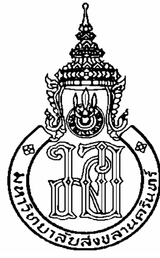

Change in Qualities and Volatile Compounds of Oyster (*Crassostrea belcheri*) during Storage and Processing

Somwang Songsaeng

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Food Technology

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ชื่อวิทยานิพนธ์ การเปลี่ยนแปลงคุณภาพและสารระเหยได้ของหอยนางรมระหว่างการแปรรูปและการเก็บรักษา

ผู้เขียน นางสาวสมหวัง สงแสง

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บทคัดย่อ

การศึกษาการเปลี่ยนแปลงคุณภาพทางด้านกายภาพ เคมี จุลินทรีย์และประสาทสัมผัส รวมทั้งสารระเหยได้ของหอยนางรมสด (*Crassostrea belcheri*) ในระหว่างการเก็บรักษาภายใต้สภาวะแตกต่างกัน และเมื่อผ่านการแปรรูปแบบแช่เยือกแข็งร่วมกับการใช้สารต้านออกซิเดชัน และการแปรรูปด้วยความร้อนในภาชนะปิดสนิท พบว่า

ผลของสภาวะการเก็บรักษาที่แตกต่างกัน ต่อการเปลี่ยนแปลงคุณภาพและสารระเหยได้ของหอยนางรมสดซึ่งประกอบด้วย การเก็บหอยนางรมทั้งเปลือกในบรรยากาศปกติและน้ำเกลือร้อยละ 2.5 ที่อุณหภูมิแช่เย็น (4 ± 2°ซ) และอุณหภูมิห้อง (30 ± 2°ซ) และหอยนางรมที่แกะเปลือกแล้วในน้ำ และน้ำเกลือร้อยละ 2.5 และ 4 ที่อุณหภูมิแช่เย็น (4 ± 2 °ซ) พบว่า ปริมาณเกลือ ความชื้น โปรตีน และไขมันของเนื้อหอยในระหว่างการเก็บรักษาอยู่ในช่วงร้อยละ 1.1-2.6 79.1-82.2 7.4-8.8 และ 2.2-2.6 ตามลำดับ การเก็บรักษาหอยนางรมทั้งเปลือกในสภาพบรรยากาศปกติและแช่ในน้ำเกลือร้อยละ 2.5 ที่อุณหภูมิแช่เย็นมีการเปลี่ยนแปลงคุณภาพทางด้านเคมี จุลินทรีย์และประสาทสัมผัสช้ากว่าที่อุณหภูมิห้อง และพบว่าหอยนางรมทั้งเปลือกซึ่งเก็บในน้ำเกลือร้อยละ 2.5 มีค่าพีเอชลดลงช้ากว่า แต่องค์ประกอบของไขมันและกรดไขมันลดลงเร็วกว่า ในขณะที่ปริมาณไนโตรเจนที่ระเหยได้ทั้งหมด เปอร์ออกไซด์ จุลินทรีย์ทั้งหมด และแบคทีเรียกลุ่มเจริญได้ที่อุณหภูมิต่ำเพิ่มขึ้นเร็วกว่าหอยนางรมที่เก็บในบรรยากาศปกติ สำหรับหอยนางรมแกะเปลือกและเก็บในน้ำเกลือร้อยละ 4 พบการเปลี่ยนแปลงของพีเอช องค์ประกอบของไขมันและกรดไขมัน เปอร์ออกไซด์ และจุลินทรีย์น้อยกว่าหอยนางรมแกะเปลือกและเก็บในน้ำเกลือร้อยละ 2.5 และน้ำภายใต้อุณหภูมิแช่เย็น คะแนนการยอมรับทางประสาทสัมผัสทุกคุณลักษณะมีแนวโน้มลดลง ในขณะที่ปริมาณจุลินทรีย์เพิ่มขึ้นในระหว่างการเก็บรักษา ดังนั้นเมื่อพิจารณาทั้งคุณภาพทางจุลินทรีย์และประสาทสัมผัส พบว่าหอยนางรมทั้งเปลือกทั้งที่เก็บในบรรยากาศปกติและในน้ำเกลือร้อยละ 2.5 ที่อุณหภูมิห้องและอุณหภูมิแช่เย็นนั้น สามารถเก็บได้ไม่เกิน 3 และ 9 วัน ตามลำดับ ในขณะที่หอยนางรมแกะเปลือกและเก็บในน้ำ และน้ำเกลือร้อยละ 2.5 และ 4 ที่อุณหภูมิแช่เย็นสามารถเก็บได้นาน 9 10 และ 10 วัน

ตามลำดับ แม้ว่าหอยนางรมที่แกะเปลือกและเก็บในน้ำเกลือร้อยละ 4 สามารถยืดอายุการเก็บได้ดีที่สุด แต่อาจพบปัญหาต่อการยอมรับรสชาติ ส่วนสารระเหยได้ในหอยนางรมสดสามารถพบได้เฉพาะสารระเหยกลุ่มแอลกอฮอล์ ได้แก่ 1-octen-3-ol และ 2-ethyl-1-hexanol แต่ในระหว่างการเก็บรักษาสามารถพบสารระเหยในกลุ่มของแอลกอฮอล์ 9 ชนิด กรดคาร์บอกซิลิก 4 ชนิด คีโตน 2 ชนิด อัลดีไฮด์ 2 ชนิด และ ไฮโดรคาร์บอน 2 ชนิด

ผลของการแช่เยือกแข็งแบบเร็ว (ไอคิวเอฟ) และแบบช้า (เพลทสัมผัส)ร่วมกับการใช้และไม่ใช้สารต้านออกซิเดชัน (บิวทิลไฮดรอกซีอะนิโซล) เข้มข้นร้อยละ 0.02 ระหว่างการเก็บรักษาหอยนางรมแช่เยือกแข็งที่อุณหภูมิ -20°ซ เป็นเวลา 12 เดือน พบว่าปริมาณของเหลวที่บีบออกจากผลิตภัณฑ์หอยนางรมแช่แข็งแบบไอคิวเอฟเพิ่มขึ้นช้ากว่าในหอยนางรมแช่เยือกแข็งแบบเพลทสัมผัส เนื่องจากในการแช่เยือกแข็งแบบเร็วมีผลในการทำลายเนื้อเยื่อน้อยกว่าการแช่เยือกแข็งแบบช้า แม้ว่าวิธีการแช่เยือกแข็งและการใช้สารต้านออกซิเดชันไม่แสดงผลอย่างมีนัยสำคัญต่อคุณภาพทั้งทางเคมี จุลินทรีย์ และประสาทสัมผัสของหอยนางรม แต่ในระหว่างการเก็บรักษาพบว่าปริมาณไขมันและไนโตรเจนที่ระเหยได้ทั้งหมดเพิ่มขึ้นอย่างช้า ๆ และจุลินทรีย์ทั้งหมดและแบคทีเรียกลุ่มเจริญได้ที่อุณหภูมิต่ำมีค่าลดลง สำหรับการเปลี่ยนแปลงขององค์ประ กอบของไขมันและกรดไขมัน พบว่าไตรกลีเซอไรด์และฟอสโฟลิปิดมีแนวโน้มลดลง ในขณะที่ ไดกลีเซอไรด์และกรดไขมันอิสระมีแนวโน้มเพิ่มขึ้น ส่วนกรดไขมันอิ่มตัวและไม่อิ่มตัวเชิงเดี่ยวค่อนข้างคงที่ แต่กรดไขมันไม่อิ่มตัวเชิงซ้อนลดลง การใช้สารต้านออกซิเดชันสามารถป้องกันการเกิดออกซิเดชันของไขมันในหอยนางรมแช่เยือกแข็งแบบไอคิวเอฟได้ดีกว่าในหอยนางรมแช่เยือกแข็งแบบเพลทสัมผัสเพียงเล็กน้อย ในระหว่างการเก็บรักษาพบว่าคะแนนการทดสอบทางประสาทสัมผัสมีแนวโน้มลดลง หอยนางรมแช่เยือกแข็งแบบไอคิวเอฟได้คะแนนการทดสอบด้านสีลดลง แต่เนื้อสัมผัสเพิ่มขึ้นมากกว่าหอยนางรมแช่เยือกแข็งแบบเพลทสัมผัส การยอมรับด้านลักษณะปรากฏและกลิ่นไม่แตกต่างกัน นอกจากนี้ยังพบว่า สารระเหยได้ในหอยนางรมแช่เยือกแข็งแบบไอคิวเอฟมีค่ามากกว่าในหอยนางรมแช่เยือกแข็งแบบเพลทสัมผัส ซึ่งสารระเหยได้ที่พบเป็นกลุ่มของแอลกอฮอล์ อัลดีไฮด์ คีโตน และสารอื่น ๆ สำหรับสารระเหยได้ที่พบหลัก ๆ ได้แก่ 1-octen-3-ol 2-ethyl-1-hexanol heptanol pentanal และ 2-undecanone

ผลของการแปรรูปด้วยความร้อนและการเก็บรักษาต่อการเปลี่ยนแปลงคุณภาพและสารระเหยได้ของผลิตภัณฑ์หอยนางรมในน้ำเกลือบรรจุกระป๋อง และถุงรีทอร์ทระหว่างการเก็บรักษาที่อุณหภูมิห้อง (28 ± 2°ซ) เป็นเวลา 12 เดือน พบว่าการให้ความร้อนไม่มีผลต่อการเปลี่ยนแปลงค่าพีเอช ในขณะที่ความชื้น โปรตีน ไขมัน และค่าเปอร์ออกไซด์เกิดการเปลี่ยนแปลงเล็กน้อย

การเปลี่ยนแปลงขององค์ประกอบไขมันและกรดไขมัน พบว่าไดกลีเซอไรด์ โมโนกลีเซอไรด์ กรดไขมันอิสระ ฟอสโฟลิปิด และกรดไขมันไม่อิ่มตัวเชิงซ้อนลดลง แต่กรดไขมันอิ่มตัว กรดไขมันไม่อิ่มตัวเชิงเดี่ยว และค่าเปอร์ออกไซด์เพิ่มขึ้นหลังการให้ความร้อน ลักษณะทางประสาทสัมผัสของผลิต ภัณฑ์ พบว่าเนื้อหอยเกิดการหดตัวเพิ่มขึ้น สีของตัวหอยเป็นสีเหลืองอมเขียว เนื้อสัมผัสนิ่มขึ้น มีกลิ่นสุกเฉพาะ และรสชาติหวานมากขึ้นหลังจากการให้ความร้อน คะแนนการทดสอบทางประสาทสัมผัสมีแนวโน้มลดลง แต่อยู่ในระดับที่ยอมรับได้ สำหรับการเปลี่ยนแปลงของสารระเหยได้หลังการแปรรูปพบว่าเกิดสารระเหยได้ดังนี้คือ แอลกอฮอล์ 3 ชนิด (hexanol 2-nonanol และ (*E,Z*)-3,6-nonadien-1-ol) อัลดีไฮด์ 2 ชนิด (ethanal และ heptanal) ไพริดีน 1 ชนิด (3-methyl pyridine) ฟูแรน 1 ชนิด (2-ethyl furan) และเอมีน 1 ชนิด (trimethylamine) แต่หลังการเก็บรักษาพบว่าแอลกอฮอล์และอัลดีไฮด์มีค่าลดลง แต่ ไพริดีน ฟูแรน และเอมีนมีค่าเพิ่มขึ้น

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ABSTRACT

Changes in physical, chemical, microbiological and sensory quality as well as volatile profile of white-scar oysters (*Crassostrea belcheri*) under different storage conditions, after freezing with and without antioxidants and after thermal processing as well as during storage were studied. The results could be indicated into 3 parts as follows:

Effect of different storage conditions on quality and volatile profile of fresh oyster i.e. shell-on oysters packed in normal air and in 2.5% brine and stored at chilled (4 $\pm$ 2°C) and ambient (30 $\pm$ 2°C) temperatures, and shucked oysters packed in water, 2.5% brine and 4% brine and stored only at chilled temperature (4 $\pm$ 2°C) were investigated. It was found that salt, moisture, crude protein and crude fat content of oyster ranged from 1.1-2.6%, 79.1-82.2%, 7.4-8.8% and 2.2-2.6%, respectively. Shell-on oyster packed in both normal air and 2.5% brine under chilled temperature were slower changed in chemical, microbiological and sensory qualities than those under ambient temperature. Shell-on oyster packed in 2.5% brine showed a slower decrease in pH but greater decreases in lipid and fatty acid compositions whereas increases in total volatile basic nitrogen (TVB-N), peroxide value (PV), total viable count (TVC) and psychrotrophic bacteria at a faster rate than those stored in normal air. Shucked oyster packed in 4% brine showed slower changes in pH, lipid composition, fatty acid composition, PV and microorganisms than those packed in 2.5% brine and water during chilled storage for 12 days. During storage, the sensory score for all attribute were decreased while TVC and psychrotrophs increased. Considering from microbiological and sensory qualities, shell-on oyster packed in normal air and in 2.5% brine could be accepted at less than 3 days at ambient temperature and 9 days under chilled storage. While shucked oysters packed in water,

2.5% brine and 4% brine were accepted until 9, 10 and 10 days of chilled storage, respectively. Though keeping shucked oyster in 4% brine showed the greatest effect on the shelf-life extension, it might affect the taste of oyster. Only two volatiles i.e. 1-octen-3-ol and 2-ethyl-1-hexanol were initially detected but more compounds including nine alcohols, four carboxylic acids, two ketones, two aldehydes and two hydrocarbons were found during storage.

Effect of freezing method, individual quick freezing (IQF) and contact plate freezing (CPF) with untreated and treated antioxidant, butylhydroxy anisole (0.02% BHA suspension) during storage at -20°C for 12 months were investigated for physical, chemical, microbiological and sensory qualities as well as volatile profile. Increasing in expressible drip of IQF oyster was slower than those of CPF oyster due to the fact that quick freezing result in less tissue damage than slow freezing. Both freezing method and antioxidant treatment did not significant affect on chemical, microbiological and sensory qualities. Nevertheless, fat and TVB-N content slowly increased but TVC and psychrotrophs of all samples decreased with increasing storage time. For changes in lipid and fatty acid composition, TG and PL were significantly changed whereas DG and FFA increased. PUFA in all samples tend to decrease while SFA and MUFA were not significantly differences during storage. Antioxidant treatment could prevent lipid oxidation in IQF oysters more effectively than those in CPF oysters. The sensory scores were decreased as the storage time increased. IQF oyster showed a greater decrease in color score but slower decrease in texture score than CPF oyster. Appearance and odor in all samples were not significantly different during storage. After storage for 12 months, the volatile compounds of IQF samples were higher than those of CPF samples consisted of alcohols, aldehydes, ketones and others with the major compounds i.e. 1-octen-3-ol, 2-ethyl-1-hexanol, heptanol, pentanal and 2-undecanone.

Effect of thermal processing on changes in chemical and sensory qualities as well as volatile profile of canned and retort pouched oyster during storage for 12 months at ambient temperature (28 $\pm$ 2°C) were studied. Thermal processing did not show significant changes in pH while slightly changes in moisture, crude protein and crude fat of oyster products and also no significant changes in pH and

chemical composition in both products during storage. Changes in lipid composition, fatty acid composition and lipid stability i.e. decrease in DG, MG, FFA, PL and PUFA but increase in SFA, MUFA and PV were also found after thermal processing as well as during storage. Changes in sensory attribute i.e. the plum and adductor of oyster was shrunken, yellowish green in color, softer in texture, cooked meat/sweet odor and strong sweet in taste were found after thermal processing. Although, all sensory attributes of canned and retort pouched oysters slowly decreased with increasing storage time, they retained acceptable in all attributes throughout the storage. There were three alcohols (hexanol, 2-nonanol and (*E,Z*)-3,6-nonadien-1-ol), two aldehydes (ethanal and heptanal), one pyridine (3-methyl pyridine), one furan (2-ethyl furan) and one amine (trimethylamine) were detected in oysters after thermal processing. Alcohols and aldehydes were significantly decreased while pyridines, furans and amines increased during storage of both canned and retort pouched oysters.

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Somwang Songsaeng

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CHAPTER 1

INTRODUCTION

1.1 Background and Rationale

Oysters have been cultured in Thailand for at least 50 years. Among local oysters, only *Crassostrea belcheri, Crassostrea iredalei,* and *Saccostrea cucullata* are being cultured commercially (Tiensongrasmee, 2000). According to the recorded statistics by the Fisheries Statistics and Information Technology Sub-Division (2001), oyster ranked first among a variety of shellfish species in terms of exported value, accounting for 53%, followed by bloody cockle (35%) and green mussel (12%). The annual production and value of these oysters in the year 2000-2006 was approximately 19,600 tons and 403 million bahts, respectively (Department of Fisheries, 2009). The main cultured area of oyster in Thailand located in Bandon Bay, Suratthani province. There are 399 oyster farms which accounting for cultured area about 12,426 Rai with the production of 2,071 tons and value of 79 million bahts in 2007 (Suratthani Provincial Fisheries Office, 2007). The production of cultured oysters in Thailand has been limited mainly due to lack of advanced techniques and the seasonal inconsistency of seed supply (Jarayabhand *et al*., 1994).

In Thailand, oysters are generally sold with shell-on and consumed fresh. However, oysters have limited market because of short shelf-life (Hu *et al*., 2008). There is no report on the shelf-life of fresh oysters in Thailand. Nevertheless, the oyster farmers recommended that the fresh oyster could maintain the quality not more than 2 days at ambient (30 $\pm$ 2°C) storage in normal air. As in fish, after death oysters pass through the following stages: rigor mortis, dissolution of rigor mortis, autolysis and bacterial spoilage. The autolytic process occurs as a result of endogenous enzymatic changes within the muscle, while spoilage is a product of bacterial growth. Methods for evaluating freshness and quality of different marine species are based on measurements of postmortem changes associated with sensory quality, chemical and physical changes, and microbiological growth (Ohashi *et al*., 1991). The quality degradation of flat oyster (*Ostrea edulis*) during storage were

shown by decrease in pH, loss of mantle fluid, retraction of the mantle and changes in plum color (Aaraas *et al*., 2004) as well as microorganisms spoilage occurred.

The post-harvest changes of oyster were depended on method of capture, handling, processing and storage condition (Sikorski *et al*., 1990). Hence, some processing methods i.e. chilling, freezing and heat processing should be applied in order to maintain the quality, to prolong the shelf-life and to increase the value of oyster products for domestic as well as export market.

Chilling is the unit operation in which the temperature of a food is reduced to between -1°C and 8°C. It is used to reduce the rate of biochemical and microbiological changes, and hence to extend the shelf-life of fresh foods (Fellows, 2000). However, undesirable changes i.e. pH drop, increase in TVB-N, lipid oxidation and loss in sensory quality as well as microbial spoilage growth have been reported in oyster during chilling storage (Aaraas *et al*., 2004; Cao *et al*., 2009; Cruz-Romero *et al*., 2008; Zhang *et al*., 2004). The shelf-life of chilled food will be influenced by the type of food, the degree of microbial destruction or enzyme inactivation achieved by the process, control of hygiene during processing and packaging, the barrier properties of the package, and temperatures during chilling and storage (Pigott and Tucker, 1990).

Freezing requires lowering the temperature to lower than –18°C and is a popular method of preserving fish and shellfish (Regenstein and Regenstein, 1991). Compared with all other methods, it has long been established as the best method of preserving high quality in color, flavor, taste, texture and nutritional value of food. However, deterioration of quality caused by chemical or physical reaction can be occurred during storage. Studies have shown that lipid oxidation is one primary cause of quality losses in frozen stored shellfish, such as crab, claw crayfish, lobster, shrimp and oyster under aerobic conditions (Jeong *et al*., 1990; Mohamed Hatha *et al*., 1998; Rebach *et al*., 1990; Tseng *et al*., 2005). In addition, the production of strong rancidity, off-flavors, discoloration, drip losses and texture change from lipid oxidation and hydrolysis also greatly reduces consumer acceptance of stored seafood (Tseng *et al*., 2005).

Thermal processing of food is one of the most effective means of preserving our food supply (Karel *et al*., 1975) by destroying the spoilage

microorganisms as well as inactivation enzymes causing undesirable chemical reaction. Therefore, sterile and safety food products have long storage life under ambient conditions (Lewis and Heppell, 2000). However, color, flavor and texture changes as well as lipid oxidation may also be more pronounced during thermal processing (Ali *et al*., 2005; Bindu *et al*., 2007; Mohan *et al*., 2006; Mohan *et al*., 2008).

The short shelf-life and seasonal production of oysters are some of the major problems which hamper the development of a more extensive oyster market. Moreover, the information about postmortem changes and preservation method of oyster is scarce. Therefore, the main aim of this research was to fine out the more appropriate ways to extend the shelf-life of fresh oyster as well as using another preservation method i.e. freezing and thermal processing to produce longer shelf-life products.

1.2 Objectives of the Research

(1) To evaluate the effect of chilling under different storage condition on the qualities and volatile profile of fresh oyster both in shell-on and shucked forms.

(2) To evaluate the effects of freezing methods and antioxidants on qualities and volatile profile of frozen shucked oyster during storage.

(3) To evaluate the effect of thermal processing and storage on qualities and volatile profile of processed shucked oyster in brine.

1.3 Scope of the Research

Changes in physical, chemical, microbiological and sensory quality as well as volatile profile of fresh white-scar oysters (*C. belcheri*) under different storage conditions at ambient (30 ± 2°C) and chilled (4 ± 2°C) temperature, after freezing with and without antioxidants, and after thermal processing as well as during storage were studied.

1.4 Expecting Outcomes

The outcomes from this research will be information on the effect of chilling under different storage condition on the qualities and volatile profile of fresh oyster both in shell-on and shucked forms, the effect of freezing methods and antioxidants on qualities and volatile profile of frozen shucked oyster and during storage as well as the effect of thermal processing and storage on qualities and volatile profile of canned and retort pouched shucked oyster in brine. Those findings could be useful as guideline practice and preservation for farmers, wholesalers, retailers, manufacturers and consumers to provide and consume better quality and safety oyster.

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CHAPTER 2

LITERATURE REVIEW

2.1 Biological Characteristics of Oyster

White-scar oyster (*Crassostrea belcheri*), bivalve (two-shelled) mollusk, is categorized in the family *Ostreidae* and subfamily *Crassostreinae* (Vaught, 1989). It is the main oyster species cultured in Thailand, which is considered to have high potential for domestic and export markets. It is mainly cultured in Bandon bay, Suratthani province, south of Thailand (Department of Fisheries, 2009). It consists of a soft body enclosed between two triangular shells with large, thick and oval shapes. The external shell color is grayish white often with shades of light to dark purple in irregular markings and lamellose surface. The left or lower valve is generally deeply cupped and is the area where the oyster attaches to the substrate. The shells are attached at the hinge and held together by an adductor muscle. The interior shell color is chalky white. They have a rather small, white and almost central adductor muscle scar crescentic (Figure 2.1) (Yoosukh and Duangdee, 1999).

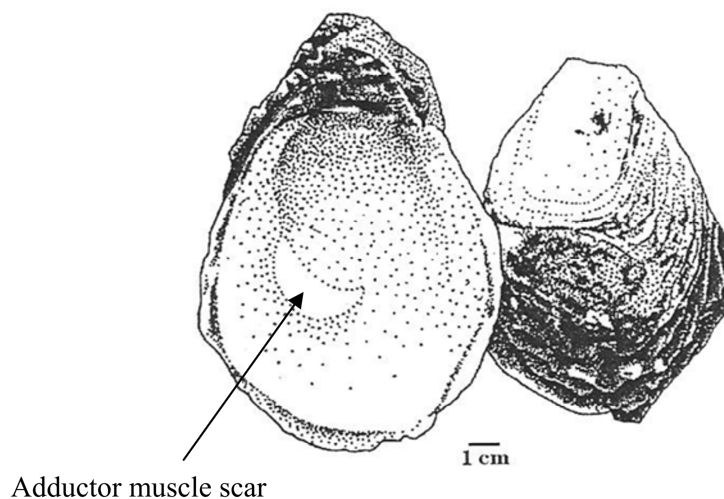

Figure 2.1 Shell morphology of oysters (*C. belcheri*)
Source: Yoosukh and Duangdee (1999)

White-scar oyster is protandrous and generally develop first as males and then may change to females. Change of sex may occur between spawning seasons. The water salinity is the most important factor to the oyster growth. The lower or higher levels of water salinity than the sea water salinity may affect the growth and the filter rate of the oysters, resulting in the oyster death (Galtsoff, 1964). Thitikulrat and Wongwiwatthanawut (1984) reported that white-scar oysters cultured in Bandon bay, Suratthani province could grow at the water salinity and the water temperature, ranging from 9-35 part per thousand (ppt) and 25-31°C, respectively. On the other hand, the Department of Fisheries, Thailand (1997) reported that white-scar oysters could thrive well at the water salinity and the water temperature, ranging from 15-30 ppt and 25-30°C, respectively.

Oyster is filter feeders and obtains food by sifting either microscopic plants from the surrounding water or organic material from the substratum. They have enlarged gills, which show the dual function of food collection and gaseous exchange. Water is drawn through the inhalant aperture into the mantle cavity by the action of hair-like cilia, which cover the gills, and pumped out through the exhalant aperture. Food material is passed along the gill surface to ciliated libial palps, one pair on each side of the mouth. The food in mucous strings passes into the stomach, which is surrounded by digestive producing enzymes. The anus is positioned so that waste material and faeces are expelled into the water, which passes out through the exhalant aperture (Figure 2.2) (King, 1995).

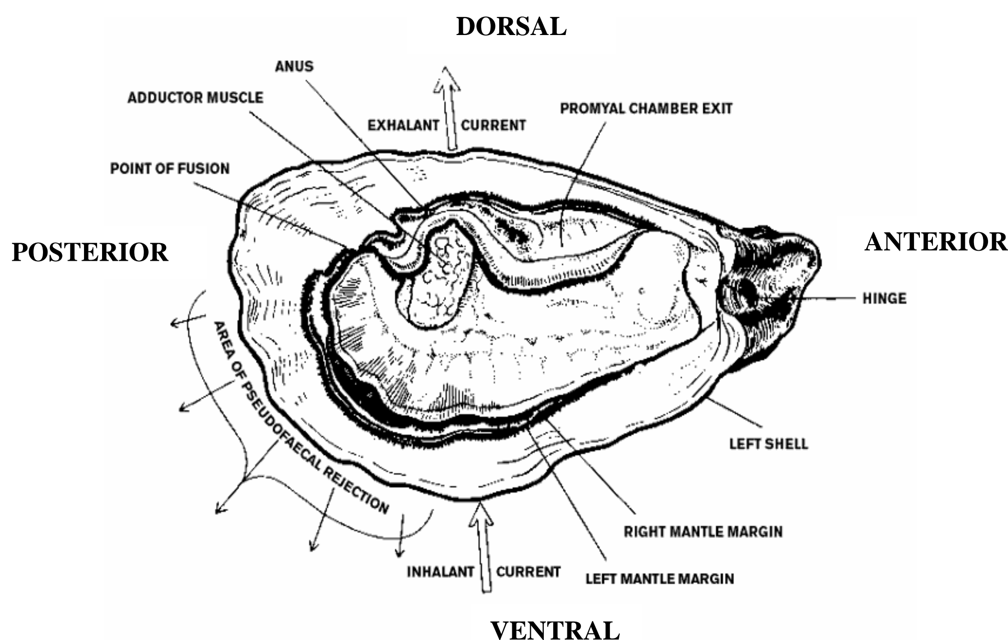

Figure 2.2 A lateral view of the oyster (*Crassostrea gigas*) with the right-hand shell, mantle and gill lobes removed.

Source: King (1995)

The proximate compositions of some species of bivalve mollusks are shown in Table 2.1. All species contained almost the same moisture content but varied in protein, fat, carbohydrate and ash. Oysters contained higher content of carbohydrate and fat but lower protein than other bivalve mollusks.

Table 2.1 Proximate composition (% by wet weight basis) in some species of bivalve mollusks.

	Moisture	Protein	Fat	Carbohydrate	Ash
Oyster species	80.5	9.8	2.1	5.6	2.0
Clam species	80.3	12.8	1.4	3.4	2.1
Scallop species	80.3	14.8	0.1	3.4	1.4

Source: Modified from Jay (2000)

2.2 Post-Mortem Changes in Oyster

As in fish, after death oysters pass through the following stages: rigor mortis, dissolution of rigor mortis, autolysis and bacterial spoilage. The autolytic process occurs as a result of endogenous enzymatic changes within the muscle, while spoilage is a product of bacterial growth. Methods for evaluating freshness and quality of different marine species are based on measurements of post-mortem changes associated with physical, chemical and sensory quality changes as well as microbiological growth (Ohashi *et al*., 1991).

2.2.1 Physical changes

Post-mortem reduction in the pH of fish muscle has an effect on the physical properties of the muscle. As the pH drops, the net surface charge on the muscle proteins is reduced, causing them to partially denature and lose some of their water-holding capacity. Some investigator has associated low muscle pH with tough texture and high drip loss (De Vido *et al*., 2001). He *et al*. (2002) reported that loss of moisture, soft and mushy in texture, and yellow/light brown was found in oyster (*Crassostrea gigas*) when decrease in pH at 2-4°C.

2.2.2 Chemical changes

(1) Glycogen

Glycogen is oxidized by the tissue enzymes in a series of reactions which ultimately produce carbon dioxide (CO_2), water (H_2O) and the energy-rich organic compound adenosine triphosphate (ATP) (Huss, 1995). Glycolysis is the only possible pathway for the production of energy once the heart stops beating. This more inefficient process has principally lactic and pyruvic acids as its end-products (Huss, 1995). Thus, post-mortem glycolysis results in the accumulation of lactic acid which in turn lowers the pH of the tissue. It was reported that pH of oyster (*Ostrea edulis*) dropped from 5.6-6.3 to an ultimate pH of 5.2-5.4 (Aaraas *et al*., 2004).

(2) Protein

Many proteases, such as cathepsins, calpains and collagenases, have been isolated from fish as well as oyster muscle. The proteolytic breakdowns by those proteases are often related to extensive softening of the tissue (Huss, 1995). Autolysis has been shown to accelerate the growth environment of spoilage bacteria (Aksnes and Brekken, 1988). The induction of bacterial spoilage in capelin by autolysis also resulted in the decarboxylation of amino acids, producing biogenic amines and lowered the nutritive value of the fish significantly (Huss, 1995). The autolytic changes in small uneviscerated fish and shellfish are caused not only by the endogenous muscle enzymes but also by kidney and liver cathepsins, as well as by the digestive enzymes of the alimentary tract. The effect depends on the activity of the enzymes, pH in the muscles, properties of the connective tissues, and the presence of proteinase inhibitors (Sikorski *et al*., 1990).

(3) Nucleotide

The content of the major adenine nucleotides and their related compounds in post-mortem muscle correlates well with the loss of freshness in a wide range of fish (Luong *et al*., 1991). The total concentration of adenosine triphosphate (ATP) and related compounds in muscle, as well as the rates and patterns of changes in their levels during storage is species and muscle dependent (Ocano-Higuera *et al*., 2006). Regardless of the species and muscle type, ATP decreases rapidly within the first 24 h postmortem (Haard, 1992). In oyster (*C. gigas*), ATP is metabolized as ATP to adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), inosine (HxR) and then hypoxanthine (Hx), respectively (Qi *et al*., 2007; Yokoyama *et al*., 1992). There are reports that decrease in ATP, ADP and AMP whereas increase in IMP, HxR and Hx were detected in iced and frozen oysters (*C. gigas*) during storage (Qi *et al*., 2007; Yokoyama *et al*., 1992). Similar results were reported by Ocano-Higuera *et al*. (2006) for the adductor muscle of scallop (*Argopecten ventricosus*).

(4) Lipid

Changes in oyster lipid are occurring after oyster death due to tissue lipids is 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) (Ohshima *et al*., 2001) which are considerably susceptible to oxidation. Both oxidation and hydrolysis of lipids in oysters are associated with quality deterioration (Jeong *et al*., 1990; Ohshima *et al*., 2001).

- Lipid oxidation: Both chemical and enzymatic oxidations could probably occur in oysters during chilled and frozen storage. The chemical oxidation is due to the combination of triplet oxygen and singlet oxygen reaction (Erickson, 1998). The endogenous enzymes called lipoxygenase which was 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 as primary oxidation products (Samson and Stodolnik, 2001). Then, 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 (Frankel, 1998). The thiobarbituric acid reactive substances (TBARS) gradually decreased but the concentration of hexadiene, hexanol and 2,5-hexanedione increased during iced (0°C) storage in oyster for 11 days (Zhang *et al*., 2004). On the other hand, Balasundari *et al*. (1997) reported that TBARS increased with increasing storage time at -18°C. Jeong *et al*. (1990) also reported that peroxide value (PV) and TBARS increased during storage at -20 and -35°C.

- Lipid hydrolysis: Post-mortem lipid degradation proceeds mainly due to enzymatic hydrolysis. During chilled storage, triglycerides (TG) and phospholipids (PL) decrease in oyster lipids due to action of endogenous lipolytic enzyme systems, resulting in the significant increase in free fatty acid (FFA) in the oyster tissues (Jeong *et al*., 1990; Jeong *et al*., 1991). Similar result has been reported

in oyster (*C. belcheri*) during storage at ambient (30 $\pm$ 2°C) and chilled (4 $\pm$ 2°C) temperatures (Songsaeng *et al*., 2009a).

2.2.3 Microbiological changes

The microflora of shellfish is closely related to microorganisms presence in environmental water affecting by temperature and salt content. Furthermore, it may depend upon the nature of shellfish, methods of catch and handling conditions (Felhusen, 2000). Jay (2000) reported that microflora could be found in oyster including *Serratia*, *Pseudomonas*, *Vibrio, Proteus*, *Clostridium*, *Bacillus*, *Escherichia*, *Enterobacter*, *Lactobacillus*, *Flavobacterium* and *Micrococcus.* After death, the microflora of oyster could be dramatically increased depending on rigor mortis stage of oyster, methods of catch, handling conditions and storage temperature. Hu *et al*. (2008) reported that increase in total viable counts (TVC) of oyster (*C. gigas*) after stored at 4°C for 14 days was ranged from 6.6 x 10^4 to 5.5 x 10^6 CFU/g while a large increase in TVC from 6.6 x 10^4 to 9.7 x 10^8 CFU/g were found in oyster after stored at 7°C for 14 days. While, Cruz-Romero *et al.* (2008) reported that increase in TVC of oyster (*C. gigas*) was ranged from 3 to 4 log CFU/g after 31 days of 2°C storage. At lower temperature (frozen temperature), microorganism could be reduced. Supporting result that TVC of frozen oyster (*Crassostrea madrasensis*) decreased from 2.2 x 10^4 to 3.4 x 10^3 CFU/g after 150 days of -18°C storage (Balasundari *et al*., 1997).

2.2.4 Sensory changes

Sensory changes are those perceived with the senses i.e. appearance, odor, texture and taste. After death, plum color of oyster changed from cream white to yellow/light brown and texture may change by softening and by development of mushiness due to enzymatic and chemical reactions (Hu *et al*., 2008). Seaweed, fish and mud odor, and more shrunken and contracted mantle appearance of the mantle of oyster were pronounced in oyster (Aaraas *et al*., 2004). The flavor deteriorates gradually due to oxidation and development of off-odors occurs as the result of enzymatic and bacterial processes (Zhang *et. al*., 2009).

2.3 Assessment of Oyster Quality

Most often "quality" refers to the aesthetic appearance and freshness or degree of spoilage which the oyster has undergone. It may also involve safety aspects such as being free from harmful bacteria or chemicals. Therefore, the parameters of oyster quality assessment are conveniently divided into four categories: physical, chemical, microbiological and sensory parameters as follows:

2.3.1 Physical parameters

(1) Color

The color of fresh oyster body is normally cream white and become yellow/light brown and transparent when spoilage. Hunter L-(lightness), a-(red/green color), and b-(blue/yellow color) values are well-known parameter to evaluate the color change of the products using a colorimeter. Cruze-Romero *et al*. (2008a) reported that L-, a- and b-values of fresh oyster (*C. gigas*) were approximately 57.0, -2.5 and 11.5, respectively and increased in L-value (indicating that brighter and less transparent appearance) and b-value (indicating that more yellow) was found in oyster (*C. gigas*) during storage at 2°C for 31 days whereas a-value did not change throughout the storage time. On the other hand, L-, a- and b-values of fresh scallop (*A. ventricosus*) were 54.1, -4.0 and 1.3, respectively and did not significant change during storage at 0°C for 15 days (Ocano-Higuera *et al*., 2006).

(2) Texture

Fresh oyster had firm and elastic texture but it become soft, less elastic and mushy when it deteriorated. Shear force measurement was used to evaluate texture in oyster (*C. gigas*) and adductor muscle of scallop (*A. ventricosus*) using texture analyzer. It was reported that initial cutting strength of oyster was 30 g/mm and significantly increased after storage at 2°C for 31 days (Cruz-Romero *et al*., 2008a), while it was not significant changed in shear force of adductor muscle of scallop (*A. ventricosus*) during storage at 0°C for 15 days (Ocano-Higuera *et al*., 2006).

2.3.2 Chemical parameters

(1) K value

As the rate and pattern of post-mortem changes in nucleotides and their related compounds differ considerably for fish and shellfish species (Ryder, 1984), muscle types (Obatake *et al*., 1988), as well as handling and storage conditions (Surette *et al*., 1988). After death, ATP degradation could occur in oyster by endogenous enzyme (Huss, 1995). ATP of oyster (*C. gigas*) was degraded to adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), inosine (HxR) and then hypoxanthine (Hx), respectively (Qi *et al*., 2007). The K value is widely accepted as a freshness index for fishery products during handling and processing. It could calculate by percent ratio of inosine (HxR) and hypoxanthine (Hx) to the sum of ATP and all products of ATP degradation (Ryder, 1984) as follows:

$$\text{K value (\%)} = \frac{[\text{HxR}] + [\text{Hx}]}{[\text{ATP}] + [\text{ADP}] + [\text{AMP}] + [\text{IMP}] + [\text{HxR}] + [\text{Hx}]} \times 100$$

Saito *et al*. (1959) described the freshness of fishery products with K values less than 20% as “very fresh”, less than 50% as “moderately fresh”, and greater than 70% as “not fresh”. Based on these K value categories, the adductor muscle of scallop could be considered as “very fresh” on day 1 (K value = 20.5%), “moderately fresh” up to day 7 (K value = 50.2%) and borderline for freshness at day 15 (K value = 68.5%) (Ocano-higuera *et al*., 2006). While, the adductor muscle of oyster (*C. gigas*) could be considered as “very fresh” on day 0 (K value = 1.6%), and still “moderately fresh” up to day 21 (K value = 37.2%) in ice (Yokoyama *et al*., 1992).

(2) pH

Post-mortem glycolysis results in the accumulation of lactic acid which in turn lowers the pH of the tissue. pH as a basis for determining microbial quality in oyster could be classified into pH 5.9-6.2 (good), pH 5.8 (off), pH 5.5-5.7 (musty)

and pH 5.2 and below (sour or putrid) (Pottinger, 1948). In addition, pH scale could be used to indicate the survival of oyster i.e. pH 5.6-6.3 (live oyster) and pH 5.2-5.4 (dead oyster) (Aaraas *et al*., 2004).

(3) Total Volatile Basic Nitrogen (TVB-N)

In the post-mortem muscle, decomposition both protein and non-protein nitrogenous compounds causing by bacterial and endogenous proteolytic enzymatic actions acumulated ammonia, trimethylamine (TMA), dimethylamine (DMA) and methylamine (Hernandez-Herrero *et al*., 1999). TVB-N (a decomposed both protein and non-protein nitrogenous compounds) analysis has been traditionally used as indicators of quality in the fisheries products during chilled and frozen storage. A limit of below 30 mg N/100 g of oyster tissue has been considered acceptable for human consumption (Lopez-Caballero *et al*., 2000). Rafrafi and Uglow (2009) reported that the initial TVB-N of oyster (*C. gigas*) tissue was 5.9 mg N/100 g of dry soft tissue, and remained unchanged up to 66 h at 4°C. While, increase in TVB-N of oyster (*C. madrasensis*) tissue were ranged from 8.5 to 12.3 mg N/100g for up to 150 days at -20°C storage (Balasundari *et al*., 1997). Similar results were reported that TVB-N of scallop (*A. ventricosus*) increased in ranging of 13.5 and 21.4 mg N/100 g after 15 days of 0°C storage (Ocano-higuera *et al*., 2006).

(4) Trimethylamine nitrogen (TMA)

TMA was produced from reduction of trimethylamine oxide (TMAO) by enzymatic activity of microorganisms (Ocano-higuera *et al*., 2006). Changes in TMA content with time of storage led to loss of sensory quality. The acceptable limit is not above 10 mg TMA/100 g muscle (Sikorski *et al*., 1990). Murata and Skaguchi (1986) reported that TMA in the adductor muscle of oyster (*C. gigas*) did not exceed 4 mg/100 g after storage for 13 days in ice. Similar results were reported in the adductor muscles of scallop (*A. ventricosus*), TMA value did not exceed 3.4 mg/100 g on day 15 at 0°C (Ocano-Higuera *et al*., 2006). Ruiz-Capillas *et al*. (2001) also reported that TMA of adductor muscle of scallop (*Pecten maximus*) did not exceed

6 mg/100 g on day 16 at 0°C. At lower temperature, it could be retard the accumulation of TMA content.

(5) Peroxide value (PV)

The highly unsaturated fatty acids found in oyster lipids are very susceptible to oxidation. The primary oxidation products are the lipid hydroperoxides. These compounds can be detected by chemical methods, generally making use of potential capability to oxidize iodide to iodine or to oxidize ferrous to ferric. The concentration of the hydroperoxides can be determined by titrimetric (Lea, 1952) or by spectrophotometric (Chapman and Mckay, 1949) methods, giving the peroxide value (PV) as milliequivalents (meq) peroxide/kg lipid. The acceptable limit is not above 20 meq/kg fish lipid (Connell, 1995). Jeong *et al*. (1990) reported that PV of frozen oyster (*C. gigas*) increased with increasing storage time but did not exceed 2 mol/kg lipid for up to 12 months of -20 and -35°C storage. While, Songsaeng *et al*. (2009a) reported that increased in PV of shell-on oyster (*C. belcheri*) packed in normal air were ranged from 7.8 to 20.4 meq/kg lipid after 9 days of chilled (4 $\pm$ 2°C) storage.

(6) Thiobarbituric acid reactive substances (TBARS)

In later stages of oxidation, secondary oxidation products (i.e. alcohols, aldehydes, ketones and short chain fatty acid) will usually be present and thus be indicative of a history of autoxidation. These compounds have very unpleasant odors and flavors. Some of secondary oxidation products i.e. aldehydes can react with thiobarbituric acid, forming a reddish colored product that can be determined spectrophotometrically (Buege and Aust, 1978). Using this principle, a measure of thiobarbituric acid reactive substances (TBARS) can be obtained and expressed as mg malonaldehyde/g lipid or μmol malonaldehyde/kg. A value of not above 2 mg malonaldehyde/kg has been considered acceptable in odor/taste of fish (Connell, 1995). Jeong *et al*. (1990) reported that TBARS values of frozen oyster (*C. gigas*) increased at -20 and -35°C of storage but did not exceed a value of 1-2 mg malonaldehyde/kg tissue up to 12 months. Similar results were reported in oyster

(*C. gigas*) during 2°C of storage for 31 days (Cruz-Romero *et al*., 2008a). While, increased in TBARS of frozen oyster (*C. madrasensis*) were ranged from 8.7 to 22.3 μmol malonaldehyde/kg tissue after stored at -18°C for 150 days (Balasundari *et al*., 1997).

(7) Volatile oxidation products

The large amount of polyunsaturated fatty acid found in oyster lipids makes them highly susceptible to oxidation (Ohshima *et al*., 2001). The secondary autoxidation products of shorter carbon chain-length mostly aldehydes, ketones, alcohols, small carboxylic acids and alkanes were later produced (Huss, 1995). Kawai (1996) reported that storage and processing enhanced generation of various volatile compounds in fish, suggesting that enzymatic and non-enzymatic oxidative reactions occurred during treatments. In addition, some volatile compound i.e. ketones can arise due to microbially-induced oxidation, lipid oxidation or amino acid degradation (Cruz-Romero *et al*., 2008b). Volatile compounds were extracted by several extraction methods including, dynamic headspace (Piveteau *et al*., 2000), vacuum steam distillation (Pennarun *et al*., 2002) and headspace solid phase microextraction (Songsaeng *et al*., 2009b), and further characterize by gas chromatography-mass spectrometry (GC/MS). Josephson *et al*. (1985) and Ohshima *et al*. (2001) reported that (*E*)-2-nonenal, (*E,Z*)-2,6-nonadienal and 3,6-nonadien-1-ol, 1,5-octadiene-3-ol, 1-octen-3-one, 1-octen-3-ol, 1,5-octadien-3-one and 2,5-octadien-1-ol were found in fresh oysters (*C. virginica* and *C. gigas*). During improperly chilled storage, fish or fish oils generated strong oxidized odors that have been described as rancid, stale, fishy and cod liver oil-like. For example, (*Z*)-4-heptenal can be generated from (*E,Z*)-2,6-nonadienal to the off odor of cold-stored cod (Josephson and Lindsay, 1987).

2.3.3 Microbiological parameters

(1) Total viable counts (TVC)

Major microorganisms were reported in oyster including, *Serratia*, *Pseudomonas*, *Proteus*, *Vibrio*, *Clostridium*, *Bacillus*, *Escherichia*, *Enterobacter*, *Lactobacillus*, *Flavobacterium* and *Micrococcus* (Jay, 2000). Under improper

handling and storage conditions, the microflora of oyster could be dramatically increased. The standard for freshly chilled and frozen bivalve meat did not exceed 5 x 10^5 CFU/g (Department of Fisheries, Thailand, 2004; ICMSF, 1980). He *et al.* (2002) reported that TVC of oyster (*C. gigas*) exceeded the refer of 5 x 10^5 CFU/g after 9 days at 2-4°C as well as at 2°C of storage for 31 days (Cruz-Romero *et al.*, 2008a). Moreover, decrease in temperature could reduce microorganisms as well as the report of Balasundari *et al.* (1997), they reported that initial TVC of frozen oyster (*C. madrasensis*) were 2.2 x 10^4 CFU/g, and decreased up to 3.4 x 10^3 CFU/g after 150 days of -18°C storage.

(2) Pathogenic bacteria

The pathogenicity of certain microorganisms is a major safety concern in the processing and handling of foods in that they produce chemicals in foods that are toxic to human. Their growth on foods may also result in undesirable appearance and off-flavors. The standard value of the pathogenic bacteria for chilled and frozen bivalve mollusks based on countries which shown in Table 2.2.

Table 2.2 The microbiological quality standard of chilled and frozen bivalve mollusks.

Countries	Pathogens	Product Category	Maximum level
EU	*E. coli*	Ready to eat (consumption	< 230 MPN/100 g
	Salmonella	without further cooking)	ND/25 g
USA	*E. coli*	Ready to eat (consumption	< 230 MPN/100 g
	Salmonella	without further cooking)	ND/25 g
	V. parahaemolyticus		< 1 x 10^4 MPN/g
	V. cholerae		ND/25 g
Japan	*E. coli*	Ready to eat (consumption	< 230 MPN/100 g
	V. parahaemolyticus	without further cooking)	< 100 MPN/g
	V. cholerae		ND/25 g

ND: not detect.

Source: Department of Fisheries (2004)

(3) Spoilage bacteria

In general, mollusks differ in their chemical composition from fish and crustacean shellfish in that they contain significant levels of carbohydrate (glycogen) and a lower total quantity of nitrogen. For this reason, fermentative activities may be expected to occur as a part of microbial spoilage (Jay, 2000). Moreover, under improper handling and storage conditions, microflora eventually leads to spoilage of oysters resulting in development of undesirable sensory characteristics and unsafe for consumption. Lopez-Caballero *et al*. (2000) reported that *Pseudomonas*, *Shewanella putrefaciens* and *Moraxella/Acinetobacter* were found during aerobic iced storage.

2.3.4 Sensory parameters

Sensory quality of fresh oysters for consumer acceptance was evaluated using appearance, odor and texture attributes. Several researchers presented the guideline for freshness evaluation of oyster as shown in Table 2.3, 2.4 and 2.5.

Table 2.3 Freshness guideline for oysters (*C. gigas*).

parameters	0	1	2	3
Odor	Hay/crisp	Strong seaweedy	Spoiled with slight sour smell	Sour and putrid smell
Plum color and appearance	Cream white	White, a few striations	Tan/beige, some striations	Yellow/light brown, many striations
Liquid	Clear	Clear with small amount of debris	Clear with large amount of debris	Cloudy
Texture	Firm and elastic	Soft and less elastic	Slightly mushy	Mushy
Mantle	Strong color brown/black	Slight fading	Mostly faded	Faded
Gill	Gill filaments well defined	Filaments less defined	Filaments poorly defined	Filaments undefined
Adductor	Pale white, translucent	Light-gray, translucent	Light-gray, partially opaque	White, opaque

Source: He *et al*. (2002)

Table 2.4 Attributes and their description used in freshness evaluation of oysters (*O. edulis*)[a].

Attributes	Left end of scale (1)	Midpoint (4)	Right end of scale (7)
Smell			
- Sea/seaweed	Fresh	Neutral[b]	Seaweed at low tide
- Fishy	Fresh	Neutral[b]	Ammonia
- Shellfish, crab	Fresh	Neutral[b]	Sour shrimp
- Mud	None		Much
Appearance			
- Gills	Intact		Dissolved
- Mantle	Outstretched		Shrunked, contracted
- Body, color	White, creamy		Transparent
- Body, form	Well rounded		Sunken

[a]A 7-points scale were used. [b]No perceptible taste of this attribute.

Source: Aaraas *et al*. (2004)

Table 2.5 Freshness grade guide for oysters (*C. gigas*).

Score	Odor	Body color	Fluid	Texture
3[a]	Hay	Cream white	Clear	Firm and elastic
2	Stronger seaweedy	White	Clear with small amount of debris	Soft and less elastic
1	Slight sour smell	Tan/beige	Clear with large amount of debris	Slightly mushy
0[b]	Sour and putrid smell	Yellow/light brown	Cloudy	Mushy

[a]Extremely desirable. [b]Extremely undesirable.

Source: Cao *et al*. (2009)

2.4 Effect of Various Storage Conditions and Processing on Qualities of Oyster

2.4.1 Effect of chilled storage on qualities of fresh oyster

Chilling is the unit operation in which the temperature of a food is reduced to between -1°C and 8°C, which can reduce the rate of biochemical and microbiological changes, and hence to extend the shelf-life of fresh and processed foods. Changes in physical, chemical, microbiological and sensory quality as well as volatile profile of oyster during chilled storage could be described as follows:

(1) Physical and chemical quality

Chilling is an essential requirement for quality retention for oyster. However, loss of color and texture qualities were found in oyster (*C. gigas*) during storage at 2°C for 31 days (Cruze-Romero *et al*., 2008a) whereas it was not significant changed in scallop (*A. ventricosus*) during storage at 0°C for 15 days (Ocano-Higuera *et al*., 2006).

Yokoyama *et al*. (1992) reported that decreased in ATP level but increased in K value of oyster (*C. gigas*) were found after stored at 0°C for 21 days. Ocano-higuera *et al*. (2006) also found that K value of scallop (*A. ventricosus*) increased with the ranged of 20.5 and 68.5% after stored at 0°C for 15 days.

Increase in TVB-N of oyster (*O. edulis*) was ranged from 13.3 to 25-30 mg N/100 g after 10 days of 2°C (Lopez-Caballero *et al*., 2000). It was also found that TVB-N increased with ranging of 5.3 and 70 mg N/100 g in oyster (*C. gigas*) after 18 days of 5 $\pm$ 1°C (Cao *et al*., 2009). On the other hand, TVB-N of oyster (*C. gigas*) remained unchanged at 5.9 mg N/100 g throughout the storage for up to 66 h at 4°C (Rafrafi and Uglow, 2009).

Upon death, pH of muscle decreased due to the formation of lactic acid from glycogen whereas rising of pH in the later storage period was due to the formation of DMA and TMA from trimethylamine oxide (TMAO) which exhibited the base properties. These changes in pH could affect connective tissue properties and cause loss of myofibrillar protein solubility (Rodger *et al*., 1980). On the other hand,

He *et al*. (2002) reported that pH value of oyster (*C. gigas*) decreases with ranging of 6.3 and 4.1 after 27 days of 2-4°C. Similar results had been reported in oyster (*O. edulis*) during chilled storage (Aaraas *et al*., 2004; Lopez-Caballero *et al*., 2000).

Lipid oxidation may occur to develop the deterioration during improperly chilled storage of food product. Generally, peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) are used to assess the acceptability index of lipid oxidation. Zhang *et al*. (2004) reported that TBARS of oyster (*C. gigas*) gradually decreased during iced (0°C) storage for 11 days while TBARS of oyster (*C. gigas*) slightly increased at 2°C but did not exceed a value of 1-2 mg malonaldehyde/ kg oyster (Cruz-Romero *et al*., 2008a). Increase in PV was found of both shell-on and shucked oyster (*C. belcheri*) during storage at 4 $\pm$ 2°C (Songsaeng *et al*., 2009a).

(2) Microbiological quality

The activity of microorganisms is a main factor limiting the shelf-life of fresh oyster. Especially, total viable count (TVC) is used as an acceptability index in standards, guidelines and specification of oyster. Department of Fisheries, Thailand (2004) and ICMSF (1980) recommended that freshly chilled and frozen bivalve meat should have TVC below 5 x 10^5 CFU/g. He *et al*. (2002) reported that TVC of oyster (*C. gigas*) exceeded 5 x 10^5 CFU/g after stored at 2-4°C for 9 days while TVC of oyster (*C. gigas*) did not exceed level of 5 x 10^5 CFU/g for up to 31 days at 2°C (Cruz-Romero *et al*., 2008a). In addition, the microbial counts of oyster (*O. edulis*) increased dramatically during iced (1°C) and cold (5°C) storage for 19 days (Aaraas *et al*., 2004). Similar results were reported in oyster (*C. gigas*) during refrigerated (5 $\pm$ 1°C) storage (Cao *et al*., 2009). Generally, the results from the microbiological analyses related to the findings of the sensory evaluation. Therefore, Aaraas *et al.* (2004) recommended that oyster (*O. edulis*) should not be consumed after 12 days of chilled (1°C and 5°C) storage. The shelf-life of this oyster was 8-9 days but this shelf-life could prolong up to 14-15 days during low temperature storage associated with chitosan treatment (Cao *et al*., 2009).

(3) Sensory quality

Storage conditions may also influence the sensory profile especially the smell of seaweed, fish and mud, and the appearance of the mantle and plum color. Oyster (*O. edulis*) significantly increased in smell of seaweed, fish, and mud, and shrunken and contracted mantle appearance during storage at 1°C and 5°C for 19 days (Aaraas *et al*., 2004). Similar results were reported in oyster (*C. gigas*) during storage at 5 $\pm$ 1°C (Cao *et al*., 2009).

(4) Volatile profile

Songsaeng *et al*. (2009a) reported that two alcohols (1-octen-3-ol and 2-ethyl-1-ol) were found only in fresh oyster (*C. belcheri*) and increased with increasing storage time. During chilled (4 $\pm$ 2°C) increases in 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 in their oyster for up to 6 days. During improperly chilled storage, fish or fish oils generated strong oxidized odors that have been described as rancid, stale, fishy and cod liver oil-like. For example, (*Z*)-4-heptenal can be generated from (*E,Z*)-2,6-nonadienal to the off odor of cold-stored cod (Josephson and Lindsay, 1987). In addition, Zhang *et al*. (2004) reported that increases in hexadiene, hexanol and 2,5-hexanedione, as off-flavor compounds, during iced (0°C) storage for 11 days were found in oyster (*C. gigas*).

2.4.2 Effect of freezing and frozen storage on qualities of oyster

Freezing provides a significantly extended shelf-life and has been successfully employed for the long term preservation of many foods. The major function of freezing is to change the physical state of water into ice when energy is removed at freezing temperature or further reduced to –18°C or lower (Rahman and Labuza, 1999). The selection of any specific freezing method on the quality of product depends on rate of freezing, shape and size of the product, and feasibility governed by location and type of product (Garthwaite, 1997). Typically, foods can be

frozen either by quick or slow freezing method. Quick freezing is usually achieved within 30 min by reducing the temperature at more than 50°C/h and form a small ice crystal in both extracellular and intracellular compartments. Whereas slow freezing may take about 3 to 72 h to reduce the temperature at 1-10°C/h and a large ice crystal will be formed (Jay, 1996).

The undesirable changes during freezing are associated with formation of large ice crystals in extracellular compartment, mechanical damage by the ice crystals to cellular structures through distortion and volume changes, and chemical damage arising from changes in concentrations of solutes. Drip loss during thawing of the quick frozen product is thus considerably low, and the surface reflects lighter than that of slow frozen product. Consequently, the cut surface appearance is more acceptable (Farias *et al*., 2005; Jiang and Lee, 2004).

The extent on quality of frozen food is dependent upon many factors including, storage temperature and time, packaging, rate of freezing and thawing, temperature fluctuations and freeze-thaw abuse (Srinivasan *et al*., 1997). Improper freezing and frozen storage could affect physical, chemical, microbiological and sensory qualities as well as volatile profile of oyster as follows:

(1) Physical and chemical quality

Decreased in ATP level but increased in K value of oyster (*C. gigas*) were found during storage at -20°C and -30°C (Qi *et al*., 2007). whereas pH of oyster (C. *gigas*) unchanged throughout the storage at -20°C and -35°C (Jeong *et al*., 1990).

Balasundari *et al*. (1997) reported that decreases in moisture, protein, glycogen and alpha amino nitrogen whereas increase in TVB-N was found in oyster (*C. madrasensis*) during frozen storage at -18°C.

Jeong *et al*. (1990) reported that increases in PV and TBARS of oyster (*C. gigas*) were ranged from 1.3 to 2 meq/kg lipid and 0.5 to 1.5 mg malonaldehyde/kg muscle, respectively, and changes in lipid and fatty acid compositions were found after 12 months of -20°C and -35°C storage. Similar results were reported in oyster (*C. madrasensis*) during storage at -18°C (Balasundari *et al*., 1997). In addition, increases in aldehyde compounds and FFA were found in oyster (*C. gigas*) during frozen storage at -8°C (~10°C) and -20°C (Hatano *et al*., 1990).

(2) Microbiological quality

Bacterial activity can be retarded by freezing and frozen storage. Balasundari *et al*. (1997) reported that the process of freezing brought about 89.9% reduction in TVC immediately after freezing, from 2.2 x 10^4 CFU/g recorded on shucked meat. During storage at -18°C, TVC decreased into 3.4 x 10^3 CFU/g after 150 days. In addition, slightly decreased in staphylococci, motile aeromonads, total coliforms and *E. coli* were also found in oyster (*C. madrasensis*) (Balasundari *et al*., 1997). Decreases in *Salmonella* and *E. coli* of oyster (*C. gigas*) were found after freezing and frozen storage (Digirolamo *et al*., 1969).

(3) Sensory quality

During frozen storage, changes in texture and flavor occur continuously though at a slower rate. The effects of frozen temperature were demonstrated by Chapman *et al*. (1993) who compared the effects of freezing temperature of -20, -30 and -40°C on the quality of fillets and minces over 24 months storage period. Both sensory and textural deteriorations increased over the storage period. However, samples stored at lower temperature developed less fishy taste and aroma and retained more original sweetness and fresh fish taste. Jeong *et al*. (1990) also reported that decreases in color and flavor scores of oyster (*C. gigas*) stored at -35°C were slower than those stored at -20°C. Qi *et al*. (2007) also reported that decreases in odor, appearance and texture scores of oyster (*C. gigas*) stored at -30°C were slower than those stored at -20°C.

(4) Volatile profile

It has been well known that oxidation and hydrolysis of lipids in fish and shellfish during frozen storage can cause serious quality deterioration especially, lipid oxidation (Jeong *et al*., 1990). Thus the accumulated aldehydes, ketones, alcohols, short-chain fatty acids and hydrocarbons were found (Sikorski, 1990). Songsaeng *et al*. (2009b) reported that only two alcohols (1-octen-3-ol and 2-ethyl-1-hexanol) were found in fresh oyster (*C. belcheri*) whereas eight alcohols ((*E*)-2-penten-1-ol, hexanol, heptanol, 1-octen-3-ol and 2-ethyl-1-hexanol, 1-octanol, 1-penten-3-ol and 3-hexanol),

six aldehydes (pentanal, hexanal, (*E*)-2-pentenal, (*E,E*)-2,4-heptadienal, (*E*)-2-methyl-2-butenal and 2-methyl-2-pentenal), two carboxylic acids (pentanoic acid and 3-methylbutanoic acid), one ketones (2-undecanone), and one hydrocarbons (1-octadecene) were found during storage at -20°C for 12 months. Volatile carbonyl compounds are thought to be responsible for the rancid odor and flavor. It is mainly (*Z*)-4-heptenal in frozen-stored cod and 2,4,7-decatrienals in oxidized mackerel oil (Sikorski, 1990).

2.4.3 Effect of thermal processing and storage on qualities of oyster

Thermal processing of food is one of the most effective means of preserving our food supply (Karel *et al.*, 1975) by destroying the spoilage microorganism as well as inactivation enzymes causing undesirable chemical reaction. Therefore, sterile and safety food products have long storage life under ambient conditions (Lewis and Heppell, 2000; Regenstein and Regenstein, 1991). Factors affecting the quality and safety of sterile food products are the heat resistance of microorganisms or enzymes likely to be present in food, heating conditions, pH of food, size of container and physical state of food (Footitt and Lewis, 1995).

For the purpose of canning, food is divided into three pH groupings: high acid foods (pH < 3.7), acid foods (pH 3.7-4.5) and low acid foods (pH > 4.5) as shown in Table 2.6.

The important terms to determine the thermal destruction include D value, Z value and the lethality value (F value) as follows:

D value: Time required at any temperature to destroy 90% or 1 log cycle of the spores or vegetative cells of a given organism.

Z value: Number of Fahrenheit or Celsius degrees required for the thermal destruction curve to traverse one log cycle.

F value: The equivalent, in minutes at some given reference temperature, of all heat considered, with respect to its capacity to destroy spores or vegetative cells of a particular organism.

Table 2.6 Classification of canned foods on basis of processing requirements.

Acidity Classification	pH value	Food Item	Food Groups	Spoilage Agents	Heat and Processing Requirements
Low acid	7.0	Crabmeat, eggs, oysters, milk, corn, duck, chicken, codfish, beef, sardines	Meat, fish, milk, poultry	Mesophilic spore-forming anaerobic bacteria	High temperature processing 116-121°C (240-250°F)
	6.0	Corned beef, lima beans, peas, carrots, beets, asparagus, potatoes	Vegetables	Thermophiles Naturally occurring enzymes in certain processes	
	5.0	Tomato soup	Soup		
Acid	4.5		Manufactured foods	Lower limit for growth of *Cl. botulinum*	
		Potato salad, tomatoes, pears, apricots, peaches, oranges	Fruit	Non-spore forming aciduric bacteria Acidic spore-forming bacteria	Boiling water processing 100°C (212°F)
High acid	3.7	Pineapple, apple, strawberry, grapefruit, sauerkraut	Berries	Natural occurring enzymes	
	3.0	Pickles, cranberry juice, lemon juice, lime juice	High acid foods (pickles) High acid-high solids foods (jam-jelly)	Yeasts Molds	
	2.0		Very acid foods		

Source: Desrosier (1970)

Generally, F_0 value can be used to evaluate the amounts of microbial inactivation in thermal sterilization, which is the equivalent exposure time at 121°C or

250°F of the actual exposure time at a variable temperature, calculated for an ideal microorganism with a temperature coefficient of destruction equal to 10. The factors influencing the F_0 value are the initial microbial population in the product, type of product, size of the container, types of heating and cooling media (Heldman and Hartel, 1998).

In order to determine the process time for a given food, it is necessary to have information about both the heat resistance of microorganisms, particularly heat resistant spores, or enzymes that are likely to be present and the rate of heat penetration into the food. In low acid foods (pH > 4.5), the heat resistant, spore forming microorganisms, *Clostridium botulinum* is the most dangerous pathogen likely to be present. Under anaerobic conditions inside a sealed container it can grow to produce a powerful exotoxin, botulin, which is sufficiently potent to be 65% fatal to humans. *Cl. botulinum* is ubiquitous in soil and therefore likely to be found in small numbers on any raw material that has contact with soil. Because of the extreme hazard from botulin, the destruction of this micro-organism is therefore a minimum requirement of commercial heat sterilization. Normally, foods receive more than this minimum treatment as other more heat-resistant spoilage bacteria may also be present (Table 2.7). In acid foods (pH 4.5-3.7), other microorganisms (for example yeasts and fungi) or heat-resistant enzymes are used to establish processing times and temperatures. In acid foods (pH < 3.7), enzyme inactivation is the main reason for processing and heating conditions are less severe (sometimes referred to as pasteurization) (Fellows, 2000).

From food safety and human health perspective, the commercial sterilization of seafood products as low-acid foods (pH > 4.5) concern to the survival of *Cl. botulinum*, an anaerobic spore-forming microorganism that produces an extremely potent exotoxin. It is generally recognized that a thermal process sufficient to eliminate *Cl. botulinum* as a health hazard is sufficient to eliminate the health hazards associated with all other pathogens likely to be found in a raw food commodity (Stumbo, 1973). Different F_0 value reported for different sterilized seafood products such as, 7 min at 121°C for canned (size 6.52 cm i.d. x 3 cm) albacore tuna (*Thunnus alalunga*) in oil (Aubourg *et al*., 1997) and 9 min at 121°C for black clam (*Villorita cyprinoides*) in masala medium packed in retort pouched

(18 cm x 11 cm) (Bindu *et al*., 2007). Durance and Collins (1991) investigated that F_0 value for canned at 121°C was 6.8 min for canned and 8.2 min for retort pouched chum salmon (*Oncorhynchus keta*) in 1% non-iodized salt packed in tinplate cans (307 x 200) and in retort pouches (176 mm x 250 mm).

Table 2.7 Heat resistance of some spore-forming bacteria used as a basis for heat sterilization of typical pH of foods.

Type of foods and bacterial groups	Approximate range of heat resistance	
	D value (min)	Z value (°F)
Low acid foods (pH > 4.5)		
Thermophiles (spores)	D 250°F	
Flat-sour (*Bacillus stearothermophilus*)	4.0-5.0	14-22
Gaseous-spoilage (*Cl. thermosaccharolyticum*)	3.0-4.0	16-22
Sulfide stinkers (*Cl. nigrificans*)	2.0-3.0	16-22
Mesophiles (spores)		
Putrefactive anaerobes		
Cl. botulinum (type A & B)	0.1-0.2	14-18
Cl. sporogenes (PA3679)	1.0-1.5	14-18
Acid foods (pH 3.7-4.5)		
Thermophiles (spores)		
B. coagulans (facultatively mesophile)	0.01-0.07	14-18
Mesophiles (spores)	D 212°F	
B. polymyxa and *B. macerans*	0.1-0.5	12-16
(*Cl. pasteurianum*)	0.1-0.5	12-16
High acid foods (pH ≤ 3.7)		
Mesophilic non-spore forming	D 150°F	
Lactobacillus spp., *Leuconostoc* spp., yeasts and molds	0.5-1.0	8-10

Source: Stumbo (1973)

The main goal of thermal processing is inactivation or destroying the pathogenic microorganisms to make food safe to the consumer (Bellara *et al*., 1999).

However, this needs to be done without the over-processing which may result in undesirable physical, chemical and sensory qualities as well as volatile compound.

(1) Physical and chemical quality

Heat processing may cause quality deterioration depending upon various factors such as poor quality raw material, unsuitable heat treatment etc. In case of canned tuna, the discoloration known as 'greening', relates to the TMAO, myoglobin, cysteine concentration, and the cooking operation itself. The greening of canned tuna can occur due to the combination between sulhydryl group of denatured metmyoglobin and cysteine under oxidized condition by TMAO. Therefore, the combined content of TMAO and TMA in raw fish that can subsequently be used to indicate the probability of 'greening' which occurs during the heating process (Nagaoka *et al*., 1971). Browning in canned fish can occur due to the reaction of 5-carbon reducing sugar, ribose and amino acids. This type of color change is more pronounced especially in spoiled fish by the chemical reaction between riboside hydrolase and ribonucleic acid. However, with ribose being soluble, pre-cooking the fish and decanting the cook-out help avoid the problem (Tai *et al*., 2000).

Abuse heating might lower the nutritional value of the food as vitamins and micro-nutrients (Miri *et al*., 2008). In addition, the changing of proximate composition (moisture, fat and protein) of processed product may occur after heat processing. Some undesirable changes due to the thermal process are associated with increased protein and fat content, and decreased moisture content (Garcia-Arias *et al*., 1994; Garcia-Arias *et al*., 2004). The presence of unsaturated fatty acids enhances lipid oxidation, through the development of FFA and TBARS during heating and storage (Bindu *et al*., 2007; Mohan *et al*., 2006).

(2) Sensory quality

Chia *et al*. (1983) reported that darker in color and softer in texture were found in canned and retort pouched rainbow trout and pollock after thermal processing whereas did not significant change in both samples after stored at 22°C for 120 days. On the other hand, changes in odor of both samples were found during

storage. Bindu *et al*. (2007) also reported that changes in texture and odor were found in retort pouched black clam (*Villorita cyprinoides*) in masala sauce while did not significant change in taste during storage at 28 ± 2°C for 12 months.

(3) Volatile profile

Upon heat processing, many low-molecular-weight aroma-active compounds are formed via lipid oxidation, strecker degradation and maillard reaction (Girard and Durance, 2000; Rodriguez-Bernaldo de Quiros *et al*., 2001). In addition, maillard and strecker degradation reactions play predominant role in developing the meaty aromas of cooked fish and shellfish, especially sulfur containing compounds, pyrone, pyrazine, oxazole and pyrrolidine compounds. The main volatile compounds in cooked mussels include 2,3-butanedione (buttery, caramel-like odor), (*Z*)-4-heptenal (boiled potato-like odor), (*E*)-2-penten-1-ol (mushroom-like odor), 2-ethylpyrazine (nutty odor), methional (boiled potato-like odor) and *(E,E)*-2,4-octadienal (cucumber-like odor) (Le Guen *et al*., 2000; Le Guen *et al*., 2001).

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CHAPTER 3

EFFECT OF DIFFERENT STORAGE CONDITIONS ON QUALITY AND VOLATILE PROFILE OF FRESH OYSTER (*CRASSOSTREA BELCHERI*)

3.1 Introduction

White-scar oyster (*Crassostrea belcheri*) is mainly cultured in Thailand and generally sold with shell-on and consumed fresh. However, some constraints of limiting domestic and export markets may occur because of their short shelf-life (Hu *et al*., 2008). There is no report on the shelf-life of fresh white-scar oyster, nevertheless Thai oyster farmers recommended that the fresh oysters could maintain the quality not more than 2 days at ambient temperature (30 $\pm$ 2°C) storage in normal air. Pacific oysters (*Crassostrea gigas*) stored in air with water sprinkling at 7°C had 52-80% of survival after 20 weeks storage (Seaman, 1991). 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 oysters (Aaraas *et al*., 2004). Like other seafood, quality deterioration and spoilage of fresh oysters occur very rapidly due to enzymatic autolysis, microbial growth and physical alterations. The post-harvest changes of oyster tissue depend very significantly upon factors affecting the concentration of substrates and metabolite in the tissue, the activity of endogenous enzymes, the microbial contamination and the conditions after harvesting (Sikorski *et al*., 1990).

Chilling is an essential requirement for quality retention for fish and shellfish. However, the microbial counts of flat oyster (*O. edulis*) increased dramatically during iced (1°C) and chilled (5°C) storage for 19 days. It was also reported that tissue pH in all live oysters ranged from 5.6-6.3 and tissue pH of dead oysters ranged from 5.2 to 5.4, and decreased during iced (1°C) and chilled (5°C) storage and had a tissue pH of 5.5 at day 16 (Aaraas *et al*., 2004). Aerobic plate count and total volatile basic nitrogen (TVB-N) of raw oyster (*C. gigas*) increased, but pH and freshness score decreased during refrigerated (5 $\pm$ 1°C) storage. The shelf-life of

this oyster was 8-9 days but this shelf-life could prolong up to 14-15 days during low temperature storage associated with chitosan treatment (Cao *et al*., 2009). Storage conditions may also influence the sensory profile especially the smell of seaweed, fish and mud, and the appearance of the mantle and plum color. Iced flat oysters and chilled flat oysters had significantly more pronounced smell of seaweed, fish, and mud, and more shrunken and contracted mantle appearance than those kept in running seawater (Aaraas *et al*., 2004). High-pressure-treated oyster (*C. gigas*) showed more inactivate microorganisms, delay microbial growth, maintain color, texture and pH changes, but increased in lipid oxidation than untreated oyster during chilled (2°C) storage (Cruz-Romero *et al*., 2008).

Changes in oyster lipid are noteworthy phenomena occurring after harvesting and storage, due to tissue lipids are 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) (Ohshima *et al*., 2001; Passi *et al*., 2002; Pennarun *et al*., 2002) which are considerably susceptible to oxidation. Both oxidation and hydrolysis of lipids in oysters are associated with quality deterioration (Jeong *et al*., 1990; Jeong *et al*., 1991; Ohshima *et al*., 2001). 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 (Frankel, 1998). The thiobarbituric acid reactive substances (TBARS) gradually decreased but the concentration of hexadiene, hexanol and 2,5-hexanedione, as off-flavor compounds, increased during iced (0°C) storage in oyster for 11 days (Zhang *et al*., 2004). Triglycerides (TG) and phospholipids (PL) decrease in oyster lipids due to action of endogenous lipolytic enzyme systems, resulting in the significant increase in free fatty acid (FFA) in the oyster tissues (Jeong *et al*., 1990; Jeong *et al*., 1991).

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 (Takiguchi, 1989). Although salt allows a prolonged storage, it has been reported to enhance lipid oxidation of highly unsaturated fatty acid (Ackman, 1989). 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 prooxidants or antioxidants. The addition of salt could increase the rate of lipid oxidation in fish muscle stored at -12°C (Apgar and Hultin, 1982), and in poultry meat (King and Earl, 1988). On the other hand, it was reported that 5% NaCl could slightly inhibit the oxidation of lard (Chang and Watts, 1950), whereas its content of above 2.3% showed the inhibition of lipid oxidation in deheaded and gutted sardine dipped in salt solutions (Nambudiry, 1980). The addition of salt at 0.29% and 0.58% in cod phospholipids (Mozuraityte, 2007) as well as at 1.5% in frankfurters (Wimmer *et al*., 1993) 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 at the content above 3 M (Srinivasan and Xiong, 1996). Furthermore, NaCl at 10.7, 21.4 and 46 g/kg of butter fat produced a protective effect on fat oxidation, while the salts at 6.6 g/kg of butter fat showed prooxidant effect (Al-Ismail and Humeid, 2004).

Information about the postmortem changes and preservation method of oysters is scarce which limits its commercialization as a fresh product. Therefore, the present study was undertaken to investigate the effect of different storage conditions on the quality and volatile profile of fresh white-scar oyster (*C. belcheri*) both in shell-on and shucked forms.

3.2 Research Methodology

3.2.1 Chemicals and media

All chemicals for chemical quality analysis were analytical grade including, trichloroacetic acid (TCA) (Merck, Darmstadt, Germany), chloroform (BDH, Poole, England), methanol, acetic acid (Lab-Scan, Bangkok, Thailand), ferrous sulfate, barium chloride, ferric chloride (Fluka, Buchs, Switzerland), anhydrous sodium sulfate, ammonium thiocyanate and benzene (Merck, Darmstadt, Germany). For lipid composition standards containing tripalmitin, triolein, dipalmitin, diolein, monopalmitin, monoolein, palmitic acid, oleic acid and phosphatidylcholine (Sigma, St. Louis, MO, USA), fatty acid standards containing C14:0 to C24:1 (PUFA-1,

Marine source, Supelco Inc., Bellefonte, PA, USA) and volatile standards including, 3-methyl-1-butanol, (*E*)-2-penten-1-ol, hexanol, (*Z*)-3-hexen-1-ol, 1-octen-3-ol, 2-ethyl-1-hexanol, 1,5-octadien-3-ol, 1-octanol, (*E*)-2-octen-1-ol, (*E,Z*)-3,6-nonadien-1-ol, propanal, (*E,E*)-2,4-heptadienal, 4-ethylbenzaldehyde, 2,3-pentanedione, (*E,E*)-3,5-octadien-2-one, 2-undecanone, propanoic acid, 3-methylbutanoic acid, pentanoic acid, octanoic acid, nonanoic acid, (*Z*)-7-tetradecene, 1-hexadecene, 2,4,6,10-tetramethylpentadecane, 1-octadecene (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) and straight-chain alkenes standard containing C5 to C21 (Sigma, St. Louis, MO, USA) were analytical grade. All media for microbiological analysis were analytical grade including, plate count agar, lauryl sulfate tryptose, EC broth, eosin methylene blue (EMB) agar, sodium chloride, alkaline peptone water (APW), glucose salt teepol broth (GSTB) and thiosulfate citrate bile salts sucrose (TCBS) agar (Merck, Darmstadt, Germany).

3.2.2 Raw material and sample preparation

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 oyster culture farm in Bandon bay, Suratthani province, south of Thailand in June 2005. The farm located 3 km from the shore with salinity and temperature of seawater approximately 25 part per thousand (ppt) and 30.2°C, respectively. After harvesting, the oysters were placed in nylon sacks and transported to the laboratory at Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, within 4-5 h at ambient temperature (30 $\pm$ 2°C), which was a normal procedure used in the region. Live oysters were washed with running water and drained for 3 min. The oysters were prepared and stored at different conditions as follows: (1) shell-on oysters packed in normal air (one hundred oysters were packed in a hemp sack, size 70 x 100 cm), and stored at ambient (30 $\pm$ 2°C) (SSA) and chilled (4 $\pm$ 2°C) (SSC) temperatures; (2) shell-on oysters packed in plastic bucket containing 2.5% brine (25 ppt table salt solution) at a ratio of oysters : brine; 7 : 3 w/w, and stored at ambient (SBA) and chilled (SBC) temperatures, with the brine changed every day during storage and (3) shucked oyster packed in different media, i.e. water (SHW), 2.5% brine (25 ppt table salt solution) (SHB2.5) and 4% brine (40 ppt table salt solution) (SHB4) in polypropylene cup at the ratio of shucked

oysters : media; 7 : 3 w/w with net weight of 180 g, covered with polypropylene film and heat sealed, then stored at chilled (4 ± 2°C) temperature.

Table 3.1 Storage conditions for fresh oyster.

Code	Form of oyster	Storage condition	Storage temperature
SSA	Shell on	Air in sack	30 ± 2°C
SSC	Shell on	Air in sack	4 ± 2°C
SBA	Shell on	2.5% brine in bucket	30 ± 2°C
SBC	Shell on	2.5% brine in bucket	4 ± 2°C
SHW	Shucked	Water in PP cup	4 ± 2°C
SHB2.5	Shucked	2.5% brine in PP cup	4 ± 2°C
SHB4	Shucked	4% brine in PP cup	4 ± 2°C

3.2.3 Effect of different storage conditions on qualities of fresh oysters

The two trials of each treatment were kept under different storage conditions (Table 3.1) and removed for chemical, microbiological and sensory analyses. The shell-on oysters stored only at ambient temperature (30 ± 2°C) for 4 days were removed every 12 h for chemical and microbiological analyses (20 oysters each time), and every 24 h for sensory evaluation (20 oysters each time), whereas both shell-on and shucked oysters stored at chilled temperature (4 ± 2°C) for 10 and 12 days, respectively, were removed every 24 h (40 oysters each time) for all analyses. The oysters for day 0 were analyzed immediately after arrival in the laboratory. Chemical, microbiological and sensory qualities of fresh oysters under different storage conditions were determined as follows:

(1) Chemical quality

pH: Ten g of oyster tissue was homogenized with 20 ml of distilled water at 12,000 rpm for 1 min using an Ultra Turrax model T18 basic homogenizer (IKA, St Augustin, Germany). The pH of the homogenate was measured using a Suntex model SP-701 pH meter (Taipei, Taiwan) (Woyewoda *et al*., 1986).

Chemical composition: The oyster tissue were subjected to the analyses of salt content, moisture, crude protein and crude fat as per AOAC (2000) with method No. 937.09, 950.46, 940.25 and 991.36, respectively.

Total volatile basic nitrogen (TVB-N): Two g of oyster tissue was homogenized with 8 ml of 4% TCA at 12,000 rpm for 2 min and kept at ambient temperature for 30 min. The homogenate was centrifuged at 3,000 rpm for 10 min using a Sorvall model RC-5B Plus centrifuge (Newtown, CT, USA). The supernatant was made up to 10 ml using 4% TCA and TVB-N was determined according to the method of Hasegawa (1987).

Lipid composition: Lipid was extracted from 30 g of oyster tissue with chloroform/methanol according to the method of Bligh and Dyer (1959) and then determined for lipid composition using a thin layer chromatography/flame ionization detection analyzer (Iatroscan 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 Chrmatorod-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 standards 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 composition: Fatty acid composition of extracted lipid was determined as fatty acid methyl esters (FAMEs). The FAMEs were prepared according to the method of Jham *et al.* (1982). The prepared methyl ester was injected into the gas chromatography (Perkin-Elmer Autosystem XL, The Perkin-Elmer Corporation, Norwalk, CT, USA) equipped with the 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 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.

Peroxide value (PV): Peroxide value of extracted lipid was determined using the ferric thiocyanate method (Chapman and Mckay, 1949). The lipid sample (1.0-5.0 mg) was treated with 9.9 ml of organic solvent mixture (benzene:methanol, 7:3 v/v) in a 16 x 150 mm test-tube (Pyrex, Corning Incorporated, NY, USA). The mixture was shaken vigorously, followed by addition of one drop of ammonium thiocyanate solution and one drop of ferrous chloride solution. The tube was shaken and placed in a water bath (Memmert, Schwabach, Germany) at 50°C for 2 min. It is then cooled to ambient temperature and the absorbance was measured at 510 nm using a Genesys 10 UV spectrophotometer (Thermo-Spectronic, NY, USA). A standard curve was prepared using ferric chloride at concentrations ranging from 0 to 90 μg/ml. The PV was calculated and expressed as milliequivalents of oxygen/kg lipid.

(2) Microbiological quality

Total viable count (TVC) and psychrotrophic count: Twenty five g of oyster tissue was blended with 225 ml of sterile Butterfield's phosphate-buffered water using a sterile blender jar (Warring, Torrington, CT, USA) for 2 min. From the 10^{-1} dilution, other decimal dilutions of 10^{-2} to 10^{-6} were prepared with sterile Butterfield's phosphate diluent. TVC and psychrotroph were determined by pour plate method using plate count agar and incubated at 35°C for 48 h and at 7°C for 10 days, respectively (BAM, 2001). The TVC and psychrotroph were expressed as log CFU/g.

***Escherichia coli*:** Twenty five g of oyster tissue was blended with 225 ml of sterile Butterfield's phosphate-buffered water. Serial decimal dilutions of 10^{-1}, 10^{-2} and 10^{-3} were prepared with sterile Butterfield's phosphate diluent. Then, one ml

portions were transferred to 10 ml of LST tube for 5 tubes in each dilution. The LST tubes were incubated at 35°C for 24-48 h. All presumptive positive (gas) tubes were performed confirmed test and completed test using EC broth and EMB agar, respectively. Typical *E. coli*-like colonies were performed biochemical test using IMViC reaction (BAM, 2002) (Appendix D1). The *E. coli* was determined using a 5-tube MPN and expressed as MPN/g.

Vibrio parahaemolyticus**:** Twenty five g of oyster tissue was blended with 225 ml of APW at pH 8.6 for 2 min. Serial decimal dilutions of 10^{-1}, 10^{-2} and 10^{-3} were prepared with APW diluent. After that, 1 ml portions were transferred to 9 ml of GSTB broth for 3 tubes in each dilution. The GSTB tubes were incubated at 35°C for 18-24 h. A loopful of suspension from the top 1 cm of a positive GSTB tube was transferred on to a TCBS plate and streak to obtain isolated colonies after incubation at 35°C for 18-24 h. The typical colonies of *V. parahaemolyticus*, large and blue-green with a dark center, were performed biochemical test (BAM, 2004) (Appendix D2). The present of *V. parahaemolyticus* was determined using a 3-tube MPN and expressed as MPN/g.

(3) Sensory quality

Sensory evaluation of oysters was carried out by a ten trained panelists using the modified guidelines from Aaraas *et al*. (2004), Cao *et al*. (2009), He *et al*. (2002) and Qi *et al*. (2007) with the scale from 1 to 9: 1, reject; 2, extremely poor; 3, very poor; 4, poor; 5, acceptable (borderline); 6, good; 7, very good; 8, extremely good and 9, excellent (Table E1). Panelists were asked to score without consuming for appearance, color of plum, texture and odor of oysters.

3.2.4 Effect of different storage conditions on volatile profile of fresh oysters

The two trials of each treatment were kept under different storage conditions and removed for volatile profile analysis. The shell-on oysters (10 oysters each time) stored at ambient temperature (30 $\pm$ 2°C) were sampled at 0, 2 and 3 days of storage. The shell-on and shucked oysters (10 oysters each time) stored at chilled

temperature were sampled at 0, 3 and 6 days of storage. Volatile profile of fresh oysters under different storage conditions was determined as follows:

(1) Headspace – solid phase microextraction

The oyster tissue was 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, which was then crimp-capped with a cap fitted with a PTFE/Silicone-lined septum (Supelco Inc., Bellefonte, PA, USA). Ten µl of 3-methyl-1-butanol (100 ml/l of ethanol) was added evenly to the samples as an internal standard, and stirred using a IKA® RET basic hotplate stirrer (IKA, St Augustin, Germany) for 10 min at ambient temperature.

The SPME device equipped with a fused-silica fiber coated with 100 µm of polydimethyl siloxane (PDMS) (Supelco Inc., Bellefonte, PA, USA) was used in this experiment. The fiber was pushed out of the needle (after penetrating the septum) and exposed for 2 h at 30°C in the headspace above the samples.

(2) Volatile profile analysis

The SPME fiber was desorbed in a gas chromatograph (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; Supelco Inc., Tokyo, Japan) and a flame ionization detector (FID). The carrier gas (helium) flow rate was 1 ml/min. The injection port and detector temperature 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 and held at 220°C for 20 min. The data acquisition and processing were carried out with a Shimadzu C-R7A Plus Chromatopac (Kyoto, Japan).

The retention indices (RI) of each compound were calculated according to the method of Van den Dool and Kratz (1963) using a series of straight-chain alkanes (C5-C21). The volatile compounds were identified by matching their retention indices with those proposed in the literature (Pennarun *et al*., 2002; Pennarun *et al*., 2003; Piveteau *et al*., 2000), and confirmed with the authentic

standards under the same experimental conditions. The relative concentration of each compound was estimated by using an internal standard (3-methyl-1-butanol) and expressed as μg/g oyster. The concentrations of total volatile compounds were calculated by combining of the identified volatile compounds in each sample.

3.2.5 Statistical analysis

The data from two trials (three replicates from each trial) was subjected to analysis of variance (ANOVA) according to the experimental design in CRD. Comparison of means was carried out by Duncan's multiple range test for significant differences at $P < 0.05$ (Steel and Torrie, 1980).

3.3 Results and Discussion

3.3.1 Effect of different storage conditions on qualities of fresh oysters

(1) Changes in chemical quality

pH: Changes in pH can be used as indicator for the postmortem changes of glycogen to lactic acid and the degradation of muscle components e.g. proteins and nucleotides during long term of storage (Jay, 2000). The initial pH of white-scar oyster (*C. belcheri*) was 6.1 and gradually decreased ($P < 0.05$) in all treatments during storage (Figure 3.1). Similar results on pH of live flat oyster (*O. edulis*) were reported ranging from 5.6 to 6.3 and after death from 5.2 to 5.3 (Aaraas *et al*., 2004). The shell-on oysters in different media showed slower change in pH when stored at chilled temperature than those stored at ambient temperature (Figure 3.1a). This was probably due to the lower temperature storage could retard the decomposition of glycogen (Huss, 1995). Using 2.5% brine resulted in a slower decrease in pH of shell-on oyster than those packed in normal air under both ambient and chilled storage. Because ionic strength of salt solution could affect the solubility of myofibrillar proteins and hence produced volatile bases which could delay the decreasing rate of pH (Huss, 1995). It was found that pH of shell-on oysters both packed in normal air and in 2.5% brine at ambient temperature after 4 days were lower than the acceptable limit (< 5.5) (Pottinger, 1948), similar to the result of

samples packed in 2.5% brine at chilled storage after 9 days. However, samples packed in normal air at chilled storage obtained pH not lower than the acceptable limit throughout the storage. Shucked oyster packed in 4% brine showed a slower decrease in pH than those packed in 2.5% brine and in water during storage at chilled temperature (Figure 3.1b) and only shucked oyster packed in water after 12 days of chilled storage was lower than the acceptable limit (< 5.5) (Pottinger, 1948).

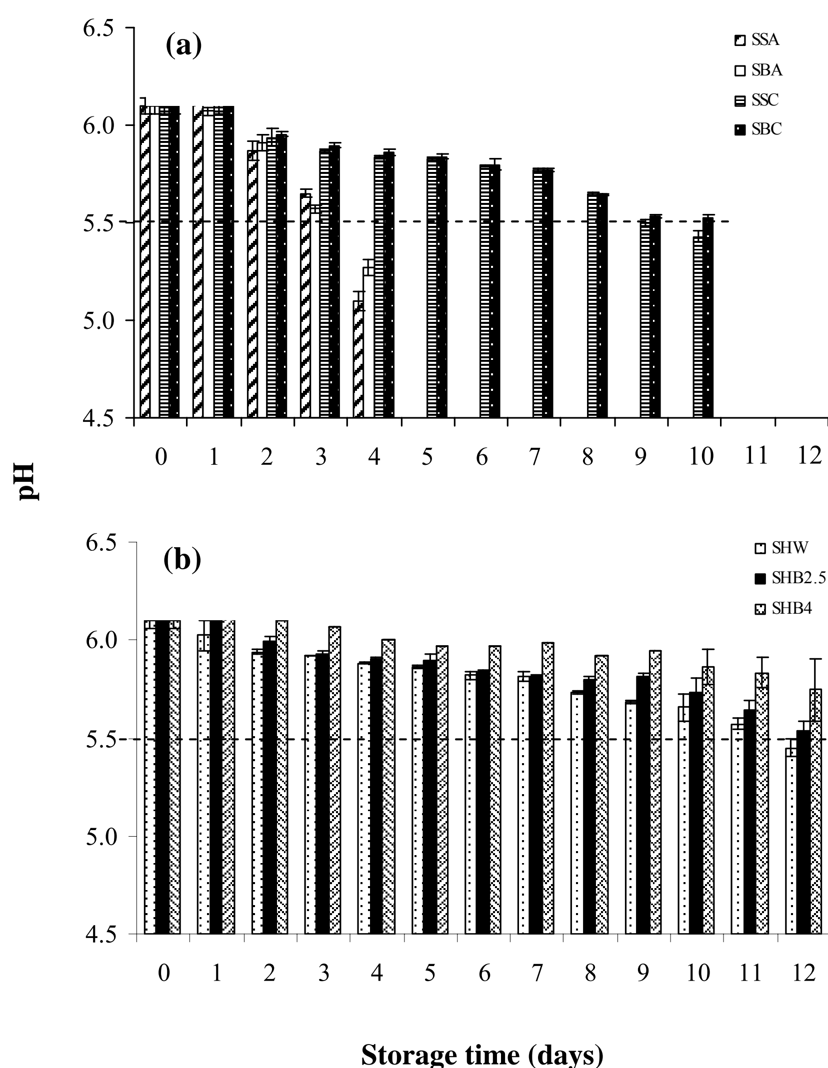

Figure 3.1 Changes in pH of (a) shell-on oyster stored in normal air at ambient (SSA) and chilled temperatures (SSC), and in 2.5% brine at ambient (SBA) and chilled temperatures (SBC), and (b) shucked oyster stored in water (SHW), 2.5% brine (SHB2.5) and 4% brine (SHB4) at chilled temperature.

Salt content: The initial salt content of fresh oyster tissue was approximately 1.5% w/w. During storage, salt contents of shell-on oyster packed in normal air (SSA and SSC) were not significantly different ($P \geq 0.05$), whereas those of shell-on oyster packed in 2.5% brine (SBA, SBC) and shucked oyster packed in different media (SHB2.5 and SHB4) gradually increased ($P < 0.05$) (Figure 3.2). Salt content of shucked oyster packed in water (SHW) decreased ($P < 0.05$) with the increase in storage time, possibly because the water media diluted the salt content in the oyster tissue. The final salt contents of oyster under different packing media and storage temperature were 1.5, 2.3, 1.5 and 1.8% w/w for shell-on oyster (SSA, SBA, SSC and SBC) and 1.1, 2.1 and 2.6% w/w for shucked oyster (SHW, SHB2.5 and SHB4), respectively. Shucked oysters packed in 4% brine showed the highest final salt contents which possibly affect the taste of oyster. Nevertheless, it was reported that surimi products with 73-76% moisture and 2.5-3% salt were still accepted (Bledsoe and Rasco, 2006). Therefore, the acceptable salt content may depend on moisture content of the product.

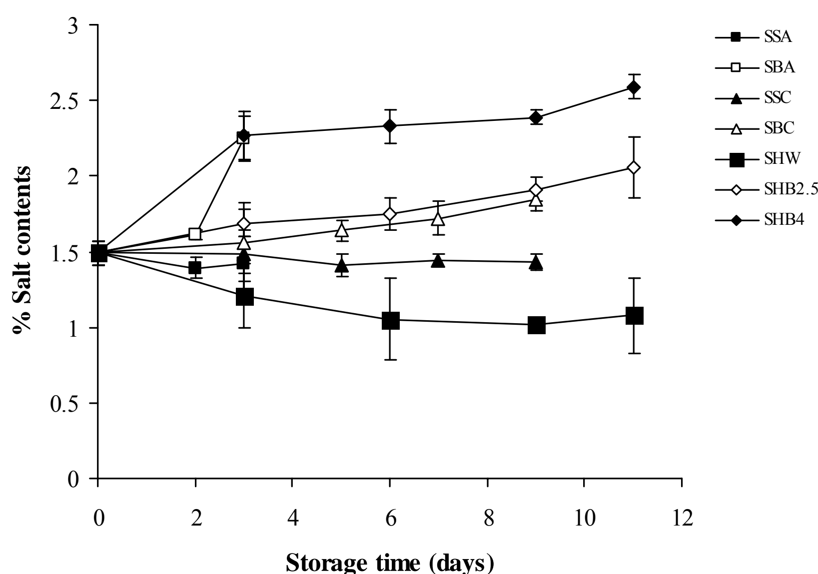

Figure 3.2 Changes in salt content of shell-on oyster stored in normal air at ambient (SSA) and chilled temperatures (SSC), and in 2.5% brine at ambient (SBA) and chilled temperatures (SBC), and shucked oyster stored in water (SHW), 2.5% brine (SHB2.5) and 4% brine (SHB4) at chilled temperature.

Chemical composition: The fresh white-scar oyster (*C. belcheri*) was composed of 81.6% moisture, 8.3% crude protein and 2.4% crude fat (Table 3.2 and 3.3). The moisture, crude protein and crude fat of all samples ranged from 79.1-82.2%, 7.4-8.8% and 2.2-2.6%, respectively during storage. Mangrove oyster (*Crassostrea rhizophorae*) was found to contain moisture, crude protein and crude fat at 82.0%, 9.7% and 1.7% of wet weight, respectively (Martino and Cruz, 2004). Pacific oyster (*C. gigas*) and Atlantic oyster (*Crassostrea virginica*) had similar compositions (Jay, 2000).

TVB-N: Total volatile base-nitrogen (TVB-N), a decomposed both protein and non-protein nitrogenous compounds (trimethylamine (TMA), dimethylamine (DMA) and ammonia), of all samples increased with storage time, probably caused by bacterial and endogenous proteolytic enzymatic actions (Hernandez-Herrero *et al*., 1999). The initial TVB-N value of white-scar oyster (*C. belcheri*) samples was 1.8 mg N/100 g (Figure 3.3). It was slightly unusual, probably due to the delay time of oyster transportation and product preparation. The considerably high initial TVB-N values were also reported in purified and nonpurified with high pressure flat oysters (*O. edulis*) with 11.2 and 13.3 mg N/100 g, respectively (Lopez-Caballero *et al*., 2000). The TVB-N values of the shell-on oyster in different media stored at chilled temperature increased at a slower rate than those stored at ambient temperature (Figure 3.3a), probably due to the low temperature most likely delay the growth of protease-producing bacteria and inhibit the protease activity (Huss, 1995). Shell-on oyster packed in 2.5% brine showed an increase in TVB-N at a faster rate than those packed in normal air, probably because the higher level of NaCl in the samples supported higher growth of halophilic bacteria and thus contributed to the higher contents of TVB-N (Tsai *et al*., 2005). The TVB-N values of shucked oysters packed in different media were not significantly changed ($P \geq 0.05$) during chilled storage (Figure 3.3b) and did not exceed the acceptable limit (30 mg N/100 g) (Lopez-Caballero *et al*., 2000). Although these values were considerably lower than the acceptable limit, the oyster showed spoilage due to the microbiological quality (Table 3.6). Similar results were reported in scallop (*Argopecten ventricosus*) during storage at 0°C for 15 days (Ocano-higuera *et al*., 2006).

Table 3.2 Chemical composition of shell-on oysters during ambient and chilled storage.

Storage time (days)	Composition (% wet basis)					
	SSA			SBA		
	Moisture	Crude protein	Crude fat	Moisture	Crude protein	Crude fat
0	$81.61 \pm 0.11^{b,*}$	8.30 ± 0.18^{b}	2.44 ± 0.03^{a}	81.61 ± 0.11^{d}	8.30 ± 0.18^{b}	2.44 ± 0.03^{a}
1	81.53 ± 0.29^{b}	8.26 ± 0.18^{ab}	2.48 ± 0.41^{a}	79.81 ± 0.16^{b}	7.70 ± 0.40^{ab}	2.34 ± 0.08^{a}
2	79.23 ± 0.49^{a}	8.03 ± 0.14^{a}	2.56 ± 0.10^{a}	79.07 ± 0.41^{a}	8.36 ± 0.37^{b}	2.45 ± 0.58^{a}
3	81.71 ± 0.49^{b}	8.22 ± 0.09^{ab}	2.37 ± 0.01^{a}	79.82 ± 0.09^{b}	8.15 ± 0.32^{ab}	2.38 ± 0.30^{a}
4	81.14 ± 0.36^{b}	8.78 ± 0.02^{c}	2.49 ± 0.08^{a}	80.94 ± 0.43^{c}	7.62 ± 0.37^{a}	2.43 ± 0.35^{a}
	SSC			SBC		
0	81.61 ± 0.11^{d}	8.30 ± 0.18^{abc}	2.44 ± 0.03^{a}	81.61 ± 0.11^{c}	8.30 ± 0.18^{abc}	2.44 ± 0.03^{bc}
1	80.08 ± 0.65^{ab}	8.15 ± 0.37^{abc}	2.48 ± 0.05^{a}	79.76 ± 0.10^{ab}	8.41 ± 0.23^{bc}	2.33 ± 0.10^{ab}
2	79.73 ± 0.16^{a}	8.10 ± 0.04^{abc}	2.48 ± 0.05^{a}	80.31 ± 0.23^{b}	8.50 ± 0.10^{bc}	2.57 ± 0.09^{c}
3	80.13 ± 0.35^{ab}	8.00 ± 0.64^{abc}	2.35 ± 0.07^{a}	81.83 ± 0.17^{c}	8.61 ± 0.11^{c}	2.40 ± 0.17^{abc}
4	80.03 ± 0.41^{ab}	8.55 ± 0.36^{bc}	2.50 ± 0.25^{a}	79.45 ± 0.08^{a}	8.62 ± 0.30^{c}	2.52 ± 0.10^{bc}
5	79.77 ± 0.54^{a}	8.74 ± 0.18^{c}	2.42 ± 0.10^{a}	80.33 ± 0.16^{b}	8.22 ± 0.31^{abc}	2.54 ± 0.01^{bc}
6	79.99 ± 0.24^{ab}	7.95 ± 0.59^{ab}	2.48 ± 0.08^{a}	81.31 ± 0.31^{c}	8.17 ± 0.39^{abc}	2.38 ± 0.11^{abc}
7	80.47 ± 0.35^{bc}	7.55 ± 0.16^{a}	2.47 ± 0.11^{a}	79.52 ± 0.38^{a}	7.78 ± 0.53^{a}	2.34 ± 0.15^{ab}
8	80.96 ± 0.10^{c}	7.93 ± 0.20^{ab}	2.39 ± 0.28^{a}	79.84 ± 0.81^{ab}	7.90 ± 0.51^{ab}	2.20 ± 0.12^{a}
9	79.94 ± 0.17^{ab}	7.87 ± 0.15^{ab}	2.37 ± 0.28^{a}	81.57 ± 0.34^{c}	8.24 ± 0.29^{abc}	2.33 ± 0.41^{ab}
10	80.46 ± 0.25^{bc}	8.43 ± 0.77^{bc}	2.35 ± 0.10^{a}	81.58 ± 0.29^{c}	8.63 ± 0.14^{c}	2.34 ± 0.04^{ab}

Mean ± SD from two trials. Values in the same column for each sample followed by different superscript letters are significantly different ($P < 0.05$*).

SSA, SBA and SSC, SBC = shell-on oyster stored in normal air, in 2.5% brine at ambient and chilled temperatures, respectively.

Table 3.3 Chemical composition of shucked oysters during chilled storage

Storage Time (days)	Composition (% wet basis)								
	SHW			SHB2.5			SHB4		
	Moisture	Crude protein	Crude fat	Moisture	Crude protein	Crude fat	Moisture	Crude protein	Crude fat
0	81.61 ± 0.11^{ef,*}	8.30 ± 0.18^{bc}	2.44 ± 0.03^{bc}	81.61 ± 0.11^{cd}	8.30 ± 0.18^{defg}	2.44 ± 0.03^{bcde}	81.61 ± 0.11^{g}	8.30 ± 0.18^{ab}	2.44 ± 0.03^{abc}
1	79.10 ± 0.96^{a}	8.84 ± 0.31^{c}	2.18 ± 0.02^{a}	81.46 ± 0.19^{cd}	7.53 ± 0.25^{a}	2.18 ± 0.02^{ab}	79.92 ± 0.57^{abc}	8.16 ± 0.08^{ab}	2.38 ± 0.01^{abc}
2	82.15 ± 0.23^{f}	8.65 ± 0.13^{c}	2.18 ± 0.03^{a}	80.27 ± 0.25^{b}	7.80 ± 0.10^{abcd}	2.32 ± 0.05^{abcd}	81.58 ± 0.34^{g}	8.68 ± 0.02^{b}	2.55 ± 0.10^{c}
3	81.47 ± 0.24^{ef}	7.43 ± 0.10^{a}	2.47 ± 0.17^{bc}	81.54 ± 0.38^{cd}	8.58 ± 0.07^{g}	2.30 ± 0.20^{abcd}	81.45 ± 0.36^{fg}	8.66 ± 0.39^{b}	2.28 ± 0.04^{ab}
4	81.34 ± 0.38^{def}	7.41 ± 0.24^{a}	2.42 ± 0.48^{abc}	81.77 ± 0.68^{de}	8.10 ± 0.10^{bcdefg}	2.16 ± 0.01^{a}	80.46 ± 0.15^{cde}	8.44 ± 0.10^{ab}	2.52 ± 0.18^{bc}
5	81.76 ± 0.26^{ef}	8.84 ± 0.37^{c}	2.47 ± 0.10^{bc}	82.27 ± 0.25^{e}	8.30 ± 0.10^{defg}	2.47 ± 0.56^{de}	79.53 ± 0.22^{a}	8.11 ± 0.78^{ab}	2.44 ± 0.16^{abc}
6	81.41 ± 0.24^{ef}	8.68 ± 0.66^{c}	2.23 ± 0.05^{ab}	81.79 ± 0.13^{de}	8.02 ± 0.29^{abcdef}	2.20 ± 0.01^{abc}	80.82 ± 0.12^{def}	8.29 ± 0.23^{ab}	2.55 ± 0.47^{c}
7	80.12 ± 0.30^{bc}	8.43 ± 0.50^{c}	2.53 ± 0.06^{c}	80.23 ± 0.21^{b}	7.67 ± 0.15^{ab}	2.45 ± 0.12^{cde}	79.27 ± 0.72^{a}	8.29 ± 0.63^{ab}	2.51 ± 0.18^{bc}
8	82.09 ± 0.17^{ef}	8.53 ± 0.08^{c}	2.30 ± 0.22^{abc}	80.47 ± 0.47^{b}	7.73 ± 0.21^{abc}	2.63 ± 0.14^{e}	79.64 ± 0.18^{ab}	8.75 ± 0.24^{b}	2.58 ± 0.05^{c}
9	81.49 ± 0.37^{ef}	7.82 ± 0.27^{ab}	2.42 ± 0.08^{abc}	80.14 ± 0.12^{b}	7.89 ± 0.71^{abcde}	2.32 ± 0.03^{abcd}	79.44 ± 0.10^{a}	8.29 ± 0.67^{ab}	2.38 ± 0.05^{abc}
10	80.51 ± 0.91^{cd}	7.58 ± 0.18^{a}	2.44 ± 0.28^{bc}	81.13 ± 0.15^{c}	8.23 ± 0.21^{cdefg}	2.30 ± 0.12^{abcd}	81.29 ± 0.25^{fg}	7.69 ± 0.55^{a}	2.19 ± 0.32^{a}
11	81.18 ± 0.71^{de}	8.56 ± 0.11^{c}	2.23 ± 0.01^{ab}	80.17 ± 0.15^{b}	8.33 ± 0.25^{efg}	2.58 ± 0.26^{e}	80.20 ± 0.18^{bcd}	8.35 ± 0.12^{ab}	2.27 ± 0.08^{ab}
12	79.56 ± 0.50^{ab}	7.67 ± 0.15^{a}	2.31 ± 0.12^{abc}	79.30 ± 0.30^{a}	8.40 ± 0.21^{fg}	2.26 ± 0.00^{abcd}	80.95 ± 0.57^{efg}	8.40 ± 0.95^{ab}	2.45 ± 0.12^{bc}

*Mean ± SD from two trials. Values in the same column followed by different superscript letters are significantly different ($P < 0.05$).

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

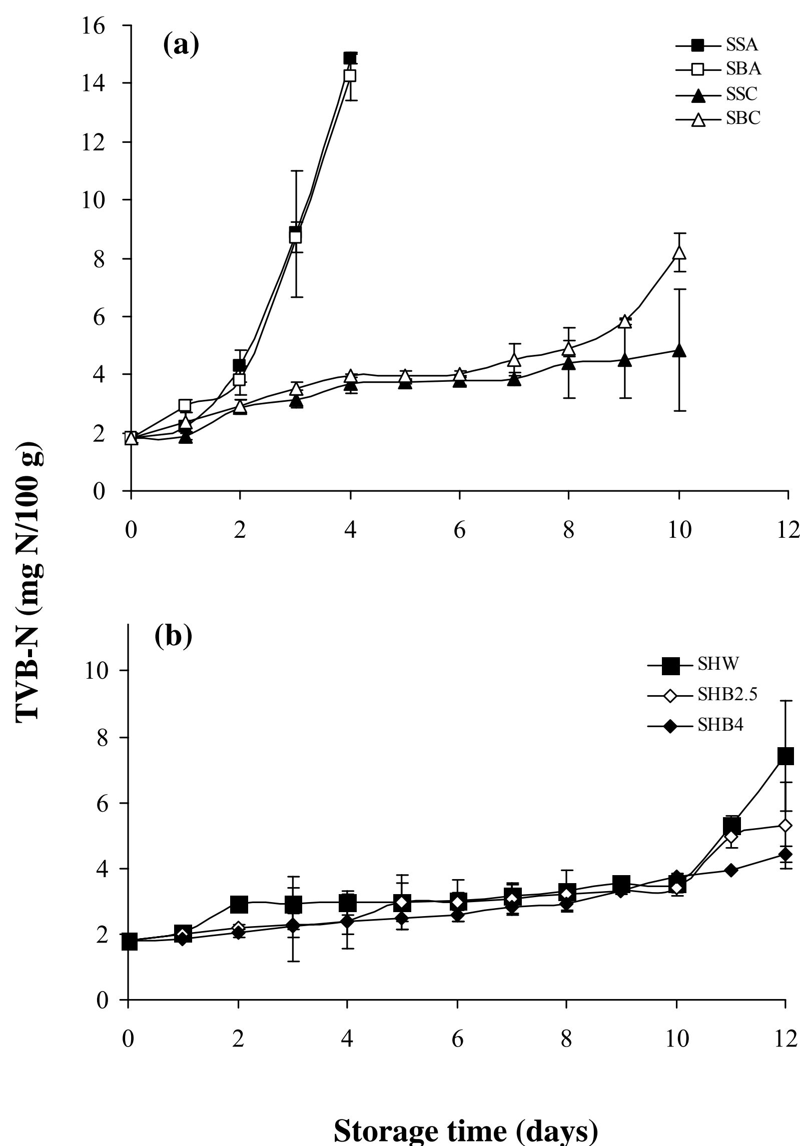

Figure 3.3 Changes in TVB-N of (a) shell-on oyster stored in normal air at ambient (SSA) and chilled temperatures (SSC), and in 2.5% brine at ambient (SBA) and chilled temperatures (SBC), and (b) shucked oyster stored in water (SHW), 2.5% brine (SHB2.5) and 4% brine (SHB4) at chilled temperature.

Lipid composition: The lipid composition of white-scar oyster (*C. belcheri*) was 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 were 59.4, 4.1, 0.1, 0.5, and 36.0 g/100 g

oil, respectively. During storage, both TG and PL contents of all treatments decreased ($P < 0.05$) (Table 3.4 and 3.5), possibly due to the activity of both endogenous and microbial lipases and/or phospholipases (Erickson, 1998). DG, MG and FFA contents increased ($P < 0.05$) with the storage time increase, due to the hydrolysis of TG and PL.

Shell-on oysters in different media showed slower decrease in TG and PL but increase in DG, MG and FFA when stored at chilled temperature than those stored at ambient temperature (Table 3.4). It was well-known that activities of endogenous and microbial lipases and/or phospholipases could probably occur in oyster at a faster rate during ambient storage. Shell-on oysters packed in 2.5% brine showed a higher increase in FFA than those packed in normal air during storage. Whereas, shucked oysters packed in 4% brine showed a slower increase in FFA than those packed in 2.5% brine and water during storage (Table 3.5), probably due to a certain high salt content (~ 4%) could inhibit lipase and phospholipase. Therefore, it could be shown that salt content of less than 2.5% could accelerate enzyme activity but at higher salt content, it could inhibit enzyme activity. However, it was reported that FFA of horse mackerel (*Trachurus trachurus*) muscle with salt content of 0.8-1.1% slower increased than those with salt content of 0.1-0.4% after 270 days of frozen storage (Aubourg and Ugliano, 2002).

Table 3.4 Changes in lipid composition of shell-on oysters during ambient and chilled storage.

Storage time (days)	Lipid composition (g/100 g oil)									
	SSA					SBA				
	TG	DG	MG	FFA	PL	TG	DG	MG	FFA	PL
0	59.44 ± 0.01^{b,*}	4.06 ± 0.14^{a}	0.06 ± 0.01^{a}	0.47 ± 0.25^{a}	35.97 ± 1.30^{c}	59.44 ± 0.01^{b}	4.06 ± 0.14^{a}	0.06 ± 0.01^{a}	0.47 ± 0.25^{a}	35.97 ± 1.30^{d}
1	58.51 ± 0.32^{b}	5.08 ± 0.11^{b}	0.20 ± 0.25^{ab}	0.62 ± 0.04^{a}	35.59 ± 0.05^{c}	59.53 ± 0.25^{b}	4.26 ± 0.01^{a}	0.16 ± 0.05^{a}	0.86 ± 0.11^{a}	35.19 ± 0.49^{cd}
2	58.80 ± 0.66^{b}	5.62 ± 0.01^{c}	0.33 ± 0.03^{b}	1.72 ± 0.01^{b}	33.53 ± 0.85^{b}	58.08 ± 2.20^{b}	5.83 ± 0.46^{b}	0.38 ± 0.39^{a}	2.50 ± 0.56^{b}	33.21 ± 0.98^{bc}
3	57.70 ± 1.26^{ab}	5.87 ± 0.03^{c}	0.61 ± 0.01^{c}	4.67 ± 0.06^{c}	31.15 ± 0.51^{b}	56.07 ± 3.50^{b}	6.27 ± 0.21^{b}	0.52 ± 0.09^{a}	5.19 ± 0.40^{c}	31.95 ± 0.81^{b}
4	55.90 ± 2.40^{a}	6.58 ± 0.39^{d}	1.21 ± 0.06^{d}	7.83 ± 0.03^{d}	28.48 ± 0.65^{a}	50.86 ± 0.51^{a}	7.89 ± 0.36^{c}	1.35 ± 0.43^{b}	12.13 ± 1.00^{d}	27.77 ± 1.88^{a}
	SSC					SBC				
0	59.44 ± 0.01^{b}	4.06 ± 0.14^{a}	0.06 ± 0.01^{a}	0.47 ± 0.25^{a}	35.97 ± 1.30^{e}	59.44 ± 0.01^{e}	4.06 ± 0.14^{a}	0.06 ± 0.01^{a}	0.47 ± 0.25^{a}	35.97 ± 1.30^{g}
1	58.38 ± 0.88^{ab}	5.07 ± 0.20^{b}	0.21 ± 0.10^{ab}	0.52 ± 0.01^{a}	35.82 ± 0.47^{de}	58.37 ± 0.51^{de}	5.38 ± 0.26^{b}	0.15 ± 0.01^{ab}	0.56 ± 0.02^{a}	35.54 ± 1.06^{g}
2	58.03 ± 1.49^{ab}	6.07 ± 0.12^{c}	0.22 ± 0.09^{ab}	0.62 ± 0.21^{ab}	35.06 ± 0.65^{cde}	57.98 ± 2.10^{de}	6.56 ± 0.15^{c}	0.18 ± 0.01^{b}	0.62 ± 0.09^{a}	34.66 ± 0.55^{fg}
3	57.82 ± 2.23^{ab}	6.69 ± 0.19^{cd}	0.24 ± 0.08^{ab}	0.95 ± 0.22^{bc}	34.30 ± 1.21^{cde}	57.72 ± 0.46^{cd}	7.79 ± 0.49^{d}	0.20 ± 0.04^{b}	0.64 ± 0.48^{a}	33.65 ± 1.79^{ef}
4	57.64 ± 0.68^{ab}	6.95 ± 0.57^{de}	0.25 ± 0.07^{ab}	1.07 ± 0.14^{c}	34.09 ± 0.85^{cd}	57.68 ± 1.0^{cd}	8.37 ± 0.16^{de}	0.35 ± 0.06^{c}	1.08 ± 0.01^{b}	32.52 ± 2.14^{de}
5	57.08 ± 1.27^{ab}	7.62 ± 0.42^{ef}	0.27 ± 0.06^{ab}	1.16 ± 0.41^{c}	33.87 ± 2.02^{c}	58.18 ± 0.5^{de}	8.72 ± 0.50^{e}	0.58 ± 0.06^{d}	1.56 ± 0.11^{c}	30.96 ± 0.39^{cd}
6	57.76 ± 0.79^{ab}	8.09 ± 0.41^{fg}	0.30 ± 0.12^{ab}	1.88 ± 0.06^{d}	31.97 ± 0.11^{b}	57.55 ± 0.29^{cd}	8.89 ± 0.65^{e}	0.61 ± 0.06^{d}	2.61 ± 0.13^{d}	30.34 ± 0.57^{c}
7	57.65 ± 0.52^{ab}	8.27 ± 0.90^{fg}	0.40 ± 0.03^{ab}	2.45 ± 0.04^{e}	31.23 ± 0.83^{b}	56.22 ± 0.05^{bc}	9.58 ± 0.27^{f}	0.63 ± 0.09^{d}	3.74 ± 0.06^{e}	29.83 ± 0.29^{bc}
8	56.92 ± 2.37^{ab}	8.39 ± 0.30^{fg}	0.41 ± 0.05^{ab}	3.92 ± 0.21^{f}	30.36 ± 1.06^{b}	55.73 ± 1.17^{b}	9.62 ± 0.29^{f}	0.76 ± 0.09^{e}	4.77 ± 0.20^{f}	29.12 ± 0.61^{abc}
9	56.96 ± 5.50^{ab}	8.72 ± 0.59^{gh}	0.49 ± 0.55^{b}	5.82 ± 0.27^{g}	28.01 ± 0.45^{a}	54.83 ± 0.11^{b}	9.98 ± 0.42^{f}	0.91 ± 0.01^{f}	6.12 ± 0.10^{g}	28.16 ± 0.38^{ab}
10	54.97 ± 1.48^{a}	9.26 ± 0.59^{h}	0.50 ± 0.22^{b}	7.43 ± 0.30^{h}	27.84 ± 0.48^{a}	51.38 ± 0.09^{a}	11.53 ± 0.1^{g}	1.82 ± 0.10^{g}	7.75 ± 0.25^{h}	27.52 ± 0.03^{a}

*Mean ± SD from two trials. Values in the same column for each sample followed by different superscript letters are significantly different ($P < 0.05$).

SSA, SBA and SSC, SBC = shell-on oyster stored in normal air, in 2.5% brine at ambient and chilled temperatures, respectively.

TG, triglyceride; DG, diglyceride; MG, monoglyceride; FFA, free fatty acid; PL, phospholipid.

Table 3.5 Changes in lipid composition of shucked oysters during chilled storage.

Treatments	Storage time (days)	Lipid composition (g/100 g oil)				
		TG	DG	MG	FFA	PL
SHW	0	59.44 ± 0.01$^{d, *}$	4.06 ± 0.14^{a}	0.06 ± 0.01^{a}	0.47 ± 0.25^{a}	35.97 ± 1.30^{e}
	1	58.72 ± 0.7^{cd}	4.60 ± 0.27^{ab}	0.14 ± 0.13^{ab}	0.65 ± 0.26^{ab}	35.89 ± 0.67^{e}
	2	58.05 ± 1.65^{cd}	5.25 ± 1.24^{bc}	0.13 ± 0.03^{ab}	1.03 ± 0.19^{b}	35.54 ± 0.99^{e}
	3	58.11 ± 0.91^{cd}	5.66 ± 0.25^{c}	0.18 ± 0.08^{ab}	1.13 ± 0.18^{b}	34.92 ± 0.38^{de}
	4	58.10 ± 0.69^{cd}	6.82 ± 0.13^{d}	0.23 ± 0.06^{ab}	2.25 ± 0.23^{c}	32.60 ± 0.68^{cd}
	5	57.69 ± 1.07^{cd}	6.88 ± 0.36^{de}	0.42 ± 0.02^{bc}	3.89 ± 0.20^{d}	31.12 ± 0.86^{bc}
	6	56.61 ± 0.92^{bcd}	7.83 ± 0.43^{ef}	0.54 ± 0.04^{cd}	4.52 ± 0.04^{e}	30.50 ± 1.10^{abc}
	7	56.97 ± 3.67^{bcd}	7.84 ± 0.96^{ef}	0.62 ± 0.07^{cd}	4.78 ± 0.58^{e}	29.79 ± 4.14^{abc}
	8	56.71 ± 2.35^{bcd}	8.76 ± 0.31^{fg}	0.74 ± 0.04^{d}	5.05 ± 0.49^{e}	28.74 ± 1.99^{ab}
	9	56.96 ± 1.31^{bcd}	8.35 ± 0.74^{fg}	1.02 ± 0.19^{e}	5.76 ± 0.54^{f}	27.91 ± 2.32^{a}
	10	55.76 ± 2.97^{bc}	9.21 ± 0.10^{gh}	1.12 ± 0.01^{e}	5.95 ± 0.12^{f}	27.96 ± 0.16^{a}
	11	53.60 ± 0.87^{ab}	9.75 ± 0.56^{h}	1.53 ± 0.37^{f}	7.29 ± 0.04^{g}	27.83 ± 0.88^{a}
	12	52.28 ± 2.96^{a}	9.93 ± 0.26^{h}	1.87 ± 0.35^{g}	8.41 ± 0.12^{h}	27.51 ± 0.50^{a}
SHB2.5	0	59.44 ± 0.01^{g}	4.06 ± 0.14^{a}	0.06 ± 0.01^{a}	0.47 ± 0.25^{a}	35.97 ± 1.30^{d}
	1	58.95 ± 0.74^{fg}	4.82 ± 0.44^{b}	0.10 ± 0.06^{a}	0.75 ± 0.24^{ab}	35.38 ± 0.15^{d}
	2	57.80 ± 1.01^{ef}	5.45 ± 0.23^{c}	0.12 ± 0.01^{ab}	1.23 ± 0.17^{b}	35.40 ± 0.42^{d}
	3	57.92 ± 0.72^{e}	5.61 ± 0.03^{c}	0.28 ± 0.10^{bc}	1.30 ± 0.00^{b}	34.89 ± 5.38^{d}
	4	56.80 ± 0.82^{d}	6.82 ± 0.50^{d}	0.33 ± 0.02^{c}	2.45 ± 0.02^{c}	33.60 ± 0.84^{cd}
	5	56.11 ± 0.77^{cd}	7.48 ± 0.24^{e}	0.40 ± 0.09^{cd}	2.89 ± 0.19^{c}	33.12 ± 0.61^{cd}
	6	55.72 ± 0.95^{cd}	7.73 ± 0.45^{e}	0.53 ± 0.03^{d}	3.52 ± 0.16^{d}	32.50 ± 0.10^{bcd}
	7	55.80 ± 0.67^{cd}	7.94 ± 0.45^{e}	0.72 ± 0.07^{e}	4.75 ± 0.46^{e}	30.79 ± 0.60^{abc}
	8	55.59 ± 0.56^{cd}	8.78 ± 0.36^{f}	0.84 ± 0.11^{e}	5.05 ± 0.78^{e}	29.74 ± 0.48^{abc}
	9	54.76 ± 0.60^{bc}	9.25 ± 0.27^{fg}	1.22 ± 0.04^{f}	5.86 ± 0.10^{f}	28.91 ± 2.82^{ab}
	10	54.63 ± 0.96^{bc}	9.55 ± 0.49^{gh}	1.32 ± 0.11^{f}	6.90 ± 0.47^{g}	27.60 ± 2.31^{a}
	11	53.81 ± 0.79^{b}	9.75 ± 0.07^{gh}	1.50 ± 0.26^{g}	7.31 ± 0.50^{g}	27.63 ± 3.37^{a}
	12	52.33 ± 0.68^{a}	9.89 ± 0.40^{h}	1.97 ± 0.13^{h}	8.40 ± 0.09^{h}	27.41 ± 0.55^{a}
SHB4	0	59.44 ± 0.01^{d}	4.06 ± 0.14^{a}	0.06 ± 0.01^{a}	0.47 ± 0.25^{a}	35.97 ± 1.30^{e}
	1	59.05 ± 3.40^{cd}	4.49 ± 0.22^{ab}	0.10 ± 0.09^{ab}	0.68 ± 0.05^{a}	35.68 ± 1.82^{de}
	2	59.01 ± 0.06^{cd}	4.63 ± 0.15^{ab}	0.16 ± 0.07^{ab}	0.71 ± 0.01^{a}	35.49 ± 0.62^{de}
	3	58.57 ± 0.27^{cd}	5.06 ± 0.21^{b}	0.24 ± 0.01^{abc}	1.20 ± 0.03^{b}	34.93 ± 2.10^{cde}
	4	58.89 ± 1.04^{cd}	5.17 ± 0.46^{b}	0.34 ± 0.03^{abc}	1.45 ± 0.05^{b}	34.15 ± 0.38^{bcde}
	5	57.51 ± 0.27^{bcd}	6.04 ± 1.11^{c}	0.67 ± 0.51^{bc}	2.10 ± 0.13^{c}	33.68 ± 0.18^{bcde}
	6	56.78 ± 0.90^{abc}	6.58 ± 0.19^{c}	0.78 ± 0.28^{cd}	2.87 ± 0.42^{d}	32.99 ± 0.54^{abcde}
	7	56.10 ± 1.01^{ab}	6.50 ± 0.38^{c}	0.83 ± 0.39^{cd}	4.06 ± 0.37^{e}	32.51 ± 0.60^{abcd}
	8	55.56 ± 0.69^{ab}	6.71 ± 0.36^{cd}	1.28 ± 0.31^{de}	4.36 ± 0.21^{ef}	32.09 ± 3.44^{abc}
	9	54.94 ± 0.01^{a}	7.34 ± 0.51^{de}	1.34 ± 0.42^{de}	4.65 ± 0.08^{f}	31.73 ± 0.30^{abc}
	10	55.34 ± 0.42^{ab}	7.43 ± 0.23^{de}	1.36 ± 0.38^{de}	4.60 ± 0.13^{f}	31.27 ± 1.82^{ab}
	11	55.38 ± 1.19^{ab}	7.56 ± 0.08^{e}	1.45 ± 0.59^{e}	5.26 ± 0.24^{g}	30.35 ± 3.24^{a}
	12	54.78 ± 1.67^{a}	7.76 ± 0.19^{e}	1.53 ± 0.28^{e}	5.76 ± 0.14^{h}	30.17 ± 1.57^{a}

*Mean ± SD from two trials. Values in the same column for each sample followed by different superscript letters are significantly different ($P < 0.05$).

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

TG, triglyceride; DG, diglyceride; MG, monoglyceride; FFA, free fatty acid; PL, phospholipid.

Fatty acid composition: The fatty acid composition of white-scar oysters (*C. belcheri*) was classified into 3 groups i.e. saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA) (Table A1-3). The predominant SFA was palmitic acid (C16:0), stearic acid (C18:0) and myristic acid (C14:0). Whereas the predominant MUFA was oleic acid (C18:1), palmitoleic acid (C16:1) and eicosanoic acid (C20:1) and the predominant PUFA was eicosapentaenoic acid (EPA, C20:5), docosahexaenoic acid (DHA, C22:6), arachidonic acid (C20:4), linoleic acid (C18:2) and stearidonic acid (C18:4). The high levels of PUFA (EPA and DHA), found in lipids, are noteworthy. They are very sensitive to oxidation because of their high level of unsaturation and can give rise to the formation of carbonyl compounds during oxidation reactions (Pennarun *et al*., 2002).

Fresh oyster lipids were composed of 33.3% PUFA, 29.7% SFA, and 15.7% MUFA (Figure 3.4 and 3.5). 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 (Erickson, 1998). Decreases in the PUFA and MUFA of the shell-on oysters packed in 2.5% brine were greater than those packed in normal air (Figure 3.4). 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 3.5).

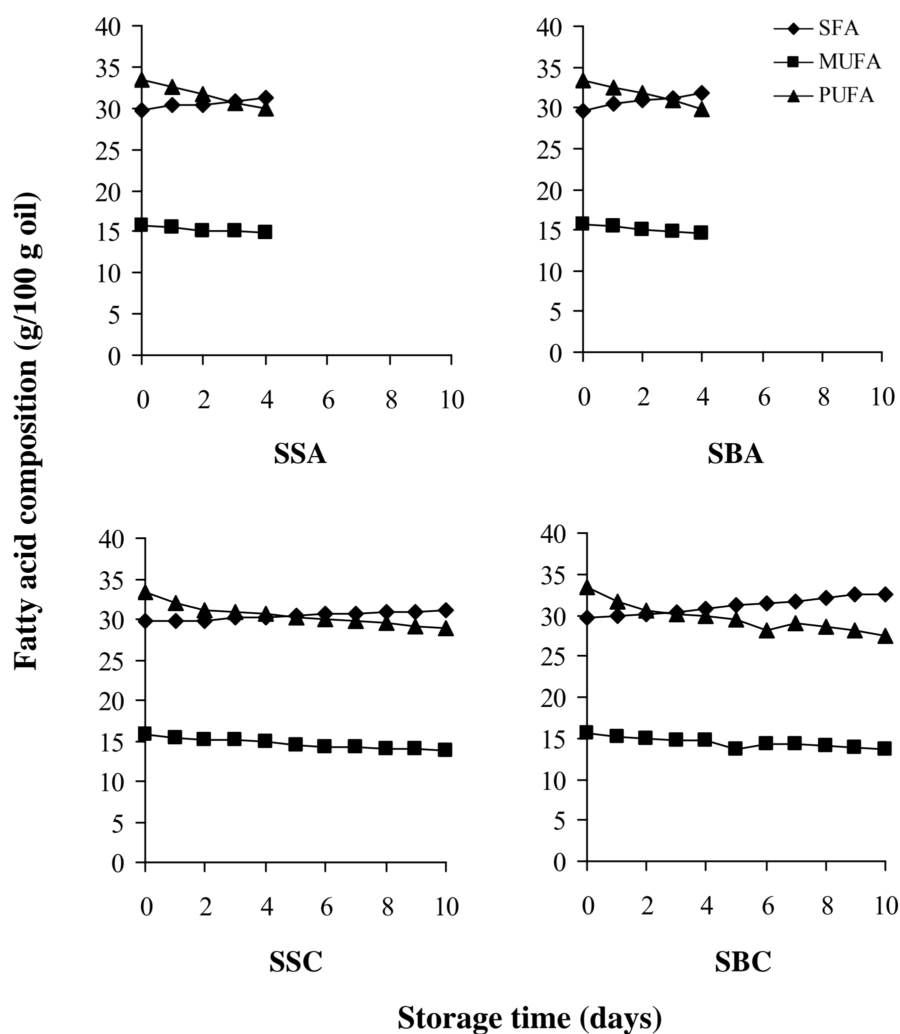

Figure 3.4 Changes in fatty acid composition of shell-on oyster stored in normal air at ambient (SSA) and chilled temperatures (SSC), and in 2.5% brine at ambient (SBA) and chilled temperatures (SBC). SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

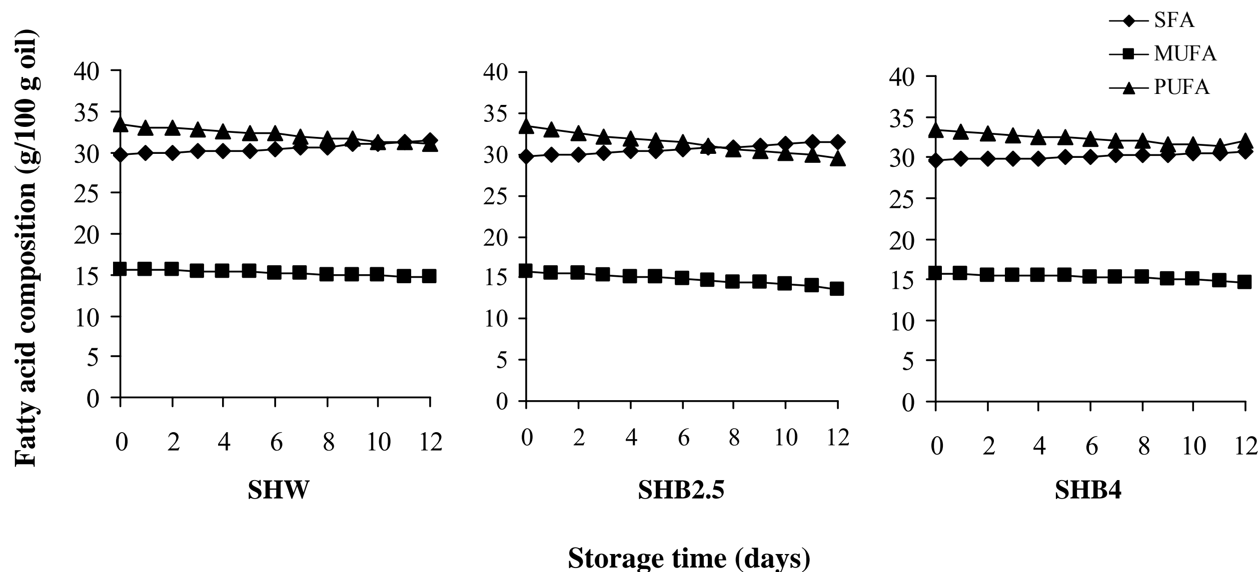

Figure 3.5 Changes in fatty acid composition of shucked oyster stored in water (SHW), 2.5% brine (SHB2.5) and 4% brine (SHB4) at chilled temperature. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

PV: Oyster lipids are highly susceptible to oxidation because they contain high levels of PUFA especially, EPA and DHA (Ohshima *et al.*, 2001; Passi *et al.*, 2002; Pennarun *et al.*, 2002). The basic mechanisms of lipid oxidation can be characterized by three steps: initiation, propagation and termination reactions (Nawar, 1996). 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 (Frankel, 1998; Hultin, 1994). Both chemical and enzymatic oxidations could probably occur in oysters during chilled storage. The chemical oxidation is due to the combination of triplet oxygen and singlet oxygen reaction (Erickson, 1998). The endogenous enzymes called lipoxygenase which was 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 (Samson and Stodolnik, 2001). The oxidized lipids could react with oxygen producing peroxides as primary oxidation products. The initial peroxide value (PV) of white-scar

oyster (*C. belcheri*) was 7.8 meq/kg lipids. During storage, most of the treatments showed increase in PV with extended storage time ($P < 0.05$) (Figure 3.6). On the other hand, PV of SSC and SBC decreased after 9 days of storage, probably due to a higher rate of hydroperoxide decomposition than the rate of oxidation (Nawar, 1996). 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 (Figure 3.6a) and was higher than the acceptable limit of not above 20 meq/kg lipid (Connell, 1995) after 4 days of ambient storage whereas shell-on oyster packed in normal air at ambient storage and shell-on oyster stored at chilled temperature were not higher than the acceptable limit throughout the storage. The shucked oyster packed in 4% brine showed a slower increase in PV than those in 2.5% brine and water (Figure 3.6b), and also was not higher than the acceptable limit throughout the storage. On the other hand, PV of shucked oyster packed in water and 2.5% brine after chilled storage for 7 and 6 days were higher than the acceptable limit, respectively. 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 (Kanner *et al*., 1991). 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% NaCl during chilled storage, respectively (Takiguchi, 1989). NaCl could also catalyze some enzyme activities i.e. 0.43 M NaCl in mackerel could catalyze the activity of lipoxygenase (Osinchak *et al*., 1992) and 0.15 M NaCl in flatfish muscle could accelerate the enzymatic oxidation (Apgar and Hultin, 1982). On the other hand, the salt content at 2.3% and above in sardine could inhibit lipid oxidation (Nambudiry, 1980) and salt at 0.29% and 0.58% could inhibit lipid oxidation in cod phospholipids (Mozuraityte, 2007).

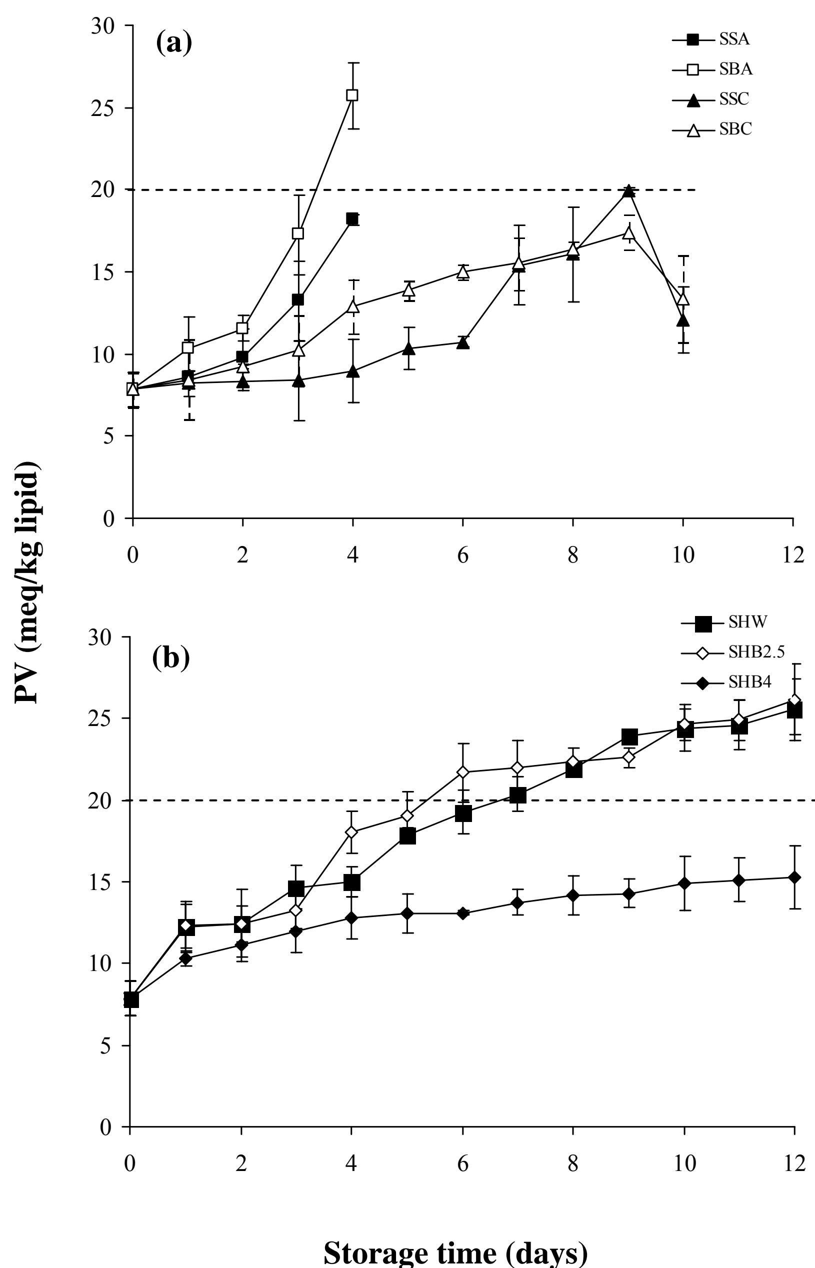

Figure 3.6 Changes in PV of (a) shell-on oyster stored in normal air at ambient (SSA) and chilled temperatures (SSC), and in 2.5% brine at ambient (SBA) and chilled temperatures (SBC), and (b) shucked oyster stored in water (SHW), 2.5% brine (SHB2.5) and 4% brine (SHB4) at chilled temperature.

(2) Changes in microbiological quality

Changes in TVC of shell-on and shucked white-scar oysters (*C. belcheri*) during storage under chilled and ambient temperature are shown in Table 3.6. The initial TVC of fresh oysters in this study was 3.7 log CFU/g and gradually increased throughout the duration of storage under the studied media. It was reported that the initial TVC in mussels (*Mytilus galloprovincialis*) was 4.5 log CFU/g and dramatically increased during storage at 4°C under modified atmosphere packaging (Goulas *et al*., 2005). Refer to the acceptable limit of TVC for chilled bivalve mollusks (not more than 5.7 log CFU/g, Department of Fisheries, 2004), shell-on oysters packed in both normal air and 2.5% brine, stored at ambient temperature (SSA and SBA) and chilled temperature (SSC, SBC) could be kept for less than 3 and 10 days, respectively. With the same reference, shucked oyster packed in water, 2.5% brine and 4% brine (SHW, SHB2.5 and SHB4) stored at chilled temperature could be kept for 12 days.

After storage, salt content of oyster increased from initially 1.5% w/w to different value under different packing media and storage temperature as shown in Figure 3.2. The increase in salt content may affect the growth of microorganisms showing from some microorganisms could survive in the presence of 2.5% salt (Huss, 1995). Furthermore, the inhibition of microbial growth in shucked oysters stored in both 2.5% brine and 4% brine was not significantly different throughout the duration of storage. The higher salt contents (> 5.0%) in salted mackerel apparently had some inhibitory effect on bacterial growth (Tsai *et al*., 2005). Wheaton and Lawson (1985) showed that fish with salt content above 1%, the bacteria associated with fish spoilage were increasingly stressed. Most of these bacteria would die or at least stop growing as the salt content of the fish was increased from 6% to 8%.

The psychrotrophs of shell-on and shucked oysters in different storage conditions gradually increased during chilled storage (Table 3.7). The psychrotrophs of shell-on oyster packed in 2.5% brine increased faster than those packed in normal air during chilled storage, probably due to some of the spoilage bacteria can grow well in the sodium-containing condition (Huss, 1995). Whereas those microorganisms of shucked oysters packed in 4% brine increased with a slower rate than those packed

in 2.5% brine and in water. The result suggested that the growth of the spoilage bacteria might be inhibited by a high salt concentration.

E. coli and *V. parahaemolyticus* of shell-on and shucked oysters under various media, storage temperatures were less than 1.8 and 3 MPN/g, respectively, throughout the storage time (Table 3.8). Those results did not exceed the acceptable limit for raw chilled bivalve mollusks (not above 2.3 and 10^4 MPN/g for USA market and 2.3 and 100 MPN/g for Japanese and Thai markets, respectively) (Department of Fisheries, 2004). The pathogenic bacteria of all treatments were lower than the standard value, probably due to the storage conditions were not suitable for their growth.

(3) Changes in sensory quality

The sensory qualities of oyster tissue after storage were observed from shrunken and contracted mantle, dissolved gill, soft in texture, dark in color and strong odor. Decrease in sensory scores of shell-on oysters both packed in normal air and in 2.5% brine was not significantly different ($P \geq 0.05$) but under ambient temperature were greater than those stored at chilled temperature during storage (Figure 3.7). The appearance, color, texture and odor scores of the shucked oysters in water slightly decreased at a greater rate than those packed in 2.5% and 4% brine during chilled storage (Figure 3.8). The acceptable shelf-life will be considered from the sensory score of more than 5.0. The results showed that shell-on oysters packed in normal air and in 2.5% brine could be accepted at less than 3 days at ambient temperature and 9 days under chilled storage. Whereas shucked oysters packed in water was up to 9 days and those in 2.5% and 4% brine were up to 10 days. Aaraas *et al*. (2004) recommended that the immersed flat oysters (*O. edulis*) could be consumed within a week but should not be used after 12 days of storage.

Table 3.6 Changes in total viable count of shell-on and shucked oysters during storage.

Storage time (days)	Total viable count (log CFU/g)						
	SSA	SBA	SSC	SBC	SHW	SHB2.5	SHB4
0	$3.69 \pm 0.13^{a,*}$	3.69 ± 0.13^{a}	3.69 ± 0.13^{a}	3.69 ± 0.13^{a}	3.69 ± 0.13^{a}	3.69 ± 0.13^{a}	3.69 ± 0.13^{a}
1	3.76 ± 0.31^{ab}	4.10 ± 0.02^{a}	3.72 ± 0.13^{a}	3.72 ± 0.11^{a}	3.71 ± 0.21^{a}	3.69 ± 0.36^{a}	3.75 ± 0.26^{a}
2	4.01 ± 0.28^{ab}	4.19 ± 0.16^{a}	3.94 ± 0.05^{a}	4.00 ± 0.37^{a}	3.91 ± 0.26^{a}	3.89 ± 0.06^{a}	3.81 ± 0.10^{a}
3	5.83 ± 0.06^{c}	6.33 ± 0.29^{c}	4.40 ± 0.02^{b}	4.80 ± 0.06^{b}	4.50 ± 0.51^{ab}	4.41 ± 0.06^{ab}	4.26 ± 0.34^{ab}
4	6.62 ± 0.47^{c}	6.74 ± 0.03^{c}	4.94 ± 0.23^{c}	5.21 ± 0.10^{bc}	4.48 ± 0.17^{bc}	4.57 ± 0.69^{abc}	4.68 ± 0.24^{bc}
5	NT	NT	5.25 ± 0.19^{d}	5.29 ± 0.02^{cd}	5.27 ± 0.18^{bcd}	5.01 ± 0.16^{bcd}	4.88 ± 0.40^{cd}
6	NT	NT	5.36 ± 0.11^{de}	5.72 ± 0.22^{de}	5.41 ± 0.32^{cd}	5.09 ± 0.06^{bcd}	5.08 ± 0.22^{cde}
7	NT	NT	5.40 ± 0.13^{de}	5.78 ± 0.21^{e}	5.59 ± 0.77^{cd}	5.43 ± 0.14^{cd}	5.19 ± 0.16^{cdef}
8	NT	NT	5.45 ± 0.04^{def}	5.94 ± 0.37^{e}	5.65 ± 0.11^{cd}	5.17 ± 0.85^{bcd}	5.38 ± 0.46^{def}
9	NT	NT	5.61 ± 0.03^{ef}	5.99 ± 0.14^{e}	5.66 ± 0.35^{cd}	5.43 ± 0.47^{cd}	5.49 ± 0.08^{ef}
10	NT	NT	5.72 ± 0.20^{f}	6.09 ± 0.16^{e}	5.69 ± 0.34^{cd}	5.66 ± 0.11^{d}	5.58 ± 0.20^{ef}
11	NT	NT	NT	NT	5.78 ± 0.63^{cd}	5.55 ± 0.56^{d}	5.64 ± 0.18^{ef}
12	NT	NT	NT	NT	5.89 ± 0.34^{d}	5.78 ± 0.04^{d}	5.73 ± 0.10^{f}
Standard value	5.7 log CFU/g						

*Mean $\pm$ SD from two trials. Values in the same column with different superscript letters are significantly different ($P < 0.05$).

NT, no test.

SSA, SBA and SSC, SBC = shell-on oyster stored in normal air, in 2.5% brine at ambient and chilled temperatures, respectively.

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

Table 3.7 Changes in psychrotrophic bacteria of shell-on and shucked oysters during storage.

Storage time	Psychrotrophic bacteria (log CFU/g)						
(days)	SSA	SBA	SSC	SBC	SHW	SHB2.5	SHB4
0	NT	NT	$3.69 \pm 0.13^{a, *}$	3.69 ± 0.13^{a}	3.69 ± 0.13^{a}	3.69 ± 0.13^{a}	3.69 ± 0.13^{a}
1	NT	NT	3.73 ± 0.11^{a}	3.74 ± 0.06^{a}	3.72 ± 0.04^{ab}	3.72 ± 0.05^{a}	3.71 ± 0.05^{ab}
2	NT	NT	3.90 ± 0.12^{ab}	4.96 ± 0.38^{a}	4.94 ± 0.08^{abc}	3.90 ± 0.23^{ab}	4.86 ± 0.03^{abc}
3	NT	NT	4.39 ± 0.12^{bc}	4.51 ± 0.13^{b}	4.03 ± 0.63^{abcd}	4.15 ± 0.07^{bc}	4.02 ± 0.13^{abc}
4	NT	NT	4.47 ± 0.22^{bc}	4.89 ± 0.47^{bc}	4.12 ± 0.20^{abcd}	4.21 ± 0.26^{bc}	4.09 ± 0.41^{abc}
5	NT	NT	4.67 ± 0.68^{c}	4.94 ± 0.20^{bc}	4.32 ± 0.16^{abcd}	4.23 ± 0.19^{bcd}	4.12 ± 0.12^{abc}
6	NT	NT	4.70 ± 0.38^{c}	4.98 ± 0.06^{bc}	4.38 ± 0.46^{abcd}	4.24 ± 0.18^{bcd}	4.15 ± 0.01^{abc}
7	NT	NT	4.82 ± 0.15^{c}	5.02 ± 0.05^{c}	4.41 ± 0.18^{bcd}	4.25 ± 0.18^{bcd}	4.23 ± 0.21^{abc}
8	NT	NT	4.86 ± 0.13^{c}	5.05 ± 0.17^{c}	4.43 ± 0.21^{cd}	4.30 ± 0.18^{cde}	4.28 ± 0.34^{abc}
9	NT	NT	4.87 ± 0.24^{c}	5.08 ± 0.14^{c}	4.49 ± 0.27^{cd}	4.38 ± 0.01^{cde}	4.30 ± 0.03^{bc}
10	NT	NT	4.90 ± 0.14^{c}	5.11 ± 0.01^{c}	4.50 ± 0.14^{cd}	4.45 ± 0.00^{cde}	4.33 ± 0.45^{c}
11	NT	NT	NT	NT	4.64 ± 0.42^{cd}	4.59 ± 0.05^{de}	4.37 ± 0.32^{c}
12	NT	NT	NT	NT	4.66 ± 0.22^{d}	4.63 ± 0.15^{e}	4.42 ± 0.31^{c}

*Mean ± SD from two trials. Values in the same column with different superscript letters are significantly different ($P < 0.05$).

NT, no test.

SSA, SBA and SSC, SBC = shell-on oyster stored in normal air, in 2.5% brine at ambient and chilled temperatures, respectively.

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

Table 3.8 Changes in *Escherichia coli* and *Vibrio parahaemolyticus* of shell-on and shucked oysters during storage.

Storage time (days)	*Escherichia coli* (MPN/g)						
	SSA	SBA	SSC	SBC	SHW	SHB2.5	SHB4
0-12	< 1.8	< 1.8	< 1.8	< 1.8	< 1.8	< 1.8	< 1.8
Storage time (days)	*Vibrio parahaemolyticus* (MPN/g)						
0-12	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0

SSA, SBA and SSC, SBC = shell-on oyster stored in normal air, in 2.5% brine at ambient and chilled temperatures, respectively.

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

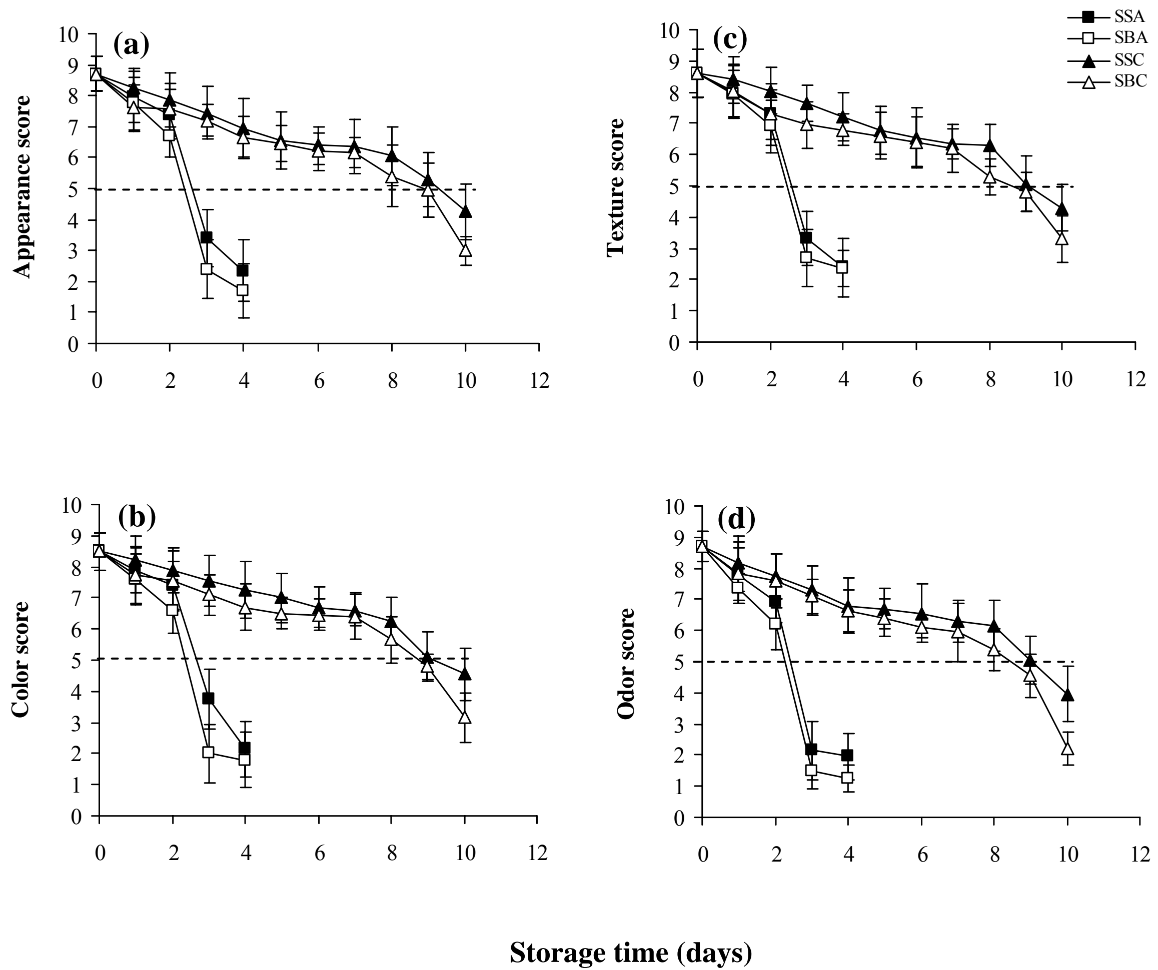

Figure 3.7 Sensory quality of shell-on oyster stored in normal air at ambient (SSA) and chilled temperatures (SSC) and in 2.5% brine at ambient (SBA) and chilled temperatures (SBC).

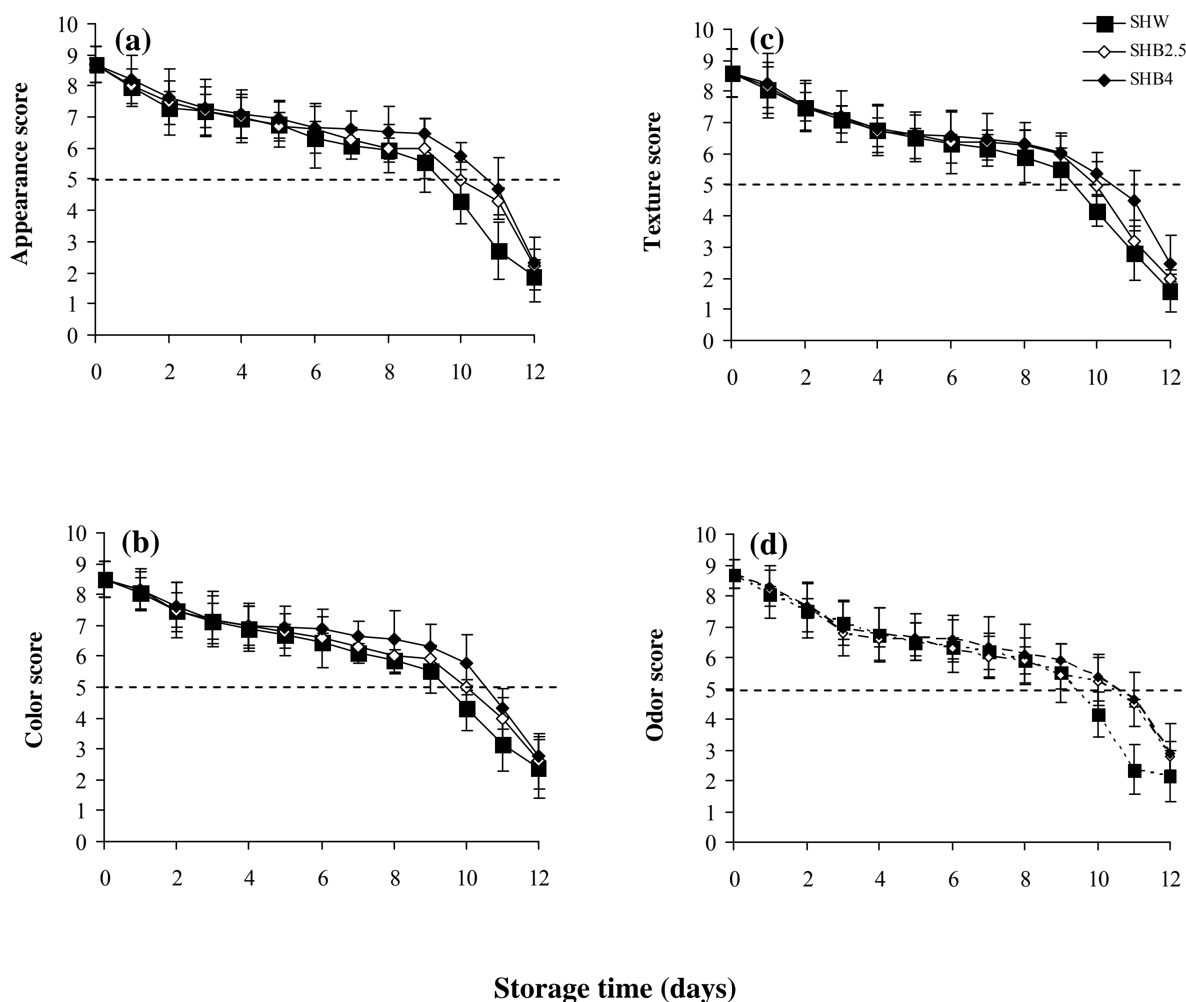

Figure 3.8 Sensory quality of shucked oyster stored in water (SHW), 2.5% brine (SHB2.5) and 4% brine (SHB4) at chilled temperature.

3.3.2 Effect of different storage conditions on volatile profile of fresh oysters

Changes in volatile profile

The volatile compounds produced from lipid oxidation include esters, aldehydes, alcohols, ketones, lactones, acid compounds and hydrocarbons (Mozuraityte, 2007) which are influenced by the composition of the hydroperoxides and the types of oxidative cleavage of double bonds in the fatty acids (Min, 1998). In the current research, nine alcohols (hexanol, (*E*)-2-penten-1-ol, 1-octen-3-ol, 2-ethyl-1-hexanol, 3-(*Z*)-hexen-1-ol, 1,5-octadien-3-ol, 1-octanol, (*E*)-2-octen-1-ol and (*E,Z*)-3,6-nonadien-1-ol), four

carboxylic acids (propanoic acid, pentanoic acid, octanoic acid and nonanoic acid), two ketones (2-undecanone and (*E,E*)-3,5-octadien-2-one), two aldehydes (propanal and (*E,E*)-2,4-heptadienal) and two hydrocarbon (1-hexadecene, and 2,4,6,10-tetramethylpentadecane), as oxidized volatile compounds, were found during storage (Table 3.9 and 3.10).

Two alcohols i.e. 1-octen-3-ol and 2-ethyl-1-hexanol were detected in fresh oyster. They have been found, in several earlier studies, in the raw Atlantic oyster (*C. virginica*) (Cha, 1995) and Pacific oyster (*C. gigas*) (Pennarun *et al*., 2002; Pennarun *et al*., 2003). The eight-carbon alcohols contribute distinct mushroom, heavy and plant-like aromas to both species of Atlantic and Pacific oysters (Josephson *et al*., 1985), and also have been observed to contribute strongly to the aromas of all species of fresh fish (Josephson and Lindsay, 1986). At day 0 of the storage, the amounts of 1-octen-3-ol and 2-ethyl-1-hexanol in oyster were 8.6 and 4.2 μg/g oyster, respectively. These compounds showed significant increase ($P < 0.05$) throughout the storage in each treatment.

During storage, alcohols in shell-on oyster packed in different media stored at chilled temperature increased at a slower rate than those stored at ambient temperature. Increase in alcohols of shell-on oyster packed in 2.5% brine were greater than those of shell-on oyster packed in normal in both ambient and chilled temperature for up to 3 and 6 day of storage, respectively. For shucked oyster, increases in alcohols of shucked oyster packed in 4% brine were greater than those of shucked oyster packed in water and 2.5% brine.

Increases in aldehydes were found only in shell-on oyster packed in 2.5% brine during ambient storage while increases in these compounds of shell-on oyster packed in 2.5% brine were greater than those of oyster packed in normal air during chilled storage. Increases in aldehydes of shucked oyster packed in 4% brine were greater than those shucked oyster packed in water and 2.5% brine after 6 days of chilled storage.

Increases in ketones were found only in shell-on oyster packed in 2.5% brine in both ambient and chilled storage. Ketones of shucked oyster packed in 2.5% brine increased at a faster rate than those shucked oyster packed in 4% brine, while

these compounds were not found in shucked oyster packed in water throughout the storage time.

Increases in carboxylic acids and hydrocarbons were found only in shell-on oyster packed in 2.5% brine in both ambient and chilled storage, while these compounds were not found in all samples of shucked oysters during storage.

Considering from total volatile concentrations, total volatiles of shell-on oyster in different media stored at chilled temperature increased at a slower rate than those stored at ambient temperature. Increase in total volatile concentrations of shell-on oyster packed in 2.5% brine were greater than those of shell-on oyster packed in normal air, possibly due to the ability of NaCl to induce lipid oxidation (Goulas and Kontominas, 2005; Goulas and Kontominas, 2007). For shucked oyster, increase in total volatile concentrations of shucked oyster packed in 4% brine were greater than those packed in water and 2.5% brine during chilled storage for up to 6 days. This was probably because the higher salt content could promote the formation of oxidized volatile compounds (Aubourg and Ugliano, 2002).

Table 3.9 Relative concentration (µg/g oyster) of volatile profile of shell-on oysters during ambient and chilled storage.

	Compound	Storage time (days)					
		SSA			SBA		
		0	2	3	0	2	3
Alcohols	(*E*)-2-Penten-1-ol	0.00 ± 0.00^{a,*}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	2.91 ± 1.73^{a}
	Hexanol	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.87 ± 0.08^{b}
	(*Z*)-3-Hexen-1-ol	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	2.91 ± 1.38^{b}
	1-Octen-3-ol	8.61 ± 1.16^{a}	8.67 ± 0.47^{a}	14.69 ± 2.69^{b}	8.61 ± 1.16^{a}	41.40 ± 9.07^{b}	54.91 ± 4.45^{c}
	2-Ethyl-1-hexanol	4.21 ± 0.46^{a}	4.25 ± 0.26^{a}	7.71 ± 0.95^{b}	4.21 ± 0.46^{a}	21.78 ± 2.97^{b}	31.39 ± 1.67^{c}
	1,5-Octadien-3-ol	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.75 ± 0.36^{b}
	1-Octanol	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	4.20 ± 0.71^{b}	6.83 ± 1.96^{b}
	(*E*)-2-Octen-1-ol	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	3.10 ± 1.79^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	5.28 ± 0.94^{b}
	(*E,Z*)-3,6-Nonadien-1-ol	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	3.93 ± 0.42^{b}
Aldehydes	Propanal	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	17.26 ± 2.89^{b}	33.09 ± 29.20^{c}
	(*E,E*)-2,4-Heptadienal	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	15.58 ± 4.73^{b}	20.26 ± 3.77^{b}
	4-Ethylbenzaldehyde	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.15 ± 0.33^{b}	2.55 ± 0.64^{c}
Ketones	2,3-Pentanedione	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	5.76 ± 3.19^{b}
	(*E,E*)-3,5-Octadien-2-one	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.13 ± 0.63^{b}
	2-Undecanone	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	4.86 ± 0.66^{b}	10.78 ± 4.87^{b}

Table 3.9 *Continued*

	Compound	Storage time (days)					
		SSA			SBA		
		0	2	3	0	2	3
Carboxylic acids	Propanoic acid	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	3.37 ± 1.02[b]	5.29 ± 1.42[b]
	3-Methylbutanoic acid	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	10.83 ± 4.66[b]	15.27 ± 0.39[b]
	Pentanoic acid	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	2.66 ± 0.74[b]	2.67 ± 0.39[b]
	Octanoic acid	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.96 ± 0.28[b]
	Nonanoic acid	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	6.04 ± 1.61[b]
Hydrocarbons	(*Z*)-7-Tetradecene	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	5.09 ± 4.77[b]	11.25 ± 2.25[b]
	1-Hexadecene	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	2.41 ± 0.70[b]	2.52 ± 0.21[b]
	2,4,6,10-Tetramethylpentadecane	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.80 ± 0.15[b]	1.07 ± 0.39[b]
	1-Octadecene	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	1.36 ± 0.57[b]	5.12 ± 3.39[b]
	Total volatiles	12.82	12.92	25.50	12.82	132.75	234.54
		SSC			SBC		
		0	3	6	0	3	6
Alcohols	(*Z*)-3-Hexen-1-ol	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.84 ± 0.30[b]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
	1-Octen-3-ol	8.61 ± 1.16[a]	10.10 ± 0.18[a]	11.91 ± 0.77[b]	8.61 ± 1.16[a]	10.81 ± 0.40[b]	13.17 ± 0.98[c]
	2-Ethyl-1-hexanol	4.21 ± 0.46[a]	4.97 ± 0.14[a]	8.96 ± 0.53[b]	4.21 ± 0.46[a]	5.47 ± 0.20[b]	10.76 ± 0.48[c]

Table 3.9 *Continued*

	Compound	Storage time (days)					
		SSC			SBC		
		0	3	6	0	3	6
Aldehydes	Propanal	0.00 ± 0.00[a]	26.77 ± 6.57[b]	44.61 ± 7.63[c]	0.00 ± 0.00[a]	31.41 ± 4.01[b]	83.73 ± 22.09[c]
	(*E,E*)-2,4-Heptadienal	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	3.45 ± 0.52[b]
Ketones	2-Undecanone	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	2.29 ± 0.79[b]
Carboxylic acids	Propanoic acid	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	1.31 ± 0.77[b]
	3-Methylbutanoic acid	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	7.86 ± 0.79[b]
Hydrocarbons	1-Hexadecene	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	1.49 ± 0.09[b]
	2,4,6,10-Tetramethylpentadecane	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.91 ± 0.07[b]
	Total volatiles	12.82	41.84	66.32	12.82	47.69	124.97

*Mean ± SD from two trials. Values in the same row for each sample followed by different superscript letters are significantly different ($P < 0.05$).

SSA, SBA and SSC, SBC = shell-on oyster stored in normal air, in 2.5% brine at ambient and chilled temperatures, respectively.

Table 3.10 Relative concentration (μg/g oyster) of volatile profile of shucked oysters during chilled storage.

	Compound	Storage time (days)								
		SHW			SHB2.5			SHB4		
		0	3	6	0	3	6	0	3	6
Alcohols	(*Z*)-3-Hexen-1-ol	$0.00 \pm 0.00^{a,*}$	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.76 ± 0.20^{b}
	1-Octen-3-ol	8.61 ± 1.16^{a}	9.68 ± 3.69^{a}	11.22 ± 1.04^{a}	8.61 ± 1.16^{a}	9.34 ± 0.96^{a}	10.53 ± 0.81^{a}	8.61 ± 1.16^{a}	9.03 ± 0.97^{a}	10.86 ± 1.59^{a}
	2-Ethyl-1-Hexanol	4.21 ± 0.46^{a}	5.22 ± 1.86^{a}	5.38 ± 0.64^{a}	4.21 ± 0.46^{a}	4.77 ± 0.56^{ab}	5.17 ± 0.24^{b}	4.21 ± 0.46^{a}	4.68 ± 0.34^{ab}	5.77 ± 0.89^{b}
	1,5-Octadien-3-ol	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.06 ± 0.62^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Aldehydes	Propanal	0.00 ± 0.00^{a}	20.61 ± 4.65^{b}	27.51 ± 10.13^{b}	0.00 ± 0.00^{a}	7.06 ± 3.78^{b}	13.34 ± 8.53^{b}	0.00 ± 0.00^{a}	8.81 ± 2.25^{b}	33.82 ± 11.60^{c}
Ketones	2-Undecanone	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	4.06 ± 1.60^{b}	4.30 ± 0.71^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	2.71 ± 1.66^{b}
	Total volatiles	12.82	35.51	44.11	12.82	25.23	34.40	12.82	22.52	53.92

*Mean ± SD from two trials. Values in the same row for each sample followed by different superscript letters are significantly different ($P < 0.05$).

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

3.4 Conclusion

Shell-on oysters, under chilled storage in both normal air and 2.5% brine showed slower changes in chemical, microbiological and sensory qualities than those under ambient temperature. Shell-on oysters packed in 2.5% brine showed slower decrease in pH but greater decreases in TG, PL, PUFA and MUFA whereas increase in TVB-N, PV, TVC and psychrotrophs than those packed in normal air during the storage. Considering from microbiological and sensory qualities, it was found that shell-on oysters packed in normal air and in 2.5% brine could be accepted at less than 3 days at ambient temperature and 9 days under chilled storage. Shucked oysters packed in 4% brine showed a decrease in pH and increases in TVB-N, TVC and psychrotrophs at a slower rate than those packed in 2.5% brine and water when stored at chilled temperature. The lipid hydrolysis and oxidation were slower in shucked oysters packed in 4% brine than those packed in 2.5% brine and water during storage. Considering from microbiological and sensory qualities, it was found that shucked oysters packed in water, 2.5% brine and 4% brine were accepted at 9, 10 and 10 days of chilled storage, respectively. Though keeping shucked oyster in 4% brine showed the greatest effect on the shelf-life extension, it might affect to the acceptable taste of oyster. Only two volatiles i.e. 1-octen-3-ol and 2-ethyl-1-hexanol were initially detected, which could contribute to mushroom, heavy and plant-like odor (Josephson *et al*., 1985; Piveteau *et al*., 2000). On the other hand, more compounds including nine alcohols, four carboxylic acids, two ketones, two aldehydes and two hydrocarbons were found during storage. A large increase of propanal was found in oyster during storage, which could contribute to pungent odor (Olafsdottir and Kristbergsson, 2006).

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CHAPTER 4

EFFECT OF FREEZING AND ANTIOXIDANTS ON QUALITIES AND VOLATILE PROFILE OF FROZEN OYSTER (*CRASSOSTREA BELCHERI*) DURING STORAGE

4.1 Introduction

Freezing provides a significantly extended shelf-life and has been successfully employed for the long term preservation of many foods. The major function of freezing is to change the physical state of water into ice when energy is removed at freezing temperature or further reduced to –18°C or lower (Rahman and Labuza, 1999). The selection of any specific freezing method on the quality of product depends on rate of freezing, producing cost, shape and size of the product, and feasibility governed by factors of location and type of product (Garthwaite, 1997). Typically foods can be frozen either by quick or slow freezing method. Quick freezing is usually achieved within 30 min by reducing the temperature at more than 50°C/h and form a small ice crystal in both extracellular and intracellular compartments. Whereas slow freezing may take about 3 to 72 h to reduce the temperature at 1-10°C/h and a large ice crystal will be formed (Jay, 1996). In commercial practice, freezing rates based on the rate of movement of the ice front which vary between 0.2 and 100 cm/h; 0.2 cm/h (slow freezing) for bulk freezing in blast rooms, 0.5 to 3 cm/h (quick freezing) for retail packages in air blast or plate freezers, 5 to 10 cm/h (rapid freezing) for individual quick freezing of small sized products, e.g., in a fluidized bed, and 10 to 100 cm/h (ultrarapid freezing) by spraying with or immersion in liquid gases (Jiang and Lee, 2004). The undesirable changes during freezing are associated with formation of large ice crystals in extracellular compartment, mechanical damage by the ice crystals to cellular structures through distortion and volume changes, and chemical damage arising from changes in concentrations of solutes. Drip loss during thawing of the quick frozen product is thus considerably low, and the surface reflects lighter than that of slow frozen product. Consequently, the cut surface appearance is more acceptable (Farias *et al*., 2005; Jiang and Lee, 2004).

The extent on quality of frozen food is dependent upon many factors including, storage temperature and time, packaging, rate of freezing and thawing, temperature fluctuations and freeze-thaw abuse (Srinivasan *et al*., 1997). Difference in freezing and frozen storage could affect the quality of oyster i.e. adenosine 5′-triphosphate (ATP) decreased in oyster (*Crassostrea gigas*) during frozen storage at -20°C and -30°C (Qi *et al*., 2007) whereas pH of oyster (*C. gigas*) unchanged throughout the storage at -20°C and -35°C (Jeong *et al*., 1990). Decrease in moisture, protein, glycogen and alpha amino nitrogen but increase in total volatile basic nitrogen (TVB-N), thiobarbituric acid reactive substances (TBARS) and free fatty acids (FFA) were found in oyster (*Crassostrea madrasensis*) during storage at -18°C (Balasundari *et al*., 1997). Jeong *et al*. (1990) also reported that the lipid hydrolysis and oxidation took place in oyster (*C. gigas*) during storage at -20°C and -35°C. For microbiological quality, total viable count (TVC), staphylococci, motile aeromonads, total coliforms and *Escherichia coli* decreased in oyster (*C. madrasensis*) during storage at -18°C (Balasundari *et al*., 1997). The unacceptable odor, taste, color and texture of oyster (*C. gigas*) occurred at the decomposed stage during frozen storage (Qi *et al*., 2007).

To minimize color and flavor changes due to lipid oxidation in the shellfish products prior to storage, some pretreatments have been applied i.e. antioxidant dipping. Butylated hydroxytoluene (BHT) and natural vitamin E were used to minimize color and flavor changes or lipid oxidation in oysters (*C. gigas*) during storage at -20°C and -35°C (Jeong *et al*., 1990). Ascorbic acid and chelating agents were effectively used to inhibit progression of oxidative rancidity and sensory deterioration (aroma, flavor and texture) of mussel (*Mytilus edulis*) during storage at -30°C (Ablett *et al*., 1986). In addition, flavonols (myricetin, quercetin and morin) exhibited stronger antioxidant activity than butylated hydroxyanisole (BHA) and BHT in marine oil (Wanasundara and Shahidi, 1998).

However, information was limited regarding to the effect of freezing and antioxidants on quality and volatile profile of frozen white-scar oysters (*Crassostrea belcheri*) during frozen storage. Therefore, the objective of this research was to study the effect of freezing and antioxidant on quality and volatile profile of frozen oyster during storage at -20°C.

4.2 Research Methodology

4.2.1 Chemicals and media

All chemicals, volatile standards and media were analytical grade as previously described in section 3.2.1. Other volatile standards were analytical grade including, 1-penten-3-ol, 3-hexanol, heptanol, pentanal, hexanal, (*E*)-2-methyl-2-butenal, (*E*)-2-pentenal, 2-methyl-2-pentenal and 2,4,6-trimethylpyridine (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan). Butylated hydroxyanisole was food grade (NU, Tokyo, Japan).

4.2.2 Raw material and sample preparation

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 oyster culture farm in Bandon bay, Suratthani province, south of Thailand in August 2005. The farm located 3 km from the shore with salinity and temperature of seawater approximately 17 part per thousand (ppt) and 24.4°C, respectively. After harvesting, the live oysters were placed in nylon sacks and transported to the laboratory (4-5 h at ambient temperature of 30 $\pm$ 2°C) and then mechanically shucked. The shucked oysters were equally divided into 4 lots (450 each). Two lots of shucked oysters were individually placed on a conveyer and frozen in a tunnel individually quick freezer (IQF) (Asian Seafood Frozen Co. Ltd., Suratthani, Thailand) using liquid CO_2 at -60°C for 10 min. The other two lots of shucked oysters were packed in blocks (size: 14 x 18 x 6 cm), some cold water (approximately 4°C) added to 30% of net weight, and frozen at -40°C in a contact plate freezer (CPF) (Samifi Babcock, Italy) for 3 h (Appendix B). After freezing, both individual and blocked frozen oysters were immediately glazed in water without BHA or in BHA suspension (0.02% w/w BHA) for 5-10 s, packed in polyethylene (PE) bags and stored in polystyrene boxes at -20°C. The frozen samples were designated as follows: Individual Quick Frozen (IQF) oyster without BHA (SHI) and with BHA (SHIB), and Contact Plate Frozen (CPF) oyster without BHA (SHC) and with BHA (SHCB).

Table 4.1 Different treatments for frozen oyster.

Code	Treatments	
	Freezing method	Antioxidant
SHI	Individually quick freezing	Without
SHIB	Individually quick freezing	BHA
SHC	Contact plate freezing	Without
SHCB	Contact plate freezing	BHA

4.2.3 Effect of freezing and antioxidants on qualities of frozen oysters

The two trials of each treatment were kept for 12 months at -20°C. Sixty oysters were removed from each individual and blocked frozen samples for qualities analyses every 3 months. At each pull, frozen samples were thawed for 18 h at 4 $\pm$ 2°C before analyses. Physical, chemical, microbiological and sensory qualities of frozen oysters during storage were determined as follows:

(1) Physical quality

Expressible drip: Known weight (A) of oyster samples were placed between 2-Whatman No. 4 filter paper on top and 3-filter paper below. A 5 kg weight was then placed on top within 30 sec and held for 2 min. The samples were then removed and weighed (B). The drip under pressure was determined as [(A-B)/A] x 100 (Hasegawa, 1987).

(2) Chemical quality

pH, chemical composition (moisture, crude protein and crude fat), TVB-N, lipid composition, fatty acid composition and PV were determined as previously described in section 3.2.3 (1).

(3) Microbiological quality

TVC, psychrotrophs, *E. coli* and *V. parahaemolyticus* were determined as previously described in section 3.2.3 (2).

(4) Sensory quality

Sensory scores of appearance, color of plum, texture and odor of oysters were evaluated as previously described in section 3.2.3 (3).

4.2.4 Effect of freezing and antioxidants on volatile profile of frozen oysters

The two trials of each treatment were kept for 12 months at -20°C. Thirty oysters were removed from each individual and blocked frozen samples for volatile profile analysis at 0, 3, 9 and 12 months of storage as previously described in section 3.2.4. At each pull, frozen samples were thawed for 18 h at 4 ± 2°C before analyses.

4.2.5 Statistical analysis

The data from two trials (three replicates from each trial) was subjected to the analysis of variance (ANOVA) according to the experimental design in two-way factorial (2 freezing methods x 2 antioxidants) by univariate method. Comparison of means was carried out by Duncan's multiple range test for significant differences at $P < 0.05$ (Steel and Torrie, 1980).

4.3 Results and Discussion

4.3.1 Effect of freezing and antioxidants on qualities of frozen oysters

(1) Changes in physical quality

Expressible drip: Loss of liquid from any frozen food after thawing was probably due to the rupture of cell structure and tissue by the ice crystal growth during freezing (Fellows, 2000). Frozen oyster by quick method (SHI and SHIB) showed lower expressible drip than those of frozen oyster by slow method (SHC and

SHCB) indicating the value of 16.6, 18.5, 27.5 and 26.5% w/w, respectively (Figure 4.1). During frozen storage, the expressible drip in all samples increased as the storage time increased ($P < 0.05$), probably due to ice crystal could damage the tissue and resulted in leakage of various organelles (Benjakul *et al*., 2003). Furthermore, storage at frozen temperature may lower the water holding capacity of proteins. In both untreated and treated with BHA of IQF and CPF oysters, the expressible drip was not significantly different ($P \geq 0.05$) during the 12-month frozen storage.

(2) Changes in chemical quality

pH: Changes in pH can be used as indicator for the postmortem change of glycogen to lactic acid and the degradation of muscle components e.g. proteins and nucleotides during long term storage. Both freezing method and antioxidant treatment did not show significant changes in pH of all white-scar oysters (*C. belcheri*) which ranged from 6.2 to 6.4 during frozen storage (Figure 4.1) and also were not lower than the acceptable limit (< 5.5) (Pottinger, 1948). Similar results were found in pH of oyster (*C. gigas*) which remained almost unchanged at 6.45 during storage at -20°C for 12 months (Jeong *et al*., 1990).

Chemical composition: Changes in oyster composition are shown in Table 4.2. After freezing, moisture contents of SHI, SHIB, SHC and SHCB were 81.2, 81.5, 80.0 and 80.4%, respectively but they were not significantly changed ($P \geq 0.05$) during the frozen storage over 12 months. Japanese oysters (*C. gigas*) also had similar results during storage at -20°C for 12 months (Jeong *et al*., 1990). Crude protein contents of frozen oyster i.e. SHI, SHIB, SHC and SHCB were 8.3, 8.3, 8.6 and 8.6%, respectively. There was no noteworthy change in the protein of all samples during frozen storage for 12 months, similar to the results for mussels and abalones during storage at -25°C and -10-(-35)°C for 12 months, respectively (Haard, 1992). After freezing, crude fat contents of SHI, SHIB, SHC and SHCB were 2.3, 2.4, 2.5 and 2.5%, respectively and very slightly increased during frozen storage.

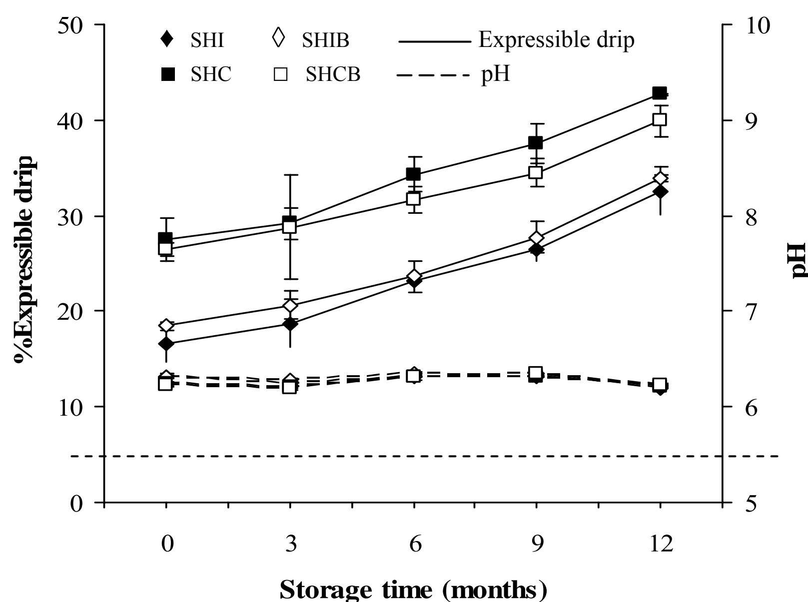

Figure 4.1 Changes in expressible drip and pH during storage at -20°C of frozen oyster by IQF without BHA (SHI) and with BHA (SHIB) and CPF without BHA (SHC) and with BHA (SHCB).

TVB-N: The TVB-N, a decomposed both protein and non-protein nitrogenous compounds (Huss, 1995) of all samples increased with storage time ($P < 0.05$) which probably caused by bacterial and endogenous proteolytic enzymatic actions (Hernandez-Herrero *et al*., 1999). After freezing, TVB-N of SHI, SHIB, SHC and SHCB were 3.3, 4.2, 4.3 and 4.5 mg N/100 g, respectively (Figure 4.2). The initial TVB-N value of all frozen product were slightly unusual, probably due to the delay time of oyster transportation and product preparation. The TVB-N of both IQF and CPF oysters treated and untreated with BHA were not significantly changed ($P \geq 0.05$) and did not exceed the acceptable limit (30 mg N/100 g) (Lopez-Caballero *et al*., 2000) during the 12-month frozen storage. Similar results were reported in oyster (*Crassostrea madrasensis*) during stored at -20°C for 150 days (Balasundari *et al*., 1997).

Table 4.2 Chemical composition changes of IQF and CPF oysters during storage at -20°C.

Composition (% wet basis)	Storage time (months)	SHI	SHIB	SHC	SHCB
Moisture	0	$81.2 \pm 0.8^{aA,*}$	81.5 ± 0.2^{aA}	80.0 ± 0.3^{aA}	80.4 ± 0.4^{aA}
	3	81.4 ± 0.5^{aA}	80.4 ± 1.0^{aA}	80.2 ± 0.7^{aA}	81.5 ± 0.8^{aA}
	6	79.9 ± 0.2^{aA}	80.1 ± 0.2^{aA}	79.6 ± 0.0^{aA}	80.3 ± 0.2^{aA}
	9	79.7 ± 0.6^{aA}	80.0 ± 0.6^{aA}	80.0 ± 0.5^{aA}	80.0 ± 0.3^{aA}
	12	80.0 ± 0.8^{aA}	80.3 ± 0.3^{aA}	79.5 ± 0.2^{aA}	80.3 ± 0.3^{aA}
Crude protein	0	8.3 ± 0.4^{aA}	8.3 ± 0.2^{aA}	8.6 ± 0.2^{aA}	8.6 ± 0.1^{aA}
	3	8.4 ± 0.1^{aA}	8.3 ± 0.1^{aA}	8.5 ± 0.1^{aB}	8.5 ± 0.1^{aB}
	6	8.5 ± 0.2^{aA}	8.3 ± 0.1^{aA}	8.5 ± 0.1^{aA}	8.5 ± 0.3^{aA}
	9	8.5 ± 0.1^{aB}	8.2 ± 0.3^{aA}	8.5 ± 0.0^{aB}	8.5 ± 0.0^{aB}
	12	8.3 ± 0.1^{aA}	8.3 ± 0.1^{aA}	8.6 ± 0.3^{aB}	8.6 ± 0.0^{aB}
Crude fat	0	2.3 ± 0.1^{aA}	2.4 ± 0.0^{aA}	2.5 ± 0.0^{aA}	2.5 ± 0.1^{aA}
	3	2.5 ± 0.8^{bA}	2.5 ± 0.3^{bA}	2.5 ± 0.0^{aA}	2.7 ± 0.0^{bB}
	6	2.5 ± 0.1^{bA}	2.5 ± 0.0^{bA}	2.7 ± 0.1^{bB}	2.7 ± 0.0^{bB}
	9	2.6 ± 0.0^{bA}	2.6 ± 0.1^{bA}	2.8 ± 0.0^{cB}	2.8 ± 0.0^{cB}
	12	2.8 ± 0.0^{cB}	2.7 ± 0.0^{cA}	2.8 ± 0.0^{cB}	2.9 ± 0.0^{cB}

*Means ± SD from two trials. ^{abc}Mean values in the same column for each parameter followed by different letters are significantly different (*P < 0.05*). ^{ABC}Mean values in the same row followed by different letters are significantly different (*P < 0.05*).

SHI and SHIB = IQF oyster untreated and treated with BHA, respectively.

SHC and SHCB = CPF oyster untreated and treated with BHA, respectively.

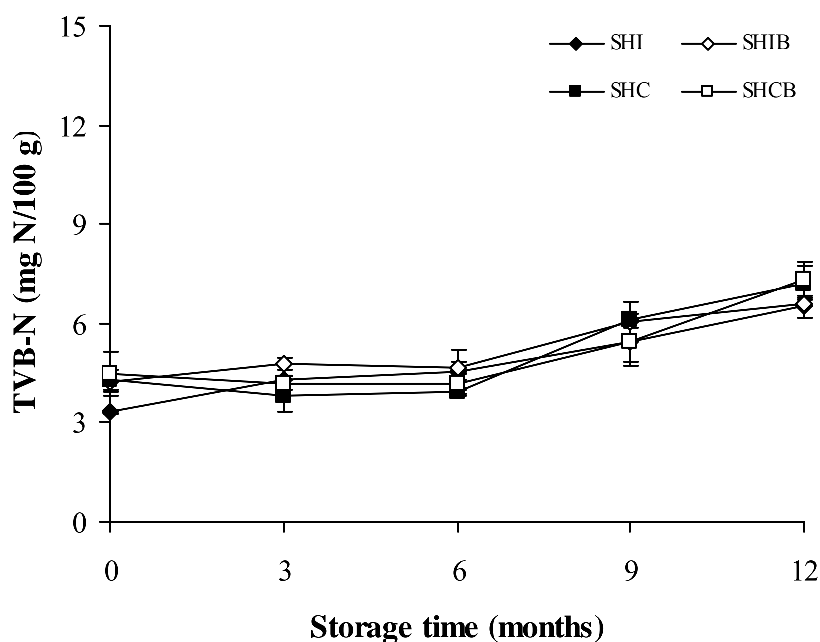

Figure 4.2 Changes in TVB-N during storage at -20°C of frozen oyster by IQF without BHA (SHI) and with BHA (SHIB) and CPF without BHA (SHC) and with BHA (SHCB).

Lipid composition: The lipid composition of white-scar oyster (*C. belcheri*) was classified into 5 groups i.e. triglyceride (TG), diglyceride (DG), monoglyceride (MG), free fatty acid (FFA) and phospholipids (PL). Both freezing method and antioxidant treatment did not show significant changes in lipid composition of all frozen oysters ($P \geq 0.05$) (Figure 4.3). During frozen storage, the TG was significantly changed whereas DG and FFA increased but PL decreased ($P < 0.05$) with the storage time increase. MG was not found in all treatments throughout the storage time. The increase of DG and FFA could be due to the hydrolysis of TG and PL by endogenous and/or microbial lipase and/or phospholipase depend upon the extent of those two enzymes (Erickson, 1998). It was shown by other researcher that the activity of lipase of Pacific oysters (*C. gigas*) stored at -20°C and -35°C were suppressed much more than of phospholipase (Jeong *et al.*, 1990). Decrease in PL but increase in DG and FFA of IQF oyster were higher than those of CPF oyster during storage, probably due to the effect of quick freezing at extremely lower temperature and more surface area of IQF samples. Oysters frozen by the same

method and treated with BHA treatment showed slower changes in lipid composition than those untreated with BHA treatment.

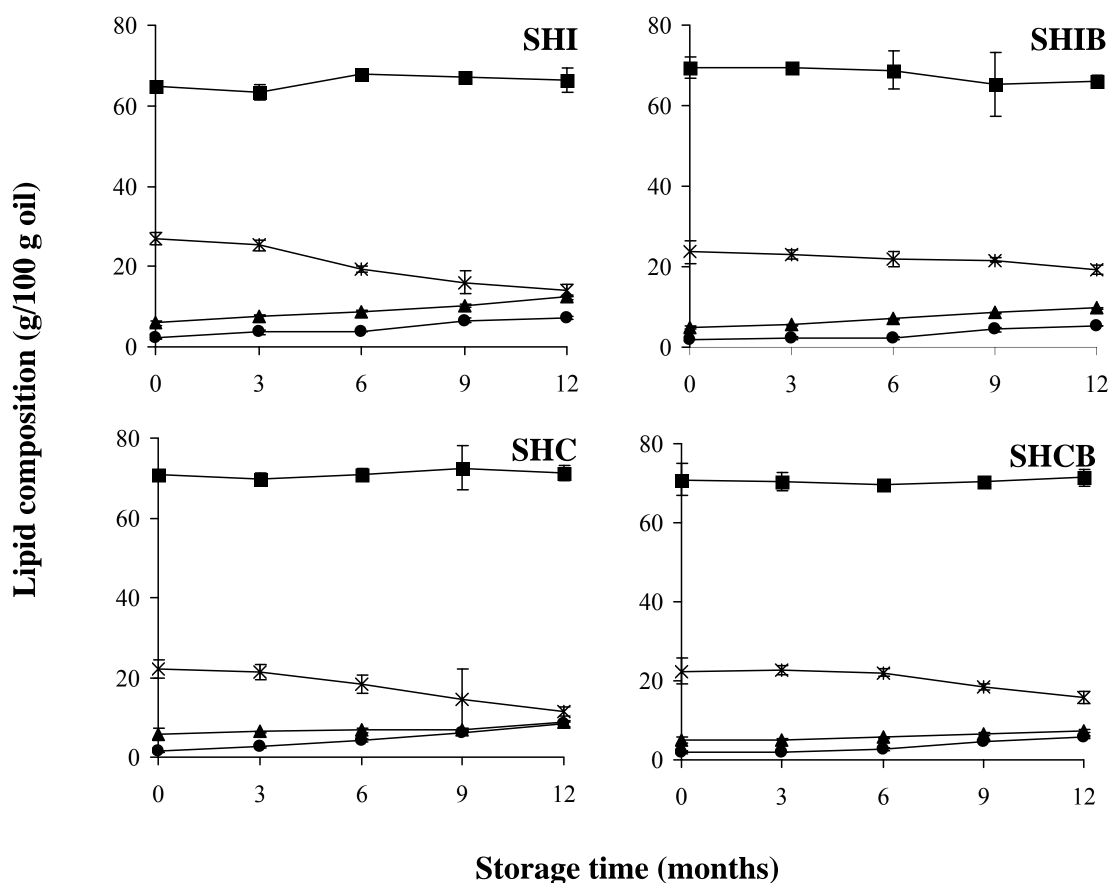

Figure 4.3 Changes in lipid composition during storage at -20°C of frozen oyster by IQF untreated with BHA (SHI) and treated with BHA (SHIB) and CPF untreated with BHA (SHC) and treated with BHA (SHCB). -■-: triglyceride; -▲-: diglyceride; -●-: free fatty acid; -✳-: phospholipid.

Fatty acid composition: Both freezing method and antioxidant treatment did not show significant changes ($P \geq 0.05$) in fatty acid composition of frozen white-scar oysters (*C. belcheri*) i.e. saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA) (Figure 4.4). The predominant fatty acid in SFA was palmitic acid (C16:0), in MUFA was oleic acid (C18:1) and in PUFA included eicosapentaenoic acid (C20:5), docosahexaenoic acid (C22:6) with the amount of 22.0-22.4, 9.3-9.8, 13.0-13.7 and

9.2-9.9 g/100 g oil, respectively (Table A4-5). Similar to the report of Piveteau *et al.* (2000) showing that Pacific oysters (*C. gigas*) contained major fatty acid of C16:0 (18.7 g/100g oil), C20:5 (18.2 g/100 g oil), C22:6 (17.8 g/100 g oil) and C18:1 (8.1 g/100 g oil).

During storage, MUFA in all samples were almost unchanged, while PUFA decreased but SFA increased, probably due to unsaturated fatty acids being converted to saturated fatty acids by hydrogenation reaction (Erickson, 1998). These results were similar to those reports for frozen Pacific oysters (*C. gigas*) (Jeong *et al.*, 1990). It was also found that IQF samples without antioxidant (SHI) showed higher decreases in PUFA and increases in SFA than those in samples with antioxidant (SHIB) during storage ($P < 0.05$). Similar changes in PUFA and SFA were shown in Pacific oysters (*C. gigas*) treated with natural vitamin E and BHT during frozen storage (Jeong *et al.*, 1990). Moreover, decreases in PUFA and increases in SFA occurred at higher rates in oysters stored at -20°C than those at -35°C (Jeong *et al.*, 1990). The decreases in PUFA and increases in SFA of CPF oysters without antioxidant (SHC) and with antioxidant (SHCB) were not significantly different ($P \geq 0.05$) during storage.

PV: Oyster lipids are highly susceptible to oxidation because they contain high levels of PUFA especially, EPA and DHA (Ohshima *et al.*, 2001). The lipid oxidation can be influenced by fatty acid composition, amount of oxygen, pro-oxidant and antioxidant, endogenous ferrous ion, enzymes, salt, pH, light, and temperature (Frankel, 1998; Hultin, 1994). After freezing, frozen oyster by quick method (SHI and SHIB) showed significant lower PV than those of frozen oyster by slow method (SHC and SHCB) ($P < 0.05$) indicating the value of 12.3, 10.6, 13.8 and 12.8 meq/kg lipid, respectively (Figure 4.5). During storage, the PV of all samples increased continuously as storage time increased ($P < 0.05$), indicating oxidative deterioration. Similar observations have been made in Pacific oysters (*C. gigas*) during storage at -20°C (Jeong *et al.*, 1990). Moreover, oysters untreated with antioxidant showed higher rate of increases in PV than oysters treated with BHT and natural vitamin E during storage at -20°C for 12 months (Jeong *et al.*, 1990). The PV of the untreated IQF oyster increased at a greater rate than those of treated with BHA,

while those of CPF oyster both untreated and treated with BHA were not significant difference ($P \geq 0.05$) during frozen storage. This was probably because of the instability of BHA, much more contact surface area and thinner layer of glazing occurring in IQF samples than in CPF samples. It was also found that PV value of IQF oysters treated and untreated with BHA after storage for 6 and 9 months were higher than the acceptable limit of not above 20 meq/kg lipid (Connell, 1995) while those of CPF samples did not exceeded the acceptable limit throughout the storage time.

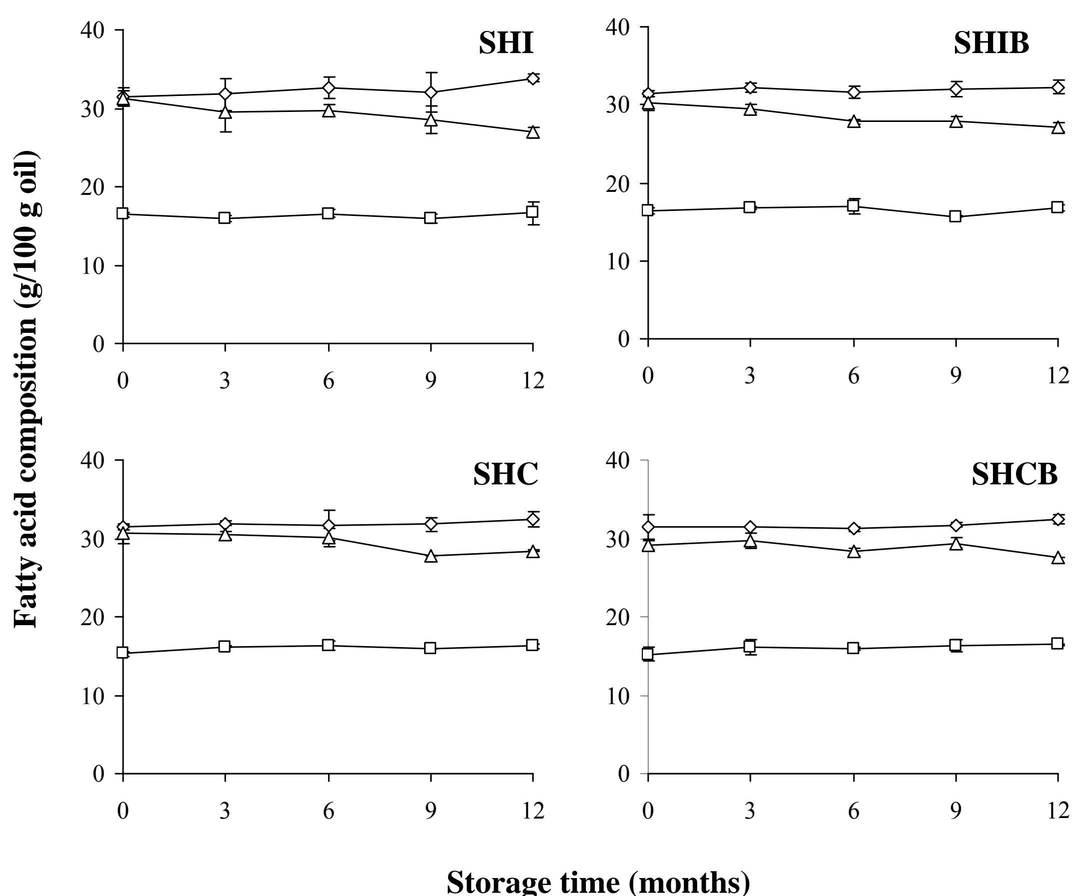

Figure 4.4 Changes in fatty acid composition during storage at -20°C of frozen oyster by IQF untreated with BHA (SHI) and treated with BHA (SHIB) and CPF untreated with BHA (SHC) and treated with BHA (SHCB). –◇– : saturated fatty acid; –□– : monounsaturated fatty acid; –△– : polyunsaturated fatty acid.

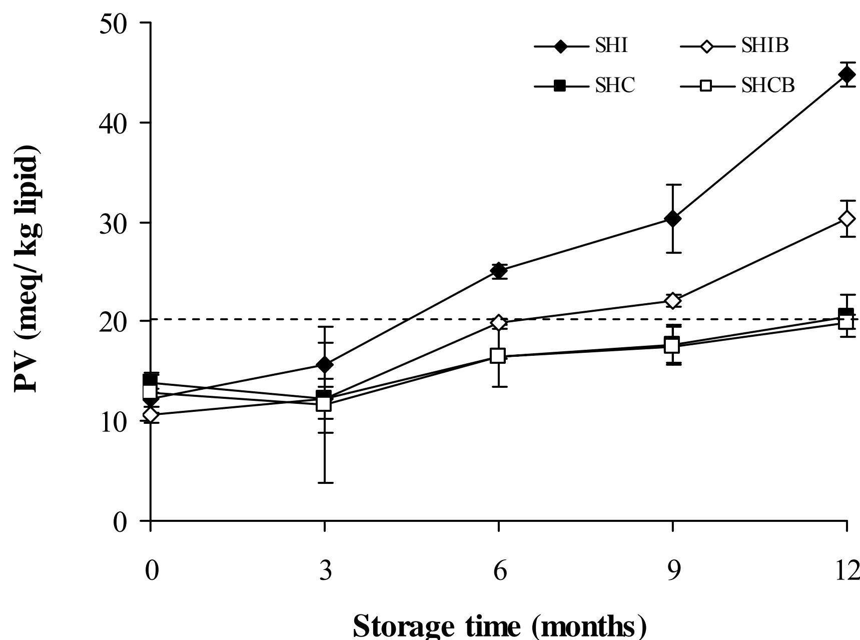

Figure 4.5 Changes in PV during storage at -20°C of frozen oyster by IQF untreated with BHA (SHI) and treated with BHA (SHIB) and CPF untreated with BHA (SHC) and treated with BHA (SHCB).

(3) Changes in microbiological quality

The TVC and psychrotrophs of all samples decreased with storage time (Figure 4.6), probably due to the lethality of microorganisms as affected by freezing rate and low storage temperature (Golden and Arroyo-Gallyoun, 1997). The TVC of all samples decreased remarkably during the first 3 months of storage. Similar results were found that TVC in oyster (*C. madrasensis*) decreased during storage at -18°C for 150 days (Balasundari *et al*., 1997). Rebach *et al*. (1990) also reported that the aerobic plate count during storage of whole Jonah crabs (*Cancer borealis*) at -23°C for 50 weeks were slowly decreased throughout the end of storage. The results in this study showed that the decreases in TVC were greater rate than in psychrotrophs, probably due to the fact that psychrotrophs could be more tolerated at frozen storage than mesophilic bacteria (Moharram and Rofael, 1993). However, the TVC value of all samples during storage did not exceed the acceptable limit for raw frozen bivalve mollusks (not more than 5.7 log CFU/g) (Department of Fisheries, 2004).

E. coli and *V. parahaemolyticus* of all samples were less than 1.8 and 3 MPN/g, respectively throughout the storage time (Table 4.3). Those results did not exceed the acceptable limit for raw frozen bivalve mollusks (not above 2.3 and 10^4 MPN/g for USA market and 2.3 and 100 MPN/g for Japanese and Thai markets, respectively) (Department of Fisheries, 2004). Thus, the control of time and temperature during freezing and frozen storage may help eliminate the organisms from oysters. On the other hand, total coliforms and *E. coli* in oyster (*C. madrasensis*) decreased during storage at -18°C for 150 days (Balasundari *et al.*, 1997).

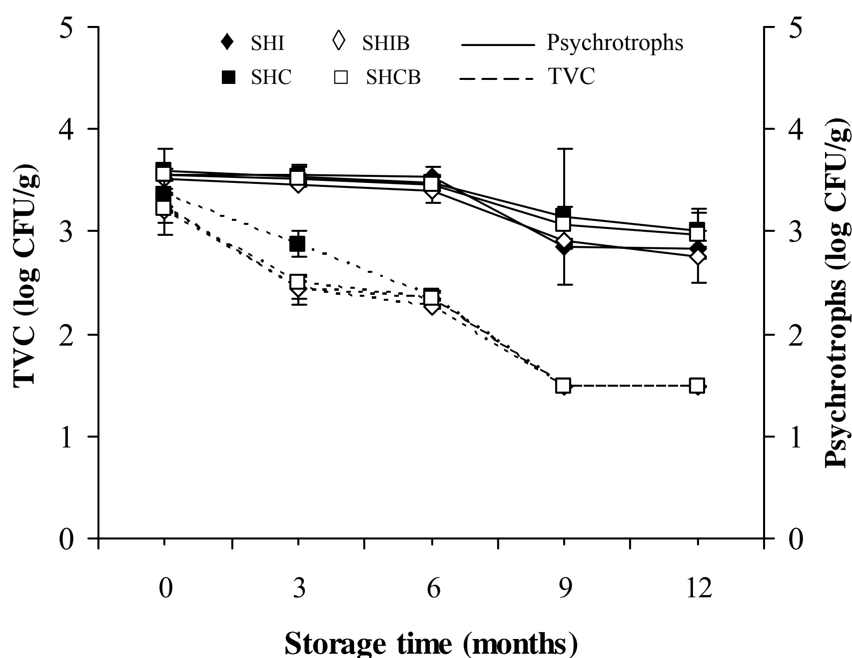

Figure 4.6 Changes in TVC and psychrotrophs during storage at -20°C of frozen oyster by IQF without BHA (SHI) and with BHA (SHIB) and CPF without BHA (SHC) and with BHA (SHCB).

Table 4.3 Changes in *Escherichia coli* and *Vibrio parahaemolyticus* of frozen oysters during storage.

Storage time (months)	*Escherichia coli* (MPN/g)			
	SHI	SHIB	SHC	SHCB
0-12	< 1.8	< 1.8	< 1.8	< 1.8
Storage time (months)	***Vibrio parahaemolyticus* (MPN/g)**			
0-12	< 3.0	< 3.0	< 3.0	< 3.0

SHI and SHIB = IQF oyster untreated and treated with BHA, respectively.
SHC and SHCB = CPF oyster untreated and treated with BHA, respectively.

(4) Changes in sensory quality

The scores of sensory quality i.e. appearance (shrunken and contacted mantle as well as dissolved gill), color of plum (yellowness), texture (softness), and odor (strong fishy odor) in the oysters during frozen storage are shown in Figure 4.7. It was found that all sensory attributes of all oysters slowly decreased during 3 months and then rapidly decreased until the end of storage. Both freezing method and antioxidant treatment did not significantly affect all attributes, but during storage they tended to decrease over storage time. The decreases in appearance and odor scores were not affected by freezing method and antioxidant. Whereas, color and texture scores of both treated and untreated with BHA were significantly affected by freezing method. Considering from the acceptable score of 5.0, it was shown that all frozen oysters could be kept for approximately 9 months depend upon different attributes.

The appearance in all samples was not significantly changed ($P \geq 0.05$) during frozen storage and still accepted (scores of more than 5.0) after 12 months of storage. Similar results were found for odor scores but accepted only after 9 months. The color in IQF oyster became more yellow at faster rate than that of CPF oyster during frozen storage. The discoloration could be resulted from lipid oxidation. The pigments were probably formed by ionic condensation of primary

amino groups of protein with conjugated unsaturated aldehydes or similar reactive lipid oxidation products that are produced by cleavage of unsaturated hydroperoxides (Thanonkaew *et al.*, 2006). The texture in CPF oysters were softer than those in IQF oysters during frozen storage and still accepted only up to 6 months. This was probably due to the difference of microstructure of small or large ice crystals depending of the freezing method (Jiang and Lee, 2004).

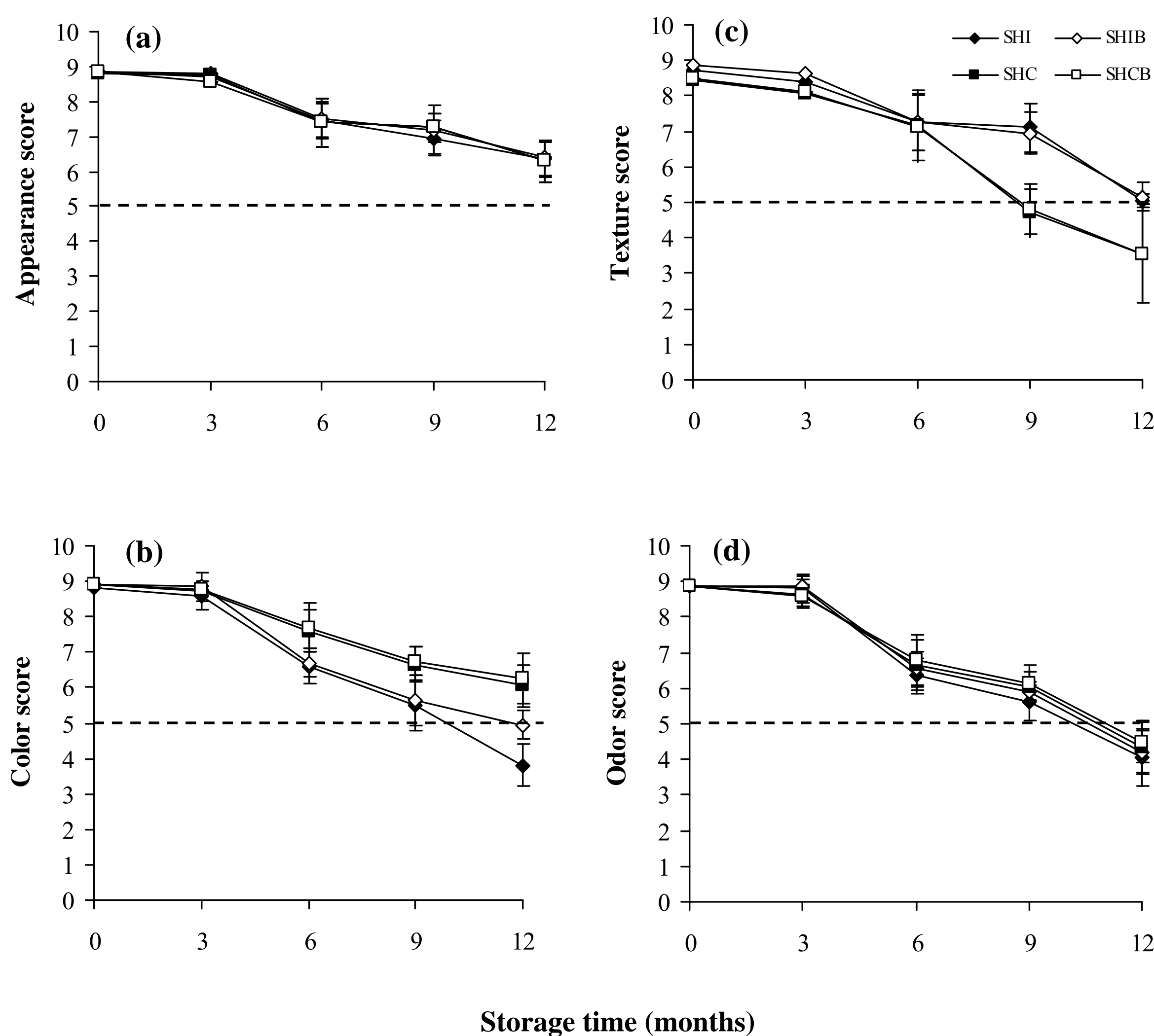

Figure 4.7 Changes in sensory scores during storage at -20°C of frozen oyster by IQF without BHA (SHI) and with BHA (SHIB) and CPF without BHA (SHC) and with BHA (SHCB).

4.3.2 Effect of freezing and antioxidants on volatile profile of frozen oysters

Changes in volatile profile

The volatile compounds produced from lipid oxidation including esters, aldehydes, alcohols, ketones, lactones, acid compounds and hydrocarbons (Mozuraityte, 2007) are influenced by the composition of the hydroperoxides and the types of oxidative cleavage of double bonds in the fatty acids (Min, 1998). After freezing, only two alcohols i.e. 1-octen-3-ol (9.7, 9.2, 6.8 and 5.6 μg/g oyster) and 2-ethyl-1-hexanol (5.1, 4.9, 4.9 and 4.7 μg/g oyster) were detected in IQF (SHI and SHIB) and CPF (SHC and SHCB) oysters (Table 4.4). These compounds, eight-carbon alcohols, contributed to the distinct mushroom, heavy and plant-like aromas (Josephson *et al*., 1985). During storage, those two compounds of both IQF and CPF samples were lesser increased in treated BHA than those in untreated oyster for up to 12 months of storage ($P < 0.05$), which probably due to the autoxidation of PUFA (Josephson *et al*., 1985), as well as the degradation of antioxidants occurring during processing and storage (Decker *et al*., 2000). Alcohols i.e. 1-penten-3-ol, 3-hexanol, hexanol, (*E*)-2-penten-1-ol, heptanol and 1-octanol were found in IQF oysters during storage whereas 1-penten-3-ol, 3-hexanol and hexanol were not found in CPF oysters throughout the storage, probably due to the effect of longer freezing time of slow freezing. During storage, increases in total alcohols of IQF oyster treated with BHA were higher rate than those untreated with BHA whereas those volatile compounds of CPF oyster untreated with BHA were higher rate than those untreated with BHA. 1-Penten-3-ol was derived from autoxidation of unsaturated fatty acids (Cruz-Romero *et al*., 2008) which related to the fresh sweet flavor of oyster (Zhang *et al*., 2009). (*E*)-2-Penten-1-ol was the products of the degradation of n-3 PUFA (Edirisinghe *et al*., 2007; Pennarun *et al*., 2003) whereas heptanol and 1-octanol were derived from autoxidation of oleic acid (C18:1, n-9), contributed to cucumber odor (Josephson *et al*., 1985; Pennarun *et al*., 2002).

Aldehydes i.e. pentanal, (*E*)-2-methyl-2-butenal, 2-methyl-2-pentenal, hexanal, (*E*)-2-pentenal and (*E,E*)-2,4-heptadienal were found in IQF oysters during storage whereas pentanal, (*E*)-2-methyl-2-butenal and 2-methyl-2-pentenal were not found in CPF oysters throughout the storage. During storage, increases in total

aldehydes of both IQF and CPF oysters treated with BHA were lower rate than those untreated with BHA. Hexanal was formed after n-6 PUFA degradation during storage whereas (*E*)-2-pentenal was the products of the degradation of n-3 PUFA (Edirisinghe *et al*., 2007; Pennarun *et al*., 2003). (*E,E*)-2,4-Heptadienal were contributed to mushroom and moss odor, derived from oxidation of polyunsaturated fatty acids (Josephson *et al*., 1985; Pennarun *et al*., 2002). Ketone i.e. 2-undecanone, contributed to cucumber and fresh odor of oysters (*C. gigas*) (Josephson *et al*., 1985), was found in both IQF and CPF oysters during storage. Increases in ketone of IQF untreated with BHA were lower rate than those treated with BHA whereas ketone in untreated with BHA of CPF oysters increased at a higher rate than those in treated with BHA. Pentanoic acid was found in both IQF and CPF oysters whereas 1-octadecene was only found in IQF oyster treated with BHA during storage. During storage, increases in total other compounds of both IQF and CPF oysters treated with BHA were lower rate than those untreated with BHA. The organic acids were considered arising from lipid oxidation in oyster during storage which related with the sour and offensive odor (Zhang *et al*., 2009).

Table 4.4 Relative concentration (μg/g oyster) of volatile profile of IQF and CPF oysters during storage at -20°C.

	Compound	Storage time (months)							
		SHI				SHIB			
		0	3	9	12	0	3	9	12
Alcohols	1-Penten-3-ol	0.00 ± 0.00$^{a, *}$	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.80 ± 0.09^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	3-Hexanol	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.77 ± 0.02^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	Hexanol	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.47 ± 0.46^{b}	1.83 ± 1.08^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.61 ± 0.35^{b}	2.95 ± 0.52^{c}
	(*E*)-2-Penten-1-ol	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	6.49 ± 3.88^{b}	8.06 ± 2.82^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	8.35 ± 1.47^{b}	11.10 ± 0.75^{b}
	1-Octen-3-ol	9.66 ± 2.52^{a}	18.53 ± 2.85^{ab}	23.17 ± 8.46^{b}	35.93 ± 1.74^{c}	9.24 ± 2.59^{a}	14.62 ± 4.67^{a}	31.77 ± 3.63^{b}	33.97 ± 11.55^{t}
	Heptanol	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	12.08 ± 3.67^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	18.57 ± 4.42^{b}
	2-Ethyl-1-hexanol	5.12 ± 0.90^{a}	10.88 ± 1.73^{b}	15.64 ± 1.81^{c}	17.02 ± 3.91^{c}	4.88 ± 0.61^{a}	8.80 ± 1.24^{b}	15.83 ± 1.35^{c}	20.19 ± 2.64^{d}
	1-Octanol	0.00 ± 0.00^{a}	1.58 ± 1.16^{b}	2.22 ± 0.74^{b}	2.37 ± 0.36^{b}	0.00 ± 0.00^{a}	1.04 ± 0.15^{b}	1.65 ± 0.28^{b}	2.84 ± 0.61^{c}
Aldehydes	Pentanal	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	10.37 ± 0.12^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	4.47 ± 4.87^{b}
	(*E*)-2-Methyl-2-butenal	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.90 ± 0.13^{b}
	2-Methyl-2-pentenal	0.00 + 0.00a	0.00 + 0.00a	0.00 + 0.00a	0.90 + 0.20b	0.00 + 0.00a	0.00 + 0.00a	0.00 + 0.00a	0.87 + 0.26b
	Hexanal	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.46 ± 0.50^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.98 ± 0.29^{b}
	(*E*)-2-Pentenal	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.72 ± 0.10^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.69 ± 0.16^{b}
	(*E,E*)-2,4-Heptadienal	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.97 ± 0.30^{b}
Ketones	2-Undecanone	0.00 ± 0.00^{a}	5.85 ± 4.29^{ab}	10.96 ± 2.36^{bc}	15.01 ± 5.06^{c}	0.00 ± 0.00^{a}	3.30 ± 0.47^{ab}	7.11 ± 2.16^{b}	18.34 ± 3.66^{c}
Others	Pentanoic acid	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.03 ± 0.30^{b}	1.70 ± 0.33^{c}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	1-Octadecene	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.73 ± 0.16^{b}
		SHC				SHCB			
Alcohols	(*E*)-2-Penten-1-ol	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	6.59 ± 1.32^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	4.76 ± 2.83^{b}	4.71 ± 2.70^{b}
	1-Octen-3-ol	6.84 ± 0.34^{a}	11.55 ± 1.05^{a}	23.23 ± 3.48^{b}	23.43 ± 3.67^{b}	5.63 ± 1.43^{a}	5.52 ± 0.76^{a}	17.79 ± 0.48^{b}	24.17 ± 9.73^{b}
	Heptanol	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	4.63 ± 0.29^{b}	14.17 ± 0.39^{c}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	9.69 ± 0.78^{b}
	2-Ethyl-1-hexanol	4.89 ± 0.58^{a}	7.97 ± 1.12^{b}	10.51 ± 0.58^{b}	16.24 ± 2.39^{c}	4.71 ± 0.90^{a}	5.50 ± 0.38^{a}	9.72 ± 0.58^{b}	11.76 ± 1.04^{c}
	1-Octanol	0.00 ± 0.00^{a}	1.18 ± 0.17^{b}	1.90 ± 0.29^{c}	2.86 ± 0.42^{d}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	2.08 ± 1.24^{b}	2.26 ± 0.36^{b}

Table 4.4 *Continued*

	Compound	Storage time (months)							
		SHC				SHCB			
		0	3	9	12	0	3	9	12
Aldehydes	Hexanal	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.87 ± 0.23^{b}	2.05 ± 0.75^{c}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	(*E*)-2-Pentenal	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.27 ± 0.16^{b}	1.54 ± 0.18^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.20 ± 0.09^{b}
	(*E,E*)-2,4-Heptadienal	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.86 ± 0.10^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Ketones	2-Undecanone	0.00 ± 0.00^{a}	4.05 ± 0.34^{b}	11.26 ± 1.84^{c}	20.94 ± 2.90^{d}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	14.60 ± 3.06^{b}	16.31 ± 6.30^{c}
Others	Pentanoic acid	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.18 ± 0.51^{b}	1.34 ± 0.65^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.91 ± 0.16^{b}

*Mean ± SD from two trials. Values in the same row for each sample followed by different superscript letters are significantly different ($P < 0.05$).

SHI and SHIB = IQF untreated and treated with BHA. SHC and SHCB = CPF untreated and treated with BHA.

4.4 Conclusion

The increase in expressible drip of IQF oyster was slower than those of CPF oyster due to the fact that quick freezing (IQF) resulted in less tissue damage than slow freezing (CPF). Both freezing method and antioxidant treatment did not show significant changes in moisture, crude protein, crude fat, pH, TVB-N, lipid composition, fatty acid composition, TVC and pyschrotrophs of all frozen oysters. During frozen storage, TVC and psychrotrophs of all samples decreased with the storage time. TG was significantly changed, whereas DG and FFA increased but PL decreased with the storage time increase. PUFA in all samples tend to decrease while SFA and MUFA were not significantly differences during storage. Antioxidant treatment could prevent lipid oxidation in IQF oysters more effectively than those in CPF oysters which exhibit non-significantly differences between treated and untreated samples. The sensory scores were decreased as the storage time increased. IQF oyster showed a greater decrease in color score, i.e., more yellow, but slower decrease in texture score, i.e., more firmness, than CPF oyster. Appearance and odor in all samples were not significantly different during storage. After storage for 12 months, the volatile compounds of IQF samples (SHI and SHIB) were higher than those of CPF samples (SHC and SHCB) consisted of alcohols, aldehydes, ketones and others Only two alcohols i.e. 1-octen-3-ol and 2-ethyl-1-hexanol were detected in oyster after freezing, which could contribute to mushroom, heavy and plant-like odor (Josephson *et al*., 1985; Piveteau *et al*., 2000). During storage, both 1-octen-3-ol and 2-ethyl-1-hexanol increased with increasing storage time. In addition, a large increase of 2-undecanone was found in frozen oyster during storage,which could contribute to cucumber and fresh odor (Josephson *et al*., 1985; Pennarun *et al*., 2002; Pennarun *et al*., 2003).

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CHAPTER 5

EFFECT OF THERMAL PROCESSING AND STORAGE ON QUALITY AND VOLATILE PROFILE OF CANNED AND RETORT POUCHED OYSTERS (*CRASSOSTREA BELCHERI*)

5.1 Introduction

To compensate for the decline of oyster production (particularly in Bandon Bay, Suratthani province, Thailand) due to overfishing, pollution and diseases, processing of oysters for extended shelf-life has become extremely important. The demand for better quality and safety processed food is ever increasing. This led to the development of a large food preservation industry aiming to supply sterile, nutritious and economical food products. Thermal processing of food is one of the most effective means of preserving our food supply (Karel *et al*., 1975) by destroying the spoilage microorganism as well as inactivation enzymes causing undesirable chemical reaction. Therefore, sterile and safety food products have long storage life under ambient conditions (Lewis and Heppell, 2000; Regenstein and Regenstein, 1991).

Factors affecting the quality and safety of sterile food products are the heat resistance of microorganisms or enzymes likely to be present in food, heating conditions, pH of food, size of container and physical state of food storage (Footitt and Lewis, 1995). The important terms to determine the thermal destruction i.e. the decimal reduction time (D value), the thermal resistance constant (Z value) and the lethality value (F value). D value indicates time required at any temperature to destroy 90% or one log cycle of the spores or vegetative cells of a given organism, whereas Z value means number of Fahrenheit or Celsius degrees require for the thermal destruction curve to traverse one log cycle and F value means time require to achieve a stated reduction in the microbial population at a given temperature. Generally, a number of parameters can be used to evaluate the amounts of microbial inactivation in thermal sterilization i.e. F_0 value, which is the equivalent exposure time at 121°C or 250°F of the actual exposure time at a variable temperature, calculated for an ideal

microorganisms with a temperature coefficient of destruction equal to 10. The factors influencing the F_0 value are the microbial population in the product, type of product, size of the container, types of heating and cooling media (Heldman and Hartel, 1998).

From food safety and human health perspective, the commercial sterilization of seafood products as low-acid foods (pH > 4.5) concern to the survival of *Clostridium botulinum*, an anaerobic spore-forming microorganism that produces an extremely potent exotoxin. It is generally recognized that a thermal process sufficient to eliminate *Cl. botulinum* as a health hazard is sufficient to eliminate the health hazards associated with all other pathogens likely to be found in a raw food commodity (Stumbo, 1973). Different F_0 value reported for different sterilized seafood products such as, 7 min at 121°C for canned (size 6.52 cm i.d. x 3 cm) albacore tuna (*Thunnus alalunga*) in oil (Aubourg *et al*., 1997) and 9 min at 121°C for black clam (*Villorita cyprinoides*) in masala medium packed in retort pouched (180 mm x 110 mm) (Bindu *et al*., 2007). Durance and Collins (1991) investigated that F_0 value for canned at 121°C was 6.8 min for canned and 8.2 min for retort pouched chum salmon (*Oncorhynchus keta*) in 1% non-iodized salt packed in tinplate cans (307 x 200) and in retort pouches (176 mm x 250 mm).

The main goal of thermal processing is inactivation or destroying the pathogenic microorganisms to make food safe to the consumer (Bellara *et al*., 1999). However, this needs to be done without the over-processing which may result in low and undesirable quality characteristics. Color, flavor and texture changes may also be more pronounced during heating (Ali *et al*., 2005; Mohan *et al*., 2006; Mohan *et al*., 2008). Furthermore, abuse heating might lower the nutritional value of the food as vitamins and micro-nutrients (Miri *et al*., 2008). The presence of unsaturated fatty acids enhances lipid oxidation, through the development of free fatty acid (FFA) and thiobabituric acid reactive substances (TBARS) during heating and storage (Bindu *et al*., 2007; Mohan *et al*., 2006). Upon heat processing, many low-molecular-weight aroma-active compounds are formed via lipid oxidation, strecker degradation and maillard reaction. In addition, maillard and strecker degradation reactions play predominant role in developing the meaty aromas of cooked fish and shellfish, especially sulfur containing compounds, pyrone, pyrazine, oxazole and pyrrolidine compounds. The main volatile compounds in cooked mussels include 2,3-butanedione

(buttery, caramel-like odor), *(Z)*-4-heptenal (boiled potato-like odor), *(E)*-2-penten-1-ol (mushroom-like odor), 2-ethylpyrazine (nutty odor), methional (boiled potato-like odor) and *(E, E)*-2,4-octadienal (cucumber-like odor) (Le Guen *et al*., 2000; Le Guen *et al*., 2001).

Various researchers have compared the quality attributes of canned and retort pouched seafood products. Dymit (1973) reported that shrimps in retort pouch were superior in flavor and color to canned products. Chia *et al*. (1983) showed that the shorter processing time of the retort pouch was responsible for firmer texture and lighter color than those of canned rainbow trout, pollack and shrimp products. Durance and Collins (1991) reported significantly less off-flavor and greater acceptance of chum salmon in retort pouches than cans. Ali *et al*. (2005) showed an improved texture of canned sardine in oil in retort pouched compared to cans. Mohan *et al*. (2006) reported that shrimp kuruma processed in retort pouches were lighter in color, more succulent, and firmer in texture compared to samples in cans. Moreover, thermal processing resulted in a 14% loss of water in canned shrimp kuruma compared to 9% in retort pouched products (Mohan *et al*., 2006). Mohan *et al*. (2008) showed that shrimp kuruma processed in retort pouches had better color, texture, and overall acceptability than when processed in cans.

However, the information about processing times required and effect of thermal processing on quality throughout storage period of lacquer-tin can and retort pouch for white-scar oyster (*Crassostrea belcheri*) is scarce. Therefore, the aim of this research was to study the effects of thermal processing and storage on chemical and sensory qualities as well as volatile profile of heat sterilized white-scar oyster (*C. belcheri*) during ambient temperature storage for 12 months.

5.2 Research Methodology

5.2.1 Chemicals and media

All chemicals, volatile standards and media were analytical grade as previously described in section 3.2.1. Other volatile standards were analytical grade including, 1-propanol, 2-hexanol, hexanol, (*Z*)-3-hexen-1-ol, 1-heptanol, 2-nonanol, 1-octanol, (*E,Z*)-3,6-nonadien-1-ol, ethanal, 2-methyl propanal, butanal, heptanal,

(*Z*)-4-heptenal, 2,3-butanedione, 2,3-pentanedione, 3-methyl pyridine, 2-ethyl furan and trimethylamine (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) and a series of straight-chain alkanes (C10-C28) (Sigma, St. Louis, MO, USA).

5.2.2 Raw material and sample preparation

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 oyster culture farm in Bandon bay, Suratthani province, south of Thailand in November 2006. The farm located 3 km from the shore with salinity and temperature of seawater approximately 11 part per thousand (ppt) and 27.8°C, respectively. After harvesting, the live oysters were placed in nylon sacks and transported to the laboratory at Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, within 4-5 h at ambient temperature (28 $\pm$ 2°C). Upon arrival, live oysters were washed with running water and then drained for 3 min. The shell-on oysters were steamed for 30 min and cooled immediately in cold water (0-2°C) for 3 min. Then, they were shucked by hands and steamed again for 10 min before further processing.

5.2.3 Packaging materials

Two-piece lacquer-tin cans of 220 g capacity (307 x 113), manufactured by Crown Food Packaging (Songkhla, Thailand), were used for the producing canned oyster. Stand-up retort pouches of 3-ply laminates consisting of 12 μm polyester (outer layer), 15 μm aluminum foil (middle layer) and 70 μm cast polypropylene (inner layer) of sizes 140 x 180 x 40 mm, manufactured by Royalcan Industry Company (Samutsakorn, Thailand), were used for retort pouched oyster.

5.2.4 Thermal processing

The steamed oyster meat (160 $\pm$ 2 g) was placed in cans and retort pouches. About 40 $\pm$ 2 g of hot (85-90°C) 1 % brine was added, the cans and retort pouches were double-seamed and heat-sealed, respectively. Then the cans and retort pouches were heat-processed in vertical-steam air retort (Tropical Canning, Inc., Thailand) and horizontal-steam water spray automated batch retort (FMC, Belgium),

respectively. To study heat penetration, an adequate number of cans and retort pouches were fixed with glands and thermocouple tips were inserted into the oyster meat to record its core temperature during heat processing using a data recorder (Ellab CMC 821, Kopenhegen, Denmark). The lowest core temperature from all inserted thermocouples at each minute of heating was chosen and considered as the cold point. The results showed in Table C1 and C2 for canned and retort pouched oyster products, respectively. The retort temperature (RT) was maintained at 121°C and air pressure was maintained at 1.14 bar and 2 bar throughout the heating and cooling period of canned and pouched products, respectively. F_0 values of the thermal processed canned and retort pouched oysters were calculated by the formula method of Ball (1923) (Appendix C2). The thermally processed cans and pouches were tested for sterility according to the method of BAM (2001) (Appendix D3).

5.2.5 Effect of thermal processing and storage on qualities of canned and retort pouched oysters

The two trials of each treatment i.e. canned (can) and retort pouched oysters (pouch) under the studied F_0, were kept at ambient temperature (28 $\pm$ 2°C) for 12 months. Five canned and five retort pouched samples were taken for chemical and sensory analysis every 3 months. The liquid was drained off the cans and retort pouches carefully for 3 min before analysis. Chemical and sensory qualities of canned and retort pouched oysters during storage were determined as follows:

(1) Chemical quality

pH, chemical composition (moisture, crude protein and crude fat), lipid composition, fatty acid composition and PV were determined as previously described in section 3.2.3 (1).

(2) Sensory quality

The sensory quality of canned and retort pouched oysters was assessed by ten trained panelists using scoring method with the scale from 1 to 7: 1, strong fail; 2, middle fail; 3, borderline fail; 4, borderline; 5, borderline pass; 6, middle pass;

7, high pass with the details shown in Table 5.1. Acceptability of oyster products during storage was scored in appearance, color, texture, odor, taste and overall acceptability. The sample of caned and retort pouched oysters were prepared by placing them in a water bath at 85°C for 10 min before opening and kept at 55 $\pm$ 2°C during serving.

Table 5.1 Guideline of sensory evaluation of thermal processed oyster.

Sensory Quality Indicators of thermal processed oyster						
Score	**Quality**	**Appearance**	**Color**	**Texture**	**Odor**	**Taste**
7	High Pass	Uniform texture Clearly Defined Shape	White Creamy	Firm Moist	Sweet Neutral Strong Characteristic Cooked Meat	Sweet
6	Middle Pass	Clearly Defined Shape	Yellowish Green	Slightly firm Slightly Moist	Neutral Moderate Characteristic Cooked Meat	Slight Sweet Neutral
5	Borderline Pass	Slight Clearly Defined Shape	Slightly Yellow or Brown Slightly Green	Slightly Soft Slight Mushy Slightly Dry	Moderate Fishy Cardboardy Oxidized Slight Sulfide	Moderate Sweet Slight Sulfide Cardboard Oxidized
4	Borderline					
3	Borderline Fail	Loss of Shape	Moderately Brown Moderately Green	Moderately Tough (or) Slight Mushy Moderately Dry	Slightly Sour Slightly Rancid Moderate-Strong Fishy Slightly Pungent Slight Sickly Sweet Slight Ammonia	Slightly Sour Slightly Bitter Moderate Strong Fishy Slightly Rancid Slight Vegetable/Sulfur
2	Middle Fail	Loss of Shape Integrity	Moderate-Strong Brown Moderate-Strong Green	Moderately Mushy Moderate-Strongly Tough Strongly Dry	Moderately Sour Moderately Rancid Moderate Pungent Moderate Sickly Sweet Moderate Ammonia	Moderately Sour Moderately Bitter Strong Fishy Moderate Ammonia Moderately Rancid Moderate-Strong Vegetable (Sulfur)
1	Strong Fail	No Shape Identity	Strongly Brown Strongly Green	Strongly Tough (or) Strong Mushy	Strongly Sour Strong Putrid Strong Pungent Strong Sickly Sweet Strong Ammonia	Strong Sour Strong Bitter Strong Ammonia Strong Rancid Strong Sulfur

Source: Modified from U.S. Food and Drug Administration (2005)

5.2.6 Effect of thermal processing and storage on volatile profile of canned and retort pouched oysters

The two trials of each treatment i.e. canned (can) and retort pouched oysters (pouch) under the studied F_0, were kept at ambient temperature (28 $\pm$ 2°C) for 12 months. Three canned and three retort pouched samples (each trial) were taken for volatile profile analysis every 3 months of storage as follows:

(1) Headspace-solid phase microextraction

The volatile compound of the canned and retort pouched oysters with medium were extracted as previously described in section 3.2.4 (1).

(2) Volatile profile analysis

The SPME fibers were desorbed in a gas chromatograph (Perkin-Elmer Autosystem XL, The Perkin-Elmer Corporation, Norwalk, CT, USA) equipped with a DBwax fused silica open tubular capillary column (0.32 mm i.d. x 60 m, 0.25 μm in film thickness; J&W Scientific, USA) and a flame ionization detector (FID). The carrier gas (helium) flow rate was 1 ml/min. The injection port and detector temperature were set at 250°C. The oven temperature involved an initial stage at 35°C for 5 min in splitless mode, followed by an increase from 35°C to 220°C at rate of 2°C/min and held at 220°C for 40 min. The data acquisition and peak analysis were undertaken using TotalChrom Chromatography Data Systems (version 6.3.1; Perkin Elmer, Inc., Norwalk, CT, USA) software.

The retention indices (RI) of each compound were calculated according to the method of Van den Dool and Kratz (1963) using a series of straight-chain alkanes (C10-C28). The volatile compounds were identified by matching their retention indices with those proposed in the literature (Chung *et al*., 2001; Chung *et al*., 2002; Kim *et al*., 2000; Le Guen *et al*., 2000; Le Guen *et al*., 2001; Tanchotikul and Hsieh, 1991), and confirmed with the authentic standards under the same experimental conditions. The relative concentration of each compound was estimated by using an internal standard (3-methyl-1-butanol) and expressed as μg/g oyster. The

concentrations of total volatile compounds were calculated by combining of the identified volatile compounds in each sample.

5.2.7 Statistical analysis

The data from two trials (three replicates from each trial) was subjected to the analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple range tests for significant differences at $P < 0.05$ (Steel and Torrie, 1980).

5.3 Results and Discussion

5.3.1 Thermal processing

The results from thermal processing showed that F_0 value of canned (307 x 113) and retort pouched (140 x 180 x 40 mm) white-scar oyster (*C. belcheri*) in 1% brine was 30.78 and 30.91 min at 121°C, respectively. Whereas, Durance and Collins (1991) reported that the F_0 value for canned (307 x 200) and retort pouched (176 x 250 mm) chum salmon (*Oncorhynchus keta*) in 1% non-iodized salt was 6.8 and 8.2 min, respectively at 121°C. The F_0 values of canned and retort pouched oyster (*C. belcheri*) were longer than those of canned and retort pouched salmon (*O. keta*), probably due to more unevenness of shape and size, and more thickness in oyster than salmon. Moreover, different structure and protein composition in whole oyster including, adductor, body trunk (myofibrillar protein i.e. paramyosin and actin), and mantle (connective tissue) were processed whereas only myofibrillar protein (especially, myosin and actin) were processed in canned and retort pouched salmon, leading to different heat distribution during thermal processing (Akahane *et al*., 1985; Paredi *et al*., 1998). It was also reported that many factors influenced F_0 value such as the microbial population in the product, density of product, heat penetration characteristic, initial temperature of product, size of container, and the types of heating and cooling media (Heldman and Hartel, 1998).

5.3.2 Effect of thermal processing and storage on qualities of canned and retort pouched oysters

(1) Changes in chemical quality

pH: pH value of white-scar oyster (*C. belcheri*) before and after thermal processing was 6.2 and 6.5, respectively. Therefore, thermal processing did not significantly affect pH of oysters. It was clearly shown that there were no significantly changes ($P \geq 0.05$) in pH of both canned and retort pouched oysters throughout the 12 months storage (Figure 5.1) and were not lower than the acceptable limit (< 5.5) (Pottinger, 1948).

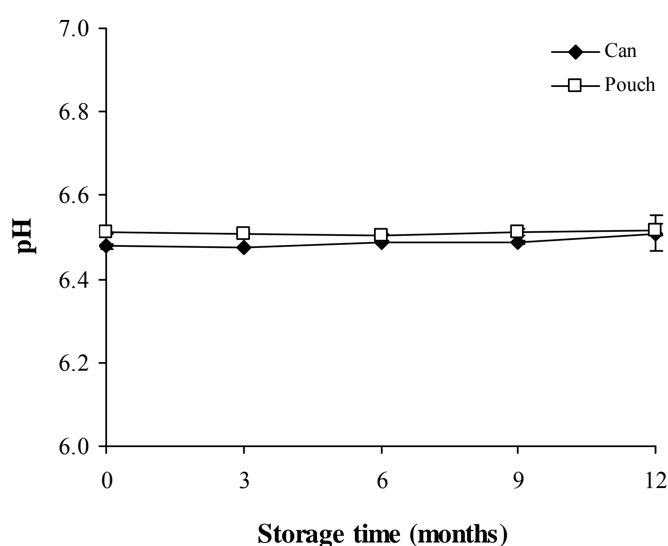

Figure 5.1 Changes in pH of canned and retort pouched oysters during ambient (28 $\pm$ 2°C) storage.

Chemical composition: Moisture, crude protein and crude fat contents of fresh white-scar oyster (*C. belcheri*) before thermal processing were 81.3, 8.4 and 2.5% of wet weight, respectively but they were changed to 72.3, 14.2 and 4.5% of wet weight, respectively after thermal processing. The decrease in the moisture content has been described as the most prominent change that makes the protein and fat

contents increase significantly in canned albacore tuna (*Thunnus alalunga*) after thermal processing (Garcia-Arias *et al*., 1994; Garcia-Arias *et al*., 2004). The results showed that the processing method did not significant affect moisture, crude protein and crude fat of both canned and retort pouched oysters which ranged from 72.25-72.33, 14.19-14.26 and 4.41-4.51% of wet weight, respectively (Table 5.2). During ambient storage, moisture slightly decreased but protein and fat tended to increased.

Table 5.2 Chemical composition of canned and retort pouched oysters during ambient (28 ± 2°C) storage.

Parameter (% of wet weight)	Storage time (months)	Can	Pouch
Moisture	0	72.25 ± 0.08[b,*]	72.33 ± 0.04[b]
	3	72.21 ± 0.02[b]	71.91 ± 0.37[ab]
	6	71.58 ± 0.20[a]	71.55 ± 0.54[a]
	9	71.56 ± 0.56[a]	71.64 ± 0.44[a]
	12	71.52 ± 0.10[a]	71.41 ± 0.07[a]
Crude protein	0	14.26 ± 0.06[a]	14.19 ± 0.06[a]
	3	14.60 ± 0.26[ab]	14.61 ± 0.13[b]
	6	14.72 ± 0.20[ab]	15.02 ± 0.11[b]
	9	14.78 ± 0.40[ab]	15.46 ± 0.32[c]
	12	14.90 ± 0.37[b]	15.66 ± 0.35[c]
Crude fat	0	4.51 ± 0.04[a]	4.41 ± 0.01[a]
	3	4.57 ± 0.01[b]	4.74 ± 0.02[b]
	6	4.63 ± 0.01[c]	5.16 ± 0.01[c]
	9	4.82 ± 0.02[d]	5.15 ± 0.01[c]
	12	4.86 ± 0.01[d]	5.51 ± 0.01[d]

*Mean ± SD from two trials. Values in the same columns for each parameter followed by different superscript letters are significantly different ($P < 0.05$).

Lipid composition: Lipids in white-scar oysters (*C. belcheri*) were classified into 5 groups i.e. triglyceride (TG), diglyceride (DG), monoglyceride (MG), free fatty acid (FFA) and phospholipids (PL). The TG, DG, MG, FFA and PL contents of fresh white-scar oyster (*C. belcheri*) were 59.4, 4.1, 0.1, 0.5 and 36 g/100 g oil, respectively (Table 3.4). After thermal processing, it was only found TG, DG and PL contents in canned (68.2, 2.2 and 29.6 g/100 g oil) and retort pouched (68.6,

2.3 and 29.0 g/100 g oil) oysters, respectively (Table 5.3). TG contents of canned and retort pouched oysters were higher than those of fresh oyster. Decrease in the DG and PL contents after thermal processing, probably due to lipid hydrolysis can occur with heating or action of enzymes (Sampels *et al*., 2004). While MG and FFA in oysters were not found after thermal processing, these contents may lose under high temperature and long time of thermal processing.

During ambient storage, TG of both canned and retort pouched oysters were not significantly different ($P \geq 0.05$). DG increased slightly with the storage time increases, but the difference was not significant in retort pouched oysters during the 12 months of storage. Small amount of FFA was found in both canned and retort pouched oysters after 12 months of storage. Although PL decreased slightly, the difference were not significant in both canned and retort pouched oysters during the 12 months of storage. The slightly decrease in TG, FFA and PL of this study were similar to the report in canned tuna in brine during 12 months of storage (Siriamornpun *et al*., 2008).

Table 5.3 Changes in lipid composition of canned and retort pouched oysters during ambient temperature (28 ± 2°C) storage.

Samples	Storage time (months)	Lipid composition (g/100 g oil)			
		TG	DG	FFA	PL
Can	0	68.23 ± 0.16[a, *]	2.19 ± 0.09[a]	0.00 ± 0.00[a]	29.58 ± 0.24[a]
	3	69.78 ± 0.59[a]	2.26 ± 0.01[a]	0.00 ± 0.00[a]	27.96 ± 0.60[a]
	6	68.96 ± 0.75[a]	2.94 ± 0.69[ab]	0.00 ± 0.00[a]	28.10 ± 0.07[a]
	9	69.91 ± 0.26[a]	2.61 ± 0.26[ab]	0.00 ± 0.00[a]	27.48 ± 0.71[a]
	12	68.60 ± 0.81[a]	3.24 ± 0.06[b]	0.25 ± 0.17[b]	27.91 ± 1.04[a]
Pouch	0	68.64 ± 0.13[a]	2.34 ± 0.19[a]	0.00 ± 0.00[a]	29.02 ± 0.32[a]
	3	68.45 ± 0.60[a]	2.46 ± 0.50[a]	0.00 ± 0.00[a]	29.09 ± 0.10[a]
	6	68.45 ± 0.06[a]	2.98 ± 0.44[a]	0.00 ± 0.00[a]	28.57 ± 0.30[a]
	9	68.45 ± 0.30[a]	2.86 ± 0.57[a]	0.00 ± 0.00[a]	28.69 ± 0.86[a]
	12	68.73 ± 0.37[a]	3.13 ± 0.00[a]	0.18 ± 0.26[a]	27.96 ± 0.70[a]

*Mean ± SD from two trials. Values in the same columns for each sample followed by different superscript letters are significantly different ($P < 0.05$).

TG, triglyceride; DG, diglyceride; FFA, free fatty acid; PL, phospholipid.

Fatty acid composition: The fatty acid composition of white-scar oyster (*C. belcheri*) was classified into 3 groups : (1) saturated fatty acid (SFA) i.e. palmitic acid (C16:0), stearic acid (C18:0), myristic acid (C14:0) and arachidic acid (C20:0), (2) monounsaturated fatty acid (MUFA) i.e. oleic acid (C18:1), palmitoleic acid (C16:1) and eicosanoic acid (C20:1), and (3) polyunsaturated fatty acid (PUFA) i.e. eicosapentaenoic acid (EPA, C20:5), docosahexaenoic acid (DHA, C22:6), arachidonic acid (C20:4), linoleic acid (C18:2) and stearidonic acid (C18:4). The predominant fatty acids in both canned and retort pouched oysters were palmitic acid, EPA, oleic acid, and DHA (Table A6). The fatty acid composition of fresh oyster included 29.7% (w/w oil) SFA, 15.7% MUFA and 33.3% PUFA (Table A1). After heating, the lipid of canned oyster consisted of 34.2% SFA, 16.9% MUFA and 30.4% PUFA while those of retort pouched oyster consisted of 33.9% SFA, 16.9% MUFA and 30.7% PUFA (Figure 5.2) which were not significant difference ($P \geq 0.05$). From the result, fatty acid composition was altered due to thermal processing, resulting in a large increase in SFA, some increase in MUFA, and a significant decrease in PUFA (Aubourg *et al*., 1990; Garcia-Arias *et al*., 1994).

During ambient storage, SFA gradually increased whereas slightly decrease in MUFA and a large decrease in PUFA in both canned and retort pouched oysters during 12-month storage. However, decrease significantly in PUFA of retort pouched oysters was greater than those of canned oysters. Decrease in PUFA levels might be caused by processing and heating, in which the protein in the membrane structure will be decomposed, PL will be released, and PUFA will be oxidized gradually during the storage because long-chain PUFA is predominantly located in PL (Sinclair and O'Dea, 1987). The high levels of PUFA (EPA and DHA) found in lipids are noteworthy and sensitive to oxidation because of their high level of unsaturation which could form carbonyl compounds during oxidation (Pennarun *et al*., 2002). After thermal processing, EPA and DHA of canned (13.5 and 10.1 g/100 g oil, respectively) and retort pouched (13.7 and 10.0 g/100 g oil, respectively) oysters were found (Table A6), and EPA slightly decreased whereas DHA rapidly decreased in both canned and retort pouched oysters during storage. Similar results were found in canned albacore tuna (*T. alalunga*) in oil (Aubourg *et al*., 1997) and canned tuna in brine (Siriamornpun *et al*., 2008).

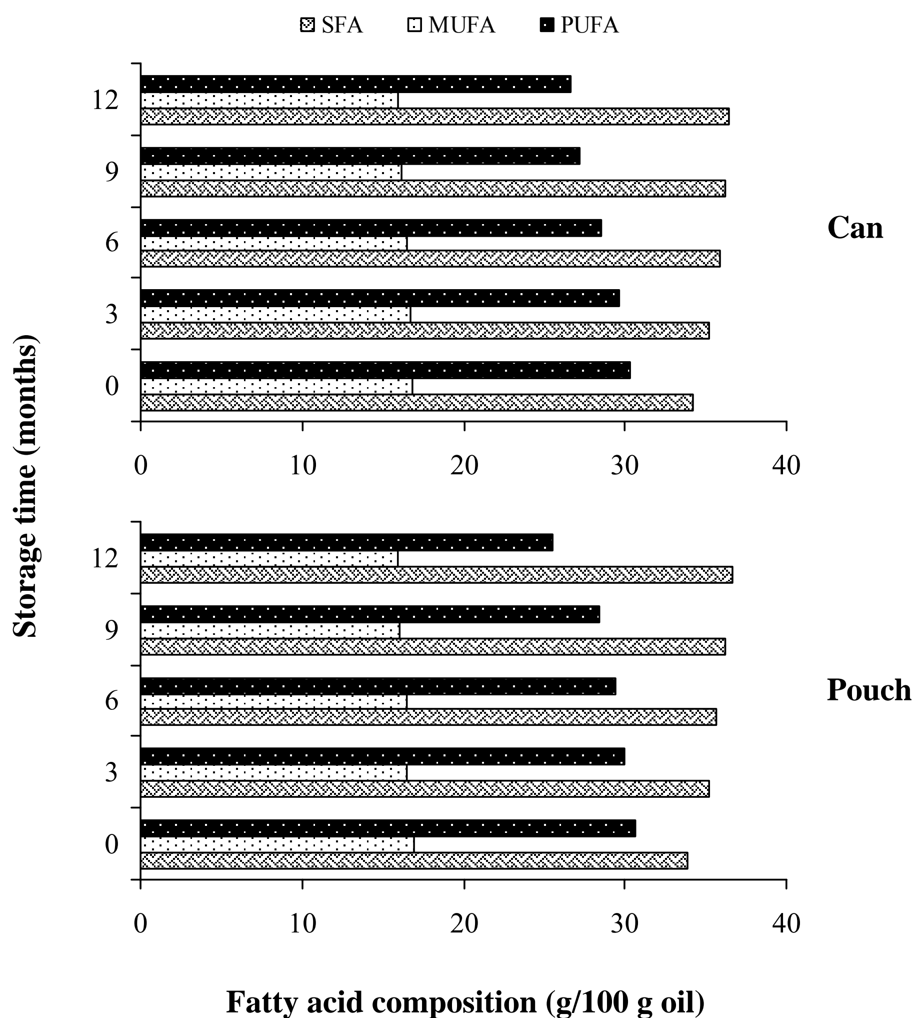

Figure 5.2 Changes in fatty acid composition of canned and retort pouched oysters during ambient (28 $\pm$ 2°C) storage.
SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

PV: The stability of lipids in canned and retort pouched oysters in brine was evaluated by peroxide value (PV). The PV of fresh white-scar oyster (*C. belcheri*) was 7.8 meq/kg lipids and changed to 13.4 and 16.1 meq/kg lipid for canned and retort pouched oyster, respectively (Figure 5.3). Increase in PV of oysters during thermal processing may reflect the oxidation of PUFA (Eboh *et al*., 2006).

During ambient storage, the PV in both canned and retort pouched oysters increased with increasing storage time ($P < 0.05$) and a large increase in PV

starting from 6 months of storage. The PV of retort pouched oysters increased at a higher rate than those of canned oysters, probably due to the presence of residual oxygen in the head space of retort pouches (Table C3) may induce lipid oxidation. It was also found that PV of retort pouched and canned oysters after storage for 9 and 12 months were higher than the acceptable limit of not above 20 meq/kg lipid (Connell, 1995).

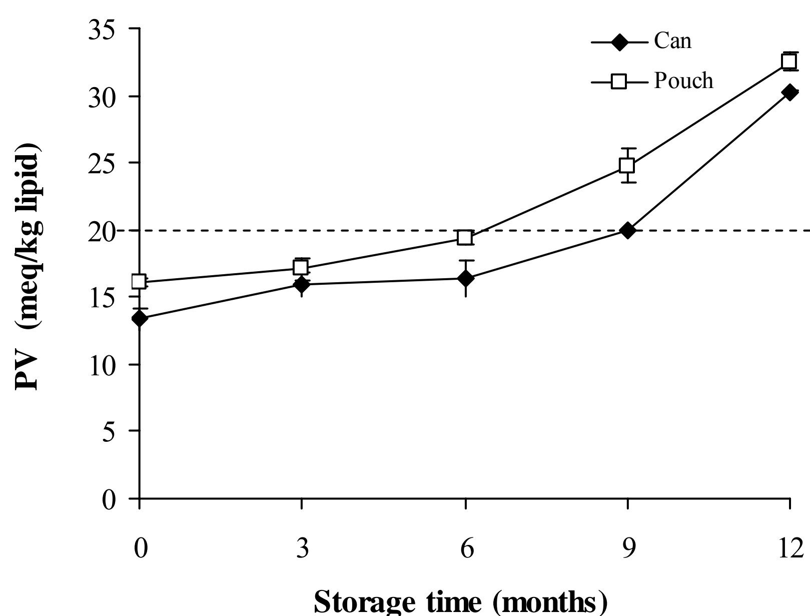

Figure 5.3 Changes in PV of canned and retort pouched oyster during ambient (28 ± 2°C) storage.

(3) Changes in sensory quality

The thermal processing resulted in obviously changing of appearance, color, texture, odor and taste in oyster. The fresh white-scar oysters (*C. belcheri*) were uniform texture, white/creamy in color, firm/elastic, melon-like/seaweed odor and lightly sweet. After thermal processing, all attributes of canned and retort pouched oysters were changed including, the plum and adductor of oyster was shrunken,

yellowish green in color, softer in texture, cooked meat/sweet odor and strong sweet in taste.

During ambient storage, the scores of sensory quality i.e. appearance, color, texture, odor, taste and overall acceptability in the canned and retort pouched oysters during ambient storage are presented in Table 5.4. It was found that all sensory attributes of canned and retort pouched oysters slowly decreased with increasing storage time ($P < 0.05$). The decrease in the appearance, color, texture, odor and overall acceptability scores of canned oysters was at a greater rate than that of retort pouched oysters whereas the decrease in taste score of canned and retort pouched oysters was not significantly different ($P \geq 0.05$), and also retained accept (scores of more than 5.0) in all attributes throughout the storage.

Table 5.4 Sensory evaluation of canned and retort pouched oysters during ambient temperature (28 ± 2°C) storage.

Treatment	Storage time (months)	Sensory scores					
		Appearance	Color	Texture	Odor	Taste	Overall acceptability
Can	0	6.40 ± 0.63^{b,*}	6.27 ± 0.46^{c}	6.40 ± 0.63^{c}	6.67 ± 0.49^{a}	6.73 ± 0.46^{b}	6.67 ± 0.49^{c}
	3	6.20 ± 0.77^{b}	6.20 ± 0.68^{c}	6.20 ± 0.94^{bc}	6.20 ± 0.56^{a}	6.67 ± 0.62^{ab}	6.20 ± 0.77^{ab}
	6	5.87 ± 0.83^{ab}	5.87 ± 0.83^{bc}	6.07 ± 0.80^{c}	6.07 ± 1.10^{a}	6.40 ± 0.51^{ab}	6.00 ± 1.00^{bc}
	9	5.87 ± 0.64^{ab}	5.60 ± 0.51^{b}	5.67 ± 0.62^{ab}	6.07 ± 0.80^{a}	6.33 ± 0.49^{ab}	6.00 ± 0.38^{ab}
	12	5.47 ± 0.83^{a}	5.07 ± 0.26^{a}	5.40 ± 0.51^{a}	6.07 ± 0.59^{a}	6.27 ± 0.46^{a}	5.60 ± 0.51^{a}
Pouch	0	6.53 ± 0.52^{c}	6.67 ± 0.49^{b}	6.73 ± 0.46^{b}	6.73 ± 0.46^{b}	6.73 ± 0.46^{b}	6.80 ± 0.41^{c}
	3	6.33 ± 0.72^{bc}	6.13 ± 0.64^{a}	6.33 ± 0.82^{ab}	6.67 ± 0.62^{ab}	6.60 ± 0.63^{ab}	6.47 ± 0.52^{bc}
	6	6.07 ± 0.59^{abc}	6.33 ± 0.72^{ab}	6.13 ± 0.83^{a}	6.20 ± 0.94^{ab}	6.47 ± 0.52^{ab}	6.13 ± 0.52^{ab}
	9	5.87 ± 0.64^{ab}	6.13 ± 0.64^{a}	6.00 ± 0.93^{a}	6.20 ± 0.86^{ab}	6.40 ± 0.63^{ab}	6.07 ± 0.59^{ab}
	12	5.80 ± 0.68^{a}	6.07 ± 0.70^{a}	5.80 ± 0.41^{a}	6.13 ± 0.74^{a}	6.20 ± 0.86^{a}	5.93 ± 0.70^{a}

*Mean ± SD from two trials.

Values in the same columns for each sample followed by different superscript letters are significantly different ($P < 0.05$).

5.3.3 Effect of thermal processing and storage on volatile profile of canned and retort pouched oysters

Changes in volatile profile

From previous study, only two alcohols i.e. 1-octen-3-ol and 2-ethyl-1-hexanol were found in fresh white-scar oyster (*C. belcheri*) (Table 3.9). These two compounds were reported to contribute distinct mushroom, heavy and plant-like odor to oysters (Josephson *et al*., 1985). After thermal processing, those two alcohols were not found but three alcohols (hexanol, 2-nonanol and (*E,Z*)-3,6-nonadien-1-ol), two aldehydes (ethanal and heptanal), one pyridine (3-methyl pyridine), one furan (2-ethyl furan) and one amine (trimethylamine) were detected in processed oysters (Table 5.5). This was probably due to many low-molecular-weight aroma-active compounds are produced via lipid oxidation, strecker degradation and maillard reaction influencing by thermal processing (Girard and Durance, 2000; Rodriguez-Bernaldo de Quiros *et al*., 2001). The straight-chain aldehydes (i.e. ethanal and heptanal) and 2-ethyl furan had herbaceous/grassy/pungent odor and burnt/sweet odor, respectively in canned sockeye (*Oncorhynchus nerka*) and pink (*Oncorhynchus gorbuscha*) salmons (Girard and Durance, 2000). While 3-methyl pyridine considered to have fishy/green odor in roasted shrimp (*Sergia lucens* Hansen) (Ishizaki *et al*., 2005), and the potent odorants trimethylamine had fishy/ammonia-like odor in cooked spiny lobster (*Panulirus argus*) (Cadwallader *et al*., 1995).

During storage, the volatile profile of canned and retort pouched white-scar oysters (*C. belcheri*) consisted of eight alcohols (1-propanol, 2-hexanol, hexanol, (*Z*)-3-hexen-1-ol, 1-heptanol, 2-nonanol, 1-octanol and (*E,Z*)-3,6-nonadien-1-ol), five aldehydes (ethanal, 2-methyl propanal, butanal, heptanal and (*Z*)-4-heptenal), two ketones (2,3-butanedione and 2,3-pentanedione), one pyridine (3-methyl pyridine), one furan (2-ethyl furan) and one amine (trimethylamine) (Table 5.5). The increases in quantitative and qualitative qualities of alcohols, aldehydes and ketones were found in processed oysters during storage probably due to lipid oxidation progressing (Mottram, 1998). Similar results have been reported that aldehydes and ketones in cooked turkey breast meat showed large increases during storage (Brunton *et al*., 2002). While, pyridine, furan and amine increased during storage, probably due to

maillard reaction and strecker degradation (Rodriguez-Bernaldo de Quiros *et al*., 2001).

Alcohols i.e. hexanol and 2-nonanol significantly decreased but (*E,Z*)-3,6-nonadien-1-ol increased ($P < 0.05$) in both canned and retort pouched oysters with increasing storage time. In addition, 1-propanol, (Z)-3-hexen-1-ol and 1-octanol were found in both canned and retort pouched oysters after 0 month of storage and significantly increased ($P < 0.05$) throughout the storage. The other alcohols i.e. 2-hexanol and 1-heptanol were found only in retort pouched oyster after 6 months of storage and significantly increased ($P < 0.05$) throughout the storage. Decrease in alcohols of canned oyster was greater than those of retort pouched oyster during storage.

Aldehydes i.e. ethanal significantly increased but heptanal decreased ($P < 0.05$) in both canned and retort pouched oysters with increasing storage time. 2-Methyl propanal and (*Z*)-4-heptenal were found in both canned and retort pouched oysters after 0 months of storage and significantly increased ($P < 0.05$) throughout the storage. Butanal was found only in retort pouched oyster after 3 months of storage but was not significant changed throughout the storage. Decrease in aldehydes of canned oyster was greater than those of retort pouched oyster during storage.

Ketone i.e. 2,3-butanedione was found in both canned and retort pouched oysters after 9 and 6 months of storage, respectively and significantly increased ($P < 0.05$) with increasing storage time. While 2,3-pentanedione was found only in retort pouched oyster after 12 months of storage. Increase in ketones of retort pouched oyster was greater than those of canned oyster during storage.

3-Methyl pyridine and 2-ethyl furan gradually increased throughout the storage ($P < 0.05$) in both canned and retort pouched oysters, and increase in pyridines and furans of canned oyster were lower than those of retort pouched oyster during storage. On the other hand, trimethylamine dramatically increased as storage time increased ($P < 0.05$) in both canned and retort pouched oysters, and increase in amines of canned oyster was greater than those of retort pouched oyster during storage.

Table 5.5 Relative concentration (μg/g oyster) of volatile profile in canned and retort pouched oysters during ambient temperature (28 ± 2°C) storage.

Can		Compounds	Storage time (months)				
			0	3	6	9	12
	Alcohols	1-Propanol	$0.00 \pm 0.00^{a,*}$	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.25 ± 0.07^{b}
		Hexanol	10.65 ± 0.04^{c}	8.58 ± 1.23^{c}	5.89 ± 1.25^{b}	1.34 ± 0.36^{a}	1.63 ± 0.32^{a}
		(*Z*)-3-Hexen-1-ol	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.30 ± 0.05^{b}	0.35 ± 0.11^{b}	0.71 ± 0.07^{c}
		2-Nonanol	2.12 ± 1.09^{b}	1.59 ± 0.59^{b}	1.22 ± 0.13^{ab}	0.51 ± 0.29^{a}	0.57 ± 0.28^{a}
		1-Octanol	0.00 ± 0.00^{a}	0.23 ± 0.26^{ab}	0.56 ± 0.08^{b}	0.65 ± 0.13^{b}	1.48 ± 0.48^{c}
		(*E,Z*)-3,6-Nonadien-1-ol	0.25 ± 0.14^{a}	0.26 ± 0.17^{a}	0.34 ± 0.07^{a}	0.37 ± 0.07^{a}	0.40 ± 0.17^{a}
	Aldehydes	Ethanal	0.11 ± 0.01^{a}	0.16 ± 0.04^{ab}	0.21 ± 0.02^{b}	0.35 ± 0.06^{c}	0.36 ± 0.05^{c}
		2-Methyl propanal	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.11 ± 0.06^{b}	0.27 ± 0.07^{c}
		Heptanal	1.11 ± 0.54^{b}	1.08 ± 0.21^{b}	0.73 ± 0.37^{b}	0.13 ± 0.11^{a}	0.00 ± 0.00^{a}
		(*Z*)-4-Heptenal	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.27 ± 0.27^{b}
	Ketones	2,3-Butanedione	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.14 ± 0.02^{b}	0.43 ± 0.12^{c}
	Pyridines	3-Methyl pyridine	0.17 ± 0.11^{a}	0.30 ± 0.08^{b}	0.43 ± 0.09^{c}	0.53 ± 0.13^{c}	0.66 ± 0.06^{d}
	Furans	2-Ethyl furan	0.40 ± 0.10^{a}	0.59 ± 0.12^{a}	0.54 ± 0.07^{a}	0.95 ± 0.23^{b}	0.99 ± 0.15^{b}
	Amines	Trimethylamine	4.76 ± 0.75^{a}	8.84 ± 0.44^{a}	13.76 ± 0.82^{a}	25.57 ± 0.81^{b}	43.54 ± 0.29^{c}

Table 5.5. *Continued*

Pouch		Compounds	Storage time (months)				
			0	3	6	9	12
	Alcohols	1-Propanol	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.20 ± 0.11^{b}
		2-Hexanol	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.49 ± 0.10^{b}	0.60 ± 0.03^{c}	0.75 ± 0.06^{d}
		Hexanol	11.87 ± 0.90^{b}	9.49 ± 2.66^{b}	7.92 ± 0.82^{b}	2.27 ± 0.69^{a}	1.00 ± 0.43^{a}
		(*Z*)-3-Hexen-1-ol	0.00 ± 0.00^{a}	0.22 ± 0.25^{b}	0.30 ± 0.07^{b}	0.41 ± 0.10^{bc}	0.50 ± 0.06^{c}
		1-Heptanol	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.50 ± 0.06^{b}	0.60 ± 0.06^{c}	0.58 ± 0.05^{c}
		2-Nonanol	1.94 ± 0.83^{c}	1.20 ± 0.33^{bc}	1.65 ± 0.64^{c}	0.76 ± 0.10^{ab}	0.31 ± 0.12^{a}
		1-Octanol	0.00 ± 0.00^{a}	0.08 ± 0.07^{ab}	0.59 ± 0.29^{bc}	0.67 ± 0.40^{c}	0.68 ± 0.46^{c}
		(*E,Z*)-3,6-Nonadien-1-ol	0.23 ± 0.03^{a}	0.34 ± 0.06^{b}	0.38 ± 0.09^{bc}	0.46 ± 0.02^{cd}	0.48 ± 0.01^{d}
	Aldehyde	Ethanal	0.18 ± 0.04^{a}	0.21 ± 0.03^{a}	0.27 ± 0.05^{a}	0.27 ± 0.02^{a}	0.37 ± 0.09^{b}
		2-Methyl propanal	0.00 ± 0.00^{a}	0.12 ± 0.01^{b}	0.12 ± 0.07^{b}	0.15 ± 0.09^{c}	0.22 ± 0.05^{d}
		Butanal	0.00 ± 0.00^{a}	0.11 ± 0.02^{b}	0.12 ± 0.13^{b}	0.13 ± 0.01^{b}	0.13 ± 0.09^{b}
		Heptanal	1.01 ± 0.27^{c}	0.86 ± 0.18^{c}	0.71 ± 0.74^{bc}	0.11 ± 0.09^{ab}	0.00 ± 0.00^{a}
		(*Z*)-4-Heptenal	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.35 ± 0.01^{b}
	Ketones	2,3-Butanedione	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.12 ± 0.25^{b}	0.22 ± 0.07^{c}	1.15 ± 0.09^{d}
		2,3-Pentanedione	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.22 ± 0.05^{b}
	Pyridines	3-Methyl pyridine	0.50 ± 0.08^{a}	0.67 ± 0.15^{b}	0.64 ± 0.07^{ab}	0.98 ± 0.03^{c}	1.08 ± 0.04^{c}
	Furans	2-Ethyl furan	0.84 ± 0.07^{a}	1.06 ± 0.05^{b}	1.32 ± 0.20^{c}	2.08 ± 0.05^{d}	2.10 ± 0.08^{d}
	Amines	Trimethylamine	2.45 ± 0.05^{a}	8.49 ± 0.82^{b}	12.00 ± 1.83^{b}	20.24 ± 0.66^{c}	30.52 ± 0.33^{d}

*Mean ± SD from two trials. Values in the same rows followed by different superscript letters are significantly different ($P < 0.05$).

5.4 Conclusion

Thermal processing did not show significant changes in pH but slightly affected in moisture, crude protein and crude fat of the products. Changes in lipid composition, fatty acid composition and lipid stability i.e. decrease in DG, MG, FFA, PL and PUFA but increase in SFA, MUFA and PV were also found after thermal processing. Changes in sensory attribute i.e. the plum and adductor of oyster was shrunken, yellowish green in color, softer in texture, cooked meat/sweet odor and strong sweet in taste were found after thermal processing. Only two alcohols i.e. 1-octen-3-ol and 2-ethyl-1-hexanol were found in fresh oyster but three alcohols (hexanol, 2-nonanol and (*E,Z*)-3,6-nonadien-1-ol), two aldehydes (ethanal and heptanal), one pyridine (3-methyl pyridine), one furan (2-ethyl furan) and one amine (trimethylamine) were detected after thermal processing. There were no significantly changes in pH, moisture, crude protein and crude fat in both canned and retort pouched oyster during storage. While changes in lipid i.e. increases in DG, FFA, SFA and PV but decreases in PL, MUFA and PUFA were found in both canned and retort pouched oysters. There were no significant difference in lipid composition of both canned and retort pouched oysters during storage whereas decrease in PUFA but increase in PV of retort pouched oysters were greater than those of canned oysters. Although, all sensory attributes of canned and retort pouched oysters slowly decreased with increasing storage time. Nevertheless, the canned and retort pouched oysters retained acceptability in all attributes throughout the storage. Alcohols and aldehydes were significantly decreased while ketones, pyridine, furan and amine increased during ambient storage of both canned and retort pouched oysters.

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CHAPTER 6

SUMMARY AND FUTURE WORKS

6.1 Summary

There were three studies for this dissertation including changes in physical, chemical, microbiological and sensory quality as well as volatile profile of fresh white-scar oysters (*C. belcheri*) under different storage conditions, after freezing with and without antioxidants and after thermal processing as well as during storage. The results could be summarized as follows:

Effect of different storage conditions i.e. temperature and keeping media of both shell-on and shucked oysters showed that shell-on oysters kept in both normal air and 2.5% brine were slower changed in chemical, microbiological and sensory qualities than those under ambient temperature. Shell-on oysters kept in 2.5% brine showed greater changes than those kept in normal air during ambient and chilled storage. Shucked oysters kept in 4% brine showed slower changes in quality than those kept in 2.5% brine and water when stored at chilled temperature. Considering from microbiological and sensory qualities, it was found that shell-on oysters kept in normal air and in 2.5% brine could be accepted at less than 3 days at ambient temperature and 9 days under chilled storage. While shucked oysters kept under chilled temperature in water, 2.5% brine and 4% brine were accepted at 9, 10 and 10 days, respectively. Though keeping shucked oyster in 4% brine showed the greatest effect on the shelf-life extension, it might affect to the acceptable taste of oyster. Only two volatiles i.e. 1-octen-3-ol and 2-ethyl-1-hexanol were initially detected but more compounds including nine alcohols, four carboxylic acids, two ketones, two aldehydes and two hydrocarbons were found during storage.

Effect of freezing and antioxidant on qualities and volatile profile of frozen oysters and during storage resulted that both freezing method and antioxidant treatment did not show significantly changes in physical, chemical and microbiological qualities of all frozen oysters. Freezing by quick method (IQF) resulted in less tissue damage than slow freezing (CPF). Although CPF showed a

large in tissue damage, it could maintain lipid and fatty acid compositions as well as lipid oxidation better than IQF oyster during 12 months of frozen storage. In addition, antioxidant treatment could prevent lipid oxidation more effectively than non-antioxidant treatment in both contact plate and individual quick frozen oysters. Volatile compounds of both CPF and IQF oysters were significantly changed during frozen storage for 12 months. Treatment with BHA of IQF oyster could prevent volatile changes more effectively than untreated samples at early storage but not at the later. In contrast, BHA treating in CPF oyster showed more effectively prevent volatile compound changes than IQF samples.

Effect of thermal processing and storage on qualities and volatile profile of canned and retort pouched oysters were shown that using the thermal processing did not show significant changes in pH while slightly changes in moisture, crude protein, crude fat and PV of the products were found. Changes in lipid composition, fatty acid composition, sensory attribute and volatile profile were also found after thermal processing and during ambient storage. There were no significantly changes in pH, moisture, crude protein and crude fat of both canned and pouched oyster during storage. Whereas lipid composition and peroxide value were significantly changed. Although, all sensory attributes of canned and pouched oysters slowly decreased with increasing storage time, they retained acceptable in all attributes throughout the ambient storage. Alcohols and aldehydes were significantly decreased while ketones, pyridines, furans and amines increased during ambient storage of both canned and pouched oysters.

6.2 Recommendation for future works

1. In the analysis of volatile compound, different extraction methods i.e. dynamic headspace and vacuum steam distillation should be further studied to be able to extract more volatile compounds than our method (headspace solid phase microextraction).

2. Highly advanced technique for volatile analysis to characterize the most potent odorants in oysters should be involved such as GC/MS for qualitative and quantitative analysis of volatile compounds, relation of individual volatile compound with sensory perception and gas chromatography/olfactometry.

3. Although high salt content (4% brine) showed the greatest effect on the shelf-life extension, it might affect the taste of oyster for fresh consumption. Therefore, further processing for any products or other seasoning products of oyster should be developed.

4. More type of antioxidants with different properties as well as method of applying/treating should be further studied in order to get more efficiency in prevention of lipid deterioration during freezing and frozen storage.

5. Sterilization is considerably general method in preserving food with shelf stable for a long time but the softer texture and darker color may occur after abused storage. Therefore, another hurdle processing and storage conditions should be investigated, such as pasteurization combines with chilled storage.

APPENDIX

APPENDIX A

FATTY ACID COMPOSITION OF OYSTERS

Table A1. Changes in fatty acid composition (g/100 g oil) of shell-on oysters during ambient temperature (30 ± 2°C) storage.

Fatty acid	Storage time (days)									
	SSA					SBA				
	0	1	2	3	4	0	1	2	3	4
C14:0	2.58 ± 0.14	2.43 ± 0.33	2.56 ± 0.16	2.63 ± 0.08	2.71 ± 0.01	2.58 ± 0.14	2.60 ± 0.08	2.62 ± 0.09	2.77 ± 0.04	2.88 ± 0.12
C16:0	20.89 ± 0.87	21.42 ± 2.37	21.36 ± 0.74	21.52 ± 1.00	21.86 ± 0.28	20.89 ± 0.87	21.41 ± 0.38	21.70 ± 0.32	21.76 ± 0.25	22.25 ± 0.31
C16:1	2.64 ± 0.03	2.57 ± 0.23	2.33 ± 0.10	2.39 ± 0.09	2.25 ± 0.03	2.64 ± 0.03	2.52 ± 0.04	2.34 ± 0.04	2.32 ± 0.02	2.12 ± 0.08
C18:0	6.24 ± 0.18	6.58 ± 0.58	6.51 ± 0.11	6.66 ± 0.55	6.75 ± 0.10	6.24 ± 0.18	6.51 ± 0.03	6.55 ± 0.04	6.68 ± 0.03	6.73 ± 0.03
C18:1	10.56 ± 0.05	10.49 ± 0.49	10.46 ± 0.01	10.47 ± 0.29	10.34 ± 0.08	10.56 ± 0.05	10.54 ± 0.04	10.35 ± 0.61	10.29 ± 0.01	10.26 ± 0.05
C18:2	2.35 ± 0.03	2.16 ± 0.14	1.75 ± 0.01	1.68 ± 0.10	1.59 ± 0.04	2.35 ± 0.03	2.16 ± 0.00	1.85 ± 0.11	1.78 ± 0.01	1.68 ± 0.01
C18:4	2.29 ± 0.01	1.83 ± 0.13	1.71 ± 0.05	1.37 ± 0.13	1.35 ± 0.03	2.29 ± 0.01	1.78 ± 0.00	1.72 ± 0.05	1.64 ± 0.00	1.44 ± 0.06
C20:1	2.47 ± 0.25	2.46 ± 0.15	2.38 ± 0.19	2.33 ± 0.00	2.30 ± 0.23	2.47 ± 0.25	2.37 ± 0.06	2.28 ± 0.08	2.20 ± 0.09	2.18 ± 0.09
C20:4	5.57 ± 0.04	5.50 ± 0.35	5.39 ± 0.03	4.96 ± 0.23	4.88 ± 0.03	5.57 ± 0.04	5.48 ± 0.04	5.40 ± 0.03	4.92 ± 0.01	4.72 ± 0.02
C20:5	11.80 ± 0.20	11.76 ± 1.13	11.68 ± 0.11	11.48 ± 1.23	11.18 ± 0.03	11.80 ± 0.20	11.72 ± 0.13	11.69 ± 0.08	11.47 ± 0.05	11.03 ± 0.15
C22:6	11.33 ± 0.42	11.28 ± 0.14	11.27 ± 0.10	11.19 ± 0.12	11.03 ± 0.03	11.33 ± 0.42	11.23 ± 0.25	11.18 ± 0.16	11.06 ± 0.11	11.03 ± 0.23
ΣSFA	29.70	30.43	30.42	30.80	31.32	29.70	30.52	30.87	31.21	31.86
ΣMUFA	15.67	15.52	15.16	15.19	14.89	15.67	15.44	14.97	14.81	14.56
ΣPUFA	33.34	32.53	31.80	30.68	30.03	33.34	32.38	31.83	30.87	29.89

Mean ± SD from two trials.

SSA and SBA= shell-on oyster stored in normal air and 2.5% brine at ambient temperature.

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

Table A2. Changes in fatty acid composition (g/100 g oil) of shell-on oysters during chilled (4 ± 2°C) storage.

Treatment	Fatty acid	Storage time (days)										
		0	1	2	3	4	5	6	7	8	9	10
SSC	C14:0	2.58 ± 0.14	2.59 ± 0.10	2.60 ± 0.04	2.61 ± 0.10	2.63 ± 0.13	2.66 ± 0.19	2.67 ± 0.06	2.68 ± 0.15	2.70 ± 0.00	2.72 ± 0.07	2.73 ± 0.10
	C16:0	20.89 ± 0.87	20.92 ± 0.33	20.95 ± 0.17	21.19 ± 0.53	21.32 ± 0.49	21.44 ± 0.81	21.50 ± 0.11	21.55 ± 0.85	21.59 ± 0.08	21.65 ± 0.40	21.74 ± 0.41
	C16:1	2.64 ± 0.03	2.62 ± 0.10	2.60 ± 0.01	2.58 ± 0.05	2.56 ± 0.07	2.54 ± 0.08	2.53 ± 0.05	2.52 ± 0.41	2.48 ± 0.02	2.46 ± 0.05	2.45 ± 0.04
	C18:0	6.24 ± 0.18	6.25 ± 0.05	6.31 ± 0.04	6.33 ± 0.06	6.35 ± 0.00	6.42 ± 0.03	6.43 ± 0.01	6.45 ± 0.14	6.55 ± 0.01	6.58 ± 0.02	6.64 ± 0.02
	C18:1	10.56 ± 0.05	10.24 ± 0.04	10.20 ± 0.06	10.17 ± 0.02	9.89 ± 0.02	9.62 ± 0.01	9.61 ± 0.04	9.58 ± 0.13	9.54 ± 0.07	9.53 ± 0.01	9.50 ± 0.00
	C18:2	2.35 ± 0.03	1.87 ± 0.01	1.81 ± 0.01	1.80 ± 0.03	1.76 ± 0.02	1.74 ± 0.02	1.73 ± 0.00	1.72 ± 0.04	1.70 ± 0.04	1.67 ± 0.00	1.64 ± 0.01
	C18:4	2.29 ± 0.01	2.25 ± 0.03	2.17 ± 0.05	2.11 ± 0.04	2.05 ± 0.06	1.97 ± 0.03	1.96 ± 0.00	1.89 ± 0.06	1.86 ± 0.01	1.83 ± 0.01	1.80 ± 0.02
	C20:1	2.47 ± 0.25	2.44 ± 0.16	2.41 ± 0.07	2.40 ± 0.63	2.37 ± 0.22	2.20 ± 0.07	2.15 ± 0.14	2.13 ± 0.07	1.98 ± 0.11	1.96 ± 0.01	1.87 ± 0.09
	C20:4	5.57 ± 0.04	4.92 ± 0.00	4.32 ± 0.01	4.18 ± 0.06	4.08 ± 0.01	4.04 ± 0.05	3.97 ± 0.02	3.88 ± 0.05	3.80 ± 0.01	3.77 ± 0.03	3.74 ± 0.02
	C20:5	11.80 ± 0.20	11.72 ± 0.01	11.71 ± 0.16	11.68 ± 0.01	11.64 ± 0.06	11.52 ± 0.27	11.49 ± 0.06	11.47 ± 0.22	11.35 ± 0.05	11.32 ± 0.08	11.28 ± 0.05
	C22:6	11.33 ± 0.42	11.30 ± 0.04	11.21 ± 0.22	11.19 ± 0.09	11.08 ± 0.17	11.05 ± 0.62	10.97 ± 0.07	10.84 ± 0.00	10.83 ± 0.03	10.48 ± 0.16	10.40 ± 0.16
	ΣSFA	29.70	29.77	29.86	30.13	30.31	30.52	30.60	30.68	30.85	30.96	31.11
	ΣMUFA	15.67	15.30	15.20	15.16	14.82	14.36	14.29	14.23	14.00	13.95	13.82
	ΣPUFA	33.34	32.06	31.22	30.97	30.61	30.32	30.11	29.80	29.54	29.07	28.86

Table A2. *Continued*

Treatment	Fatty acid	Storage time (days)										
		0	1	2	3	4	5	6	7	8	9	10
SBC	C14:0	2.58 ± 0.14	2.61 ± 0.06	2.63 ± 0.02	2.65 ± 0.00	2.70 ± 0.05	2.73 ± 0.01	2.78 ± 0.01	2.79 ± 0.02	2.81 ± 0.16	2.85 ± 0.19	2.88 ± 0.01
	C16:0	20.89 ± 0.87	20.96 ± 0.21	21.14 ± 0.16	21.3 6 ± 0.29	21.66 ± 0.33	21.96 ± 0.46	22.17 ± 0.10	22.38 ± 0.13	22.53 ± 1.73	22.88 ± 0.59	22.93 ± 0.05
	C16:1	2.64 ± 0.03	2.53 ± 0.04	2.49 ± 0.06	2.38 ± 0.06	2.33 ± 0.00	2.32 ± 0.05	2.27 ± 0.04	2.26 ± 0.04	2.11 ± 0.18	2.05 ± 0.09	2.00 ± 0.04
	C18:0	6.24 ± 0.18	6.36 ± 0.04	6.37 ± 0.02	6.40 ± 0.11	6.41 ± 0.03	6.44 ± 0.09	6.51 ± 0.09	6.54 ± 0.00	6.67 ± 0.60	6.70 ± 0.01	6.75 ± 0.00
	C18:1	10.56 ± 0.05	10.22 ± 0.04	10.14 ± 0.09	10.10 ± 0.03	10.07 ± 0.04	9.99 ± 0.01	9.86 ± 0.07	9.75 ± 0.06	9.73 ± 0.37	9.70 ± 0.04	9.65 ± 0.01
	C18:2	2.35 ± 0.03	1.72 ± 0.02	1.68 ± 0.06	1.65 ± 0.01	1.50 ± 0.00	1.44 ± 0.03	1.42 ± 0.02	1.39 ± 0.01	1.33 ± 0.14	1.32 ± 0.04	1.25 ± 0.00
	C18:4	2.29 ± 0.01	1.85 ± 0.01	1.79 ± 0.00	1.70 ± 0.01	1.69 ± 0.03	1.59 ± 0.03	1.47 ± 0.00	1.44 ± 0.01	1.39 ± 0.12	1.38 ± 0.02	1.35 ± 0.01
	C20:1	2.47 ± 0.25	2.36 ± 0.04	2.33 ± 0.06	2.33 ± 0.00	2.25 ± 0.05	1.26 ± 0.02	2.21 ± 0.13	2.18 ± 0.02	2.14 ± 0.31	2.12 ± 0.04	1.97 ± 0.00
	C20:4	5.57 ± 0.04	4.98 ± 0.07	4.22 ± 0.01	4.06 ± 0.06	4.03 ± 0.04	3.94 ± 0.02	3.93 ± 0.03	3.91 ± 0.05	3.66 ± 0.42	3.50 ± 0.04	3.48 ± 0.01
	C20:5	11.80 ± 0.20	11.73 ± 0.08	11.67 ± 0.03	11.59 ± 0.21	11.57 ± 0.05	11.53 ± 0.12	10.51 ± 0.06	11.47 ± 0.07	11.42 ± 1.02	11.36 ± 0.11	11.25 ± 0.04
	C22:6	11.33 ± 0.42	11.30 ± 0.00	11.24 ± 0.40	11.18 ± 0.31	11.15 ± 0.21	10.96 ± 0.06	10.90 ± 0.14	10.88 ± 0.07	10.87 ± 0.07	10.53 ± 0.11	10.18 ± 0.00
	ΣSFA	29.70	29.93	30.14	30.41	30.78	31.14	31.46	31.71	32.01	32.43	32.56
	ΣMUFA	15.67	15.11	14.96	14.81	14.66	13.57	14.34	14.19	13.98	13.87	13.62
	ΣPUFA	33.34	31.58	30.60	30.18	29.94	29.47	28.24	29.08	28.67	28.09	27.50

Mean ± SD from two trials.

SSC and SBC= shell-on oyster stored in normal air and 2.5% brine at chilled temperature.

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

Table A3. Changes in fatty acid composition (g/100 g oil) of shucked oysters during chilled (4 ± 2°C) storage.

Treatment	Fatty acid	Storage time (days)												
		0	1	2	3	4	5	6	7	8	9	10	11	12
SHW	C14:0	2.58 ± 0.14	2.59 ± 0.14	2.60 ± 0.02	2.62 ± 0.19	2.63 ± 0.04	2.66 ± 0.01	2.67 ± 0.06	2.68 ± 0.04	2.72 ± 0.12	2.75 ± 0.11	2.80 ± 0.05	2.84 ± 0.12	2.88 ± 0.11
	C16:0	20.89 ± 0.87	20.93 ± 0.51	21.07 ± 0.05	21.09 ± 0.82	21.18 ± 0.19	21.19 ± 0.13	21.24 ± 0.83	21.51 ± 0.08	21.53 ± 0.64	21.59 ± 0.06	21.67 ± 0.11	21.69 ± 0.19	21.73 ± 0.50
	C16:1	2.64 ± 0.03	2.63 ± 0.05	2.62 ± 0.02	2.60 ± 0.08	2.58 ± 0.02	2.55 ± 0.02	2.53 ± 0.06	2.51 ± 0.01	2.47 ± 0.09	2.46 ± 0.04	2.43 ± 0.01	2.40 ± 0.07	2.39 ± 0.06
	C18:0	6.24 ± 0.18	6.28 ± 0.07	6.30 ± 0.01	6.32 ± 0.04	6.33 ± 0.04	6.34 ± 0.04	6.35 ± 0.09	6.37 ± 0.02	6.37 ± 1.61	6.56 ± 0.04	6.62 ± 0.05	6.66 ± 0.13	6.76 ± 0.07
	C18:1	10.56 ± 0.05	10.52 ± 0.01	10.50 ± 0.12	10.45 ± 0.11	10.43 ± 0.04	10.40 ± 0.15	10.39 ± 0.72	10.34 ± 0.02	10.30 ± 0.06	10.29 ± 0.12	10.26 ± 0.03	10.22 ± 0.01	10.18 ± 0.02
	C18:2	2.35 ± 0.03	2.26 ± 0.02	2.25 ± 0.00	2.24 ± 0.00	2.21 ± 0.01	2.18 ± 0.03	2.17 ± 0.15	2.15 ± 0.01	2.14 ± 0.03	2.12 ± 0.04	2.09 ± 0.01	2.04 ± 0.04	2.02 ± 0.01
	C18:4	2.29 ± 0.01	2.24 ± 0.04	2.23 ± 0.03	2.18 ± 0.03	2.15 ± 0.00	2.14 ± 0.02	2.11 ± 0.00	2.05 ± 0.01	2.03 ± 0.04	2.00 ± 0.00	1.98 ± 0.00	1.94 ± 0.01	1.92 ± 0.02
	C20:1	2.47 ± 0.25	2.46 ± 0.05	2.45 ± 0.07	2.41 ± 0.08	2.39 ± 0.31	2.38 ± 0.16	2.33 ± 0.25	2.33 ± 0.04	2.27 ± 0.04	2.24 ± 0.12	2.21 ± 0.04	2.19 ± 0.05	2.16 ± 0.22
	C20:4	5.57 ± 0.04	5.46 ± 0.05	5.38 ± 0.00	5.37 ± 0.02	5.31 ± 0.02	5.26 ± 0.05	5.25 ± 0.04	5.14 ± 0.02	5.12 ± 0.09	5.06 ± 0.02	5.01 ± 0.02	4.96 ± 0.01	4.92 ± 0.05
	C20:5	11.80 ± 0.20	11.78 ± 0.20	11.76 ± 0.05	11.65 ± 0.08	11.61 ± 0.11	11.60 ± 0.07	11.59 ± 0.06	11.51 ± 0.01	11.49 ± 0.25	11.46 ± 0.08	11.37 ± 0.07	11.36 ± 0.02	11.24 ± 0.14
	C22:6	11.33 ± 0.42	11.29 ± 0.43	11.24 ± 0.03	11.20 ± 0.81	11.16 ± 0.08	11.09 ± 0.06	11.08 ± 0.09	10.95 ± 0.07	10.93 ± 0.18	10.89 ± 0.10	10.85 ± 0.04	10.83 ± 0.27	10.79 ± 0.30
	ΣSFA	29.70	29.81	29.97	30.02	30.14	30.19	30.26	30.56	30.61	30.90	31.09	31.19	31.37
	ΣMUFA	15.67	15.61	15.57	15.46	15.40	15.33	15.26	15.18	15.05	14.99	14.90	14.81	14.73
	ΣPUFA	33.34	33.04	32.86	32.64	32.45	32.27	32.20	31.80	31.71	31.54	31.30	31.13	30.89

Table A3. *Continued*

Treatment	Fatty acid	Storage time (days)												
		0	1	2	3	4	5	6	7	8	9	10	11	12
SHB2.5	C14:0	2.58 ± 0.14	2.61 ± 0.03	2.62 ± 0.00	2.64 ± 0.06	2.67 ± 0.04	2.72 ± 0.06	2.74 ± 0.09	2.75 ± 0.00	2.78 ± 0.08	2.85 ± 0.06	2.87 ± 0.01	2.90 ± 0.01	2.96 ± 0.11
	C16:0	20.89 ± 0.87	20.94 ± 0.06	21.03 ± 0.04	21.17 ± 0.43	21.19 ± 0.21	21.27 ± 0.30	21.30 ± 0.63	21.35 ± 0.03	21.45 ± 0.41	21.56 ± 0.28	21.64 ± 0.01	21.74 ± 0.08	21.80 ± 0.99
	C16:1	2.64 ± 0.03	2.62 ± 0.00	2.56 ± 0.02	2.51 ± 0.08	2.44 ± 0.04	2.39 ± 0.05	2.38 ± 0.12	2.36 ± 0.01	2.32 ± 0.08	2.25 ± 0.05	2.23 ± 0.01	2.17 ± 0.02	2.11 ± 0.20
	C18:0	6.24 ± 0.18	6.31 ± 0.00	6.39 ± 0.05	6.40 ± 0.08	6.43 ± 0.04	6.50 ± 0.03	6.55 ± 0.21	6.63 ± 0.02	6.65 ± 0.06	6.68 ± 0.00	6.71 ± 0.04	6.75 ± 0.02	6.79 ± 0.35
	C18:1	10.56 ± 0.05	10.50 ± 0.02	10.48 ± 0.03	10.37 ± 0.06	10.34 ± 0.07	10.30 ± 0.02	10.25 ± 0.33	10.21 ± 0.02	10.11 ± 0.10	10.08 ± 0.03	10.01 ± 0.10	9.96 ± 0.04	9.82 ± 0.26
	C18:2	2.35 ± 0.03	2.25 ± 0.01	2.24 ± 0.02	2.22 ± 0.02	2.15 ± 0.05	2.07 ± 0.03	2.04 ± 0.06	1.95 ± 0.01	1.87 ± 0.03	1.73 ± 0.14	1.68 ± 0.13	1.63 ± 0.00	1.60 ± 0.09
	C18:4	2.29 ± 0.01	2.18 ± 0.03	2.15 ± 0.07	2.09 ± 0.05	2.07 ± 0.08	2.05 ± 0.00	1.91 ± 0.01	1.87 ± 0.01	1.83 ± 0.02	1.82 ± 0.17	1.78 ± 0.17	1.74 ± 0.00	1.66 ± 0.09
	C20:1	2.47 ± 0.25	2.43 ± 0.00	2.40 ± 0.07	2.36 ± 0.02	2.34 ± 0.08	2.32 ± 0.00	2.21 ± 0.01	2.12 ± 0.04	2.05 ± 0.06	2.04 ± 0.02	1.93 ± 0.04	1.87 ± 0.03	1.68 ± 0.19
	C20:4	5.57 ± 0.04	5.50 ± 0.10	5.24 ± 0.18	5.11 ± 0.13	5.05 ± 0.08	5.04 ± 0.26	4.91 ± 0.04	4.90 ± 0.07	4.85 ± 0.24	4.81 ± 0.08	4.79 ± 0.06	4.72 ± 0.05	4.69 ± 0.91
	C20:5	11.80 ± 0.20	11.73 ± 0.05	11.65 ± 0.07	11.59 ± 0.04	11.54 ± 0.04	11.53 ± 0.03	11.48 ± 0.02	11.36 ± 0.02	11.27 ± 0.08	11.26 ± 0.02	11.19 ± 0.00	11.12 ± 0.01	11.08 ± 0.30
	C22:6	11.33 ± 0.42	11.28 ± 0.21	11.26 ± 0.29	11.15 ± 0.12	11.11 ± 0.19	11.07 ± 0.27	11.03 ± 0.11	10.94 ± 0.07	10.85 ± 0.07	10.75 ± 0.18	10.71 ± 0.05	10.69 ± 0.05	10.53 ± 0.05
	ΣSFA	29.70	29.86	30.03	30.21	30.30	30.48	30.59	30.73	30.88	31.09	31.22	31.39	31.55
	ΣMUFA	15.67	15.55	15.44	15.25	15.12	15.01	14.84	14.69	14.48	14.37	14.17	13.99	13.60
	ΣPUFA	33.34	32.94	32.54	32.15	31.92	31.77	31.37	31.03	30.66	30.37	30.16	29.90	29.55

Table A3. *Continued*

Treatment	Fatty acid	Storage time (days)												
		0	1	2	3	4	5	6	7	8	9	10	11	12
SHB4	C14:0	2.58 ± 0.14	2.59 ± 0.01	2.61 ± 0.02	2.62 ± 0.12	2.63 ± 0.06	2.64 ± 0.01	2.65 ± 0.16	2.67 ± 0.39	2.69 ± 0.06	2.70 ± 0.01	2.74 ± 0.09	2.76 ± 0.03	2.77 ± 0.13
	C16:0	20.89 ± 0.87	20.91 ± 0.04	20.93 ± 0.17	20.95 ± 0.48	20.99 ± 0.03	21.03 ± 0.04	21.06 ± 0.20	21.13 ± 1.07	21.14 ± 0.48	21.17 ± 0.11	21.25 ± 0.32	21.30 ± 0.12	21.32 ± 0.44
	C16:1	2.64 ± 0.03	2.63 ± 0.04	2.61 ± 0.01	2.57 ± 0.08	2.56 ± 0.00	2.55 ± 0.05	2.51 ± 0.11	2.49 ± 0.01	2.47 ± 0.06	2.46 ± 0.01	2.44 ± 0.01	2.42 ± 0.04	2.40 ± 0.06
	C18:0	6.24 ± 0.18	6.26 ± 0.02	6.28 ± 0.00	6.30 ± 0.01	6.31 ± 0.00	6.32 ± 0.03	6.35 ± 0.04	6.36 ± 0.21	6.40 ± 0.01	6.42 ± 0.01	6.47 ± 0.00	6.50 ± 0.00	6.54 ± 0.05
	C18:1	10.56 ± 0.05	10.54 ± 0.02	10.51 ± 0.01	10.48 ± 0.08	10.46 ± 0.01	10.45 ± 0.06	10.40 ± 0.02	10.37 ± 0.43	10.35 ± 0.04	10.32 ± 0.09	10.28 ± 0.00	10.27 ± 0.03	10.26 ± 0.11
	C18:2	2.35 ± 0.03	2.33 ± 0.01	2.26 ± 0.01	2.23 ± 0.04	2.22 ± 0.00	2.19 ± 0.01	2.16 ± 0.01	2.12 ± 0.04	2.11 ± 0.01	2.10 ± 0.01	2.06 ± 0.01	2.04 ± 0.01	2.03 ± 0.00
	C18:4	2.29 ± 0.01	2.24 ± 0.01	2.21 ± 0.01	2.19 ± 0.05	2.17 ± 0.04	2.14 ± 0.03	2.09 ± 0.05	2.05 ± 0.14	2.02 ± 0.02	1.97 ± 0.01	1.96 ± 0.02	1.95 ± 0.00	1.91 ± 0.02
	C20:1	2.47 ± 0.25	2.46 ± 0.05	2.45 ± 0.05	2.42 ± 0.06	2.40 ± 0.20	2.39 ± 0.06	2.37 ± 0.16	2.35 ± 0.12	2.33 ± 0.11	2.26 ± 0.08	2.24 ± 0.22	2.22 ± 0.06	1.98 ± 0.08
	C20:4	5.57 ± 0.04	5.49 ± 0.02	5.46 ± 0.03	5.42 ± 0.03	5.39 ± 0.02	5.37 ± 0.01	5.34 ± 0.02	5.32 ± 0.25	5.26 ± 0.00	5.17 ± 0.03	5.15 ± 0.03	5.10 ± 0.01	5.03 ± 0.05
	C20:5	11.80 ± 0.20	11.73 ± 0.02	11.66 ± 0.01	11.64 ± 0.13	11.62 ± 0.01	11.61 ± 0.09	11.58 ± 0.03	11.53 ± 0.69	11.51 ± 0.03	11.46 ± 0.12	11.43 ± 0.16	11.41 ± 0.06	11.35 ± 0.16
	C22:6	11.33 ± 0.42	11.28 ± 0.04	11.26 ± 0.15	11.24 ± 0.56	11.15 ± 0.02	11.14 ± 0.10	11.11 ± 0.09	11.09 ± 0.04	11.06 ± 0.30	10.95 ± 0.20	10.90 ± 0.13	10.86 ± 0.18	10.81 ± 0.14
	ΣSFA	29.70	29.75	29.82	29.87	29.93	29.99	30.06	30.17	30.23	30.30	30.45	30.55	30.63
	ΣMUFA	15.67	15.63	15.57	15.46	15.43	15.39	15.28	15.21	15.15	15.04	14.96	14.91	14.64
	ΣPUFA	33.34	33.07	32.85	32.70	32.55	32.45	32.29	32.12	31.97	31.65	31.50	31.35	32.12

Mean ± SD from two trials.

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

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

Table A4. Changes in fatty acid composition (g/100 g oil) of IQF oyster during storage at -20°C.

Fatty acid	Storage time (months)									
	SHI					SHIB				
	0	3	6	9	12	0	3	6	9	12
C14:0	3.21 ± 0.16	2.97 ± 0.44	2.93 ± 0.13	2.91 ± 0.13	3.06 ± 0.27	3.34 ± 0.01	3.07 ± 0.12	2.75 ± 0.10	2.84 ± 0.04	2.98 ± 0.09
C16:0	22.40 ± 0.80	22.71 ± 1.60	23.21 ± 1.86	23.45 ± 0.99	24.83 ± 0.31	22.11 ± 0.31	22.88 ± 0.43	23.01 ± 0.61	23.33 ± 0.81	23.63 ± 0.69
C16:1	4.65 ± 0.11	4.11 ± 0.09	4.34 ± 0.32	4.01 ± 0.18	4.64 ± 0.37	4.60 ± 0.03	4.65 ± 0.00	3.97 ± 0.07	4.13 ± 0.02	4.44 ± 0.10
C18:0	5.82 ± 0.01	5.68 ± 0.21	5.89 ± 0.21	5.52 ± 0.17	5.74 ± 0.43	5.94 ± 0.22	5.73 ± 0.04	5.67 ± 0.11	5.71 ± 0.10	5.44 ± 0.14
C18:1	9.85 ± 0.15	9.90 ± 0.41	10.05 ± 0.01	9.50 ± 0.18	9.64 ± 0.44	9.90 ± 0.20	10.14 ± 0.07	10.78 ± 0.20	9.28 ± 0.12	10.12 ± 0.13
C18:2	2.02 ± 0.46	1.77 ± 0.07	1.83 ± 0.03	1.70 ± 0.06	1.93 ± 0.27	1.77 ± 0.07	1.84 ± 0.03	2.10 ± 1.67	1.64 ± 0.00	1.80 ± 0.05
C18:4	1.94 ± 0.38	1.59 ± 0.08	1.69 ± 0.08	1.64 ± 0.09	1.60 ± 0.04	1.64 ± 0.09	1.70 ± 0.22	1.46 ± 0.01	1.64 ± 0.09	1.85 ± 0.06
C20:0	0.00 ± 0.00	0.40 ± 0.22	0.63 ± 0.06	0.15 ± 0.01	0.22 ± 0.02	0.00 ± 0.00	0.47 ± 0.09	0.16 ± 0.03	0.17 ± 0.01	0.19 ± 0.01
C20:1	1.91 ± 0.09	1.97 ± 0.35	2.17 ± 0.09	2.39 ± 0.10	2.39 ± 0.16	1.88 ± 0.82	1.99 ± 0.03	2.22 ± 0.07	2.25 ± 0.07	2.20 ± 0.05
C20:4	3.78 ± 0.21	3.81 ± 0.35	3.75 ± 0.17	3.54 ± 0.25	3.44 ± 0.11	3.47± 0.30	3.47 ± 0.45	3.44 ± 0.18	3.41 ± 0.04	3.54 ± 0.08
C20:5	13.68 ± 0.15	13.06 ± 1.46	13.60 ± 0.60	12.84 ± 0.40	11.97 ± 0.92	13.73 ± 0.35	13.55 ± 0.03	12.19 ± 0.73	12.27 ± 0.17	11.87 ± 0.25
C22:6	9.78 ± 0.10	9.35 ± 0.53	8.79 ± 0.04	8.79 ± 0.04	7.95 ± 1.33	9.58 ± 0.86	8.97 ± 0.39	8.68 ± 0.17	8.93 ± 0.43	8.05 ± 0.25
ΣSFA	31.43	31.77	32.66	32.03	33.86	31.39	32.15	31.59	32.05	32.24
ΣMUFA	16.41	15.98	16.55	15.90	16.66	16.38	16.78	16.97	15.66	16.76
ΣPUFA	31.20	29.58	29.66	28.51	26.90	30.19	29.53	27.87	27.89	27.11

Mean ± SD from two trials.

SHI and SHIB = IQF oyster untreated and treated with BHA, respectively.

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

Table A5. Changes in fatty acid composition (g/100 g oil) of CPF oyster during storage at -20°C.

Fatty acid	Storage time (months)									
	SHC					SHCB				
	0	3	6	9	12	0	3	6	9	12
C14:0	3.53 ± 0.32	3.28 ± 0.01	2.91 ± 0.14	2.99 ± 0.04	3.08 ± 0.02	3.44 ± 0.34	3.09 ± 0.04	2.84 ± 0.05	2.91 ± 0.08	3.17 ± 0.02
C16:0	22.00 ± 0.23	22.81 ± 0.22	22.89 ± 1.11	23.20 ± 0.67	23.77 ± 0.99	22.36 ± 1.13	22.05 ± 0.04	22.52 ± 0.15	22.76 ± 0.71	23.39 ± 0.55
C16:1	3.97 ± 0.04	4.56 ± 0.08	4.19 ± 0.53	4.37 ± 0.04	3.95 ± 0.37	4.07 ± 0.15	4.51 ± 0.42	4.33 ± 0.07	4.53 ± 0.16	4.33 ± 0.01
C18:0	5.34 ± 0.25	5.35 ± 0.36	5.51 ± 0.30	5.41 ± 0.06	5.35 ± 0.06	5.63 ± 0.06	5.62 ± 0.02	5.31 ± 0.09	5.41 ± 0.12	5.35 ± 0.01
C18:1	9.39 ± 0.05	9.77 ± 0.21	10.11 ± 0.12	9.34 ± 0.01	10.19 ± 0.01	9.16 ± 0.06	9.84 ± 0.31	9.53 ± 0.06	9.74 ± 0.26	10.13 ± 0.07
C18:2	2.63 ± 0.07	2.47 ± 0.99	1.85 ± 0.08	1.63 ± 0.05	1.85 ± 0.08	1.72 ± 0.10	1.72 ± 0.04	1.66 ± 0.09	1.75 ± 0.21	1.88 ± 0.01
C18:4	1.63 ± 0.12	1.62 ± 0.07	1.84 ± 0.01	1.48 ± 0.11	1.58 ± 0.25	1.55 ± 0.02	1.65 ± 0.01	1.48 ± 0.11	1.67 ± 0.25	1.46 ± 0.01
C20:0	0.50 ± 0.70	0.46 ± 0.05	0.38 ± 0.41	0.17 ± 0.01	0.18 ± 0.00	0.00 ± 0.00	0.64 ± 0.01	0.54 ± 0.01	0.64 ± 0.67	0.50 ± 0.01
C20:1	1.95 ± 0.07	1.88 ± 0.03	1.97 ± 0.12	2.15 ± 0.00	2.11 ± 0.04	1.98 ± 0.69	1.75 ± 0.14	2.12 ± 0.06	2.07 ± 0.08	2.00 ± 0.00
C20:4	3.68 ± 0.03	3.76 ± 0.06	3.56 ± 0.19	3.64 ± 0.01	3.60 ± 0.04	3.45 ± 0.20	3.85 ± 0.01	3.52 ± 0.07	3.79 ± 0.09	3.26 ± 0.05
C20:5	13.41 ± 0.74	13.67 ± 0.23	13.82 ± 0.63	12.75 ± 0.09	12.83 ± 0.21	13.03 ± 0.28	14.54 ± 0.11	13.19 ± 0.19	13.62 ± 0.27	12.53 ± 0.09
C22:6	9.29 ± 0.00	8.89 ± 0.33	9.02 ± 0.31	8.31 ± 0.07	8.52 ± 0.20	9.44 ± 0.00	8.03 ± 1.03	8.45 ± 0.02	8.55 ± 0.12	8.39 ± 0.49
ΣSFA	31.37	31.90	31.69	31.77	32.38	31.43	31.40	31.21	31.72	32.41
ΣMUFA	15.31	16.21	16.27	15.86	16.25	15.21	16.10	15.98	16.34	16.46
ΣPUFA	30.64	30.41	30.09	27.81	28.38	29.19	29.79	28.30	29.38	27.52

Mean ± SD from two trials.

SHC and SHCB = CPF oyster untreated and treated with BHA, respectively.

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

Table A6. Changes in fatty acid composition (g/100 g oil) of canned and retort pouched oysters during ambient temperature (28 ± 2°C) storage.

Fatty acids	Storage time (months)									
	Can					Pouch				
	0	3	6	9	12	0	3	6	9	12
C14:0	2.77 ± 0.00	2.96 ± 0.07	3.00 ± 0.02	3.06 ± 0.27	3.09 ± 0.18	2.88 ± 0.02	2.92 ± 0.14	3.00 ± 0.06	3.04 ± 0.11	3.05 ± 0.01
C16:0	22.16 ± 0.20	22.89 ± 0.44	23.24 ± 0.37	23.42 ± 1.43	23.56 ± 1.18	22.13 ± 0.12	22.93 ± 0.94	23.21 ± 0.08	23.58 ± 0.05	23.90 ± 0.70
C16:1	4.22 ± 0.20	3.95 ± 0.02	3.93 ± 0.12	3.89 ± 0.21	3.81 ± 0.18	4.01 ± 0.01	4.05 ± 0.08	3.98 ± 0.05	3.91 ± 0.01	3.89 ± 0.26
C18:0	6.45 ± 0.00	6.55 ± 0.09	6.73 ± 0.17	6.74 ± 0.26	6.80 ± 0.43	6.10 ± 0.04	6.53 ± 0.13	6.58 ± 0.06	6.62 ± 0.27	6.67 ± 0.22
C18:1	10.43 ± 0.06	10.45 ± 0.01	10.36 ± 0.34	10.22 ± 0.44	10.15 ± 0.20	10.58 ± 0.09	10.28 ± 0.07	10.27 ± 0.00	9.95 ± 0.11	9.85 ± 0.04
C18:2	1.93 ± 0.02	1.90 ± 0.00	1.89 ± 0.14	1.80 ± 0.02	1.74 ± 0.04	1.95 ± 0.07	1.84 ± 0.12	1.79 ± 0.11	1.74 ± 0.03	1.63 ± 0.10
C18:4	1.56 ± 0.11	1.62 ± 0.03	1.58 ± 0.11	1.46 ± 0.05	1.43 ± 0.02	1.73 ± 0.01	1.51 ± 0.08	1.50 ± 0.07	1.44 ± 0.02	1.39 ± 0.05
C20:0	2.82 ± 0.03	2.79 ± 0.07	2.88 ± 0.05	2.95 ± 0.17	2.98 ± 0.07	2.70 ± 0.01	2.87 ± 0.17	2.88 ± 0.09	2.93 ± 0.48	3.05 ± 0.09
C20:1	2.23 ± 0.07	2.28 ± 0.10	2.21 ± 0.04	2.07 ± 0.02	2.00 ± 0.31	2.30 ± 0.07	2.20 ± 0.10	2.19 ± 0.03	2.17 ± 0.00	2.17 ± 0.06
C20:4	3.28 ± 0.12	3.23 ± 0.02	3.20 ± 0.11	3.16 ± 0.20	3.01 ± 0.09	3.37 ± 0.15	3.26 ± 0.10	3.26 ± 0.06	3.24 ± 0.04	2.91 ± 0.13
C20:5	13.50 ± 0.32	13.30 ± 0.08	13.22 ± 0.07	13.01 ± 0.06	12.96 ± 0.33	13.66 ± 0.68	13.44 ± 0.14	13.32 ± 0.24	13.17 ± 0.49	13.02 ± 0.14
C22:6	10.09 ± 0.13	9.61 ± 0.06	8.65 ± 0.09	7.81 ± 3.26	7.53 ± 0.16	9.96 ± 0.01	9.98 ± 0.18	9.56 ± 0.13	8.76 ± 0.30	6.57 ± 2.63
ΣSFA	34.20	35.19	35.85	36.18	36.44	33.82	35.26	35.66	36.17	36.67
ΣMUFA	16.88	16.67	16.49	16.18	15.95	16.88	16.54	16.44	16.03	15.90
ΣPUFA	30.35	29.66	28.53	27.24	26.67	30.69	30.02	29.42	28.36	25.52

Mean ± SD from two trials.

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

APPENDIX B
FREEZING TIME OF OYSTER

The freezing time of contact plate frozen white-scar oyster was obtained from the study as follows: the oyster meats were packed in block (size: 14 x 18 x 6 cm), some cold water (approximately 4°C) was added to 30% of net weight, and then frozen in contact plate freezer (Samifi Babcock, Italy) at -40°C. For an adequate number of blocks, thermocouple probes were inserted into the oyster meat to record its core temperature during freezing. The temperature was recorded by a data logger (Presica 2002) every 5 min for 180 min (Table B1). The mean values of core temperature were plotted for freezing curve (Figure B1).

The results showed that to freeze white-scar oyster in block by contact plate freezer at -40°C, it should be kept for not less than 135 min.

Table B1. Freezing temperature and time of white-scar oysters (*C. belcheri*) during freezing by contact plat freezer.

Time (min)	Temperature (˚C)						
	Probe number						
	1	2	3	4	5	6	7
0	17.4	15.2	13.9	13.0	17.2	21.6	17.8
5	17.8	13.9	14.4	13.5	15.0	19.9	13.5
10	18.6	2.7	14.6	13.3	13.4	14.7	13.6
15	17.5	-4.1	13.9	12.4	10.3	7.7	13.2
20	15.9	-7.8	13.4	11.3	7.0	4.1	12.5
25	13.5	-10.7	12.6	9.7	4.7	-0.1	11.6
30	11.7	-14.5	11.6	7.9	2.5	-4.5	10.4
35	9.7	-15.7	10.2	6.0	0.9	-6.2	9.1
40	7.7	-18.1	8.7	4.3	-0.5	-9.2	7.6
45	5.4	-16.3	6.8	2.6	-1.4	-10.3	5.7
50	3.5	-19.6	5.6	1.4	-2.1	-10.2	4.2
55	2.3	-20.0	4.6	0.2	-2.5	-11.1	3.0
60	0.7	-21.6	3.5	-0.7	-4.2	-13.0	1.7
65	-0.3	-19.6	2.4	-0.8	-5.9	-13.5	0.5
70	-1.1	-17.7	1.6	-1.1	-8.2	-13.6	-0.3
75	-1.5	-16.6	0.6	-1.6	-10.1	-12.7	-1.2
80	-2.1	-15.3	-0.2	-1.6	-10.7	-13.4	-1.3
85	-3.3	-22.2	-0.6	-1.6	-12.2	-14.1	-1.6
90	-4.2	-22.6	-0.9	-2.1	-14.4	-15.4	-1.6

Table B1. *Continued*

Time (min)	Temperature (˚C)						
	Probe number						
	1	2	3	4	5	6	7
95	-4.7	-20.8	-1.2	-1.9	-16.2	-14.8	-2.1
100	-5.8	-19.1	-1.6	-2.3	-17.1	-15.3	-2.4
105	-6.3	-18.7	-1.3	-2.0	-18.2	-15.0	-2.6
110	-7.8	-17.6	-1.7	-2.6	-18.5	-14.4	-3.9
115	-8.4	-25.8	-1.4	-3.3	-20.2	-14.4	-4.4
120	-9.2	-28.0	-1.4	-4.0	-23.0	-15.5	-5.2
125	-10.7	-30.0	-1.6	-7.6	-26.2	-16.8	-6.2
130	-14.2	-35.6	-1.5	-11.8	-29.4	-18.0	-6.9
135	-21.2	-36.8	-1.7	-16.8	-32.4	-19.1	-7.9
140	-25.7	-37.2	-1.4	-21.1	-34.1	-18.6	-7.8
145	-29.9	-35.9	-2.6	-24.3	-35.5	-19.3	-10.0
150	-31.8	-35.2	-2.1	-26.4	-36.1	-19.3	-11.9
155	-33.4	-34.1	-2.4	-28.4	-36.0	-19.2	-15.3
160	-34.7	-33.0	-4.9	-29.2	-35.4	-19.3	-19.0
165	-36.7	-36.7	-9.3	-29.9	-34.9	-19.0	-23.9
170	-37.9	-37.7	-13.2	-31.6	-35.7	-21.3	-28.8
175	-38.5	-38.6	-16.4	-33.2	-36.4	-21.7	-32.1
180	-38.9	-39.0	-18.9	-34.2	-37.3	-21.6	-34.1

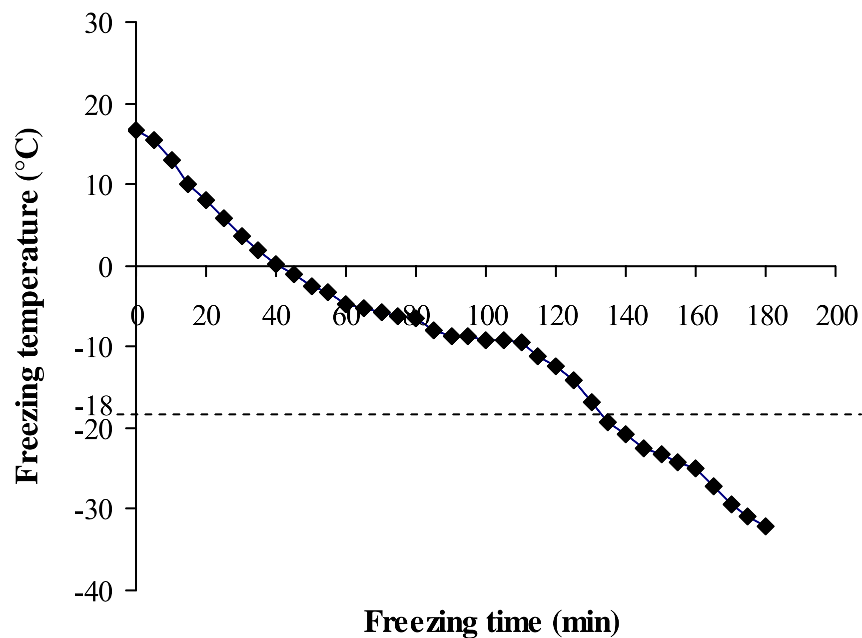

Figure B1. Freezing curve of frozen white-scar oyster (*C. belcheri*) using contact plate freezer.

APPENDIX C
HEAT PENETRATION STUDY OF OYSTER

C1. Heat penetration study of canned and retort pouched oysters

The steamed oyster meat (160 ± 2 g) was placed in cans and retort pouches. About 40 ± 2 g of hot (85-90°C) 1 % brine was added, the cans and retort pouches were double-seamed and heat-sealed, respectively. Then the cans were heat-processed in vertical-steam air retort (Tropical Canning, Inc., Thailand) where retort pouches were processed in horizontal-steam water spray automated batch retort (FMC, Belgium). To study heat penetration, an adequate number of cans and retort pouches were fixed with glands and thermocouple tips were inserted into the oyster meat to record its core temperature during heat processing using a data recorder (Ellab CMC 821, Kopenhegen, Denmark). The lowest core temperature from all inserted thermocouples at each minute of heating was chosen and considered as the cold point. The results showed in Table C1 and C2 for canned and pouched oyster products, respectively. The retort temperature (RT) was maintained at 121°C and air pressure was maintained at 1.14 bar and 2 bar throughout the heating and cooling period of canned and pouched products, respectively.

Table C1. The lowest core temperature (cold point) of canned oyster during heat penetration study.

Time (min)	Temp. (°C)	Time (min)	Temp. (°C)	Time (min)	Temp. (°C)
0	32.1	19	112.4	38	120.1
1	32.0	20	113.9	39	120.1
2	32.5	21	115.0	40	120.2
3	33.5	22	116.0	41	120.3
4	36.8	23	116.7	42	120.3
5	42.8	24	117.4	43	120.3
6	50.2	25	117.9	44	120.4
7	58.2	26	118.3	45	120.5
8	65.6	27	118.6	46	120.5
9	71.2	28	118.9	47	120.5
10	77.3	29	119.1	48	120.6
11	83.0	30	119.3	49	120.6
12	88.6	31	119.4	50	120.6
13	94.0	32	119.6	51	120.7
14	98.7	33	119.7	52	120.7
15	102.6	34	119.8	53	120.8
16	105.9	35	119.8	54	120.8
17	108.6	36	119.9	55	120.8
18	110.8	37	120.0		

Table C2. The lowest core temperature (cold point) of retort pouched oyster during heat penetration study.

Time (min)	Temp. (°C)	Time (min)	Temp. (°C)	Time (min)	Temp. (°C)
0	35.6	18	109.7	36	121.0
1	35.3	19	111.7	37	121.1
2	35.1	20	113.7	38	121.3
3	35.6	21	115.2	39	121.4
4	37.2	22	116.2	40	121.4
5	39.8	23	117.0	41	121.4
6	43.6	24	117.2	42	121.4
7	48.2	25	118.5	43	121.4
8	56.6	26	119.0	44	121.5
9	61.1	27	119.5	45	121.5
10	69.0	28	119.7	46	121.6
11	77.1	29	120.0	47	121.7
12	84.2	30	120.2	48	121.7
13	90.0	31	120.5	49	121.6
14	95.3	32	120.7	50	121.7
15	100.0	33	120.8	51	121.7
16	104.0	34	121.0		
17	107.2	35	121.0		

C2. F_0 calculation

The data from Table C1 and Table C2 were plotted in semi-logarithmic paper to get a pseudo-initial temperature (IT') value, time required for the straight line portion of the heating curve plotted on semi-log paper to pass through one log cycle (f_h), time required for the second portion of the curve to pass through the point (RT-n) and (RT-10n) where n can have any convenient value (f_2) and time from the corrected beginning of the process to the point of break in the heating curve (X_{bh}), which necessary for the calculation of F_0 values according to Ball model (1923)

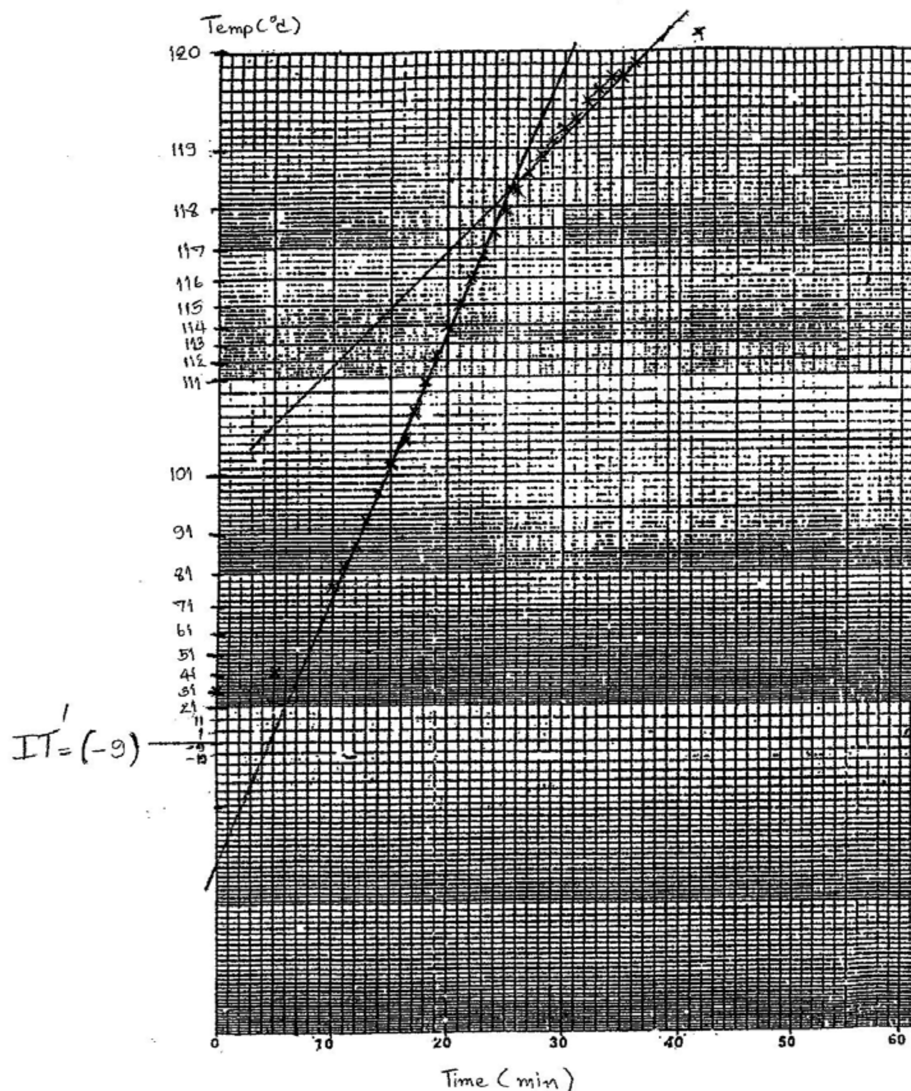

Figure C1. Plot of lowest core temperature of canned oyster during heat penetration study.

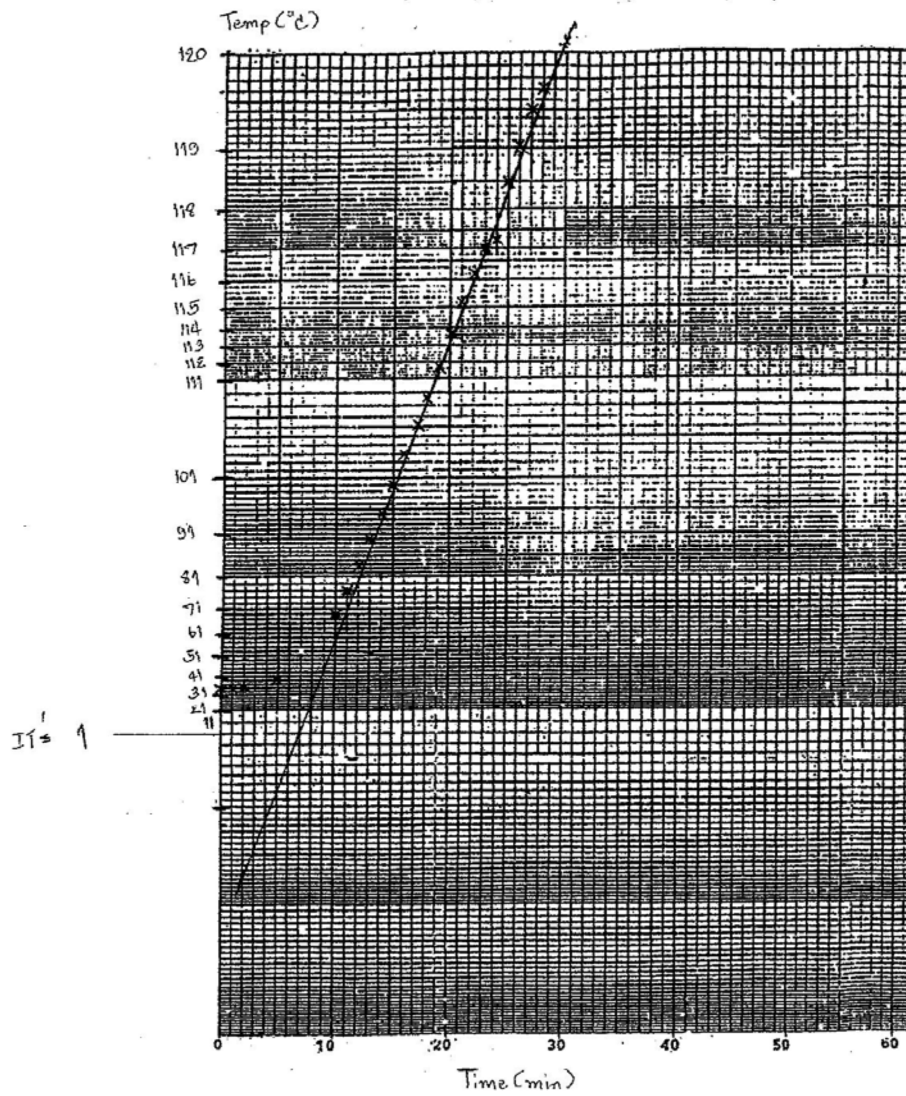

Figure C2. Plot of lowest core temperature of retort pouched oyster during heat penetration study.

C2.1 The calculation for F_0 value of canned oyster (Ball, 1923)

Come up time = 8 min

Corrected Zero Time = 8 x 0.58 = 4.64 min

Retort temperature (RT) = 121°C

Process time (B) = 55 min

Initial temperature of product (IT) = 32.1°C

Pseudo-initial temperature (IT') = -9.0°C

Time required for the straight line portion of the heating curve plotted on semi-log paper to pass through one log cycle (f_h) = 12.5

Time required for the second portion of the curve to pass through the point (RT-n) and (RT-10n) where n can have any convenient value (f_2) = 28.3

Heating lag factor (j) = (RT-IT') / (RT-IT)

= (121-(-9.0) / (121-32.1)

= 130 / 88.9

= 1.462

Factor related to RT (Fi) = 1.026

Time from the corrected beginning of the process to the point of break in the heating curve (X_{bh}) = 20.9

Temperature below retort temperature at which the heating curve exhibits a change of slope (g_{bh})

Log g_{bh} = Log (1.8 x j x (RT-IT)) - (Xbh/fh)

= Log (1.8 x 1.462 x (121-32.1)) - (20.9/12.5)

= 0.6972

(fh/U_{bh}) = 4.44

r_{bh} = 0.8062

Temperature below retort temperature on a broken heating curve at the end of the heating period (g_{h2})

Log g_{h2} = [f_h x Log(j x 1.8 (RT-IT)) + (f_2-f_h) x Log g_{bh} - B] / f_2

= [12.5 x Log(1.462 x 1.8 (121-32.1)) + (28.3-12.5) x 0.6972 - 55] / 28.3 = -0.5077

f_h/U_{h2} = 0.822

F_0	=	$f_2/(f_h/U_{h2} \times Fi) - r_{bh} \times (f_2-f_h)/(f_h/U_{bh}) \times Fi$
	=	[28/(0.822 x 1.026)] – [0.8062 x (28.3-12.5)/(4.44 x 1.026)]
	=	33.5721 – 2.7965
	=	30.78 min

C2.2 The calculation for F_0 value of retort pouched oyster (Ball, 1923)

Come up time	=	12 min
Corrected Zero Time	=	12 x 0.58 = 6.96 min
RT	=	121°C
B	=	51 min
IT	=	35.6°C
IT’	=	1.0°C
f_h	=	11.1
j	=	(RT-IT’) / (RT-IT)
	=	(121-(1.0) / (121-35.6)
	=	120 / 85.4
	=	1.405
Fi	=	1.026

Temperature of degrees below retort temperature on a simple heating curve at the end of the heating period (g)

Log g	=	Log (1.8 x j x (RT-IT)) - (B/f_h)
	=	Log (1.8 x 1.405 x (121-35.6)) - (51/11.1)
	=	-2.2601
f_h/U	=	0.35
F_0	=	f_h / ((f_h/U) x Fi)
	=	11.1 / (0.35 x 1.026)
	=	30.91 min

The results of heat penetration study of canned and retort pouched oysters from 2 replicates are shown in Table C3.

Table C3. Information on heat penetration of canned and retort pouched oysters.

Parameter	Canned Oyster	Retort pouched Oyster
Can/Pouch size	307 x 113 (2-pcs)	140 x 180 x 40 mm
Number of cans/pouches	12	12
Style of stacking	Stacked with divider plate	Stacked with divider plate
Max. filling weight (g)	160	160
Net weight (g)	200	200
Headspace (mm)	6.3	-
Vacuum (inch Hg)	5.3	-
Residual air (ml)	0	45
pH	6.4	6.4
Process Temp./Time	121°C / 55 min	121°C / 51 min
Initial Temp. (°C)	32.1	35.6
Come-up-time (min)	8	12
Heating parameter		
f_h	12.5	11.1
f_2	28.3	-
j	1.462	1.405
X_{bh}	20.9	-
Lethality (F_0) (min)	30.78	30.91

APPENDIX D
MICROBIOLOGICAL ANALYSIS

D1. Detection of *Escherichia coli* in shellfish (BAM, 2002)

- Presumptive test

Twenty five g of oyster tissue was blended with 225 ml of sterile Butterfield's phosphate-buffered water. Serial decimal dilutions of 10^{-1}, 10^{-2} and 10^{-3} were prepared with sterile Butterfield's phosphate diluent. Then, one ml portions were transferred to 10 ml of lauryl sulphate tryptose (LST) tube for 5 tubes in each dilution. The LST tubes were incubated at 35°C for 24-48 h. All presumptive positive (gas) tubes were performed confirmed test as follows:

- Confirmed test

A loopful from each LST tube (with gas-positive) was transferred into a tube of EC broth and incubated at 44.5 $\pm$ 0.2°C for 24-48 h. All confirmed positive (gas) EC tubes were performed completed test as follows:

- Completed test

A loopful from each EC tube (with gas-positive) was streaked on to L-EMB agar plate and incubated at 35°C for 18-24 h. The typical colonies of *E. coli*, dark centered and flat with or without metallic sheen, were transferred to PCA slants and incubated at 35°C for 18-24 h. To perform Gram stain, all cultures appearing as Gram-negative, short rods should be tested for the IMViC reactions below and also re-inoculated back into LST to confirm gas production.

Indole production: The stain was inoculated in tryptone broth and incubated at 35°C for 24 $\pm$ 2 h. Indole was tested by adding 0.2-0.3 ml of Kovacs' reagent. The appearance of distinct red color in upper layer is positive test.

Voges-Proskauer (VP)-reactive compounds: The stain was inoculated in MR-VP broth, incubated at 35°C for 48 $\pm$ 2 h and transferred 1 ml to 13 x 100 mm tube. The 0.6 ml alpha-naphthol solution and 0.2 ml 40% KOH were added in tube and

shaked. A few crystals of creatine was added, shaked and let stand for 2 h. Test is positive if eosin pink color develops.

Methyl red-reactive compounds: After VP test, the MR-VP tube was additionally incubated at 35°C for 48 $\pm$ 2 h. Five drops of methyl red solution were added to each tube. Distinct red color is positive test. Yellow is negative reaction.

Citrate utilization: The stain was inoculated in Koser's citrate broth and incubated at 35°C for 96 h. Development of distinct turbidity is positive reaction.

Interpret results as follows:

	Indole	MR	VP	Citrate
Typical *E. coli*	+	+	-	-
Atypical *E. coli*	-	+	-	-

Using the MPN tables (Table D1), the MPN of *E. coli* were calculated base on the proportion of EC tubes in 3 successive dilutions which were shown to contain *E. coil.*

D2. Detection of *Vibrio parahaemolyticus* in food (BAM, 2004)

Twenty five g of oyster tissue was blended with 225 ml of alkaline peptone water (APW) at pH 8.6 for 2 min. Serial decimal dilutions of 10^{-1}, 10^{-2} and 10^{-3} were prepared with APW diluent. After that, 1 ml portions were transferred to 9 ml of GSTB broth for 3 tubes in each dilution. The GSTB tubes were incubated at 35°C for 18-24 h. A loopful of suspension from the top 1 cm of a positive GSTB tube was transferred on to a TCBS plate and streak to obtain isolated colonies after incubation at 35°C for 18-24 h. The typical colonies of *V. parahaemolyticus*, large and blue-green with a dark center, were performed biochemical test as follows:

- Screening

Screen suspected isolated colony by inoculating on the following media and incubate at 35°C for 24 h.

Test	Results
TSI + 0.5% NaCl	
- Butt/slant	acid/alkaline
- Gas	-
- H_2S	-
LIM + 0.5% NaCl	
- Lysinedecaboxylase	+
- Indole	+
- Motility	+

- Biochemical test

Culture from the TSI slant was inoculated into peptone water (3% NaCl) and nutrient agar (3% NaCl), and incubated at 35°C for 24 h. The oxidase test from the nutrient agar slant and were performed biochemical test as follows:

Test	Results
Oxidase	+
Peptone water + 0% NaCl	-
Peptone water + 3% NaCl	+
Peptone water + 6% NaCl	+
Peptone water + 8% NaCl	+
Peptone water + 10% NaCl	-

Using the MPN tables (Table D2), the MPN of *V. parahaemolyticus* were calculated base on the proportion of GSTB tubes in 3 successive dilutions which were confirmed for the presence of *V. parahaemolyticus*.

Table D1. Most probable number/g of sample, using 3 tubes with each of 0.1, 0.01 and 0.01 g portions.

Positive tubes			MPN/g	Positive tubes			MPN/g
0.1	0.01	0.001		0.1	0.01	0.001	
0	0	0	<3.0	2	2	0	21
0	0	1	3	2	2	1	28
0	1	0	3	2	2	2	35
0	1	1	6.1	2	3	0	29
0	2	0	6.2	2	3	1	36
0	3	0	9.4	3	0	0	23
1	0	0	3.6	3	0	1	38
1	0	1	7.2	3	0	2	64
1	0	2	11	3	1	0	43
1	1	0	7.4	3	1	1	75
1	1	1	11	3	1	2	120
1	2	0	11	3	1	3	160
1	2	1	15	3	2	0	93
1	3	0	16	3	2	1	150
2	0	0	9.2	3	2	2	210
2	0	1	14	3	2	3	290
2	0	2	20	3	3	0	240
2	1	0	15	3	3	1	460
2	1	1	20	3	3	2	1100
2	1	2	27	3	3	3	>1100

Source: BAM. 2006. Bacteriological Analytical Manual Appendix 2: Most Probable Number from Serial Dilutions. *In* FDA Bacteriological Analytical Manual (Online). Available: http://www.cfsan.fda.gov/~ebam/bam-9.html (February 2007)

Table D2. Most probable number/g of sample, using 5 tubes with each of 0.1, 0.01 and 0.01 g portions.

Positive tubes				Positive tubes			
0.1	0.01	0.001	MPN/g	0.1	0.01	0.001	MPN/g
0	0	0	<1.8	4	0	2	21
0	0	1	1.8	4	0	3	25
0	1	0	1.8	4	1	0	17
0	1	1	3.6	4	1	1	21
0	2	0	3.7	4	1	2	26
0	2	1	5.5	4	1	3	31
0	3	0	5.6	4	2	0	22
1	0	0	2	4	2	1	26
1	0	1	4	4	2	2	32
1	0	2	6	4	2	3	38
1	1	0	4	4	3	0	27
1	1	1	6.1	4	3	1	33
1	1	2	8.1	4	3	2	39
1	2	0	6.1	4	4	0	34
1	2	1	8.2	4	4	1	40
1	3	0	8.3	4	4	2	47
1	3	1	10	4	5	0	41
1	4	0	11	4	5	1	48
2	0	0	4.5	5	0	0	23
2	0	1	6.8	5	0	1	31
2	0	2	9.1	5	0	2	43
2	1	0	6.8	5	0	3	58
2	1	1	9.2	5	1	0	33
2	1	2	12	5	1	1	46
2	2	0	9.3	5	1	2	63
2	2	1	12	5	1	3	84
2	2	2	14	5	2	0	49
2	3	0	12	5	2	1	70
2	3	1	14	5	2	2	94
2	4	0	15	5	2	3	120
3	0	0	7.8	5	2	4	150
3	0	1	11	5	3	0	79
3	0	2	13	5	3	1	110
3	1	0	11	5	3	2	140
3	1	1	14	5	3	3	180
3	1	2	17	5	3	4	210
3	2	0	14	5	4	0	130
3	2	1	17	5	4	1	170

Table D2. *Continued*

Positive tubes				Positive tubes			
0.1	0.01	0.001	MPN/g	0.1	0.01	0.001	MPN/g
3	2	2	20	5	4	2	220
3	3	0	17	5	4	3	280
3	3	1	21	5	4	4	350
3	3	2	24	5	4	5	430
3	4	0	21	5	5	0	240
3	4	1	24	5	5	1	350
3	5	0	25	5	5	2	540
4	0	0	13	5	5	3	920
4	0	1	17	5	5	4	1600
				5	5	5	>1600

Source: BAM. 2006. Bacteriological Analytical Manual Appendix 2: Most Probable Number from Serial Dilutions. *In* FDA Bacteriological Analytical Manual (Online). Available: http://www.cfsan.fda.gov/~ebam/bam-9.html (February 2007)

D3. Sterility test (BAM, 2001)

D3.1 Incubation test

Ten cans and ten retort pouches were divided into 2 groups and then incubated at 35°C for 14 days and at 55°C for 7 days. The defect or unusual appearance of incubated cans and retort pouches were observed for the following defects

Flat - a can with both ends concave; it remains in this condition even when the can is brought down sharply on its end on a solid, flat surface.

Flipper - a can that normally appears flat; when brought down sharply on its end on a flat surface, one end flips out. When pressure is applied to this end, it flips in again and the can appears flat.

Springer - a can with one end permanently bulged. When sufficient pressure is applied to this end, it will flip in, but the other end will flip out.

Soft swell - a can bulged at both ends, but not so tightly that the ends cannot be pushed in somewhat with thumb pressure.

Hard swell - a can bulged at both ends, and so tightly that no indentation can be made with thumb pressure. A hard swell will generally "buckle" before the can bursts. Bursting usually occurs at the double seam over the side seam lap, or in the middle of the side seam.

D3.2 Microbiological analysis

Three cans and three retort pouches from each incubating temperature (35°C and 55°C) were randomly sampled for mesophilic aerobic bacteria, mesophilic anaerobic bacteria, thermophilic aerobic bacteria and thermophilic anaerobic bacteria analyses (BAM,2001).

For aerobic bacteria testing, 1-2 g of sample from each container were transferred into dextose tryptone bromocresol purple broth (DTB) for 4 tubes and incubated at 35°C and 55°C for 5 days. Test is positive if yellow color developed.

For anaerobic bacteria testing, 1-2 g of sample from each container were transferred into liver broth for 4 tubes, covered with agar and incubated at 35°C and 55°C for 5 days. Test is positive if gas production occurred.

The results showed that to test for sterility of canned and retort pouched oysters, they were not detected the mesophilic aerobic bacteria, mesophilic anaerobic bacteria, thermophilic aerobic bacteria and thermophilic anaerobic bacteria.

APPENDIX E
SENSORY EVALUATION

E1. Sensory evaluation for chilled and frozen oyster

E1.1 Sample preparation for training

After arriving the Faculty of Agro-Industry laboratory, Prince of Songkla University, Hat Yai, within 4-5 h after harvesting, the fresh oysters were mechanically shucked. The shucked oysters were left at ambient temperature (30 $\pm$ 2°C) and sampled at 0, 12 and 30 h. Those oysters were used as good fresh oyster, accepted borderline and spoiled samples for training, respectively.

E1.2 Trained panelist

A group of ten panelist, three males and seven females, graduate students in the Food Technology program of age ranging from 20 to 35 years, Faculty of Agro-Industry, Prince of Songkla University were trained for sensory attributes using group discussion with the prepared samples from E1.1.

E1.3 Description for sensory attributes

The sensory description on appearance, color of plum, texture and odor qualities for fresh and frozen oysters were modified from Aaraas *et al*. (2004), Cao *et al*. (2009), He *et al*. (2002) and Qi *et al*. (2007) as shown in Table E1 with scaling from 1 to 9: 1, reject; 2, extremely poor; 3, very poor; 4, poor; 5, acceptable (borderline); 6, good; 7, very good; 8, extremely good and 9, excellent .

E1.4 Sensory evaluation

Individual samples of each treatment were placed on cup (diameter 3.0 inch), covered with aluminium foil and presented to the panelists in a random order at chilled temperature (5-7°C). Panelists were asked to score without consuming for appearance, color of plum, texture and odor of oysters according to description in Table E1.

Table E1. Guideline of sensory evaluation of fresh white-scar oyster (*C. belcheri*).

Score	Quality	Appearance	Color of plum	Texture	Odor
9	Excellent	- Mantle: outstretched 100% and strong brown/dark - Gill: filaments well defined 100% - Adductor: pale white and strong translucent	Cream white	Firm and elastic 100%	Strong melon-like
8	Extremely good	- Mantle: outstretched 80-90% and moderate brown/dark - Gill: filaments well defined 80-90% - Adductor: pale white and moderate translucent	Moderate cream white	Firm and elastic 80-90%	Moderate melon-like
7	Very good	- Mantle: outstretched 70-80% and slight brown/dark - Gill: filaments well defined 70-80% - Adductor: pale white and slight translucent	Slight cream white	Firm and elastic 70-80%	Slight melon-like/slight seaweed
6	Good	- Mantle: outstretched 60-70% and slight faded - Gill: filaments well defined 60-70% - Adductor: pale white and slight translucent	Moderate white	Firm and elastic 60-70%	Moderate seaweed
5	Acceptable (borderline)	- Mantle: outstretched 50-60% and slight faded - Gill: filaments well defined 50-60% - Adductor: pale white and slight translucent	Slight white	Firm and elastic 50-60%	Strong seaweed

Table E1. *Continued*

Score	Quality	Appearance	Color of plum	Texture	Odor
4	Poor	- Mantle: slight shrunken and contracted and slight faded - Gill: filaments slightly undefined - Adductor: light-gray and slight opaque	Slight yellow	Soft and less elastic	Spoiled with slight sour
3	Very poor	- Mantle: slight shrunken and contracted, and slight faded - Gill: filaments slightly undefined - Adductor: white and slight opaque	Moderate yellow	Slight mushy	Moderate sour
2	Extremely poor	- Mantle: moderate shrunken and contracted, and moderate faded - Gill: filaments moderately undefined - Adductor: white and moderate opaque	Tan/beige	Moderate mushy	Moderate sour and putrid
1	Reject	- Mantle: strong shrunken and contracted, and strong faded - Gill: filaments strongly undefined - Adductor: white and strong opaque	Yellow/ brown	Strong mushy	Strong sour and putrid

Source: Modified from Aaraas *et al*. (2004), Cao *et al*. (2009), He *et al*. (2002) and Qi *et al*. (2007)

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List of Publication and Proceeding

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