CHAPTER I
INTRODUCTION

1.1 Introduction

Thailand is a leader in the world's tropical surimi production. Most of Thailand's surimi comes from threadfin bream (*Nemipterus spp*), lizardfish (*Saurida spp*), and goatfish (*Mullidae spp*) (Guenneugues and Lanelli, 2014). Currently, demand for surimi products throughout the world is increasing but traditional resources are limited. Surimi can be produced from freshwater fish like tilapia (*Oreochromis niloticus*), providing good color and gel-forming ability (Rohani, Indon and Yunus, 1995). Tilapia is a prolific species that is easy to cultivate, grows quickly and has a high yield. It has become an important species in terms of help boosting the local aquaculture industry.

Storage of fish in ice is an important post-harvest operation. Biochemical changes of fish at postharvest is species-specific and depends on metabolites in fish tissue, microbial contamination, and post-harvest handlings (Pacheco-Aguilar, Lugo-Sánchez and Robles-Burgueño, 2000). Biochemical changes of tropical fish species at the post-harvest are not well studied as compared to cold water species. Especially, changes in quality of flavor/off-flavor related to biochemical changes have never been systematically investigated despite that fishy note is one of negative attributes of tropical surimi perceived by international market.

Lipoxygenase (LOX, linoleate: oxygen oxidoreductase, EC 1.13.11.12), is a dioxygenase that oxygenates polyunsaturated fatty acids (PUFA) containing a cis, cis-1,4 - pentadiene structure (–CH=CH–CH_2–CH=CH–), converting them into conjugated unsaturated fatty acid hydroperoxides (ROOH) (Grechkin, 1998). The hydroperoxy fatty acids turn into alcohols, aldehydes, ketones, and hydrocarbons, all of which contribute to an unpleasant taste and odor (Josephson et al, 1984). Marine, and freshwater species are rich in omega-3 and omega-6 polyunsaturated fatty acids (PUFAs), such as docosahexaenoic (DHA) eicosapentaenoic acids (EPA), linoleic acid, and linolenic acid

(Strobel, Jahreis, and Kuhnt, 2012). Fish lipids are more prone to oxidation than lipids found in other foods because of their high degree of unsaturation and low antioxidant content. Lipoxygenase-induced oxidation of tropical fish species has not been characterized. It would be important to realize changes of LOX activity from various parts of fish tissues, namely gill, skin, and muscle at post-harvest. A better process control can be designed and implemented for minimizing lipid oxidation and fishy note formation based on this fundamental information.

Previous studies on volatile compounds presume that lipoxygenase (LOX) is a major contributor to the generation of free radicals that cause lipid peroxidation in fish tissue (German, Chen, and Kinsella, 1985). LOX is classified, based on different position of hydroperoxide groups on a fatty acid chain after attacking the 1, 4-cis, cis-pentadiene group. LOX oxidizes at the C13 position of arachidonic acid (AA, 20:4) resulting in hydroperoxide at C15 position, would be called a 15-LOX (Brash, 1999). 1-Octen-3-one, 1-octen-3-ol, 1, (Z)-1,5-octadien-3-ol, and 1, (Z)-1,5-octadien-3-one occurred by action of 12-LOX on EPA, whereas hexanal and (Z)-2-hexenal could occur from **ω**-6 fatty acids and **ω**-3 fatty acids (Cadwallader, 2000). Site specificity of LOXs is a crucial factor in the formation of aroma compounds in fish. Differences in LOX isoenzymes between species are responsible for the distinctive aroma profiles of each species. LOX isozymes have been reported in tissues of various fish, including 12-LOX in trout skin and 15-LOX in the gill of teleost fishes (Hsieh, German, and Kinsella, 1988; German and Creveling, 1990). 5-LOX, 12-LOX, and 15-LOX are present in the gray mullet (Hsu and Pan, 1996). In addition, LOX has a different preference for PUFA, which means that it makes different volatile compounds. Information about LOX specificity of tropical fish used for surimi production is limited thus far.

Typically, surimi has bland taste and less fishy note than its respective mince. However, tropical surimi is known to have strong fishy odor and sometime is considered as off-odor. This would be due to poor post-harvest handling of raw material. Although washing can remove odorous compounds, the extent of strong off-note is much higher than cold water species surimi (An, Qian, Alcazar Magana, Xiong, and Qian, 2020). Hexanal, heptanal, and 1-octen-3-ol are the major volatile compounds of grass carp surimi (Liu et al., 2021). The cause of an off-odor in tropical

surimi has not well understood. Washing is ineffective at removing membrane phospholipids from fish muscle. These phospholipids are PUFA-rich, regularly in contact with muscle heme iron, and highly susceptible to oxidation. (Eymard, Baron, and Jacobsen, 2009). These phospholipids can serve as a substrate of LOX, causing off-flavors. However, it is not known how much LOX residual activity remains after washing. Understanding the role of LOX in flavor characteristic of surimi would lead to better control strategies of surimi processing and storage.

1.2 Research objectives

The objectives of this study were:

1.2.1 To investigate changes of lipoxygenase activity, fatty acid profiles, and volatile compounds of tilapia in various tissues, namely muscle, gill, and skin during ice storage.

1.2.2 To partially purify and characterize lipoxygenase from lizardfish gill (LG-LOX)

1.2.3 To determine the effect of surimi washing process on LOX activity and fatty acid composition.

1.3 Research hypotheses

Ice storage of tilapia would affect LOX activity and extent of lipid oxidation. Volatile compound profiles of muscle, gill, and skin of tilapia are different during ice storage. Principal component analysis could be applied to correlate between volatile compounds and extent of lipid oxidation. Purified LOX from lizardfish gill could be obtained its properties can be characterized. Washing process of lizardfish (LZ) surimi production might reduce LOX activity and fatty acid composition. Residual LOX activity remained after washing would contribute to oxidative off-flavor of surimi.

1.4 Scope of the study

LOX activity, fatty acid profiles, and volatile compounds of tilapia in various tissues, namely muscle, gill, and skin during ice storage were evaluated. Tilapia were stored in ice for 0, 3, 6, and 9 days. Lipid oxidation products were measured by peroxide value. Fatty acid profiles and volatile compounds of various tissues were evaluated.

LOX activity and fatty acid contents of raw threadfin bream, goatfish and lizardfish in various tissues, namely gill, skin, and muscle were evaluated. The effect of industrial production on LOX activity and fatty acid composition was evaluated. Change of surimi LOX activity during LZ surimi processing, namely mince fish, the first washed, second washed, and third washed mince, mince from refiner, screw press, and surimi were followed. LOX of fishes exhibiting the highest activity was selected for purification and characterization. pH optimum and stability, temperature optimum and stability and substrate affinity of purified LOX were evaluated. LOX inhibitors were also tested with various chemicals.

1.5 References

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