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Changes of lipids in sardine (*Sardinella gibbosa*) muscle during iced storage

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Abstract

Changes in lipids of sardine (*Sardinella gibbosa*) muscle during 15 days of iced storage were investigated. Lipid deterioration, lipolysis and lipid oxidation, occurred throughout the storage. The progressive peroxide formation was monitored by the increase in the absorbance band at 3600–3200 cm^{-1} in Fourier transform infrared (FTIR) spectra and increased peroxide values were observed in sardine muscle up to 6 days of iced storage, followed by a continuous decrease from then for 9 days ($P < 0.05$). The increase in thiobarbituric acid reactive substances (TBARS) was noticeable throughout the iced storage ($P < 0.05$). However, no difference in conjugated diene (CD) of sardine muscle was found within the first 12 days of iced storage ($P > 0.05$). Marked decreases in unsaturated fatty acids, especially eicosapentaenoic acid (EPA; C20:5($n - 3$)) and docosahexaenoic acid (DHA; C22:6($n - 3$)) were observed as the storage time increased. Those changes indicated that lipid oxidation occurred in sardine muscle. A gradual increase in free fatty acid formation, with decreases in triglyceride and phospholipid contents, was found during iced storage ($P < 0.05$), suggesting hydrolysis induced by lipases and phospholipases.

Keywords: Lipid oxidation; Lipolysis; Muscle; Sardine; Iced storage

1. Introduction

Fresh fish is susceptible to spoilage caused by both microbiological and chemical reactions. Lipid deterioration easily takes place and limits the shelf-life of oily fish during storage (Cho, Endo, Fujimoto, & Kaneda, 1989; McDonald & Hultin, 1987). Both hydrolytic and oxidative rancidities in fish muscle are associated with quality deterioration (Pacheco-Aguilar, Lugo-Sanchez, & Robles-Burgueno, 2000). Hydrolysis, induced by lipases and phospholipases, produces free fatty acids that undergo further oxidation to produce low-molecular-weight compounds that are responsible for the rancid off-flavour and taste of fish and fish products (Toyomizu, Hanaoka, & Yamaguchi, 1981).

The lipid components of *post-mortem* fish muscle tissue are prone to oxidation because fatty acids of fish lipids are much more unsaturated than those of mammals and birds (Foegeding, Lanier, & Hultin, 1996). The basic mechanisms of lipid oxidative reactions can be characterised by three distinctive steps: initiation, propagation and termination reactions (Nawar, 1996; Underland, 2001). This phenomenon can be influenced by both intrinsic and extrinsic factors, such as the fatty acid composition, the concentration of pro-oxidants, endogenous ferrous iron, myoglobin, enzymes, pH, temperature, ionic strength and oxygen consumption (Andreo, Doval, Romero, & Judis, 2003; Harris & Tall,

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1994a; Renerre & Labas, 1987; Underland, 2001). Metmyoglobin formation is positively correlated with lipid oxidation (Chan, Faustman, Yin, & Decker, 1997; Lee, Phillips, Liebler, & Faustman, 2003). Myoglobin and other heme compounds, at high concentration in red meats, function as prooxidants in muscle tissue (Love, 1983). Chaijan, Benjakul, Visessanguan, and Faustman (2005) reported that the release of non-heme iron in sardine and mackerel during iced storage might be associated with the induced oxidation process in the muscle. Enzymatic and non-enzymatic reducing systems, converting iron from the inactive ferric form to the active ferrous state, can accelerate oxidation of meat (Foegeding et al., 1996). In addition, membrane components, such as cytochromes or non-heme iron proteins, are able to convert species, such as superoxyl radicals into more reactive species, such as the hydroxyl radical (Hultin & Kelleher, 2000). The conversion of superoxide ($O_2^{\bullet -}$) to the more reactive $HOO^{\bullet}$ favours the oxidative reactions (McDonald & Hultin, 1987).

Due to the lack of lean fish, which are commonly used for surimi production, dark-fleshed fish, such as sardine, have recently received more attention as the raw material for surimi production (Chaijan, Benjakul, Visessanguan, & Faustman, 2004). However, the large quantity of lipids and myoglobin in the muscle tissue limits the production of surimi, from those fatty dark-fleshed fish. During *post-mortem* handling or storage, several reactions take place, particularly lipid deterioration (Cho et al., 1989; McDonald & Hultin, 1987). Recently, sardine myoglobin extractability has been reported to be lowered during extended iced storage (Chaijan et al., 2005). This might be associated with increased lipid oxidation and the occurrence of oxidation products, such as aldehydes, associated with protein cross-linking (Li & King, 1999; Tironi, Tomas, & Anon, 2002). However, no information regarding the changes in lipid in muscle of sardine caught in Thailand has been reported. Thus, this study aimed to investigate the changes of lipid components, lipolysis and lipid oxidation, in whole sardine (*Sardinella gibbosa*) meat during extended storage in ice.

2. Materials and methods

2.1. Chemicals

Palmitic acid, cupric acetate and pyridine were purchased from Sigma (St. Louis. MO, USA). Sodium thiosulfate, potassium iodide, trichloroacetic acid, anhydrous sodium sulfate, isooctane and benzene were obtained from Merck (Darmstadt, Germany). Chloroform was purchased from BDH (Poole, England). 2-Thiobarbituric acid and 1,1,3,3-tetramethoxypropane were procured from Fluka (Buchs, Switzerland). Methanol and acetic acid were obtained from Lab-Scan (Bangkok, Thailand).

2.2. Fish samples

Sardine (*Sardinella gibbosa*), with an average weight of 55–60 g, was caught from Songkhla-Pattani Coast along the Gulf of Thailand in November, 2004. The fish, off-loaded approximately 12 h after capture, were placed in ice with a fish/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai within 2 h. Whole fish were immediately washed and kept in ice with a fish/ice ratio of 1:2 (w/w). The fish were placed and distributed uniformly between the layers of ice. The box containing fish and ice was kept at 4 °C for 15 days. To maintain the ice content, melted ice was removed and replaced with an equal amount of ice every 2 days. During storage, 1 kg of fish was randomly taken as the composite sample at day 0, 3, 6, 9, 12 and 15 for analyses. The fish samples were washed and filleted. The flesh was chopped to uniformity and used for analyses.

2.3. Lipid extraction

Lipid was extracted by the Bligh and Dyer method (1959). Sample (25 g) was homogenised with 200 ml of a chloroform:methanol:distilled water mixture (50:100:50) at the speed of 9500 rpm for 2 min at 4 °C using an IKA Labortechnik homogeniser (Selangor, Malaysia). The homogenate was treated with 50 ml of chloroform and homogenised at 9500 rpm for 1 min. Then, 25 ml of distilled water were added and the homogenised again for 30 s. The homogenate was centrifuged at 3000 rpm at 4 °C for 15 min using a RC-5B plus centrifuge (Sorvall, Norwalk, CT, USA), and transferred into a separating flask. The chloroform phase was drained off into a 125 ml Erlenmeyer flask containing about 2–5 g of anhydrous sodium sulfate, shaken very well, and decanted into a round-bottom flask through a Whatman No. 4 filter paper. The solvent was evaporated at 25 °C, using an EYELA rotary evaporator N-100 (Tokyo, Japan), and the residual solvent was removed by flushing with nitrogen. The total lipid content ranged from 4.62% to 6.70%.

2.4. Measurement of peroxide value (PV)

Peroxide value was determined according to the method of Low and Ng (1978). The lipid sample (1.0 g) was treated with 25 ml of organic solvent mixture (chloroform:acetic acid mixture, 2:3). The mixture was shaken vigorously, followed by addition of 1 ml of saturated potassium iodide solution. The mixture was kept in the dark for 5 min and 75 ml of distilled water were added and the mixture was shaken. To the mixture, 0.5 ml of starch solution (1%, w/v) was added as an indicator. The peroxide value was determined by titrating the iodine liberated from potassium iodide with standardised

0.01 N sodium thiosulfate solution. The PV was expressed as milliequivalents of free iodine per kg of lipid.

2.5. Measurement of conjugated diene (CD)

Conjugated diene was measured according to the method of Frankel and Huang (1996). Oil sample (0.1 g) was dissolved in 5.0 ml of isooctane and the absorbance was measured at 234 nm using a UV-1601 spectrophotometer (Shimadzu, Japan).

2.6. Measurement of thiobarbituric acid-reactive substances (TBARS)

Thiobarbituric acid-reactive substances (TBARS) assay was performed as described by Buege and Aust (1978). Ground sample (0.5 g) was homogenised with 2.5 ml of a solution containing 0.375% thiobarbituric acid, 15% trichloroacetic acid and 0.25 N HCl, using an IKA Labortechnik homogeniser (Selangor, Malaysia). The mixture was heated in a boiling water bath (95–100 °C) for 10 min to develop a pink colour, cooled with running tap water and centrifuged at 3600*g* at 25 °C for 20 min using a RC-5B plus centrifuge (Sorvall, Norwalk, CT, USA). The absorbance of the supernatant was measured at 532 nm. A standard curve was prepared using 1,1,3,3-tetramethoxypropane at concentrations ranging from 0 to 10 ppm. TBARS was calculated and expressed as mg malonaldehyde/kg sample.

2.7. Measurement of free fatty acid (FFA)

Free fatty acid content was determined according to the method of Lowry and Tinsley (1976). The lipid sample (0.1 g) was treated with 5 ml of isooctane and swirled vigorously to dissolve the sample. The mixture was then treated with 1 ml of 5% (w/v) cupric acetate–pyridine reagent, prepared by dissolving reagent grade cupric acetate, filtering and adjusting the pH to 6.0–6.2 using pyridine. The mixture was shaken vigorously for 90 s using a Vortex-Genie2 mixer (Bohemia, NY, USA) and allowed to stand for 10–20 s. The upper layer was subjected to absorbance measurement at 715 nm, using a UV-1601 spectrophotometer (Shimadzu, Japan). A standard curve was prepared using palmitic acid in isooctane at concentrations ranging from 0 to 50 μmol/5 ml. FFA content was expressed as g FFA/100 g lipid.

2.8. Lipid compositions

Lipid classes were determined using a thin layer chromatography/flame ionisation detection analyser (IATROSCAN® TLC/FID Analyser, IATRON Laboratories, Inc., Tokyo, Japan). One μl of lipid sample (0.25 mg/ml) was spotted onto the scanned quartz rod (silica powder coated Chrmatorod-S III, IATRON Laboratories, Inc., Tokyo, Japan) and separated using a mixture of benzene:chloroform:acetic acid (50:20:0.7) for 35 min. The developed sample was dried in an oven at 105 °C for 5 min and immediately scanned with the TLC-FID analyser with a scanning speed of 30 s/scan. The analytical conditions were: H_2, flow rate of 160 ml/min; air, flow rate of 2000 ml/min. Retention times of lipid standards were used to identify chromatographic peaks of the samples. Each lipid content was calculated, based on peak area ratio and expressed as mg lipid/kg meat.

2.9. Fatty acid profile

Fatty acid profile was determined as fatty acid methyl esters (FAMEs). The FAMEs were prepared according to the method of AOAC (2000). The prepared methyl ester was injected to the gas chromatography (Shimadzu, Kyoto, Japan) equipped with the flame ionisation detector (FID) at a split ratio of 1:20. A fused silica capillary column (30 m × 0.25 mm), coated with bonded polyglycol liquid phase, was used. The analytical conditions were: injection port temperature of 250 °C and detector temperature of 270 °C. The oven was programmed from 170 to 225 °C at a rate of 1 °C/min (no initial or final hold). Retention times of FAME standards were used to identify chromatographic peaks of the samples. Fatty acid content was calculated, based on the peak area ratio and expressed as g fatty acid/100 g oil.

2.10. Fourier transform infrared (FTIR) spectra analysis

FTIR analysis of crude sardine oil was performed in a horizontal ATR Trough plate crystal cell (45° ZnSe; 80 mm long, 10 mm wide and 4 mm thick) (PIKE Technology, Inc., Madison, WI) equipped with a Bruker Model Vector 33 FTIR spectrometer (Bruker Co., Ettlingen, Germany). Prior to analysis, the crystal cell was cleaned with acetone, wiped dry with soft tissue and the background scan was run. For spectra analysis, oil sample (200 μl) was applied directly onto the crystal cell and the cell was clamped into the mount of the FTIR spectrometer. The spectra, in the range of 4000–400 cm^{-1} (mid-IR region) with automatic signal gain, were collected in 16 scans at a resolution of 4 cm^{-1} and were ratioed against a background spectrum recorded from the clean, empty cell at 25 °C. Analysis of spectral data was carried out using the OPUS 3.0 data collection software programme (Bruker Co., Ettlingen, Germany).

2.11. Statistical analysis

Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple range test (Steel & Torrie, 1980). Statistical analysis was performed using the Statistical Package

for Social Science (SPSS 11.0 for windows, SPSS Inc., Chicago, IL).

3. Results and discussion

3.1. Changes in PV

Marked increase in PV was observed in sardine muscle throughout the iced storage up to 6 days ($P < 0.05$) (Fig. 1). No changes in PV were found from days 6 to 9 ($P > 0.05$). Thereafter, a decrease in PV was noticeable ($P < 0.05$). The decreased PV observed with extended storage time was presumed to be due to the decomposition of hydroperoxide. Hydroperoxides break down in several steps, yielding a wide variety of decomposition products, including aldehydes (Nawar, 1996). From the result, the lipid oxidation occurred in sardine muscle rapidly during iced storage, probably due to the high content of unsaturated fatty acids and prooxidants in the muscle, especially dark muscle. Pacheco-Aguilar et al. (2000) reported that the shelf life of oily Monterey sardine was limited by lipid oxidation, as shown by the increase of PV during storage at 0 °C up to 15 days. Myoglobin and other heme compounds in red meats functioned as prooxidants in muscle tissue (Han, Mcmillin, & Godber, 1994; Love, 1983). A high concentration of polyunsaturated fatty acids made Monterey sardine susceptible to oxidation (Pacheco-Aguilar et al., 2000). Lipid oxidation is a complex process in which unsaturated fatty acids react with molecular oxygen, usually via a free radical mechanism, to form hydroperoxides, the primary oxidation products (Simic & Taylor, 1987). Apart from plenty of unsaturated fatty acids, heme protein, as well as reactive iron in the muscle, might contribute to the accelerated oxidation of sardine. Kisia (1996) reported that sardine contained more dark muscle, and greater quantities of mitochondria, myoglobin, fats, glycogen and cytochromes, than did with white fleshed-fish species. Dark muscle from sardine had a much greater pigment content than ordinary muscle (Chaijan et al., 2005). The release of non-heme iron in sardine muscle during extended iced storage might enhance the oxidation process in the muscle (Chaijan et al., 2005).

3.2. Changes in CD

No difference in CD of sardine muscle was observed within the first 12 days of iced storage ($P > 0.05$) (Fig. 2). From this result, it was presumed that the formation and decomposition of CD took place at equal rates. After 15 days of iced storage, lipid oxidation proceeded to a great extent, as evidenced by the slight increase in CD. The rate of CD formation could be greater than the decomposition rate, leading to increase in CD accumulated in the lipid fraction. Almost immediately after peroxides are formed, the non-conjugated double bonds (C=C–C–C=C) that are present in natural unsaturated lipids are converted to conjugated double bonds (C=C–C=C) (Gunstone & Norris, 1983). Pérez-Alonso, Arias, and Aubourg (2003) reported no changes in CD in dorsal muscle of Atlantic pomfret within the first 9 days of chilled storage, followed by a gradual increase up to 19 days of storage. For horse mackerel (*Trachurus trachurus*), CD formation showed no clear trend during frozen storage with or without previous chilled storage (Aubourg, Lehmann, & Gallardo, 2002).

3.3. Changes in TBARS

Changes in TBARS of sardine meat during iced storage are shown in Fig. 3. TBARS value in sardine muscle increased as the storage time increased ($P < 0.05$). The initial value of TBARS was 17.2 mg/kg meat, suggesting that lipid oxidation occurred during *post-mortem* handling to some extent. From this result, TBARS slightly increased within the first 3 days of iced storage. Thereaf-

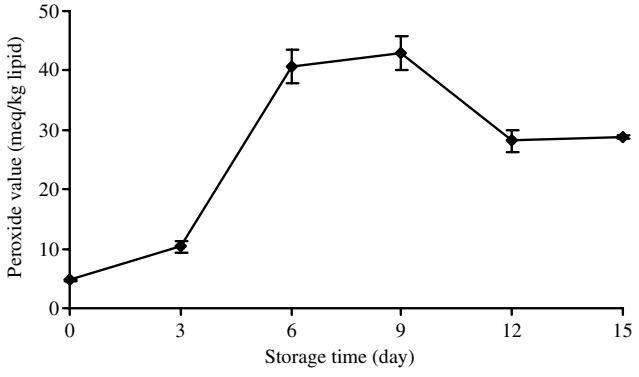

Fig. 1. Changes in peroxide values of sardine muscle during iced storage. Bars indicate standard deviations from triplicate determinations.

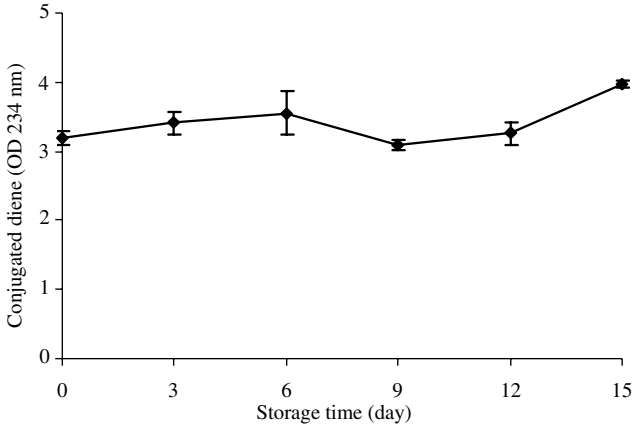

Fig. 2. Changes in conjugated dienes of sardine muscle during iced storage. Bars indicate standard deviations from triplicate determinations.

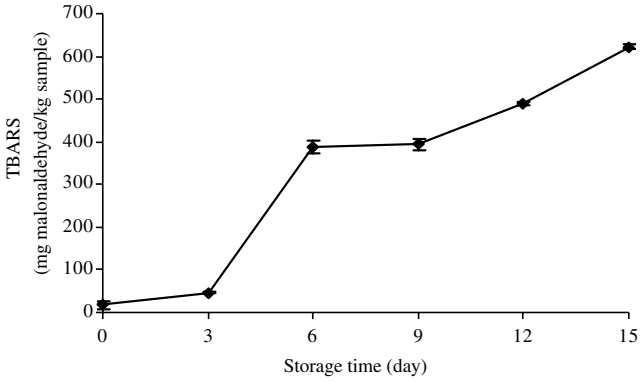

Fig. 3. Changes in TBARS values of sardine muscle during iced storage. Bars indicate standard deviations from triplicate determinations.

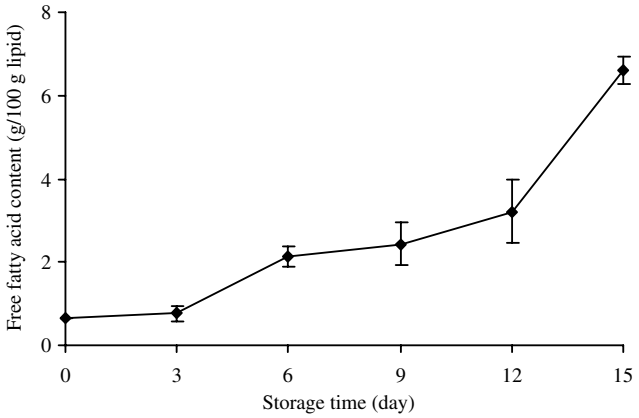

Fig. 4. Changes in free fatty acid contents of sardine muscle during iced storage. Bars indicate standard deviations from triplicate determinations.

ter, it increased sharply up to 6 days and gradually increased throughout the storage ($P < 0.05$). After 15 days of iced storage, TBARS value in sardine muscle increased by 97% when compared with that found in fresh muscle. The increase in TBARS indicated formation of secondary lipid oxidation products (Kolakowska, 2002). TBARS has been used to measure the concentration of relatively polar secondary reaction products, especially aldehydes (Nawar, 1996). Due to a high content of phospholipids, possibly from the skin and subdermal fat layer, oxidation could take place rapidly (Ke, Ackman, Linke, & Nash, 1977). A gradual increase in TBARS was found during frozen storage of horse mackerel (*Trachurus trachurus*) (Aubourg et al., 2002). The marked increase in TBARS during 9–15 days of storage was coincidental with a decrease in PV (Fig. 1). This was probably due to the destruction of hydroperoxides into secondary oxidation products, especially aldehydes in the later stages of lipid oxidation. It was most likely that a higher rate of lipid oxidation might be taking place at the end of storage period (days 9–15). This was probably due to greater release of free iron and other prooxidants from the muscle which was excessively degraded when storage time increased. Muscle proteins, as well as heme proteins, might undergo degradation with increasing storage times in ice. Benjakul, Visessanguan, and Turksuban (2003) reported a marked increase in protein hydrolysis, especially myosin heavy chain, in lizardfish during extended iced storage. Additionally, the loss in natural antioxidants during extended storage might contribute to the increased lipid oxidation. Thus, lipid oxidation became more pronounced in sardine muscle when iced storage time increased. This resulted in the deterioration and unacceptability of the sardine meat.

3.4. Changes in FFA

The changes in FFA in sardine muscle during iced storage are depicted in Fig. 4. No changes in FFA content were observed within the first 3 days of iced storage ($P > 0.05$). Subsequently, gradual increase was found up to day 12 and the highest FFA content was obtained on day 15. Possibly, lipid hydrolysis occurred to a great extent at the end of the storage period. Hydrolysis of glycerol–fatty acid esters is one important change that occurs in fish muscle lipids *post-mortem* with the release of free fatty acids. This is catalysed by lipases and phospholipases (Pacheco-Aguilar et al., 2000). In general, lipase activity is greater in dark muscle than in ordinary muscle of the same fish species (Foegeding et al., 1996). Since the whole fish were stored in ice, lipases from internal organs might be released into muscle, where lipids are localised. Intestinal lipase was found in sardine oil (*Sardinella longiceps* Linnaeus) (Nayak, Nair, Ammu, & Mathew, 2003). Kolakowska, Olley, and Dunstan (2002) also found active phospholipase in fish pyloric caeca. In addition, extracellular lipase, produced by certain microorganisms, such as *Pseudomonas fragi* also contribute to the lipolytic breakdown of fish lipids (Nayak et al., 2003). The accumulation of FFA in sardine muscle could be attributable to the lipase and phospholipase activity in sardine muscle, digestive organs as well as microorganisms, which were enhanced with extended storage.

3.5. Changes in lipid compositions

Lipid compositions in sardine muscle were affected by iced storage (Table 1). Lipids in fresh sardine muscle were composed of triglycerides and phospholipids as major constituents. Small amounts of free fatty acids and diglycerides were noticeable. During iced storage, both triglyceride and phospholipid contents decreased, while the free fatty acid, diglyceride and monoglyceride contents increased, particularly with increasing storage time ($P < 0.05$). This suggested that triglycerides and phospholipids were hydrolysed into free fatty acids,

Table 1
Changes in lipid compositions of sardine muscle during iced storage

Storage time (days)	Lipid composition (g/kg meat)				
	Triglyceride	Free fatty acid	Diglyceride	Monoglyceride	Phospholipid
0	32.6 ± 0.11c[a]	0.03 ± 0.02a	0.14 ± 0.02a	ND[b]	21.7 ± 1.15d
3	31.3 ± 0.69bc	0.20 ± 0.17a	0.20 ± 0.01b	ND	17.3 ± 1.19c
6	30.4 ± 1.73b	0.18 ± 0.17a	0.19 ± 0.03b	ND	13.5 ± 0.53b
9	31.0 ± 0.72bc	0.17 ± 0.05a	0.19 ± 0.03b	0.00 ± 0.01a	12.9 ± 0.06ab
12	29.8 ± 0.41b	0.77 ± 0.37b	0.21 ± 0.00b	0.02 ± 0.02a	12.4 ± 0.71ab
15	19.6 ± 1.37a	1.57 ± 0.19c	0.27 ± 0.02c	0.41 ± 0.38b	11.3 ± 1.39a

Values are given as means ± SD from triplicate determinations.
[a] Different letters in the same column indicate significant differences ($P < 0.05$).
[b] ND, non-detectable.

diglycerides and/or monoglycerides during extended storage in ice. Those decreases were in accordance with the increase in FFA content of sardine muscle (Fig. 4). At the end of the storage period (day 15), triglyceride and phospholipid contents decreased by 39.9% and 48%, respectively, when compared with those of fresh sardine. This result indicated that the decreasing rate of phospholipid was comparatively higher than that of triglyceride. This was possibly due to the greater activity of phospholipase in sardine than lipase activity. Our result was in agreement with Sikorski, Kolakowska, and Burt (1990) who reported that about 20% of fish lipids were hydrolysed during iced storage. Those free fatty acids released were prone to oxidation. As a result, lipid oxidation was more intense, as shown by the marked increase in TBARS (Fig. 3) or CD (Fig. 2).

3.6. Changes in fatty acid profile

Fatty acid compositions in sardine meat during iced storage are present in Table 2. Changes in saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were observed in sardine meat throughout the iced storage. Fresh sardine muscle lipid comprised 45.9% SAT, 16.7% MUFA and 35.7% PUFA. Among PUFA, docosahexaenoic acid C22:6($n-3$) (DHA) was the most abundant, followed by eicosapentaenoic acid C20:5($n-3$) (EPA). Kolakowska et al. (2002) reported that DHA is usually more abundant than EPA (up to 2–3 times). From the result, DHA was 3.21 times greater than EPA. The high content of DHA was coincidental with the high content of phospholipids, which normally contain a high amount of polyunsaturated fatty acids (Table 1). During iced storage, decreases in PUFA, especially EPA were observed, particularly as the storage time increased. During iced storage, EPA decreased by 13.2% and 19.2% at day 6 and day 15, respectively. For DHA, it decreased by 5.5% and 5.9% at day 6 and day 15, respectively. The marked decrease in EPA and DHA might be due to their susceptibility to oxidation. At day 15 of iced storage, MUFA and PUFA contents decreased by 9.7% and 8.1%, respectively, whereas SFA content increased by 2.3%, compared with that found at day

Table 2
Changes in fatty acid profile of sardine muscle during iced storage

Fatty acids (g/100 g oil)	Storage time (days)		
	0	6	15
C8:0	0.19	0.25	0.12
C11:0	0.14	0.17	0.22
C12:0	0.47	0.42	1.94
C14:0	5.96	5.48	5.06
C15:0	0.91	0.98	0.98
C16:0	28.5	28.1	27.1
C16:1 $n-7$	7.66	6.97	6.61
C17:0	1.41	1.68	1.60
C17:1	0.44	0.37	0.35
C18:0	6.96	7.87	8.28
C18:1 $n-9$	3.90	3.97	3.79
C18:1 $n-7$	3.59	3.53	3.23
C18:2 $n-6$	1.29	1.40	1.20
C18:3 $n-3$	0.63	0.63	0.66
C18:3 $n-6$	0.25	0.25	ND
C18:4 $n-3$	0.49	0.45	0.36
C20:0	0.24	0.24	0.22
C20:1 $n-7$	0.51	0.54	0.47
C20:1 $n-9$	0.27	0.27	0.41
C20:2 $n-6$	0.20	0.21	0.28
C20:3 $n-6$	0.11	0.13	0.14
C20:3 $n-3$	ND[a]	0.08	ND
C20:4 $n-6$	2.70	2.62	2.77
C20:4 $n-3$	0.34	0.31	0.25
C20:5 $n-3$ (EPA)	6.14	5.33	4.96
C21:0	0.18	0.16	0.16
C21:5 $n-3$	0.19	0.21	0.29
C22:0	0.30	0.32	0.38
C22:1 $n-9$	0.14	0.16	0.13
C22:4 $n-6$	0.34	0.33	0.36
C22:5 $n-6$	*2.32*	*2.40*	*2.40*
C22:5 $n-3$	*1.06*	*0.95*	*0.79*
C22:6 $n-3$ (DHA)	19.7	18.6	18.5
C23:0	0.10	0.12	0.19
C24:0	0.23	0.25	0.27
C24:1	0.14	0.09	ND
Saturated fatty acid (SFA)	45.9	46.4	46.9
Monounsaturated fatty acid (MUFA)	16.7	15.9	15.1
Polyunsaturated fatty acid (PUFA)	35.7	33.9	32.9

[a] ND = non-detectable.

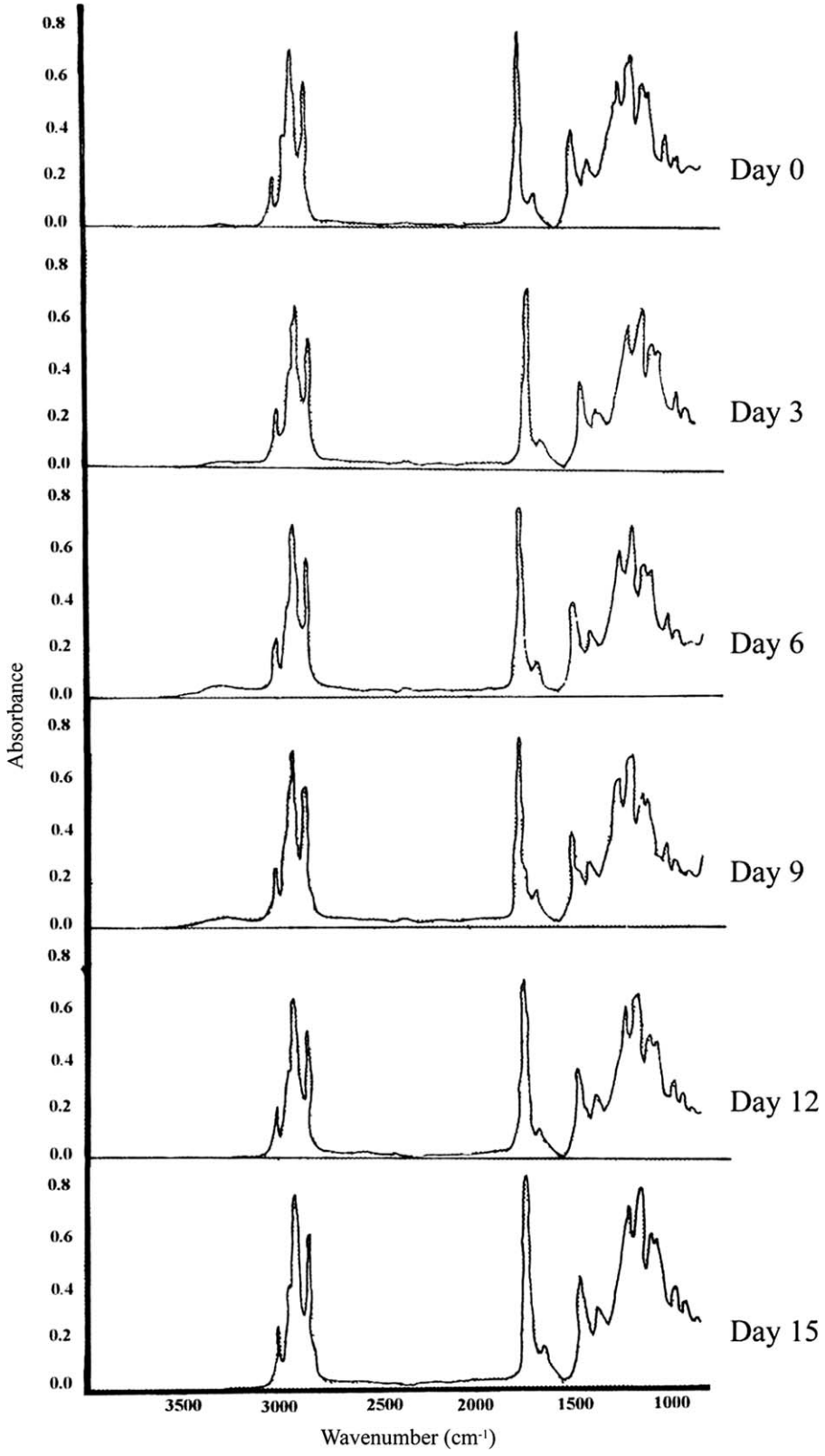

Fig. 5. Changes in FTIR spectra of crude oil extracted from sardine muscle during iced storage.

0. Since triglycerides and phospholipids underwent hydrolysis into free fatty acids during iced storage, free PUFA and MUFA possibly undergo oxidation, to a greater extent than SFA. This result was evidenced by the decrease in PUFA and MUFA during storage. Harris and Tall (1994b) reported that fish

Table 3
Changes in absorbance at 1741 and 1711 cm^{-1} of crude oil extracted from sardine muscle during iced storage

Storage time (days)	Absorbance at 1741 cm^{-1}	Absorbance at 1711 cm^{-1}
0	0.785	0.053
3	0.721	0.161
6	0.729	0.179
9	0.711	0.182
12	0.691	0.204
15	0.676	0.256

contain high levels of highly unsaturated fatty acids and they are susceptible to oxidative rancidity.

3.7. Changes in FTIR spectra

Changes in FTIR spectra of crude oil extracted from sardine muscle during iced storage are depicted in Fig. 5. The dominant spectral features in the region from 3050 to 2800 cm^{-1} were the CH stretching absorptions (*cis* C=CH, CH_2, CH_3 and CH_2/CH_3 stretching bands). The bands associated with the fingerprint region observed between 1500 and 1000 cm^{-1} were not different throughout the storage period. Generally, the carbonyl absorption of the triglyceride ester linkage was observed at 1741 cm^{-1} whereas a value of 1746 cm^{-1} was reported by Setiowaty, Che Man, Jinap, and Moh (2000). Smith (1998) reported that changes in infrared band positions were typically caused by changes in the electronic structure, as well as changes in force constant of a molecule. A gradual decrease in the absorbance at 1741 cm^{-1} was obtained during iced storage (Table 3). These changes were associated with an increase in the absorption band at 1711 cm^{-1}, which represents FFAs (Table 3). The C=O carboxylic group exhibiting a stretching band at 1711 cm^{-1} represents FFAs (Guillén & Cabo, 1997; Van de Voort, Ismail, Sedman, & Emo, 1994b). The increase in absorbance at 1711 cm^{-1} suggested the accumulation of free fatty acid due to the hydrolysis of lipid caused by lipase and phospholipase. This observation was coincidental with the increase in free fatty acid determined by both colorimetric and TLC/FID methods, as shown in Fig. 4 and Table 1, respectively.

Increase of the absorbance band at 3600–3200 cm^{-1} was observed during the first 9 days of iced storage, indicating the presence of hydroperoxide in the sample. Thereafter, the absorbance in this region was not detectable up to 15 days of storage, suggesting the decomposition of hydroperoxide to yield secondary lipid oxidation products. These results were coincidental with the increase in PV during the first 6 days, followed by a gradual decrease in PV with a concomitant increase in TBARS value until the end of the storage time (Figs. 1 and 3). Van de Voort et al. (1994b) reported absorbance at 3800–3100 cm^{-1} in the ATR/FTIR spectra, referred to as the OH stretching region. Hydroperoxide moieties exhibit characteristic absorption bands between 3600 and 3400 cm^{-1} due to their –OO–H stretching vibrations (Van de Voort, Ismail, Sedman, Dubois, & Nicodemo, 1994a).

4. Conclusion

Whole sardine lipids were susceptible to hydrolysis and oxidation during iced storage. Hydroperoxides and conjugated dienes were generated and underwent some changes to yield secondary oxidation products, especially aldehydes. An increase in lipolysis, indicated by free fatty acid formation, and decrease in triglycerides and phospholipids was observed throughout the storage period. Those lipid changes showed a detrimental effect on sardine meat quality and might be associated with the lowered heme protein removal during the washing process of surimi production.

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