CHAPTER III

CHANGES OF VOLATILE COMPOUNDS FROM LIPID OXIDATION OF NILE TILAPIA DURING ICE STORAGE

3.1 Abstract

Changes in the lipid oxidation and the generation of volatile compounds of various tissues of Nile tilapia, including muscle, gill, skin during ice storage was investigated. Lipoxygenase (LOX) activity and peroxide value were detected in gill, skin, and muscle throughout 9 days of storage and increasing with the extended storage time. The highest LOX was found in gill, whereas the highest PV was found in skin. Individual fatty acid contents decreased during storage. Oleic acid was predominant monounsaturated fatty acid (MUFA), whereas linoleic acid (LA) and docosahexaenoic acid (DHA) were the main polyunsaturated fatty acid (PUFA) in all tissues. Twenty-seven volatile compounds were identified in muscle followed by 45 compounds in gill and 30 compounds in the skin based on headspace solid phase microextraction coupled with gas chromatography and mass spectrometry (HS-SPME-GC/MS). Principal component analysis showed gradual changes in the volatile composition with increasing storage time. 2-Butanone, nonanal, 6-methyl-2-haptanone,1-haptanol, and 1-nonanol can be used as freshness indicators. In addition, 1-penten-3-ol and hexanal were potential markers for measuring degree of lipid oxidation in tilapia stored in ice.

Keywords: Nile tilapia, Lipid oxidation, Lipoxygenase, Solid phase microextraction coupled with gas chromatography and mass spectrometry (HS-SPME-GC/MS), Volatile compound

3.2 Introduction

Tilapia is the world's second most farmed fish, with 6.93 million tons harvested in 2020. (FAO, 2021). It is inexpensive and has white-colored meat, a mild flavor, high protein, and absence of intermuscular bones, making it an acceptable substitute for more expensive choices such as salmon. Fresh fillets of premium-quality tilapia must be processed as soon as possible after harvest, as ice storage reduces the shelf life of the final product (Jimenez-Ruiz et al., 2020). Iced storage is method to maintain the quality of fish during handling and storage. However, fatty fishes are particularly susceptible to lipid degradation which can cause severe problem on postmortem, producing a range of substances having unpleasant taste and smell. Quality deterioration during ice storage of fish is associated with strong fishy note. Hexanal and nonanal are an important compound contributing to fishy odor of seabass skin store in ice for an extended time (Sae-leaw, and Benjakul, 2014). Changes of volatile compounds during ice storage of tilapia have not been well characterized. In this study, changes of volatile compounds during ice storage were monitored. The volatile compounds in tilapia could be useful for determining the freshness and deterioration of the fish during transport/storage.

Fish is a rich in omega-3 polyunsaturated fatty acids (PUFAs), such as docosahexaenoic (DHA) and eicosapentaenoic acids (EPA) (Strobel, Jahreis, and Kuhnt, 2012). Compared to other food lipids, fish lipids are more susceptible to oxidation due to their high degree of unsaturation and low antioxidant content. Lipid oxidation is associated to the formation of unpleasant odors (Olaoye, 2016). As both lipolysis and lipid oxidation in fish are associated with quality loss, lipid deterioration still occurs readily during storage and limits the shelf life of fish (Pacheco-Aguilar, Lugo-Sanchez, and Robles-Burgue-no, 2000). It is well recognized that various tissues of the fish contain different lipid contents, leading to varied degree of lipid oxidation during ice storage.

Lipoxygenase (LOX) is a dioxygenase that oxygenates polyunsaturated fatty acids (PUFA) containing a 1-cis, 4-cis-pentadiene system converting them into conjugated unsaturated fatty acid hydroperoxides (ROOH) (Grechkin, 1998). The hydroperoxy fatty acids can be further metabolized to produce alcohols, aldehydes,

ketones and hydrocarbons, resulting in off-flavor and off-odors. Previous studies on volatile compounds presumed LOX is a main source of initiating radicals and subsequent lipid peroxidation in fish tissue (German and Kinsella (1985). Mostly LOX has been discovered in muscle, skin, and gill tissue of fish (Tolasa, Çaklı, Şen Yılmaz, Kırlangıç, and Lee 2018; Sae-Leaw, Benjakul, Gokoglu, and Nalinanon 2013; Hsu and Pan 1996). Iglesias et al. (2009) reported that Z-4-heptenal is an important compound contributing to off-note of gilthead sea bream fish, which is produced by the action of LOX on EPA. Moreover, strong fishy odor in silver carp is caused by 2, 4-heptadienal (E, E) resulted from the action of -LOX on linoleic acid (Fu et al, 2009). However, quality changes of tilapia during ice storage as related LOX activity and formation of volatile compounds has not been explored. The aim of this work was to investigate changes of LOX activity tilapia at various tissues, namely gill, skin, and muscle during ice storage.

3.3 Materials and methods

3.3.1 Chemicals and reagents

Bovine serum albumin (BSA), 2-thiobarbituric acid, cumene hydroperoxide, guanidine hydrochloride, trichloroacetic and ferrous chloride were purchased from Fluka (Buchs, Switzerland). Linoleic acid (≥99%), heptadecanoic acid (≥99%), Tween-20, L-glutathione reduced (≥98.0%), phosphatidylcholine, cumene hydroperoxide, cyclohexanol, and 2,4-dinitrophenylhydrazine (DNPH) were purchased from Sigma-Aldrich Canada Ltd. (Oakville, province, Canada). Other chemicals and reagents were of analytical grade.

3.3.2 Sample preparation

Live tilapia (*Oreochromis niloticus*) with an average weight of 400 – 600 g were purchased from the fish market in Nakhon Ratchasima, Thailand. Fish were placed in ice with a ratio of fish to ice of 1:2 (w/w), packed in polystyrene foam boxes, and immediately transported to the laboratory within 20 min. Upon arrival, the molten ice was drained off and additional ice was added. All polystyrene foam containers were kept in a cold room (4 ℃) throughout the experiment and ice was replaced every 2 days. Temperature was around 0 – 4 $^{\circ}$C throughout the storage of 9 days. At 0, 3, 6,

and 9 d of storage, 6 fishes were randomly taken for analyses. Skins were manually removed, gill and flesh were also collected, the skins were washed with cold water, and cut into small pieces using a scissor. Skin, gill, and muscle samples were immediately stored at -70 ° C until use.

3.3.3 Enzyme extraction

Each tissue was homogenized in 0.05 M phosphate buffer (pH 6.5) with 2 mM reduced L-glutathione and 0.04% Tween-20 at ratio of 1:4 (w/v) using an IKA homogenizer (IKA Works Asia, Bhd, Selangor, Malaysia). The homogenate was centrifuged at 15,000 g (Sorvall Legend MACH 1.6/R, Thermo Electron LED GmbH, Lengensellbold, Germany) for 20 min at 4 ° C The supernatant was collected and brought to 40% ammonium sulfate, stirred for 30 min and centrifuged at 15,000 g for 20 min. The supernatant was then brought to 70% ammonium sulfate and stirred for 45 min. The enzyme extract was collected from centrifugation at 15,000 g for 30 min. Pellets were collected and dissolved with 0.05 M phosphate buffer (pH 6.5) with 2 mM L-glutathione reduced (≥98.0%) and 0.04% Tween-20, and dialyzed overnight against the same buffer with two changes of the buffer. The extract produced was used and referred to as LOX extract. The temperature was maintained between 0 - 4 ° C at all steps. Protein determination was carried out by the modified Bradford method using bovine serum albumin (BSA) as a standard (Bradford 1976).

3.3.4 Lipoxygenase assay

LOX activity was measured by monitoring conjugated dienes. Linoleic acid was prepared according to the method of Patel, Patel & Thakkar (2015). Linoleic acid (45 mg) was mixed with 90 mg of Tween-20 in 1 ml of DI water. One hundred and fifty microliters of 1 N NaOH was added and the final volume of 20 ml was brought up with 0.05 M phosphate buffer (pH 6.5). The reaction mixture was prepared by adding 1.3 ml 50 mM phosphate buffer, pH 6.5, containing 1 mM glutathione and 0.04% Tween-20 and 100 µl LOX extract. The reaction was initiated by adding 100 µl of substrate. Absorbance at 234 nm of the reaction was recorded every 30 sec. within 3

min. One unit of LOX activity was defined as an increase in absorbance at 234 nm of 0.001 per min.

3.3.5 Peroxide value

Peroxide value (PV) was determined according to the method of Richards and Hultin (2002). Samples (1 g) were homogenized in 11 ml of chloroform/methanol (2:1, v/v). Homogenates were then filtered using a Whatman No. 1 filter paper. Two milliliters of 0.5% NaCl were added to 7 ml of the filtrate. The mixtures were vortexed and then was centrifuged to separate the sample into two phases. To 3 ml of lower phase, 25 µl of 30% (w/v) ammonium thiocyanate and 25 µl of 20 mM iron (II) chloride was added to the mixture. The reaction mixture was kept for 20 min at room temperature prior to reading the absorbance at 500 nm. Blanks were prepared in the same manner, except that deionized water was used instead of ferrous chloride. A standard curve was prepared using cumene hydroperoxide at concentrations ranging from 0.5–2 ppm. PV was expressed as mg cumene hydroperoxide/kg sample after blank subtraction.

3.3.6 Total lipid content

Total lipid content of skin, gill and muscle was analyzed according to Bligh & Dyer method (Bligh & Dyer, 1959). Samples (12 g) were homogenized with 60 ml chloroform and methanol solution (2:1, v/v) at 8000 rpm for 2 min at 4 $^{\circ}$ C using an IKA homogenizer (IKA Works Asia, Bhd, Selangor, Malaysia). After 30 min, the homogenates were filtered through Whatman No. 1 filter paper into a separating funnel. Thereafter, 24 ml of chloroform, 24 ml of distilled water and 2 ml of 0.58% (v/v) sodium chloride solution were added. The mixture was gentle shaken. After separation, the chloroform phase was drained off into a 125-ml Erlenmeyer flask containing about 2–5 g of anhydrous sodium sulfate and was shaken very well to remove any traces of water that might be present in the chloroform phase. The solution was filtered through Whatman No. 1 filter paper into a pre-weighed 125-ml Erlenmeyer flask and solvent was removed by flushing with nitrogen. The extracted lipid was stored at -80 $^{\circ}$ C for further analysis.

3.3.7 Phospholipid content

Phospholipid content of extracted lipid was evaluated by a colorimetric method as described by Chaijan, Klomklao and Benjakul (2013) , based on the formation of a complex between phospholipids and ammonium ferrothiocyanate. One ml of thiocyanate reagent (a mixture of 0.10 M ferric chloride hexahydrate and 0.40 M ammonium thiocyanate) was added to 2 ml of extracted lipid in chloroform solution (0.25 mg/ml). After mixing for 1 min, the lower layer was removed and the absorbance at 488 nm was measured. A standard curve was prepared using phosphatidylcholine (0–0.1 mg/ml) as a standard. Phospholipid content was expressed as mg/100 g lipid.

3.3.8 Fatty acid profile

Fatty acid profiles of extracted lipids from skin, gill and muscle were determined as fatty acid methyl esters (FAMEs) using gas chromatography (GC) according to the AOAC method (AOAC, 2010). Aliquots of the extracted lipids were used to prepare the FAME according to the method of AOAC (2010). In brief, the sample was mixed with 0.5 M methnolic solution (2% sodium hydroxide in methanol), heated at 85 ° C for 30 min, cooled and extracted with 2,2,4-trimethylpentane (Isooctane). Heptadecanoic acid (C17:0) was used as an internal standard. FAMEs were injected, separated and identified on an Agilent/HP 7890 gas chromatograph (Agilent, Palo Alto, CA, USA) with flame ionization detector (FID) equipped with a fused silica capillary column (100 m x 0.25 mm x 0.2 μm film thickness) (Supelco, Bellefonte, PA, USA), using helium as the carrier gas at a flow rate of 1 ml/min. The analytical conditions were: injection port temperature of 250 ° C and detector temperature of 270 ° C. Retention times of FAME standards were used to identify chromatographic peaks of the samples. Fatty acid contents were calculated, based on the peak area ratio and expressed as g fatty acid/100 g lipid.

3.3.9 HS-SPME-GC/MS analysis

Each sample (gill, skin, and muscle) was cut into small pieces (0.5x 0.5 cm^2) and 2 g were placed in a 10 ml flat bottom headspace vial fitted with a PTFE/silicone septum and crimp cap (Supleco, Bellefonte, PA, USA) that contained 3

ml of saturated sodium chloride, 30 µl of internal standard (1ppm cyclohexanol) and 10 µl of 7.2% butylated hydroxyanisole (BHA). The samples were analyzed immediately by headspace solid-phase microextraction combined with gas chromatography mass spectrometry (HS-SPME-GC/MS). The sample was pre-incubated at 40 °C for 10 min with agitation. Then, a SPME fiber (50/30 µm DVB/carboxen/ polydimethylsiloxane fiber; Supelco, Bellefonte, PA, USA) was exposed to the vial headspace for 20 min. The fiber was desorbed by splitless injection (injector temperature, 260°C; splitless time, 4min; vent flow, 50 mL/min) into a GC-MS system.

Analyses were carried out using an Agilent GC 7890A series instrument (Agilent Technologies, Inc., Santa Clara, CA, USA). Compounds were separated on a fused silica DB-wax column (60 m x 0.3 mm x 0.25 µm) capillary column. The GC oven temperature program was: 35° C for 3 min, followed by an increase of 3 ° C/min to 70 ° C; then an increase of 10 ° C/min to 200 ° C and finally an increase of 20 ° C/min to a final temperature of 250 ° C, held for 5 min. Helium, with a constant flow of 1.5 ml/min, was used as the carrier gas. Transfer line temperature was maintained at 265 ° C. The identification of the volatile compounds was achieved by comparing their mass spectra with those stored in the National Institute of Standards and Technology (NIST) US Government library. Retention indices of all the volatile compounds were determined by the modified Kovats method reported by Van den Dool and Kratz. MS identification was confirmed by comparing Kovats retention indices (RI) to RI reported in the literatures.

3.3.10 Statistical analyses

All experiments were conducted in triplicate, and the results were analyzed by one-way analysis of variance (ANOVA). Significant differences in mean values were analyzed by Duncan's multiple range mean comparison test within the 95% confidence interval using All data were analyzed with the SPSS 17.0 statistical package (SPSS Ltd., Working, Surrey, UK). Principal component analysis (PCA) of all measured parameters were performed on means results using the XLSTAT software (Addinsoft, New York, NY, USA).

3.4 Results and discussion

3.4.1 LOX activity and peroxide values of various tissues

LOX activities during ice storage of muscle, gill, and skin of tilapia is demonstrated in Figure 3.1. LOX activities varied with fish tissues. A significant increase in LOX activities was detected in gill, skin, and muscle throughout 9 days of storage ($p < 0.05$). An increase in LOX activities associated with the extended storage time indicated either the increase in extractability of LOX or the activation of LOX in fish tissues. Gill reached its maximum level of LOX activities by 9 days of storage time with a 3.30-fold increase. Moreover, the gill showed the highest LOX activity among other tissues. The increasing rate of muscle LOX activity was slower than that of skin, and reached the maximum level at the end of storage time with a 1.9-fold increase. In the present study, ranking of LOX activity (gill > skin > muscle) agreed with the findings of Wang et al. (2012) who reported that LOX activity of grass carp gradually increased in the muscle and skin less than gill. At 3 days of storage time, muscle and skin showed LOX activity increased significantly, whereas gill showed a significant increase at 6 days of storage time. Membranes and/or subcellular structures of muscle and skin are easily damaged, rendering an increase in LOX activity. Cao et al. (2019) reported LOX activity of grass carp muscle increased significantly during 0–4 days of chilled storage. An increase in LOX activities was also observed in tilapia skin, Baltic herring muscle, and gray mullet gill (Sae-leaw et al., 2013; Samson and Stodolnik, 2001; Hsu and Pan, 1996). Moreover, Medina, Saeed, and Howell (1999) reported that LOX activity of skin tissue of sardine was detected for up to 2 days of chilled storage. Hsieh et al. (1988) reported the high activity of gill LOX at near 0 °C, which is significant in the initiation of fish lipid oxidation under refrigeration conditions. Wu, Forghani, Abdollahi, and Undeland (2022) reported that head showed the highest LOX activity among other fractions of sorted herring and LOX had a significant impact on the lipid oxidation susceptibility. Our results indicate that gill is a main source of LOX in tilapia.

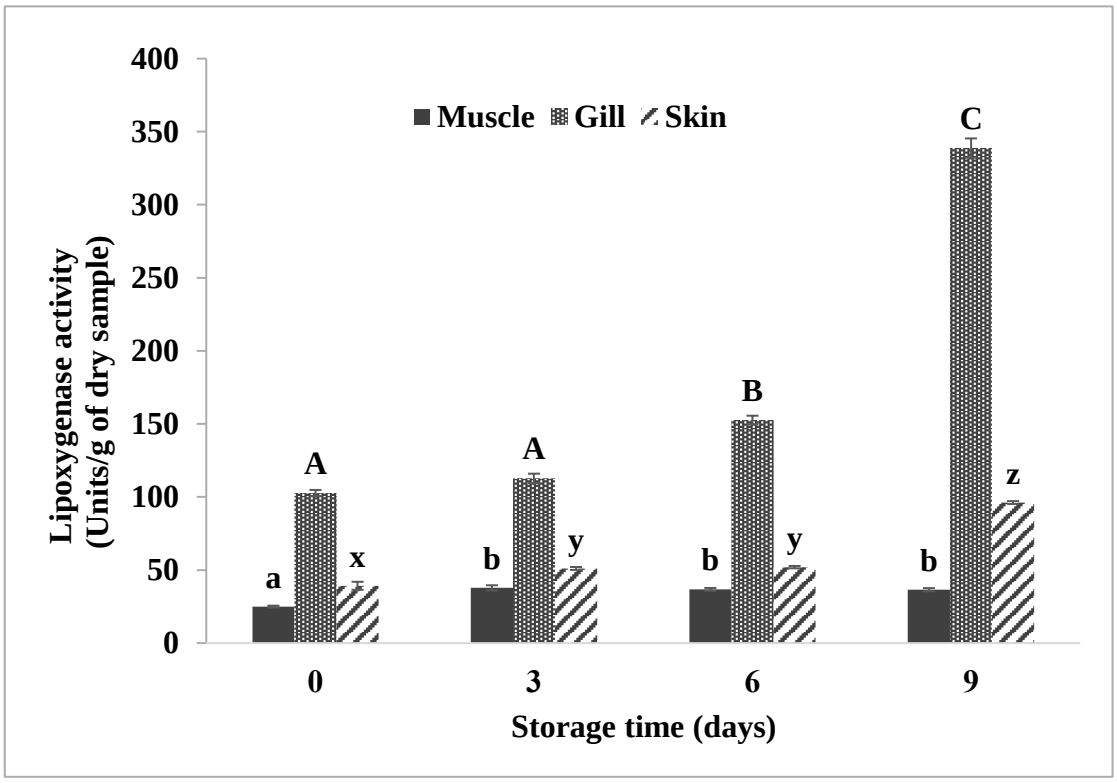

Figure 3.1 Lipoxygenase activity of various tissues of tilapia during ice storage. Means with different superscripts differ significantly ($P < 0.05$) as $^{a-b}$ within the muscle, $^{A-B}$ within gill, and $^{x-z}$ within the skin.

Figure 3.2 showed changes of peroxide values (PV) (mg hydroperoxide/kg of dry sample) of muscle, gill, and skin of tilapia during ice storage. The initial PV of gill, skin and muscle were 2.67, 3.11, and 1.48 mg hydroperoxide/kg of dry sample respectively, which agreed with previously reported values of 1-2 mg hydroperoxide/kg sample in tilapia muscle (Yarnpakdee et al., 2012), and 3-5 mg hydroperoxide/kg sample in sardine muscle (Chaijan et al., 2006). Lipid oxidation occurred during postmortem handling and may also be influenced by muscle type, and age. Fresh fish is known to contain peroxide which is naturally produced and can react with biological molecules. A significant increase in PV was detected in all tissues with storage time ($p<0.05$), suggesting that even at low temperatures, lipid oxidation is always active. Skin showed the highest PV followed by gill and muscle at any storage time. PV values of skin and gill increased by 257% and 247% at 9 days of storage as compared to day 0. Lipid hydroperoxides are formed by either the reaction of singlet oxygen with unsaturated lipids or the lipoxygenase-catalyzed oxidation of PUFA (Nawar, 1996). High

concentrations of polyunsaturated fatty acids (PUFA) were found in all studied tissues, but gill containing the highest PUFA content (Table 1). Yarnpakdee et al. (2012) reported that the increase in PV correlated with the increased amount of non-heme iron, which more likely acted as a pro-oxidant of lipid oxidation. Thus, the degree of oxidation of the muscle, gill, and skin of tilapia kept for an extended time might vary based on a variety of factors, including as the concentration of unsaturated fatty acids, the type and amount of iron, and other pro-oxidants. These lipid oxidation products were detected in the tissues and could have contributed to quality degradation, particularly in fish with an unpleasant flavor. In this study, changes of LOX activity doses not correspond with PV. LOX activity was highest in gill, but PV was highest in skin. It might be that LOX are not the main factor in lipid oxidation. The extent of lipid oxidation can be influenced by various factors, including oxygen consumption, the concentration of prooxidants, pH, temperature, and fatty acid content, which affect skin more than gill tissue.

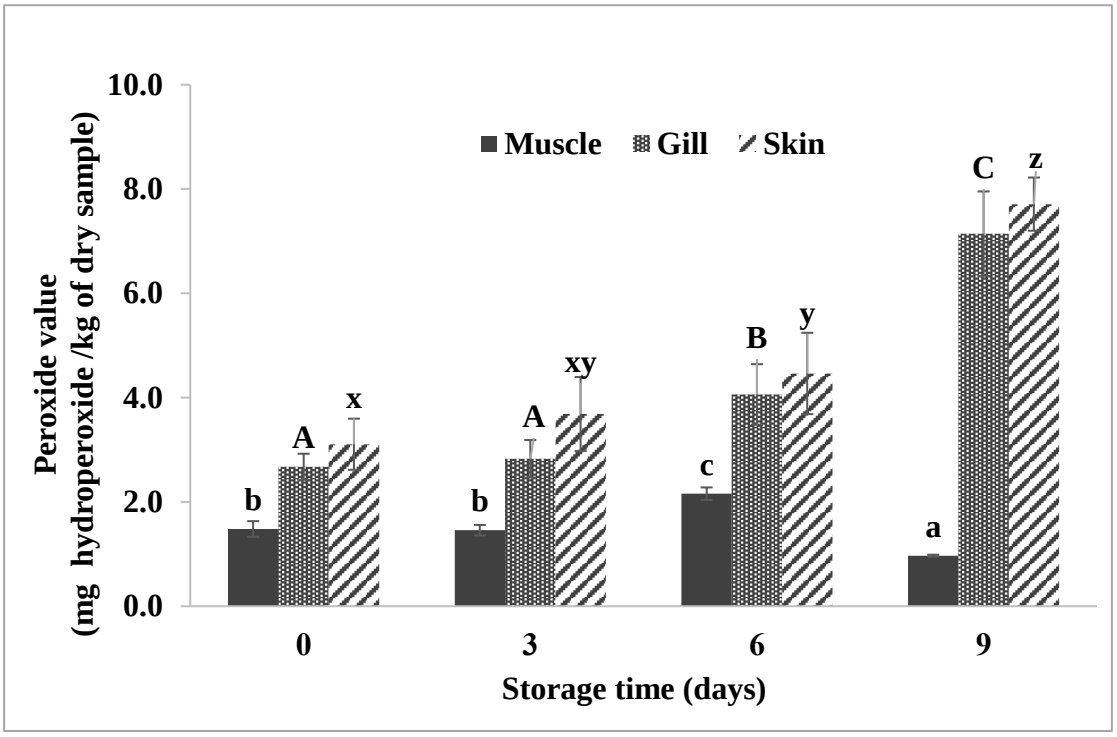

Figure 3.2 Peroxide values of various tissues of tilapia during ice storage. Means with the different superscripts differ significantly ($P < 0.05$) as $^{a-b}$ within the muscle, $^{A-B}$ within gill, and $^{x-z}$ within the skin

3.4.2 Changes of fatty acids at various tissues during ice storage

Changes in the content of various fatty acids in different tissues of tilapia could indicate the extent of lipid oxidation. Changes of fatty acids during ice storage of muscle, gill, and skin and of tilapia are listed in Table 3.1. Gill showed the highest fatty acid contents followed by skin and muscle. A decrease in fatty acid was detected in all tissues at day 9 of storage time ($p<0.05$). Lipid oxidation is likely to be a main reason for the decrease in fatty acids, which coincided with an increase in PV content (Fig. 3.2). Moreover, activation of lipoxygenase is believed to have caused such decreases in fatty acids (Joaquin, Tolasa, Oliveira, Lee, and Lee, 2008). However, Sae-leaw et al. (2013) reported that fatty acid content of tilapia skin increased significantly during 18-day storage. It was possibly due to lipase activity and phospholipase activity. Gill and skin showed a decrease in ∑MUFA and ∑PUFA in the first 3 days, whereas muscle showed a decrease in 6 days ($p<0.05$). Skin showed the highest rate of decrease in ∑MUFA and ∑PUFA among other tissues, coinciding with the highest PV. The decreasing rate of ∑PUFA was higher than ∑MUFA in all tilapia tissues studied, indicating that a high degree of unsaturated lipid were easily oxidized. Lipid oxidation includes autooxidation and enzymatic oxidation. LOX is a significant endogenous enzyme related to free fatty acid oxidation in fish (German, Chen, and Kinsella, 1985). In our study, palmitic acid as the predominant saturated fatty acid (SFA), oleic acid is highest monounsaturated fatty acid (MUFA), whereas linoleic acid (LA) and docosahexaenoic acid (DHA) are the main polyunsaturated fatty acid (PUFA) in all tilapia tissues. Xu et al. (2019) reported that unsaturated fatty acid in silver carp mince, particularly PUFA, significantly decreased during cold storage. Since the whole fish were stored in ice, LOX from intracellular might be released and initiated lipid oxidation, converting DHA and EPA into more polar products (German and Kinsella, 1985). Mori, Cho, Endo, and Fujimoto (1992) found LOX in sardine skin oxidized linoleic acid more efficiently than arachidonic acid and EPA. LOX in mackerel gill exhibited the highest reactivity toward EPA followed by arachidonic acid and linoleic acid (Hong, Shim, and Byun, 1994). LOX in rabbit fish muscle with an arachidonic acid (ARA) substrate specificity contributes to volatile lipid oxidation products that explain the unpleasant smell of rabbit fish (Jiarpinijnun et al., 2022). Wu et al. (2022) reported that LOX had a significant impact

on lipid oxidation susceptibility, although the quantity of lipids or polyunsaturated fatty acids had no significant correlation.

3.4.3 Changes of volatile compounds

Figure 3.3 shows GC-MS chromatograms obtained from the SPME analysis of muscle, gill, and skin from tilapia at day 9 of ice storage. Twenty-seven volatile compounds were identified in muscle followed by 45 compounds in gill and 30 compounds in the skin (represented by relative content %), grouped by chemical families (Table 3.2): alcohols, aldehydes, ketones, furans, acid, ester, hydrocarbons, and aromatic compounds. In our study, the majority of these compounds showed significant statistical difference ($p<0.05$) after ice storage compared to initial values, indicating that ice storage affects the volatile nature of tilapia.

Alcohols were found to be the compounds showing the highest peak areas during ice storage in muscle, gill, and skin. Among them, 1-hexanol showed the highest level in the muscle and skin at the end of storage.1-Hexanol has been reported as a major volatile compound of fish, derived from the degradation products of oleic acid and palmitic acid, which were the major fatty acids in tilapia tissue. Whereas, 1-octen-3-ol and 1-nonanol showed major alcohols in gill. 1-Octen-3-ol is an important contributor to off-flavor in fish due to its low odor score and generated from the oxidation of arachidonic and eicosapentaenoic acids (EPA) by gill lipoxygenase and degradation of linoleic acid hydroperoxide (Hsieh and Kinsella, 1989; Iglesias et al., 2009; and Mu et al., 2017). Jiarpinijnun et al. (2022) reported that 1-octen-3-ol was the main volatile compound associated with the unpleasant smell in rabbit fish generated from the reaction of LOX and arachidonic acid (ARA).

Table 3.1 Fatty acid compositions of muscle, gill, and skin from Nile tilapia during iced storage.

Fatty acids (mg/ g of dry sample)	Muscle				Gill				Skin			
	0 day	3 days	6 days	9 days	0 day	3 days	6 days	9 days	0 day	3 days	6 days	9 days
C10 : 0	ND	ND	ND	ND	ND	ND	ND	0.06 ± 0.01	ND	ND	0.01 ± 0.01	0.01 ± 0.02
C11 : 0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.01 ± 0.02	0.01 ± 0.01
C12 : 0	0.29 ± 0.02	0.26 ± 0.01	0.27 ± 0.02	0.29 ± 0.01	1.04 ± 0.04	0.95 ± 0.04	0.8 ± 0.02	0.84 ± 0.07	0.48 ± 0.07	0.38 ± 0.04	0.34 ± 0.02	0.27 ± 0.02
C13 : 0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C14 : 0	2.45 ± 0.17	2.31 ± 0.13	1.88 ± 0.19	2.25 ± 0.17	9.29 ± 0.49	8.47 ± 0.99	7.72 ± 0.31	6.53 ± 0.49	3.94 ± 0.76	2.73 ± 0.34	2.81 ± 0.13	2.04 ± 0.19
C15 : 0	0.14 ± 0.02	0.21 ± 0.01	0.1 ± 0.02	0.12 ± 0.01	0.55 ± 0.04	0.57 ± 0.04	0.42 ± 0.06	0.46 ± 0.03	0.23 ± 0.05	0.18 ± 0.02	0.16 ± 0.01	0.12 ± 0.01
C16 : 0	21.51 ± 0.68	21.67 ± 0.22	18.17 ± 0.86	20.49 ± 1.4	81.6 ± 1.54	73.44 ± 1.46	63.58 ± 0.84	62.29 ± 2.02	36.09 ± 7.35	23.99 ± 2.61	22.9 ± 0.61	17.78 ± 0.63
C17 : 0	4.87 ± 0.07	4.32 ± 0.14	4.64 ± 0.06	2.98 ± 0.02	16.34 ± 0.31	14.52 ± 0.5	11.9 ± 0.37	17.24 ± 0.35	6.62 ± 0.05	7.09 ± 0.12	6.92 ± 0.1	5.86 ± 0.13
C18 : 0	5.63 ± 0.56	5.17 ± 0.19	4.56 ± 0.21	5.13 ± 0.41	22.6 ± 1.35	18.16 ± 0.88	14.98 ± 0.3	15.72 ± 0.76	9.89 ± 2.18	5.98 ± 0.51	5.55 ± 0.14	4.58 ± 0.24
C20 : 0	ND	ND	ND	ND	ND	ND	ND	0.53 ± 0.01	ND	0.79 ± 0.07	0.81 ± 0.06	0.6 ± 0.04
C21 : 0	0.21 ± 0.05	0.23 ± 0.03	0.18 ± 0.02	0.16 ± 0.03	0.87 ± 0.03	0.76 ± 0.07	0.62 ± 0.12	0.29 ± 0.03	0.34 ± 0.02	0.12 ± 0.02	0.11 ± 0.02	0.09 ± 0.01
C22 : 0	0.09 ± 0.02	0.07 ± 0.01	0.06 ± 0.02	0.07 ± 0.01	0.25 ± 0.04	0.31 ± 0.08	0.27 ± 0.02	0.04 ± 0.07	0.08 ± 0.06	0.78 ± 0.06	0.75 ± 0.03	0.61 ± 0.07
C23 : 0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C24 : 0	0.65 ± 0.05	ND	ND	0.31 ± 0.15	ND	ND	ND	ND	0.76 ± 0.1	ND	ND	ND
ΣSFA	**35.8 ± 1.58^{c}**	**34.21 ± 0.7^{bc}**	**29.83 ± 1.36^{a}**	**31.76 ± 2.18^{ab}**	**132.52 ± 2.18^{d}**	**117.15 ± 1.75^{c}**	**100.26 ± 1.85^{b}**	**103.93 ± 2.05^{a}**	**58.4 ± 10.62^{b}**	**41.99 ± 3.75^{a}**	**40.34 ± 1.11^{a}**	**31.92 ± 1.32^{a}**
C14 : 1	0.12 ± 0.01	0.13 ± 0.01	0.08 ± 0.02	0.11 ± 0.01	0.41 ± 0.06	0.38 ± 0.04	0.36 ± 0.04	0.34 ± 0.04	0.18 ± 0.02	0.13 ± 0.02	0.15 ± 0.02	0.11 ± 0.01
C15 : 1	ND	ND	ND	ND	ND	ND	ND	0.04 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.03	0.01 ± 0.01
C16 : 1	4 ± 0.15	4.7 ± 0.2	3.71 ± 0.42	3.95 ± 0.4	14.44 ± 0.65	14.17 ± 0.79	12.49 ± 0.12	12.43 ± 0.84	6.57 ± 1.27	4.46 ± 0.54	4.6 ± 0.29	3.47 ± 0.18
C17 : 1	0.14 ± 0.02	0.21 ± 0.04	0.16 ± 0.05	0.13 ± 0.01	0.51 ± 0.08	0.62 ± 0.11	0.46 ± 0.03	ND	0.24 ± 0.03	ND	ND	ND
C18 : 1n9t	0.46 ± 0.06	0.23 ± 0.05	0.19 ± 0.05	0.38 ± 0.06	1.45 ± 0.14	1.11 ± 0.11	0.67 ± 0.06	0.72 ± 0.42	0.68 ± 0.08	0.35 ± 0.04	0.37 ± 0.02	0.16 ± 0.14
C18 : 1n9c	**32.24 ± 0.19**	**31.9 ± 0.53**	**29.53 ± 0.87**	**28.31 ± 2.01**	**113.6 ± 1.36**	**108.52 ± 2.73**	**92.91 ± 1.74**	**88.48 ± 2.14**	**51.32 ± 8.77**	**32.86 ± 2.88**	**31.31 ± 1.31**	**24.83 ± 0.99**
C20 : 1	1.1 ± 0.07	0.99 ± 0.02	0.96 ± 0.07	0.99 ± 0.04	3.67 ± 0.35	4.15 ± 0.2	2.94 ± 0.09	2.41 ± 0.11	1.88 ± 0.34	1.77 ± 0.18	1.57 ± 0.06	1.02 ± 0.31
C22 : 1n9	0.19 ± 0.01	0.11 ± 0.03	0.09 ± 0.04	0.16 ± 0.01	0.67 ± 0.02	0.69 ± 0.14	0.46 ± 0.04	0.67 ± 0.03	0.28 ± 0.05	0.13 ± 0.02	0.12 ± 0.01	0.09 ± 0.01
C24 : 1	ND	0.72 ± 0.06	0.34 ± 0.05	ND	1.96 ± 0.08	1.2 ± 0.16	1.13 ± 0.07	0.21 ± 0.02	0.00 ± 0.00	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.02
ΣMUFA	**38.23 ± 0.47^{b}**	**38.96 ± 0.91^{b}**	**35.03 ± 1.54^{a}**	**33.98 ± 2.52^{a}**	**136.67 ± 1.85^{d}**	**130.8 ± 2.22^{c}**	**111.39 ± 1.84^{b}**	**105.26 ± 2.42^{a}**	**61.11 ± 10.53^{c}**	**39.74 ± 3.66^{b}**	**38.19 ± 1.71^{ab}**	**29.73 ± 1.63^{a}**
C18 : 2n6t	ND	ND	ND	ND	ND	ND	ND	0.08 ± 0.06	0.00 ± 0.00	0.03 ± 0.03	0.04 ± 0.04	0.02 ± 0.02
C18 : 2n6c	**14.75 ± 0.89**	**14.24 ± 0.17**	**13.23 ± 0.77**	**12.99 ± 0.15**	**48.99 ± 1.32**	**50.31 ± 1.23**	**40.14 ± 0.39**	**39.4 ± 1.73**	**22.8 ± 3.89**	**15.72 ± 1.41**	**13.38 ± 1.5**	**10.99 ± 0.57**
C18 : 3n6	1.38 ± 0.14	1.3 ± 0.09	0.63 ± 0.02	0.79 ± 0.1	4.64 ± 0.41	3.4 ± 0.22	2.26 ± 0.23	2.04 ± 0.1	1.67 ± 0.42	0.21 ± 0.02	0.18 ± 0.01	0.14 ± 0.01
C18 : 3n3	1.5 ± 0.11	1.23 ± 0.04	1.08 ± 0.1	1.33 ± 0.09	5.1 ± 0.28	4.57 ± 0.12	3.92 ± 0.13	3.28 ± 0.2	2.3 ± 0.34	1.33 ± 0.12	1.17 ± 0.05	0.9 ± 0.06
C20 : 2	0.73 ± 0.05	0.64 ± 0.01	0.63 ± 0.05	0.64 ± 0.03	2.43 ± 0.1	2.29 ± 0.15	1.83 ± 0.07	2.03 ± 0.07	1.05 ± 0.18	0.06 ± 0.01	0.06 ± 0.01	0.26 ± 0.26
C20 : 3n6	0.79 ± 0.07	0.77 ± 0.03	0.58 ± 0.06	0.72 ± 0.03	2.43 ± 0.21	2.04 ± 0.15	1.64 ± 0.08	2.2 ± 0.11	1.07 ± 0.19	ND	ND	ND
C20: 3n3	1.03 ± 0.14	1.19 ± 0.05	0.54 ± 0.1	1.01 ± 0.01	3.06 ± 0.1	3.19 ± 0.42	2.35 ± 0.12	0.32 ± 0.01	1.7 ± 0.32	0.29 ± 0.03	0.26 ± 0.02	0.19 ± 0.02
C20 : 4n6	0.11 ± 0.01	0.12 ± 0.02	0.06 ± 0.01	0.11 ± 0.01	0.43 ± 0.13	0.34 ± 0.08	0.3 ± 0.04	2.46 ± 0.22	0.16 ± 0.02	1.06 ± 0.05	1.23 ± 0.02	0.87 ± 0.05
C22 : 2	ND	ND	ND	ND	ND	ND	ND	0.14 ± 0.05	ND	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
C20 : 5n3	0.09 ± 0.02	0.14 ± 0.02	0.09 ± 0.02	0.12 ± 0.04	0.38 ± 0.1	0.38 ± 0.07	0.22 ± 0.04	0.74 ± 0.07	0.19 ± 0.07	0.19 ± 0.02	0.18 ± 0.01	0.16 ± 0.04
C22 : 6n3	**1.58 ± 0.08**	**1.41 ± 0.12**	**0.65 ± 0.22**	**1.33 ± 0.08**	**4.71 ± 0.18**	**4.88 ± 1.24**	**2.11 ± 0.17**	**3.08 ± 0.23**	**3.8 ± 1.95**	**1.25 ± 0.08**	**1.18 ± 0.01**	**0.84 ± 0.05**
ΣPUFA	**21.92 ± 1.48^{b}**	**20.98 ± 0.53^{b}**	**17.46 ± 1.3^{a}**	**19.02 ± 0.51^{a}**	**72.13 ± 1.32^{b}**	**71.37 ± 0.71^{b}**	**54.75 ± 0.51^{a}**	**55.72 ± 2.57^{a}**	**34.7 ± 7.35^{c}**	**20.14 ± 1.74^{b}**	**17.69 ± 1.64^{ab}**	**14.37 ± 1.04^{a}**

*SFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids.

$^{a-d}$ Mean values in the same row with different superscripts differ significantly (P < 0)

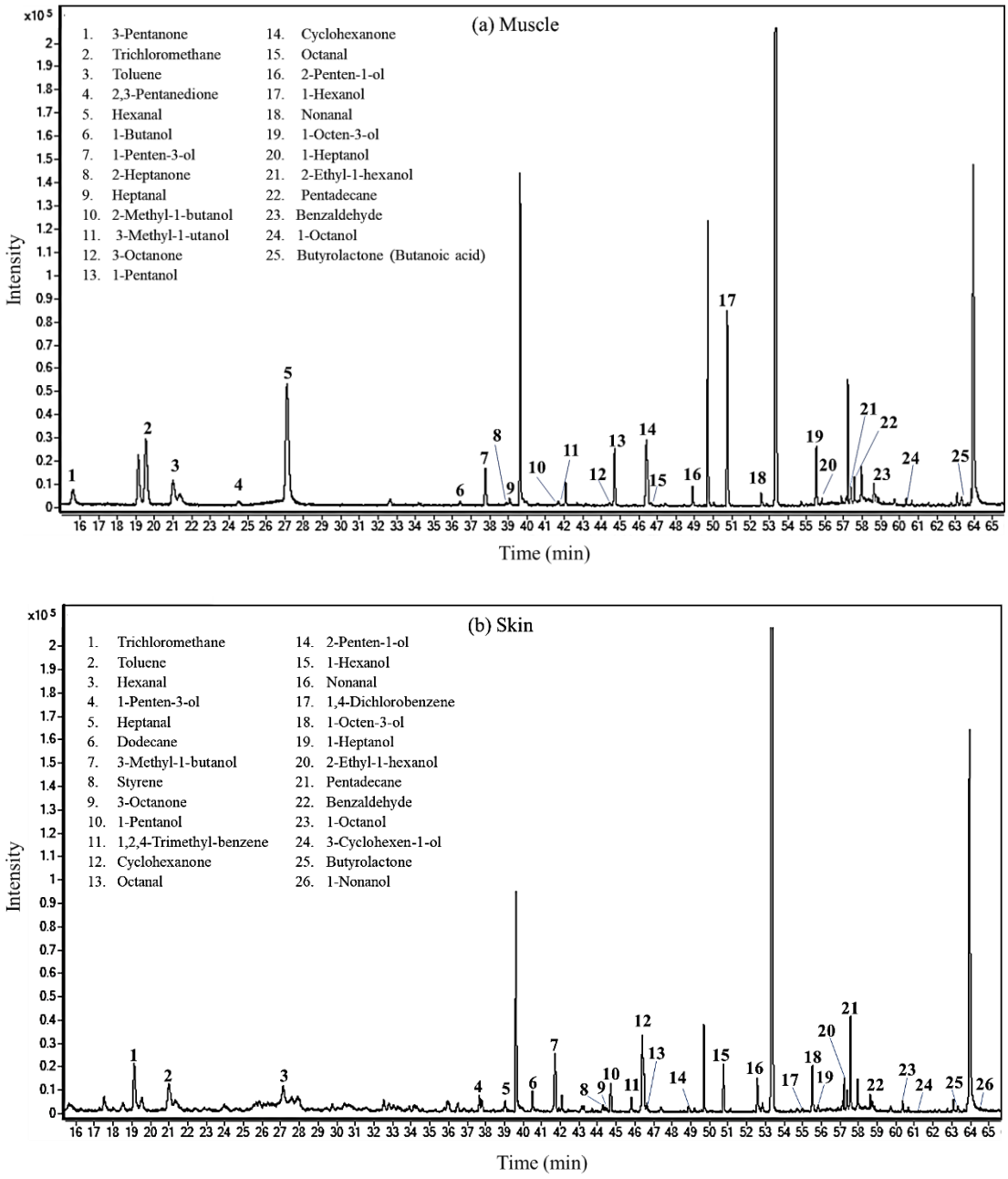

Figure 3.3 Representative GC–MS (full scan) chromatogram of muscle (a), skin (b), and gill (c) from Nile tilapia at day 9 of ice storage.

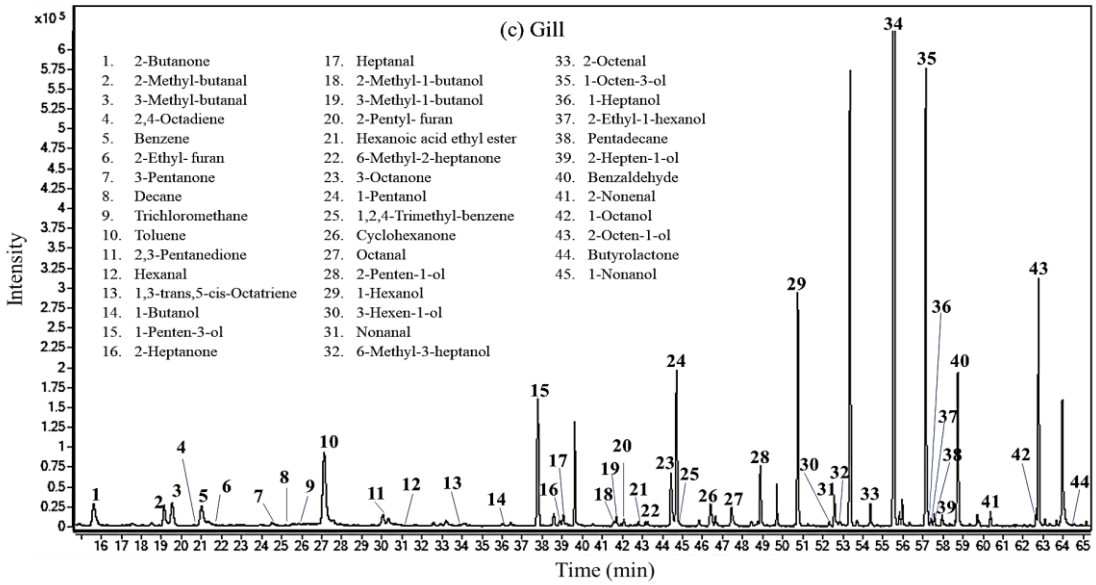

Figure 3.3 Representative GC–MS (full scan) chromatogram of muscle (a), skin (b), and gill (c) from Nile tilapia at day 9 of ice storage. (Continued).

A significant increase in 1-penten-3-ol and 2-penten-1-ol was detected in gill, skin, and muscle at day 9 of storage ($p<0.05$, Table 3.2). An increase of 1-penten-3-ol can be explained by lipoxygenase acting on EPA and hydroperoxide lyases, which is used as a marker of lipid oxidation in chilled Atlantic horse mackerel muscle (Iglesias and Medina, 2008). The increase of 2-penten-1-ol can be explained by oxidized lipid, which was the most potent odorant of raw sardine (Prost et al., 2004). 3-Methy-1-butanol was identified in muscle, gill, and skin of tilapia with the increase of storage time ($p<0.05$). Similar trend has already been observed in previous studies, which caused an unpleasant smell and was derived from oxidative deamination of leucine by spoilage bacteria (Wang, Chen, Zhang, Wang, and Shi, 2019).

Table 3.2 Relative amount of volatile compounds detected by HS-SPME-GC/MS in tilapia during ice storage.

Compound name by class	RIL	RIC	Muscle				Gill				Skin			
			0	3	6	9	0	3	6	9	0	3	6	9
Alcohols (17)														
1-Butanol	1158	1156	0.43 ± 0.06^{ab}	0.33 ±0.07^{a}	0.56 ± 0.03^{c}	0.49 ± 0.09^{bc}	0.10 ± 0.01	0.1 ± 0.01	0.11 ± 0.07	0.13 ± 0.02	ND	ND	ND	ND
1-Penten-3-ol	1166	1169	1.56 ± 0.20^{a}	2.08 ± 0.23^{a}	4.32 ± 0.21^{a}	3.97 ± 0.38^{b}	4.07 ± 0.31^{a}	5.11 ±0.85^{ab}	7.91 ± 0.63^{b}	5.29 ± 1.11^{a}	2.27 ± 0.46^{a}	2.26 ± 0.63^{a}	1.27 ± 0.04^{a}	5.32 ± 0.95^{b}
1-Butanol, 2-methyl-	1205	1211	ND	0.25 ± 0.07^{b}	ND	0.18 ± 0.01^{a}	0.14 ± 0.02	0.13 ± 0.04	0.13 ± 0.03	0.08 ± 0.01	ND	ND	ND	ND
1-Butanol, 3-methyl-	1208	1212	0.15 ± 0.00^{a}	0.11 ± 0.01^{a}	ND	0.32 ± 0.08^{b}	0.05 ± 0.01^{ab}	0.06 ± 0.02^{ab}	0.10 ± 0.01^{b}	0.30 ± 0.01^{a}	0.40 ± 0.05^{a}	0.5 ± 0.07^{a}	0.94 ± 0.11^{a}	7.63 ± 0.77^{b}
1-Pentanol	1259	1257	4.64 ± 0.75	5.17 ± 0.72	3.89 ± 0.49	4.68 ± 0.96	3.71 ± 0.37	3.48 ± 0.22	4.78 ± 0.83	5.55 ± 1.12	1.91 ± 0.06^{a}	1.87 ± 0.28^{a}	2.12 ± 0.05^{a}	3.19 ± 0.32^{b}
2-Penten-1-ol, (Z)-	1325	1324	0.84 ± 0.02^{a}	0.49 ± 0.03^{a}	0.88 ± 0.07^{a}	1.47 ± 0.23^{b}	1.06 ± 0.18^{b}	1.09 ± 0.17^{b}	1.36 ± 0.06^{b}	1.89 ± 0.39^{a}	0.22 ± 0.06^{a}	0.38 ± 0.09^{a}	0.25 ± 0.00^{a}	0.78 ± 0.03^{b}
1-Hexanol	1359	1358	34.21 ± 7.11^{b}	34.67 ± 4.38^{b}	25.71 ± 2.46^{a}	7.69 ± 0.10^{a}	9.05 ± 3.19	7.67 ± 1.07	7.97 ± 1.14	6.74 ± 1.79	7.05 ± 1.66^{ab}	7.60 ± 1.13^{b}	4.22 ± 0.45^{ab}	3.86 ± 0.11^{a}
3-Hexen-1-ol, (Z)-	1387	1387	ND	ND	ND	ND	0.12 ± 0.03	0.1 ± 0.01	0.18 ± 0.02	0.11 ± 0.02	ND	ND	ND	ND
3-Heptanol, 6-methyl-	1398	1397	ND	ND	ND	ND	0.16 ± 0.14^{b}	0.12 ± 0.01^{ab}	0.10 ± 0.01^{a}	0.12 ± 0.01^{ab}	ND	ND	ND	ND
1-Octen-3-ol	1456	1454	4.79 ± 0.70	5.08 ± 1.63	3.09 ± 0.59	4.51 ± 0.69	40.30 ± 14.09	57.11 ± 13.83	50.23 ± 7.92	42.40 ± 22.21	3.51 ± 0.13	6.40 ± 0.82	4.27 ± 0.66	4.81 ± 0.61
1-Heptanol	1461	1461	1.01 ± 0.10^{a}	1.08 ± 0.14^{a}	0.59 ± 0.06^{b}	0.51 ± 0.10^{a}	0.47 ± 0.19	0.43 ± 0.11	0.43 ± 0.03	0.28 ± 0.10	1.02 ± 0.10^{ab}	1.07 ± 0.19^{b}	0.64 ± 0.13^{a}	0.79 ± 0.11^{ab}
1-Hexanol, 2-ethyl-	1497	1494	0.94 ± 0.12	2.22 ± 0.70	1.92 ± 0.13	1.78 ± 0.63	0.32 ± 0.07	0.25 ± 0.02	0.36 ± 0.01	0.26 ± 0.06	4.01 ± 0.91	4.04 ± 1.20	5.36 ± 1.01	2.99 ± 0.45
2-Hepten-1-ol, (E)-	1517	1516	ND	ND	ND	ND	0.1 ± 0.01^{a}	ND	ND	ND	ND	ND	ND	ND
1-Octanol	1558	1564	1.46 ± 0.36	1.82 ± 0.50	1.88 ± 0.03	1.26 ± 0.11	0.4 ± 0.13^{b}	0.39 ± 0.08^{b}	0.32 ± 0.01^{ab}	0.24 ± 0.05^{a}	1.90 ± 0.23^{b}	1.70 ± 0.19^{ab}	1.15 ± 0.07^{a}	1.78 ± 0.16^{b}
3-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-	1582	1585	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.07 ± 0.19	ND
2-Octen-1-ol, (E)-	1626	1631	ND	ND	ND	ND	5.64 ± 1.58	7.87 ± 1.63	6.17 ± 0.90	7.99 ± 1.23	ND	ND	ND	ND
1-Nonanol	1666	1668	ND	ND	ND	ND	22.05 ± 5.07^{b}	0.11 ± 0.02^{a}	1.45 ± 0.19^{a}	0.04 ± 0.01^{a}	0.83 ± 0.25^{a}	0.51 ± 0.13^{a}	ND	ND
Aldehydes (9)														
Butanal, 2-methyl-	910	909	ND	ND	ND	ND	0.11 ± 0.02^{a}	ND	ND	0.35 ± 0.01^{b}	ND	ND	ND	6.30 ± 1.02^{a}
Butanal, 3-methyl-	908	913	ND	ND	ND	ND	ND	ND	ND	0.69 ± 0.11^{a}	ND	ND	ND	7.72 ± 1.33^{a}
Hexanal	1078	1073	2.45 ± 0.57^{a}	1.53 ± 0.20^{a}	15.8 ± 1.62^{b}	28.94 ± 2.44^{c}	2.73 ± 0.24^{ab}	2.83 ± 0.11^{a}	2.73 ± 0.15^{ab}	4.89 ± 0.52^{b}	1.92 ± 0.18^{a}	1.97 ± 0.28^{a}	1.68 ± 0.20^{a}	4.55 ± 0.66^{b}
Heptanal	1182	1183	1.89 ± 0.38^{ab}	2.07 ± 0.19^{b}	0.88 ± 0.09^{a}	0.87 ± 0.25^{a}	0.16 ± 0.03^{a}	0.25 ± 0.09^{ab}	0.16 ± 0.03^{a}	0.33 ± 0.10^{b}	1.07 ± 0.29^{a}	1.00 ± 0.22^{a}	0.44 ± 0.02^{a}	1.88 ± 0.24^{b}
Octanal	1291	1286	0.75 ± 0.07^{a}	1.84 ± 0.38^{b}	0.58 ± 0.07^{a}	0.37 ± 0.06^{a}	0.14 ± 0.03^{ab}	0.19 ± 0.05^{b}	0.10 ± 0.01^{a}	0.13 ± 0.01^{ab}	0.94 ± 0.25	1.07 ± 0.13	0.56 ± 0.08	1.20 ± 0.41
Nonanal	1397	1392	4.15 ± 0.30^{b}	4.55 ± 0.41^{b}	1.71 ± 0.26^{a}	2.61 ± 0.55^{a}	0.39 ± 0.10^{bc}	0.11 ± 0.02^{a}	0.32 ± 0.09^{b}	0.55 ± 0.12^{a}	3.35 ± 0.67^{a}	3.35 ± 0.43^{a}	2.19 ± 0.46^{a}	5.17 ± 0.98^{b}
2-Octenal, (E)-	1413	1429	ND	ND	ND	ND	0.85 ± 0.08	1.13 ± 0.24	0.86 ± 0.07	0.74 ± 0.16	ND	ND	ND	ND
Benzaldehyde	1520	1525	ND	ND	ND	ND	0.24 ± 0.03^{a}	0.59 ± 0.04^{a}	0.24^{a}	7.51 ± 0.57^{b}	0.64 ± 0.09^{a}	0.52 ± 0.06^{a}	0.45 ± 0.04^{a}	1.74 ± 0.33^{b}
2-Nonenal, (E)-	1538	1540	ND	ND	ND	ND	0.1 ± 0.01^{b}	0.13 ± 0.03^{b}	ND	0.06 ± 0.01a	ND	ND	ND	ND

Values are means ± standard deviations of three individual samples. Mean values with different superscript across the column are significantly different (p> 0.05).

ND, Not detected; RIL, retention index; RIC, retention indices and their mass spectra with NIST mass spectral library.

Table 3.2 (continued)

Compound name by class	RIL	RIC	Muscle				Gill				Skin			
			0	3	6	9	0	3	6	9	0	3	6	9
Ketones (8)														
2-Butanone	904	900	0.49 ± 0.08^{a}	0.47 ± 0.08^{a}	ND	ND	0.14 ± 0.02^{b}	0.15 ± 0.03^{b}	ND	0.09 ± 0.01^{a}	ND	ND	ND	ND
3-Pentanone	974	971	0.83 ± 0.01^{a}	1.49 ± 0.07^{a}	0.81 ± 0.11^{a}	3.15 ± 0.32^{b}	0.76 ± 0.07	0.71 ± 0.08	0.98 ± 0.03	3.07 ± 1.06	ND	ND	ND	ND
2,3-Pentanedione	1054	1053	0.78 ± 0.12	0.63 ± 0.02	1.03 ± 0.19	1.05 ± 0.14	0.11 ± 0.11^{a}	0.14 ± 0.01^{a}	0.23 ± 0.03^{ab}	0.37 ± 0.05^{b}	ND	ND	ND	ND
2-Heptanone	1184	1181	0.27 ± 0.03	0.35 ± 0.01	0.37 ± 0.03	0.28 ± 0.06	0.18 ± 0.08	0.13 ± 0.01	0.15 ± 0.01	0.16 ± 0.03	ND	ND	ND	ND
2-Heptanone, 6-methyl-	1237	1236	ND	ND	ND	ND	0.15 ± 0.04^{a}	0.12 ± 0.02^{a}	ND	ND	ND	ND	ND	ND
3-Octanone	1253	1253	0.26 ± 0.01^{a}	0.26 ± 0.06^{a}	ND	0.31 ± 0.02^{a}	1.20 ± 0.47^{ab}	1.18 ± 0.19^{ab}	0.96 ± 0.10^{a}	1.55 ± 0.29^{b}	0.23 ± 0.05^{a}	0.32 ± 0.04^{b}	ND	0.31 ± 0.01^{b}
Cyclohexanone	1285	1282	1.9 ± 0.13^{a}	2.12 ± 0.42^{a}	2.55 ± 0.19^{a}	3.34 ± 0.54^{b}	0.22 ± 0.05^{a}	0.3 ± 0.04^{a}	0.44 ± 0.02^{b}	0.55 ± 0.12^{b}	2.15 ± 0.15^{a}	3.45 ± 0.29^{b}	3.66 ± 0.16^{b}	6.91 ± 0.14^{c}
1-Hepten-3-one	1298	1298	ND	ND	ND	ND	1.25 ± 0.23	1.16 ± 0.00	1.09 ± 0.18	0.75 ± 0.07	ND	ND	ND	ND
Furans (2)														
Furan, 2-ethyl-	944	946	ND	ND	ND	ND	0.42 ± 0.14^{a}	0.61 ± 0.09^{b}	0.37 ± 0.03^{a}	0.55 ± 0.19^{ab}	ND	ND	ND	ND
Furan, 2-pentyl-	1231	1230	ND	ND	ND	ND	0.13 ± 0.02	0.14 ± 0.02	0.11 ± 0.02	0.13 ± 0.01	ND	ND	ND	ND
Acid (1)														
Butyrolactone (Butanoic acid, 4-hydroxy-)	1635	1637	1.21 ± 0.09^{b}	0.35 ± 0.08^{a}	0.41 ± 0.07^{a}	1.08 ± 0.11^{b}	0.18 ± 0.10	0.07 ± 0.01	0.10 ± 0.02	0.10 ± 0.01	1.66 ± 0.14^{b}	0.54 ± 0.01^{a}	0.57 ± 0.04^{a}	0.90 ± 0.13^{a}
Ester (1)														
Hexanoic acid, ethyl ester	1233	1234	ND	ND	ND	ND	0.11 ± 0.04	0.09 ± 0.01	0.06 ± 0.01	0.13 ± 0.02	ND	ND	ND	ND
Hydrocarbons (5)														
2,4-Octadiene	925	925	ND	ND	ND	ND	ND	0.07 ± 0.00^{a}	ND	0.20 ± 0.05^{b}	ND	ND	ND	ND
Decane	1000	999	0.76 ± 0.08^{a}	ND	ND	ND	0.1 ± 0.01^{a}	0.09 ± 0.00^{b}	0.2 ± 0.02^{a}	0.21 ± 0.04^{b}	5.32 ± 0.54^{b}	2.10 ± 0.42^{a}	2.52 ± 0.15^{a}	4.67 ± 0.97^{b}
1,3-trans,5-cis-Octatriene	1097	1096	ND	ND	ND	ND	0.34 ± 0.07^{a}	0.36 ± 0.05^{a}	0.58 ± 0.06^{b}	0.31 ± 0.10^{a}	ND	ND	ND	ND
Dodecane	1120	1197	ND	ND	ND	ND	ND	ND	ND	ND	1.96 ± 0.36^{a}	1.01 ± 0.13^{a}	1.14 ± 0.12^{a}	3.63 ± 0.89^{b}
Pentadecane	1500	1498	0.53 ± 0.06^{a}	1.46 ± 0.23^{b}	4.92 ± 0.28^{d}	3.18 ± 0.51^{c}	0.21 ± 0.03^{a}	0.29 ± 0.08^{a}	0.90 ± 0.10^{b}	0.34 ± 0.08^{a}	1.13 ± 0.17^{a}	2.51 ± 0.51^{a}	6.74 ± 0.57^{b}	8.37 ± 1.42^{b}
Aromatic compounds (6)														
Benzene	938	932	ND	ND	ND	ND	0.38 ± 0.10	0.27 ± 0.08	0.34 ± 0.04	0.34 ± 0.07	ND	ND	ND	ND
Trichloromethane	1015	1015	29.17 ± 4.29	25.42 ± 8.57	16.49 ± 2.06	22.56 ± 11.86	0.50 ± 0.14^{a}	4.43 ± 1.06^{b}	2.86 ± 0.23^{ab}	0.93 ± 0.10^{a}	46.85 ± 3.01^{b}	44.38 ± 0.15^{b}	41.62 ± 10.87^{b}	3.68 ± 0.54^{a}
Toluene	1037	1026	4.43 ± 0.79^{a}	3.65 ± 0.18^{a}	10.87 ± 2.26^{b}	5.14 ± 0.62^{a}	1.17 ± 0.34^{ab}	0.46 ± 0.05^{a}	3.32 ± 1.06^{ab}	3.37 ± 1.04^{b}	7.91 ± 0.50^{a}	5.57 ± 0.30^{a}	14.34 ± 2.76^{b}	9.14 ± 0.65^{a}
Styrene	1256	1251	0.14 ± 0.03^{a}	0.52 ± 0.05^{bc}	0.75 ± 0.17^{c}	0.27 ± 0.01^{ab}	ND	ND	ND	ND	0.25 ± 0.02^{a}	1.1 ± 0.16^{b}	1.21 ± 0.19^{b}	1.02 ± 0.03^{b}
Benzene, 1,2,4-trimethyl-	1275	1274	ND	ND	ND	ND	ND	ND	0.04 ± 0.00^{a}	0.20 ± 0.00^{b}	0.32 ± 0.02^{a}	0.42 ± 0.05^{a}	0.30 ± 0.01^{a}	1.13 ± 0.29^{b}
Benzene, 1,4-dichloro-	1446	1443	ND	ND	ND	ND	ND	ND	ND	ND	1.18 ± 0.06^{a}	0.92 ± 0.15^{a}	1.09 ± 0.21^{a}	ND

Values are means ± standard deviations of three individual samples. Mean values with different superscript across the column are significantly different ($p > 0.05$).

ND, Not detected; RIL, retention index; RIC, retention indices and their mass spectra with NIST mass spectral library.

Aldehydes are essential compounds giving characteristic odors, both pleasant and unpleasant, to foods. Their odor threshold values are typically lower than those of alcohols. As a result, even trace levels of aldehydes have the potential to dominate flavor of other compounds (Wang et al., 2019). 3-Methylbutanal and benzaldehyde were detected in gill and skin with the highest concentration at day 9. They are likely to produce from Strecker degradation of leucine and phenyl glycine, respectively (Mu et al., 2017). The predominant aldehydes found in tilapia tissues are hexanal, heptanal, and nonanal. Hexanal was mostly derived from the oxidation of linoleic acid (Kawai, 1996) and related to degradation of volatile compounds namely (E)-2-octenal (Josephson and Lindsay, 1987; Koelsch, Downes, and Labuza, 2006), and the levels of lipid oxidation and flavor production were reflected by its contents. Our findings are remarkably similar to those of Miyasaki, Hamaguchi, and Yokoyama (2011) who reported that hexanal in fish meat was detected early on and that the concentration of hexanal rapidly increased during ice storage. 3-Methylbutanal and hexanal both contribute to the smell of rabbit fish skin, muscle, viscera, and stomach contents (Jiarpinijnun et al, 2022). Heptanal and nonanal are generated from the oxidation of oleic acid and linoleic acid. Heptanal is the major volatile compound detected in the Asian seabass muscle and has been provided as an indicator of flavor deteriorations for fish products (Maqsood and Benjakul, 2011; Augustin, Sanguansri, and Bode, 2006). Another detected compound, (E)-2-octenal and (E)-2-nonenal which are produced by LOX on arachidonic acid. Nonanal, octanal, and (E) -2-octenal were the most powerful contributors to the fishy odor of tilapia (Liu, Hu, and Li, 2014).

The main ketone identified in our study is cyclohexanone. Enzymatic degradation of polyunsaturated fatty acids, amino acids, or microbial oxidation generate ketones (Survey, Pan, and Sahidi, 1998). Gill had the highest concentrations of ketone compounds, followed by the muscles and skin. 2,3-Pentanedione was identified in gill and muscle. Iglesias and Medida (2008) reported that 2,3-Pentanedione as an indicator of lipid oxidation in chill fish muscle.

Furan compounds were detected and identified only in the gill of tilapia including 2-ethyl furan and 2-pentyl furan. Furans and their derivatives could be produced through the oxidation of fatty acids. A mechanism for the formation of 2-ethylfuran in fish muscle has previously been proposed (Medina et al., 1999), including

omega-3 fatty acid β-oxidation to generate conjugated dienes radicals, which can react with oxygen to produce vinyl hydroperoxides; cleavage of vinyl hydroperoxide results in the formation of an alkoxyl radical, which proceeds cyclization, to form 2-ethylfuran (Medina et al., 1999). 2-Ethylfuran was detected to be the highest concentration after 3 days of ice storage. 2-Ethylfuran has been reported previously in hilsa and was found to be highest after ice storage of 168 h, indicating that the unfavorable fishy odor increased during storage due to lipid oxidation (Ganguly, Mahanty, Mitra, Raman, and Mohanty 2017; Leduc et al., 2012). 2-Ethylfuran has been considered to contribute to the distinctive sardine-fishy odor (Prost et al., 2004). Besides 2-ethylfuran, 2-pentylfuran is a well-known autoxidation product from linoleic acid and has low threshold values (Giri et al., 2010). 2-Ethylfuran and 2-pentylfuran were the main contributors to the aromatic flavor of the short-necked clam hydrolysate (Chen et al., 2016).

Hydrocarbons and aromatic compounds were also identified in our study. Although their presence has been reported in a variety of fish, the origin of these compounds has not been fully elucidated (Ganguly et al., 2017; Iglesias and Meida, 2008; Wang et al., 2019). Hydrocarbons had high sensory thresholds, thus they contribute less to the overall odor formation of fish (Zhou, Chong, Ding, Gu, and Liu, 2016).

3.4.4 Principal component Analysis (PCA)

In muscle, the first two components of PCA explained 65.35% of the variation (Figure 3.4). According to this PCA, volatile compounds were grouped by storage time. 2-Butanone, hexanal, 1-butanol, 1-penten-3-ol, heptanal, nonanal, pentadecane were closely related to PC1 while 3-methyl-1-butanol, 3-pentanone, 3-octanone, 1-heptanol were closely related to PC2. 2-Butanone and nonanal are the major volatile compounds present in 0 day. 2-Butanone was detected in day 0 and and disappeared after 3 day of storages, while nonanal was detected and its quantities decreased with increasing time period of storage. Nonanal contributes to fatty, green, fish-like odors and is found in some fish species, especially freshwater species (Jones et al., 2022). Thus, 2-butanone and nonanal can be considered a freshness indicators of tilapia muscle. 1-Penten-3-ol, hexanal and 3-methyl-1-butanol are the major volatile compounds present in day 9, which showed positive correlation and their relative quantities increased with increasing time storage ($p < 0.05$). 1-Penten-3-ol is established

when lipoxygenase reacts with EPA and hydroperoxide lyases which is used as a marker of lipid oxidation in Atlantic horse mackerel (Iglesias and Medina, 2008). Linoleic acid was primarily oxidized to produce hexanal (Kawai, 1996). According to Miyasaki, Hamaguchi, and Yokoyama (2011), hexanal was detected early in fish meat, and its concentration increased rapidly during ice storage. 3-Methyl-1-butanol has an offensive odor and is derived from the oxidative deamination of leucine by bacteria predominately responsible for spoilage (Wang, Chen, Zhang, Wang, and Shi, 2019). In this study, these compounds were potential markers for measuring the degree of lipid oxidation in tilapia muscle.

In gill, the principle component 1(PC1) explained 37.62% and principle component 2 (PC2) explained 20.28% of total variance (Figure 3.5). According to this PCA, volatile compounds were grouped according to storage time, which appeared in three quadrants: 6 days; 0 day and 3 days; 9 days. The volatile compounds 2-methyl-butanal, 3-methyl-butanal, 2,4-octadiene, 3-pentanone, decane, 2,3-pentanedione, hexanal, heptanal, 6-methyl-2-heptanone, 3-octnone, 1-pentanol, 1,2,4-trimethyl benzene, cyclohexanone, 2-penten-1-ol, nonanal, 1-haptanol, benzaldehyde, 1-octanol were closely related to PC1 while 2-butanone, 1,3-trans,5-cis-octatriene, 1-penten-3-ol, 3-methyl-1-butanol, 2-pentyl-furan, octanal, 3-hexen-1-ol, 6-methyl-3-heptanol, pentadecane, 2-nonenal were closely related to PC2. The most abundant volatile compounds in the third quadrant are 6-methyl-2-heptanone and 2-nonenal (0 day and 3 days). These volatiles were detected between 0 and 3 days and were no longer detectable after 3 days of storage. 2-Nonanal is generated by LOX on arachidonic acid and contributes to tilapia's fishy odor (Qian, Fei, and Fan, 2012). Therefore, these can be regarded as indicators of the freshness of tilapia gill. 3-Methyl-butanal, 2,4-octadiene and hexanal are the major volatiles present in day 9 which showed positive correlation with each other. As a result, these volatiles could be used to track the lipid oxidation of tilapia gill during ice storage.

In skin, the first two components of PCA explained 74.69 % of the variation (Figure 3.6). According to this PCA, volatile compounds were grouped by storage time.2-Methylbutanal, 3-methylbutanal, hexanal, 1-penten-3-ol, heptanal, 3-methyl-1-butanol, 1-pentanol, cyclohexanone, 2-penten-1-ol, nonanal, benzaldehyde were closely related to PC1. While 3-octanone, 1-heptanol, 1-octanol, 3-cyclohexen-1-ol,

butyrolactone, 1-nonanol were closely related to PC2. The PCA biplot revealed the correlation between various volatile compounds and also explains the overall variation in the data sets. 1-Heptanol and 1-nonanol are the major volatile compounds present in day 0 and 3. These two compounds detected in day 0 and their relative quantities decreased as ice storage time increased. While 1-nonanol disappeared after 3 days of storage, suggesting it as freshness indicator for tilapia skin. 2-Methylbutanal, 3-methylbutanal, hexanal, 1-penten-3-ol, heptanal, 3-methyl-1-butanol,2-penten-1-ol, and nonanal are the major volatiles, showing in day 9. 2-Methylbutanal and 3-methylbutanal were detected at 9 days of storage and they are produced from Strecker degradation of leucine (Mu et al, 2017). Hexanal, 1-penten-3-ol, heptanal, 3-methyl-1-butanol,2-penten-1-ol, heptanal and nonanal appeared after 0 day of storage, and their amount increased with storage time.

The first two components of PCA explained 65.64% of the variation (Figure 3.7). The muscle, gill and skin are clearly separated in different quadrants. The skin sample was characterized by peroxide values and the main volatile compounds, namely 1-3-methyl butanol, and 2-methyl butanal, which increased with the storage time. It should be noted that the effect of storage time on the measured parameters is well correlated for the skin sample. Our study suggests that the skin should be a target tissue for monitoring freshness quality of tilapia during ice storage. The gill was characterized by LOX activity, PUFA contents, and volatile compounds, particularly furan, 2-pentyl-, 2-octenal, 3-heptanol, 6 methyl-, and 2-octen-1-ol. The skin and muscle samples are positioned in the opposite PCA quadrant from the gill sample, indicating lower levels of PUFA contents, LOX activity, and associated volatiles in muscle. Our study indicates that gill is the main tissue of lipid oxidation induced by LOX during ice storage. Furan, 2-pentyl-, 3-heptanol, 6-methyl-, 2-octenal, (E)-, 1-octen-3-ol and 2-octen-1-ol are the main volatile compounds in tilapia gill.

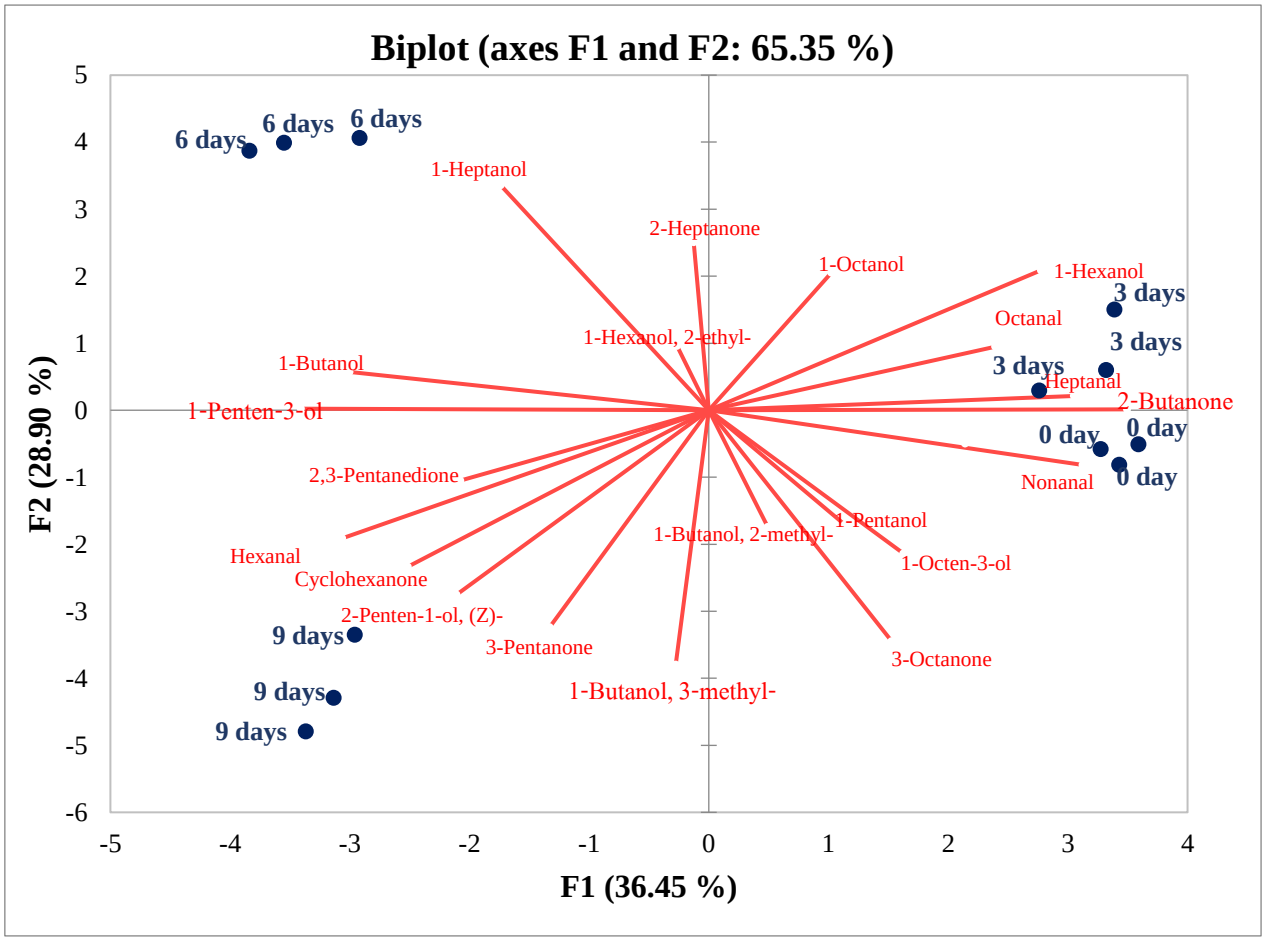

Figure 3.4 Principal component analysis (PCA) biplot of volatile compounds present in muscle of tilapia in different storage times assessed by SPME-GC/MS.

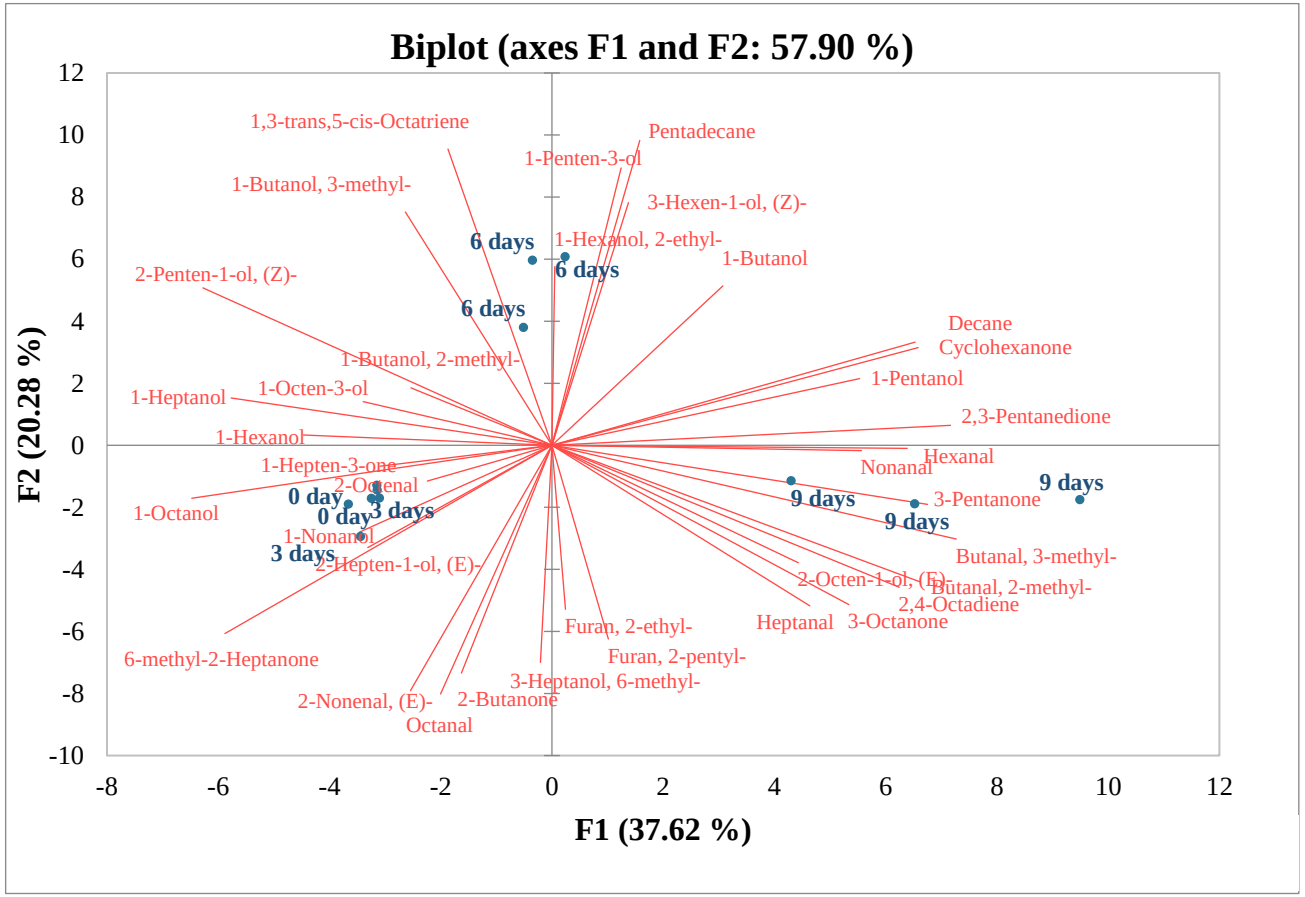

Figure 3.5 Principal component analysis (PCA) biplot of volatile compounds present in gill of tilapia in different storage times assessed by SPME-GC/MS.

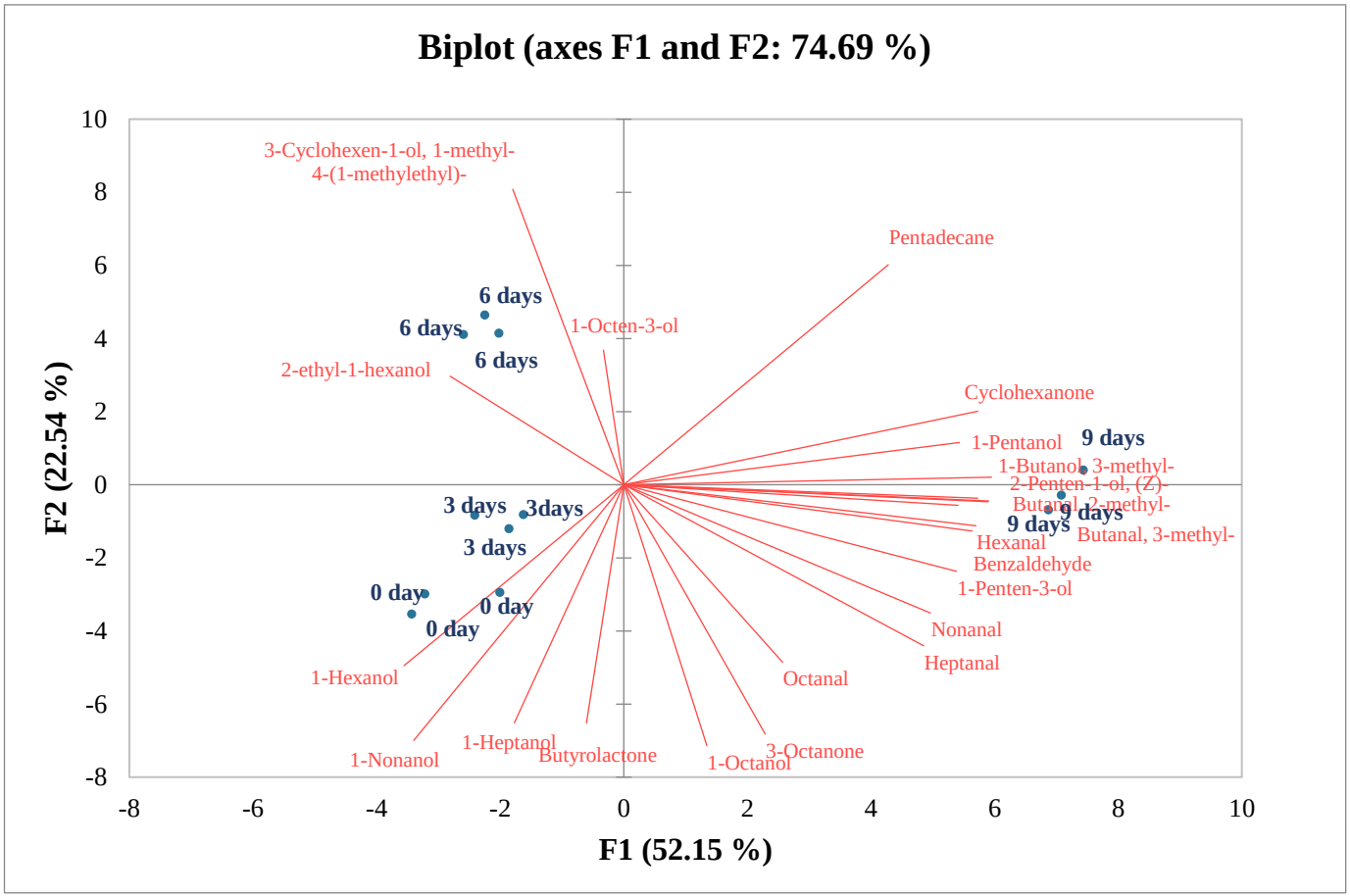

Figure 3.6 Principal component analysis (PCA) biplot of volatile compounds present in skin of tilapia in different storage times assessed by SPME-GC/MS.

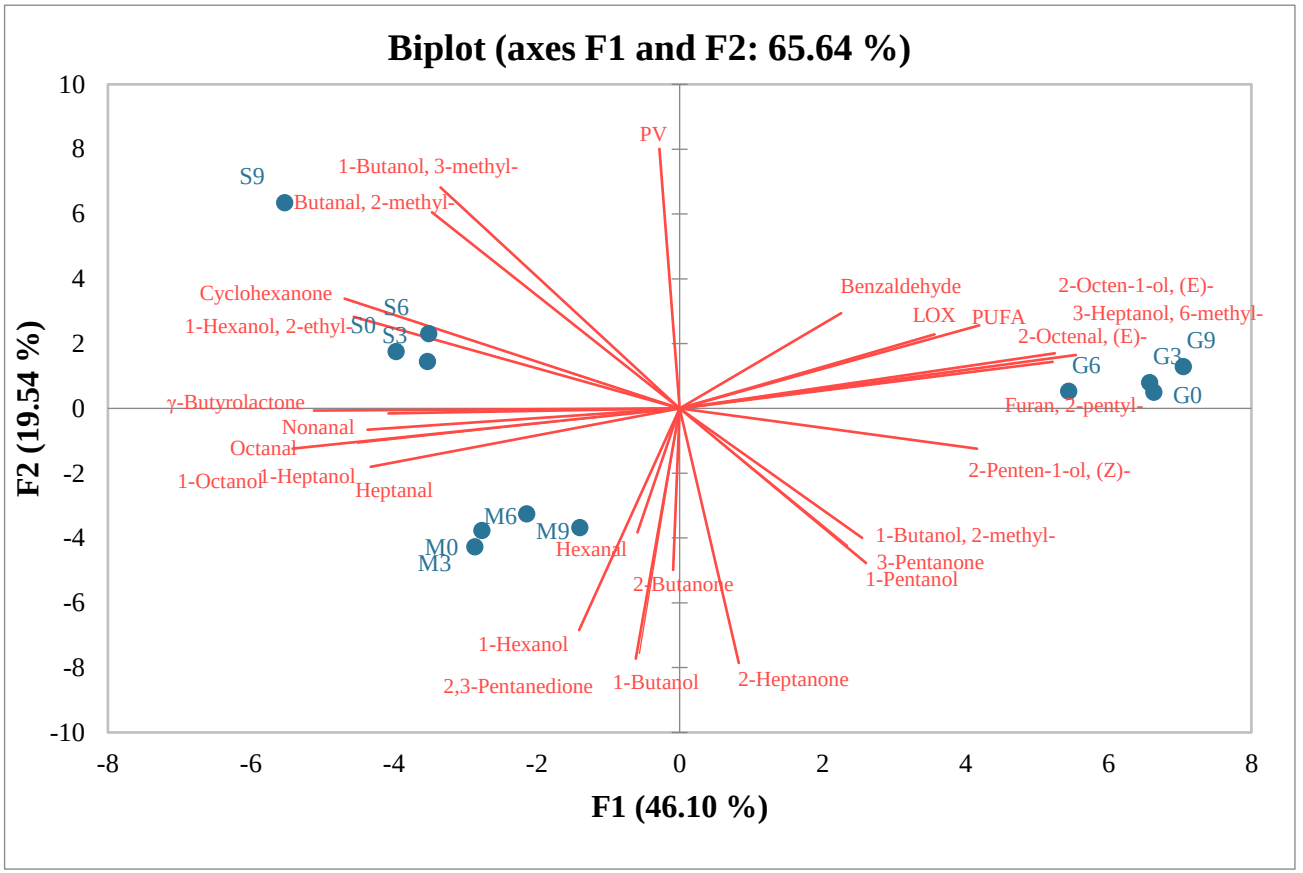

Figure 3.7 Principal component analysis (PCA) biplot describing measured quality parameters namely volatile compounds, peroxide values, lipoxygenase activity, and PUFA of muscle (M), gill (G), and skin (S) of tilapia stored in ice at different times 0, 3, 6 and 9 days. Each point is the average of three measurements of samples from a batch of three fishes. LOX, lipoxygenase activity; PV, peroxide value; PUFA, polyunsaturated fatty acids.

3.5 Conclusions

Lipid oxidation of tilapia differed among body parts. During prolonged ice storage, the gill of tilapia was the most prone to lipid oxidation. Free fatty acid composition decreased as the storage time was extended. The activity of LOX was relatively high during storage and LOX could catalyze oxidation of free fatty acids, suggesting that it was an important enzyme to promote volatile substances. The majority of volatile compounds exhibited statistically significant differences during ice storage when compared to their initial values, indicating that ice storage affected the volatile nature of tilapia. According to the PCA, 2-butanone and nonanal in muscle, 6-methyl-2-haptanone and 2-nonanal in gill, and 1-haptanol, and 1-nonanol in skin have the potential to be used as freshness indicators and hexanal was used to track the lipid oxidation of tilapia during ice storage. To preserve the quality of tilapia, gills should be removed prior to ice storage and filleting.

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