Studies on Methods and Standards for Evaluating Quality of Fresh Post-harvest Oyster (*Crassostrea Virginica*)

by

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Abstract

Oysters are one of the most popular seashell foods in the world due to high nutritive value and delicious and unique taste. The consumption of oysters has become an important part of the diet for consumers in the U.S.A. Oysters are usually consumed fresh, but deterioration of quality occurs over the time of storage, which may bring a risk of safety as well as costumers' rejection to the product. This study was aimed at developing a set of scientific methods to identify and evaluate the quality and safety of raw/live oyster, *C. virginica*. A method of solid phase microextraction (SPME) coupled with GC/MS was optimized to study the volatile profile characteristics, and chemical analyses including moisture, ash content, pH value, total volatile basic nitrogen (TVBN) and textural analyses were conducted to evaluate changes of quality during oyster storage.

Thirty-two volatile compounds including alkenes, aldehydes, alcohols, ketones, organic sulfides and others were identified in the oyster volatile profile. Nine out of them were responsible for the aroma of fresh oysters. Intensity and odor characteristics of some volatiles such as 2, 4-heptadienal, 2-nonenal, 2-decenal, 2-octen-1-ol, 7, 10, 13-hexdecatrienal, (E, E)-2, 4-decadienal, and indole, were closely related to the deterioration of oyster. The decomposition of polyunsaturated fatty acids, especially C18:3n-3, C20:5n-3 and C22:6n-3 was related to the presence of specific aldehydes.

Linear regression analyses of the storage period of oysters vs. various quality indicators showed that the strongest correlation was between storage period and the ash contents ($r = -0.976$) followed by the total volatile basic nitrogen (TVBN) ($r = 0.953$) and cutting force ($r = -0.893$). TVBN level of 11 mg /100 g could be used as the limit of acceptability for cold stored Eastern oyster. Parameters in texture analyses (cutting force and chewiness on adductor muscle) had strong correlation with storage period before the death of oysters. Further studies are required to confirm the suitability of ash content as a quality indicator of oysters. The textural and chemical results indicated a shelf-life of 20 days for Eastern oyster, *C. virginica* stored at 4°C.

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Chapter 1 Introduction

Oysters are one of the most popular seashell foods in the world due to high nutritive value and delicious and unique taste. Oysters are excellent source of marine protein, omega-3 fatty acids that are particularly beneficial for cardiovascular and cerebrovascular systems, and high amount of unsaturated fat and low amounts of saturated fat. The consumption of oyster has become an important part of diet for consumers in the U.S.A.

The oyster landings totaled 33.1 million pounds (15,045 tons) in the U.S.A., valued at $155.1 million in 2012--an increase of 4.6 million pounds (16 percent) and $23.5 million (18 percent) compared with 2011. The Gulf of Mexico region led in production with 20.4 million pounds (9,273 tons) of meat, almost 62 percent of the national total; followed by the Pacific Coast region with 9.4 million pounds (28 percent), principally Washington State, with more than 8.1 million pounds (3,682 tons) (National Marine Fisheries Service). With imports of 31.1 million pounds (14,136 tones) and exports of 10.3 million pounds, the total supply of oyster in the U.S.A. reached 53.8 million pounds (24,455 tons) in 2012.

Oysters are often sold and consumed fresh, which means alive or raw. Although oysters are considered relatively easy to keep and store alive in cold for several weeks, deterioration of quality occurs over the time of storage. The deterioration is not only associated with quality, it also represents a risk of safety. Therefore, a systematic study on the development of a set of scientific methods to identify and evaluate the quality and safety of raw/live oysters is needed for the oyster industry, as well as consumers.

The prime purpose of this study was to identify volatile organic compounds (VOCs) from fresh oysters and compare the change of volatile profile, as well as fatty acids content of oysters during cold storage. The secondary objective was to evaluate quality of oysters by texture profile analyses as well as chemical parameters during cold storage. Differences of quality between two different treatments of cultured oysters were determined by selected quality indicators, which helped to provide enhanced management practices in the aquaculture of oysters.

Chapter 2: Literature Review

Eastern Oyster (*C. virginica*)

The word oyster is used as a common name for bivalve mollusks that live in marine or brackish habitats and have two hard protective shells. The Eastern oyster, *C. virginica,* is a member of the phylum Mollusca, class Bivalvia, order Ostreoid, family Ostreidae. In North America, five species of oysters are commercially cultivated: Eastern (*Crassostrea Virginica*), Pacific (*Crassostrea gigas*), Kumamoto (*Crassostrea sikamea*), European Flat (*Ostrea edulis*) and Olympia (*Ostrea conchaphila*) (Jacobsen 2010).

The Eastern oyster, also known as Atlantic oyster, or Virginia oyster, *C. virginica,* is native to our entire east coast and Gulf of Mexico. Oyster farming in the United States has been around since the 1800's and makes up a large portion of U.S.A. marine aquaculture today.

The shell shape is asymmetrical with the left valve generally thicker and more deeply cupped than the right (Galtsoff 1964). The interior of the shell has a prominent purple-pigmented adductor muscle scar located close to the dorsal end of the valve. The purple pigmentation of the adductor muscle scar differentiates the eastern oyster from similar species (NMFS 2007).

The Eastern oysters prefer to grow on the depth of 0.6-5.0 m in water (MacKenzie 1996) of sheltered drowned river valleys and bar-built lagoonal estuaries. Optimum temperatures range from 20 to 30ºC (Stanley and Sellers 1986). The larvae normally grow in salinity range from 10-27.5 ppt, while adults survive for a wide range from 5-40 ppt (Calabrese and Davis 1970)

Oyster aquaculture is practiced in two forms: one involving in collection of wild spat that amplifies natural production for commercial harvest with minimal effect on the local gene pool, and another representing true “domestication” is reproductive isolating from wild populations (NMFS 2007). A number of good production methods and techniques applied by shellfish farmers are not well-documented in the scientific literature, which can lead to large variation in the quality of the product on the market.

Quality Assessment

Quality of the oyster can be related to various technical parameters such as total volatile base nitrogen (TVBN) and pH, but it is also connected to other parameters, which are linked to the consumer’s expectations and preferences (Aaraas et al. 2004). The University of Florida’s Sensory Profile for Raw Oysters (2010) claims: “Sensory attributes can make the difference in preference and market success of raw oysters”. Sensory parameters describing features such as texture, aroma, and taste therefore become important to be involved in the quantitative evaluation of oyster quality.

1. Chemical parameters

Quality and safety of oyster can be assessed by a number of measurable chemical parameters. For seafood, including oysters, one of the fundamental methods is the determination of total volatile basic nitrogen (TVBN). TVBN results from the degradation of nitrogenous compounds through microbial activities and is widely used as a quality and safety index for seafood. Ababouch et al. (1996) suggested that 20-25 mg/ 100g of TVBN could be used as the quality limit of acceptability for fresh seafood.

Due to formation of lactic acid from glycogen by a series of enzymatic reactions in the oyster tissue, pH value decreases with storage time. A drop in the pH value triggers the release of proteolytic enzymes such as cathepsin. Enzymes from spoilage microorganisms could metabolize the amino acids of the oyster tissue producing a wide variety of volatile compounds resulting in off-flavors and odors (Ali et al. 2009). Tissue pH value has been reported as a reliable indicator of death in oysters (Aaraas et al. 2004). Therefore pH value is an indicator of both death (safety issue) as well as a warning index for oyster unpleasant odors.

2. Aroma/Volatile organic compounds

Volatile organic compounds (VOCs) played a crucial role in the acceptance of shellfish, because the aroma was considered by consumers to be intimately connected to the quality and safety level of the product (Pennarun et al. 2002). Moreover, the change of volatile characteristics occurred earlier than that of other seafood flesh deterioration (Zhang et al. 2009). Research on the freshness quality of seafood, therefore, required addressing the importance of aroma. Pennarun (2002) identified 59 volatile compounds in oyster aroma extract. Among these, 25 were responsible for the overall odor of raw oyster. Four compounds identified in oysters were associated with freshness and marine odor: 3-(E)-hexen-1-ol, decanal, 2-undecanone and 3, 6-(E, Z)-nonadien-1-ol. Either enzymatic or auto-oxidative reactions of polyunsaturated fatty acids (PUFA) produce VOCs responsible for both fresh flavors and off-flavors, and it was shown that some character-impact compounds in oyster come from n-3 and n-6 polyunsaturated fatty acid oxidation (Pennarun et al. 2002). Aroma of oysters was evaluated by a sensory panel in Aaraas's sensory profile experiment (2004), and several sensory attributes linked to aroma were significantly influenced by storage conditions and time. They concluded that freshness of the oysters was correlated to the smell of sea, fresh fish, and shellfish (Aaraas 2004).

Studying seafood odor characteristics needs a systematic analytical method, which should include efficient sampling techniques, sensitive detection and a suitable data-processing method (Zhang et al. 2009). The accurate extraction of volatile compounds is a first and crucial step in determining those factors influencing freshness and acceptability. Several well-summarized reviews of sampling techniques of volatile components can be found in the VOCs research area (Fratini et al. 2012; Zhang et al. 2009). Those reviews included extraction methods frequently used to analyze VOCs in food, such as steam distillation (SD), purge and trap (P&T), simultaneous distillation extraction (SDE), dynamic headspace (DHS), vacuum distillation (VD), purge/microwave distillation and solid phase microextraction (SPME). There are some defects in methods based on distillation, such as time requirements, the need for large quantities of organic solvents and high temperature which could cause unstable volatiles to be thermally decomposed and degraded. Solid phase microextraction (SPME) therefore becomes a good choice of extraction method.

Solid-phase microextraction (SPME) is a fast, sensitive, solvent free and economical method for sample preparation in comparison to well established techniques for analyzing volatiles in food (Fratini et al. 2012). In SPME, volatiles can be absorbed from a liquid sample, by immersion or headspace extraction, or a solid sample, by headspace extraction, based on reaching the equilibrium between the concentration of volatile compounds in the headspace or solution and the concentration on a silica fiber coated with a polymer sorbent. Volatiles are desorbed from the fiber by exposing the fiber in the injection port of a GC or in the desorption chamber of an SPME/ HPLC interface (Sigma-Aldrich guideline). It was developed by Pawliszyn and co-workers (Arthur et al. 1990) and in the past several years it has been

successfully used for determining the volatile flavor profiles of different oyster species (Pennarun et al. 2002; Zhang et al. 2009).

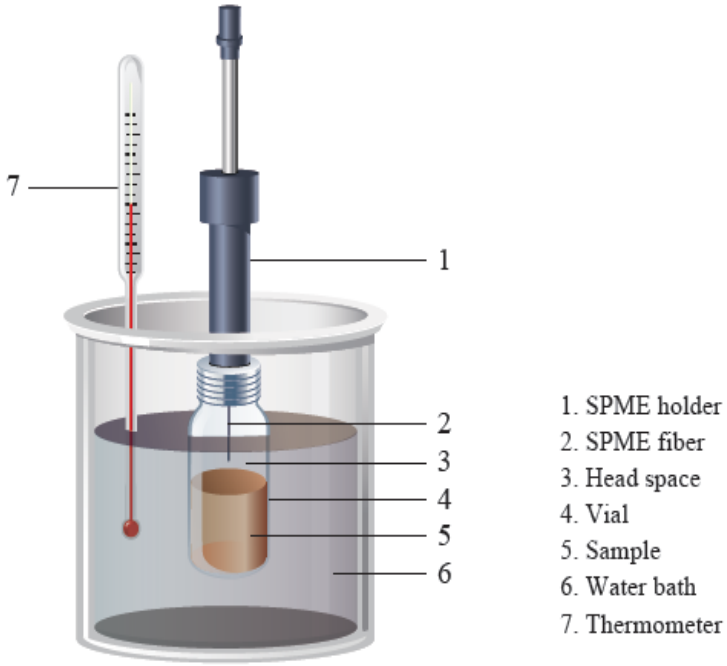

Figure 2. 1 SPME procedure

Seafood volatiles have been conventionally analyzed using gas chromatographic (GC) techniques because this modern technique is very suitable for the detection of biological VOCs and corresponding volatile profile characteristics (Zhang et al. 2009). In GC/MS, VOCs are identified by comparing their mass spectra and retention times with those of standard compounds, or by comparing their mass spectrum with those in the mass spectrum library commonly found in GC/MS software. However, the VOCs identified by GC/MS include both odor-active and non-odor-active compounds. If the contribution of odor-active VOCs to the entire aroma of biological samples is of interest, gas chromatography-olfactometry (GC-O) is often used. The potential of a compound as an odorant depends on its detection threshold, and GC-O uses human assessors as a

sensitive and selective detector for odor-active compounds' quantitative and descriptive analyses (Delahunty et al. 2006). But all humans are not biologically equal in selectivity of odor threshold and GC-O cannot provide the structural information of every VOC compound, either. GC-MS still has its strengths when objective and specific volatile characteristics researches are needed.

The data of VOCs identification and quantification obtained from GC/MS are enormous, so the suitable data-processing methods should be used for interpreting the entire biological volatile profile characteristics in statistics and distilling the potential bio-information. Normalization, principle component analysis (PCA), partial least squares (PLS), common model strategy and artificial neural network (ANN) are the techniques which have been used to manage the large numbers of experimental data. According to Zhang & Li (2010), normalization, PCA, and common model strategy were considered more efficient than others, owing to their ability for quantifying biological VOC components, interpreting statistical biological VOC characteristics and distilling potential bio-markers from statistical biological VOC characteristics, respectively.

3. Texture

Patrick McMurray described the three essential elements important to the taste of an oyster: texture, salinity, and pure taste (McMurray 2007). Rowan Jacobsen, an oyster connoisseur, also highlighted the sense of the body of quality oysters as being both firm and slippery at the same time (Jacobson 2010). Texture loss during the storage of fish products has been reported in the literature, but the nature and extent of texture change in oyster after harvest

or post-harvest treatment is still unclear. Not much literature is available analyzing the texture of oyster using well-established instrumental methods.

Cruz-Romero (2008) has reported that there was no apparent softening of the texture of oysters when measuring the shear strength on the ventral part of the oysters. As to the adductor part of the oyster, texture attributes were barely investigated by researchers. But researchers have been interested in the textural properties of scallops, which can be considering as an equivalent part to the adductor muscle of oyster in bivalves. In Ocano-Higuera's study (2005), no significant differences were obtained for texture measurement, shear force and puncture, in the adductor muscle of catarina scallops during a 15-day storage period.

The most commonly used instrumental technique for texture measurement is probably texture profile analysis (TPA), which mimics the conditions the material is subjected to during the mastication process. Moreover, TPA is a non-destructive process for food samples. TPA has been successfully applied on some other shellfishes, such as abalones (Sanchez‐Brambila et al. 2002). Sanchez‐Brambila evaluated the effect of tenderization treatment on adductor and opercular muscle of abalones by both sensory and instrumental methods. They concluded that sensory and instrumental hardness and chewiness were highly correlated and provided the best description of shellfish texture. A review of the literature, however, indicates TPA has never been used to assess textural characteristic of oysters, neither as a means of assessing oyster quality.

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Chapter 3 Identification of Volatile Compounds in Eastern Oyster (*Crassostrea Virginica*) during Cold Storage

Abstract

The study of volatile compounds of the oyster *C. virginica* is of economic interest because it is related to the freshness of oysters. Solid phase microextraction (SPME) method was optimized to study the volatile profile characteristics during oyster storage, followed by GC/MS detection. Thirty-two volatile compounds including alkenes, aldehydes, alcohols, ketones, organic sulfides, and others were identified in the oyster extract. Among them, nine of the volatile compounds were responsible for the aroma of fresh oyster. Intensity and odor characteristics of some volatiles such as 2, 4-heptadienal, 2-nonenal, 2-decenal, 2-octen-1-ol, 7, 10, 13-hexdecatrienal, (E, E)-2, 4-decadienal and indole, were closely related to the deterioration of oysters. The decomposition of polyunsaturated fatty acids, especially C18:3n-3, C20:5n-3, and C22:6n-3 was related with the presence of specific aldehydes. The analysis of volatile compounds in oysters during storage using SPME is a rapid and precise method to assess the freshness.

Introduction

Oysters are usually sold and consumed fresh/ alive, thus freshness of oysters is the most important issue among sellers, consumers, and researchers. Although the quality of oysters can be determined by numerous sensory, biochemical and chemical, physical and microbiological methods, it still lacks reliable and applicable determination of what freshness is. Volatile

compounds contributing to the characteristic odor of oysters might be related to the freshness. A European concerted action project, “Evaluation of fish freshness” (AIR3 CT94-2283), has stressed the need to further characterize “fresh” fish odors for various species to guide the development of selective sensors for specific detection of fresh odor compounds.

Some studies have explained the chemical basis of the flavor of the Eastern oyster over storage time. Among several low molecular weight compounds found in oysters, dimethyl sulfide has been recognized as one of the most important volatiles that characterize the aroma of oysters (Ronald and Thomson, 1964). Raw Eastern oysters exhibit a mild fresh, planty, seaweed-like flavor that is contributed by 1-octen-3-one, 1-octen-3-ol, 1, 5-octadien-3-one, 1, 5-octadien-3-ol, and 2, 5-octadien-1-ol (Josephson et al. 1985).

Fifty-nine volatile compounds have been identified in Pacific oyster (*Crassostrea gigas*) aroma extract, among which four compounds were associated with freshness and marine odor: 3-(E)-hexen-1-ol, decanal, 2-undecanone and 3, 6-(E, Z)-nonadien-1-ol. Either enzymatic or auto-oxidative reactions of polyunsaturated fatty acids (PUFA) produce VOCs responsible for both fresh flavors and off-flavors, and it has been shown that some character-impact compounds in oyster come from n-3 and n-6 polyunsaturated fatty acid oxidation (Pennarun et al. 2002). Zhang (2009) compared the difference of volatile profile characteristics for both fresh and deteriorated Pacific oyster to distill the top ten volatiles contributing most to the freshness of oyster. However, few studied has been done to associate volatile characteristics with freshness for Eastern oysters.

In order to carry out a research on the freshness of oysters associated with volatile characteristics, the starting point is always the determination of the volatile composition of the fresh product. Solid-phase microextraction (SPME) is a fast, sensitive, solventless and

economical method for sample preparation before gas chromatography analysis in comparison to well established techniques for analyzing volatiles in food. Research has successfully identified volatile compounds of shellfish species, including oysters by this technique (Fratini et al. 2012; Zhang et al. 2009).

The present investigation aimed to develop and optimize an experimental procedure, based on head space SPME (HSSPME) coupled with GC/MS, to study the relationship between volatile profile characteristics and freshness for evaluating quality of Eastern oyster (*C. virginica*). The optimization of the extraction conditions (salt concentration of extract in the vial, and time and temperature of incubation) for extraction of volatile organic compounds was also needed for the study. Due to the well-known relationship between the PUFA and volatile compounds, the fatty acid composition of the oyster determined by gas chromatography was also presented for both fresh and less-fresh oysters. The methodology based on SPME could provide a good method to investigate the volatile compounds of oyster and to determine the changes in the volatile profile during storage. It could help to provide precise information for the change of freshness during oyster storage.

Material and Methods

Material

Oyster samples (*C. virginica*) were provided by the Auburn University Shellfish Lab, collected at the Portersville Bay research oyster farm in Mississippi Sound near Coden, AL in December 2013. All samples were shipped immediately with overnight service in coolers with ice packs after harvest to the laboratory at Auburn University, and stored in 4 °C walk-in refrigerator with about 90% relative humility. In order to exam oyster meat quality, three oysters

each were shucked and determined for VOCs and fatty acids at selected time intervals (0, 5, 10, 15, 20, and 25 days).

Statistical analysis

One-way ANOVA was employed in effect of day of storage on quality of oyster at a significance level of 95%. When necessary ($P<0.05$), a Duncan's test was applied to compare results of a given parameter at different stored time. To test relationships between indicators with oyster freshness and quality, a correlation analysis was applied. The SAS 9.2 software package was used to perform these analyses.

Extracting volatile organic compounds (VOCs) from oyster and analyzing

Each oyster was ground with a blender for 20 s before test. An aliquot of 3.0 g of the ground oyster meat was suspended with 7.0 mL of deionized water in a 20-mL headspace vial. The vial was immersed in water bath to maintain the desired extraction temperature, and the mixture was stirred with a magnetic stir bar through the extraction. An SPME fiber was inserted into the vial and exposed to the headspace of the sample. To optimize the extraction conditions, extraction temperatures (60, 70, and 80 °C), extraction durations (50, 60, and 70 min) and NaCl concentrations (0, 10%, and 15%) were tested. After the extraction, the fiber was immediately desorbed in the injector of a gas chromatograph for 5 min. All of the analyses were performed three times for oysters.

A gas chromatography-mass spectrometry (GC-MS) system (Agilent 7890A gas chromatograph coupled with Agilent 5975C mass spectrometer, Agilent Technologies Inc., USA) equipped with a DB-5MS column (30 m × 0.25 mm, 0.25 μm film thickness) was employed to separate and identify the volatile compounds extracted from oyster using the SPME method.

Compounds were identified by comparing their mass spectrum with those in the mass spectra library (Wiley 6). Compounds were quantified with peak area of characteristic ions.

Fatty acids

Before the determination of fatty acids by GC, the fatty acid salts, as well as the acyl components in all lipid classes, such as triacylglycerols, phospholipids, sphingolipids, and waxes, need to be converted to fatty acid methyl esters (FAMEs). O'fallon et al. (2007) reported a simple, direct, 1-step esterification procedure. According to the assay, the oyster adductor muscle and body part was separately cut into 1.5-mm rectangular strips with a razor blade or scalpel. A 1.0 g of wet oyster samples were placed into a 16 × 125 mm screw-cap Pyrex culture tube to which 1.0 mL of the C19:0 internal standard (0.5 mg of C19:0/mL in MeOH), 0.7 mL of 10 N KOH in water, and 5.3 mL of MeOH were added. The tube was incubated in a 65°C water bath for 1.5 h with vigorous hand-shaking for 5 s every 20 min to properly permeate, dissolve, and hydrolyze the sample. After cooling below room temperature in a cold tap water bath, 0.58 mL of 24 N H_2SO_4 solution was added. The mixture was mixed by inversing the tube for several times. Then, the mixture was incubated again in a 55°C water bath for 1.5 h with precipitated K_2SO_4 present. In order to have a good result, a vortex shake for 5 s every 20 min was conducted during the incubation. After FAME synthesis, the tube was cooled in a cold tap water bath. Three milliliters of hexane was added, and the tube was vortex-mixed for 5 min on a multitube vortex. The tube was centrifuged for 5 min in a tabletop centrifuge at 10,000 g, and the hexane layer, containing the FAME, was removed and placed into a GC vial. The vial was capped and placed at −20°C until GC analysis.

The fatty acid composition of the FAME was determined by capillary GC on a polyalkylene glycol (PAG) 30 m x 0.25 mm with thickness of 0.25 μm capillary column

(Supelco) installed on an Agilent Technologies 7890A gas chromatograph equipped with a flame ionization detector. Fatty acids were identified and quantified by comparing their retention times with those of a standard solution of SupelcoTM 37 component FAMEs mix.

Results and Discussion

Optimization of headspace solid-phase microextraction (SPME) conditions

The type of fiber used in this study was an 85µm Polydimethylsiloxane/ Carboxen (PDMS/CAR)-coated fiber. Kataoka, Lord, and Pawliszyn (2000) found that CAR-PDMS coating is more efficient for extracting oxidation products, sulfur-aroma compounds and, more generally, low molecular mass VOCs with mid-low polarity. CAR-PDMS coating has been successfully used to identify volatile profile of many Eastern shellfish species (Fratini et al. 2012), and detect quality indicators in fish species such as whiting (Merlangius merlangus) (Duflos,Moine, Coin,& Malle, 2005) and King salmon (Oncorhynchus tshawytscha) (Wierda, Fletcher, Xu, & Dufour, 2006).

In order to obtain a good extraction efficiency and high analytical sensitivity, the HSSPME conditions impacting oyster volatiles, such as extraction temperature, extraction time and NaCL concentration in the sample solution were optimized. The amounts of major identified oyster volatiles were used as the indicators to decide the final optimum conditions.

The oyster volatiles from HSSPME were very sensitive to extraction temperature. Temperature is one of the factors most affecting the vapor pressure and equilibrium between volatile compounds, which in turn greatly influence the method's sensitivity. Extraction temperature has an important positive effect for most of the aldehydes and ketones. Among those aldehydes and ketones, the main effect of temperature was found to be significant for hexanal, heptanal, octanal, nonanal, decanal, and 2-undecanone.

Although larger numbers and greater amounts of volatiles were identified from temperature of 90℃, some of the volatiles probably resulted from thermal oxidation of fatty acids and thermal degradation of oyster volatile compounds. The results demonstrated in Fig3.1 suggested that the response of the target volatile from 80℃ is close to that from 90℃, thus the temperature herein was set at 80℃as a good compromise between sensitivity gain and thermal modification caused by heating of volatile compounds in the extraction.

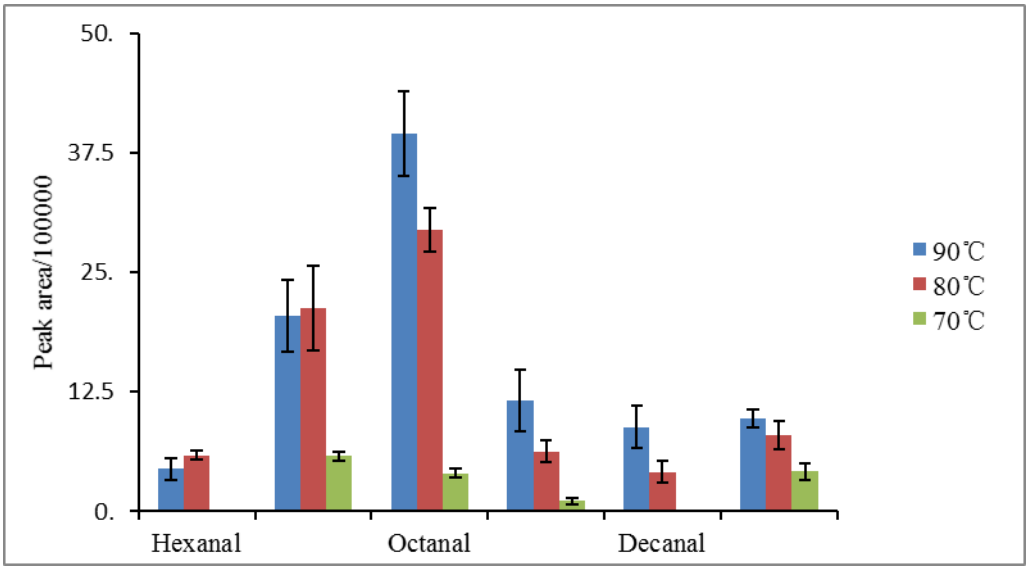

Figure 3.1 Optimization of the HSSPME temperature

It has been reported that sampling time was an important factor for alcohols. This effect was consistent with observations made in this study. The effect of time was positive for 2-octen-1-ol from 40 min to 50min. Sampling time was also an important factor for aldehydes such as hexanal, heptanal, octanal, benzaldehyde, nonanal, and 2,4-heptadienal in this study. However, the results demonstrated in Fig 3.2 suggested that there was no significant difference of detected volatiles from extraction time of 50 min and 60 min. In consideration of experiment efficiency, the shorter time of 50 min was selected as the optimum extraction time.

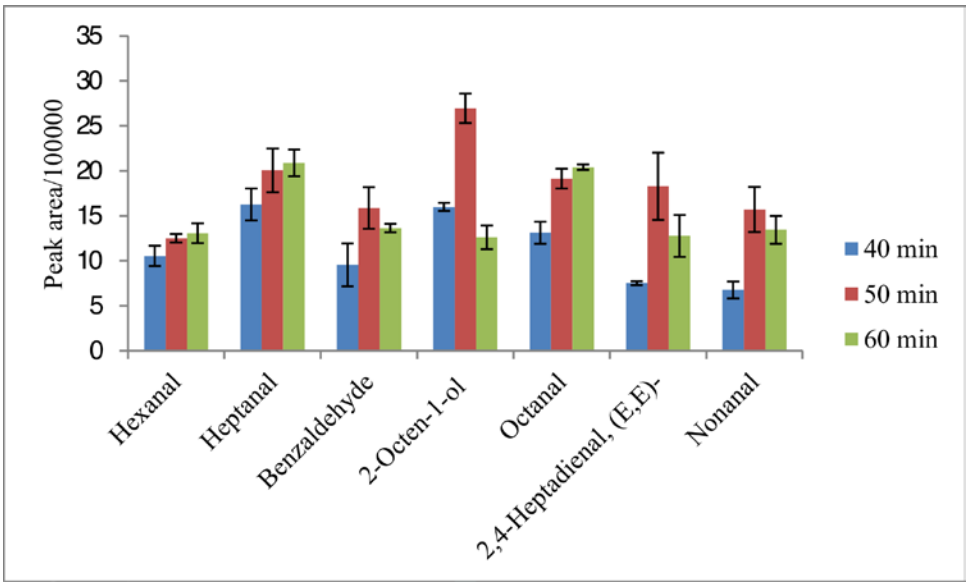

Figure 3.2 Optimization of the HSSPME time

From the result of Fig 3.3, the addition of salt reduced the extraction efficiency of some volatiles (heptanal, decanal, and octanal) and had no significant impact on the other volatiles. However, the ion strength of the sample solution was reported to improve the HSSPME efficiency, because it should reduce the affinity of organic compounds in water layer, which would facilitate the volatility of oyster volatiles. Since the oyster sample was supposed to have a degree of salinity, in this study, no salt was added to the sample solution.

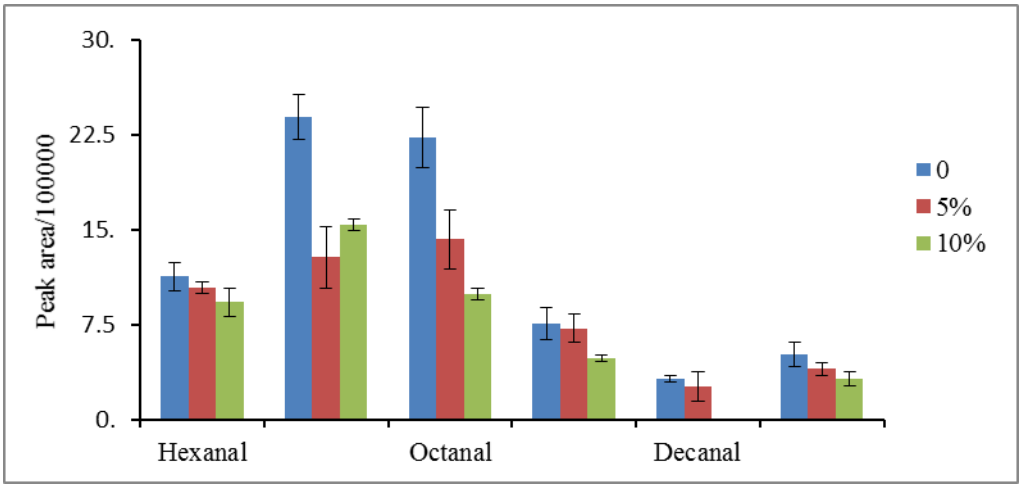

Figure 3.3 Optimization of the HSSPME NaCl concentration

In summary, we used PDMS/CAR coated fiber (85 µm) to extract volatile compounds at 80°C for 50 min without addition of salt.

Identification of VOCs by GC/MS

The optimized SPME method was then applied to identify the volatile profile of Eastern oyster. A capillary gas chromatographic separation of volatile compounds from Eastern oysters is shown in Fig 3.4.

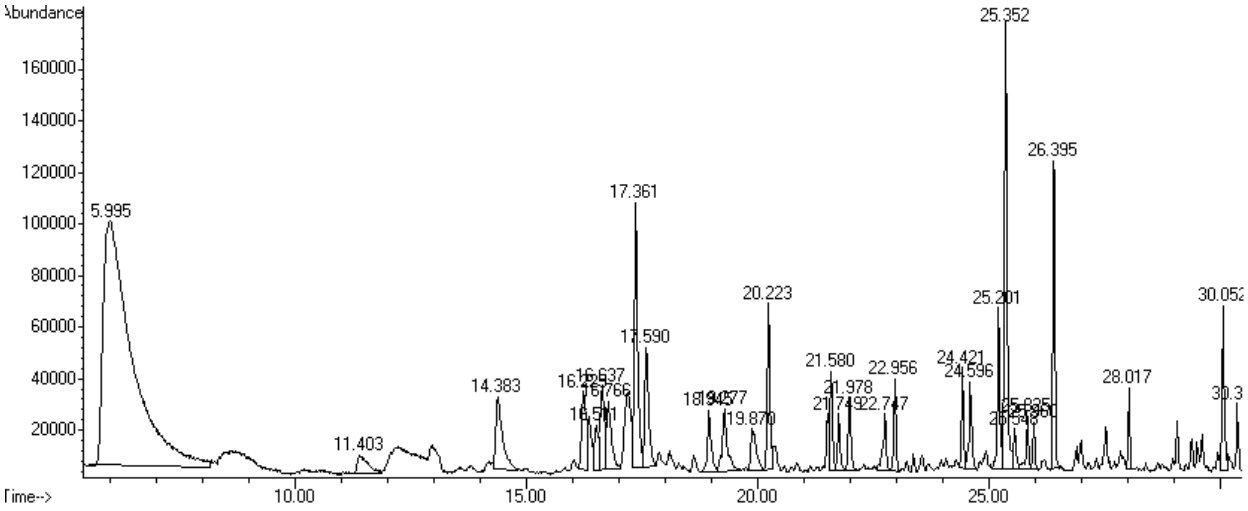

Figure 3.4 Capillary gas chromatographic separation of volatile compounds from Eastern oyster

Oyster volatiles during storage were identified according to the standard mass spectra of the spectral library (Wiley 6). The volatile compounds were tentatively considered as ‘identified’, when their mass spectral fit values were at the default value of 85 or above; otherwise the compounds were named as ‘unknown’. The oyster volatiles obtained by HSSPME are listed in table 3.1according to the retention time.

Table 3.1 Volatile organic compounds from Eastern oyster correspond to Fig 3.4

Number	Compound	Retention time (min)	Odor descriptor[a,b]	Reference threshold (ppb)[a,b,c,d]
1	Dimethyl sulfide	6.023	canned corn-like	1
2	Pentanal	8.81	grass	1.3-12
3	Hexanal	11.501	green	4.5
4	Heptanal	14.45	fatty, fishy	0.8-1
5	Benzaldehyde	16.423	almond, sweet	9960
6	Unknow	16.581		
7	2-octen-1-ol	16.718	fatty, rancid	40
8	2,3-Octanedione	16.833	plant-like, metallic-like	
9	Octanal	17.433	plant-like, metallic-like	0.7
10	2,4-Heptadienal, (E,E)-(Hexadiene)	17.662	fatty, mushroom, moss	
11	2-Octenal	19.025		3
12	2,4-Decadien-1-ol	19.322		
13	2-Nonanone	19.93		
14	3,5-Octadien-2-one	19.987	fatty,fruity	
15	Nonanal	20.29	melon-like	1
16	Unknow	21.112		
17	Unknow	21.316		
18	2,6-Nonadienal, (E,E)-	21.643	cucumber,like	0.01
19	2-Nonenal	21.809	fatty, green	
20	Benzaldehyde, 4-ethyl-	22.053		
21	7,10,13-Hexdecatrienal	22.747		
22	Decanal	23.017	marine	0.1
23	1-Undecyne	24.339		
24	2-Decenal	24.476	fatty	
25	10-Undecyn-1-ol	24.596		
26	2-Undecanone	25.25	cucumber,fresh	
27	Indole	25.352	nasty, fecal	0.3
28	Undecanal	25.604		
29	2,4-Decadienal, (E,E)-	25.825	fishy	
30	Lilac aldehyde	26.45	fresh, flowery	0.2
31	Dodecanal	28.071		
32	Tetradecanal	30.413		

a. Odor descriptor and corresponding threshold according to Pennarun et al. 2002
b. Odor descriptor and corresponding threshold according to Triqui & Bouchriti 2003
c. Threshold of indole according to Benner er al. 2003
d. Threshold of lilac aldehyde according to Kreck, M. & A. Mosandl 2003

A total of 32 volatile organic compounds were detected in the oyster extract including alkenes, aldehydes, alcohols, ketones, organic sulfides and others. Among them, the compounds associated with fresh oyster flavor are mostly 7-, 8-, 9-, 10-, and 11-carbon aldehydes and ketones. The compounds detected hadconsistence with study of Fratini et al. (2012) who detected 22 volatile compounds in flat oyster (*Ostrea edulis*) with similar extraction technique (PDMS/CAR coating fiber, 80°C of incubation temperature and 30 min of sampling time). The

main compounds in Fratini's study were: (i) aldehydes: pentanal, (E)-2-pentenal, hexanal, (E)-2-hexenal, heptanal, (EE)-2,4-heptadienal, (Z)-4-heptenal, 2-octenal, 2,6-nonadienal; (ii) alcohols: 1-penten-3-ol, (Z)-2-penten-1-ol, (Z)-1,5-octadien-3-ol, 2-octen-1-ol, 3-cyclohexene-1-ethanol; (iii) hydrocarbons: hexane; (iv) oxygenated aromatics: benzaldehyde; (v) ketones: 1-penten-3-one, 2,3-pentanedione, 3-octanone, 1-octen-3-one, and (vi) ethers: 2-ethylfuran and lilac aldehyde 2-(5-methyl-5-vinyltetrahydrofuran2-yl)propanal. Compounds found with high concentrations in this study but not in Fratini's were: Nonanal and related 9-carbon compounds, decanal and related 10-carbon compounds, undecanal and related 11-carbon compounds, and especially the dimethyl sulfide which is related to characteristic aroma of oyster. The differences in volatile profile may result from the different experimental conditions as well as different species of oyster samples.

Changes of volatile profile of oyster during storage

Changes of volatile profile of oyster during storage is shown in table 3.2

Table 3.2 Volatile organic compounds profile of oyster during storage.

VOC amount expressed in peak area/100000 (mean±s.d.).

Compounds	Day 0	Day 25	p-value of increase	p-value of decrease
Aldehydes				
Pentanal	24.40±1.27a	33.40±2.29b	0.0005	
Hexanal	14.33±1.14	14.39±5.48		
Heptanal	42.95±3.78	55.14±24.42	p>0.05	
Benzaldehyde	26.29±2.60	28.60±6.90		
Octanal	64.84±4.38	65.61±6.31		
2,4-Heptadienal	13.42±2.84a	23.98±3.54b	0.0018	
2-Octenal	8.87±0.77a	14.78±3.04b	0.0094	
Nonanal	32.39±3.22	28.32±3.11		p>0.05
2,6-Nonadienal, (E,E)-	7.86±1.18a	16.11±3.64b	0.005	
2-Nonenal	5.56±0.62a	10.27±2.20b	0.0063	
Benzaldehyde, 4-ethyl-	7.78±0.96	12.91±4.66	0.0746	
7,10,13-Hexdecatrienal	-	10.96±2.93		
Decanal	13.20±1.14	10.48±2.23		0.0729
2-Decenal	6.28±0.51a	13.65±1.90b	0.0003	
Undecanal	1.85±0.29a	7.62±0.95b	<0.0001	
2,4-Decadienal, (E,E)-	-	7.52±3.22		
Lilac aldehyde	19.54±2.94a	38.54±2.81b	<0.0001	
Dodecanal	4.31±0.43a	9.74±2.56b	0.0058	
Tetradecanal	3.33±0.11	10.99±6.47	0.0558	
Alcohols				
2-Octen-1-ol	13.23±1.90	21.96±4.30	0.01	
2,4-Decadien-1-ol	16.57±1.58a	22.51±3.59b	0.0232	
10-Undecyn-1-ol	-	15.98±2.75		
Ketones				
2,3-Octanedione	14.14±5.23a	20.88±2.07a	0.053	
2-Nonanone	4.40±0.27a	7.19±0.37b	<0.0001	
2-Undecanone	6.82±0.94a	21.62±5.49b	0.0018	
3,5-Octadien-2-one	4.75±1.06a	8.36±0.92b	0.0022	
Others				
Dimethyl sulfide	172.08±15.32a	259.46±26.12b	0.0012	
1-Undecyne	2.47±0.24	-		
Indole	-	65.83±4.60		

Twenty-eight volatile compounds detected on day 0 could be catalogued into five groups, each certain group of volatiles was typically in correspondence to one certain aroma. Fresh oysters have 1) seaside and seaweed, 2) green and planty, 3) plant-like and metallic-like, 4) melon-like, and 5) canned corn-like aroma notes base on previous studies about aroma of oysters or other seafood.

VOCs such as 2, 6-nonadienal, lilac aldehyde, 2-undecanone and decanal detected in oysters on day 0 represented for group one. Because Pennarun et al (2002) reported these four compounds responsible for seaside and seaweed aroma of fresh oysters (group one).

Pentanal and hexanal were cataloged into group two, because they had been reported to possess green odor which contributed green and planty aroma characteristic to fresh oyster (Triqui and Bouchriti 2003).

Eight-carbon volatile alcohols and ketones are existing in most seafood where they contribute distinct plant-like, metallic-like aromas, even though these compounds individually exhibit mushroom-like and crushed geranium leaf aroma qualities (Lindsay 1990). So the VOCs detected in this study belonging to group three were octanal, 2-octenal, and 3, 5-octadien-2-one.

Group four should include 9-carbon compounds such as 2-nonanone, nonanal, and 2-nonenal, which might contribute to melon-like flavor of oyster, identified in the study. To the best of our knowledge, these compounds were detected in Eastern oyster for the first time. Josephson et al. (1985) identified the volatiles of both Pacific and Eastern oysters. However, for some reasons they only found 9-carbon compounds in Pacific oyster, not in Eastern oyster.

Another volatile compound detected with high concentration in oyster extract was dimethyl sulfide (group five). Dimethyl sulfide is the main volatile constituent of fresh Pacific oyster's aroma and dimethyl-β-propiothetin is the thermally-labile precursor for this sulfur

compound (Ronald et al. 1964). It is well known for its “canned corn-like” aroma and flavor which blends nicely into milk-based oyster stew flavors. Volatile sulfur compounds which can cause putrid flavor in oyster such as dimethyl disulfide and dimethyl trisulfide, were not found in the fresh oyster extract, so the dimethyl sulfide in volatile profile could be responsible for the characteristic odor of fresh Eastern oysters in this study.

Other compounds such as benzaldehyde which link to pleasant almond odor, due to its high recognition threshold (9960 ppb) and detected with peak area of $7.78*10^5$ in oyster extract, it couldn’t be a significant contributor to the overall odor of oyster.

Based on the quantities of compounds detected in the oyster extract of day 0 (peak area>$10*10^5$) and the reference threshold of individual compounds, 9 out of the 32 volatile compounds found in this study (Table 3) were selected as odor-active compounds which have significant contributions to the odor of fresh oyster. Among them, (E,E)-2,6-nonadienal was only detected with peak area of $7.86*10^5$, but due to its low threshold (0.01 ppb) of odor detection, it is also considered as a significant contributor to the fresh odor of oyster.

Table 3.3 Odor-active compounds of fresh Eastern oyster

Number	Compound	Retention time (min)	Odor descriptor	Reference threshold (ppb)
1	Dimethyl sulfide	6.023	canned corn-like	1
2	Pentanal	8.81	grass	1.3-12
3	Hexanal	11.501	green	4.5
4	2,3-Octanedione	16.833	plant-like, metallic-like	N.A.
5	Octanal	17.433	plant-like, metallic-like	0.7
6	Nonanal	20.29	melon-like	1
7	2,6-Nonadienal, (E,E)-	21.643	cucumber,like	0.01
8	Decanal	23.017	marine	0.1
9	Lilac aldehyde	26.45	fresh, flowery	0.2

Subsequent loss in freshness was characterized by significantly increasing abundances of 2, 4-heptadienal, 2-nonenal, 2-decenal, and 2-octen-1-ol. These compounds had been reported to have fatty, fishy, and rancid odors in seafood. It is noted that there are four volatile compounds

which only were detected in oyster exact on day 25. They are 7, 10, 13-hexdecatrienal, (E, E)-2, 4-decadienal, 10-undecyn-1-ol, and indole. Some other volatile compounds with significantly increasing abundances were 4-ethyl-benzaldehyde, 2,4-decadien-1-ol. Among the fresh aroma contributors, five out of nine compounds, pentanal, (E,E)-2, 6-nonadienal , lilac aldehyde, 2,3-octanedione, and dimethyl sulfide were also shown to have significantly increasing trend during oyster's storage, while the other compounds keep at the same level or slightly decreased.

Spoiled or putrid flavors in seafood are contributed by the metabolism of microorganisms, which are often supplemented with simultaneous production of lipid oxidation products.

Losses of fresh flavors in refrigerated oyster were caused by conversions of some of the fresh compounds to less flavorful derivatives and the concomitant production of odorous microbial metabolites. Because of the relatively high threshold values exhibited by the alcohols, the alcohols compounds usually has less impact on seafood flavors and aromas than that provided by the carbonyls (Lindsay 1990). This feature may explain the fact that increasing alcohols (2, 4-decadien-1-ol and 10-undecyn-1-ol) caused losses of fresh flavors in oyster aroma.

Meijboom and Stroink (1972) had studied the fishy off-flavors occurring in strongly autoxidized fish oils, and these off-flavors might be caused by 2, 4, 7-decatrienal which were autoxidation products of long-chain PUFA n-3 fatty acids. Thus it could be speculated that 7, 10, 13-Hexdecatrienal and (E, E)-2, 4-Decadienal, detected in oyster aroma on day 25, were the autoxidation products of PUFAs and contributed some unpleasant flavor notes to the overall odor of oyster in this study.

Indole is a noxious chemical compound in seafood with its fecal and nasty odor note and usually used as aroma markers for discrimination of fresh and deteriorated seafood (Zhang et al. 2009). Indole is formed by bacterial degradation of the amino acid tryptophan. Tryptophan,

found in a large count of shrimp flesh, is broken down to indole by the enzyme tryptophanase found in some mesophilic microorganisms, frequently Gram-negative (Mendes et al. 2002). Research has shown that it is possible to have decomposed shrimp with definite odors of decomposition that contain indole at concentrations less than 25 μg/100 g (Shamshad et al. 1990). Indole is usually a product of high-temperature decomposition, but it also can be formed at low level when seafood is decomposed at low temperature. The presence of indole in our oyster samples further proved that the oysters were under deterioration and oysters at this stage may cause negative consumer reaction towards such products.

In marine fish and seafood, the production of trimethylamine (TMA) from trimethylamine oxide by microorganisms causes an enhancement of fishy flavors. It is another commonly used indicator of deteriorated seafood. Zhang et al. (2009) has detected trimethylamine from deteriorated Pacific oyster by SPME method. However, this compound was not detectable in oyster odor on day 25. The reason of this discrepancy remains unknown, but it indicates that it is unappropriated to use only one or two compounds as chemical markers for oyster freshness discrimination.

The volatile profile of oyster had significantly changes during storage. Intensity and odor characteristics of some volatiles such as 2, 4-heptadienal, 2-nonenal, 2-decenal, 2-octen-1-ol, 7, 10, 13-Hexdecatrienal, (E, E)-2, 4-Decadienal and idole, were closely related to the deterioration of oyster. They could help to provide precise information for deterioration of oyster during storage.

However, the odor characteristics and the contributions of each compound to the loss of freshness flavor of oyster need to be further investigated.

Fatty acid composition of oyster during storage

Table 3.4 Fatty acid composition of Eastern oyster (mean ± s.d.). SFAs: saturated fatty acids, MFAs: monounsaturated fatty acids, PUFAs: polyunsaturated fatty acids. They were expressed as mg/ml.

Peak	RT	Fatty Acid	Day 0		Day 25	
			mean	±s.d.	mean	±s.d.
8	33.70	Myristic acid(C14:0)	1.03	0.11	0.94	0.11
10	36.19	Pentadecanoic acid(C15:0)	0.26	0.06	0.29	0.04
12	38.66	Palmitic acid (C16:0)	7.68	1.45	7.92	1.13
14	40.90	Heptadecanoic acid (C17:0)	0.43	0.09	0.46	0.07
16	43.13	Stearic acid (C18:0)	1.09	0.18	1.15	0.11
		SFAs	10.49		10.75	
13	39.01	Palmitoleic acid (C16:1)	0.96	0.20	0.95	0.15
15	41.36	cis-10-Heptadecenoic acid (C17:1)	0.35	0.02	0.23	0.02
17	43.37	Oleic acid (C18:1n-9c)	1.88	0.12	1.80	0.15
18	43.51	Elaidic acid (C18:1n-9t)	0.96	0.23	0.97	0.17
24	48.11	cis-11-Eicosenoic acid (C20:1n-9)	0.14	0.01	0.12	0.00
		MUFAs	4.29		4.07	
19	44.15	Linoleic acid (C18:2n-6c)	0.82	0.17	0.83	0.10
22	45.35	α-Linolenic acid (C18:3n-3)	0.93	0.16	0.89	0.13
27	50.43	cis-11,14,17-Eicosatrienoic acid (C20:3n-3)	1.06	0.09	1.10	0.14
29	52.55	cis-5,8,11,14,17-Eicosatrienoic acid(C20:5n-3, EPA)	4.35	0.12	3.50	0.15
34	64.88	cis-4,7,10,13,16,19-Docosahexaenoic acid (C22:6n-3,DHA	9.20	0.52	7.55	0.30
		PUFAs	16.35		13.87	

The composition of fatty acids of oyster during storage is shown in Table 3.4. Among them, 52.5% of total fatty acids were polyunsaturated fatty acids, it was almost the same percentage level as that of Pacific oyster (Pennarun et al. 2002).The main PUFAs were n-3 PUFAs (49.9% of total fatty acids), mostly C20:5n-3 (EPA) and C22:6n-3 (DHA). However, there were also non-negligible amounts of n-9 monounsaturated fatty acids (9.57% of total fatty acids) and saturated fatty acids especially the palmitic acid (24.7% of total fatty acids).

Based on the research of aromatic precursors, some odor-active compounds in volatile profile of oyster extract could be found as products of PUFAs' oxidation. 2, 6-(E, E)-Nonadienal could arise from the oxidation of C20:5n-3 and C18:3n-3 (Josephson 1991; Schrodter 1990). According to Grosch (1987), 2-(Z)-octenal and 2, 4-(E, E)-heptadienal could result from the

oxidation of linolenic acid; Decanal, octanal, and nonanal could arise from the oxidative degradation of C18:1n-9. Hexanal and heptanal were significantly related with the content of n-6 PUFA (Fratini et al. 2012). Five out of nine odor-active compounds that have significantly contribution to the fresh aroma of oysters are derived from PUFAs.

However, only C20:5n-3 and C22:6n-3 showed a significant decreasing trend during storage, with p-value of 0.0241 and 0.0613, respectively. The decrease of C20:5n3 could be related with the increase intense of 2, 4-(E, E)-heptadienal in oyster aroma during storage. 7, 10, 13-Hexdecatrienal in oyster volatile compounds of day 25 could be derived from C22:6n-3. The decease of C22:6n-3 further suggests it is the primary precursor of flavor compounds in less-fresh oyster volatile profile.

Conclusions

The SPME method was optimized to study the volatile profile characteristics during oyster storage, followed by GC/MS detection. Using PDMS/CAR coated fiber (85 μm) to extract volatile compounds at 80°C for 50 min without addition of salt is the optimal extract condition in this study. Thirty-two volatile compounds of oyster including alkenes, aldehydes, alcohols, ketones, organic sulfides and others were identified in oyster extract. Nine out of thirty-two volatile compounds were responsible for the aroma of fresh oyster. The volatile profile of oyster significantly changed during storage. Intensity and odor characteristics of some volatiles such as 2, 4-heptadienal, 2-nonenal, 2-decenal, 2-octen-1-ol, 7, 10, 13-hexdecatrienal, (E, E)-2, 4-decadienal, and indole, were closely related to the deterioration of oysters. They could help to provide precise information for change of freshness during oyster storage. The decomposition of PUFAs, especially C18:3n-3, C20:5n-3, and C22:6n-3 was related with the presence of specific aldehydes.

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Chapter 4 Chemical and Textural Changes of Eastern Oyster (*C. virginica*) during Cold Storage

Abstract

Quality changes of oyster stored at 4°C were investigated by means of chemical analyses and textural analyses. Linear regression analyses of the storage period of oysters versus various quality indicators show that the strongest correlation was between storage period and ash contents (r = -0.976) followed by the total volatile basic nitrogen (TVBN) (r = 0.953) and cutting force (r = -0.893). TVBN level of 11 mg /100 g could be used as the limit of acceptability for cold stored oyster. Parameters in texture analyses (cutting force and chewiness off adductor muscle) have strong correlations with storage period before the death of oysters. Further study is required to confirm the suitability of ash content as a quality indicator of oysters. The textural and chemical results indicated a shelf-life of 20 days for Eastern oyster *C. virginica* stored at 4 °C. TVBN and textural parameters can be served as good indicators of evaluating quality of oysters.

Introduction

During storage of fresh oysters, some microbiological, chemical, physical, biochemical, and sensory changes may occur depending on duration and conditions of storage, as well as the initial quality of the oyster. In order to standardize and improve the measurement of quality of oysters, as well as other bivalves, there is a need for methods or instruments that can be commonly and easily used to determine the quality of oyster by the people who handle the bivalves.

Sensory methods are, perhaps, the most accurate quality predictors, which are linked to the consumer's expectations and acceptances. However, sensory parameters describing features

such as appearance, smell, texture, and taste depend on food culture, habits, and experience, which are difficult to either identify or quantify (Aaraas, R et al., 2004). Moreover, sensory assessment is a time-consuming procedure that requires for well-trained panelists. Therefore, it cannot be used for routine analysis.

Quality of oysters can be assessed by a number of measurable chemical parameters. Among them, pH is commonly used as an indicator of freshness of seafood, including mollusks. Bacterial fermentation of carbohydrates in seafood leads to production of organic acids, which resulted in a reduction in pH and thus the quality (Khan et al., 2005). Another widely used quality index for seafood is total volatile basic nitrogen (TVBN) resulting from the degradation of nitrogenous compounds by microbial activity. Ababouch et al. (1996) suggested that TVBN of 20-25 mg/ 100g could be used as the quality limit of acceptability for fresh seafood. Changes in proximate composition can also relate to the freshness. Khan et al. (2005) found a strong correlation between storage period and ash content($r=0.819$, $p=0.0002$) in blue mussels, suggesting that ash content may be another quality indicator of bivalve freshness.

Texture testing instruments can detect and quantify certain physical parameters which then can be interpreted in terms of sensory perception. Texture profiling analysis (TPA) involves compressing the tested substance at least twice and quantifying the mechanical parameters from the recorded force-deformation curves. Strong correlations between sensory and instrumental hardness and chewiness have been reported on shellfish including abalones (Sanchez-Brambila G.Y., 2002). Instrumental measurements correlated with chemical properties may aid in the establishment of quality parameters for oysters. However, few studies have been performed to analyze the texture of oysters by a well-established instrumental method.

The current study was designed to 1) examine the changes in pH, TVBN, moisture, ash content, and texture profile of Eastern oyster (*C. virginica*) during cold and dry storage (out of water with a cover of wet clothes); 2) use statistical analysis to select the most appropriate indicators for evaluating quality of fresh Eastern oysters; and 3) apply selected indicators to evaluate quality among different treatment of oyster.

Material and Methods

Material

Oyster samples (*C. virginica*) were provided by the Auburn University Shellfish Lab, collected at the Portersville Bay research oyster farm in Mississippi Sound near Coden, AL in December 2013. Major components were measured and are shown in table 4.1. Hatchery raised oysters were split into two different desiccation treatments: in one treatment, the oysters were kept constantly submerged until harvest (referred as Never), while in the second treatment, oysters were air-dried weekly for duration of 24 hrs (referred as Daily) at a time (in an effort to control bio-fouling). All samples were shipped immediately by overnight service in coolers with ice packs after harvest to the laboratory at Auburn University, and stored in 4 °C walk-in refrigerator with about 90% relative humility. At selected time intervals (0, 5, 10, 15, 20, and 25 days), oysters from both treatments were shucked to be tested of oyster meat quality by several methods. The methods and standards were established by the data obtained from oysters of Never treatment.

Table 4.1 Chemical composition of oysters from different treatments

	Daily		Never	
	Adductor	Body	Adductor	Body
Moisture	80.86%	80.55%	81.88%	80.81%
Ash content	0.99%	0.94%	0.89%	0.88%
Protein content	16.65%	N.A.	14.17%	N.A.
% Amino acid of total protein				
ASX	10.5%	N.A.	10.1%	N.A.
GLX	15.3%	N.A.	15.1%	N.A.
SER	4.6%	N.A.	4.5%	N.A.
HIS	1.5%	N.A.	1.5%	N.A.
GLY	4.0%	N.A.	3.7%	N.A.
THR	4.9%	N.A.	4.6%	N.A.
ALA	4.7%	N.A.	6.6%	N.A.
ARG	10.6%	N.A.	10.2%	N.A.
TYR	4.0%	N.A.	3.6%	N.A.
VAL	4.2%	N.A.	4.1%	N.A.
MET	3.2%	N.A.	3.0%	N.A.
PHE	3.8%	N.A.	3.6%	N.A.
ILE	4.4%	N.A.	4.3%	N.A.
LEU	9.5%	N.A.	9.3%	N.A.
LYS	11.4%	N.A.	12.8%	N.A.
PRO	3.5%	N.A.	2.9%	N.A.

Statistical analysis

One-way ANOVA was employed in effect of day of storage on quality of oyster at a significance level of 95%. When necessary ($P<0.05$), a Duncan's test was applied to compare results of a given parameter at a different storage time. Linear regression analyses were performed to establish the statistical relationships between storage days and various quality indices examined. Various statistical parameters were used to examine the strength of the examined linear relationships including the correlation coefficient (r) and level of significance (p) values. Relationships with $|r| < 0.5$ and $p > 0.05$ were considered to be poorly correlated, relationships with $|r| = 0.500\text{-}0.700$ and $p < 0.05$ were considered moderately correlated, and relationships with $|r| > 0.700$ and $p < 0.05$ were strongly correlated.

When comparing the effect of treatment and day of storage on quality, two-way ANOVA was employed at a significance level of 95%.

The SAS 9.2 software package was used to perform these analyses.

Instrumental measurements

Texture profile analysis (TPA) and cutting testing were performed at room temperature using a TA.XT2i Stable Micro Systems Texture Analyser (Stable Microsystems Ltd., Surrey, England) with the Texture Expert programmes.

TPA is a double compression cycle test which performs up to 50% compression of the original portion height with an aluminum cylinder probe P/25. A time of 5 s was allowed to elapse between the two compression cycles. Force-time deformation curves were obtained with a 5 kg load cell applied at a cross-head speed of 2 mm/s. The following parameters were determined: 1) hardness(N), maximum force required to compress the sample; 2) springiness(m), ability of the sample to recover its original form after deforming force was removed; 3) adhesiveness (N*s), area under the abscissa after the first compression; 4) cohesiveness, extent to which the sample could be deformed prior to rupture; 5) chewiness (J), work required to masticate the sample before swallowing; and 6) resilience, measurement of how a sample recovers from deformation in relation to speed and forces derived.

In the cutting test, an incisor blade (length= 34.04 mm, width =9.90 mm, thickness =1.50 mm) was applied to imitate a human tooth. The probe was attached to the Texture Analyzer with its longitudal side perpendicular to the test platform and blade side (9.90 * 1.50 mm) facing the muscle surface. Each cut was made at directions that perpendicular to the muscle. After a touch was achieved between the blade tip and oyster surface, the ‘‘tooth’’ probe was allowed to cut the muscle fiber perpendicularly at a speed of 2 mm/s with the trigger type of ‘‘button’’ and then returned to the starting position. The cutting force (N) was measured at the maximum force

required to cut through the samples (failure point). Tests were made at 4-6mm thickness for each oyster adductor muscles.

Eight to twelve oysters from each treatment were used to perform the texture analysis.

Moisture and ash

Each oyster was shucked and the whole body was dried using paper towel until the water on body and adductor surface was completely removed. After drying, oyster body part and adductor muscle was chopped separately and 2g of chopped sample each was used for moisture determination by AOAC method.

The ash content of chopped oyster body and adductor muscle was determined in triplicate; A 2 g of chopped oyster was incinerated in a muffle furnace (Model BF51842, Thermo Scientific Lindberg Blue M) at 500°C, according to AOAC method.

Protein and Free Amino Acids

pH value

A 5 g of chopped whole oyster meat was diluted 1:10 with distilled water, homogenized for 1 min and pH measured using an ATI orion perpHecT LogR meter, model 370 with a standard polymer pH electrode (12*160 mm). The process was performed at room temperature.

TVBN

TVBN was determined by Conway Microdiffusion method. A 10 g of chopped whole oysters were homogenized in 40 ml of 4% trichloroacetic acid (TCA) solution for 30 mins at room temperature and centrifuged for 15 mins at 10000g. 1 ml aliquot of the supernate was combined with 1 ml of saturated K_2CO_3 (potassium carbonate) solution in the Conway unit (SCIENCEWARE® Conway Diffusion Cell, Bel-Art) to produce ammonia, which in turn was absorbed into 1 ml of 2% H_3BO_3 (boric acid) solution. TVBN was then determined as mg per

100 g by titration with 0.01 N HCl solution. A control test was also carried out using 1ml of 1% TCA, instead of sample extract.

Three oysters from each treatment were used to perform the moisture and ash content tests. For pH value and TVBN, three oysters each were pooled and ground for triplicate tests.

Results and Discussion

Changes in moisture, ash contents, pH, and TVBN during storage (data obtained from oysters of never treatment)

Changes in various quality parameters during 4°C dry storage are summarized in Table 4.2. There was no significant difference in the moisture among oyster throughout the storage period. However, the ash contents of oyster, both for the adductor muscle and body part were significantly lower for those stored for 25 days as compared to those stored for 0 or 10 days. Loss of mantle fluid and retraction of the mantle were observed in oyster, and soft parts appeared dry on day 25. Results of moisture analysis showed that there was no significant difference between oysters through storage. The decrease of ash contents might be due to the leaching of oyster constituents by the intervalval fluid.

The initial pH value of whole fresh oyster was 6.6 with a gradual decrease of 0.2 units of pH after storage for 25 days. The actual variation of pH value between samples was smaller than the lowest resolution (0.1 units) of the pH meter. The stable pH during storage indicated that a live oyster was able to maintain extracellular pH. It is important for many intertidal bivalve mollusks to maintain respiratory gas exchange and thus tolerate emersion during low tide (Cruz-Romero et al. 2008). It has been reported that oysters (with liquor) are classified as being of good quality if their pH$\geqslant$6.0 (Cook 1991).

TVBN increased significantly with time to a final value of 11.12±0.07 mg/100g on day 25. Ababouch et al. (1996) used 20–25 mg/100 g of TVBN as the quality limit of acceptability for fish products. Lannelongue et al. (1982) listed the following values (mg per 100 g TVBN) for different degrees of freshness of fish: ⩽12, fresh fish; 12-20, edible with only slight decomposition; 20-25 borderline; and >25, inedible and decomposed. Sikorski et al. (1990) reported an acceptability level of TVB-N of 17mg per 100 g in raw oysters. Based on the previous TVBN standards, the oyster in this study could be classified as fresh on day 25. However, among the researchers having worked on cold storage of oyster, Boyd et al. (1980) observed that oysters maintained high-quality with good flavor for 13 days. Aaraas et al. (2004) suggested that 12 days of storage is the limit for emersed oyster in cold storing condition. Cao et al. (2009b) reported a shorter shelf-life of 11 days considering the sensory assessment total score. The discrepancy may due to the different initial TVBN values between this study and others. The initial TVBN value was 4.02±1.29 mg/100g in this study on day 0, which was a value much lower than those reported most similar studies such as Kim et al. (2002). They reported an initial TVBN of 19 mg/100g for fresh oyster.

Table 4.2 Changes in pH, TVBN, moisture, and ash content during storage.

Storage day	Moisture[1]		Ash[2]		pH[3]	TVBN[4]
	Adductor	Body	Adductor	Body	Whole	Whole
0	81.88±0.66a	80.81±1.11a	0.89±0.06a	0.88±0.05a	6.6	4.02±1.29d
5	-[5]	-	-	-	-	4.57±0.40cd
10	82.12±0.13a	81.45±0.98a	0.80±0.03a	0.81±0.04a	6.5	5.52±0.40c
15	-	-	-	-	-	9.13±0.54b
20	-	-	-	-	-	9.78±0.50b
25	81.51±0.06a	81.22±0.03a	0.56±0.02b	0.63±0.01b	6.4	11.12±0.07a

All values are means ± s.d. (n=3). Means with different letters in each column are significantly different from one another ($p<0.05$)

1, 2 Moisture and ash expressed as percentage of wet weight.

3 pH values have no standard deviation due to the limit of pH meter.

4 TVBN expressed as mg/100g

5 no determination of parameters in this day

Texture profile during storage

Table 4. 3 Changes in TPA parameters on body part of oysters during storage

Storage day	Hardness		Adhesiveness		Springiness		Cohesiveness		Chewiness		Resilience	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
0	698.41	134.26	-15.94	4.93	0.68	0.09	0.46	0.05	223.14	75.81	0.29	0.03
5	714.12	64.54	-18.40	5.20	0.73	0.06	0.48	0.05	252.49	54.20	0.32	0.03
10	663.39	78.69	-17.59	4.71	0.71	0.08	0.44	0.06	209.23	52.15	0.28	0.03
15	737.50	46.96	-18.74	4.85	0.71	0.07	0.49	0.04	256.47	49.22	0.30	0.04
20	745.72	105.60	-18.93	8.80	0.66	0.11	0.43	0.06	212.67	57.15	0.30	0.04
25	694.21	44.72	-17.52	8.95	0.66	0.08	0.44	0.06	203.16	46.47	0.28	0.04

Table 4. 4 Changes in TPA parameters and cutting force on adductor muscle of oysters during storage.

All values are means ± s.d. from textural experiments (n>4). Means with different letters in each column are significantly different from one another ($p<0.05$)

Storage Day	Hardness	Adhesiveness	Springiness	Cohesiveness	Chewiness	Resilience	Cutting
0	1140.03±159.29a	-2.007±0.79a	0.78±0.04a	0.61±0.03a	544.18±77.62a	0.47±0.02a	167.82±16.10a
5	875.36±147.10b	-2.153±0.58a	0.71±0.04ab	0.58±0.01ab	356.46±57.48b	0.45±0.02ab	137.48±20.63b
10	846.46±54.23b	-2.30±0.96a	0.70±0.08b	0.56±0.07ab	329.08±65.30b	0.44±0.08ab	110.64±9.11c
15	872.21±188.52b	-2.82±1.65a	0.71±0.03ab	0.57±0.04ab	348.74±65.34b	0.45±0.05ab	99.92±21.38c
20	617.10±65.52c	-2.26±0.55a	0.71±0.05ab	0.54±0.04b	236.84±41.84c	0.41±0.04ab	71.00±9.83d
25	812.31±77.97b	-2.06±0.26a	0.71±0.04ab	0.56±0.02ab	321.54±46.59b	0.44±0.04ab	134.70±20.47b

There were fluctuations, but no significant variation or trend in TPA parameters on body part of oyster during storage (Table 4.3). It suggested that the textural properties on oyster body cannot reveal quality changes during storage.

Contrarily, the statistical analysis of textural parameters on adductor muscle of oyster showed significant differences during storage (Table 4.4). The hardness, chewiness, and cutting force of oyster adductor gradually decreased from day 0 to 20. Texture loss during storage has been related to water content (Olsson et al. 2003), pH value (Ashie et al. 1996), drip loss (Vidode et al. 2001), protein content (Beltrán‐Lugo et al. 2005), and enzyme activities (Ashie et al. 1997). In this study, moisture stayed constant and pH value had no rapid decline, so the drip loss, change in protein and enzyme activities may contribute to the firmness decrease. On day 25, there were significantly increases for hardness, chewiness, and cutting force, which may relate to mortem rigidity (Rigor mortis). The occurrence of Rigor mortis was consistent with the observations that oysters were dead or close to death at the end of storage. Rigor mortis is one of the most important post mortem phases and caused by a decrease in energy levels of the major metabolite, ATP, leading to muscle changes from a relaxed to a rigid and inflexible state (Jiménez-Ruiz et al. 2013). It is known that this phase can extend from hours to days.

Adhesiveness, springiness, cohesiveness, and resilience were stable on adductor of oyster throughout the storage. Hardness is one of the 4 primary texture characteristics (hardness, adhesiveness, springiness, and cohesiveness). Chewiness is a secondary texture characteristic which is a product of hardness, springiness, and cohesiveness. Sanchez‐Brambila et al. (2002) stated that sensory and instrumental hardness and chewiness were highly correlated and provided the best description of product texture. Therefore, hardness, chewiness, as well as the cutting

force on adductor muscle of oysters may be used to indicate quality change regarding the texture loss of product during storage.

Linear regression analyses of the storage period vs. various quality parameters

During the cold storage of oyster, various chemical and textural changes occurred that might have led to reduction in the quality. Understanding these changes may help to select the most appropriate indicators for evaluating quality of seafood during storage. Linear regression analyses were used to support the selection process. Various parameters such as the correlation value and level of significance were used to examine the strength of the linear relationships (Khan et al. 2005).

Figures 4.1 and Table 4.5 summarize the results of linear regression analyses of the storage period of oyster vs. various potential quality indicators (TPA-Hardness, TPA-Chewiness, Cutting force, TVBN, Ash contents of adductor muscle and body). The strongest correlations were between the storage period and ash content of body (r=-0.976), followed by storage period and ash content of adductor (r=-0.967), TVBN (r=0.953), cutting force (r=-0.893), and TPA-chewiness (r=-0.751). The correlation between storage period and TPA-harness is moderate (r=-0.682). The level of significant for all parameters is less than 0.05.

Table 4.5 Linear regression analyses of the storage period of oyster vs. various potential quality indicators

Quality indicator	Correlation coefficient (r)	Level of significance(p)
TPA-Hardness	-0.682	<0.0001
TPA-Chewiness	-0.751	<0.0001
Cutting	-0.893	<.0001
TVBN	0.953	<.0001
Ash of adductor	-0.967	0.0017
Ash of body	-0.976	0.0009

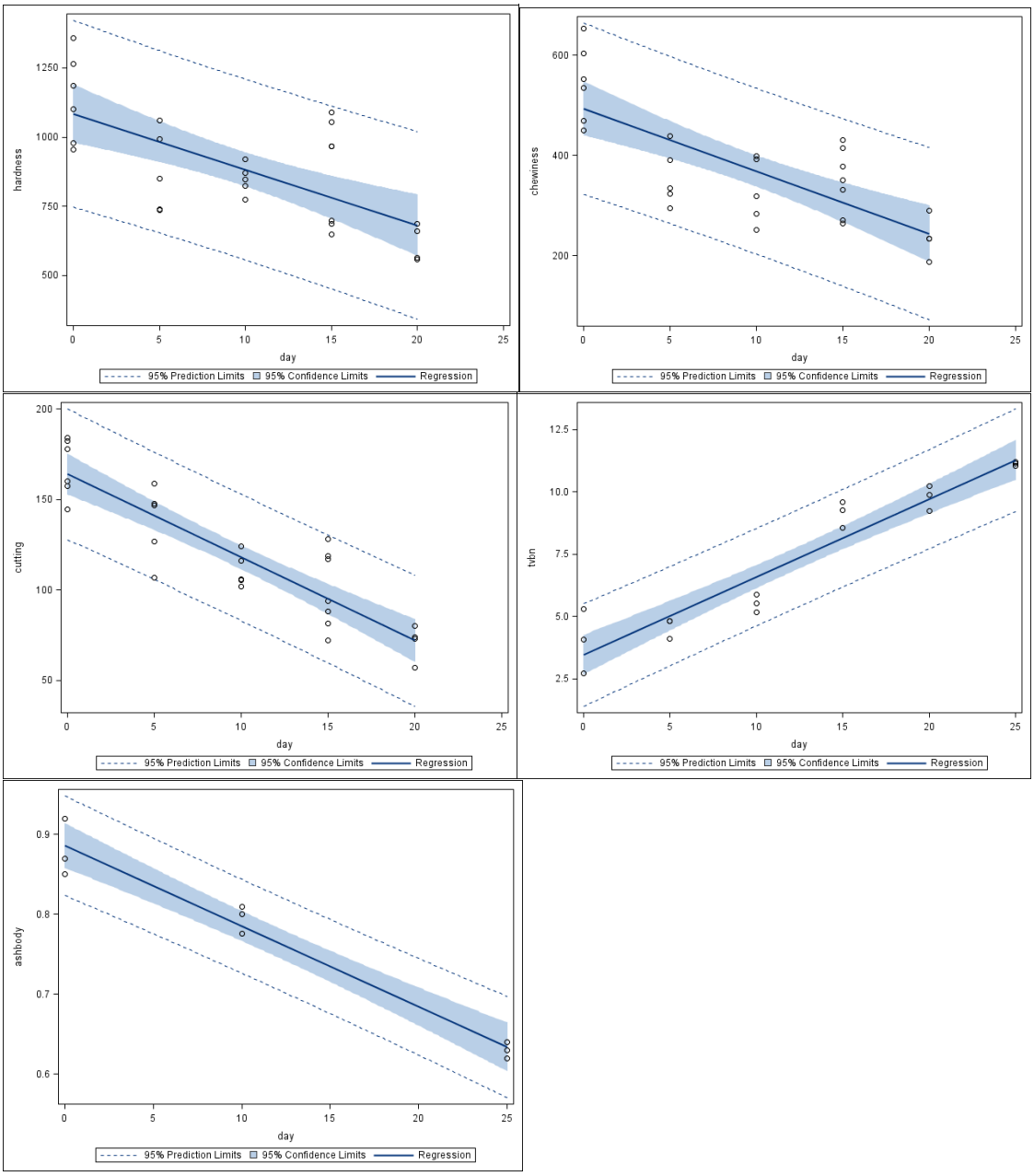

Figure 4.1 Linear regression lines of oyster storage period vs. various potential quality indicators. The dashed lines indicate the prediction interval (95%) and hatched area indicate the confidence interval (95%).

Except for one study examining the changes in proximate composition of oysters due to seasonal changes (Linehan et al. 1999), no previous study appears to have reported changes in the ash contents of oyster during cold storage. Linehan et al. (1999) has reported changes in the ash content of oyster harvested in different seasons, but has illustrated no fundamental causality

for the variability. The observed decrease in the ash content of stored oyster may be explained by the leaching of oyster constituents by the intervalval fluid. However, a decrease of the ash content of oyster during storage (r=-0.976 for body, r=-0.967 for adductor) should be confirmed by determination from more time point during the storage.

TVBN had a high correlation(r=0.953) with storage period. It was not surprising because TVBN has been widely used as an indicator of the freshness of seafood, including oysters. Though the level of TVBN remained below the rejection limits cited in the literature review, the strong correlation between the storage period and TVBN indicated the suitability of this parameter as a quality indicator.

Suddenly increase of cutting force, chewiness, and hardness existed on day 25, indicated the death and mortem rigidity effect of oyster, which would definitely affect the consumer's expectation for live oyster. Texture results on day 25 could not represent the quality of fresh oysters. Therefore data on day 25 was taken from the linear regression analyses. For the texture parameters, correlation between cutting force and storage(r=-0.893) was bigger than that of chewiness (r=-0.751) and hardness (r=-0.682). Cutting test might be more sensitive to reveal the freshness of oyster than TPA. Although chewiness is highly depended on hardness (Chewiness=Hardness*Cohesiveness*Springiness), chewiness's correlation with storage period was better than hardness. Because chewiness contains more sensory attributes (springiness and cohesiveness) so it may explain the texture changes better. Results from this study supported the use of cutting force and TPA-chewiness on the adductor muscle of oyster as quality indicators of fresh oyster, and the measures were valid until the oyster was dead.

Based on the texture results, the value of day 25 could be set as the rejection limit in this study. Acceptability limit for TVBN as freshness indicator is 11 mg/100g.

Differences of quality among treatments base on quality indicator

Two culture treatments of oyster were tested at the same time in this study for the quality assessment. Analyses of variance were done for comparing the differences of quality indicators over time as well as among treatment. The model for the change of the quality indicators were:

TVBN= Storage+Treatment+Storage*Treatment

Cutting force=Storage+Treatment+Storage*Treatment

When using TVBN as quality indicator in the whole storage period (Table 4.6), the interaction variable of treatment*storage day was significant ($p<0.0001$), indicating that both treatment and storage day had effect on the change of TVBN. When investigated the effect of treatment within each level of storage day, significantly differences were shown on day 15, 20, and 25, with p-value<0.0001. Since TVBN was related to freshness of oyster, the result suggested that two treatment of oyster were at the same level of freshness from day 0 to day 15, while the level of freshness became different from day 15 to day 25. The difference was also shown in figure 4.2. Oysters cultured by Daily method had smaller TVBN value on days 15 to 25, suggesting that longer shelf life can be expected from daily treatment oysters.

Table 4.6 Analyses of variance on interaction of culture treatment*storage day

	TVBN	Cutting force
Storage*Treatment	<0.0001	0.0211
Treatment effect on day		
0	0.9619	0.0178
5	0.2432	0.0057
10	0.1206	0.0474
15	<0.0001	0.0147
20	<0.0001	0.3902
25	<0.0001	0.5299

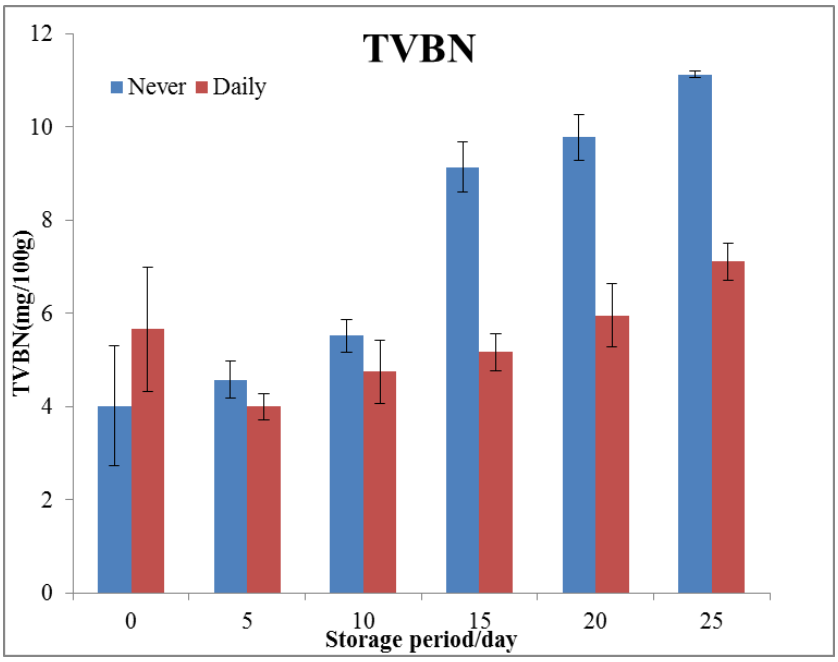

Figure 4.2 Comparison of oyster quality from two culture treatments by storage period vs. TVBN.

On behalf of texture characteristic, the statistical analysis showed that cutting force on oyster adductor muscle was depending on the effect of treatment* storage day (p=0.02). When investigated the effect of treatment within each level of storage day, significantly differences were shown on day 0, 5, 10, and 15, with p-value is 0.017, 0.0057, 0.047, and 0.0147, respectively. The difference suggested that culture treatment had impact on the texture attribute of oyster adductor muscle. The cutting force could be used to evaluate texture quality of different treatment oyster when the oysters were fresh. The difference of oyster texture from two culture treatment is shown in figure 4.3. Daily treatment oysters had larger cutting force on day 0 to day 15, indicating that the adductor muscle of daily treatment oysters has firmer texture than that of never treatment oysters.

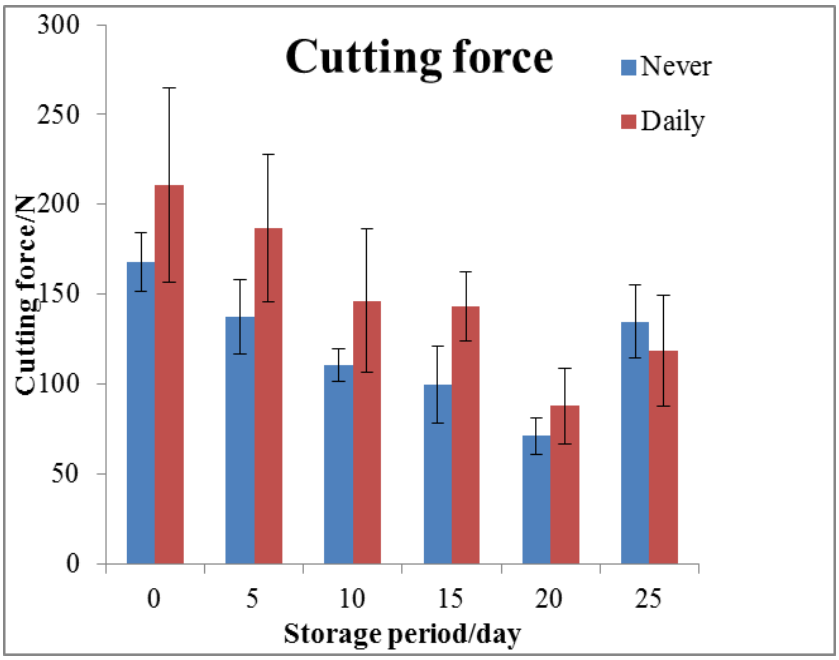

Figure 4. 3 Comparison of oyster quality from two culture treatments by storage period vs. cutting force.

Conclusions

Quality changes of oysters stored in 4°C were investigated by means of chemical analyses and textural analyses. TVBN could be used as good freshness indicator with the acceptability limit of 11 mg/100g for Eastern oyster. Parameters in texture analysis (cutting force and chewiness on adductor muscle) had strong correlation with storage period before the death of oyster and could be a quality indicator for oyster. Further studies are required to confirm the suitability of ash content as quality indicator of oyster. The textural and chemical results indicated a shelf-life of 20 days for oyster stored at 4 ◦C. The selected indicator could also evaluate the difference of quality among different culture treatment of oysters. We concluded that TVBN and textural parameters such as cutting force could serve as good indicators for evaluating quality of oysters.

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