

Research Paper

Changes in lipids of white-scar oyster (*Crassostrea belcheri*) during chilled storage under different salt concentrations

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Abstract

Changes in lipids of white-scar oyster (*Crassostrea belcheri*) during chilled storage at $4\pm 2^{\circ}\text{C}$ under different salt concentration were investigated. Shell-on oyster packed in normal air and 2.5% brine and shucked oyster packed in water, 2.5% brine and 4% brine were monitored for lipid class, fatty acid composition, peroxide value (PV) and volatile profile. The results showed a decrease in triglyceride (TG) and phospholipid (PL) but free fatty acid (FFA), as well as peroxide value increased ($P<0.05$). Decrease in polyunsaturated fatty acid (PUFA) was found in oysters during chilled storage, leading to the presence of alcohols, carbonyls, carboxylic acid and hydrocarbons. Shell-on oysters packed in 2.5% brine showed greater changes in lipid composition than those packed in normal air. Shucked oysters packed in 4% brine showed less changes in lipid composition than those packed in 2.5% brine and water. However, this might affect the acceptable taste of the oysters.

Keywords: oyster, lipid changes, salt content, chilled storage

Introduction

The main oyster species cultured in Thailand is White-scar oyster (*Crassostrea belcheri*), which present considerably high potential for domestic and export markets. Oysters in Thailand are generally sold with shell-on and are consumed fresh.

White-scar oysters (*C. belcheri*) cultured in Ban Don Bay, Suratthani province can grow at water salinity and temperature, ranging from 9-35 ppt and 25-31°C, respectively [1]. Conversely, the Department of Fisheries in Thailand has reported that White-scar oysters could thrive well at water salinity and temperature, ranging from 15-30 ppt and 25-30°C, respectively [2]. Pacific oysters (*C. gigas*) stored in air with water sprinkling at 7°C had 52-80% survival after 20 weeks storage [3]. In addition, Flat oysters (*Ostrea edulis*) stored in running seawater at 9°C, freshwater ice at 1°C and cold wood wool without ice at 5°C were mostly alive after 3 weeks with only 1-4% of dead samples [4].

Changes in oyster lipids are noteworthy phenomena occurring after harvesting and storage, due to tissue lipids being rich in highly unsaturated fatty acids. The main polyunsaturated fatty acid (PUFA) in oyster tissues are eicosapentaenoic acid (EPA, C20:5, n-3) and docosahexaenoic acid (DHA, C22:6, n-3) [5-9] which are considerably susceptible to oxidation. Both oxidation and hydrolysis of lipids in oysters are associated with quality deterioration [5, 9-11]. Generally, lipoxygenases catalyze the oxygenation of EPA and DHA to hydroperoxides. The hydroperoxides are converted by endogenous lyase and/or by classical chemical cleavage reactions to several secondary volatile compounds, including relatively short chain alcohols, aldehydes, and ketones [12]. Triglycerides (TG) and phospholipids (PL) decrease in oyster lipids due to action of endogenous lipolytic enzyme systems, resulting in a significant increase in free fatty acid (FFA) in the oyster tissues [10-11].

Sodium chloride (NaCl) is added to food products for various purposes, including a decrease in water activity, less availability to microbial attack and enhancement of functional properties, leading to an increase of the shelf-life time [13]. Although salt allows for prolonged storage, it has been reported to enhance lipid oxidation of highly unsaturated fatty acids [14]. However, little is still known about the effect of Na⁺ or Cl⁻ on the kinetics of lipid oxidation and literature data are often contradictory. NaCl has been reported to act as a pro-oxidant or an antioxidant. The addition of salt can increase the rate of lipid oxidation in fish muscle stored at -12°C [15] and in poultry meat [16]. On the other hand, it was reported that 5% NaCl could slightly inhibit the oxidation of lard [17] whereas a content of above 2.3% showed the inhibition of lipid oxidation in de-headed and gutted sardine dipped in salt solutions [18]. The addition of salt at 0.29% and 0.58% in cod phospholipids [19] as well as at 1.5% in frankfurters [20] showed the inhibitory effect of lipid oxidation. Some reports have shown that NaCl content at less than 0.1 M could inhibit the lipid oxidation in washed beef heart surimi but it showed the catalyzing of lipid oxidation when the content was above 3 M [21]. Furthermore, NaCl at 10.7, 21.4 and 46 g/kg of butter fat produced a protective effect on fat oxidation, while the higher concentration of salts at 6.6 g/kg of butter fat showed a pro-oxidant effect [22].

Therefore, this study aimed to investigate the changes in lipids of White-scar oysters (*Crassostrea belcheri*), both shell-on and shucked oysters, under different salt concentrations during chilled storage.

Materials and Methods

Storage conditions and sampling

White-scar oysters (*C. belcheri*) of market size, about two years old, 300-350 g in weight and 13-15 cm in length, were obtained from Bandon Bay, Suratthani in the south of Thailand between July and September 2005. After harvesting, the oysters were placed in nylon sacks and transported to the laboratory within 4-5 h at ambient temperature ($30\pm 2^\circ\text{C}$). Fresh oysters were washed with tap water for 10 min and drained for 5 min. Duplicate trials were carried out at different conditions as follows: shell-on oysters packed in two different media i.e. in hemp sack with normal air (designated SSC) and in plastic bucket containing 2.5% brine (25 ppt NaCl salt solution) (SBC) at a ratio of oysters:brine; 7:3 w/w, with new brine changed every day during storage. While shucked oysters were packed in 3 different media, i.e. water (SHW), 2.5% brine (25 ppt NaCl salt solution) (SHB2.5) and 4% brine (40 ppt NaCl salt solution) (SHB4) in polypropylene cup at the ratio of shucked oysters : media; 7:3 w/w with net weight 180 g. All samples were stored at chilled ($4\pm 2^\circ\text{C}$) temperature. The shell-on and shucked oysters were removed every day for salt content, lipid class, fatty acid composition and peroxide value (PV) analysis (10 oysters each time) until spoilage or microbiologically unacceptable, whereas the volatile profile analysis were carried out at 0, 3 and 6 days of storage (5 oysters each time). The oysters for day 0 were analyzed immediately after arrival in the laboratory.

Salt content determination

The edible part of oysters were subjected to the analysis of salt contents as per AOAC method No. of 937.09 [23].

Lipid extraction

Total lipids were extracted from 30 g of oyster edible part with chloroform/methanol according to the method of Bligh and Dyer [24].

Analysis of lipid composition

The lipid composition of the samples were determined using a thin layer chromatography/flame ionization detection analyzer (Iatrosan TH-10 TLC/FID analyzer, Iatron Laboratories, Inc., Tokyo, Japan). One μl of lipid sample (20 mg/ml) was spotted onto the scanned quartz rod (silica powder coated Chromarod-S III, Iatron Laboratories, Inc., Tokyo, Japan) and separated using a mixture of benzene:chloroform:acetic acid (50:20:0.7 v/v/v) for 35 min. The developed sample was dried in an oven at 105°C for 5 min and immediately scanned with the TLC-FID analyzer with a scanning speed of 30 s/scan. The analytical conditions were hydrogen flow rate of 160 ml/min and air flow rate of 2,000 ml/min. Retention times of lipid composition standards containing tripalmitin, triolein, dipalmitin, diolein, monopalmitin, monoolein, palmitic acid, oleic acid and phosphatidylcholine (Sigma, St. Louis, MO, USA) were used to identify chromatographic peaks of the samples. Each lipid composition was calculated, based on peak area ratio and expressed as g/100 g oil.

Fatty acid determination

Fatty acid composition was determined as fatty acid methyl esters (FAMES). The FAMES were prepared according to the method of Jham et al. [25]. The prepared methyl ester was injected into the gas chromatograph (Perkin-Elmer Autosystem XL, The Perkin-Elmer Corporation, Norwalk, CT, USA) equipped with a flame ionization detector (FID) at a split ratio of 1:50. An Optima-5 fused silica capillary column (0.25 mm i.d. x 25 m, 0.25 μm in film thickness) (Macherey-Nagel, Germany) was used. The analytical conditions were injection port and detector temperature of 250°C. The oven temperature was held at 150°C for 0.5 min, then programmed to 170°C at 4°C/min, further programmed to 195°C at 5°C/min and the final temperature was 215°C at 10°C/min from 195°C and held for 7.5 min. The carrier gas (helium) flow rate was 0.5 ml/min. Retention times of FAME standards containing C14:0 to C24:1 (PUFA-1, Marine source, Supelco Inc., Bellefonte, PA, USA) 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.

Analysis of peroxide value (PV)

Peroxide value of oyster lipid was determined using the ferric thiocyanate method [26]. The PV was calculated and expressed as milliequivalents of ferric ion/kg lipid.

Analysis of oxidized volatile compounds

The oysters were homogenized for 2 min at 10,000 rpm and 4°C in a Nissei AM-8 homogenizer (Nihonseiki Kaisha Ltd., Tokyo, Japan). The homogenate oyster (20 g) was placed in a 50-mL glass vial and crimp-capped with a PTFE/Silicone-lined septum. 10 μL of 3-methyl-1-butanol (100 mL/L of ethanol was used as an internal standard. The volatile compounds were analyzed by headspace Solid Phase Microextraction (HS-SPME). The SPME fibres (coated with 100 μm of polydimethyl siloxane) were exposed for 2 h at 30°C in the headspace of the samples, then the fibres were desorbed in a GC-FID (Shimadzu model 14B, Kyoto, Japan) equipped with a SUPELCOWAX-10™ fused silica open tubular capillary column (0.32 mm i.d. x 60 m, 0.25 μm in film thickness). The carrier gas used was helium set at a flow rate of 1 ml/min. The injector and detector temperatures were set at 250°C. The oven temperature involved an initial stage at 5°C for 2 min in splitless mode, followed by an increase from 5°C to 220°C at rate of 10°C/min before a final stage at 220°C for 20 min. The data acquisition and processing were carried out with a Shimadzu C-R7A Plus Chromatopac. The retention indices (RI) of each compound were calculated according to the method of Van den Dool and Kratz [27]. The volatile compounds were identified by matching their retention indices with those proposed in the literature [7-8, 28], and confirmed with the authentic standards containing 1-octen-3-ol, 2-ethyl-1-hexanol, 3-(*E*)-hexen-1-ol, 1,5-octadien-3-ol, propanal, (*E,E*)-2,4-heptadienal, propanoic acid, 1-hexadecene, 2,4,6,10-tetramethylpentadecane and 2-undecanone (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) under the same experimental conditions. The relative concentration of each compound was estimated by using internal standard (3-methyl-1-butanol) and expressed as $\mu\text{g/g}$. The concentrations of total oxidized volatile compounds were calculated by combining the identified oxidized volatile compounds in each sample.

Statistical analysis

The data from the two trials were subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple range tests for significant differences at $P < 0.05$ [29].

Results and Discussion

Salt content

The initial salt content of fresh oyster meat was approximately 1.5% w/w. During chilled storage, salt contents of SSC was not significantly different ($P \geq 0.05$), whereas in SBC, SHB2.5 and SHB4 they gradually increased ($P < 0.05$) (Figure 1). Salt content of shucked oyster stored in water (SHW) decreased ($P < 0.05$) with storage time increase, possibly due to the water media diluting the salt content in the oyster tissue. The final salt contents of oyster samples were in the range of 1.1-2.6% w/w. Shucked oysters packed in 4% brine showed the highest final salt contents, which possibly affects the acceptable taste of the oyster.

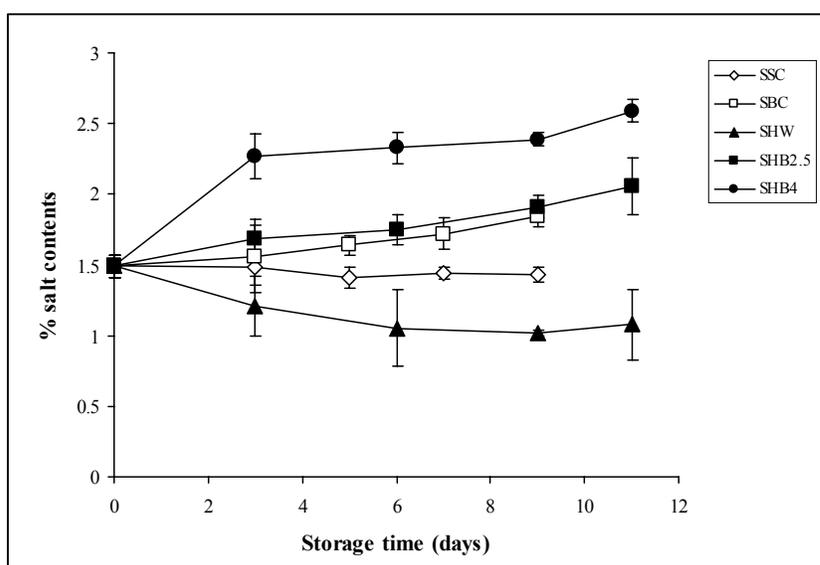


Figure 1. Changes in salt content of shell-on oysters packed in hemp sack (SSC) and 2.5% brine (SBC) and shucked oyster packed in water (SHW), 2.5% brine (SHB2.5) and 4% brine (SHB4) during chilled storage.

Changes in lipid composition

The lipid compositions were classified into 5 groups i.e. triglyceride (TG), diglyceride (DG), monoglyceride (MG), free fatty acid (FFA) and phospholipids (PL). The contents of TG, DG, MG, FFA and PL of fresh oyster accounted for 59.4, 4.1, 0.1, 0.5, and 36.0 g/100 g oil, respectively. During chilled storage, both TG and PL contents of all treatments decreased ($P < 0.05$) (Tables 1 and 2), possibly due to the activity of both endogenous and microbial lipases and/or phospholipases [30]. DG, MG and FFA contents increased ($P < 0.05$) with the storage time increases, due to the hydrolysis of TG and PL.

Shell-on oysters packed in 2.5% brine showed a higher decrease in TG and PL but a greater increase in DG, MG and FFA than those packed in normal air during storage (Table 1). The shucked oysters packed in 4% brine showed a decrease in TG and PL but increases in DG, MG and FFA at the slower rate than those packed in 2.5% brine and in water during storage (Table 2). The rates of lipid oxidation of salted sardine fillet (*Sardinops melanostictus*) increased with increase of NaCl content while lipid hydrolysis depended upon the NaCl content and storage temperature. The lower NaCl content, the faster hydrolysis proceeded. Therefore, it is clear that NaCl accelerates lipid oxidation and inhibits lipid hydrolysis in the salted sardine fillet during storage [13]. Additionally, it was shown there is a strong effect of NaCl content of horse mackerel (*Trachurus trachurus*) on rancidity development during frozen storage for 270 days [31].

Table 1. Changes in lipid composition (g/100 g oil) of shell-on oysters during chilled storage.

Storage time (days)	Lipid compositions				
	SSC				
	Triglyceride	Diglyceride	Monoglyceride	Free fatty acid	Phospholipid
0	59.44 ^b	4.06 ^a	0.06 ^a	0.47 ^a	35.97 ^c
1	58.38 ^{ab}	5.07 ^b	0.21 ^{ab}	0.52 ^a	35.82 ^{de}
2	58.03 ^{ab}	6.07 ^c	0.22 ^{ab}	0.62 ^{ab}	35.06 ^{cde}
3	57.82 ^{ab}	6.69 ^{cd}	0.24 ^{ab}	0.95 ^{bc}	34.30 ^{cde}
4	57.64 ^{ab}	6.95 ^{de}	0.25 ^{ab}	1.07 ^c	34.09 ^{cd}
5	57.08 ^{ab}	7.62 ^{ef}	0.27 ^{ab}	1.16 ^c	33.87 ^c
6	57.76 ^{ab}	8.09 ^{fg}	0.30 ^{ab}	1.88 ^d	31.97 ^b
7	57.65 ^{ab}	8.27 ^{fg}	0.40 ^{ab}	2.45 ^e	31.23 ^b
8	56.92 ^{ab}	8.39 ^{fg}	0.41 ^{ab}	3.92 ^f	30.36 ^b
9	56.96 ^{ab}	8.72 ^{gh}	0.49 ^b	5.82 ^g	28.01 ^a
10	54.97 ^a	9.26 ^h	0.50 ^b	7.43 ^h	27.84 ^a
Storage time (days)	SBC				
	Triglyceride	Diglyceride	Monoglyceride	Free fatty acid	Phospholipid
0	59.44 ^c	4.06 ^a	0.06 ^a	0.47 ^a	35.97 ^g
1	58.37 ^{de}	5.38 ^b	0.15 ^{ab}	0.56 ^a	35.54 ^g
2	57.98 ^{de}	6.56 ^c	0.18 ^b	0.62 ^a	34.66 ^{fg}
3	57.72 ^{cd}	7.79 ^d	0.20 ^b	0.64 ^a	33.65 ^{ef}
4	57.68 ^{cd}	8.37 ^{de}	0.35 ^c	1.08 ^b	32.52 ^{de}
5	58.18 ^{de}	8.72 ^e	0.58 ^d	1.56 ^c	30.96 ^{cd}
6	57.55 ^{cd}	8.89 ^e	0.61 ^d	2.61 ^d	30.34 ^c
7	56.22 ^{bc}	9.58 ^f	0.63 ^d	3.74 ^e	29.83 ^{bc}
8	55.73 ^b	9.62 ^f	0.76 ^e	4.77 ^f	29.12 ^{abc}
9	54.83 ^b	9.98 ^f	0.91 ^f	6.12 ^g	28.16 ^{ab}
10	51.38 ^a	11.53 ^g	1.82 ^g	7.75 ^h	27.52 ^a

SSC and SBC = shell-on oyster stored in normal air and in 2.5% brine, respectively.

Values in the same column followed by different superscript letters are significantly different ($P < 0.05$).

Table 2. Changes in lipid composition (g/100 g oil) of shucked oysters during chilled storage.

Storage time (days)	Lipid compositions				
	SHW				
	Triglyceride	Diglyceride	Monoglyceride	Free fatty acid	Phospholipid
0	59.44 ^d	4.06 ^a	0.06 ^a	0.47 ^a	35.97 ^c
1	58.72 ^{cd}	4.60 ^{ab}	0.14 ^{ab}	0.65 ^{ab}	35.89 ^e
2	58.05 ^{cd}	5.25 ^{bc}	0.13 ^{ab}	1.03 ^b	35.54 ^e
3	58.11 ^{cd}	5.66 ^c	0.18 ^{ab}	1.13 ^b	34.92 ^{de}
4	58.10 ^{cd}	6.82 ^d	0.23 ^{ab}	2.25 ^c	32.60 ^{cd}
5	57.69 ^{cd}	6.88 ^{de}	0.42 ^{bc}	3.89 ^d	31.12 ^{bc}
6	56.61 ^{bed}	7.83 ^{ef}	0.54 ^{cd}	4.52 ^e	30.50 ^{abc}
7	56.97 ^{bed}	7.84 ^{ef}	0.62 ^{cd}	4.78 ^e	29.79 ^{abc}
8	56.71 ^{bed}	8.76 ^{fg}	0.74 ^d	5.05 ^e	28.74 ^{ab}
9	56.96 ^{bed}	8.35 ^{fg}	1.02 ^e	5.76 ^f	27.91 ^a
10	55.76 ^{bc}	9.21 ^{gh}	1.12 ^e	5.95 ^f	27.96 ^a
11	53.60 ^{ab}	9.75 ^h	1.53 ^f	7.29 ^g	27.83 ^a
12	52.28 ^a	9.93 ^h	1.87 ^g	8.41 ^h	27.51 ^a
Storage time (days)	SHB2.5				
	Triglyceride	Diglyceride	Monoglyceride	Free fatty acid	Phospholipid
0	59.44 ^g	4.06 ^a	0.06 ^a	0.47 ^a	35.97 ^d
1	58.95 ^{fg}	4.82 ^b	0.10 ^a	0.75 ^{ab}	35.38 ^d
2	57.80 ^{ef}	5.45 ^c	0.12 ^{ab}	1.23 ^b	35.40 ^d
3	57.92 ^e	5.61 ^c	0.28 ^{bc}	1.30 ^b	34.89 ^d
4	56.80 ^d	6.82 ^d	0.33 ^c	2.45 ^c	33.60 ^{cd}
5	56.11 ^{cd}	7.48 ^e	0.40 ^{cd}	2.89 ^c	33.12 ^{cd}
6	55.72 ^{cd}	7.73 ^e	0.53 ^d	3.52 ^d	32.50 ^{bcd}
7	55.80 ^{cd}	7.94 ^e	0.72 ^e	4.75 ^e	30.79 ^{abc}
8	55.59 ^{cd}	8.78 ^f	0.84 ^e	5.05 ^e	29.74 ^{abc}
9	54.76 ^{bc}	9.25 ^{fg}	1.22 ^f	5.86 ^f	28.91 ^{ab}
10	54.63 ^{bc}	9.55 ^{gh}	1.32 ^f	6.90 ^g	27.60 ^a
11	53.81 ^b	9.75 ^{gh}	1.50 ^g	7.31 ^g	27.63 ^a
12	52.33 ^a	9.89 ^h	1.97 ^h	8.40 ^h	27.41 ^a
Storage time (days)	SHB4				
	Triglyceride	Diglyceride	Monoglyceride	Free fatty acid	Phospholipid
0	59.44 ^d	4.06 ^a	0.06 ^a	0.47 ^a	35.97 ^c
1	59.05 ^{cd}	4.49 ^{ab}	0.10 ^{ab}	0.68 ^a	35.68 ^{de}
2	59.01 ^{cd}	4.63 ^{ab}	0.16 ^{ab}	0.71 ^a	35.49 ^{de}
3	58.57 ^{cd}	5.06 ^b	0.24 ^{abc}	1.20 ^b	34.93 ^{cde}
4	58.89 ^{cd}	5.17 ^b	0.34 ^{abc}	1.45 ^b	34.15 ^{bcdde}
5	57.51 ^{bcd}	6.04 ^c	0.67 ^{bc}	2.10 ^c	33.68 ^{bcdde}
6	56.78 ^{abc}	6.58 ^c	0.78 ^{cd}	2.87 ^d	32.99 ^{abcde}
7	56.10 ^{ab}	6.50 ^c	0.83 ^{cd}	4.06 ^e	32.51 ^{abcd}
8	55.56 ^{ab}	6.71 ^{cd}	1.28 ^{de}	4.36 ^{ef}	32.09 ^{abc}
9	54.94 ^a	7.34 ^{de}	1.34 ^{de}	4.65 ^f	31.73 ^{abc}
10	55.34 ^{ab}	7.43 ^{de}	1.36 ^{de}	4.60 ^f	31.27 ^{ab}
11	55.38 ^{ab}	7.56 ^e	1.45 ^e	5.26 ^g	30.35 ^a
12	54.78 ^a	7.76 ^e	1.53 ^e	5.76 ^h	30.17 ^a

SHW, SHB2.5 and SHB4 = shucked oyster stored in water, 2.5% brine and 4% brine, respectively. Values in the same column followed by different superscript letters are significantly different ($P < 0.05$).

Changes in fatty acid composition

The fatty acid compositions of White-scar oysters were classified into 3 groups i.e. saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA). The predominant SFA were palmitic acid (C16:0), stearic acid (C18:0) and myristic acid (C14:0). The predominant MUFA were oleic acid (C18:1), palmitoleic acid (C16:1) and eicosanoic acid (C20:1) and the predominant PUFA were eicosapentaenoic acid (EPA, C20:5), docosahexaenoic acid (DHA, C22:6), arachidonic acid (C20:4), linoleic acid (C18:2) and stearidonic acid (C18:4). Fresh oyster lipids were composed of 33.3% PUFA, 29.7% SFA, and 15.7% MUFA (Figure 2). During storage, PUFA and MUFA in all oyster samples decreased while SFA increased, probably due to unsaturated fatty acids (UFA) being converted to SFA by hydrogenation reaction [30]. Decreases in the PUFA and MUFA of the shell-on oysters packed in 2.5% brine were greater than those packed in normal air. The shucked oysters packed in 4% brine showed lower decreases in PUFA and MUFA than those packed in water and 2.5% brine during storage.

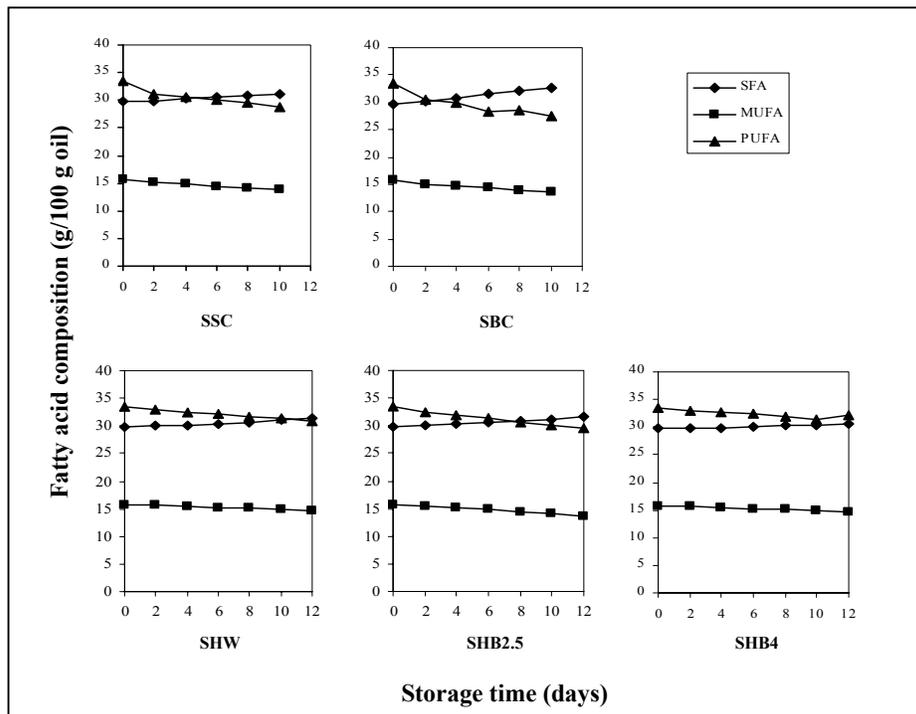


Figure 2. Changes in fatty acid composition of shell-on oysters packed in hemp sack (SSC) and in 2.5% brine (SBC), and shucked oyster packed in water (SHW), 2.5% brine (SHB2.5) and 4% brine (SHB4) during chilled storage. SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid.

Changes in peroxide value (PV)

Oyster lipids are highly susceptible to oxidation because they contain high levels of PUFA, especially EPA and DHA [5-9]. The basic mechanisms of lipid oxidation can be characterized by three steps: initiation, propagation and termination reactions [32]. This phenomenon can be influenced by fatty acid composition, concentration of pro-oxidants and antioxidants, endogenous ferrous iron, enzymes, salt concentration, pH, light, storage temperature and oxygen concentration [12, 33-34]. Both chemical and enzymatic oxidations probably occur in oysters during chilled storage. The chemical oxidation is due to the combination of triplet oxygen and singlet oxygen reaction [30]. The endogenous enzymes called lipoxygenase which were responsible for catalyzing cis, cis-1,4-pentadiene sequences through separation of a hydrogen atom from active methylene groups of PUFA. This enzyme produced conjugated diene hydroperoxides [35]. The oxidized lipids could react with oxygen producing peroxides as primary oxidation products. In this study, the initial peroxide value (PV) of oyster was 7.8 meq/kg lipids. During storage, most of treatments showed increase in PV contents with extended storage time ($P < 0.05$) (Figure 3). Whereas PV contents of SSC and SBC decreased after 9 days of storage, probably due to a higher rate of hydroperoxide decomposition than rate of oxidation [32].

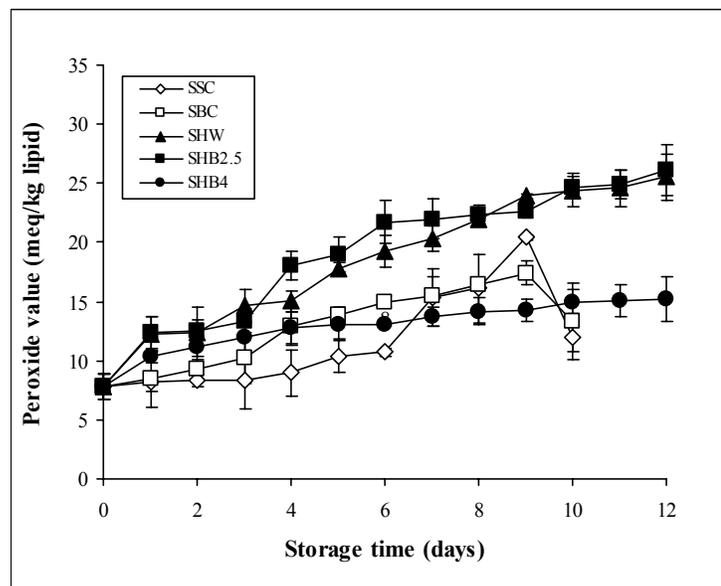


Figure 3. Changes in PV of shell-on oysters packed in hemp sack (SSC) and in 2.5% brine (SBC), and shucked oyster packed in water (SHW), 2.5% brine (SHB2.5) and 4% brine (SHB4) during chilled storage.

The PV of the shell-on oysters packed in 2.5% brine increased at a higher rate than those packed in normal air during storage. The shucked oysters packed in 4% brine showed a slower increase in PV than those in 2.5% brine and water. The oxidative activity of NaCl is due to its ability to release iron from heme pigments and other heme binding molecules, increasing the catalytic activity by free iron ions [36]. It was reported that the lipid oxidation of salted sardine fillet with 9.19% NaCl was faster

than those with 5.36%, 2.83% and 0.39% of NaCl during chilled storage, respectively [13]. NaCl could also catalyze some enzyme activities i.e. 0.43 M NaCl in mackerel could catalyze the activity of lipoxygenase [37] and 0.15 M NaCl in flatfish muscle could accelerate the enzymatic oxidation [15]. On the other hand, the salt content at 2.3% and above in sardine could inhibit lipid oxidation [18] and salt at 0.29% and 0.58% could inhibit lipid oxidation in cod phospholipids [19].

Changes in oxidized volatile compounds

The volatile compounds produced from lipid oxidation including esters, aldehydes, alcohols, ketones, lactones, acid compounds and hydrocarbons [19] are influenced by the composition of the hydroperoxides and the types of oxidative cleavage of double bonds in the fatty acids [38]. In this result, four alcohols (1-octen-3-ol, 2-ethyl-1-hexanol, 3-(*Z*)-hexen-1-ol and 1,5-octadien-3-ol), two aldehydes (propanal and (*E,E*)-2,4-heptadienal), two hydrocarbons (1-hexadecene and 2,4,6,10-tetramethylpentadecane), one carboxylic acids (propanoic acid), and one ketone (2-undecanone), as oxidized volatile compounds, were found during storage. The 1-Octen-3-ol and 2-ethyl-1-hexanol occurred from n-6 PUFA degradation [7-8, 28, 39]. The 3-(*Z*)-Hexen-1-ol, 1,5-octadien-3-ol, propanal, (*E,E*)-2,4-heptadienal, propanoic acid and 2-undecanone were derived from autoxidation of n-3 PUFA [7-8, 28, 39-42]. Whereas 1-hexadecene and 2,4,6,10-tetramethylpentadecane were the products from fatty acid degradation [43].

Only alcohols were detected in fresh oysters and rapidly increased in shell-on oysters both packed in normal air and packed in 2.5% brine with storage time increases (Table 3). Aldehydes were determined after 3 days storage of shell-on oysters and rapidly increased with storage time increases in both packed in normal air and in 2.5% brine. Ketones, carboxylic acids and hydrocarbons were only found in shell-on oysters packed in 2.5% brine after 6 days of storage. Increase in total oxidized volatile compounds of shell-on oysters packed in 2.5% brine were higher than those of shell-on oysters packed in normal air, possibly due to effect of NaCl to induce lipid oxidation [44-46].

Alcohols and aldehydes in shucked oysters gradually increased during storage (Table 3). Ketones were found only in SHB2.5 and SHB4 during storage. Increase in total oxidized volatile compounds of SHW was greater than those of SHB2.5 and SHB4 up to 3 days of storage. While total oxidized volatile compounds of SHB4 showed a higher increase than those of SHB2.5 and water for up to 6 days of storage. This was probably due to the higher salt content which may promote the formation of oxidized volatile compounds.

Table 3. Changes in total oxidized volatile compounds of oysters during chilled storage.

Treatments	Storage time (days)	Volatiles ($\mu\text{g/g}$)					Total oxidized volatiles
		Alcohol	Aldehyde	Ketone	Carboxylic acid	hydrocarbon	
SSC	0	12.82	0.00	0.00	0.00	0.00	12.82
	3	15.07	26.77	0.00	0.00	0.00	41.84
	6	21.71	44.61	0.00	0.00	0.00	66.32
SBC	0	12.82	0.00	0.00	0.00	0.00	12.82
	3	16.28	31.41	0.00	0.00	0.00	47.69
	6	26.97	87.18	2.29	9.17	2.40	128.01
SHW	0	12.82	0.00	0.00	0.00	0.00	12.82
	3	14.90	20.61	0.00	0.00	0.00	35.51
	6	16.60	27.51	0.00	0.00	0.00	44.11
SHB2.5	0	12.82	0.00	0.00	0.00	0.00	12.82
	3	14.11	7.06	4.06	0.00	0.00	25.23
	6	16.76	13.34	4.30	0.00	0.00	34.40
SHB4	0	12.82	0.00	0.00	0.00	0.00	12.82
	3	13.71	8.81	0.00	0.00	0.00	22.52
	6	17.39	33.82	2.71	0.00	0.00	53.92

SSC and SBC = shell-on oyster stored in normal air and in 2.5% brine, respectively.

SHW, SHB2.5 and SHB4 = shucked oyster stored in water, 2.5% brine and 4% brine, respectively.

Conclusions

Treated shell-on and shucked oysters with different NaCl content have shown important lipid hydrolysis and oxidation development during chilled storage. The changes of oyster lipids caused by the storage conditions have been satisfactorily assessed by chemical indices such as lipid class, fatty acid composition, PV and oxidized volatile compounds. Shell-on oysters packed in 2.5% brine (1.8% salt in oyster) showed a decrease in TG, PL, PUFA and MUFA, but an increase in PV and total oxidized volatile compounds, higher than those packed in normal air (1.4% salt in oyster) during storage. The lipid hydrolysis and oxidation were slower in shucked oysters packed in 4% brine (2.6% salt in oyster) than those packed in 2.5% brine (2.1% salt in oyster) and water (1.1% salt in oyster) during storage.

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