 10 min. The red lower layer was collected for absorbance measurement at 488 nm. Phosphatidylcholine (PC) at various concentrations, ranging from 0 to 0.1 mg/mL, was used as a standard. Phospholipid content was expressed as g PC/100 g dry sample.

Fatty acid composition and quantification was evaluated using gas chromatography (GC) according to the Association of Official Analytical Chemists (AOAC) method (AOAC, 2005). Methylation of fatty acid was performed as follows. Extracted lipid (25 mg) was weighted in a 10-mL screw cap tube, and 1.5 mL of 0.5 M NaOH in methanol was added. The mixture was flushed with nitrogen gas for 30 s and heated at 85 °C for 2 min. Subsequently, 1 mL of internal standard (C17 fatty acid) and 2 mL 14% boron trifluoride (BF3) in methanol was added. The mixture was flushed with nitrogen gas and reheated again at 85 °C for 30 min and mixed with 1 mL of isooctane. Subsequently, 5 mL of saturated NaCl solution was added to separate isooctane phase from the methanol and water phase. The mixture was reextracted with isooctane, and the isooctane phase was collected until 5 mL of the extraction was obtained. The isooctane phase containing fatty acid methyl esters (FAME) was filtered through a 0.45 μm syringe filter before GC analysis.

GC (7890A, Agilent technologies, Santa Clara, CA, USA) equipped with a flame ionization detector (FID) and an SP2560 capillary column (100 m × 0.20 μm

film thickness × 0.25 mm internal diameter, Supelco Co., Ltd., Bellefonete, PA, USA) was used for FAME analysis. The carrier gas was helium with a flow rate of 1.0 mL/min. The temperature of the injection port and detector were maintained at 250 °C. Identification and quantification of fatty acids were performed using external standards (Supelco 37 FAME, Sigma–Aldrich Co., St.Louis, MO, USA) at concentrations ranging from 0 to 10 mg/mL and was expressed as mg/g dry sample.

4.3.3 Heme iron content

Heme iron content was determined according to the method of Clark, Mahoney, and Carpenter (1997). Two grams of ground sample were added to 20 mL of acid-acetone mixture (40 mL of acetone, 9 mL of water and 1 mL of concentrated hydrochloric acid). The mixture was homogenized at 10000 rpm for 30 s. Then, 20 mL of the acid-acetone mixture was added again, and the mixture was kept in the dark for 1 h. The mixture was centrifuged at 2200×*g* for 10 min. The supernatant was collected and filtered through Whatman No.1, and the absorbance was measured at 640 nm. The concentration of total pigments in the sample (μg hematin/g sample) was calculated by multiplying the absorbance by a factor of 6800 and then dividing by the sample weight. The iron content was calculated using a factor of 0.0882 μg iron/μg hematin. The heme iron content was expressed as mg/100 g sample (Lombardi-Boccia et al., 2002).

4.3.4 Thiobarbituric acid reactive substances (TBARS)

TBARS values were determined according to Reitznerová et al. (2017). Two grams of sample were homogenized with 7.5% trichloroacetic acid (TCA) for 30 s and centrifuged at 10000×*g*, 4 °C, for 10 min. The homogenate was filtered through Whatman no. 1 filter paper. The supernatant (2 mL) was mixed with 2 mL of 0.02 M TBA solution. The sample was heated at 95 °C for 20 min and cooled in ice or at room

temperature for 10 min. The absorbance was measured at 532 nm. 1,1,3,3-Tetraethyloxypropane (TEP) was used as a standard. The TBARS value was expressed as ng of malonaldehyde/kg dry sample.

4.3.5 Determination of volatile compounds

Volatile compounds were detected by head space solid-phase microextraction gas chromatography-mass spectrometry (SPME/GC-MS). The SPME fiber was coated with carboxen–divinylbenzene–polydimethylsiloxane (CAR/DVB/PDMS) (Supelco, Bellefonte, PA, USA). One gram of ground sample was placed in a 20 mL round bottom vial and then mixed with 3 mL of deionized water, 0.7 g of NaCl, 10 μL of 7.2% butylated hydroxytoluene (BHT) in 70% ethanol and 30 μL of 100 ppm cyclohexanol as an internal standard. The vial was sealed with polytetrafluoroethylene (PTFE)/silicone septa (Agilent, Santa Clara, CA, USA). The mixture was equilibrated at 60 °C for 10 min. Volatile compounds were then analyzed on a 450-GC coupled to a 320-MS Quadrupole mass spectrometer (Bruker Daltonics, Billerica, MA, USA). The GC oven temperature program started at 3 °C for 5 min, followed by heating at 3 °C/min to 70 °C, then heating at 10 °C/min to 200 °C, and then heating at 20 °C/min to 260 °C, and this temperature was maintained for 5 min. Helium was employed as the carrier gas in the linear flow control mode with a constant column flow of 1.0 mL/min. The quadrupole mass spectrometer was operated in the electron impact (EI) mode, and the source temperature was set at 70 eV and 200 °C. Volatile compounds were identified by searching MS library and using Kovats indices (RI).

4.3.6 Vibrational spectroscopy

FT-Raman spectroscopy was performed on a Bruker RAM II FT-Raman module coupled to a Bruker Vertex 70v interferometer (Bruker Co., Ettlingen,

Germany). The excitation source was an Nd:YAG laser at 1064 nm with 500 mW of laser power. The scattered radiation was collected from the range between 4000 and 400 cm^{-1} with a resolution of 4 cm^{-1} and 256 scans. Extracted lipid samples were placed in a stainless steel cup inserted in a sample holder and monitored via video camera. A Ge detector used liquid nitrogen as the coolant. Instrument control and spectral acquisition were performed using OPUS 7.2 (Bruker Optics Ltd., Ettlingen, Germany). At least 12 Raman spectra per sample were collected, averaged and normalized using the OPUS program, version 7.2. The integrated intensity of the second derivative (13-point smoothing) was then computed to distinguish the overlapped peaks. The result was expressed as the relative integrated intensity. FT-IR spectroscopy with a single reflection attenuated total reflectance (ATR) sampling module, coupled with MCT detector and cooled with liquid nitrogen (Bruker Tensor 27, Bruker Optics Ltd., Ettlingen, Germany), was also used to collected IR spectra. Extracted lipid (20 μL) was placed in contact with a horizontal ATR plate. IR spectra were obtained from an interval of 4000-600 cm^{-1} at a 4 cm^{-1} spectral resolution with 64 scans. At least 30 spectra were collected from each sample, and they were analyzed with the OPUS 7.2 program. Spectra were taken from three replications and then averaged. Normalization and the second derivative were carried out. The results were expressed as the relative integrated intensity.

4.3.7 Statistical analyses

Statistical analyses were performed using SPSS 17.0 software (SPSS Inc, Chicago, IL, USA). Statistical evaluation was conducted using one-way analysis of variance (ANOVA). Comparison of means within each tissue at various storage time was carried out by Duncan's new multiple range tests. The significance of difference

was defined at 95% confidence interval ($P < 0.05$). Principal component analysis (PCA) of all measured parameters were performed on means results using the XLSTAT software (Addinsoft, New York, NY, USA).

4.4 Results and discussion

4.4.1 Changes in lipids

The highest lipid content was found in the belly flap (33.9–40.6%), followed by muscle (9.0–11.5%), and skin (7.0–8.8%) (**Table 4.1**). Lipids in fish are typically located in subcutaneous tissue, belly flap, muscle, mesentery, liver and head (Ackman, 1994). Moradi et al. (2011) reviewed that the lipid content in the skin of lean fish ranged from 0.2–3.9% (wet basis), while that of fatty fish could be higher than 50% (wet basis). In contrast, lipid content in fish muscle ranged from <2% in lean fish to >8% in fatty fish Moradi et al. (2011), which was equivalentto 10% and 40% (dry basis), respectively. Thilakarathne and Attygalle (2009) reported that the highest lipid content (6.52% wet basis) in Indo-Pacific sailfish (*Istiophorus platypterus*) was in the skin, followed by the belly flap, which contained 3.91%. Distribution of lipids in fish body appeared to vary with species. The study demonstrated that lipids are primarily located at the belly flap in silver carp. Among various parts of the studied raw material, muscle tissues contained the highest phospholipid content, which is an important component of membranes.

The lowest phospholipid content was found in the skin, which is composed of subcutaneous tissues that mainly contain fat cells composed of triacylglycerols. Aursand et al. (1994) reported lower contents of phospholipid in belly flap and higher proportion of neutral lipid. When washing was performed, 35–45% of lipids were

removed (**Table 4.1**). Tongnuanchan et al. (2011) reported that the lipid content of washed red tilapia mince decreased by 14.4%, in comparison with that found in unwashed mince. The removed fat was mainly triacylglycerols, which were clearly separated and appeared as floating fats after centrifugation. However, the phospholipid content of washed mince was increased when compared with unwashed mince, regardless of storage time (**Table 4.1**). Membrane lipids bind to membrane proteins, making it difficult to remove them by washing (Domínguez et al., 2019). Myofibillar proteins remained in the washed mince along with membrane lipids. Eymard et al. (2005) also reported that washing horse mackerel led to a greater reduction in neutral lipids as compared with polar lipids. Total lipid content of all tissues from raw materials decreased during ice storage ($P < 0.5$, **Table 4.1**), but those of washed mince were comparable ($P > 0.05$). A decrease in lipid content during ice storage was likely to be due to the degradation of lipid by endogenous lipases and/or lipid oxidation. Chaijan et al. (2006) reported that, during ice storage, triacylglycerols in sardine (*Sardinella gibbosa*) muscle decreased, while free fatty acid, diglycerol and mono-glycerol contents increased. This suggested that triacylglycerols were hydrolysed into free fatty acids. The phospholipid content of all samples tended to decrease with storage time, particularly in the muscle ($P < 0.05$, **Table 4.1**). This indicated oxidation of membrane lipids during ice storage.

Table 4.1 Chemical composition changes of silver carp during iced storage for 14 days and their respective washed mince (dry basis).

Storage time (days)	Skin			Belly			Muscle			Washed mince		
	0	**7**	**14**	**0**	**7**	**14**	**0**	**7**	**14**	**0**	**7**	**14**
Moisture content (%)	69.03±.62^{b}	71.79±.94^{a}	71.42±1.24^{a}	72.47±1.52	73.16±1.80	74.83±1.10	79.40±0.90	78.58±0.28	78.29±0.98	87.43±0.71^{a}	86.75±0.20^{b}	86.60±0.38^{b}
Total lipid (g /100g dry basis)	8.78±0.34^{a}	8.12±0.55^{a}	7.07±0.60^{b}	40.52±1.86^{a}	38.60±1.98^{a}	33.86±2.74^{b}	11.45±0.15^{a}	10.74±0.33^{b}	9.00±0.14^{c}	6.40±0.52	6.29±0.85	5.83±0.56
Phospholipid (g PC/100g dry basis)	0.52±0.08	0.51±0.09	0.47±0.12	1.15±0.18	1.10±0.21	0.99±0.22	1.67±0.27^{a}	1.38±0.17^{b}	1.19±0.20^{b}	2.49±0.23	2.35±0.41	2.17±0.36
TBARS (ng of MDA / kg. dry basis)	0.69±0.05^{b}	0.83±0.11^{ab}	0.94±0.06^{a}	2.08±0.10^{c}	10.12±0.90^{b}	12.51±0.21^{a}	0.59±0.03^{c}	0.70±0.02^{b}	0.86±0.06^{a}	0.77±0.08^{b}	0.96±0.05^{a}	1.06±0.05^{a}
Heme (mg/100g dry basis)	0.092±0.01	0.090±0.01	0.071±0.01	0.176±0.02	0.155±0.04	0.156±0.05	0.772±0.08^{a}	0.590±0.03^{b}	0.294±0.05^{c}	0.193±0.04^{a}	0.118±0.03^{b}	0.102±0.02^{b}

^{a,b,c} Different letters within each tissue are significantly different ($P < 0.05$).

The belly flap contained the highest saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) contents, while washed mince contained the lowest ($P < 0.05$, **Table 4.2**). These results are in agreement with the total lipid content (**Table 4.1**). The predominant SFA and MUFA in all samples were palmitic acid (C16:0) and oleic acid (C18:1n9c). The major polyunsaturated fatty acid (PUFA) in the skin and belly flap was linolenic acid (C18:3n3). The main PUFA in unwashed and washed mince was docosahexaenoic acid (DHA, C22:6n3). These results suggested that unwashed and washed mince from silver carp contained PUFA, which is prone to lipid oxidation. MUFA and PUFA contents of all samples decreased with a concomitant increase in SFA after raw materials were stored in ice for 14 days. The reduction of MUFA and PUFA was probably due to lipid oxidation during ice storage. The increase in SFA was likely to be due to the degradation of MUFA and PUFA, which, in turn, increased the proportion of SFA (Chávez-Mendoza et al., 2014). These results are in agreement with Chaijan et al. (2006), who reported that MUFA and PUFA contents in 15 days ice stored sardine (*Sardinella gibbosa*) muscle decreased by 9.7% and 8.1%, respectively, whereas SFA content increased by 2.3%. Šimat et al. (2015) also reported that PUFA in farm-affected wild bogues (*Boops boops*, Linnaeus, 1758) was reduced over a storage period of 16 days in ice.

Table 4.2 Fatty acid profiles of various tissue of silver carp and their respective washed mince (mg g^{-1} dry sample).

Fatty acids	Skin			Belly			Mince			Washed mince		
	D0	D7	D14	D0	D7	D14	D0	D7	D14	D0	D7	D14
C10:0	0.20	0.25	0.27	1.15	1.21	1.17	0.15	0.14	0.34	0.10	0.09	0.09
C12:0	0.08	0.10	0.08	0.50	0.52	0.60	0.05	0.03	0.08	0.03	0.03	0.04
C13:0	0.04	0.07	0.05	0.32	0.39	0.43	0.03	0.04	0.06	0.00	0.02	0.03
C14:0	1.30	2.06	1.59	9.55	11.28	12.08	0.92	1.17	2.04	0.56	0.65	1.20
C15:0	0.29	0.39	0.29	0.88	0.87	0.93	0.37	0.32	0.74	0.23	0.28	0.44
C16:0	8.16	8.53	10.90	58.59	63.75	65.59	7.87	8.37	10.63	6.00	6.33	10.51
C18:0	1.49	2.20	1.90	10.74	11.71	11.94	1.86	1.61	3.36	1.18	1.19	1.31
C20:0	0.14	0.28	0.15	0.89	0.90	1.09	0.11	0.11	0.23	0.08	0.08	0.16
C21:0	0.02	0.02	0.04	0.18	0.25	0.31	0.00	0.02	0.10	0.00	0.02	0.03
C22:0	0.00	0.04	0.00	0.08	0.05	0.05	0.00	0.03	0.12	0.00	0.02	0.03
C23:0	0.00	0.00	0.03	0.07	0.20	0.22	0.00	0.00	0.00	0.00	0.00	0.00
C24:0	0.86	0.95	0.90	4.26	2.52	2.52	2.43	2.30	2.43	2.03	1.82	2.29
C14:1	0.64	0.73	0.38	3.66	4.30	3.35	0.21	0.14	0.03	0.03	0.02	0.02
C16:1	3.77	3.33	3.43	24.09	24.56	23.19	2.49	2.13	2.16	1.60	1.66	1.54
C17:1	0.74	0.64	0.58	3.72	3.48	4.11	0.48	0.40	0.41	0.34	0.32	0.39
C18:1n9t	0.28	0.06	0.17	1.08	1.59	1.41	0.16	0.14	0.14	0.12	0.11	0.12
C18:1n9c	15.13	14.14	10.01	85.16	70.87	67.39	7.12	6.46	6.22	4.93	4.44	3.95
C20:1	0.92	1.56	0.87	5.38	4.72	6.21	0.58	0.57	0.60	0.38	0.33	0.59
C22:1n9	0.07	0.04	0.03	0.16	0.25	0.26	0.03	0.03	0.03	0.02	0.02	0.02
C18:2n6t	0.15	0.10	0.12	1.20	1.20	1.15	0.06	0.06	0.04	0.04	0.05	0.09
C18:2n6c	2.80	1.80	2.17	13.12	16.74	15.15	1.91	1.68	1.74	1.61	1.22	1.18
C18:3n6	0.29	0.10	0.19	1.04	1.59	1.45	0.15	0.12	0.13	0.13	0.12	0.19
C18:3n3	3.80	2.38	2.92	24.74	23.65	22.61	2.18	1.90	2.02	1.57	1.45	1.04
C20:2	0.35	0.23	0.25	1.42	2.02	1.91	0.24	0.20	0.21	0.21	0.18	0.15
C20:3n6	0.81	0.38	0.58	2.38	2.71	2.52	0.75	0.63	0.59	0.65	0.54	0.54
C20:3n3	0.45	0.36	0.42	2.74	2.90	2.63	0.35	0.31	0.34	0.24	0.29	0.41
C20:4n6	1.15	1.41	0.91	5.20	5.05	5.54	1.82	1.42	1.45	1.64	1.57	1.53
C22:2	0.98	0.00	0.00	4.48	4.30	4.37	0.00	0.00	0.00	0.00	0.00	0.00
C20:5n3	1.02	0.00	0.00	6.40	5.71	5.20	0.97	0.95	0.58	1.25	1.18	1.17

Table 4.2 Fatty acid profiles of various tissue of silver carp and their respective washed mince (mg g^{-1} dry sample). (continued)

Fatty acids	Skin			Belly			Mince			Washed mince		
	D0	D7	D14	D0	D7	D14	D0	D7	D14	D0	D7	D14
C22:6n3	1.90	2.63	1.39	13.88	9.83	9.70	3.47	2.66	2.58	3.07	2.39	2.34
SFA	12.58±1.05^{b}	14.89±0.92^{ab}	16.20±0.94^{a}	87.20±1.89^{b}	93.65±2.25^{ab}	96.93±2.53^{a}	13.78±0.98^{b}	14.14±1.23^{b}	20.13±0.62^{a}	10.21±0.53^{b}	10.55±0.67^{b}	16.11±1.08^{a}
MUFA	21.55±0.93^{a}	21.49±0.85^{a}	15.47±0.84^{b}	123.25±2.91^{a}	109.77±1.58^{b}	105.93±2.82^{b}	11.09±0.31^{a}	9.87±0.57^{ab}	9.59±0.41^{b}	7.42±0.69	6.91±0.76	6.62±0.26
PUFA	13.70±0.92^{a}	9.39±0.83^{b}	8.97±0.70^{b}	76.59±1.18^{a}	75.70±1.09^{ab}	72.23±1.41^{b}	11.91±0.32^{a}	9.94±0.29^{b}	9.69±0.34^{b}	10.41±0.45^{a}	8.99±0.39^{b}	8.64±0.46^{b}

^{a,b,c} Different letters within each tissue are significantly different ($P < 0.05$).

4.4.2 Heme iron content

Heme iron is an important catalyst of lipid oxidation. The heme iron content in muscle was significantly decreased in fish stored in ice for an extended period and in the corresponding washed mince (**Table 4.1**). This might be due to the breakdown of the heme iron complex, induced by oxidative cleavage of the porphyrin ring during ice storage. Thiansilakul, Benjakul, and Richards (2010) also reported that the heme iron content of seabass (*Lates calcarifer*) and red tilapia (*Oreochromis mossambicus* and *O. iloticus*) muscles decreased after 15 days in ice storage. The authors suggested that the disruption of heme protein and release of heme iron occurred during ice storage. Rezaei and Hosseini (2008) found that heme content in whole rainbow trout (*Oncorhynchus mykiss*) also decreased with 20 days of ice storage, which was due to the release of free iron from heme. The heme iron content in washed mince was lower than that of unwashed mince (**Table 4.1**). The washing process removed water-soluble heme proteins, leading to less heme iron in the washed mince.

4.4.3 TBARS value

The TBARS value of the different fish tissues increased with ice storage time ($P < 0.05$, **Table 4.1**). The belly flap was found to have the highest TBARS value, as it contained the highest amount of total lipid ($P < 0.05$, **Table 4.1**) and polyunsaturated fatty acids (**Table 4.2**). Moreover, various enzymes, including lipoxygenase, peroxidase and microsomal enzymes in viscera (Mei, Ma, and Xie, 2019), could potentially promote lipid oxidation in the belly flap. Although heme iron content in unwashed mince was higher than that in washed mince, the TBARS value was slightly lower (**Table 4.1**). Washed mince contained higher phospholipids content with a higher proportion of PUFA, and thus it tended to be more susceptible to lipid oxidation

despite the lower amount of the catalyst, heme iron. Addeen, Benjakul, and Maqsood (2016) reported that a higher TBARS in washed chicken mince was plausibly due to the presence of membrane lipids and the removal of natural antioxidants in muscle, such as carnosine, anserine, glutathione, and polyamines, which are water-soluble compounds. Hoke et al. (2000) also reported that the TBARS of washed mince increased during the first three months of frozen storage. In surimi and/or washed mince processing, lipid oxidation has not been as well studied as protein denaturation. However, our results suggested that lipid oxidation of washed mince produced from fish stored in ice for an extend period occurred to a greater extent than that produced from fresh fish. In addition, washed mince is prone to lipid oxidation, as it contains a higher proportion of membrane lipids and PUFA.

4.4.4 Volatile compounds

Volatile compounds, including alcohols, aldehydes and ketones, increased in the different tissues of silver carp during ice storage (**Table 4.3**). Several volatile compounds that are markers of lipid oxidation were prevalent in fish belly. These included alcohols (1-pentanol, 1-hexanol, 1-octen-3-ol, 1, -heptanol, 1-octanol) and aldehydes (hexanal, octanal, nonanal, 2-octenal), which are degradation products of peroxides. 1-Pentanol and 1-octen-3-ol are derived from the oxidation of linoleic acid. Iglesias et al. Iglesias et al. (2009) reported that 1-octen-3-ol is an important volatile contributing to off-flavor, due to its low odor threshold. 1-Heptanol, 1-octanol, 1-hexanol, nonanal and (E)-2-octenal are likely degradation products of oleic acid (Choe and Min, 2006). 2,3-Octanedione is derived from the lipid oxidation of ω-6 fatty acids (Buettner, 2017). Hexanal is a secondary product from the oxidation of linoleic acid, typically used as a lipid oxidation marker in fish (Giménez et al., 2011). On day 14,

hexanol and 1-octen-3-ol were detected at their highest level in the fish belly (**Table 4.3**). This is related to the oxidation of fatty acids, oleic acid and linoleic acid, respectively, which are abundant in the belly flap (**Table 4.2**). 1-Hexanol and 1-octen-3-ol could be used as lipid oxidation markers of belly flap of silver carp.

In skin, 1-hexanol, 1-octen-3-ol, 1-octanol and nonanal increased during the ice storage of slver carp. These compounds are derived from oxidation of oleic acid and linoleic acid, which were also found to be abundant in silver carp skin (**Table 4.3**). After 14 days of storage, 1-octen-3-ol and nonanal were predominant volatile compounds, and nonanal was found to be the highest in all tissues. Therefore, 1-octen-3-ol and nonanal could be considered lipid oxidation markers of silver carp skin.

1-Hexanol, 1-octen-3-ol, nonanal and 2,3-octanedione were found to increase in mince during ice storage (**Table 4.3**). 1-Hexanol was likely to be derived from the degradation products of oleic acid, which was the most abundant fatty acid in mince (**Table 4.2**). Several ketones have been identified in dry-cured fish and regarded as a sign of fish spoilage (Zhang et al., 2020). Lower levels of volatile compound were detected in washed mince samples. This indicated that washing can efficiently remove volatile compounds that cause off-odor. Only low levels of hexanal were detected in washed mince prepared from aged fish (14 days ice storage). Hexanal have also been found to be a predominant aldehyde in commercial silver carp surimi (An et al., 2020). Differences in volatile compounds from the different tissues of silver carp are mainly due to variations in lipid content and fatty acid composition. The belly flap of silver carp is susceptible to lipid oxidation, generating volatile compounds, especially 1-hexanol and 1-octene-3-ol and hexanal, which likely contribute to off-odor in fish stored in ice for an extended period.

Table 4.3 Relative signal intensities of volatile compounds of various part tissue of silver carp during ice storage and their respective washed mince during ice storage.

RI	Compounds	skin			belly			mince			washed mince		
		D0	D7	D14	D0	D7	D14	D0	D7	D14	D0	D7	D14
	Alcohols												
1256	1-pentanol	0.014±0.004^{a}	0.022±0.005^{a}	N.D.	0.057±0.014^{b}	0.259±0.052^{a}	0.190±0.064^{a}	0.019±0.009	0.025±0.005	0.056±0.034	N.D.	N.D.	N.D.
1360	1-hexanol	0.040±0.012^{b}	0.174±0.047^{a}	0.198±0.027^{a}	0.491±0.259^{b}	1.642±0.065^{a}	1.432±0.055^{a}	0.199±0.154^{b}	0.319±0.049$^{a}_{b}$	0.524±0.039^{a}	0.039±0.010	0.050±0.017	0.053±0.017
1456	1-octen-3-ol	0.029±0.017^{c}	0.225±0.004^{b}	0.285±0.012^{a}	0.131±0.059^{b}	0.926±0.119^{a}	1.107±0.520^{a}	0.048±0.006^{b}	0.099±0.013$^{a}_{b}$	0.175±0.073^{a}	0.041±0.013	0.052±0.028	0.048±0.022
1460	1-heptanol	0.018±0.001^{b}	0.095±0.031^{a}	0.065±0.015^{ab}	0.049±0.031^{b}	0.162±0.010^{a}	0.175±0.042^{a}	0.020±0.008^{b}	0.029±0.003^{b}	0.064±0.009^{a}	0.012±0.007	0.016±0.002	0.018±0.001
1488	1,5-octadien-3-ol, (Z)-	N.D.	N.D.	N.D.	N.D.	0.171±0.022^{a}	0.279±0.139^{a}	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1492	2-Ethyl-1-hexanol	0.062±0.015$^{a}_{b}$	0.073±0.020^{a}	0.033±0.014^{b}	0.388±0.232	0.354±0.0353	0.396±0.068	N.D.	0.111±0.049^{a}	0.042±0.035^{b}	0.230±0.039^{a}	0.037±0.015^{b}	N.D.
1562	1-octanol	0.038±0.009^{b}	0.153±0.029^{a}	0.101±0.023^{a}	0.018±0.013^{b}	0.073±0.004^{a}	0.068±0.028^{a}	N.D.	0.028±0.015^{a}	0.021±0.006^{a}	0.007±0.001^{a}	0.003±0.001^{b}	0.006±0.001^{a}
1621	2-octenol	0.007±0.002^{b}	0.031±0.002^{a}	0.025±0.011^{a}	0.021±0.004	0.048±0.008	0.087±0.012	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1665	1-nonanol	0.024±0.008^{b}	0.149±0.035^{a}	0.091±0.034^{ab}	0.041±0.003	0.371±0.005	0.027±0.011	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1689	2-octyn-1-ol	N.D.	0.039±0.003^{a}	0.014±0.002^{b}	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1777	2-ethyl-1-hexanol	N.D.	N.D.	N.D.	N.D.	0.044±0.004	0.041±0.003	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1842	3-octen-1-ol, (E)-	N.D.	N.D.	N.D.	N.D.	0.053±0.010	0.064±0.007	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	Aldehydes												
1081	hexanal	0.019±0.024	0.029±0.007	0.027±0.003	.087±0.034^{b}	0.144±0.054^{b}	0.440±0.135^{a}	N.D.	N.D.	N.D.	0.014±0.012^{b}	0.033±0.005^{b}	0.055±0.005^{a}
1281	octanal	N.D.	0.070±0.009^{a}	0.026±0.004^{b}	N.D.	0.027±0.001^{b}	0.061±0.018^{a}	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1392	nonanal	0.032±0.008^{c}	0.161±0.036^{b}	0.242±0.029^{a}	0.041±0.008^{b}	0.063±0.007^{b}	0.102±0.017^{a}	0.022±0.007^{b}	0.039±0.003$^{a}_{b}$	0.064±0.014^{a}	0.013±0.012	0.019±0.003	0.020±0.008
1430	2-octenal, (E)-	N.D.	N.D.	N.D.	N.D.	0.035±0.006^{b}	0.073±0.010^{a}	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	Ketones												
1320	2,3-octanedione	N.D.	0.023±0.002^{b}	0.035±0.007^{a}	0.049±0.003^{b}	0.234±0.053^{a}	0.291±0.089^{a}	N.D.	0.037±0.008^{b}	0.142±0.028^{a}	N.D.	N.D.	N.D.
1576	3,5-octadien-2-one, (E,E)-	N.D.	N.D.	N.D.	N.D.	N.D.	0.024±0.003	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

N.D. = not detected. ^{a,b,c} Different letters within each tissue are significantly different ($P < 0.05$).

4.4.5 FT-Raman spectroscopy

Changes in the selected FT-Raman wavenumbers of extracted lipid from the different tissues of silver carp during ice storage and in its corresponding washed mince are shown in **Table 4.4**. A decrease in the Raman band at 3015 cm^{-1} was observed in spectra from belly flap and unwashed and washed mince during ice storage. In addition, a decrease in the Raman band at 1267 cm^{-1} was observed from the analysis of the belly flap and mince during ice storage of raw material. A decrease in the two Raman bands at 3015 and 1267 cm^{-1} corresponds to *cis* =CH stretching and =CH symmetric rock *cis* double bond vibration, respectively, which indicated a reduction in unsaturated fatty acids during ice storage. Our results suggested that the Raman intensity of the cis-olefinic group =C-H stretching vibration at the 3015 cm^{-1} band and the =CH symmetric rock cis double bond at 1267 cm^{-1} can be used to monitor the oxidation of the extracted lipids. A decrease in the Raman bands of methylene groups (2935 cm^{-1} CH2 asymmetric, 2850 cm^{-1} symmetric stretching, 1438 cm^{-1} the CH2 deformation and 1301 cm^{-1} CH2 in-phase twisting) was also observed in lipids extracted from washed mince (**Table 4.4**, $P < 0.05$). An increase in the band at 1747 cm^{-1} was found in all samples during ice storage, corresponding to the ν(C=O) stretching of peroxides. Thus, the Raman band at 1747 cm^{-1} can be used to monitor the progress of the oxidation of lipids extracted from different tissues of silver carp. Another strong band at 1658 cm^{-1} corresponding to the *cis* double bond (C=C) stretching motion appeared to decrease in the spectra of the belly flap and mince during ice storage of raw material. This was likely due to the loss of conjugated double bonds, which was concomitant with a decrease in MUFA and PUFA (**Table 4.2**). Our results are in agreement with Chen et al. (2018). Chen et al. (2018) who found that the Raman band at 1655 cm^{-1}, assigned to

ν(C=C), decreased after repeated freeze-thaw of beef. They suggested that the oxidation reduced the total unsaturation of lipid, resulting in a decrease in the C=C band. Our study demonstrates that Raman spectroscopy can be potentially used to follow the progress of lipid oxidation of silver carp during ice storage, as well as that of the respective washed mince.

4.4.6 FTIR

Changes in the distinct FTIR bands of lipid extracted from the different tissues of silver carp during ice storage and those extracted from washed mince prepared from fish at various ice storage time are shown in **Table 4.5**. A decrease in the peak areas at 3013 cm cm^{-1}, corresponding to *cis* =C-H stretching, was observed in all samples ($P < 0.05$). The band observed at 3012 cm^{-1} is related to the stretching vibration of *cis* olefinic =C–H double bonds (Cebi et al., 2017; Rohman and Che Man, 2012). A continuous decrease in this band with extended storage time indicated the loss of *cis* double bonds. This also corresponded to changes in the Raman band at 3015 cm^{-1} (**Table 4.4**). Volpe et al. (2019) reported an increase in the FTIR band at 3011 cm^{-1} in trout fillets stored at 4 °C for up to 12 days. It should be noted that the changes in FTIR bands at 3011-3015 cm^{-1} of extracted lipid in this study had trends the differed from those detected in fish flesh in situ (Volpe et al., 2019). Fish flesh is composed of other components, including proteins, glycogen and nucleotides, which could interact to some extent with lipids and/or peroxides during storage. On the other hands, extracted lipid fractions contain only lipids, fatty acids and some degradation products of lipid oxidation. FTIR measurement of the extracted lipids can better reflect the extent of lipid oxidation than measuring samples from the flesh of whole fish. Changes in methylene groups at 2922 and 2853 cm^{-1}, representing asymmetric and symmetric stretching

vibrations of methylene (-CH2) and methyl (-CH3) group, respectively (Cebi et al., 2017; Maurer et al., 2012), were rather subtle. In addition, the FTIR band at 721 cm^{-1}, representing the bending vibrations of –(CH2) n–, HC=CH– (*cis*) groups (Cebi et al., 2017; Lu, 2009), were comparable in all samples. The band at 722 cm^{-1} was also assigned to the out of plane bending of a *cis*-disubstituted group. Lipids extracted from silver carp stored for an extended period tended to produce lower values at 722 cm^{-1}, suggesting the loss of cis double bonds and isomerization to a trans configuration, which commonly occurred in lipid oxidation. Changes observed in all methylene groups (2924, 2853, 722 cm^{-1}) reflect structural changes in fatty acids induced by lipid oxidation, particularly lipids extracted from washed mince, which showed a decrease in Σ methylene groups as storage time of raw material in ice increased ($P < 0.05$, **Table 4.5**). Our results indicated that silver carp stored in ice for an extended period of time exhibited a higher degree of lipid oxidation. When these raw materials were used to prepare washed mince, the resulting product contained higher oxidized lipids content, even after extensive washing (3 cycles). These changes can be monitored using either FTIR or FT-Raman spectroscopy, which is a rapid technique that requires fewer chemicals when compared with the classical peroxide/TBARS analysis. Carbonyl compounds are indicated by the wavenumber at 1744 cm^{-1}, which is related to the stretching vibration of triglyceride ester carbonyl (C=O) (Lu, 2009; Setiowaty et al., 2000). An increase in the band intensity at 1745 cm^{-1} was observed in all samples during ice storage ($P < 0.05$, **Table 4.5**). Volpe et al. (2019) reported that the FTIR band at 1743 cm^{-1} was associated with peroxidation of fatty acids, which increased over time during the storage of trout fillets. Thus, an increase in the FTIR band intensity at 1745 cm^{-1} implied the formation of peroxides and/or secondary oxidation products. This was

concomitant with an increase in TBARS value during the ice storage of all samples (**Table 4.1**). An increase in the IR band 970 cm^{-1}, implying an increase in -HC=CH- isolated trans double bonds, was observed in lipids extracted from belly flap of 14 days iced fish. In addition, lipid extracted from washed mince had higher level of trans fat than those extracted from unwashed mince ($P < 0.05$), suggesting a greater extent of lipid oxidation in the former. The higher content of phospholipids in washed mince and the washing process, in which agitation with the incorporation of air is continually applied, could be important factors that contribute to higher lipid oxidation of washed mince.

In the skin and belly, the phospholipids observed at 925 cm^{-1} and 825 cm^{-1}, corresponding to P-O-C symmetric and asymmetric stretching, respectively, appeared to undergo subtle changes with increasing storage time ($P > 0.05$, **Table 4.5**). However, these IR bands decreased in mince stored in ice for an extended period and in the respective washed minces. These FT-IR results are well correlated with phospholipid content analyzed using the colorimetric method (**Table 4.1**). This implied that oxidation of phospholipid induced by autooxidation and/or by the action of phospholipase took place during the ice storage of fish. It should be mentioned that phosphate groups were not detected in FT-Raman spectra. Therefore, the FT-IR and FT-Raman techniques can complement each other to reveal information about both polar and nonpolar moieties in lipids.

Table 4.4 Relative integrated intensity of selected regions of Raman spectra of lipids extracted from various tissues of silver carp and their respective washed mince.

Wavenumber (cm^{-1})	Band assignment	skin			belly			mince			washed mince		
		D0	D7	D14	D0	D7	D14	D0	D7	D14	D0	D7	D14
3015	cis-olefinic group =CH stretching	7.374±0.14	7.260±0.11	7.279±0.06	7.763±0.30^{A}	7.525±0.22^{B}	7.151±0.18^{C}	8.463±0.11^{x}	8.098±0.23^{xy}	7.964±0.23^{y}	9.187±0.14^{X}	9.044±0.10^{XY}	8.840±0.10^{Y}
∑methylene groups (2935, 2850,1438, 1301)		56.450±0.51	56.003±0.21	55.856±0.79	55.157±0.45	54.306±0.84	54.133±0.31	54.496±0.41	53.930±0.53	53.546±0.64	51.845±0.34^{X}	50.632±0.50^{Y}	50.645±0.79^{Y}
2935	ν_{as} CH2	9.805	9.827	9.783	9.545	9.510	9.401	9.538	9.543	9.422	9.753	9.579	9.560
2850	ν_{s} CH2	32.890	32.591	32.487	31.604	31.140	31.398	31.717	31.504	31.306	29.675	28.614	28.648
1438	δ(CH2)	9.380	9.227	9.229	9.461	9.073	8.931	8.856	8.666	8.676	8.468	8.501	8.504
1301	tCH2 in-phase twisting	4.375	4.358	4.357	4.547	4.583	4.403	4.384	4.217	4.143	3.949	3.938	3.933
1747	ν(C=O) carbonyl compounds	1.421±0.01^{b}	1.526±0.07^{ab}	1.576±0.03^{a}	1.519±0.04^{B}	1.575±0.14^{B}	1.606±0.07^{A}	1.255±0.12^{y}	1.455±0.03^{x}	1.449±0.04^{x}	0.991±0.04^{Y}	1.078±0.09^{XY}	1.152±0.04^{X}
1658	ν(C=C) conjugated double bonds	11.119±0.52	11.175±0.60	10.901±0.29	11.606±0.41^{A}	11.210±0.11^{AB}	10.739±0.07^{B}	11.790±0.38^{x}	11.287±0.07^{xy}	10.994±0.12^{y}	11.853±0.22	11.874±0.28	11.725±0.11
1267	δ(=CH) symmetric rock (cis)	2.541±0.10	2.517±0.23	2.497±0.19	3.503±07^{A}	3.231±0.09^{AB}	3.051±0.17^{B}	3.549±0.02^{x}	3.346±0.08^{xy}	3.072±0.04^{y}	3.895±0.09	3.867±0.07	3.701±0.07

Abbreviation: s, symmetric; vs, asymmetric; ν, stretch; δ, deformation; r, rock. a,b,c Different letters within the same row of each treatment are significantly different ($P < 0.05$).

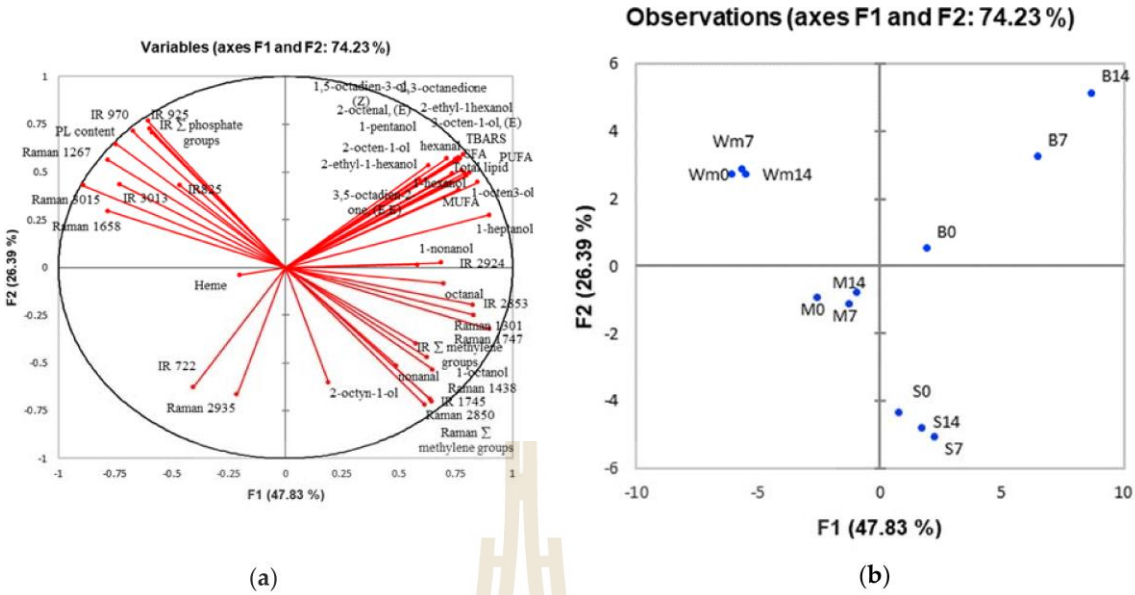
Table 4.5 Relative integrated intensity of selected regions of Fournier transforms infrared (FT-IR) spectra of extracted lipid samples from various tissues of silver carp during ice storage and their respective washed minces.

Wavenumber (cm^{-1})	Band assignment	skin			belly			mince			washed mince		
		D0	D7	D14	D0	D7	D14	D0	D7	D14	D0	D7	D14
3013	Olefinic ν(C=H) cis)	2.685±0.10^{a}	2.538±0.04^{ab}	2.508±0.10^{b}	2.980±0.12^{a}	2.743±0.15^{ab}	2.635±0.09^{b}	3.203±0.12	3.035±0.35	2.895±0.31	3.558±0.20^{a}	3.243±0.17^{ab}	2.913±0.22^{b}
∑methylene groups (2924, 2853, 722)		32.507±0.57	32.057±0.56	32.065±0.79	32.636±0.23	32.141±0.24	32.201±0.42	32.516±0.40	32.665±0.76	31.583±0.90	31.453±0.51^{a}	30.818±0.39^{bc}	29.991±0.96^{b}
2924	$\nu_{as}(CH_2)$	15.472	15.146	15.301	16.093	15.672	15.777	15.587	15.483	15.209	14.966	15.091	14.640
2853	$\nu_{s}(CH_2)$	12.019	11.968	11.745	12.038	12.000	12.322	11.457	12.221	11.457	11.294	11.217	11.002
722	$-(CH_2)-$ rocking	5.017	4.943	5.020	4.504^{a}	4.469^{a}	4.101^{b}	5.472^{a}	4.961^{b}	4.917^{b}	5.193^{a}	4.510^{b}	4.349^{b}
1745	Ester ν(C=O)	27.268±0.11^{c}	27.712±0.11^{b}	28.103±0.05^{a}	23.014±0.23^{b}	25.175±0.86^{ab}	25.455±0.85^{a}	23.292±1.21^{b}	24.068±1.01^{ab}	25.685±1.16^{a}	21.069±0.32^{b}	21.687±0.26^{bc}	22.029±0.11^{a}
970	HC=CH-(trans) Isolated double bonds	0.735±0.04	0.755±0.07	0.728±0.09	1.693±0.08^{b}	1.827±0.12^{b}	2.188±0.11^{a}	2.074±0.23	2.345±0.13	2.396±0.21	4.105±0.19	4.111±0.22	4.605±0.19
∑phosphate (925, 825)		1.104±0.05	1.169±0.08	1.128±0.17	2.050±0.35	1.866±0.13	1.883±0.23	1.598±0.05^{a}	1.453±0.04^{b}	1.458±0.04^{b}	3.815±0.12^{a}	3.203±0.09^{b}	2.962±0.13^{b}
925	ν_{s}(P-O-C)	0.717	0.889	0.605	1.326	1.213	1.282	1.296	1.183	1.171	1.957	1.934	1.947
825	ν_{as}(P-O-C)	0.386	0.280	1.047	0.724	0.653	0.601	0.302	0.270	0.287	1.858	1.269	1.014

Abbreviation: s, symmetric; vs, asymmetric; ν, stretch; δ, deformation; r, rock. ^{a,b,c} Different letters within the same row of each treatment are significantly different ($P < 0.05$).

4.4.7 Principal component analysis (PCA)

The first two components of PCA explained 74.23% of the variation (**Figure 4.1**). The skin, belly flap and unwashed and washed mince are clearly separated in different quadrants (Figure 1). The belly flap was characterized by high levels of lipid, TBARS and volatile compounds, particularly 1-octen-3-ol and 1-heptanol, which increased with storage time. Oil extracted from the belly flap was characterized by a Raman band at 1747 cm^{-1}, the summation of methylene group detected by Raman spectroscopy (Raman ∑methylene), and an IR band at 1745 cm^{-1}. The intensity of these spectra increased with the storage time of the raw material. Thus, these bands could be used as markers of lipid oxidation in the belly flap, along with 1-octen-3-ol and 1-heptanol as volatile markers. Mince samples are located in the PCA quadrant opposite to the belly flap, indicating lower contents of fat and volatile compounds. It should be noted that the effect of storage time on the measured parameters is not as well correlated for mince as it is for the belly flap. Our study suggests that the belly flap should be a target tissue for monitoring the extent of the lipid oxidation of silver carp during ice storage. For washed mince sample, phospholipid content is a distinct characteristic, while IR bands that indicate phosphate group (925, 825 cm^{-1}) are notable in the oil extracted from washed mince (Figure 1). This could be a potential marker to indicate the degree of lipid oxidation in the washed mince, which correlated with the freshness quality of raw material stored in ice.



(a) (b)

Figure 4.1 Correlation loading plot (a) and score plot (b) of principal component analysis describing measured quality parameters, namely volatile compounds, fatty acid profiles, lipid oxidation indicators and band intensity obtained from FT-IR and FT-Raman spectroscopy of skin (S), belly flap (B), and mince (M) of silver carp stored in ice for various times 0, 7 and 14 days and washed mince (Wm) prepared from fish stored in ice for 0, 7, and 14 days.

4.5 Conclusion

Lipid oxidation of silver carp varied among the different body parts of fish. The belly flap of silver carp was the most susceptible part to lipid oxidation during prolonged ice storage. Fatty acids in all parts decreased as the ice storage time was extended. 1-Hexanol and 1-octen-3-ol were key volatile compounds detected in the belly of silver carp, and they increased with storage time. Washing can efficiently remove volatile compounds that cause off-odor. FTIR and FT-Raman spectroscopy revealed changes of

cis double bonds, methylene groups, phosphate groups and ester bonds and the formation of trans isomerized fatty acids of lipids extracted from different parts of silver carp at various storage time. The Raman band at 1747 cm^{-1} could serve as a potential marker to indicate the extent of the lipid oxidation of oil extracted from the belly. The IR band at 925, 825 cm^{-1} could be used to monitor the extent of the lipid oxidation of washed mince, which is well correlated with the freshness quality of the raw material. To maintain the quality of silver carp mince and its respective washed mince, the fish belly should be removed before ice storage and before the mechanical deboning process.

4.6 References

Ackman, R. (1994). Seafood lipids. In Seafoods: **Chemistry, processing technology and quality** (pp. 34-48): Springer.

Addeen, A., Benjakul, S., & Maqsood, S. (2016). Haemoglobin-Mediated Lipid Oxidation in Washed Chicken Mince. **Indian J. Sci. Technol,** 9(2), 1-8.

An, Y., Qian, Y. L., Alcazar Magana, A., Xiong, S., & Qian, M. C. (2020). Comparative Characterization of Aroma Compounds in Silver Carp (Hypophthalmichthys molitrix), Pacific Whiting (Merluccius productus), and Alaska Pollock (Theragra chalcogramma) Surimi by Aroma Extract Dilution Analysis, Odor Activity Value, and Aroma Recombination Studies. **Journal of Agricultural and Food Chemistry,** 68(38), 10403-10413.

AOAC. (Association of Official Analytical Chemists (AOAC). Official Methods of Analysis of the Association of Official Analytical Chemists, 18th ed.; AOAC: Arlington, VA, USA, 2005.).

Aursand, M., Bleivik, B., Rainuzzo, J. R., Leif, J., & Mohr, V. (1994). Lipid distribution and composition of commercially farmed atlantic salmon (salmosalar). **Journal of the Science of Food and Agriculture**, 64(2), 239-248.

Buettner, A. Springer Handbook of Odor; Springer: Berlin/Heidelberg, Germany, 2017; p. VI, doi:10.1007/978-3-319-26932-0.

Cebi, N., Yilmaz, M. T., Sagdic, O., Yuce, H., & Yelboga, E. (2017). Prediction of peroxide value in omega-3 rich microalgae oil by ATR-FTIR spectroscopy combined with chemometrics. **Food chemistry,** 225, 188-196.

Chaijan, M., Benjakul, S., Visessanguan, W., & Faustman, C. (2006). Changes of lipids in sardine (Sardinella gibbosa) muscle during iced storage. **Food chemistry,** 99(1), 83-91.

Chávez-Mendoza, C., García-Macías, J. A., Alarcón-Rojo, A. D., Ortega-Gutiérrez, J. Á., Holguín-Licón, C., & Corral-Flores, G. (2014). Comparison of fatty acid content of fresh and frozen fillets of rainbow trout (Oncorhynchus mykiss) Walbaum. **Brazilian Archives of Biology and Technology,** 57(1), 103-109.

Chen, Q., Xie, Y., Xi, J., Guo, Y., Qian, H., Cheng, Y., . . . Yao, W. (2018). Characterization of lipid oxidation process of beef during repeated freeze-thaw by electron spin resonance technology and Raman spectroscopy. **Food chemistry,** 243, 58-64.

Choe, E., & Min, D. B. (2006). Mechanisms and factors for edible oil oxidation. **Comprehensive reviews in food science and food safety,** 5(4), 169-186.

Clark, E. M., Mahoney, A. W., & Carpenter, C. E. (1997). Heme and total iron in ready-to-eat chicken. **Journal of Agricultural and Food Chemistry,** 45(1), 124-126.

Domínguez, R., Pateiro, M., Gagaoua, M., Barba, F. J., Zhang, W., & Lorenzo, J. M. (2019). A comprehensive review on lipid oxidation in meat and meat products. **Antioxidants,** 8(10), 429.

Eymard, S., Carcouët, E., Rochet, M. J., Dumay, J., Chopin, C., & Genot, C. (2005). Development of lipid oxidation during manufacturing of horse mackerel surimi. **Journal of the Science of Food and Agriculture,** 85(10), 1750-1756.

Folch, J., Lees, M., & Stanley, G. S. (1957). A simple method for the isolation and purification of total lipides from animal tissues. **Journal of biological chemistry,** 226(1), 497-509.

Giménez, B., Gómez-Guillén, M., Pérez-Mateos, M., Montero, P., & Márquez-Ruiz, G. (2011). Evaluation of lipid oxidation in horse mackerel patties covered with borage-containing film during frozen storage. **Food chemistry,** 124(4), 1393-1403.

Hoke, M., Jahncke, M., Silva, J., Hearnsberger, J., Chamul, R., & Suriyaphan, O. (2000). Stability of washed frozen mince from channel catfish frames. **Journal of food science,** 65(6), 1083-1086.

Iglesias, J., & Medina, I. (2008). Solid-phase microextraction method for the determination of volatile compounds associated to oxidation of fish muscle. **Journal of Chromatography A**, 1192(1), 9-16.

Iglesias, J., Medina, I., Bianchi, F., Careri, M., Mangia, A., & Musci, M. (2009). Study of the volatile compounds useful for the characterisation of fresh and frozen-thawed cultured gilthead sea bream fish by solid-phase microextraction gas chromatography–mass spectrometry. **Food chemistry,** 115(4), 1473-1478.

Lombardi-Boccia, G., Martínez-Domínguez, B., Aguzzi, A., & Rincón-León, F. (2002). Optimization of heme iron analysis in raw and cooked red meat. **Food chemistry,** 78(4), 505-510.

Lu, H. (2009). A comparative study of storage stability in virgin coconut oil and extra virgin olive oil upon thermal treatment.

Maurer, N. E., Hatta-Sakoda, B., Pascual-Chagman, G., & Rodriguez-Saona, L. E. (2012). Characterization and authentication of a novel vegetable source of omega-3 fatty acids, sacha inchi (Plukenetia volubilis L.) oil. **Food chemistry,** 134(2), 1173-1180.

Mei, J., Ma, X., & Xie, J. (2019). Review on natural preservatives for extending fish shelf life. **Foods,** 8(10), 490.

Moradi, Y., Bakar, J., Motalebi, A., Syed Muhamad, S., & Che Man, Y. (2011). A review on fish lipid: composition and changes during cooking methods. **Journal of Aquatic Food Product Technology,** 20(4), 379-390.

Park, J. W., Graves, D., Draves, R., & Yongsawatdigul, J. (2013). Manufacture of Surimi. **Surimi and Surimi seafood,** 55-97.

Reitznerová, A., Šuleková, M., Nagy, J., Marcinčák, S., Semjon, B., Čertík, M., & Klempová, T. (2017). Lipid peroxidation process in meat and meat products: a comparison study of malondialdehyde determination between modified 2-Thiobarbituric acid spectrophotometric method and reverse-phase high-performance liquid chromatography. **Molecules,** 22(11), 1988.

Rezaei, M., & Hosseini, S. (2008). Quality assessment of farmed rainbow trout (Oncorhynchus mykiss) during chilled storage. **Journal of food science,** 73(6), H93-H96.

Rohman, A., & Che Man, Y. (2012). Quantification and classification of corn and sunflower oils as adulterants in olive oil using chemometrics and FTIR spectra. **The Scientific World Journal,** 2012.

Sánchez-Alonso, I., Carmona, P., & Careche, M. (2012). Vibrational spectroscopic analysis of hake (Merluccius merluccius L.) lipids during frozen storage. **Food chemistry,** 132(1), 160-167.

Setiowaty, G., Che Man, Y., Jinap, S., & Moh, M. (2000). Quantitative determination of peroxide value in thermally oxidized palm olein by Fourier transform infrared spectroscopy. Phytochemical Analysis: **An International Journal of Plant Chemical and Biochemical Techniques,** 11(2), 74-78.

Šimat, V., Bogdanović, T., Poljak, V., & Petričević, S. (2015). Changes in fatty acid composition, atherogenic and thrombogenic health lipid indices and lipid stability of bogue (Boops boops Linnaeus, 1758) during storage on ice: Effect of fish farming activities. **Journal of Food Composition and Analysis,** 40, 120-125.

Thiansilakul, Y., Benjakul, S., & Richards, M. P. (2010). Changes in heme proteins and lipids associated with off-odour of seabass (Lates calcarifer) and red tilapia (Oreochromis mossambicus× O. niloticus) during iced storage. **Food chemistry,** 121(4), 1109-1119.

Thilakarathne, L., & Attygalle, M. (2009). Lipid composition of skin and muscle of the Indo-Pacific sailfish, Istiophorus platypterus.

Tongnuanchan, P., Benjakul, S., Prodpran, T., & Songtipya, P. (2011). Characteristics of film based on protein isolate from red tilapia muscle with negligible yellow discoloration. **International Journal of Biological Macromolecules,** 48(5), 758-767.

Volpe, M. G., Coccia, E., Siano, F., Di Stasio, M., & Paolucci, M. (2019). Rapid evaluation methods for quality of trout (Oncorhynchus mykiss) fresh fillet preserved in an active edible coating. **Foods,** 8(4), 113.

Weng, W., & Zheng, W. (2015). Silver carp (Hypophthalmichthys molitrix) surimi acid-induced gel extract characteristics: A comparison with heat-induced gel. **International Journal of Food Properties,** 18(4), 821-832.

Zhang, Q., Ding, Y., Gu, S., Zhu, S., Zhou, X., & Ding, Y. (2020). Identification of changes in volatile compounds in dry-cured fish during storage using HS-GC-IMS. **Food Research International,** 137, 109339.

CHAPTER V

CHARACTERIZATION AND QUANTIFICATION OF ODOR ACTIVE COMPOUNDS OF SILVER CARP WASHED MINCE UNDER FREEZE-THAW CYCLES

5.1 Abstract

Odorant volatile compounds of fresh and freeze-thaw washed silver carp (*Hypophthalmichthys molitrix*) mince were investigated by gas chromatography-olfactometry-mass spectrometry (GC-O-MS). Washed mince gels were also prepared and analyzed for odor active compounds. Temperature abuse during freezing and heating induced lipid oxidation as indicated by an increase in TBARS value, propanal, butanal, pentanal, hexanal, 1-hexen-3-one, 1-octen-3-one, (Z)-1,5-octadien-3-one, nonanal, 1-octen-3-ol, (E)-2-nonenal and 2,4,6-nonatrienol. An increase in methanethiol, dimethyl sulfide, 3-methylbutanal, 2-acetyl-1-pyroline, and dimethyl trisulfide were observed in cooked products. (Z)-1,5-Octadien-3-one, (E)-2-nonenal and 1-octen-3-ol were possessed the highest odor active values (OAVs) in washed mince and gel. A stable isotope dilution assay (SIDA) was developed for the quantification of key odor active compounds, including hexanal, 1-octen-3-ol, propanal, nonanal and dimethyl sulfide.

Keywords: silver carp, odorant volatile compounds, gas chromatography-olfactometry-mass spectrometry (GC-O-MS), stable isotope dilution assay (SIDA)

5.2 Introduction

Surimi, a concentrated myofibrillar proteins in which contain high proportion of phospholipids. Polyunsaturated fatty acids presenting in phopsholipids inevitably serve as a substrate of lipid oxidation, particularly during frozen storage, which would ultimately lead to off-flavor. Changes of proteins during frozen storage have been well elucidated in surimi, but systematic study on lipid oxidation of surimi during frozen storage is scarce, particularly as related to flavor quality of the product.

Volatile compounds in surimi can be generated by enzymatic reactions, lipid autoxidation or microbial action (Olafsdottir et al., 1997). Taking into the action of microorganisms is negligible at frozen temperatures, volatiles formed as consequence of this activity should not be found in fresh/thawed fish and fish products. However, the progress of lipid oxidation of polyunsaturated fatty acids (PUFA) leads to the formation of several volatiles. Hexanal has been widely reported as a lipid oxidation marker in fish and fishery products (Azarbad and Jeleń, 2015). Recently, the evaluation of volatile compounds has become an additional indicator of lipid oxidation (Ross and Smith, 2006). Several volatiles, such as E-2-hexenal, Z-4-heptenal, (E,E)-2,4-heptadienal, have been associated with off-odor caused by lipid oxidation. However, there is still no report of lipid oxidation markers of washed mince during frozen storage and those cooked washed mince gel. The objective of this study was to identify and quantify the

odorant volatile compounds of silver carp washed mince during frozen storage, using SPME-GCO-MS, at various freeze-thaw cycles and their respective washed mince gel. The results obtained will be explains the formation of odor volatiles that contributed to the overall aroma in washed mince and guide the future odorants development. Moreover, lipid oxidation marker will be useful to monitor deterioration in frozen washed mince and their cooked gel sample.

5.3 Materials and methods

5.3.1 Reference standard compounds

Chemicals (listed in **Tables 5.1**) were obtained from commercial sources: no. 1–9, 11, 18–24 (Sigma-Aldrich, St. Louis, MO); 10, 12-14, 16 (Fisher Scientific, Pittsburgh, PA). 2-Acetyl-1-pyrroline (no. 15) was synthesized using the procedure described by Fuganti, Gatti, and Serra (2007) and (*Z*)-1,5-octadien-3-one (no. 17) was synthesized according to Lin et al., (1999). All solvents were of analytical grade. Odorless deionized–distilled water was prepared by boiling glass-distilled water in an open flask until its volume was reduced by one-fourth of the original volume. Fiber carboxen/polydimethylsiloxane/divinylbenzene 2 cm 50/30 μm (CAR–PDMS–DVB) was from Supelco (Bellefonte, PA, USA).

5.3.2 Isotopically labeled compounds

The labeled compounds were synthesized using previously published methods in parentheses: [^{2}H3]-Propanal, [^{2}H4]-Hexanal (Steinhaus et al., 2009), [^{2}H4]-nonanal, [^{2}H2,3]-1-Octen-3-ol (Lorjaroenphon, 2012). The isotropically-labeled [^{2}H6]-Dimethyl sulfide were obtained from Sigma-Aldrich (St. Louis, MO).

5.3.3 Preparation of washed mince

Live silver carp (*Hypophthalmichthys molitrix*) were obtained from Illinois River and were frozen and transported overnight in polystyrene foam boxes dry ice containing to the Agricultural Bioprocessing Laboratory at University of Illinois, Urbana-Champaign. Upon arrival, fish were thawed at room temperature followed by eviscerated, deboned, filleted, washed and minced using a grinder with a 5-mm perforation plate. Fish mince was washed 2 times with portable water at mince:water ratio of 1:3, temperature of water is below 5 °C. At each washing cycles, manually dewatering were performed through a filter cloth. The 3rd washing cycle was carried out using the same volume of 0.3% NaCl solution. Centrifugation was carried out at 5000×*g* for 15 min at 4 °C at last washing step. Some floating matters including muscle tissue and triglycerides were manually removed after centrifugation. Washed minces were mixed with cryoprotectants (0.3% polyphosphate, 4% sorbitol, and 4% sucrose) and were divided to 500 g portions and vacuum-packed in a polyethylene bag. Samples were subjected to a freeze-thaw cycle by freezing at -20 °C for 48 h, followed by thawed in running tap water (25-28 °C) until the core temperature reached 0-2 °C to complete one cycle. Freeze-thaw cycles were repeatedly for 10 times.

5.3.4 Thiobarbituric acid reactive substances (TBARS)

TBARS determination was carried out according to Ahn et al. (1995) with slight modifications. Two grams of samples was homogenized with 7.5% trichloroacetic acid (TCA) for 30 s. The homogenate was centrifuged at 10000*g* x 4 °C for 10 min, then filtered through Whatman no. 1 filter paper. Filtrate was mixed with 0.02 M TBA solution at equal volume and incubated in a 95 °C water bath for 20 min. The absorbance was determined at 450 nm.

5.3.5 Sample preparation for GC-O-MS

Washed mince sample was homogenized with odorless water at ratio 1:3. The 5 g of mixture sample was poured into a 20 mL headspace vial (overnight baked at 120 °C) with magnetic stirrer. After the 2 μL of internal standards (1.17 μg μL^{-1} of 6-undecanone in methanol) were added, a polytetrafluoroethylene (PTFE)-lined cap was immediately sealed. Semi-quantification of volatile compounds was determined by comparing standard compounds with its internal standards. The mass ratios at 10:1, 5:1, 1:1, 1:5, and 1:10 were used to generate internal standard calibration plots. The respond factor (1/slope) was determined by linear regression of a plot of the selected ion area ratio (reference standard/internal standard) against the mass ratio (reference standard/internal standard).

Pre-incubated time at 60 °C for 10 min with agitator speed 500 rpm, agitator on time 60 s, agitator off time 10 s using a MPS2 autosampler (Gerstel, Inc.). The SPME fiber used was 50/30 μm DVB/CarboxenTM/PDMS StableFlexTM (Supelco, Bellefonte, PA). SPME fiber was exposed to the vial headspace for 20 min. Then, fiber was immediately transferred to injection port of the GC and desorbed for 10 min for subsequent analysis by GC-O-MS.

GC-O-MS was performed using a 6890 GC-5973N mass selective detector (Agilent Technologies Inc., city, state) equipped with olfactory detection port (DATU Technology Transfer, Geneva, NY). The oven temperature was programmed from 35 to 225 °C at a heating rate of 10 °C/min with initial and final hold times of 5 and 20 min, respectively. Helium was used as carrier gas at a constant rate of 1.0 mL/min. The MSD conditions were as follows: capillary direct interface temperature, 280 °C; ionization energy, 70 eV; mass range, 35-300 amu; electron multiplier voltage

(Autotune + 200 V); scan rate, 5.27 scans/s. SIM scan mode was performed to select the valid target mass spectra compound which may not be revealed in regular scanning ion mode.

5.3.6 Quantification of volatile compounds

Samples were spiked with individual stable isotope solutions through the septum using a 10 µL syringe. Total volume of isotope solutions was less than 20 uL to minimize the interference problem of naturally sample. Subsequently, the vial was recapped with the new cap in order to prevent volatile loss through the needle holes, and was thoroughly stirred. The response factor (1/slope) was determined by linear regression of a plot of the selected ion area ratio (unlabeled analyte compounds/its isotope labeled standard) against the mass ratio (unlabeled analyte compounds/its isotope labeled standard). The mass ratios used to generate calibration plots were performed.

OAV of a compound was calculated by dividing its concentration by its published odor detection threshold in water (Buttara et al., 2014). Compound identification was based on retention indices, odor descriptions and mass spectra of unknowns with those of authentic standards. A homologous series of *n*-alkanes was used to establish retention indices according to the method of Van den Dool and Kratz (1963).

5.4 Results and discussion

5.4.1 TBARS value

Extended frozen storage and cooking process resulted in an increase in TBARS, leading to higher absorbance at 450 nm (data not shown). Generally,

malonaldehyde reacts with thiobarbituric acid at 95 °C, forming complex with pink color, which can be detected at 532 nm. However, yellow chromophores of TBA-aldehyde complex have been reported. Sun et al. (2001) reported that alkanals and alk-2-enals showed yellow solution, while alka-2,4-dienals resulted in pink color. From these results, it is likely that aldehydes (propanal, butanal, pentanal, hexanal, heptanal, octanal, nonanal and (E)-2-nonenal) formed during freeze-thaw cycle and cooking of silver carp washed mince were responded for yellow chromophores complex. It should be noted that TBARS value in raw washed mince was higher than that of cooked washed mince gel, could be due to thermal processing inactivated lipases, lipoxygenase, phopholipases and other enzymes of fish muscles that response for lipid oxidation (Erickson, 2002). Wang, Miller, and Addis (1991) reported that heating of lake herring (*Coregonus artedii*) at 80 °C for 5 min was fully inactivated lipoxygenase in which contributing to lipid oxidation increased. However, time-temperature required for inactivate enzymes is different species-specific. (Bosund and Ganrot, 1970).

5.4.2 Odorants compounds in washed mince

A total of 24 odorants were identified in raw washed mince, including 11 aldehydes, 3 sulfur-containing compounds, 6 ketones, and 4 alcohols (**Table 5.1**). 2,6-Nonadienal, (E,Z)-, (cucumber) was the most perception odorant in raw washed mince. Josephson and Lindsay (1986) reported that the odor of fresh fish is characterized as mild, green, melon-like and plant-like notes, contributing from 6, 8 and 9 carbon aldehydes, ketones and alcohols which was derived via lipoxygenase activity on polyunsaturated fatty acids.

After 10 freeze-thaw cycles, 2,6-nonadienal, (E,Z)- was still belonged the most important odorants of freeze-thaw washed mince, followed by methanethiol

(rotten cabbage, pumpkin, squash) and (Z)-1,5-octadien-3-one (green, metallic, fishy, geranium). Odorant perception raised in 2,6-nonadienal, (E,Z)-, of freeze-thaw washed mince implied the occurrences of lipid oxidation. Choe and Min (2006) reported that 2,6-nonadienal, (E,Z)- was formed through lipid oxidation of linolenic acid. (Z)-1,5-octadien-3-one was formed by the action of lipoxygenase/ hydroperoxide lyase on linolenic acids (Whitfield and Mottram, 1992). Methanethiol was a degradation product of methinonine or its Strecker degradation product methional (Devos and Rouault, 1990). Alasalvar, Taylor, and Shahidi (2005) reported that methanethiol was a volatile sulfur compound which possessed an unpleasant off odor in cultured and wild sea bream (*Sparus aurata*) which continuous increased during 23 days ice storage. Refsgaard, Haahr, and Jensen (1999) informed that deteriorated frozen fish odor was characterized by rancid/fishy, strong sulfurous and off-odor. As results, mainly chemical reaction responsible for odorants compounds in freeze-thaw washed mince were derived from lipid oxidation, enzymatic mechanism and Strecker degradation.

Cooked fresh washed mince exhibited (Z)-1,5-octadien-3-one was the major odorants perception followed by pentanal (yogurt), 3-methylbutanal (malty, dark chocolate) and dimethyl trisulfide (cabbage, sulfurous, garlic). Present of (Z)-1,5-octadien-3-one was due to mechanism of enzymatic action as mentioned above. Milo and Grosch (1993) reported that (Z)-1,5-octadien-3-one and dimethyl trisulfide were important odorants of freshly boiled trouts (*Salmo fario*). Variety odorant of cooked fresh washed mince revealed several reactions were involved. Pentanal is a breakdown lipid oxidation product from arachidonate and linolenate (Refsgaard et al., 2000). 3-Methylbutanal was formed by Strecker degradation of valine, isoleucine, leucine, phenylalanine and methionine (Whitfield and Mottram, 1992). Dimethyl trisulfide was

resulted from microbial action of *Pseudomonas* spp. or bacterial contamination (Whitfield, Freeman, and Bannister, 1981). Frozen storage have been reported as an excellent method to preserve food quality which microorganism do not grow when the temperature is -10 °C or colder (Boylston et al., 2012). However, freeze-thaw abuse during storage of washed mince could contributed negative odor from microbial development.

Pentanal, 1-octen-3-one, dimethyl trisulfide, (Z)-1,5-octadien-3-one were the main odorants formed in cooked freeze-thawed washed mince. Four odorants were increased comparrf to cooked fresh washed mince. 1-Octen-3-one was formed via lipoxygenase/hydroperoxide lyase on linoleic (Whitfield and Mottram, 1992). These results indicated that extended frozen storage period increased off odor intensity perception in cooked sample. The important reaction that responses toward odorants developing in cooked freeze-thawed washed mince were similar to those cooked raw washed mince. Milo and Grosch (1996) reported increase in odorants of 1-octen-3-one and dimethyl trisulfide were found in boiled cod after frozen stored raw material for 20 weeks, led to a change of mild fishy odor that found in boiled fresh cod into an off odor and putrid fishy odor.

Odor active volatile compounds in washed mince can be produced by various reactions. Lipid oxidation played important roles in odorant compounds formation. Unsaturated fatty acid is a prime reason of rancidity and off-odor development during frozen storage. Hexanal is odorant aldehydes product of oxidation of linoleic acid (Kawai and Sakaguchi, 1996). Propanal is derived from 16-hydroperoxide formed by autoxidation of methyl linolenate and from the 15-hydroperoxide formed by photosensitized oxidation of methyl linolenate (Frankel,

1998). Butanal and 2,4,6-nonatrienol were derived from lipid oxidation of linolenic acid (Choe and Min, 2006; Whitfield and Mottram, 1992). (Z)-4-Heptenal, (E)-2-nonenal and heptanal are derived from lipid oxidation of ω-3, ω-6 and ω-9 unsaturated fatty acids, respectively (Jónsdóttir, Bragadóttir, and Arnarson, 2005). Moreover, (Z)-4-heptenal is also produced by retro-aldol degradation of (E,Z)-2,6-nonadienal (Josephson, Lindsay, and Stuiber, 1987) and by the action of 12-lipoxygenase on EPA via 2,6-nonadienal (Aro et al., 2003). Octanal and nonanal are produced by autoxidation of oleic and/or linoleic acid (Whitfield and Mottram, 1992). 1-Octen-3-ol are generated from lipid oxidation of linoleic acid (Sullivan and Kerry, 2013) and by enzymatic reaction, via the action of 15-lipoxygenase on eicosapentaenoate or of 12-lipoxygenase on arachidonate (Hsieh and Kinsella, 1989; Kawai and Sakaguchi, 1996). Sulfur-containing compounds, dimethyl sulfide, is a degradation product of methionine or bacterial degradation of methionine (Yu and Ho, 1995). Moreover, formation of dimethyl sulfide can be formed by action of endogenous enzymes and by microbial activity of *Pseudomonas* spp. which lead to negative effect on fish odor (Alasalvar et al., 2005; Shiomi et al., 1982).

Additionally, some odorant compounds were formed via other chemical reaction. The 2-acetyl-1-pyroline, a nitrogen-containing compound, is likely produced form Strecker degradation of proline in the Maillard reaction (Blank et al., 2003). The 2,3-pentanedione, can be formed by the Maillard reaction (Yaylayan and Keyhani, 1999). 3-Methyl-nonan-2,4-dione was derived from furanoid fatty acid (Sano et al., 2017). 2-phenylethanol, is derived from phenylalanine (Acree and Teranishi, 1993).

Table 5.1 Characteristic odor active compounds in silver carp washed mince during frozen storage

No.	RI	Odorant compounds	Descriptors	Method of Identification	Odor intensity			
					0 FT		10FT	
					uncooked	cooked	uncooked	cooked
1	696	Methanethiol	rotten cabbage, squash	MS, RI, odor, STD	++	++	++++	++++
2	716	Dimethyl sulfide	sweet, corn	MS, RI, odor, STD	+	++	+	++
3	798	Propanal	sweat, alcohol	MS, RI, odor, STD	+	+	+	+
4	864	Butanal	green, pungent, dark chocolate	MS, RI, odor, STD	++	+	+++	+
5	914	3-Methylbutanal	malty, dark chocolate	MS, RI, odor, STD	+	++	+	+++
6	986	Pentanal	yogurt	MS, RI, odor, STD	+	+++	+++	++++++
7	1065	2,3-Pentanedione	buttery, sour	MS, RI, odor, STD	+	++	++	+++
8	1084	Hexanal	green	MS, RI, odor, STD	+	++	+	+++
9	1102	1-Hexen-3-one	plastic	MS, RI, odor, STD	+	+	+++	+
10	1165	Heptanal	green, orange	MS, RI, odor, STD	+	++	++	+++
11	1213	3-methylbutanal	malty, dark chocolate	MS, RI, odor, STD	++	+++	++	++
12	1222	(Z)-4-Heptenal	sweet, fishy, crabby	MS, RI, odor, STD	++	++	+++	++++
13	1257	Octanal	nutty	MS, RI, odor, STD	+	++	++	++
14	1279	1-Octen-3-one	mushroom	MS, RI, odor, STD	+	+++	+++	+++++
15	1329	2-acetyl-1-pyroline	nutty, popcorn	MS, RI, odor, STD	+	N.D.	+	N.D.
16	1346	Dimethyl trisulfide	cabbage, sulfurous, garlic	MS, RI, odor, STD	N.D.	+++	++	+++++
17	1357	(Z)-1,5-Octadien-3-one	green, metallic, fish, geranium	MS, RI, odor, STD	++	++++	++++	+++++
18	1393	nonanal	green	MS, RI, odor, STD	+	++	+++	+++
19	1429	1-Octen-3-ol	green, leaf	MS, RI, odor, STD	+	++	+++	+++
20	1518	(E)-2-Nonenal	hay-like, fatty	MS, RI, odor, STD	++	+	++	+
21	1572	2,6-Nonadienal, (E,Z)-	cucumber	MS, RI, odor, STD	+++	++	+++++	+++
22	1707	3-methyl-nonan-2,4-dione	hay-like, green, plant-like	MS, RI, odor, STD	+	++	+++	++
23	1888	2,4,6-nonatrienol	fishy, oats, fatty	MS, RI, odor, STD	+	++	++	++
24	1911	2-Phenylethanol	rosy	MS, RI, odor, STD	+	N.D.	+	N.D.

Odor intensity was evaluated by 3 judges on 5-point scale (+ = very weak odor intensity, +++++ = very strong intensity. N.D. = not detected

5.4.3 Relative concentrations and odor activity values (OAVs) in washed mince

Relative concentrations and OAVs detected in raw and cooked washed mince were given in **Tables 5.2**. Since freshly washed mince and washed mince gel prepared from the unfrozen sample contain relatively high level of moisture content, 75.30-78.00), OAVs were calculated based on corresponding odor thresholds determined in water (Grosch, 2001), demonstrated that the compounds possessing an OAV greater than 1 are considered to be potent odorants and contributed to the overall aroma in the sample. Therefore, 2-phenylethanol was not the key compounds in the overall aroma of all washed mince and washed mince gel. In addition, the OAV of 2,4,6-nonatrienol was not calculated due to unavailable odor threshold.

(Z)-1,5-Octadien-3-one, (E)-2-nonenal and 1-octen-3-ol showed the highest OAVs in all samples was due to their low odor threshold. Grosch (1994) stated that odorants with high OAVs can contribute to the characteristic off-flavor in foods. Increase of OAVs was found when washed mince was subject to temperature abuse during freeze-thaw cycles. This was in agreement with Milo and Grosch (1993) found that (Z)-1,5-octadien-3-one was the most potent odorant in boiled trouts (*Salmo fario***)** stored for 17 weeks at -13 °C and in boiled salmon (*Salmo salar*) stored for 14 weeks at -60 °C. Moreover, (E)-2-nonenal in boiled fish was increased when extended storage period of raw material (Milo and Grosch, 1996). Iglesias and Medina (2008) reported that 1-octen-3-ol is an important odorant in chilled Atlantic horse mackerel muscle due to its low odor threshold, which associated to an off-odor in fish. Cadwallader and Baek (1998) informed that (E)-2-nonenal was an important aroma

Table 5.2 Quantitative results of odor active compounds in silver carp washed mince subjected to freeze-thaw and cooking

No.	RI	Odorant compounds	Selected ion (m/z)	Rf	Concentration (μg kg^{-1})				Odor threshold (μg kg^{-1}) in water	OAV			
					0 FT		10FT			0 FT		10FT	
					uncooked	cooked	uncooked	cooked		uncooked	cooked	uncooked	cooked
1	696	Methanethiol	48	1.67	0.162	0.126	0.303	0.491	0.2[a]	810	630	1515	2455
2	716	Dimethyl sulfide	62	0.93	0.055	0.134	0.053	0.108	12.3[b]	4	11	4	9
3	798	Propanal	58	1.22	1.098	2.850	2.781	3.031	15.1[c]	73	189	184	201
4	864	Butanal	72	0.32	0.049	0.081	0.153	0.134	9[d]	5	9	17	15
5	914	3-Methylbutanal	86	0.07	0.028	0.030	0.015	0.018	1.1[c]	25	27	14	16
6	986	Pentanal	85	1.2	0.028	0.041	0.166	0.198	12[d]	2	3	14	17
7	1065	2,3-Pentanedione	100	0.35	0.394	0.208	0.157	0.131	30[e]	13	7	5	4
8	1084	Hexanal	72	0.69	27.858	33.412	42.944	48.086	5[f]	6191	7425	9543	10686
9	1102	1-Hexen-3-one	98	0.94	0.089	0.118	0.199	0.501	0.024[f]	3708	4917	8292	20875
10	1165	Heptanal	113	0.08	0.436	0.868	0.182	0.266	2.8[g]	156	310	65	95
11	1213	3-Methyl, 1-butanol,	70	0.77	0.422	0.523	0.170	0.750	4[c]	106	131	43	188
12	1222	(Z)-4-Heptenal	84	2.25	2.277	4.032	2.234	1.233	4.2[c]	542	960	532	294
13	1257	Octanal	110	0.53	0.019	0.049	0.013	0.049	0.59[g]	32	83	22	83
14	1279	1-Octen-3-one	70	3.73	0.016	0.021	0.122	0.098	0.05[h]	320	420	2440	1960
15	1329	2-acetyl-1-pyroline	83	1.92	0.014	0.017	0.031	0.062	0.1[i]	140	170	310	620
16	1346	Dimethyl trisulfide	126	1.44	1.998	5.428	3.433	5.710	1.45[g]	1378	3743	2368	3938
17	1357	(Z)-1,5-Octadien-3-one	105	46.9	0.509	0.587	1.429	2.377	0.0012[j]	424167	489167	1190833	1980833
18	1393	nonanal	114	2.33	6.959	13.825	7.783	15.179	1.1[g]	6326	12568	7075	13799
19	1429	1-Octen-3-ol	57	0.96	19.975	26.503	33.577	42.223	1.5[g]	13317	17669	22385	28149
20	1518	(E)-2-Nonenal	139	1.6	19.414	30.902	38.255	77.021	0.5[k]	38828	61804	76510	154042

[a] Ref (Guth and Grosch, 1994), [b] Ref (Gu et al., 2013), [c] Ref (Giri, Osako, and Ohshima, 2010), [d] Ref (Guadagni, Buttery, and Okano, 1963), [e] Ref (Grumezescu and Holban, 2017), [f] Ref (Buttery, Guadagni, and Ling, 1978), [g] Ref (Zhou et al., 2016), [h] Ref (Buttery and Ling, 1998), [i] Ref (Buttery, Turnbaugh, and Ling, 1988), [j] Ref (Swoboda and Peers, 1977), [k] Ref (Flament and Bessière-Thomas, 2002), [l] Ref (Masanetz and Grosch, 1998).

Table 5.2 Quantitative results of odor active compounds in silver carp washed mince subjected to freeze-thaw and cooking. (Continued)

No.	RI	Odorant compounds	Selected ion (m/z)	Rf	Concentration (μg kg^{-1})				Odor threshold (μg kg^{-1}) in water	OAV			
					0 FT		10FT			0 FT		10FT	
					uncooked	cooked	uncooked	cooked		uncooked	cooked	uncooked	cooked
21	1572	2,6-Nonadienal, (E,Z)-	137	1.97	0.095	0.291	0.079	0.239	0.8[c]	119	364	99	299
22	1707	3-Methyl-nonan-2,4-dione	170	1.23	0.132	0.228	0.136	0.333	0.03[l]	4400	7600	4533	11100
23	1888	2,4,6-Nonatrienol	205	23.9	0.841	1.667	3.346	3.300	N.A.	0	0	0	0
24	1911	2-Phenylethanol	91	1.32	0.062	0.046	0.096	0.097	564.23[c]	0	0	0	0

N.A.= not available

[a] Ref (Guth and Grosch, 1994), [b] Ref (Gu, Wang, Tao, and Wu, 2013), [c] Ref (Giri, Osako, and Ohshima, 2010), [d] Ref (Guadagni, Buttery, and Okano, 1963), [e] Ref (Grumezescu and Holban, 2017), [f] Ref (Buttery, Guadagni, and Ling, 1978), [g] Ref (Zhou, Chong, Ding, Gu, and Liu, 2016), [h] Ref (Buttery and Ling, 1998), [i] Ref (Buttery, Turnbaugh, and Ling, 1988), [j] Ref (Swoboda and Peers, 1977), [k] Ref (Flament and Bessière-Thomas, 2002), [l] Ref (Masanetz and Grosch, 1998).

component in cooked crayfish (*Procambarus clarki*) which considered as the off-odor compound.

The combination of extended freeze-thawed storage and followed by cooking process resulted in an increase in concentration of these compounds. Lorjaroenphon (2012) reported that increased in 2-acetyl-1-pyrroline content resulted formed Maillard reaction of glucose and proline in washed mince. Kubota et al. (1996) revealed that 2-phenylethanol was found in cooked squid (*Todarodes pacificus* Steenstrup). 2-Acetyl-1-pyrroline has been reported as a component of cooked tail meat of American lobster (*Homarus americanus*) (Lee, Suriyaphan and Cadwallader, 2001). They suggested that 2-acetyl-1-pyrroline was not considered as off-odor volatile in cooked sample.

5.4.4 Quantification of selected compounds

The SIDA HS-SPME-GCMS method was applied to quantify selected volatile compounds in samples. The response factor was calculated using selected ion (m/z) of unlabeled compound against the labeled compound (**Table 5.3**). R^2 values are all acceptable, ranging from 0.9963-0.9997. Hexanal is reported to be an important marker for lipid oxidation in fish and fish products. From the results, high amount of hexanal could be used to monitor the occurrence of off-odor from lipid oxidation during frozen storage. Furthermore, propanal and nonanal have been identified as lipid oxidation markers (Varlet, Prost and Serot, 2007; Zepka et al., 2014). Propanal is generated from oxidation of ω-3 fatty acids, while nonanal is a degradation products of ω-6 fatty acids (Akoh, 2017). Iglesias et al. (2009) reported that 1-octen-3-ol exhibited

Table 5.3 Selected ions (m/z), response factors and concentration (μg kg^{-1}) of selected compounds using stable isotope dilution analysis

No.	RI	unlabeled analytes	Ion	Isotope (labeled internal standard)	Ion	Rf	R^2	Uncooked		Cooked	
								0FT	10FT	0FT	10FT
2	716	dimethyl sulfide	62	[$^2H_6$]-dimethyl sulfide	68	1.10	0.9997	452.14	558.28	1589.20	1276.87
3	798	propanal	58	[$^2H_3$]-propanal	61	6.34	0.9996	154.83	275.68	161.38	286.87
8	1084	hexanal	72	[$^2H_4$]-hexanal	78	0.97	0.9963	80.60	86.17	439.86	526.15
18	1393	nonanal	114	[$^2H_4$]-nonanal	116	1.34	0.9987	2.07	7.70	11.72	24.90
19	1429	1-octen-3-ol	57	[$^2H_{2,3}$]-1-octen-3-ol	62	1.56	0.9992	157.49	189.04	193.44	232.81

the greatest correlation with the lipid oxidation and could be used for differentiation between fresh and frozen-thawed gilthead sea bream. Moreover, 1-octen-3-ol has been reported as an important contributor to off-flavors of Atlantic horse mackerel (*Trachurus trachurus*) (Iglesias and Medina, 2008). Zepka et al. (2014) found that 1-octen-3-ol and nonanal were identified as potential freshness markers of anchovy (*Engraulis anchoita*) during 120 days of frozen storage. Josephson (1991) reported that formation of dimethyl sulfide is a product from microbial degradation, contributing to off-odor in seafood. Whereas 3-methyl-nonan-2,4-dione was derived from furanoid fatty acid (fatty acids containing a furan ring) (Sano et al., 2017). Therefore, these 6 volatiles could be used as a marker of off-odor and lipid oxidation occurring during the frozen storage of silver carp washed mince.

5.5 Conclusion

(Z)-1,5-Octadien-3-one, (E)-2-nonenal and 1-octen-3-ol were the most odorants responsible for the overall washed mince note. Lipid oxidation played an important role in odorant formation. Maillard reaction and Strecker degradation, contributed to odor formation of cooked samples. Increase in hexanal and 1-octen-3-ol during frozen storage and cooking process, suggested that hexanal could be used as a marker to monitor lipid oxidation in silver carp washed mince.

5.6 References

Acree, T. E., and Teranishi, R. (1993). *Flavor science: Sensible principles and techniques*: American Chemical Society.

Ahn, D., Lutz, S., Cherian, G., Wolfe, F., and Sim, J. (1995). Lipid oxidation and sensory characteristics of fresh and cured sausage from α-linolenic acid enrich pork. **Journal of food quality**, 18(5): 397-413.

Akoh, C. C. (2017). *Food lipids: chemistry, nutrition, and biotechnology*: CRC press.

Alasalvar, C., Taylor, K. A., and Shahidi, F. (2005). Comparison of volatiles of cultured and wild sea bream (*Sparus aurata*) during storage in ice by dynamic headspace analysis/gas chromatography− mass spectrometry. **Journal of agricultural and food chemistry**, 53(7): 2616-2622.

Aro, T., Tahvonen, R., Koskinen, L., and Kallio, H. (2003). Volatile compounds of Baltic herring analysed by dynamic headspace sampling–gas chromatography–mass spectrometry. **European Food Research and Technology**, 216(6): 483-488.

Azarbad, M. H., and Jeleń, H. (2015). Determination of hexanal—an indicator of lipid oxidation by static headspace gas chromatography (SHS-GC) in fat-rich food matrices. **Food analytical methods**, 8(7): 1727-1733.

Blank, I., Devaud, S., Matthey-Doret, W., and Robert, F. (2003). Formation of odorants in Maillard model systems based on L-proline as affected by pH. **Journal of agricultural and food chemistry**, 51(12): 3643-3650.

Bosund, I., and Ganrot, B. (1970). Effect of pre-cooking on lipid oxidation and storage life of frozen fish. **Lebensmittel Wissenschaft Technologie. Food science technology**.

Boylston, T., Chen, F., Coggins, P., Hydlig, G., McKee, L., and Kerth, C. (2012). *Handbook of meat, poultry and seafood quality*: John Wiley & Sons.

Buttara, M., Intarapichet, K.-O., and Cadwallader, K. R. (2014). Characterization of potent odorants in Thai chempedak fruit (*Artocarpus integer* Merr.), an exotic fruit of Southeast Asia. **Food research international**, 66: 388-395.

Buttery, R. G., Guadagni, D. G., and Ling, L. C. (1978). Volatile aroma components of cooked artichoke. **Journal of Agricultural and Food Chemistry**, 26(4): 791-793.

Buttery, R. G., and Ling, L. C. (1998). Additional studies on flavor components of corn tortilla chips. **Journal of agricultural and food chemistry**, 46(7): 2764-2769.

Buttery, R. G., Turnbaugh, J. G., and Ling, L. C. (1988). Contribution of volatiles to rice aroma. **Journal of Agricultural and Food Chemistry**, 36(5): 1006-1009.

Cadwallader, K., and Baek, H. (1998). Aroma-impact compounds in cooked tail meat of freshwater crayfish (*Procambarus clarkii*) **Developments in Food Science.** 40:271-2780

Choe, E., and Min, D. B. (2006). Mechanisms and factors for edible oil oxidation. **Comprehensive reviews in food science and food safety**, 5(4): 169-186.

Devos, P., and Rouault, L. (1990). *Van Gemert "Standardized Human Olfactory Thresholds*, Vol. 1: New York, Oxford university Press.

Erickson, M. C. (2002). Lipid oxidation of muscle foods. **Food lipids: chemistry, nutrition, and biotechnology**, 2.

Flament, I., and Bessière-Thomas, Y. (2002). *Coffee flavor chemistry*: John Wiley & Sons.

Frankel, E. (1998). Lipid Oxidation. Dundee. **Scotland: The Oily Press LTD**.

Fuganti, C., Gatti, F. G., & Serra, S. (2007). A general method for the synthesis of the most powerful naturally occurring Maillard flavors. **Tetrahedron**, 63(22), 4762-4767.

Giri, A., Osako, K., and Ohshima, T. (2010). Identification and characterisation of headspace volatiles of fish miso, a Japanese fish meat based fermented paste, with special emphasis on effect of fish species and meat washing. **Food Chemistry**, 120(2): 621-631.

Grosch, W. (1994). Determination of potent odourants in foods by aroma extract dilution analysis (AEDA) and calculation of odour activity values (OAVs). **Flavour and Fragrance Journal**, 9(4): 147-158.

Grosch, W. (2001). Evaluation of the key odorants of foods by dilution experiments, aroma models and omission. **Chemical senses**, 26(5): 533-545.

Grumezescu, A. M., and Holban, A. M. (2017). *Natural and Artificial Flavoring Agents and Food Dyes* (Vol. 7): Academic Press.

Gu, S.-q., Wang, X.-c., Tao, N.-p., and Wu, N. (2013). Characterization of volatile compounds in different edible parts of steamed Chinese mitten crab (Eriocheir sinensis). **Food Research International**, 54(1): 81-92.

Guadagni, D. G., Buttery, R. G., and Okano, S. (1963). Odour thresholds of some organic compounds associated with food flavours. **Journal of the Science of Food and Agriculture**, 14(10): 761-765.

Guth, H., and Grosch, W. (1994). Identification of the character impact odorants of stewed beef juice by instrumental analyses and sensory studies. **Journal of Agricultural and Food Chemistry**, 42(12): 2862-2866.

Hsieh, R. J., and Kinsella, J. E. (1989). Lipoxygenase generation of specific volatile flavor carbonyl compounds in fish tissues. **Journal of Agricultural and Food Chemistry**, 37(2): 279-286.

Iglesias, J., and Medina, I. (2008). Solid-phase microextraction method for the determination of volatile compounds associated to oxidation of fish muscle. **Journal of Chromatography A**, 1192(1): 9-16.

Iglesias, J., Medina, I., Bianchi, F., Careri, M., Mangia, A., and Musci, M. (2009). Study of the volatile compounds useful for the characterisation of fresh and frozen-thawed cultured gilthead sea bream fish by solid-phase microextraction gas chromatography–mass spectrometry. **Food Chemistry**, 115(4): 1473-1478. doi: https://doi.org/10.1016/j.foodchem.2009.01.076

Jónsdóttir, R., Bragadóttir, M., and Arnarson, G. (2005). Oxidatively Derived Volatile Compounds in Microencapsulated Fish Oil Monitored by Solid-phase Microextraction (SPME). **Journal of food science**, 70(7).

Josephson, and Lindsay, R. C. (1986). *In Biogeneration of Aromas.* Paper presented at the Parliment, T.H.; Croteau, R., Eds.; ACS Symposium Series 317.

Josephson, D. (1991). Seafood. **Volatile compounds in foods and beverages**: 179-201.

Josephson, D. B., Lindsay, R. C., and Stuiber, D. A. (1987). Enzymic hydroperoxide initiated effects in fresh fish. **Journal of food science**, 52(3): 596-600.

Kawai, T., and Sakaguchi, M. (1996). Fish flavor. **Critical Reviews in Food Science & Nutrition**, 36(3): 257-298.

Kubota, K., Matsukage, Y., Sekiwa, Y., and Kobayashi, A. (1996). Identification of the characteristic volatile flavor compounds formed by cooking squid (*Todarodes*

pacificus Steenstrup). **Food Science and Technology International, Tokyo**, 2(3): 163-166.

Lee, G.-H., Suriyaphan, O., and Cadwallader, K. (2001). Aroma components of cooked tail meat of American lobster (*Homarus americanus*). **Journal of agricultural and food chemistry**, 49(9): 4324-4332.

Lorjaroenphon, Y. (2012). Identification and characterization of potent odorants responsible for typical and storage-induced flavors of cola-flavored carbonated beverages (Doctoral dissertation, University of Illinois at Urbana-Champaign).

Masanetz, C., and Grosch, W. (1998). Hay-like off-flavour of dry parsley. **Zeitschrift für Lebensmitteluntersuchung und-Forschung A**, 206(2): 114-120.

Milo, C., and Grosch, W. (1993). Changes in the odorants of boiled trout (*Salmo fario*) as affected by the storage of the raw material. **Journal of Agricultural and Food Chemistry**, 41(11): 2076-2081.

Milo, C., and Grosch, W. (1996). Changes in the odorants of boiled salmon and cod as affected by the storage of the raw material. **Journal of Agricultural and Food Chemistry**, 44(8): 2366-2371.

O'Sullivan, M. G., and Kerry, J. P. (2013). 12 - Instrumental assessment of the sensory quality of meat, poultry and fish A2 - Kilcast, David *Instrumental Assessment of Food Sensory Quality* (pp. 355-373): Woodhead Publishing

Olafsdottir, G., Martinsdóttir, E., Oehlenschläger, J., Dalgaard, P., Jensen, B., Undeland, I., . . . Nilsen, H. (1997). Methods to evaluate fish freshness in research and industry. **Trends in Food Science & Technology**, 8(8): 258-265.

Refsgaard, H. H., Haahr, A.-M., and Jensen, B. (1999). Isolation and quantification of volatiles in fish by dynamic headspace sampling and mass spectrometry. **Journal of agricultural and food chemistry**, 47(3): 1114-1118.

Refsgaard, H. H., Tsai, L., and Stadtman, E. R. (2000). Modifications of proteins by polyunsaturated fatty acid peroxidation products. **Proceedings of the National Academy of Sciences**, 97(2): 611-616.

Ross, C. F., and Smith, D. M. (2006). Use of volatiles as indicators of lipid oxidation in muscle foods. **Comprehensive Reviews in Food Science and Food Safety**, 5(1): 18-25.

Sano, T., Okabe, R., Iwahashi, M., Imagi, J., Sato, T., Yamashita, T., . . . Bamba, T. (2017). Effect of Furan Fatty Acids and 3-Methyl-2, 4-nonanedione on Light-Induced Off-Odor in Soybean Oil. **Journal of Agricultural and Food Chemistry**, 65(10): 2136-2140.

Shiomi, K., Noguchi, A., Yamanaka, H., Kikuchi, T., and Iida, H. (1982). Volatile sulfur compounds responsible for an offensive odor of the flat-head, Calliurichthys doryssus. **Comparative biochemistry and physiology. B, Comparative biochemistry**, 71(1): 29-31.

Steinhaus, M., Sinuco, D., Polster, J., Osorio, C., and Schieberle, P. (2009). Characterization of the key aroma compounds in pink guava (*Psidium guajava* L.) by means of aroma re-engineering experiments and omission tests. **Journal of agricultural and food chemistry**, 57(7): 2882-2888.

Swoboda, P. A., and Peers, K. E. (1977). Metallic odour caused by vinyl ketones formed in the oxidation of butterfat. the identification of octa-1, cis-5-dien-3-one. **Journal of the Science of Food and Agriculture**, 28(11): 1019-1024.

Van den Dool, H., and Kratz, P. D. (1963). A generalization of the retention index system including linear temperature programmed gas—liquid partition chromatography. **Journal of Chromatography A**, 11: 463-471.

Varlet, V., Prost, C., and Serot, T. (2007). Volatile aldehydes in smoked fish: Analysis methods, occurence and mechanisms of formation. **Food chemistry**, 105(4): 1536-1556.

Wang, Y.-J., Miller, L. A., and Addis, P. B. (1991). Effect of heat inactivation of lipoxygenase on lipid oxidation in lake herring (*Coregonus artedii*). **Journal of the American Oil Chemists Society**, 68(10): 752-757.

Whitfield, F., Freeman, D., and Bannister, P. (1981). Dimethyl trisulphide: an important off-flavour component in the royal red prawn (*Hymenopenaeus sibogae*). **Chemistry and industry**.

Whitfield, F. B., and Mottram, D. S. (1992). Volatiles from interactions of Maillard reactions and lipids. **Critical Reviews in Food Science & Nutrition**, 31(1-2): 1-58.

Yaylayan, V. A., and Keyhani, A. (1999). Origin of 2, 3-pentanedione and 2, 3-butanedione in D-glucose/L-alanine Maillard model systems. **Journal of agricultural and food chemistry**, 47(8): 3280-3284.

Yu, T.-H., and Ho, C.-T. (1995). Volatile compounds generated from thermal reaction of methionine and methionine sulfoxide with or without glucose. **Journal of agricultural and food chemistry**, 43(6): 1641-1646.

Zepka, L., Wagner, R., Jacob-Lopes, E., M. Daltoé, M., Santos, A., F. Torri, A., . . . I. Queiroz, M. (2014). Study of the Volatile Compounds Useful for the Characterization of Frozen Anchoita (*Engraulis anchoita*) by SPME-GC-MS. **Flavour science.** 169-172.

Zhou, X., Chong, Y., Ding, Y., Gu, S., and Liu, L. (2016). Determination of the effects of different washing processes on aroma characteristics in silver carp mince by MMSE–GC–MS, e-nose and sensory evaluation. **Food chemistry**, 207: 205-213.

CHAPTER VI

SUMMARY

Freshness of silver carp kept in ice continuously decreased as storage time increased. Extend ice storage induced protein conformational changes in both silver carp and their respective washed mince as indicated by an increase in surface hydrophobicity and a decrease in reactive sulfhydryl content. Silver carp mince and their respective washed mince underwent proteolytic degradation during ice storage, leading to greater loss of myofibrillar proteins during washing, subsequent lower total solid recovery and loss in gel-forming ability. FTIR spectra of amide I showed that α-helical structure of mince decreased in a concomitant increase of β-sheet structure. FT-Raman revealed the exposure of aromatic amino acids due to unfolding proteins. The disulfide content increased due to occurrence of oxidation. Therefore, prolong ice storage was found to be important factor. To maintain the quality of their washed mince, we suggested that silver carp should not be kept in ice for more than 7 days.

Lipid oxidation of silver carp varied with body parts of fish. Prolonged ice storage induced lipid oxidation in skin, belly, muscle and its washed mince which belly possessed the highest TBARS value. The amount of saturated fatty acids increased due to lipid oxidation of polyunsaturated fatty acids during ice storage. Total of 4, 10, 5 and 1 volatile compounds in skin, belly, muscle and washed mince, respectively, increased when ice storage extended. All of them were the lipid oxidative volatile products which carbon number was lower than 10 including 1-

pentanol, 1-hexanol, 1-octen-3-ol, 2,3-octanedione, 1-octanol and nonanal. FTIR could be used for determined the development of lipid oxidation in silver carp tissues by monitoring the changes of *cis* double bonds, methylene groups, phosphate groups, ester bonds and formation of *trans* isomerize fatty acids. These results demonstrated fish belly was very susceptibility to lipid oxidation. Thus, to carry on the quality of silver carp mince and their washed mince, gutting of fish prior ice storage or before further process such as mechanically deboning before mincing should be regard.

Freeze-thawed process and thermal cooking process induced lipid oxidation as indicated by a continuously increase in TBARS value. The results of GC-O and GC-MS indicated numerous odor active compounds can be generated from frozen-stored silver carp washed mince and it done gel. Lipid oxidation played an important role in odor active compounds formation. Maillard/Strecker degradation, retro-aldol degradation, associated reactions (sugar degradation), thermal degradation and microbial action were also took part in the odor formation. Odor active compounds, 2,6-nonadienal, (E,Z)- (cucumber) was the most predominant active compounds in both fresh prepared and frozen-stored washed mince. While (Z)-1,5-octadien-3-one (green, metallic, fish, geranium) and pentanal (yogurt) were the most odorants in frozen stored washed mince and frozen stored washed mince gel, respectively. Increase of OAVs was found when washed mince was extending frozen storage and underwent thermal cooking process. Moreover, all odor active compounds were considered as the major contributor of overall aroma in the sample due to possessed high OAVs (>1), except 2-phenylethanol and 2,4,6-nonatrienol that odor threshold was unavailable. (Z)-1,5-Octadien-3-one was played the major off-odor characteristic in washed mince and washed mince gel.

BIOGRAPHY

Sasinee Kunyaboon was born in February 25, 1980, at Mahasarakham Province, Thailand. She studied at Prachak Silapakarn School and graduated in 1998. In 2003, she received B.Sc. (Food Technology and Nutrition) from Mahasarakham University, Mahasarakham, Thailand. Master degree in M.Sc. (Food technology) from Mahasarakham University, Mahasarakham, Thailand in 2006. In 2011, she received scholarship from The Royal Golden Jubilee (RGJ) Ph.D. Programme, The Thailand Research Fund (TRF) for the Ph.D. at Suranaree University of Technology. In the meantime, she received D.B.A. degree (Doctor of Business Administration at North Eastern University, Khonkaen, Thailand in 2015. In 2016, she got a chance to be a visiting scholar at The University of Illinois at Urbana-Champaign, United State to work on effects of freeze-thaw cycles on lipid oxidation and volatile compounds of surimi.

Part of her thesis work was presented in the poster presentation at The 3rd SUT International agricultural colloquium (Suranaree University of Technology, Thailand, Sep 14-15, 2015) She also presented oral presentation including: 1) The 4th SUT International agricultural colloquium (Suranaree University of Technology, Thailand, June 28-29, 2016. 2) RGJ Seminar Series 116 (Khonkaen University, Thailand, Aug 9-10, 2016). 3) The 5th SUT International agricultural colloquium (Suranaree University of Technology, Thailand, Aug 15-16, 2017).