CHAPTER II
LITERATURE REVIEWS

2.1 Lipoxygenase

Lipoxygenase (LOX, linoleate: oxygen oxidoreductase, EC 1.13.11.12) is a family of iron-containing enzymes which catalyse the dioxygenation of polyunsaturated fatty acids (PUFA) containing a cis, cis-1,4- pentadiene structure such as linoleic, linolenic, arachidonic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) to yield hydroperoxides (Gardner, 1991). In 1928, LOX in soybean discovered by Bohn and Haas (Bohn and Haas, 1928 cited by Christopher, Pistorius, and Axelrod, 1970). LOX is found in plants, mammals and microorganisms, and is abundant in legumes. LOX in animal tissues has been reported in various aquatic species, showed in Table 2.1. These contain high level of polyunsaturated fatty acids. Skin tissues of 31 species of fish were found to contain pro-oxidant activity, some of which may be attributable to LOX (Mohri, Tokuori, Endo, and Fujimoto, 1999).

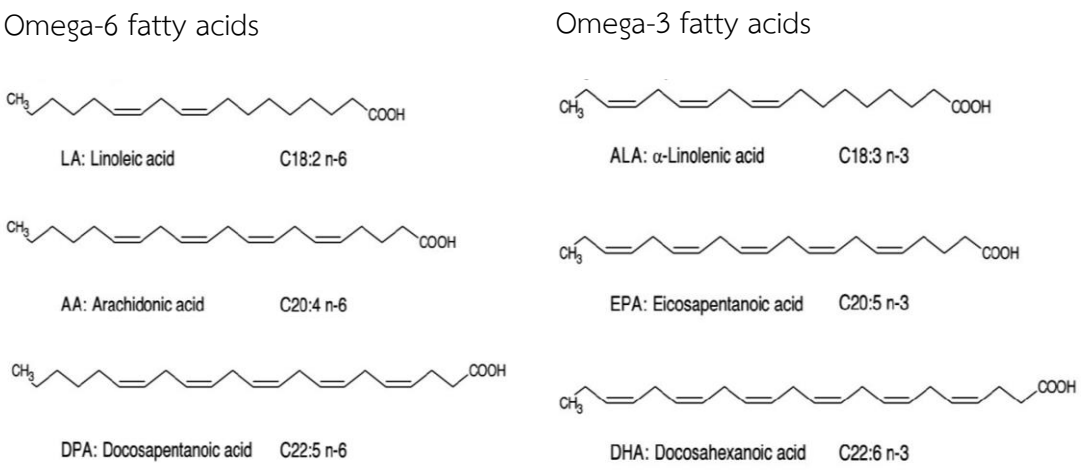

Figure 2.1 Lipoxygenase substrates, omega-6 and omega-3 fatty acids (Kashiwagi, and Huang, 2012).

Table 2.1 Lipoxygenase in various aquatic species

Common name	Scientific name	Part of fish	References
Mackerel	*Scomber scombrus*	Muscle	(BANERJEE, KHOKHAR, and APENTEN, 2002)
White Amur bream	*Parabramis pekinensis*	Muscle	(Ren, et al., 2017)
Rainbow trout	*Oncorhynchus mykiss*	Skin	(German, Chen, and Kinsella, 1985)
Scallop	*Argopecten irradians*	Muscle	(Xie, Yue, and Fan, 2022)
Sardines	*Sardinops melanosticus*	Skin	(Mohri, Cho, Endo, and Fujimoto, 1992)
Nile tilapia	*Oreochromis niloticus*	Skin	(Sae-Leaw, Benjakul, Gokoglu, and Nalinanon, 2013)
Seabass	*Lates calcarifer*	Skin	(Sae-leaw, and Benjakul, 2014)
Baltic herring	*Clupea harengus membras*	Muscle	(Samson, and Stodolnik, 2001)
Sardine	*Sardina pilchardus*	Muscle	(Tolasa Yılmaz, Çaklı, Şen Yılmaz, Kırlangıç, and Lee, 2018)
Grass carp	*Ctenopharyngodon idellus*	Muscle	(Wang, et al., 2012; Cao, et al., 2019)
Lake herring	*Coregonus artedii*	Skin, Muscle	(Wang, Miller, and Addis,1991)
Sorted herring	Clupea harengus	Muscle	(Wu, Forghani, Abdollahi, and Undeland, 2022)
Gray mullet	*Mugil cephalus*	Gill	(Hsu, and Pan, 1996)
Trout	*Salmo gairdneri*	Gill	(German, and Creveling, 1990)
Rockfish	*Sebastes flavidus*	Gill	(German, and Creveling, 1990)
Silver carp	*Hypophthalmichthys molitrix*	Muscle	(Qiu, Xia, and Jiang, 2013; Fu, Xu, and Wang, 2009)
Carp	*Cyprinus carpio*	Gill	(Iijima, Chosa, Hada, and Kayama,2000)

Table 2.1 Lipoxygenase in various aquatic species**.** (Continues)

Common name	Scientific name	Part of fish	References
Rabbit fish	*Siganus fuscescens*	Viscera	(Jiarpinijnun et al., 2022)
Ayu	*Plecoglossus altivelis*	Gill, Skin	(Zhang, Hirano, Suzuki, and Shirai, 1992)
Snakehead	*Channa argus*	Muscle	(Sun et al., 2022)

2.1.1 Structure

LOXs have a two-domain structure of a smaller -barrel domain (N-terminal domain) and a larger α-helical catalytic domain (C-terminal domain) containing a single atom of non-heme iron essential for activity (Newcomer, and Brash, 2015). In three-dimensional structure, the catalytic domain is primarily an α-helix with a single atom of non-heme iron near its center. The center of the domain consists of two long helices, which donate four iron-binding histidine groups. The fifth group that coordinates the non-heme catalytic iron is the carboxyl group of the C-terminal isoleucine (Figure 2.2). The enzymes from soybean contain 838-865 amino acids, with molecular mass of 94–104 kDa, while 550-600 amino acids, with molecular mass of 70-80 kDa in animal (Brash, 1999). German and Creveling (1990) discovered LOX activity in the gill tissue of teleost fish with molecular mass of 70 kDa. Saeed and Howell (2001) found LOXs with two prominent molecular mass of 119 and 125 kDa in Atlantic mackerel muscle.

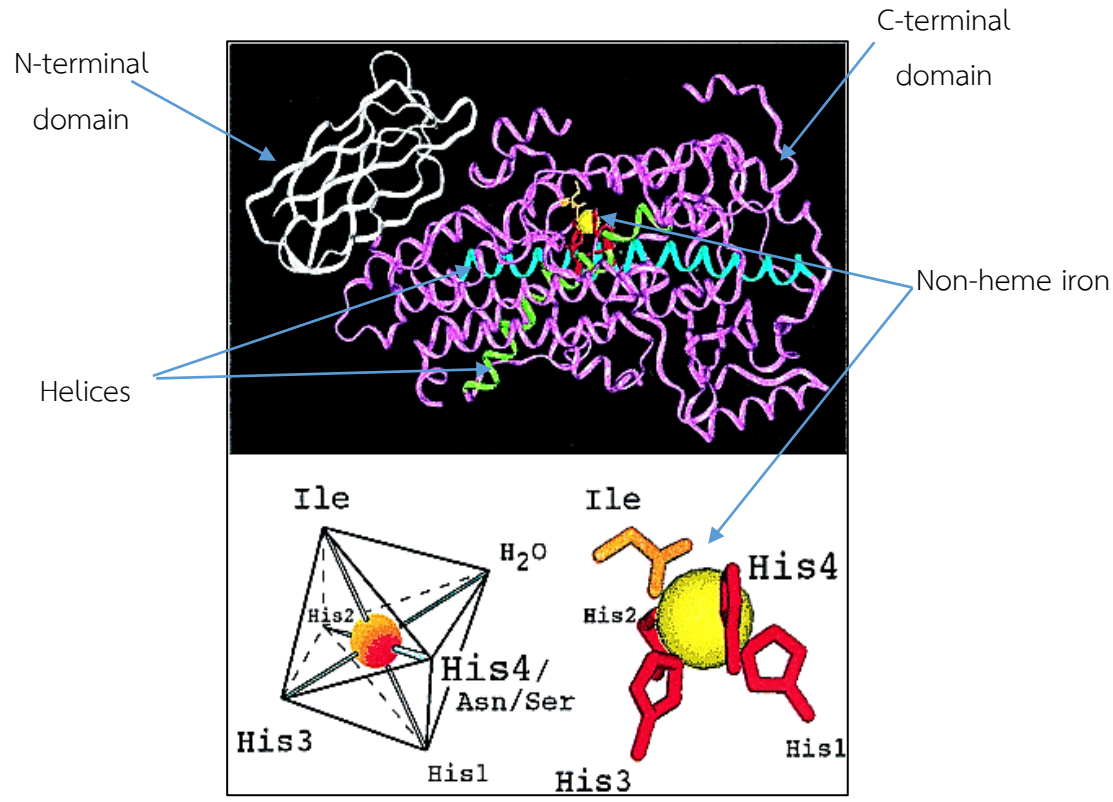

Figure 2.2 Lipoxygenase proteins structure (Brash, 1999)

LOXs are classified, based on different chain lengths of the most prevalent substrates for plants (linoleate) and animals (arachidonate) are used to categorize LOXs. After attacking the 1, 4-cis, cis-pentadiene group, LOX is able to place hydroperoxide groups in various positions along a fatty acid chain. In Figure 2.3, the positional specific of LOX-catalyzed reaction is shown with the main products. In animal tissues, enzymes with specificities for four different positions, including 5-LOX, 8-LOX, 12-LOX, and 15-LOX. For example, LOX oxidizes at the C13 position of arachidonic acid (C20:4), counting from the carboxyl end of the chain, resulting in hydroperoxide at C15 position, would be called a 15-LOX. The products from reaction are hydroperoxy-eicosatetraenes (HPETEs) (Ivanov et al., 2010).

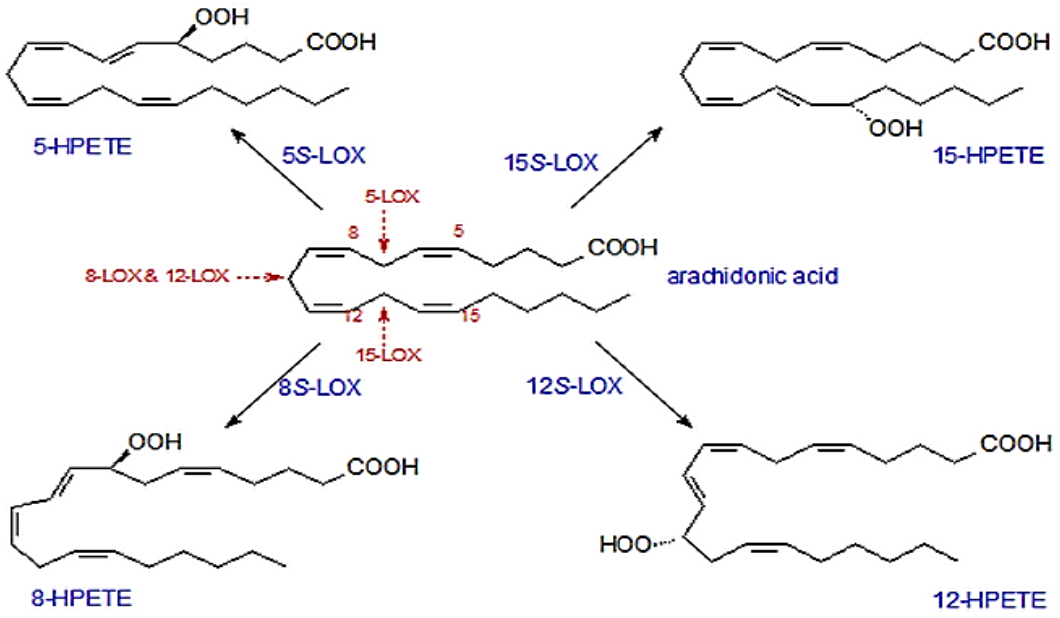

Figure 2.3 Positional specific of LOX-catalyzed reaction with the main products (Brash, 1999)

Hsieh and Kinsella (1988) suggested that LOX activity was present in the gill and skin tissues of several species of fish, and trout exhibited activity of 12-LOXs. German and Creveling (1990) discovered an arachidonic acid 15- LOX activity in the gill tissue of teleost fishes. Moreover, Cadwallader (2000) found that 12-LOX is widely distributed in different organs/tissues of rainbow trout. Three types of arachidonate LOX (5-LOX, 12-LOX, and 15-LOX) are present in the grey mullet (Hsu, and Pan, 1996). Arachidonic acid 12-LOX activity was found in Atlantic mackerel muscle (Saeed and

Howell, 2001). Arachidonic acid 12-LOX protein plays an important role in the occurrence and development of diseases in human (Zheng, Li, Jin, Huang, Zou, and Duan, 2020). Rabbit reticulocyte 15-LOX oxidizes plasma to produce cholesteryl ester and phosphatidylcholine hydroperoxides (Yamashita, Nakamura, Noguchi, Niki, and Kühn, 1999; Morita, Naito, Yoshikawa, and Niki, 2016)

2.1.2 pH optimum and stability

LOX purified from mackerel muscle is stable over the pH range of 4.0-11.0 with pH optimum at 5.6 (Banerjee et al., 2002). German and Creveling (1990) found that gill LOX exhibited activity from pH 7 to 9 with the optimum pH at 7.5. In sardine's skin, the enzyme had an optimum pH at 7.0 and was stable over the pH range of 6.0-9.0 (Mohri et al., 1992). In mackerel's gill; LOX exhibited the highest reactivity toward eicosapentaenoic acid (EPA) with the optimum pH of 4.5. The enzyme was stable at pH 5.5 (Hong et al., 1994). The 12-LOX is widely distributed in different organs/tissues of grey mullet with different optimum pH (gill at pH 8.0, platelet and ovary at pH 6.5) (Cadwallader, 2000). Meanwhile, LOX from soybean exhibited activity at different pHs, depending on isozymes; optimum of pH 9 for LOX 1. pH optimum of LOX 3 is very broad and is centered around pH 7, while the reported pH optimum of seed LOX 2 is 6.8 (Grayburn et al., 1990). Thiansilakul, Benjakul, and Richards (2011) reported the highest lipid oxidation and off-odor were observed in washed Asian seabass mince at pH 6.0.

2.1.3 Thermal optimum and thermal stability

Hsieh et al. (1988) found that at temperatures above 40 $^{\circ}$ C, LOX in trout gills was rapidly inactivated and glutathione improved the stability of gill LOX. glutathione can reduce hydroperoxides to its stable hydroxyl analogues and thus removes excessive hydroperoxides to keep the LOX active (Hsu and Pan, 1996). Banerjee et al. (2002) reported that mackerel muscle LOX was stable at 0-50 $^{\circ}$ C but lost activity completely at 70 $^{\circ}$ C. A decrease in activity is probably due to irreversible changes in the tertiary structure of the enzyme. Moreover, Harris and Tall (1994) reported that After being incubated at 50 $^{\circ}$ C for 10 minutes, LOX from mackerel muscle lost 40% of its activity. In mackerel's gill, LOX showed optimum temperature at 25 $^{\circ}$ C for EPA, arachidonic acid, and linoleic acid. When linoleic and arachidonic

acid were used as substrates, the highest thermal stability was observed at 8 ° C. When EPA was used as substrates, the highest thermal stability was observed at 20 ° C, indicating that double bonds of EPA were easily oxidized by LOX of mackerel's gill compared to other fatty acids (Hong et al., 1994). Meanwhile, LOX from soy whey protein was inactivated more than 99.9% at 75 ° C for 3 min (Zhang, and Chang, 2022). LOX activity was high in the silver carp surimi gel at 40 ° C, and rapidly decreased at temperature 90 ° C for 10 min (An et al., 2022). Tolasa Yılmaz, Çaklı, Şen Yılmaz, Kırlangıç, and Lee (2018) found that increasing temperature (from 0 to 10 ° C) increased LOX activity significantly in fresh and frozen sardine fillets and mince.

2.1.4 Substrate specificity

The activity of LOX varies with PUFA substrates. Banerjee et al. (2002) reported that the highest activity of mackerel muscle was observed when linoleic acid was used as a substrate, followed by linolenic acid, docosahexaenoic acid (DHA) and arachidonic acid. Substrate specificities of the enzyme appear to vary with sources of LOX. LOX from trout and mackerel efficiently oxidized n-3 fatty acids such as DHA and eicosapentaenoic acid (EPA) (Hsieh al. 1988; Harris and Tall 1994). LOX from carp gill showed maximal activity toward arachidonic acid (Iijima et al., 2000), while sardine skin LOX preferred linoleic acid (Mohri et al., 1990). Gill LOX of rainbow trout exhibited reactivity toward arachidonic, eicosapentaenoic, and docosahexaenoic acids, but low reactivity toward linoleic acid (German and Creveling, 1990). LOX has a different preference for PUFA, which means that it makes different volatile compounds. 1-Octen-3-ol, 1-octen-3-one, 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 n-6 polyunsaturated fatty acids and n-3polyunsaturated fatty acids (Cadwallader, 2000).

2.1.5 Lipoxygenase isozymes

LOX appears to have various isoforms; the products from each isoform are unique. The different LOXs are named related to their positional specificity for the dioxygenation of arachidonic acid: 15-, 12- or 5-LOX, corresponding to an attachment of hydroperoxide to carbon atom 15, 12, or 5 of arachidonic acid, respectively. The 12-hydroxyeicosatetraenoic acid was found to be the major product of LOX (Fu et al., 2009). The 12-LOX is the main LOX in various fish species including trout, sliver carp,

and Atlantic mackerel (Fu et al., 2009; German and John, 1985; Saeed and Howell, 2001). Moreover, 15-LOX is dominant in chicken muscle (Grossman et al., 1988) and trout (German and Richard, 1990). Three form of LOX including, 5-, 12-, and 15-LOX were found in gray mullet gill (Hsiu and Pan, 1996).

2.1.6 Inhibition of lipoxygenase oxidation in fish

The most widely used fish LOX inhibitors are nordihydroguaiaretic acid (NDGA), butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), esculetin, ascorbic acid and α-tocopherol. (Grun and Barbeau, 1995; Harris and Tall, 1994; Hsieh, et al., 1988; Mohri, et al., 1999; Saeed and Howell, 2001). NDGA is competitive lipoxygenase inhibitor. Esculetin, known inhibitor of LOX, inhibited the production of 12-hydroxyeicosatetraenoic acid by non-competitive inhibitor as a similar structure to phenolic antioxidants (Saeed and Howell, 2001). Ethylene diamine tetraacetic acid (EDTA) inhibits enzyme-catalyzed oxidation by removing iron. EDTA is more effective in minced meat. Similarly, potassium cyanide (KCN) which acts as a general enzyme inhibitor as well as metal chelator, is also a very efficient heme protein inhibitor. BHT and BHA is an antioxidant which prevents rancidification of food. The conjugated aromatic ring is able to stabilize free radicals by acting as free radical scavengers. In addition, ascorbic acid is a reducing agent for inhibition of enzyme-catalyzed oxidation. Inhibition of crude mackerel muscle LOX by α-tocopherol and ascorbic acid (Saeed and Howell, 2001), inhibition of trout gill LOX by flavonoids (Hsieh et al., 1988) and tilapia and grey mullet gill LOX by green tea extract have been reported (Liu and Pan, 2004). Tea catechins were reported to completely inhibit LOX of all the skin extracts from sardine (Mohri et al., 1999). Carnosine is a dipeptide that reports as a natural antioxidant, which is facilitated by metal ion chelating and peroxyl radical scavenging. Sun et al. (2022) reported the combined application of ultra-high pressure and carnosine on snakehead meat is effective in inhibiting LOX and reducing the fish odor.

2.2 Volatile compounds of fish species

Fish flavor can be divided into three groups: odorless representing in saltwater fish, follow by pyrrolidine an earthy-odor compound representing in freshwater fish and a variety of unsaturated carbonyls and alcohols derived from enzymatic and non-enzymatic oxidation of polyunsaturated fatty acids representing in euryhaline fish.

(Kawai, and Sakaguchi, 1996). Jones et al. (2022) reported different flavor has affect consumer acceptance. Mild ‘fishlike’, ‘marine’, ‘crustacean- like’, ‘seafood’ and ‘sweet’ odors are pleasant. However, "fish-like," "fatty," and "earthy" odors are given as unpleasant. LOX has been reported to contribute to the biogeneration of volatile flavor compounds from the initial oxidation of polyunsaturated fatty acid in fish tissues to produce acyl hydroperoxide (Hsieh, and Kinsella, 1989). The hydroperoxides can be derived from linoleic acid (octadecadienoic acid hydroperoxides, HPODs) acting as substrates for the subsequent enzymatic activities (Zhang et al., 1992; Bisakowski et al., 1997). Hydroperoxide lyase (HPL), reported to cleave the hydroperoxide, producing a volatile compound such as an alcohol or aldehyde (Kermasha, 2002b; Matsui, 2006). Figure 2.4 shows the bioconversion of linoleic acid into alcohol and carboxyl compound by the sequential enzymatic activity of LOX and HPL. LOX is considered as the primary enzyme in the sequential biocatalytic pathway involved in the production of many desirable flavor compounds (Gardner, 1991; Schrader et al., 2004). The first product is the generation of specific HPODs, 9-HPOD and 13-HPODs, considered as flavor precursors. The subsequent catalytic cleavage by the hydroperoxide lyase (HPL), HPODs resulted in their conversion into their corresponding oxoacid and volatile flavor compounds, with distinct flavor characteristics (Figure 2.5).

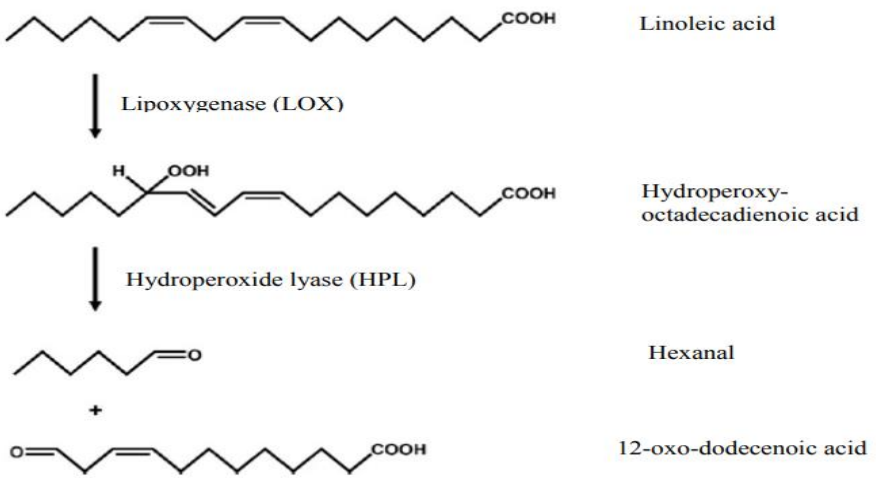

Figure 2.4. Bioconversion of linoleic acid into short-chain alcohols and carbonyl compounds by sequential enzymatic activities of LOX and HPL (Gardner, 1989).

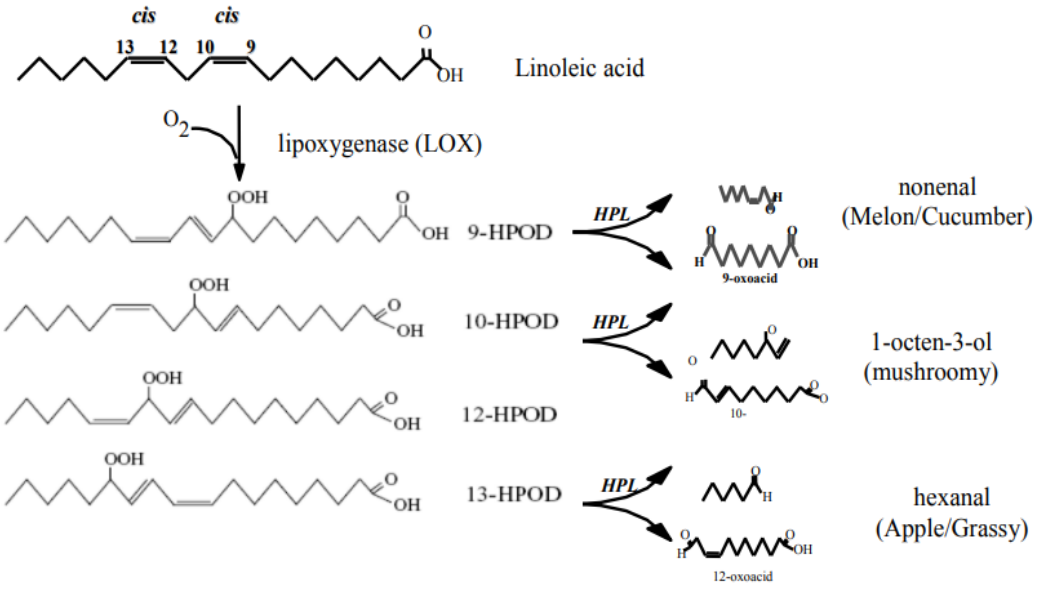

Figure 2.5. The application of lipoxygenase in the production of aroma (Gardner, 1989).

Different species of fish contain different types and activity of LOX, which accounts for different aroma as summarized in Table 2.2. Selli and Cayhan (2009) reported a total of 46 compounds were identified and quantified in gilthead sea bream (*Sparus aurata*) by simultaneous distillation-extraction (SDE) and GC-MS, which aldehydes and alcohols were the most dominant volatiles. (E)-2-Nonenal and decanal were the most powerful contributors to aroma of sea bream. Iglesias et al. (2009) reported that 51compounds were identified and quantified by dynamic headspace with solid phase microextraction (SPME) and gas chromatography–mass spectrometry (GC-MS) in Gilthead sea bream fish. They indicated that 1-octen-3-ol, 1-penten-3-ol, and Z-4-heptenal were markers for the differentiation between fresh and frozen-thawed fish over 226 days. Iglesias and Medina (2008) founded that Atlatic horse mackerel (*Trachurs trachurus*) holding at -20 ° C for 7 months initially contained 15-LOX, while the 12-LOX was barely detected. They also founded a total of 79 compounds identified and quantified by dynamic headspace with SPME and GC-MS. The most dominant volatiles are 1-penten-3-ol and 2, 3-pentanedione (1-octen-3-ol), which are useful indicator of fish rancidity. In silver carp, the major lipoxygenase was 12-LOX. The LOXs caused faster lipid oxidation in the initial phase and it was affiliated with strong fishy odor, which was likely caused by 2, 4-haptadienal (E, E) from the enzymatic oxidation of linoleic acid (Fu et al, 2009). Volatile compounds of fresh

whitefish were identified and quantified by static headspace with SPME and GC-MS. Two distinct families of compounds are cucumber-like note aroma and plant-like aroma. These aromas were identified to be (E)-2-nonenal, (E, Z)-2,6-nonadienal, 6-nonen-1-ol, 1-octen-3-ol, 1-octen-3-one, 1,5-octadien-3-ol, 1,5-octadien-3-one, 2,5-octadien-1-ol (Josephson et al., 1983). Ganeko et al., (2008) postulated that sardine skin contained high levels of polyunsaturated fatty acids and lipoxygenase. Carbonyl compounds may be generated more easily than trimethylamine. They identified 33 volatile compounds of sardine by GC-MS, including 2,3-pentanedione, hexanal, and 1-penten-3-ol. Forty-seven compounds were detected by gas chromatography-olfactometry. Among them, paint-like (1-penten-3-one), caramel-like (2,3-pentanedione), green-like (hexanal), shore-like ((Z)-4-heptenal), citrus note (octanal), mushroom-like (1-octen-3-one), potato-like (methional), insect-like ((E, Z)-2,6-nonadienal), and bloody note (not identified) were strongly detected. It can be concluded that these compounds rather than trimethylamine contributed to fresh sardine flavor. In yellowfin tuna, Edirisinghe et al., (2006) developed a new rapid indicator for determining the quality of fish. Changes in the aroma composition of yellowfin tuna were monitored using SPME GC–MS. The results showed that, hexanal and 2-nonanone were relatively high in fresh fish, whereas 3-methyl-1-butanol and 3-hydroxy-2-butanone increased with storage time.

Table 2.2 Volatile compounds of fish reported in literatures

Speices	Extraction method	No. of detected compounds	Volatile compounds	Charaterization	Reference
Gilthead seabream (*Spaus aurata*)	Simultaneous distillation-extraction gas chromatography–mass spectrometrometryand SDE/GC-MS	46	Decanal (E)-2-Nonenal	Sea bream aroma	(Selli, and Cayhan, 2009)
Gilthead seabream (*Spaus aurata*)	Solid-phase microextraction gas chromatography–mass spectrometrometry (SPME/GC-MS)	51	1-Penten-3-ol 1-Octen-3-ol Z-4-Heptenal	Fresh and frozen-thawed different markers	(Iglesias et al., 2009)
Silver carp (*Hypophthalmichthys molitrix*)	Solid-phase microextraction gas chromatography–mass spectrometrometryspectrometry–olfactometry (SPME/GC-MS-O)	21	2,4-Heptadienal (E,E) Hexanal Nonanal	Fishy odor Oxidated oil odor	(Fu, Xu, and Wang, 2009)
Atlantic horse mackerel (*Trachurus trachurus*)	Dynamic headspace extraction (DHS) and SPME/GC-MS	79	1-Penten-3-ol 1-Octen-3-ol 2,3-Pentandione	Fishy rancidity markers	(Iglesias, and Medina, 2008)
Sardine (*Sardinops melanostica*)	Gas chromatograph-olfactometry (GC-O) with SPME/GC-MS	33	2,3-Pentanedione Hexanal 1-Penten-3-ol	Sardine aroma	(Ganeko et al., 2008)
Yellowfin tuna (*Thunnus albacares*)	SPME/GC-MS	17	3-methyl-1-butanol 3-hydroxy-2-butanone Hexanal 2-nonanone	Fresh fish markers	(Edirisinghe, Graffham,and Taylor, 2007)

2.3 Analysis of volatile compounds

Sampling methods should be as mild as possible and represent the actual composition of the original sample for analysis of volatiles. One of the methods for volatile compound analysis is headspace analysis. The benefits of using headspace analysis are time saving and simple equipment for sampling. In addition, artefacts caused by heat and contact with solvent and sampling equipment are avoided and volatiles are collected in concentrations presenting their actual vapor pressure in the sample. Temperature has a direct influence on the volatility of the headspace components and sampling conditions and should be chosen so that artefacts induced by temperature will be minimized. Headspace analysis includes static and dynamic headspace. In static headspace, a sample is sealed into a vessel, warmed, and volatile compounds in the sample diffuse into the headspace. Once equilibrium is reached, the sample is withdrawn and injected into the GC column. (Zhao and Barron, 2006). Dynamic headspace is carried out by purge and trap which a flow of carrier gas is passed through the sample vessel to reduces matrix effect and increase the headspace sample size, thus increasing the sensitivity. Volatile compounds are purged from the sample into the headspace above and through a trap, which is rapidly heated and injected into the GC column. Headspace analysis was used to characterize volatile compounds in sea bream (Grigorakis et al., 2013), mackerel (Alasalvar et al., 1997), tuna (Medina et al., 1999), boiled salmon, cod, and trout, and salted-dried white herring (Chung et al., 2007).

Solid phase microextraction (SPME) is another extraction technique developed by Pawliszyn and co-workers in the early 90s that combines sampling and sample preparation in one step (Arthur and Pawliszyn, 1999). SPME can be automated and is easily of use, relatively low cost, and shows high affinity for a large group of compounds. It has become a widely used technique for the isolation of aroma volatiles (Kataoka, 2005). The needle is pushed through a septum and the fiber is contacted to the headspace above the food sample, which is sealed in a suitable container. Volatile compounds are adsorbed onto the fiber and, once equilibrium is reached; the fiber can be removed from the sample vessel and directly desorbed into the split/splitless injector of a gas chromatograph.

Type of fiber separated according to the molecular weights and polarity of the analytes. Non-polar organic volatile compounds with low molecular weight usually require a polydimethylsiloxane (PDMS)-coated fiber. To extract very polar compounds with low molecular weight from polar samples, such as alcohols or amines, polyacrylate (PA)-coated fiber is recommended. Polydimethylsiloxane/ divinylbenzene (PDMS/DVB) fiber is used for aromatic hydrocarbons with small volatile analyses. Larger molecular weight or semivolatile compounds require a fiber with high adsorbtivity and desorptivity including polydimethylsiloxane/ divinylbenzene (PDMS/DVB), Carboxen/ PDMS or DVB/carboxen/PDMS-coated fiber. Several of SPME fibers have been used for volatile of fish. A PDMS fiber was used to examine the effects of storage time on yellowfin tuna (Edirisinghe et al., 2007), while a polyacrylate (PA) fiber was found to be the most suitable for comparing smoked with non- smoked black bream and rainbow trout, included smoked cod and swordfish (Guillen and Erreecalde, 2002, 2006). Song et al. (2002) discovered that aroma of uncooked hepatic tissue was stronger than that of uncooked muscle for carp, flounder, mackerel, and skipjack based on a PDMS/DVB fiber, while DVB/carboxen/PDMS fibers was used to examine sardine freshness (Ganeko et al.,2008). Carboxen/ PDMS fibers was used for analysis of aroma compounds in sea bream, chum salmon, mackerel, sardine, tuna, prawn, and shrimp (Mansur et al., 2003). The HS-SPME has been widely used for the analysis of flavor and freshness in several foods including seafood. These methods have been applied to determine the concentration of aliphatic amines (Zeng et al, 2004), volatiles of yellowfin tuna, sardine (Edirisinghe et al, 2006, Ganeko et al., 2004), differences in volatiles of raw and smoked fish species (Guill and Errecalde, 2002), monitoring the volatile basic nitrogenous species including methylamine, dimethylamine and trymetthylamine for freshness assessment (Chan et al., 2006), differences in volatiles of fresh and fozen-thawed cultured gilthead sea bream fish (Iglesias et al., 2009), the kinetic of lipid oxidation and off-odor formation in silver carp mince (Fu et al., 2009). Iglesias and Medina (2008) applied HS-SPME to determine volatile compounds associated to oxidation of Atlantic house mackerel mince muscle.

2.4 Lipid component in fish muscle

Lipid content and fatty acid profile of fish vary with species and many factors including temperature, salinity, season, size, age, habitat, and others. Freshwater fish in general contain higher proportions of n-6 PUFA (2.42-21.92%) than marine species (0.43–14.2%) (Özogul et al., 2007). Fish can be categorized into fatty, intermediate and lean fish. In fatty fish, fat content is around 8-35 g fat/100g of fish such as herring, sardine and mackerel. In intermediate fatty fish, fat content is 2-8 g fat/100g of fish, such as tilapia and trout. Lean species, such as threadfin bream and Alaska pollack contain 0-2 g fat/100 g of fish (Murray and Burt, 2001). Fish contain high levels of cellular unsaturated lipids which can be converted to hydroperoxides by LOX. Lipids in fish are divided into two main groups, 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 head group of nitrogen-containing alcohol. Triaclyglycerols are less polar hydrophobic, whereas phospholipids are more polar due to phosphate group (Burri et al., 2012).

Phospholipids are divided into three groups: glycerophospholipids, ether glycerolipids and sphingophospholipids. Glycerophospholipids are phospholipids with different polar head 18 groups. For example, phosphatidylcholine (PC) has choline as a head group; while phosphatidylethanolamine (PE) has ethanolamine as a head group, etc. Phosphatidylcholine is a major group in fish, whereas phosphatidylethanolamine (PE) is shown to be the second most abundant. Phospholipids are important component for cell membranes and they function as precursors in structural fat, but the neutral lipids play a role as an energy source (Henderson and Tocher, 1987).

2.5 Surimi

Surimi is a uniquely functional food ingredient. Production of surimi involves heading, gutting and mincing washing, dewatering, and freezing. Good quality surimi is judged by gel-forming ability, water-holding capacity, odorless and creamy white appearance (Park and Morrissey, 2014). Thailand is the leading country for the production of threadfin bream surimi. In addition, surimi can be produced from freshwater fish, like tilapia with good gel-forming ability and white appearance. In addition, odorless is important attribute of surimi (Nopianti, Huda and Ismail, 2010).

2.5.1 Washing process

The washing process is a key step, not only because it removes water-soluble substances, mainly sarcoplasmic proteins, fat and other impurities, like pigments and many metabolic enzymes that reduce the stability of functional proteins during storage. Washing also increases concentration of myofibrillar protein, consequently improving the gel-forming ability. Surimi requires several washing steps to ensure maximum gelling, as well as colorless and odorless surimi (Hall and Ahmad, 1997). However, over-washing lead to substantial loss of fine particles and high moisture content. The number of washing cycles and the volume of water depend on fish species, freshness quality, type of washing solution and the desired quality of the surimi. Normally, two washing cycles using a 3:1 (v/w; water to mince) ratio with a five minutes agitation in each of cycles can be operated to adequate for surimi production (Lee, 1986). Pacheco-Aguilar, Crawford and Lampila (1989) 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 a typical wash ratio of 0.9–1.2 parts water in the first wash and 0.7–0.8 in a second wash. Washing process is resulting in substantial loss of gel quality during frozen storage. Hossain et al., (2002) investigated effect of washing solution, washing period and salt concentration on the gel properties of silver carp, they reported that fish mince needed to be washed once with 0.1% NaCl for 10 minutes to obtain a good quality surimi. Manna et al., (2009) reported improving the mince characteristics of marine catfish required to be washed with chilled water (meat: water ratio 1:2) for single time.

2.5.2 Effect of washing on lipid removal

Several research on washing able to decreased lipid concentration around 20-60%. Turan and S**Ö**nmez (2008) reported that the third washing lipid levels decreased approximately 30%. Twice washing reduced lipid level of 64% in the red tilapia and 70% in the Nile tilapia (Biscalchin-Gry ˆschek et al., 2003). In sardine, washing decreased approximately 40% in lipid (Ramirez-Suárez et al., 2000). Acheco-Aguilar et

al. (1989) reported that a single washing at a 3:1 water–flesh ratio and washing at pH 5.0–5.3 were the most effective in removing lipid. Washing three cycles can reduced the fat content by 23% of its initial value in shark meat (Mathew et al., 2002). Pattaravivat et al., (2008) investigated method to decrease the lipid content to less than 1% by washing escolar meat with palmitic sucrose ester (P-1670). After the seconds wash with a 0.25% (w/v) these solutions, the lipid and wax contents decreased to 0.32% to obtain good strength and whiteness of the gel. Barrero and Bello (2000) reported that minced sardine was washed with 0.5% sodium bicarbonate, which removed 57% fat.

Higher phospholipid content in surimi than in mince could be explained by the membrane polar lipids, interacting with proteins and consequently being less easily removed than neutral lipids during washing process (Eymard et al., 2005). Eymard et al., (2005) reported that most of the 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. Rhee et al., (1998) reported that washing with high pH (8.2) tap water for 4 times can reduced total fat content, particularly neutral lipids.

2.6 References

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