Quality Evaluation of Live Eastern Oyster (*Crassostrea virginica*) based on Textural Profiling Analysis, Free Amino Acids Analysis, and Consumer Sensory Evaluation

by

Jue Wang

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
August 1, 2015

Keywords: live eastern oyster, texture, free amino acids, consumer preference, linear regression

Approved by

Yifen Wang, Chair, Professor of Biosystems Engineering and Affiliate Professor of Fisheries, Aquaculture and Aquatic Sciences
William Walton, Associate Professor of Fisheries, Aquaculture and Aquatic Sciences
Douglas White, Associate Professor of Nutrition, Dietetics, and Hospitality Management
Peng Zeng, Associate Professor of Statistics, Mathematics and Statistics

Abstract

The consumption of live eastern oyster (*Crassostrea virginica*) has become an important part of the diet for consumers in the United States. Because large amounts of oysters are grown every year, it is necessary for oyster farmers to understand quality differences caused by different aquaculture methods, as well as quality changes over the time of cold storage. The objective of this study is to develop a set of systematic methods for quality evaluation of live eastern oysters.

Qualities evaluation of three aquaculture-treated oysters (daily, weekly, and never) were by means of: 1) textural analysis, 2) free amino acids (FAAs) analysis and 3) consumer preferences by means of 1) textural analyzer, 2) high-performance liquid chromatography (HPLC), and 3) consumer sensory evaluation. Besides, linear regression analysis with stepwise selection method was conducted to establish relationship between instrumental parameters (textural parameters and FAAs concentrations) and consumer preferences (texture likeability, flavor likeability and overall likeability) obtained from sensory evaluation.

For texture characteristics among three treatments, hardness, gumminess and chewiness were important parameters for oyster texture. Daily treatment had firmest body when people biting them caused by more times of desiccation during production. Weekly treatment had strongest adductor muscle and longest survival time. As to flavor characteristics, predominant FAAs (free glycine, free alanine, free glutamic acid, free arginine, free cysteine and free methionine) and bitter FAAs (free leucine, free tyrosine and free phenylalanine) were identified and considered as main parameters of oyster flavor. Daily treatment had more sweet and sulfurous flavor than other

two treatments. However, consumers were not able to statistically distinguish differences among three treatments.

For changes during storage, quality of oyster texture decreased during cold storage at 4°C. The recommended shelf-life was less than 21 days due to the apparent increases of bitter FAAs of body on day 21, and the best consumption time was within 7 days due to the increase of free leucine of adductor muscle on day 7. Consumers couldn't figure out differences among fresh oysters (0 day of storage period), oysters stored for 7 days and 14 days.

The results of linear regression analysis indicated the effect of flavor was stronger than texture on oyster consumption. Sweet FAAs of body and sulfurous FAAs of adductor muscle made positive ($r = 0.010$) and negative effect ($r = -0.025$) to flavor likeability, respectively. Sweet FAAs of body also made positive ($r = 0.008$) effect to overall likeability. Sweet FAAs of body and sulfurous FAAs of adductor muscle was indicators for eastern oyster flavor and overall sensory characteristics.

Acknowledgments

I want to express my sincere appreciation to my advisor Dr. Yifen Wang for guiding me through this project, and giving advices on my study and research. His mentorship has helped me to grow up as a food scientist and as an individual. I would like to thank Dr. William Walton for oyster supply and guidance. Thank Dr. Douglas White and Dr. Peng Zeng for their advices on my project and thesis. Thank Dr. Luxin Wang for her helps in designing and conducting the sensory evaluation.

Thanks also extend to all the Biosystems Engineering graduate staffs and students who helped me in the study and research, especially those who participated in the sensory evaluation. Special thanks go to Mr. Zhouhong Wang for his help and advices for operation of HPLC instrument, and Ms. Jinglin Zhang for her experience and help for the operation of the texture analyzer instrument. I gratefully acknowledge Xiaofei Wang, Pengmin Pan, Bangping Wang,Hua Bian, Jing Yuan and Chao Liao for their expert assistances in my experiment.

My most sincere thanks go to my parents for their unconditional love and financial support. Thank my dear friends Yaru Lin, Jinghua Cao, Ziyun Chen, Yang Zhao, Juan Hu, Shiqi Gao, and Yuan Zeng for their continued encouragement and great friendship.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

TPA	Textural Profiling Analysis
FAA	Free Amino Acid
HPLC	High Performance Liquid Chromatography
OPA	o-Phthaldialdehyde
FMOC	9-Fluorenyl-methylchloroformate
UV	Ultraviolet

Chapter 1 Introduction

Oyster is one of the most popular seashell foods in the world due to its high nutritive value and delicious taste. One popular way of consuming oysters is eating them raw. Various species of oysters have been harvested for centuries. Among various species of oysters, eastern oyster is one of the most economically and ecologically important species with abundant resource along the eastern seaboard (Wallace 2001). In 2013, U.S. oyster landings have yielded nearly 44.8 million pounds with the value of 217.5 million dollars. Both yield and unit are increasing compared with 2012 (David Van Voorhees et al. 2013).

Since large amounts of oysters have been grown, it is necessary for oyster farmers to understand quality differences of oysters caused by different aquaculture methods. In addition, deterioration of live eastern oysters often occurs over the time of cold storage. The deterioration of quality not only affects consumer preferences of foods, but also affects the food safety. It is also necessary to determine changes of oyster quality during storage. Multiple characteristics of oyster quality have been reported related to deterioration, including total viable count (TVC) (Songsaeng et al. 2010), total volatile basic nitrogen (TVB-N) (Songsaeng et al. 2010), fatty acids (Cruz-Romero and Cruz-Romero 2008), volatile compounds (Cruz-Romero et al. 2008), free amino acid content (Je et al. 2005), and sensory evaluation (Chen 2011).

Texture and flavor are two important contributors of oyster quality. Texture can affect the quality of food, and the most commonly used instrumental technique for texture measurement is the textural profiling analysis (TPA). As to flavor, free amino acids (FAAs) are known to account for a major part of the non-protein nitrogenous compounds and have been found to evoke a marked taste sensation (Konosu et al. 1988; Fuke and Konosu 1991). Amino acids

provide sweetness, bitterness, sourness, saltiness and umami flavor and have been used in food processing to enhance the flavor of natural characteristics of foods (Kirimura et al. 1969). Therefore, a set of systematic methods to identify and evaluate the quality of live eastern oysters, as well as to explore the relationships of instrumental parameters versus consumer sensory scores, is increasingly important for the oyster industry.

Overall Objective

The overall objectives of this study were to develop a set of systematic methods for oyster quality evaluation, which include three main experimental parts as textural analysis, free amino acid analysis and consumer sensory evaluation; and then, to use aforementioned methods to investigate quality changes of raw oysters during cold storage.

Sub-objectives

1. Perform textural analysis using textural profiling analysis (TPA) and cutting force of three aquaculture-treated eastern oysters. Determine differences among three treatments and their changes during storage.

2. Optimize detecting methods of free amino acid analysis of three aquaculture-treated eastern oysters. Determine differences of free amino acid profiles among three treatments and their changes during storage.

3. Perform consumer sensory evaluation of three aquaculture-treated eastern oysters. Establish relationships between instrumental parameters of oyster texture and free amino acids versus likeability scores of consumer sensory evaluation.

References

Chen, L. (2011). "Sensory and Chemical Characteristics of Eastern Oysters (*Crassostrea virginica*)." Virginia Polytechnic Institute and State University.

Cruz-Romero, M. C., J. P. Kerry, et al. (2008). "Fatty acids, volatile compounds and colour changes in high-pressure-treated oysters (*Crassostrea gigas*)." Innovative Food Science and Emerging Technologies 9(1): 54-61.

David Van Voorhees, Alan Lowther, Michael Liddel, Penny Pritzker, Kathryn D. Sullivan, Eileen Sobeck (2013). "Fisheries of the United States Current Fishery Statistics No. 2013." Silver Spring, MD, September 2014.

Je, J.Y., P.J. Park, et al. (2005). "Amino acid changes in fermented oyster (*Crassostrea gigas*) sauce with different fermentation periods." Food Chemistry 91(1): 15-18.

Kirimura, J., A. Shimizu, et al. (1969). "Contribution of peptides and amino acids to the taste of foods." Journal of Agricultural and Food Chemistry 17(4): 689-695.

Konosu, S., K. Watanabe, et al. (1988). "Taste-active components of scallop, 1: Extractive components of scallop and identification of its taste-active components by omission test." Journal of the Japanese Society for Food Science and Technology (Japan).

Songsaeng, S., P. Sophanodora, et al. (2010). "Quality changes in oyster (*Crassostrea belcheri*) during frozen storage as affected by freezing and antioxidant." Food Chemistry 123(2): 286-290.

Wallace, R. K. (2001). "Cultivating the eastern oyster, *Crassostrea virginica*."

Chapter 2 Literature Review

2.1 Eastern Oyster (*Crassostrea virginica*)

The eastern oyster (*Crassostrea virginica*) is also known as the American oyster, Atlantic oyster, or Virginia oyster. It belongs to Animalia kingdom, Mollusca phylum, Bivalvia class, Ostreoida order, Ostreidae family, *Crassostrea* genus, and *C. virginica* species. Eastern oyster has been harvested by native American Indians before Columbus discovered America (Wheaton 2007). In fact, oysters are easily cultured by farmers and widely available. The latest data shows, in 2013, U.S. oyster landings have yielded nearly 44.8 million pounds and valued at 217.5 million dollars. This is an increase of more than 35 percent of yield and over 40 percent of value compared with 2012 (David Van Voorhees et al. 2013).

Oysters marketed in the United States are always sold as whole in-the-shell oysters or as shucked meats. The whole in-the-shell oysters are shucked by consumers themselves; the shucked meats are shucked at the retail site (usually a restaurant) before being served to the consumers.

2.2 Adductor Muscle

Adductor muscle is the connective tissue of oysters, which has strength to hold two half-shells by attaching oyster body to the shells. Adductor muscle is usually in the big end of the whole oyster body (Fig 2.1). The exact location of adductor muscle and hinge line in relations to shell exterior dimensions was studied by Shays et al. (1980). The adductor muscle consists of two parts, including a fast reacting muscle tissue and a slow acting tissue (Wheaton 1970). For consumer general experiences and research evidences, adductor muscle has unique texture and flavor characteristics comparing with body. It has been reported that qualities of body and adductor muscle of oyster (Tanimoto et al. 2013) and scallop (Naidu and Botta 1978, Kawashima

and Yamanaka 1995) change differently during cold storage. It is therefore of interest to separate body and adductor muscle for determination of their characteristics.

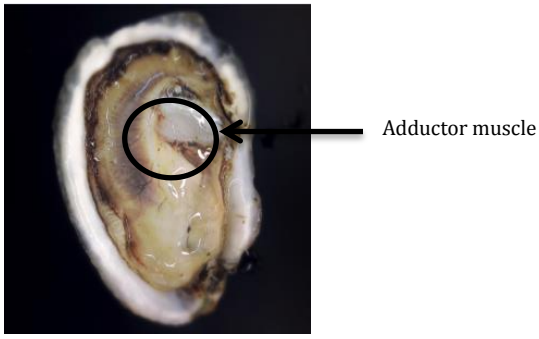


Figure 2.1 Oyster structure as seen from the inside of the shell valve

2.3 Aquaculture Method

The eastern oyster is ecological and economical specie along the coast and gulf. Oyster farmers now produce a large amount of oysters every year. But the traditional aquaculture method, which is never exposing oysters into air, always causes biofouling problem together with physical damage, mechanical interference, biological competition and environmental modification (Martin Wahl 1989). Biofouling is that gradual accumulation of organism such as bacteria attaching marine cultivated animals, and the organism may cause quality deterioration of marine food (Arakawa 1990). The main impacts of biofouling to aquaculture are restricting water exchange, increasing disease risk, and producing deformation of cages (Willemsen 2005). Thus biofouling is a problem and impediment to the oyster industry (C Enright 1993). Oyster desiccation, which is exposing oysters into air when culturing, appears to be a practical and cost-effective method to control biofouling (Campbell and Kelly 2002, Lane and Willemsen, 2004). One of current recommendations in the northern part of the Gulf of Mexico is desiccation every week for 24 hours.

2.4 Quality Evaluation

Sensory assessment by experienced people is wildly used on quality control in the oyster industry used to rely on a sensory assessment by experienced people. This way lacks standardization, and disagreements about quality evaluation of producers, buyers, and distributors (Andersonand Anderson 1991). Therefore, it is essential to develop a set of systematic methods to better control the quality of oysters in the processes of harvesting, distributing and consuming. The quality of oysters can be assessed by various physical and chemical properties analyzed by instruments, or by sensory properties adjunctively (Barnes et al. 2007).

2.4.1 Textural Analysis

A general agreement has been reached on the definition of texture, which states that texture is a major sensory property of food. Texture is detected by several senses, and the most important ones are the senses of touch and pressure (Gibson 1962). The classification and descriptions of textural parameters, which can be used to encompass textural senses, are shown in Table 2.1. These definitions and descriptions can be used in both instrumental measurement and sensory evaluation. It is reported that textural profiling analysis (TPA) and Warner-Bretzler shear (WBS) test can be performed as proper methods to determine the physical characteristics of scallop (Beltrán-Lugo et al. 2005). TPA contains the following seven parameters, 1) hardness, 2) adhesiveness, 3) cohesiveness, 4) springiness, 5) chewiness, 6) gumminess, and 7) resilience. The seven parameters of TPA could be determined through two cycles of penetration of an oyster sample and be calculated by computer software using values observed from the force-by-time plot (figure 2.2).

TPA is successfully applied to fishes and shellfishes. Zhu (Zhu et al. 2013) had reported that hardness and chewiness were the two important parameters for crisp grass carp fillets. Cruz-Romero and Kerry (2008) reported that there was no visible softening of the texture of oysters by shear strength measurement. Beltrán-Lugo et al. (2005, 2006) had revealed that changes of texture of restructured adductor muscle and adductor muscle were explained by decrease of collagen content, which is an important composition of connective muscle. The change of fish or shellfish muscle was also related to muscle moisture (Dunajski 1979; Beltrán-Lugo et al. 2006).

Table 2.1 Classification of textural characteristics (Szczesniak 1963 and 2002)

Primary parameters	Secondary parameters	Definition	Popular terms
Hardness		Force necessary to attain a given deformation	Soft→Firm→Hard
Cohesiveness	Brittleness	Extent to which a material can be deformed before it ruptures	Crumbly→Crunchy→Brittle
	Chewiness	Energy required to masticate a solid food to a state ready for swallowing: a product of hardness, cohesiveness and springiness	Tender→Chewy→Tough
	Gumminess	Energy required to disintegrate a semi-solid food to a state ready for swallowing: a product of a low degree of hardness and a high degree of cohesiveness	Short→Mealy→Pasty→Gummy
Viscosity		Rate of flow per unit force	Thin→Viscous
Springiness		Rate at which a deformed material goes back to its un-deformed condition after the deforming force is removed	Plastic→Elastic
Adhesiveness		Work necessary to overcome the attractive forces between the surface of the food and the surface of the other materials with which the food comes in contact.	Sticky→Tacky→Gooey

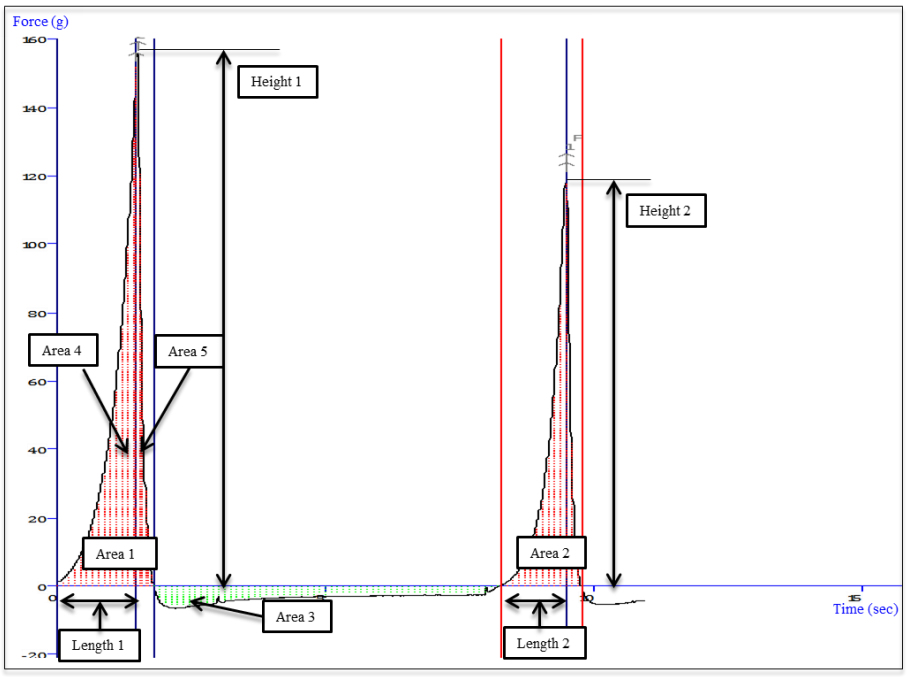


Figure 2.2 Typical force-by-time plot of textural profiling analysis (TPA) through two cycles of penetration of an oyster muscle sample to determine parameters, 1) hardness, peak force of the first compression, 2) cohesiveness = area2/area1, 3) springiness=length2/length1, 4) resilience = (area1- area2)/2, 5) chewiness = hardness*cohesiveness*springiness, 6) adhesion=area3, and 7) gumminess = area2/area1*hardness

2.4.2 Free Amino Acid (FAA) Analysis

Amino acids, along with a side-chain specific to each amino acid, are biologically important organics to food nutrition and flavor (Campos 2006). FAAs can provide the flavors of sweetness, bitterness, sourness, saltiness, and umami, and they are very important substances added to foods to enhance their flavors (Solms 1969). Three are three groups of FAAs classified and shown in table 2.1. The three groups are: group 1, those who have no flavor at all, or only a barely perceptible flavor; group 2, those who have complex flavor sensations, which are difficult to be evaluated in the pure state; group 3, those have distinctive flavors, either bitter or sweet, which are compared quantitatively with pure caffeine and sucrose solutions, respectively (Solms 1969).

Table 2.2 Taste of amino acids in 0.3% aqueous solutions [a, b] (Solms. 1969)

Amino acid	L-form	D-form
Group 1. Amino acids without taste		
Arginine	Flat	Sl. Sweet (D, L)
Aspartic acid	Flat	Flat
Isoleucine*	Flat	Flat
Lysine*	Flat	Flat
Proline	Flat, sl. Sweet	Flat (D, L)
Serine	Flat	Flat
Threonine	Flat	Flat
Valine*	Flat	Flat
Group 2. Amino acids with varying tastes		
Cysteine	Sulfurous	Sulfurous (D, L)
Glutamic acid	Unique, "glutamate"	---
Methionine*	Sulfurous, meaty	Sulfurous, meaty
Group 3. Amino acids with bitter or sweet tastes (as compared with solutions of caffeine or sucrose)		
Alanine	Sweet, 0.54% sucrose	Flat
Histidine	Flat	Sweet, 2.23% sucrose
Leucine*	Bitter, 0.011% caffeine	Sweet, 1.30% sucrose
Phenylalanine*	Bitter, 0.069% caffeine	Sweet, 2.20% sucrose
Tryptophan*	Bitter, 0.133% caffeine	Sweet, 11.00% sucrose
Tyrosine[c]*	Bitter, 0.017% caffeine	Sweet, 1.65% sucrose
Glycine	Sweet, 0.45% sucrose	Sweet, 0.45% sucrose

[a] The amino acids marked (D, L) were not available in pure D-isomer form; therefore, the race mates were used in the tests.
[b] pH adjusted to pH 6.0 with NaOH or HCl.
[c] Due to the low solubility of tyrosine, the tests were conducted at elevated temperatures.
[*] Essential amino acids.

Amino acids become zwitterion structures when dissolved in water. Their zwitterion structures have poor solubility near their isoelectric points, and most of them have poor UV absorbance. Therefore, high-performance liquid chromatography (HPLC) technology combined with the derivatization of the amino acid has become an important method for the FAA analysis (Jajić et al. 2013). Derivatization reagents can act on and modify the side-chain-structure of

amino acid (Figure 2.3), and then improve their solubility and UV absorbance. One of the most popular derivatizative reagents is o-phthaldialdehyde (OPA), which is suitable for the analysis of primary amines only, including aspartic acid, glutamic acid, asparagine, serine, glutamine, histidine, glycine, threonine, arginine, alanine, tyrosine, cysteine, valine, methionine, norvaline, tryptophan, phenylalanine, isoleucine, leucine and lysine (Turnell 1982). Derivatizative reagent 9-Fluorenyl-methyl chloroformate (FMOC) is another popular reagent used for the analysis of secondary amino acids, including hydroxyproline, sarcosine and proline (Einarsson 1985). OPA and FMOC reactions with amines and the absorbance wavelength of deviate are shown in figure 2.3. The wavelengths of HPLE detection are set at 338 nm and 262nm after derivatization using diode array detection (DAD).

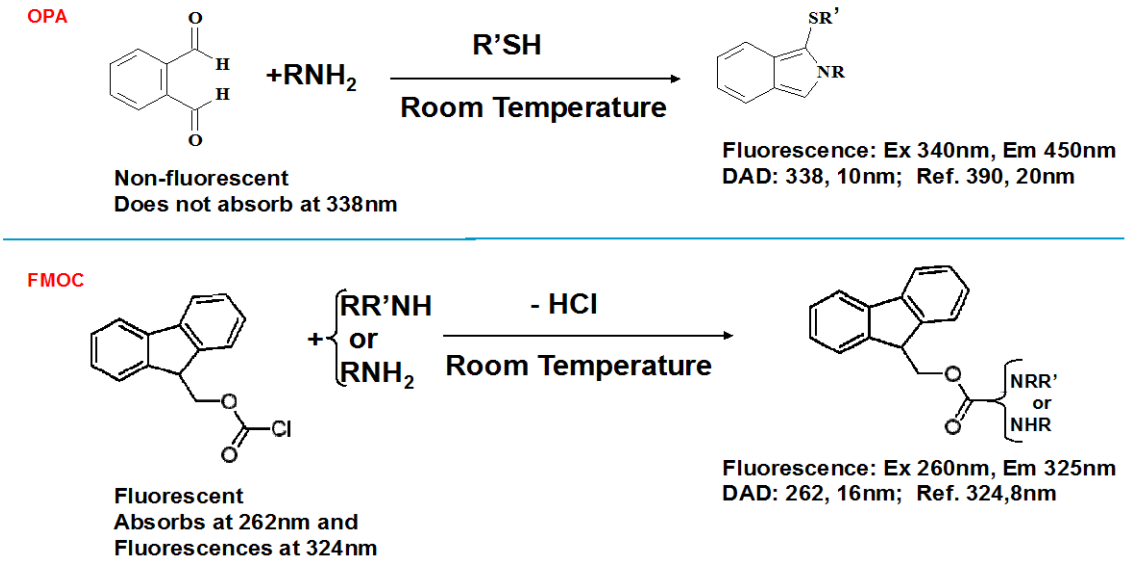


Figure 2.3 Ortho phthalaldehyde (OPA) and fluorenylmethoxy chloroformate (FMOC) reactions with amines (Henderson2010)

Many papers were published on FAA detection. FAAs of shellfish, scallop (Atungulu et al. 2007), Sydney rock oysters (*Saccostrea glomerata*) (Parker et al. 2009), European flat oysters (*Ostrea edulis*) (Jarayabhand and Newkirk 1989), Chinese mitten crab (*Eriocheir sinensis*) (Chen

et al. 2007), and snow crab (*Chionoecetes opilio*) (Miyagawa et al. 1990) were determined before. Different animals have different FAA profiles. Comparison of individual FAAs revealed that the levels of free threonine, free methionine, free arginine, and free lysine were greater of abalone than terrestrial domestic animals (King et al. 1996). Different species of one animal have similar FAA profiles, but the concentrations of FAAs are different. Vilasoa-Martınez (2007) compared FAAs between snow crab (*Chionoecetes opilio*) and other species. It found that snow crab (*Chionoecetes opilio*) has higher contents of free arginine and free lysine than other species.

The characteristic flavors of seafood depended on the presence of FAAs with high concentrations, such as high glycine reproducing the sweet flavor of scallop (Beltrán-Lugo 2006) and high glutamic acid and aspartic acid reproducing the famous sweet and umami flavor of Chinese mitten crab (*Eriocheir sinensi*) (Hanifah 2005; Chen 2007), high methionine reproducing the flavor of sea urchin (Kurihara 2009). In a study about FAAs of the Pacific oyster (*Crassostrea gigas*), the predominant FAAs were free glutamic acid, free alanine, free glycine, free taurine, and free proline for both body and adductor muscle similarly (Tanimoto et al. 2013).

The changes of FAAs are related to quality deterioration of food. Decrease of FAA is caused by amino acid degradation during storage, while protein degradation releases FAAs at the same time. The total FAA concentrations play important roles as functional substrates for animals to sustain prolonged and intensified cold stress. When temperature decreased, the balance between protein degradation and synthesis changed, which leads to the production of more FAAs (Zhou 2011). The study published by Shota Tanimoto et al. (2013) reported no significant changes of seventeen FAAs of shucked oyster body stored in the condition of salt water at 3°C for 7, with the exception of aspartic acid and tyrosine decreasing. These results suggested that FAAs were eluted from the cutting surface of the oyster.

2.4.3 Consumer Sensory Evaluation

Sensory evaluation is a scientific method used to measure and analyze how food products simulate our senses, such as sight, smell, taste, touch and hearing (Olafsdottir 1997). Consumer sensory evaluation, which is also known as affective test, is a measurement of preferences or acceptances to food products (O' Mahony1986). Due to the essential role of sensory activities in food industry, scientific sensory techniques are used to evaluate and control food quality. The information obtained from consumer sensory evaluation is extremely useful in food industry. Acceptance or preference measurements are widely used in sensory evaluation. They can be done on single product without comparison with another product. The consumers' acceptance or preference scores can express their degrees of likeability directly.

Consumer acceptance and preference tests of oysters were determined in previous studies based on paired comparison tests and acceptability ratings using half-shelled oysters from the same homogenous aquaculture and storage condition. The tests were conducted to understand the sensory characteristics of oysters. Jacobsen (2007) described the main attributes to oysters were sensation of saltiness, firmness and slipperiness and sweetness. Otwell and Garrido (2011) reported differences among four validated PHP operations (high pressure, low temperature freezing, gamma irradiation and mild heat) according to key sensory attribute saltiness and less earthy aroma during 14 days of storage. Chen (2011) studied about oyster (*Crassostrea virginica*) and compared the differences among oysters cultured in different locations. It was found by trained descriptive panel that the differences of salty attribute were significant among different locations, while the differences of sweet and umami attribute were not significant. Elliott (2010) also found differences among the sensory properties of oysters produced in different growing conditions and regions.

2.4.4 Linear Regression Analysis

Linear regression is defined “an approach for modeling the relationship between a scalar dependent variable y and one or more explanatory variables or independent variable denoted x” (Freedman 2009). Linear regressions have been used to develop mathematical models of sensory impact of food aroma and texture. Venkateshwarlu (2004) used linear regression to find the volatile lipid oxidation product (E,Z)-2,6-nonadienal and 1-penten-3-one having significant contribution toward off-flavors of fish oil and fish oil enriched foods. Caine (2002) established the relationship of TPA (hardness, cohesiveness and chewiness) and Warner-Bratzler shear (WBS) force of beef rib steaks with sensory characteristics (tenderness and palatability) obtained from trained panel (Caine et al. 2003). Another research found strong correlations between texture instrumental measurements (springiness, cohesiveness, hardness and chewiness) and texture sensory attributes (springiness, cohesiveness, hardness and chewiness) of abalones were published on 2002 (Sanchez-Brambila et al. 2002). There is little research about linear regression analysis on food flavor.

References

Anderson, J. G. and J. L. Anderson (1991). "Seafood quality: issues for consumer researchers." Journal of Consumer Affairs 25(1): 144-163.

Andrews, L. (2003). Consumer acceptability of post harvest processed and value added oysters. Proceedings of the 27th Annual Tropical and Subtropical Fisheries Technological Conference of the Americas, November.

Arakawa, K. Y. (1990). "Competitors and fouling organisms in the hanging culture of the Pacific oyster, *Crassostrea gigas* (Thunberg)". Marine and Freshwater Behaviour and Phy, 17(2): 67-94.

Atungulu, G., S. Koide, et al. (2007). "Ion-exchange membrane mediated electrodialysis of scallop broth: ion, free amino acid and heavy metal profiles." Journal of food engineering 78(4): 1285-1290.

Barnes, T., A. Volety, et al. (2007). "A habitat suitability index model for the eastern oyster (*Crassostrea virginica*), a tool for restoration of the Caloosahatchee Estuary, Florida." Journal of Shellfish Research 26(4): 949-959.

Beltrán-Lugo, A. I., Maeda-Martínez, A. N., Pacheco-Aguilar, R., Nolasco-Soria, H. G., and Ocaño-Higuera, V. M. (2005). "Physical, textural, and microstructural properties of restructured adductor muscles of 2 scallop species using 2 cold-binding systems." Journal of food science, 70(2): E78-E84.

Beltrán-Lugo, A. I., Maeda-Martínez, A. N., Pacheco-Aguilar, R., and Nolasco-Soria, H. G. (2006). "Seasonal variations in chemical, physical, textural, and microstructural properties of adductor muscles of Pacific lions-paw scallop (*Nodipecten subnodosus*)." Aquaculture, 258(1): 619-632.

Blinn, J. F. (1978, August). "Simulation of wrinkled surfaces." ACM SIGGRAPH computer graphics 12(3): 286-292).

Caine, W. R., Aalhus, J. L., Best, D. R., Dugan, M. E. R., and Jeremiah, L. E. (2003). "Relationship of texture profile analysis and Warner-Bratzler shear force with sensory characteristics of beef rib steaks." Meat Science, 64(4): 333-339.

Calvo, G. W., M. W. Luckenbach, et al. (2001). "A comparative field study of *Crassostrea ariakensis* and *Crassostrea virginica* in relation to salinity in Virginia." Journal of Shellfish Research 20(1): 221-230.

Campos, P., Martino, R. C., and Trugo, L. C. (2006). "Amino acid composition of Brazilian surubim fish (*Pseudoplatystoma coruscans*) fed diets with different levels and sources of fat." Food chemistry, 96(1): 126-130.

Cao, R., C.-H. Xue, et al. (2009). "Microbiological, chemical, and sensory assessment of Pacific oysters (*Crassostrea gigas*) stored at different temperatures." Czech J Food Science 27(2): 102-108.
Chen, D.-W., M. Zhang, et al. (2007). "Compositional characteristics and nutritional quality of Chinese mitten crab (*Eriocheir sinensis*)." Food Chemistry 103(4): 1343-1349.

Chen, L. (2011). Sensory and chemical characteristics of eastern oysters (*Crassostrea virginica*) doctoral dissertation, Virginia Polytechnic Institute and State University.

Civilie, G. V. and A. S. Szczesniak (1973). "Guidelines to training a texture profile panel." Journal of texture studies 4(2): 204-223.

Cruz-Romero, M., J. Kerry, et al. (2008). "Changes in the microbiological and physicochemical quality of high-pressure-treated oysters (*Crassostrea gigas*) during chilled storage." Food Control 19(12): 1139-1147.

Cruz-Romero, M. C., J. P. Kerry, et al. (2008). "Fatty acids, volatile compounds and colour changes in high-pressure-treated oysters (*Crassostrea gigas*)." Innovative Food Science and Emerging Technologies 9(1): 54-61.

David A. Freedman (2009). "Statistical models: theory and practice." Cambridge University Press: 26.

Durr, S. and D. I. Watson (2009). "Biofouling and antifouling in aquaculture." Biofouling: 267-282.

E Otter, D. (2012). "Standardised methods for amino acid analysis of food." British Journal of Nutrition 108(S2): S230-S237.

Einarsson, S. (1985). "Selective determination of secondary amino acids using precolumn derivatization with 9-fluorenylmethylchloroformate and reversed-phase high-performance liquid chromatography." Journal of Chromatography A 348: 213-220.

Enright, C. (1993). "Control of fouling in bivalve aquaculture: Biofouling has been identified as a major impediment to the development of a commercial oyster culture industry." World aquaculture-baton rouge 24: 44.

Frazier, J. M. (1975). "The dynamics of metals in the American oyster, *Crassostrea virginica*. I. seasonal effects." Chesapeake science 16(3): 162-171.

Fuke, S. and S. Konosu (1991). "Taste-active components in some foods: a review of Japanese research." Physiology and behavior 49(5): 863-868.

Gottschalk, G. (2012). "Bacterial metabolism." Springer science and business media.

Gibson, J. J. (1962). "Observations on active touch." Psychological review, 69(6): 477.

Henderson, J. W., and Brooks, A. (2010). "Improved amino acid methods using Agilent ZORBAX Eclipse Plus C18 columns for a variety of Agilent LC instrumentation and separation goals." Agilent Technologies, Wilmington, DE. http://www. chem. agilent. com/Library/applications/5990-4547EN. pdf.

Jacobsen, R. (2007). "A geography of oysters." Bloomsbury, New York, NY.

Jajić, I., S. Krstović, et al. (2013). "Validation of an HPLC method for the determination of amino acids in feed." Journal of the Serbian Chemical Society 78(6): 839-850.

Jarayabhand, P. and G. F. Newkirk (1989). "Effects of intraspecific competition on growth of the European oyster, Ostrea edulis Linnaeus, 1750." J. Shellfish Res 8(2): 359-365.

Je, J.-Y., P.-J. Park, et al. (2005). "Amino acid changes in fermented oyster (*Crassostrea gigas*) sauce with different fermentation periods." Food Chemistry 91(1): 15-18.

Jiang, S. T. and T. C. Lee (1985). "Changes in free amino acids and protein denaturation of fish muscle during frozen storage." Journal of Agricultural and Food Chemistry 33(5): 839-844.

Kawashima, K. and H. Yamanaka (1995). "Effects of freezing and thawing on post-mortem biochemical changes in scallop adductor muscle." Fisheries science 61(4): 691-695.

King, R. H., C. J. Rayner, et al. (1996). "The composition and amino acid balance of abalone (*Haliotis rubra*) tissue." Aquaculture 140(1): 109-113.

Kirimura, J., A. Shimizu, et al. (1969). "Contribution of peptides and amino acids to the taste of foods." Journal of Agricultural and Food Chemistry 17(4): 689-695.

Konosu, S., K. Watanabe, et al. (1988). "Taste-active components of scallop, 1: extractive components of scallop and identification of its taste-active components by omission test." Journal of the Japanese society for food science and technology (Japan).

Lawless, H. T., and Heymann, H. (2010). "Sensory evaluation of food: principles and practices (Vol. 5999)." Springer science and business media.

McMurray, P. and S. Ingber (2007). "Consider the oyster: a shucker's field guide." Australia.

O'Mahony, M. (1986). "Sensory evaluation of food: statistical methods and procedures (Vol. 16)." CRC Press.

Miyagawa, M., Y. Tabuchi, et al. (1990). "Changes in the free amino acid profile of snow crab *Chionoecetes opilio* muscle during storage in ice." Agricultural and biological chemistry 54(2): 359-364.

Naidu, K. and J. Botta (1978). "Taste panel assessment and proximate composition of cultured and wild sea scallops, *Placopecten magellanicus* (Gmelin)." Aquaculture 15(3): 243-247.

Nick Elliott (2010). "Pacific oysters, abalone undergo sensory evaluation." Global aquaculture advocate November/December: 40-50.

Olafsdottir, G., Martinsdóttir, E., Oehlenschläger, J., Dalgaard, P., Jensen, B., Undeland, I., and Nilsen, H. (1997). "Methods to evaluate fish freshness in research and industry." Food science and technology, 8(8): 258-265.

Onwulata, C. I., M. H. Tunick, et al. (2011). "5 Extrusion texturized dairy proteins: processing and application." advances in food and nutrition research 62: 173.

Otwell, S., L. Garrido, et al. (2011). "Assessment studies for Post-Harvest (PHP) oysters: part 1. consumer and expert sensory assessments." report to the interstate shellfish and sanitation conference, Food Science and Nutrition Department, University of Florida.

Parker, L. M., P. M. Ross, et al. (2009). "The effect of ocean acidification and temperature on the fertilization and embryonic development of the Sydney rock oyster *Saccostrea glomerata* (Gould 1850)." Global change biology 15(9): 2123-2136.

Peryam, D. R. and N. F. Girardot (1952). "Advanced taste-test method." Food engineering 24(7): 58-61.

Sanchez-Brambila, G. Y., Lyon, B. G., Huang, Y. W., Lyon, C. E., and Gates, K. W. (2002). "Sensory characteristics and instrumental texture attributes of abalones, *Haliotis fulgens* and *cracherodii*." Journal of food science, 67(3), 1233-1239.

Shays, D. and F. Wheaton (1980). "Biological properties of shellfish shells."

Solms, J. (1969). "Taste of amino acids, peptides, and proteins." Journal of agricultural and food chemistry 17(4): 686-688.

Songsaeng, S., P. Sophanodora, et al. (2010). "Quality changes in oyster (*Crassostrea belcheri*) during frozen storage as affected by freezing and antioxidant." Food chemistry 123(2): 286-290.

Szczesniak, A. S. (1963). "Classification of textural characteristics." Food science 28(4): 385-389.

Szczesniak, A. S. (2002). "Texture is a sensory property." Food quality and preference 13(4): 215-225.

Tanimoto, S., K. Kawakami, et al. (2013). "Changes in the free amino acid content of the shucked oyster *Crassostrea gigas* stored in salt water at 3℃." Fisheries and aquatic sciences 16(2): 63-69.

Turnell, D. and J. Cooper (1982). "Rapid assay for amino acids in serum or urine by pre-column derivatization and reversed-phase liquid chromatography." Clinical chemistry 28(3): 527-531.

Venkateshwarlu, G., Let, M. B., Meyer, A. S., and Jacobsen, C. (2004). "Modeling the sensory impact of defined combinations of volatile lipid oxidation products on fishy and metallic off-flavors." Journal of agricultural and food chemistry, 52(6): 1635-1641.

Vilasoa-Martínez, M., J. López-Hernández, et al. (2007). "Protein and amino acid contents in the crab, *Chionoecetes opilio*." Food Chemistry 103(4): 1330-1336.

Wahl, M. (1989). "Marine epibiosis. I. fouling and antifouling: some basic aspects." Marine ecology progress series 58: 175-189.

Wallace, R. K. (2001). "Cultivating the eastern oyster, *Crassostrea virginica.*"

Wheaton, F. (1970). "An engineering study of the Chesapeake bay oyster industry." Proceedings of the national shellfish association.

Wheaton, F. (1974). "Design constraints on an oyster shucking machine." Proceedings of the national shellfisheries association.

Wheaton, F. (2007). "Review of the properties of eastern oysters, *Crassostrea virginica*:part I. physical properties." Aquacultural engineering 37(1): 3-13.

Wheaton, F. W. and T. B. Lawson (1985). "Processing aquatic food products, Wiley."

Willemsen, P. (2005). "Biofouling in European aquaculture: is there an easy solution." European aquaculture society special publ(35): 82-87.

Zhou, M., A.-L. Wang, et al. (2011). "Variation of free amino acid and carbohydrate concentrations in white shrimp, *Litopenaeus vannamei*: effects of continuous cold stress." Aquaculture 317(1): 182-186.

Chapter 3 Textural Analysis of Three Aquaculture-Treated Live Eastern Oyster (*Crassostrea virginica*) and Changes during Cold Storage

Abstract

Eastern oyster (*Crassostrea virginica*) is an important oyster species with large harvest. In this study, textural analysis of live eastern oyster (*Crassostrea virginica*) was used to quantify the quality of eastern oysters. Differences among three treatments, as well as their changes during cold storage at 4°C were studied. Textural analysis was investigated for oyster body and adductor muscle separately by means of texture profiling analysis (TPA), and cutting force. The hardness and gumminess were important textural parameters for comparison of three treatments. Daily treatment had been studied having firmest, gummiest and chewiest body when people biting them, while weekly treatment having firmest, gummiest and chewiest adductor muscle when clamping two half-shells. Adductor muscle was found firmer and gummier than body. Loss of texture of both body and adductor muscle occurred during cold storage at 4°C.

Introduction

Oyster is one of the most abundant harvested mollusks in the world. It is a commercially important mollusk with large amount of yield. In 2013, oyster farmers grow nearly 44.8 million pounds valued at 217.5 million dollars of oysters. The consumption of oysters is also large due to their high nutritive values and unique textures.

Texture is a multi-parameter for the quality of oysters. Textural profiling analysis (TPA) and cutting force are commonly used for texture measurement of food products. TPA involves compressing the tested samples at least for two circles and quantifying the mechanical parameters from the recorded force-deformation curves. The mechanical parameters of TPA

consist of the following seven parameters: hardness (N), springiness (m), adhesiveness (N*s), cohesiveness, gumminess, chewiness (J), and resilience. The cutting force (N), which is also important textural parameter for food products, is measured at the maximum force required to cut through the samples (failure point).

TPA has been successfully applied to the textural analysis of shellfish. It has reported that no apparent softening of the texture of the ventral part of oysters (*Crassostrea gigas*) by measuring the shear strength (Cruz-Romero and Kerry et al. 2008). Beltrán-Lugo (2005 and 2006) has used TPA to compare textural properties of adductor muscle and restructured adductor muscles of Pacific lions-paw scallop (*Nodipecten subnodosus*), and found the textural properties were affected by seasonal variations and cold-binding systems. However, there is few investigation of TPA for the adductor muscle of oysters. Therefore, it is meaningful to determine the textural characteristics of oyster adductor muscle, which is separated from oyster body.

Materials and Methods

1. Oyster Samples

Eastern oysters were provided by the Auburn University Shellfish Lab, collected at the Portersville Bay Oyster Farm Park in Mississippi Sound near Coden, Alabama in November 2014. The oyster samples were split into three desiccation treatments. The three desiccation treatments were 1) daily exposure (referred as daily); 2) weekly exposure for 24 hrs, otherwise submerged (in an effort to control for biofouling) (referred as weekly); and 3) submerged everyday (never exposed to the air) (referred as never). The procedures of storing and handling samples are shown in figure 3.1. Eastern oysters were shipped by overnight service in coolers with ice packs covered by wet clothes but without preventing access of air to the oysters. In Biosystems Engineering Research Laboratory (BERL) of Auburn University, they were stored in

4 °C walk-in cold room with referred name marked on the coolers until further analysis. Before experiments, all eastern oysters were washed with clean water and shucked manually. The adductor muscle was separated from body of oyster by cutting the adductor muscle close to valves carefully.

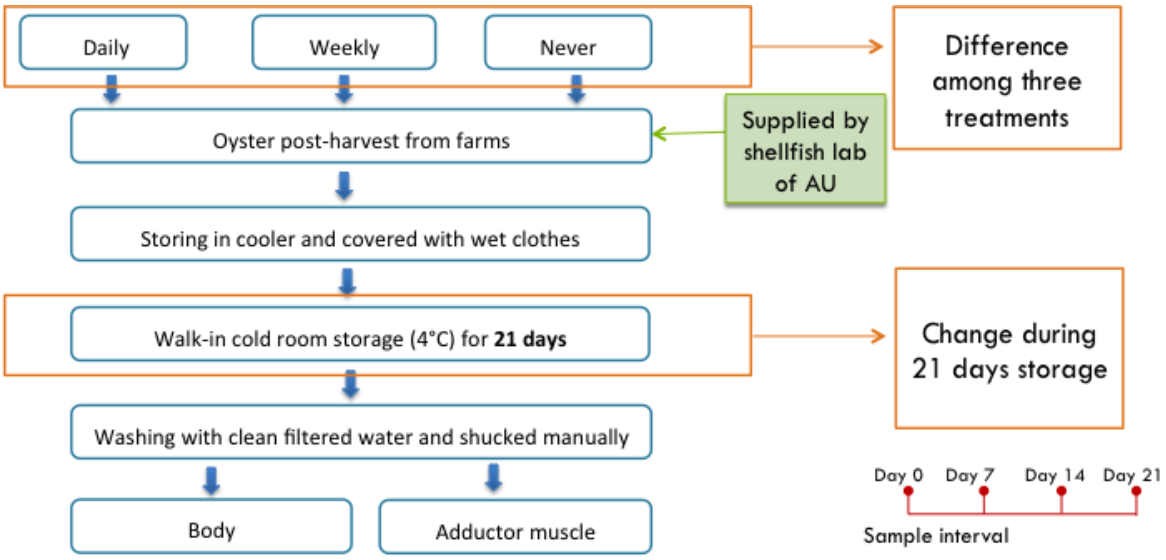


Figure 3.1 Procedures of storing and handling oyster samples

Textural analyses were carried out on day 0, day 7, day 14 and day 21 of cold storage. There were six oysters of each treatment left after finishing the experiments on day 21. On day 24, oysters began to die and the first dead oyster was from daily treatment. On day 25, six oysters of daily treatment died, and five oysters of never treatment died, while none of weekly treatment died. On day 25, six oysters of never treatment died, while four oysters of weekly treatment died. On day 26, five oysters of weekly treatment died. On day 27, all six oysters of weekly treatment died.

2. Textural Analysis Parameters

Textural profiling analysis (TPA) and cutting force test were performed at room temperature using a TA.XT.Plus Stable Micro Systems Texture Analyzer (Stable Microsystems Ltd., Surrey, England) with the Texture Expert software.

TPA was a double compression cycle test with the time period of 5.00s between two cycles of compression. P-25 probe was pressed into the adductor muscle of oyster samples at a constant speed of 1.00mm/s, until it reach 30% compression of the original height of the oyster samples. TA-30 probe was pressed into the body of oyster samples at the same speed and compression percentage. Force-time deformation curves were obtained with a 5 kg load cell applied at a cross-head speed of 2 mm/s. All samples were placed at the center of the plate when testing. Textural parameters were decided according the method of Gine (2004).

Cutting force test was a human-tooth-imitated test with a knife blade probe (length= 34.04 mm, width =9.90 mm, thickness =1.50 mm) to cut oyster samples. The blade side of probe (9.90 * 1.50 mm) was used to cut the sample 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 knife probe was performed to cut the muscle fiber perpendicularly at a speed of 2 mm/s. The cutting force (N) was measured at the maximum force required to cut through the samples (failure point). According to different initial thickness of oyster adductor muscle and body, tests were made at 3mm thickness for each oyster adductor muscle, and 5mm thickness for each oyster body, respectively. Three oysters from each treatment were used to perform the textural analysis.

3. Statistical Analysis

A two-way-factorial (3 treatments*4 storage periods) experimental design was applied to the experiment. All data were subjected to a statistical analysis. The reported data were the averages of samples from each of three individual oysters. All data are presented as means ± standard deviations (S.D.). Differences among means were carried out using Turkey's tests at a confidence level of 95% (p value < 0.05). The SAS software (version 9.3) package was used to perform these analyses.

Results and Discussion

1. Differences among Three treatments

As table 3.1 showed, for body, daily treatment had the highest hardness, gumminess and chewiness, followed by never treatment, and then weekly treatment. Because the oysters from daily treatment might be desiccated more times than oysters from other two treatments. For adductor muscle, weekly treatment had the highest hardness, gumminess and chewiness, followed by never treatment, and then daily treatment. The texture of adductor muscle affected its strength to hold two half-shells. Because the sizes of body and adductor muscle from daily treatment were much smaller than the other two treatments. In summary, daily treatment had firmest, gummiest and chewiest body when biting them, while weekly treatment had firmest, gummiest and chewiest adductor muscle when clamping two half-shells.

Table 3.1 Comparison of TPA and cutting force among three treatments on day 0

	Body			Adductor muscle		
Parameter	**Daily**	**Weekly**	**Never**	**Daily**	**Weekly**	**Never**
Hardness	462.51±209.43a	164.41±40.61b	278.12±31.70ab	1178.73±520.93abc	1806.91±320.87a	1305.05±62.80ab
Adhesiveness	-18.39±5.08a	-26.27±8.13a	-15.33±3.04a	-6.21±3.30a	-6.68±1.49a	-6.27±1.16a
Springiness	0.86±0.02a	0.75±0.09a	0.83±0.04a	0.51±0.21a	0.58±0.07a	0.67±0.03a
Cohesiveness	0.56±0.08a	0.60±0.04a	0.58±0.03a	0.29±0.04a	0.51±0.12a	0.53±0.13a
Gumminess	267.13±147.30a	88.01±14.55b	161.71±11.38ab	323.31±93.38bcde	897.53±141.04a	689.03±145.88abc

Chewiness	229.10±126.36a	65.50±11.08b	134.85±15.06ab	159.08±57.65b	521.05±130.87a	458.67±95.45a
Resilience	0.36±0.06a	0.27±0.02a	0.34±0.03a	0.24±0.04a	0.44±0.12a	0.44±0.15a
Cutting	81.66±25.27a	45.26±8.82a	74.03±11.83a	448.58±369.06a	132.60±29.63a	111.00±42.52a

*All values are means ± S.D. from textural analysis (n=3). Means with different letters in each column were significantly different from one another ($p < 0.05$)

Among seven TPA parameters and cutting force, only hardness, gumminess and chewiness indicated differences among three treatments. Zhu (Zhu et al. 2013) had reported that hardness and chewiness were the two important parameters for crisp grass carp fillets. The description of hardness and chewiness has been given as "Hardness was a force necessary to attain a given deformation, indicated firmness of oyster muscles; chewiness, which was an energy required to masticate a solid food to a state ready for swallowing" (Szczesniak 1963; Civille and Szczesniak 1973). Oyster muscles were soft and semi-solid food samples, which were much softer than fillets. Gumminess, an energy required to disintegrate a semi-solid food to a state ready for swallowing, was also important to oyster texture. Therefore, the three parameters hardness, gumminess and chewiness could be recommended as indicators to compare the differences of fresh (0 day of cold storage) eastern oysters among three treatments.

2. Differences between Body and Adductor Muscle

Figure 3.2 was drawn by data of three indicators hardness, gumminess and chewiness selected from table 3.1, in order to provide a clear pattern for comparison of differences between oyster body and adductor muscle. It showed that for daily treatment, hardness and gumminess of body were lower than adductor muscle on day 0, while chewiness of body was higher than adductor muscle. For weekly and never treatment, three parameters hardness, gumminess and chewiness of body were all lower than adductor muscle.

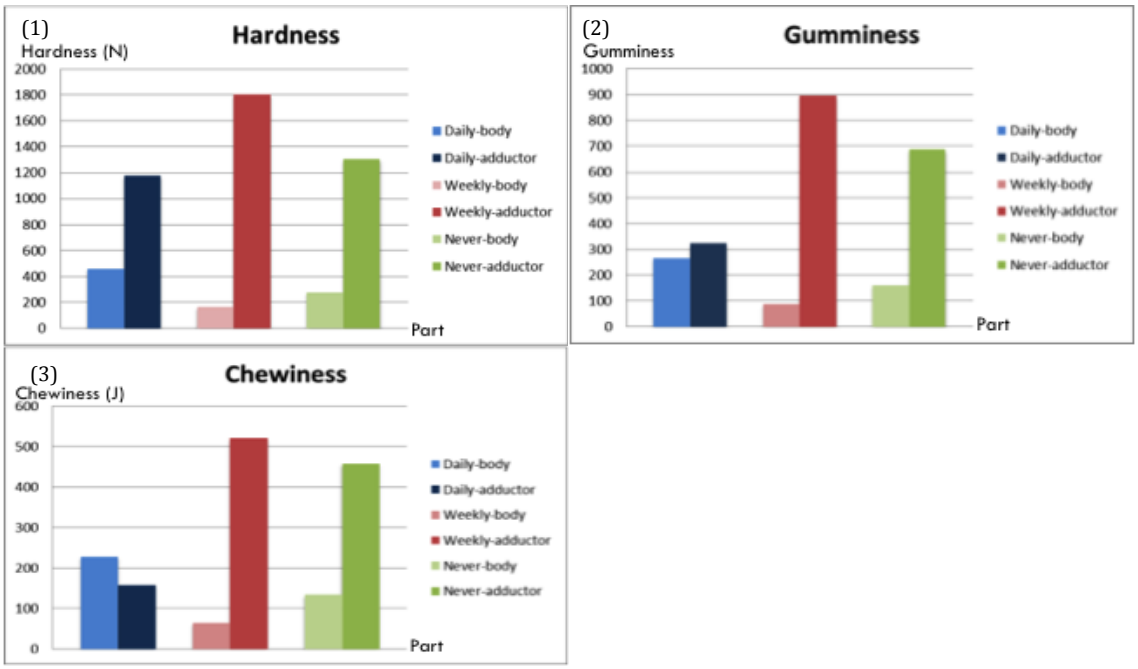


Figure 3.2 Comparison of textural parameters 1) hardness (N), 2) gumminess, and 3) chewiness (J) between body and adductor muscle on day 0; points with different letters in each curve were significantly different from one another ($p < 0.05$)

The results of hardness and gumminess from textural profiling analysis indicated firmer and gummier texture in oyster adductor muscle than body for three treatments. The low chewiness of daily adductor muscle showed it was easy for adductor muscle to reach the state for swallowing. Oyster adductor muscle has been studied having strong clamping strength. The differences of texture were explained being related to collagen contents of muscle by Beltrán-Lugo (2006). As to the connective tissue like adductor muscle of mollusk, the collagen content is an important component having a lower density of muscle fibers per surface area. This might be the reason why oyster adductor muscle was always being firmer than body.

3. Changes during Cold Storage

As shown in figure 3.3, with the delay of storage time, hardness, gumminess and chewiness of body of daily treatment decreased most during 21 days of storage, especially dropped dramatically from day 0 to day 7. The hardness, gumminess and chewiness of weekly treatment

remained unchanged during 21 days of storage. The hardness, gumminess and chewiness of never treatment significantly decreased from day 0 to day 7, then almost kept stable after day 7. Hardness is one of the basic parameters of food texture to show the extent of firmness when they are handled and chewed in mouth. Both gumminess and chewiness are properties of food when being swallowed. So the drop of hardness, gumminess and chewiness indicated that body of daily and never treatments became less firm and easier to chew and swallow during storage. It has been reported that texture of fish muscle is affected by its water-holding capacity, which meant more moisture relatively leads to softer texture during storage (Dunajski 1980). Additionally, muscle with lower protein content also has been studied with softer texture (Beltrán-Lugo et al. 2006). The increase of moisture and deterioration of protein could be related to drop of texture when storage time increased. For fresh (0 day of cold storage) oysters, hardness, gumminess and chewiness of body of daily treatment was highest, but it decreased most during 21 days of storage. On day 21, hardness, gumminess and chewiness were all the same no matter which treatment was (points marked with the same letter).

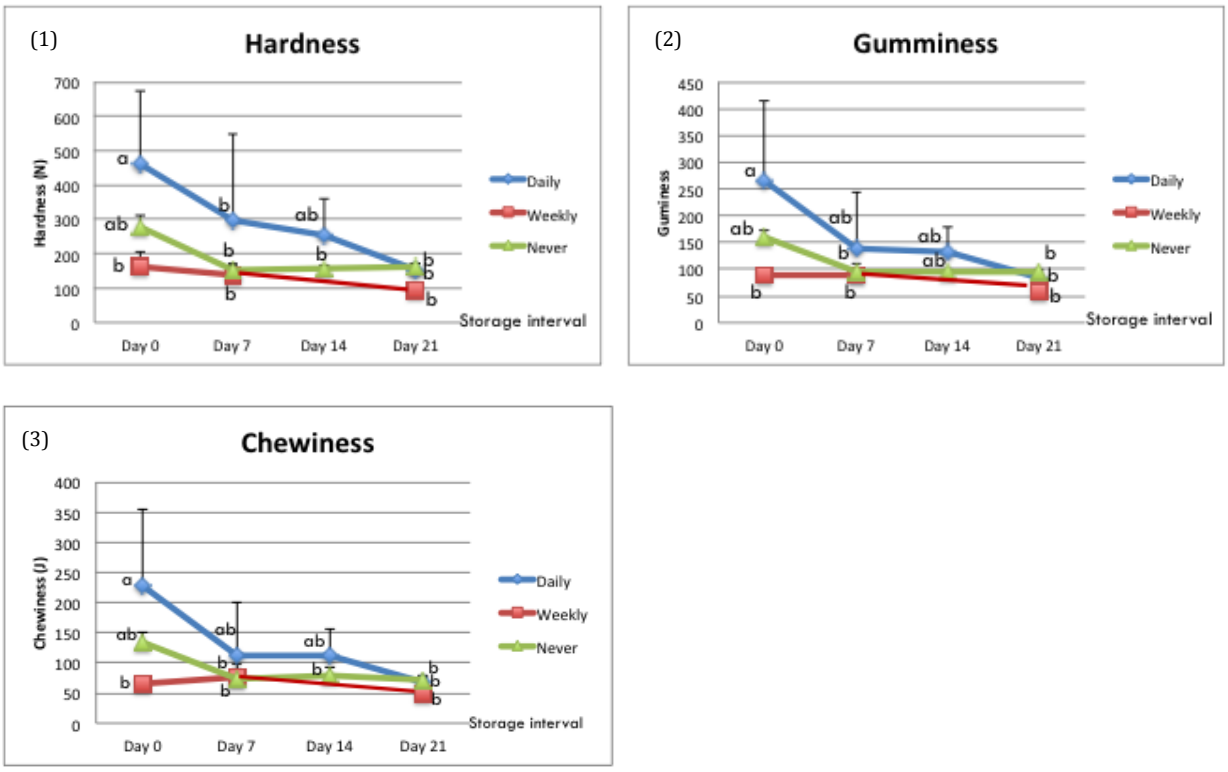


Figure 3.3 Changes of textural parameters 1) hardness, 2) gumminess, and 3) chewiness of body during 21 days of storage; points with different letters in each curve were significantly different from one another ($p < 0.05$)

Texture changes of adductor muscle during 21 days of cold storage are shown in figure 3.4, both of hardness and gumminess kept decreased. The chewiness increased on day 7, and then decreased. The results of textural profiling analysis indicated that oyster adductor muscle became less firm during 21days of cold storage. Texture loss of oyster adductor muscle is related to its water-holding capacity (Beltrán-Lugo et al. 2006) and collagen content (Beltrán-Lugo et al. 2005), and it could affect the clamping strength of adductor muscle as well as the freshness of oysters. Additionally, adductor muscle of weekly treatment had highest hardness and gumminess both at the beginning (day 0) and the end (day 21) of cold storage.

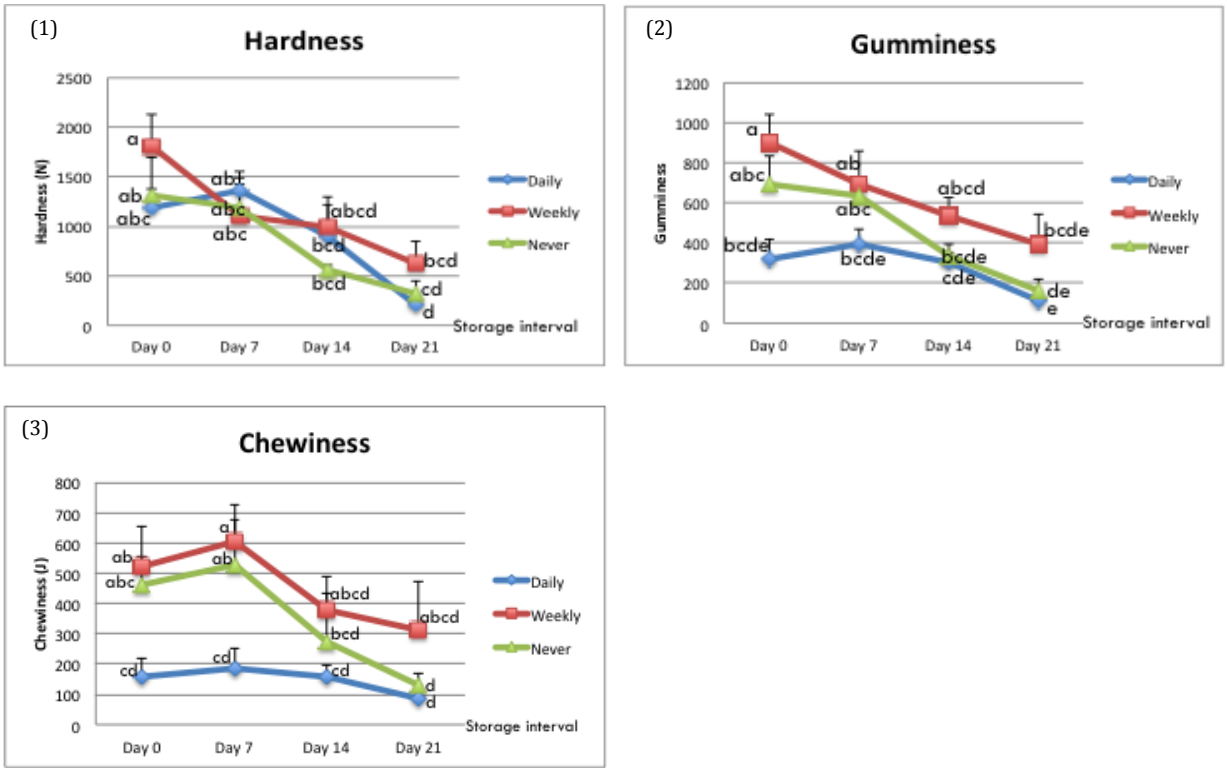


Figure 3.4 Changes of 1) hardness, 2) gumminess, and 3) chewiness of adductor muscle during 21 days of storage; points with different letters in each curve were significantly different from one another ($p < 0.05$)

Textural parameters, including adhesiveness, springiness, cohesiveness, resilience and cutting force of both body and adductor muscle didn't change significantly during 21 days of storage.

Conclusions

For three treatments, hardness, gumminess and chewiness could be recommended as indicators for comparison. Moreover, daily treatment had firmest, gummiest and chewiest body when people eating them, while weekly treatment had firmest, gummiest and chewiest adductor muscle when clamping two half-shells. For body and adductor muscle, adductor muscle was always firmer and gummier than body due to its strength to hold two half-shells. For sample

periods, loss of texture happened of both body and adductor muscle of three aquaculture-treated oysters, which was related to increase of moisture and decrease of protein content.

References

Beltrán-Lugo, A. I., Maeda-Martínez, A. N., Pacheco-Aguilar, R., Nolasco-Soria, H. G., and Ocaño-Higuera, V. M. (2005). "Physical, textural, and microstructural properties of restructured adductor muscles of 2 scallop species using 2 cold-binding systems." Journal of food science, 70(2): E78-E84.

Beltrán-Lugo, A. I., Maeda-Martínez, A. N., Pacheco-Aguilar, R., and Nolasco-Soria, H. G. (2006). "Seasonal variations in chemical, physical, textural, and microstructural properties of adductor muscles of Pacific lions-paw scallop (*Nodipecten subnodosus*)." Aquaculture, 258(1): 619-632.

Caine, W. R., J. L. Aalhus, D. R. Best, M. E. R. Dugan, and L. E. Jeremiah (2003). "Relationship of texture profile analysis and Warner-Bratzler shear force with sensory characteristics of beef rib steaks." Meat science 64(4): 333–39.

Civille, G. V., and Szczesniak, A. S. (1973). "Guidelines to training a texture profile panel. Journal of texture studies." 4(2): 204-223.

Chen, De-Wei, Min Zhang, and Sundar Shrestha. (2007). "Compositional characteristics and nutritional quality of Chinese mitten crab (*Eriocheir sinensis*)." Food Chemistry 103(4): 1343–49. Retrieved June 22, 2015

Dunajski, E. (1980). "Texture of fish muscle. Journal of texture studies." 10(4): 301-318.

Fitridge, Isla, Tim Dempster, Jana Guenther, and Rocky de Nys. (2012). "The impact and control of biofouling in marine aquaculture: a review." Biofouling 28(7): 649–69.

Gine, Rafael. (2004). "Effects of rearing temperature and strain on sensory characteristics , texture, colour and fat of *Arctic charr* (*Salvelinus alpinus*)." 15: 177–85.

Hough, Guillermo et al. (1996). "Partial Least Squares correlations between sensory and instrumental measurements of flavor and texture for Reggianito grating cheese." Food quality and preference 7(1): 47-53.

Jiang, S. T., B. S. Hwang, and C. Y. Tsao. (1987). "Protein denaturation and changes in nucleotides of fish muscle during frozen storage." Journal of the agricultural and food chemistry 32: 22-27.

Martin, Daniel E., John Supan, Uma Nadimpalli, and Steven G. Hall. (2007). "Effectiveness of a heat/cool technique for shucking oysters." Aquacultural engineering 37(1): 61–66.

Papadopoulos, V., I. Chouliara, A. Badeka, I.. Savvaidis, and M.. Kontominas. (2003). "Effect of gutting on microbiological, chemical, and sensory properties of aquacultured sea bass (*Dicentrarchus labrax*) stored in ice." Food microbiology 20(4): 411–20. Retrieved May 2, 2015.

Sanchez-Brambila, G. Y., B. G. Lyon, Y. W. Huang, C. E. Lyon, and K. W. Gates. 2002. "Sensory characteristics and instrumental texture attributes of abalones, *Haliotis Fulgens* and *Cracherodii*." Journal of food science 67(3): 1233–39.

Szczesniak, A. S. (1963). "Classification of textural characteristics." J. Food Science 28(4): 385-389.

Wheaton, Fred. (2007). "Review of the properties of eastern oysters, *Crassostrea virginica*. part I. physical properties." Aquacultural engineering 37(1): 3–13.

Willemsen, Pr. (2005). "Biofouling in european aquaculture: is there an easy solution." Aquaculture Europe 2005'conference, Trondheim, 1–6. Retrieved

Zhang, Jinglin. (2014). "Studies on methods and standards for evaluating quality of fresh post-harvest oyster (*Crassostrea virginica*)."

Zhu, Zhiwei, Zheng Ruan, Biansheng Li, Mingyan Meng, and Qingxiao Zeng. (2013). "Quality loss assessment of crisp grass carp (ctenopharyngodon Idellus C. et v) fillets during ice storage." Journal of food processing and preservation 37(3): 254–61.

Chapter 4 Fee Amino Acid Analysis of Three Aquaculture-treated Live Eastern Oysters (*Crassostrea virginica*) and Changes during Cold Storage

Abstract

The eastern oysters are abundant resources of shellfish along the seaboard. The study of free amino acid (FAA) detection is of economic interest because it is related to the quality and freshness of the eastern oysters (*Crassostrea virginica*). Predominant FAAs were identified and considered as main parameters of oyster flavor, including free glycine, free alanine, free glutamic acid, free arginine, free cysteine and free methionine. The predominant FAAs made the eastern oysters stand out as more sweet, 'glutamic' and sulfurous flavor. The bitter FAAs were also identified as main FAAs for the eastern oyster. For differences between body and adductor muscle, adductor muscle had more sweet and sulfurous flavor due to higher level of free glycine, free alanine, free cysteine, and free methionine contents. Body had more glutamic acid, which has unique and complex flavor and flavor-enhancing capacity. FAA analysis of differences among three treatments indicated daily treatment had more sweet and sulfurous flavor than other two treatments. FAA analysis of changes during storage showed, for the eastern oysters stored at 4 °C, the recommended shelf-life was less than 21 days due to the apparent increases of bitter FAAs of body on day 21; the best consumption time of daily treatment and never treatment was within 7 days, and it of weekly treatment was within 14 days due to the increases of free leucine of adductor muscle.

Introduction

Oysters are considered a delicacy around the world (Martin et al. 2007). Among all the species, the consumption of eastern oysters has become an important part of diet for consumers

in the United States. The oysters are often severed to consumers as live ones popularly. It is necessary to know flavor characteristics of live eastern oysters not only for oyster retails but also for consumers.

Mollusks such as oysters contain various types of non-protein nitrogenous compounds, including FAAs and peptides (Sakaguchi and Murata 1989). The characteristic flavors of mollusks depend on the composition and concentration of FAAs, and their changes mainly influence flavor quality. The purpose of this study is to identify predominant FAAs of the eastern oysters, and used them to determine differences among three aquaculture-treated oysters as well as their changes during cold storage.

FAA detection method in this study is high-performance liquid chromatography (HPLC) combined with derivatization. The zwitterion structure of FAAs has poor solubility near isoelectric point, and most of them have poor UV absorbance when dissolved in water (Fukumoto et al. 2005). Derivatizative with reagents OPA and FMOC was a decent method, which has been applied to amino acid analysis of scallop (Atungulu et al. 2007) and crab (Barrento et al. 2010). Both OPA and FMOC could act on and modify the side-chain-structure of amino acid, and then improve their solubility and UV absorbance in HPLC detection.

Materials and Methods

1. Oyster Samples

The oysters were the same as which in chapter 3.

2. Chemicals

2.1 Amino Acid Standard

Amino acid standard solution containing seventeen amino acids was purchased from Sigma-Aldrich Co. (St. Louis, MO, United States), transferred into 2-mL vial, and refrigerated at

4 °C once opened. The standard solution contained L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, L-valine and L-cystine. The concentrations of amino acid standards were 2.50 µMoles/mL, except for L-cystine with 1.25 µMoles/mL. Amino acid standard solution was diluted into seven concentrations (0.125, 0.25, 0.50, 1.00, 1.50, 2.00, and 2.50 µMoles/mL of single amino acid) for calibration curves detection. Particularly, the concentrations of L-cystine were 0.0625, 0.125, 0.25, 0.50, 0.75, 1.00, and 2.50 µMoles/mL.

2.2 Borate Buffers, OPA and FMOC

Borate buffers, OPA and FMOC were ready-made solutions purchased by Agilent Technologies (Logistics Center Americas, DE, United States). Borate buffer was refrigerated at 4 °C before experiment, and transferred into 2-mL vial in experiment. Both OPA and FMOC were unstable reagents, and they were transferred into 2-mL vial and refrigerated at 4 °C once opened to keep potent. All OPA, FMOC and borate buffer were replaced every 7 days during 21 days of experimental time.

2.3 Mobile Phase

The Na_2HPO_4, $Na_2B_4O_7 \cdot 10H_2O$ and NaN_3 used for the mobile phases were purchased from VWR International LLC. (United States). All solution preparations were made using deionized water. All the chemicals used for mobile phases were of HPLC reagent grade were used without further purification.

Mobile phase A was composed of 1.4g anhydrous Na_2HPO_4 and 3.8g $Na_2B_4O_7 \cdot 10H_2O$ dissolving in 100mL deionized water. After reagent crystals complete dissolution, the solution was filtered through 0.45-µm regenerated cellulose membranes, then 900mL deionized water

was added. Afterwards, 32mg NaN_3 was added, and adjusted to about pH 8.4 with 1.2mL concentrated HCl, and then several drops of concentrated HCl was added until pH reaching 8.2.

Mobile phase B was composed of acetonitrile, methanol, and water with the volume ratio of 45:45:10 (v:v:v).

3. Sample Pretreatment

Every two oysters of the same part (body or adductor muscle) and the same treatment (daily, weekly or never) were grounded together for 30s using blender. 0.2g grounded oyster together with 1mL 10% trichloroacetic acid (TCA) were mixed in 1.5-mL micro-centrifugal tubes. Then the mixture was centrifuged at 10,000 rpm for 15mins at 4 °C by VWR symphony 241 Microcentrifuge (VWR International LLC., United States). After complete precipitation of proteins, the supernatant was filtered through 0.45-μm regenerated cellulose membranes and transferred into 2-mL vials for HPLC detection.

4. Optimization of HPLC condition (Henderson and Brooks 2010)

4.1 Instruments

HPLC detection was conducted on Agilent 1260 Infinity Quaternary LC System (Agilent Technologies, Santa Clara, CA, United States) equipped with a 1260 quaternary pump (model G1311B), a 1260 vacuum degasses (model G1316A), a 1260 diode array detector (DAD) (model G1315D), a 1260 auto samplers (model G1329B), and a Chemstation software (Agilent Technologies, Santa Clara, CA, United States). An Agilent ZORBAX Eclipse Plus C_{18} Columns (25cm × 4.6mm, 5um) was used in HPLC detection. Two signals A and B were set. Signal A was set at 338nm wavelength and 10nm bandwidth, with 390nm reference wavelength and 20nm bandwidth. Signal B was set at 262nm wavelength and 16nm bandwidth, with 324nm reference

wavelength and 8nm bandwidth. All samples were derivatizated and injected by an automatic injection program.

4.2 Parameters

Sample injection volume was 10μL. HPLC analysis flow was 1.5mL/min of two mobile phases. The gradient elution was performed by varying the proportion of mobile phase A to mobile phase B. The mobile phase composition started at 2% mobile phase B for 0.84min, followed by an increase to 57% in 33.4mins, increased to 100% in 33.5mins, kept 100% until 39.3mins, and then decreased back to 2% in 39.4mins, continued decreasing to 0% and ended in 40mins.

5. Statistical analysis

The methods were the same as statistical analysis in chapter 3.

Results and Discussion

1. Calibration Curves

Different FAAs were identified according to different retention times. Seventeen calibration curves of amino acid standard were obtained. R-squares of linear calibration equations of FAA standards were in a range from 0.988 to 0.999, which showed the linearity was good. Thirteen to sixteen free amino acids were detected in a single sample.

2. Identification of Predominant FAAs

Predominant FAAs of seafood were usually identified as the FAAs with largest proportions of total FAAs. The characteristic flavors of seafood depended on the presence of predominant FAAs, such as high glycine concentration reproducing the flavor of scallops (Atungulu 2006) and high glutamic acid reproducing the flavor of Chinese mitten crab (*Eriocheir sinensis*) (Chen

et al. 2007). As shown in table 4.1, five FAAs with highest molar proportions accounted for a range of 82.81-95.67% of the total FAAs.

Table 4.1 Total molar proportions of predominant FAAs

Treatment	Part	Predominant FAA*	Total molar proportion (%)
Daily	Body	GLY, ALA, GLU, ARG, MET	82.81
	Adductor muscle	GLY, ALA, GLU, ARG, CYS	88.90
Weekly	Body	ALA, GLY, GLU, ARG, MET	87.48
	Adductor muscle	GLY, ALA, ARG, MET, CYS	93.00
Never	Body meat	ALA, GLY, ARG, GLU, MET	89.32
	Adductor muscle	GLY, ALA, ARG, GLU, CYS	95.67

* The order of bitter FAAs is from high to low in terms of concentration, from left to right.

Table 4.1 shows that body and adductor muscle of the eastern oysters had similar FAA profiles, but different concentrations of single FAAs. Thus free glycine (GLY), free alanine (ALA), free glutamic acid (GLU), free arginine (ARG), free cysteine (CYS) and free methionine (MET) had highest concentrations, and could be treated as predominant FAAs in both body and adductor muscle of the eastern oysters. Free glycine and free alanine were determined with the highest concentrations among all the FAAs. Both free glycine and free alanine were reported as sweet FAAs. Glutamic acid has a unique and complex 'glutamic' flavor, which can synergically act with nucleotide and enhance umami flavor. Free arginine does not contribute to the flavor. Free cysteine and free methionine belong to the sulfur-containing group of FAAs, especially free methionine has meaty flavor (Solms 1967). Particularly, the Maillard reaction of free cysteine with sugars can also produce meaty flavor (Van Boekel 2006). Therefore, sweet, 'glutamic' and sulfurous flavor were the major flavors of the eastern oysters.

The investigation on the Pacific oyster (*Crassostrea gigas*) showed that its predominant FAAs were free glutamic acid, free alanine, free glycine, free taurine, and free proline for both body and adductor muscle (Morihiko Sakaguchi and Michiyo Murata 1989, Tanimoto et al. 2013). The FAA profile of eastern oyster (*Crassostrea virginica*) (table 4.2) was similar to the Pacific oyster (*Crassostrea giga*). Free glutamic acid, free alanine and free glycine were abundant in both eastern oyster (*Crassostrea virginica*) and the Pacific oyster (*Crassostrea giga*). Moreover, free glutamic acid has also been studied as an important FAA attribute to the flavors of fish (Rabie et al. 2015) and crab (Chen et al. 2007). It could be found that sweetness and 'glutamic' flavor were the common attributes to oysters flavor, especially the 'glutamic' flavor also played important role in fish and shellfish.

3. Identification of Bitter FAAs

Bitter FAAs contains free leucine, free tyrosine and free phenylalanine. For these three treatments, the total molar proportions of bitter FAAs ranged in 0.15-1.25% of total FAA concentrations. Although the proportions of bitter FAAs were much lower than predominant FAAs, consumers were sensitive to bitterness due to their low taste thresholds. When dissolved in aqueous solutions as 0.3% (w/v), leucine, tyrosine and phenylalanine were equal to 0.011%, 0.017% and 0.069% caffeine, respectively (Solms 1969). Among three bitter FAAs, phenylalanine had the highest degree of bitterness. Schiffman (1979) reported that the taste threshold of bitter leucine and phenylalanine was much lower compared with sweet glycine and alanine. Therefore, concentrations of bitter FAAs could be considered as parameters in FAA analysis for the eastern oysters.

Table 4.2 Total molar proportions of bitter FAAs

Treatment	Part	Bitter FAA*	Total molar proportion (%)
Daily	Body	LEU,TYR, PHE	1.25
	Adductor muscle	LEU,TYR, PHE	0.18
Weekly	Body	LEU,TYR, PHE	0.67
	Adductor muscle	LEU,TYR, PHE	0.15
Never	Body	LEU,TYR, PHE	0.94
	Adductor muscle	LEU,TYR, PHE	0.43

* The order of bitter FAAs is from high to low in terms of concentration, from left to right.

4. Differences between Body and Adductor Muscle

Sixteen FAAs were detected in body of three aquaculture-treated oysters, but only thirteen to fourteen FAAs were detected in adductor muscle. Free threonine and free valine, which have no attributes to flavor, were not detected in adductor muscle. Free phenylalanine, which has bitter flavor, was not detected in both body and adductor muscle for most of the time during storage. In the study of scallop adductor muscle, free phenylalanine was also not detected when samples obtained in summer (Beltrán-Lugo et al. 2006). The presence of bitter free phenylalanine might depend on storage condition, storage time, and seasonal variation. But there was no report about the absence of free threonine and free valine for shellfish adductor muscle.

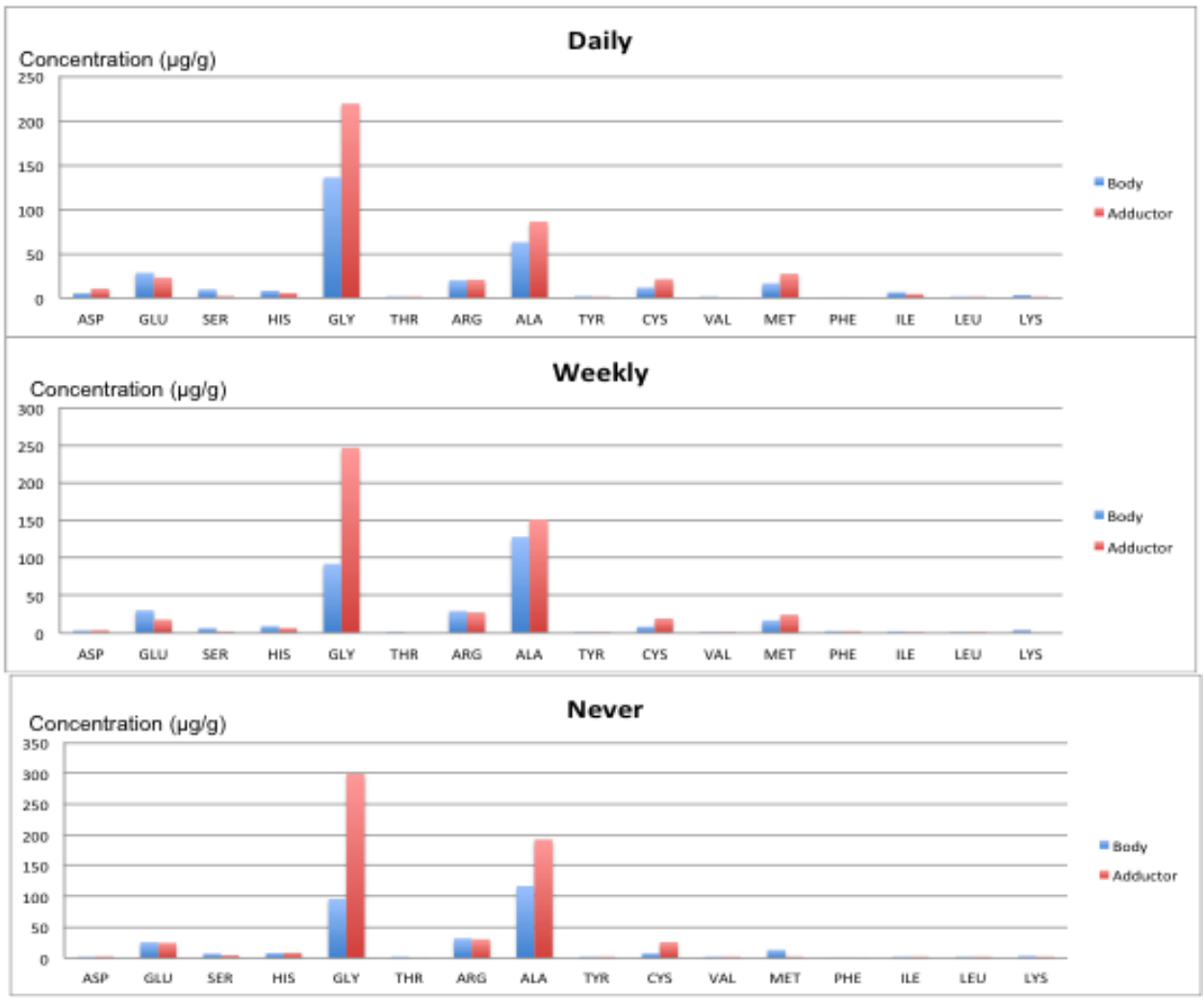


Figure 4.1 Comparison of FAA profiles between body and adductor muscle on day 0

For the comparison of FAA profiles in oyster body and adductor muscle on day 0 (figure 4.1), adductor muscle stood out with more sweet flavor and sulfurous flavor with higher level of free glycine, free alanine, free cysteine, and free methionine contents. The high concentrations of these FAAs made adductor muscle extraordinary delicious, as well as an enjoyable attribute to overall flavor. However, only free glutamic acid, which has unique and complex flavor and flavor-enhancing capacity, was higher in body than in adductor muscle. Scallop adductor muscle has been studied had abundant free glycine, free taurine, and free arginine contents accounting

for large proportions of the total FAAs (Beltrán-Lugo et al. 2006). Free glycine, which is already known as sweet FAA, is important for shellfish adductor muscle.

5. Differences among Three Treatments

Differences among three aquaculture-treated oysters were compared for predominant FAAs and bitter FAAs. The concentrations of predominant FAAs (μg/g) are illustrated in table 4.3. FAA constituents did not vary greatly in compositions among three treatments, but the amount of single FAA varied significantly. For oyster body, daily treatment stood out with significantly higher levels of free glycine and free cysteine, which attribute to sweet and sulfurous flavor (Solms 1967). For adductor muscle, daily and weekly treatment had much more sulfurous and meaty flavor from free methionine. The results showed that daily treatment had more sweet, sulfurous and meaty flavor than other two treatments in terms of FAAs of body and adductor muscle.

Table 4.3 Comparison of predominant FAAs among three treatments on day 0

AA	Attribute	Concentration (μg/g) in body			Concentration (μg/g) in adductor muscle		
		Daily	Weekly	Never	Daily	Weekly	Never
GLU	Unique, "glutamate"	28.58±3.85a	30.46±4.12a	25.84±0.07a	23.21±7.58a	18.25±2.15a	24.78±2.33a
GLY	Sweet	136.08±13.29a	92.23±10.71b	95.98±10.28b	219.27±52.25a	246.75±25.89a	299.74±27.15a
ARG	Flat	20.05±3.37a	29.40±9.34a	32.08±2.89a	219.27±52.25a	246.75±25.89a	299.74±27.15a
ALA	Sweet	62.94±12.48a	128.07±76.94a	117.21±50.52a	20.74±5.23a	27.88±5.13a	30.52±3.84a
CYS	Sulfurous	11.88±1.38a	8.80±0.38b	7.78±0.89b	21.44±4.53a	19.70±2.96a	26.08±1.88a
MET	Sulfurous, meaty	16.64±1.16a	17.18±1.03a	13.34±3.60a	27.49±11.66a	24.68±8.17a	2.36±1.51b

*All values are means ± S.D. from textural analysis (n=3). Means with different letters in each column were significantly different from one another ($p < 0.05$)

Table 4.4 shows the results of comparison of bitter FAAs among three treatments. For body, no significant difference of bitter FAAs existed among three treatments. For adductor muscle,

never treatment had more bitter FAAs. The increases of bitter FAAs are probably related to quality deterioration caused by bacteria growth and enzyme action. The desiccation method used in weekly and daily treatment can prevent this kind of quality deterioration to some extent.

Table 4.4 Comparison of bitter FAAs among three treatments on day 0

AA	Attribute	Concentration (μg/g) in body			Concentration (μg/g) in adductor muscle		
		Daily	Weekly	Never	Daily	Weekly	Never
LEU	Bitter	1.69±0.86a	1.33±0.56a	2.26±1.07a	0.72±0.05b	0.36±0.14c	1.14±0.04a
TYR	Bitter	2.29±1.54a	0.94±1.04a	0.72±0.15a	0.068±0.04a	0.23±0.13a	0.33±0.02a
PHE	Bitter	0a	0a	0a	0b	0.16±0.01a	1.13±0.67a

*All values were means ± S.D. from textural analysis (n=3). Means with different letters in each column were significantly different from one another ($p < 0.05$)

6. Changes of predominant FAAs during storage

In order to investigate the action of FAAs changes, figure 4.2 (1-6) provides valuable information. Figure 4.2 (1) and figure 4.2 (2) shows the changes of free glycine and free alanine, which always have the largest proportion of total FAAs. Free alanine concentration of daily treatment was determined lower than other two treatments, but its free glycine concentration was higher. The total sweet FAAs were not significantly different among three treatments if both of free glycine and free alanine were taken into consideration. During storage, sweet FAAs increased of daily treatment and never treatment on day 21, but decreased of weekly treatment on day 21. In figure 4.2 (3), free glutamic acid of weekly treatment and never treatment almost unchanged, but it of daily treatment sharply dropped on day 14. Fischer reported that glutamic acid at first tasted sour and then developed a peculiar “insipid” flavor (Kurihara 2009). Subsequently, Ikeda recognized the peculiar “insipid” flavor was umami taste (Kurihara 2009). The synergism between glutamate and nucleotides enhancing umami flavor was examined in by

Yamaguchi (1980). Based on the flavor-enhancing property of glutamic acid, drop of free glutamic acid concentration during cold storage weakened the flavor of daily treatment. The flat arginine (figure 4.2 (4)) and sulfurous cysteine (figure 4.2 (5)) concentrations of body were roughly constant during storage. They were related to the stability of muscle proteins during storage (Jiang et al. 1987). Arginine is a semi-essential amino acid that can be self-provided by live organisms. The concentration of arginine, a free basic amino acid, always keeps stable in live organism, but decreases during postmortem storage (Mackie et al. 1997). Cysteine forms disulfide bonds by covalently bonding their residues from each other. The disulfide bonds are related to folding and stability of proteins. It was reported that arginine and cysteine were also constant in body and adductor muscle of oyster (*Crassostrea gigas*) for 7 days of storage in salt water at 3 °C (Tanimoto et al. 2013). As shown in figure 4.2 (6), free methionine with sulfurous and meaty flavor dramatically decreased from day 14 to day 21 of all the three treatments. The results of FAA changes for oyster body showed that sweet flavor of daily treatment and never treatment became less, sweet flavor of weekly treatment became more; meaty and sulfurous flavor of three treatments became less during 21 days of storage. Thus the changes of predominant FAAs and their flavor sensation were complicated for oyster body.

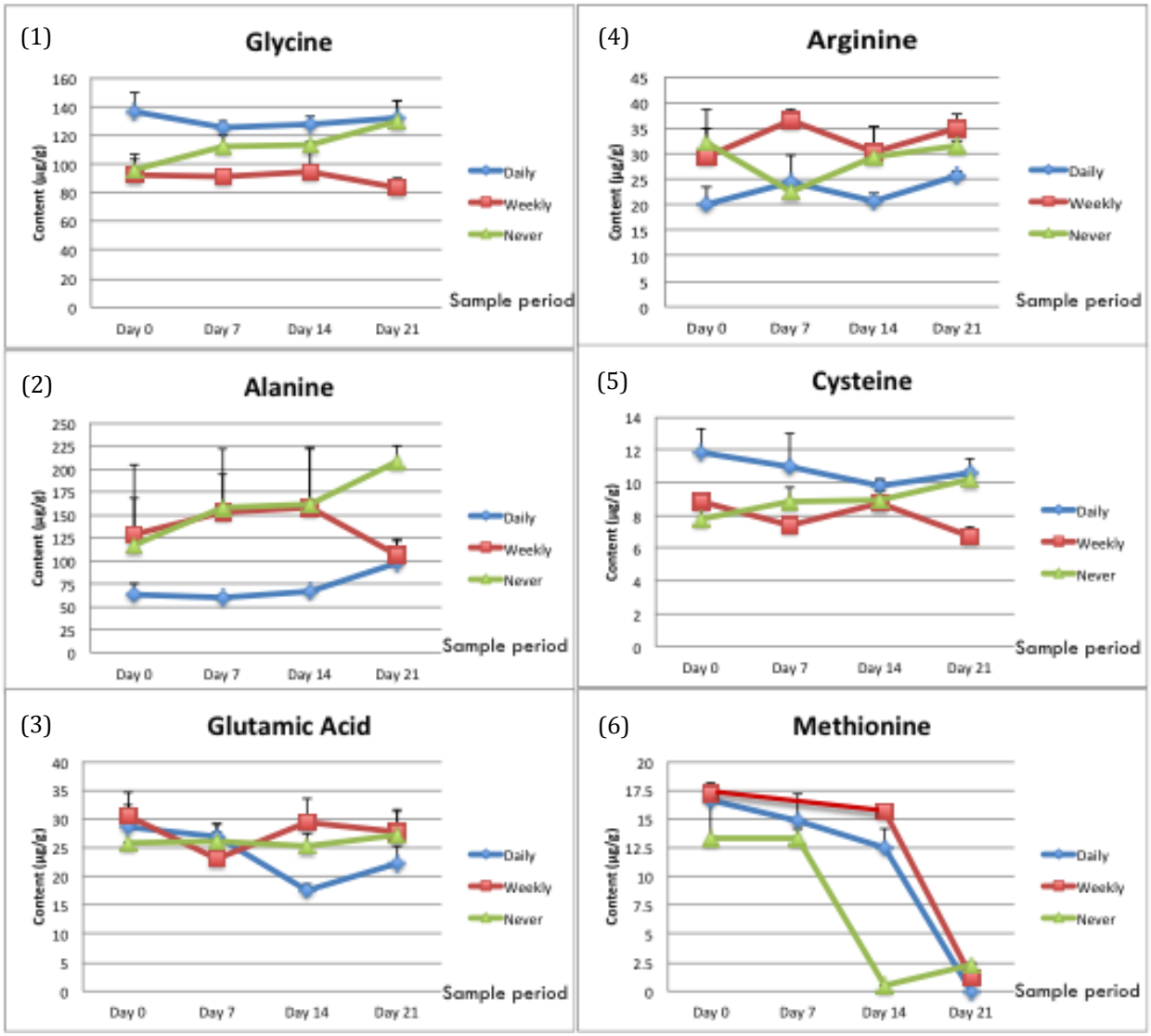


Figure 4.2 Change of predominant FAAs, 1) glycine, 2) alanine, 3) glutamic acid, 4) arginine, 5) cysteine, and 6) methionine of body during cold storage

The study published by Tanimoto (2013) reported, in the condition of salt water and at 3°C, there were no significant changes of fifteen FAAs or ammonia in shucked Pacific oyster (*Crassostrea gigas*) for 7 days of storage, only free aspartic acid and free tyrosine decreased. The results were the same with this study for 7 days of storage time. However, there is no study about changes of oyster FAAs for more than 7 days.

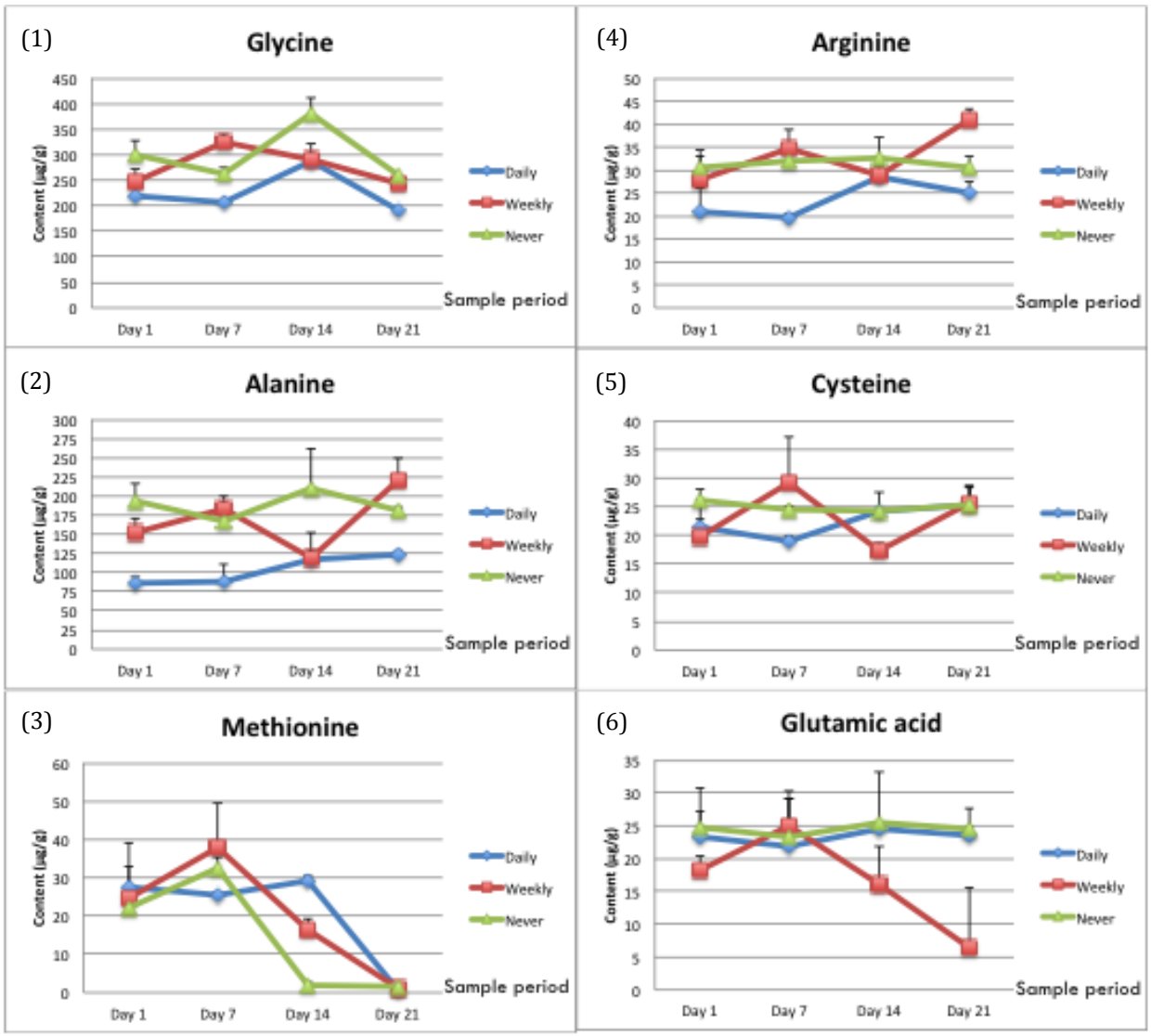


Figure 4.3 Change of predominant FAAs, 1) glycine, 2) alanine, 3) glutamic acid, 4) arginine, 5) cysteine, and 6) methionine of oyster adductor muscle during cold storage

The changes of single predominant FAAs in adductor muscle are showed in figure 4.3 (1-6). Free glycine, free alanine, free arginine and free cysteine of adductor muscle almost kept stable during storage. The trends were the same as these of body. It indicated sweet and sulfurous flavor of adductor muscle were almost constant during storage. As shown in figure 4.3 (3), free methionine concentration of three treatments all decreased during 21 days of storage, especially a great loss occurred from day 7 to day 21. It indicated adductor muscle lost sulfurous and meaty

flavor during storage. For free glutamic acid, it did not change much both in daily treatment and never treatment whereas it decreased in weekly treatment after day 7, which meant only weekly treatment lost 'glutamic' flavor after day 7. For adductor muscle, the flavor qualities of three treatments all decreased during storage.

7. Changes of Bitter FAAs during Storage

Free leucine, free tyrosine and free phenylalanine are three bitter FAAs, and their changes are shown in figure 4.4. Although the molar proportions of bitter FAAs are much lower than predominant FAAs, their presences influence oyster flavor due to their low threshold values. For 14 days of storage period, free phenylalanine was not detected in body of three treatments, and then, on day 21 it appeared in daily treatment with 0.15 μg/g of concentration, in never treatment with 0.21 μg/g of concentration. Free phenylalanine had bitter flavor, which was unhappy flavor for consumers. The increase of free phenylalanine showed the flavor quality of daily treatment and never treatment became worse on day 21, while it of weekly treatment didn't change. Besides, leucine and tyrosine concentrations fluctuated during the first 14 days of storage, but significantly increased on day 21 (Figure 4.4 (1-2)). Therefore, less than 21 days of shelf-life was recommended based on apparent increase of bitter FAAs on day 21.

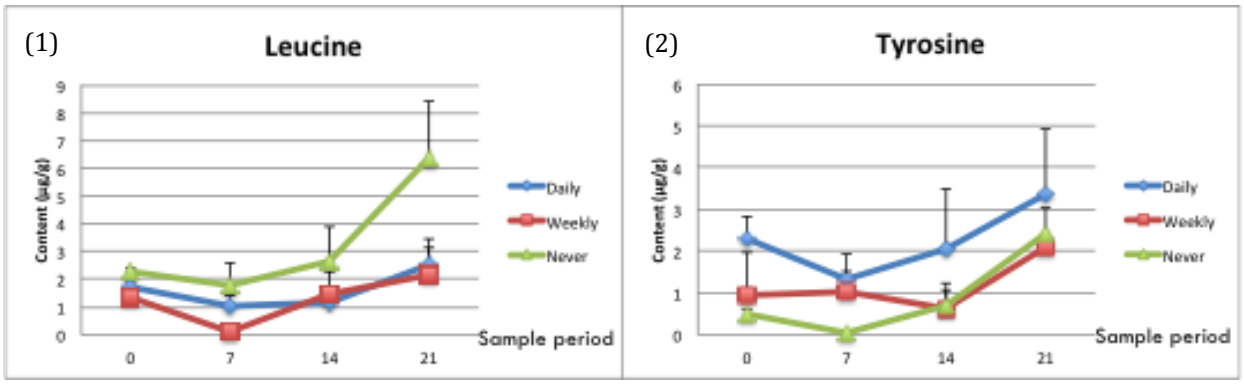


Figure 4.4 Changes of bitter FAAs, 1) leucine and 2) tyrosine of oyster body during storage

Changes of free leucine and free tyrosine od adductor muscle are shown in figure 4.5 (1-2). Free leucine of adductor muscle of daily treatment and never treatment increased from day 7, while it of weekly treatment increased from day 14. Free tyrosine of three treatments fluctuated during 21 days of storage. Free phenylalanine was not detected of three treatments for 21 days of storage. The increases of bitter FAAs of adductor muscle weakened its delicious flavor. It recommended that the best consumption time of daily treatment and never treatment was eating them within 7 days, and it of weekly treatment was within 14 days due to the increases of free leucine of adductor muscle,

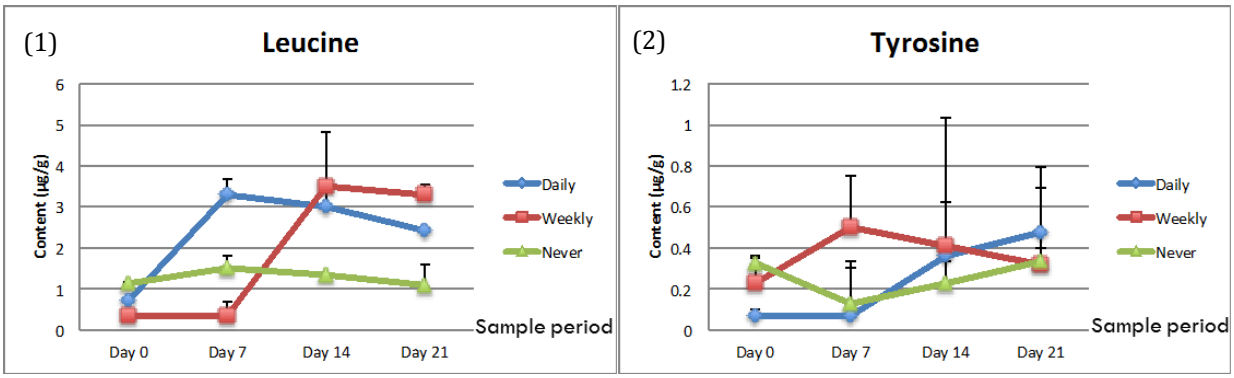


Figure 4.5 Changes of bitter FAAs, 1) leucine and 2) tyrosine of adductor muscle during storage

Conclusions

Predominant FAAs were free glycine, free alanine, free glutamic acid, free arginine, free cysteine and free methionine, which indicated that the eastern oysters stand out as sweet, 'glutamic' and sulfurous flavor. Bitter FAAs free leucine, free tyrosine and free phenylalanine were also important to oyster flavor due to their low taste threshold values. For differences between body and adductor muscle, adductor muscle had more sweetness and sulfurous flavor with higher level of free glycine, free alanine, free cysteine, and free methionine contents. Only free glutamic acid, which has unique and complex flavor and flavor-enhancing capacity, was higher in body than in adductor muscle. For three treatments, daily treatment had more sweet and

sulfurous flavor. For changes during storage, less than 21 days of shelf-life was recommended, because of apparent increases of bitter FAAs of oyster body on day 21. Moreover, the best consumption time of daily treatment and never treatment was eating them within 7 days, and it of weekly treatment was within 14 days due to the increases of free leucine of adductor muscle,

References

Atungulu, G., Koide, S., Sasaki, S., and Cao, W. (2007). "Ion-exchange membrane mediated electrodialysis of scallop broth: ion, free amino acid and heavy metal profiles." Journal of food engineering, 78(4): 1285-1290.

Barrento, S., Marques, A., Teixeira, B., Mendes, R., Bandarra, N., Vaz-Pires, P., and Nunes, M. L. (2010). "Chemical composition, cholesterol, fatty acid and amino acid in two populations of brown crab Cancer pagurus: Ecological and human health implications." Journal of Food Composition and Analysis, 23(7): 716-725.

Beltrán-Lugo, A. I., Maeda-Martínez, A. N., Pacheco-Aguilar, R., Nolasco-Soria, H. G., and Ocaño-Higuera, V. M. (2005). "Physical, textural, and microstructural properties of restructured adductor muscles of 2 scallop species using 2 cold-binding systems." Journal of food science, 70(2): E78-E84.

Beltrán-Lugo, A. I., Maeda-Martínez, A. N., Pacheco-Aguilar, R., and Nolasco-Soria, H. G. (2006). "Seasonal variations in chemical, physical, textural, and microstructural properties of adductor muscles of Pacific lions-paw scallop (Nodipecten subnodosus)." Aquaculture, 258(1): 619-632.

Chen, De-Wei, Min Zhang, and Sundar Shrestha. (2007). "Compositional characteristics and nutritional quality of Chinese mitten crab (*Eriocheir sinensis*)." Food chemistry 103(4): 1343–49. Retrieved June 22, 2015

E. Otter, Don. (2012). "Standardised methods for amino acid analysis of food." British journal of nutrition 108(S2): S230-37.

Fuke, Shinya and Shoji Konosu. (1991). "Taste-active components in some foods: a review of Japanese research." Physiology and behavior 49(5): 863–68. Retrieved June 22, 2015

Fukumoto, Kenta, Masahiro Yoshizawa, and Hiroyuki Ohno. (2005). "Room temperature ionic liquids from 20 natural amino acids." Journal of the American chemical society 127(8): 2398–99.

Gine, Rafael. (2004). "Effects of rearing temperature and strain on sensory characteristics , texture , colour and fat of *Arctic charr* (*Salvelinus alpinus*)." 15: 177–85.

Henderson, J. W. and a. Brooks. (2010). "Improved amino acid methods using Agilent ZORBAX Eclipse Plus C18 columns for a variety of Agilent LCiInstrumentation and separation goals." Agilent Technologies 1-16.

Je, Jae Young, Pyo Jam Park, Won Kyo Jung, and Se Kwon Kim. (2005). "Amino acid changes in fermented oyster (*Crassostrea gigas*) sauce with different fermentation periods." Food chemistry 91(1): 15–18.

Jiang, S. T., B. S. Hwang, and C. Y. Tsao. (1987). "Protein denaturation and changes in nucleotides of fish muscle during frozen storage." Journal of the agricultural and food chemistry 32: 22–27.

Kurihara, Kenzo. (2009). "Glutamate: from discovery as a food flavor to role as a basic taste (umami)." American journal of clinical nutrition 90(3): 1–8.

Mackie, I. M., Pirie, L., Ritchie, A. H., and Yamanaka, H. (1997). "The formation of non-volatile amines in relation to concentrations of free basic amino acids during postmortem storage of the muscle of scallop (*Pecten maximus*), herring (*Clupea harengus*) and mackerel (*Scomber scombrus*)." Food chemistry, 60(3): 291-295.

Martin, Daniel E., John Supan, Uma Nadimpalli, and Steven G. Hall. (2007). "Effectiveness of a heat/cool technique for shucking oysters." Aquacultural engineering 37(1): 61–66.

Morihiko Sakaguchi and Michiyo Murata. (1989). Seasonal variations of free amino acids in oyster whole body and adductor muscle. Nippon suisan magazines, 55(11): 2037-2041.

Papadopoulos, V., I. Chouliara, A. Badeka, I. Savvaidis, and M. Kontominas. (2003). "Effect of gutting on microbiological, chemical, and sensory properties of aquacultured sea bass (*Dicentrarchus labrax*) stored in ice." Food microbiology 20(4): 411–20. Retrieved May 2, 2015

Rabie, Mohamed, Livia Simon-Sarkadi, Hassan Siliha, Soher El-seedy, and Ahmed-Adel El Badawy. (2009). "Changes in free amino acids and biogenic amines of egyptian salted-fermented fish (*Feseekh*) during ripening and storage." Food chemistry 115(2): 635–38. Retrieved June 22, 2015

Rifkin, Bathsheva and Linda M. Bartoshuk. (1980). "Taste synergism between monosodium glutamate and disodium 5′-guanylate." Physiology and behavior 24(6): 1169–72. Retrieved June 22, 2015

Sakaguchi, Morihiko and Michiyo Murata. (1989). "Seasonal variations of free amino acids in oyster whole body and adductor muscle." Nippon suisan gakkaishi 55(11): 2037–41.

Sanchez-Brambila, G. Y., B. G. Lyon, Y. W. Huang, C. E. Lyon, and K. W. Gates. (2002). "Sensory characteristics and instrumental texture attributes of abalones, *Haliotis fulgens* and *Cracherodii*." Journal of food science 67(3): 1233–39.

Schiffman, S. S., Hornack, K., and Reilly, D. (1979). "Increased taste thresholds of amino acids with age." The American journal of clinical nutrition 32(8): 1622-1627.

Solms, Juerg, Swiss Federal. (1967). "The taste of amino acids, peptides, and proteins."

Tanimoto, Shota, Koji Kawakami, and Satoshi Morimoto. (2013). "Changes in the free amino acid content of the shucked oyster *Crassostrea gigas* stored in salt water at 3 ° C." 16(April): 63–69.

Van Boekel, M. A. J. S. (2006). "Formation of flavour compounds in the Maillard reaction." Biotechnology advances, 24(2): 230-233.

Wheaton, Fred. (2007). "Review of the properties of eastern oysters, *Crassostrea virginica*. part I. physical properties." Aquacultural engineering 37(1): 3–13.

Willemsen, Pr. (2005). "Biofouling in European aquaculture: is there an easy solution." Aquaculture Europe 2005'conference, Trondheim, 1–6. Retrieved.

Zhang, Jinglin. (2014). "Studies on methods and standards for evaluating quality of fresh post-harvest oyster *(Crassostrea virginica)*. master's thesis"

Zhu, Zhiwei, Zheng Ruan, Biansheng Li, Mingyan Meng, and Qingxiao Zeng. (2013). "Quality loss assessment of crisp grass carp (ctenopharyngodon Idellus C. et v) fillets during ice storage." Journal of food processing and preservation 37(3): 254–61.

Chapter 5 Linear Regression Relationships between Instrumental Parameters and Consumer Preferences of Live Eastern Oysters (*Crassostrea virginica*)

Abstract

Eastern oysters (*Crassostrea virginica*) are important consumptions in the United States. Consumer sensory evaluation can be conducted to better understand the consumer preferences of the eastern oysters, and provide useful information to oyster farmers and retails. In this study, it was performed to detect the differences among three treatments and changes when storage time increased. The results of consumer sensory evaluation showed that consumer preferences were not statistically different among three treatments (daily, weekly and never), either among three sample periods (0 day, 7 days and 14 days) in terms of texture likeability, flavor likeability and overall likeability. Additionally, linear regression analysis and stepwise model selection method were conducted to establish relationships between instrumental parameters and consumer preferences from sensory evaluation. The results of this study indicated no textural parameter affected texture likeability; sweet FAAs of body and sulfurous FAAs of adductor muscle positively correlated ($r = 0.010$) and negatively correlated ($r = -0.025$) with flavor likeability, respectively; sweet FAAs of body also positively ($r = 0.008$) correlated with overall likeability. The flavor attributes were more important than texture attributes to oyster consumptions.

Introduction

The eastern oyster (*Crassostrea virginica*) is one of the primary oyster species, which supports the oyster industry all over the world. Since the yield of oysters is increasing and the market of oysters is expanding every year, it is necessary for oyster farmers and retails to

understand the quality of the eastern oysters and the consumer attitudes towards the eastern oysters.

Sensory evaluation is a scientific method used to measure and analyze how food products simulate our sense, such as sight, smell, taste, touch and hearing (G. Olafsdottir 1997). Consumer sensory evaluation, which is also known as affective test, is a measurement of preferences or acceptances to food products (O' Mahony, M. 1986). The consumer sensory evaluation can be conducted to guide quality control and consumer researches of foods. The primary sensory attributes affecting consumer preferences are flavor and texture.

The quality of foods is not only derived from sensory properties, but also derived from chemical and physical properties. Instrumental methods are easy to perform on chemical and physical properties analysis. However, measuring a single parameter is not able to well explain the complicated interactions of sensory, chemical and physical properties. Therefore, studying the relationships between instrumental parameters and sensory evaluation is necessary.

It has been reported that linear regression analysis can be conducted to relate sensory properties to instrumental parameters. Moreover, stepwise method is widely used to select correlated predictors from various predictors. The stepwise model selection method has been applied to roast beef. Relationship between textural parameters and the sensory ratings from trained-panelist was established (Caine et al. 2003). Another research about abalone found strong correlations between its texture instrumental measurements and texture sensory attributes (Sanchez-Brambila et al. 2002). However, there was little study published about the relationship between FAA concentrations and sensory evaluation. It is meaningful to establish linear regression models in both texture and flavor aspects.

Materials and Methods

1. Oyster Samples

The oyster samples were split into three desiccation treatments (daily, weekly and never) as mentioned in chapter 3. All oysters were immediately refrigerated in plastic cup individually at 4 °C once being shucked manually. Three rounds of sensory evaluation were conducted on three sample periods (0 day, 7 days, and 14 days). In order to ensure oyster safety, consumer sensory evaluation was not conducted on day 21.

2. Consumer Participants

Auburn University Institutional Review Board (IRB) certified this sensory evaluation involving human subjects. 90 consumers (30 each round) participated in the sensory evaluation based on their availability and willingness to participate and acceptability of live oysters moderately or extremely. An information consent form describing the details of the study was given to consumer participants to review and sign on, in order to ensure all participants were not allergic to any seafood. The consumer participants consisted of faculties (approximate 35%) and students (approximate 65%) of Auburn University. The participants were those who did consume live oysters, and would be willing to buy live eastern live oysters. Their nationalities were United States, China, Indian, Korean and Nepal et al..

3. Sensory Evaluation

Oysters of three treatments (daily, weekly and never), were separated from each other in individual plastic cups, and served to consumers randomly. Distilled water and unsalted soda crackers were provided to purge the palate of residual flavor notes between samples. Consumers were asked to mark all scores of three samples on the same scale for each index (texture likeability, flavor likeability and overall likeability). Nine-point hedonic scale was used for

consumers to show their degrees of likeability. The nine points are shown as follows: 1 typically represents dislike extremely, 2 represents dislike very much, 3 represents dislike moderately, 4 represents dislike slightly, 5 represents neither like or dislike, 6 represents like slightly, 7 represents like moderately, 8 represents like very much, and 9 represents like extremely.

4. Statistical Analysis

Statistical analysis was conducted with SAS software 9.3. A two-way-factorial (3 treatments*3 storage periods) experimental design was applied to the data of consumer sensory evaluation. All data were presented as means ± standard deviations (S.D.). Differences among means were carried out using Turkey's tests at the confidence level of 95% (p value < 0.05).

Mean, minimum, maximum and standard deviations of the linear regression variables were calculated by the proc mean procedure. Pearson correlation coefficients were generated to describe the relationship between textural parameters and consumer preference scores by the proc corr procedure. Linear regression equations established with correlated indicators were developed by the proc reg procedure. Stepwise model selection method was conducted at the confidence level of 85%

5. Linear regression analysis

5.1 Variables

Description, mean, standard deviation (S.D.), minimum and maximum of each variable in multiple linear regression models were shown in table 5.1. In chapter 3, hardness, gumminess and chewiness were considered as important parameters for oyster texture. In chapter 4, the predominant free glycine, free alanine, free glutamic acid, free arginine, free cysteine, and free methionine were considered as important parameters for oyster flavor. In addition, bitter free leucine, free tyrosine, and free phenylalanine were also investigated important to the flavor of

eastern oysters. Therefore, the major flavors obtained from FAAs were sweet, 'glutamic', sulfurous, and bitter flavors. Concentrations of FAAs with the same flavor attributes were added together. Sweet FAAs and bitter FAAs were recalculated in terms of the quantitative amount (obtained from table 2.1) of pure sucrose and caffeine solutions, respectively. When dissolved in aqueous solutions as 0.3% (w/v), glycine and alanine are equal to 0.45 and 0.54% sucrose, respectively; leucine, tyrosine and phenylalanine are equal to 0.011%, 0.017% and 0.069% caffeine, respectively (Solms 1969). The equations of recalculation are shown as follows, in which GLY, ALA, LEU, TYR, and PHE represents the concentration of single FAA.

$$\text{SWE} = \frac{\text{GLY} \times 0.45 + \text{ALA} \times 0.54}{0.45+0.54}$$

$$\text{BIT} = \frac{\text{LEU} \times 0.011 + \text{TYR} \times 0.017 + \text{PHE} \times 0.069}{0.011+0.017+0.069}$$

Table 5.1 Descriptive statistics (n=9) for instrumental parameters

Variable	Description	Mean	S.D.	Minimum	Maximum
HAR-B	Hardness of body	245.56	105.90	138.69	462.51
GUM-B	Gumminess of body	137.95	58.60	88.01	267.13
CHE-B	Chewiness of body	113.72	51.37	65.50	229.10
HAR-A	Hardness of adductor muscle	1156	341.54	559.20	1807
GUM-A	Gumminess of adductor muscle	534.27	208.53	299.13	897.53
CHE-A	Chewiness of adductor muscle	363.23	174.97	157.60	605.96
GLU-B	'Glutamic' FAA of body	25.89	3.90	17.44	30.46
SUL-B	Sulfurous FAAs of body	22.03	5.59	9.41	28.52
SWE-B	Sweet FAAs of body	114.32	18.93	138.92	89.48
BIT-B	Bitter FAAs of body	0.35	0.13	0.19	0.60
GLU-A	'Glutamic' FAA of adductor muscle	22.50	3.30	15.99	25.59
SUL-A	Sulfurous FAAs of adductor muscle	24.20	10.39	1.72	37.80
SWE-A	Sweet FAAs of adductor muscle	206.88	46.99	141.78	287.66
BIT-A	Bitter FAAs of adductor muscle	0.21	0.10	0.08	0.52

Description, standard deviation (S.D.), minimum and maximum of each response in multiple linear regression models are showed in table 5.2. The responses are texture likeability, flavor likeability and overall likeability given by consumer participants.

Table 5.2 Descriptive statistics (n=9) for responses of consumer likeability scores

Variable	Description	Mean	S.D.	Minimum	Maximum
TL	Texture likeability score	6.59	0.43	6.12	7.15
FL	Flavor likeability score	6.64	0.52	5.96	7.41
OL	Overall likeability score	6.57	0.38	6.08	7.19

5.2 Models

It studied the question of sufficiency of instrumental parameter to the consumer preferences. Multiple models (figure 5.1) were used to establish linear regression relationships, as follows: 1) effects of hardness (HAR-B), gumminess (GUM-B), adhesiveness (ADH-B), springiness (SPR-B), cohesiveness (COH-B), chewiness (CHE-B), resilience (RES-B) and cutting force (CUT-B) in body to texture likeability (TL); 2) effects of hardness (HAR-A), gumminess (GUM-A), adhesiveness (ADH-A), springiness (SPR-A), cohesiveness (COH-A), chewiness (CHE-A), resilience (RES-A) and cutting force (CUT-A) in adductor muscle to texture likeability (TL); 3) effects of sweet FAA concentration (SWE-B), sulfurous FAA concentration (SUL-B), ‘glutamic’ FAA concentration (GLU-B) and bitter FAA concentration (BIT-B) in body to flavor likeability (FL); 4) effects of sulfurous FAA concentration (SUL-A) in adductor muscle to flavor likeability (FL); 5) effects of texture likeability (TL) and flavor likeability (FL) to overall likeability (OL); and 6) effects of sweet FAA concentration (SWE-B) in body and sulfurous FAA concentration (SUL-A) in adductor muscle (selected from model 1-4) to overall likeability (OL).

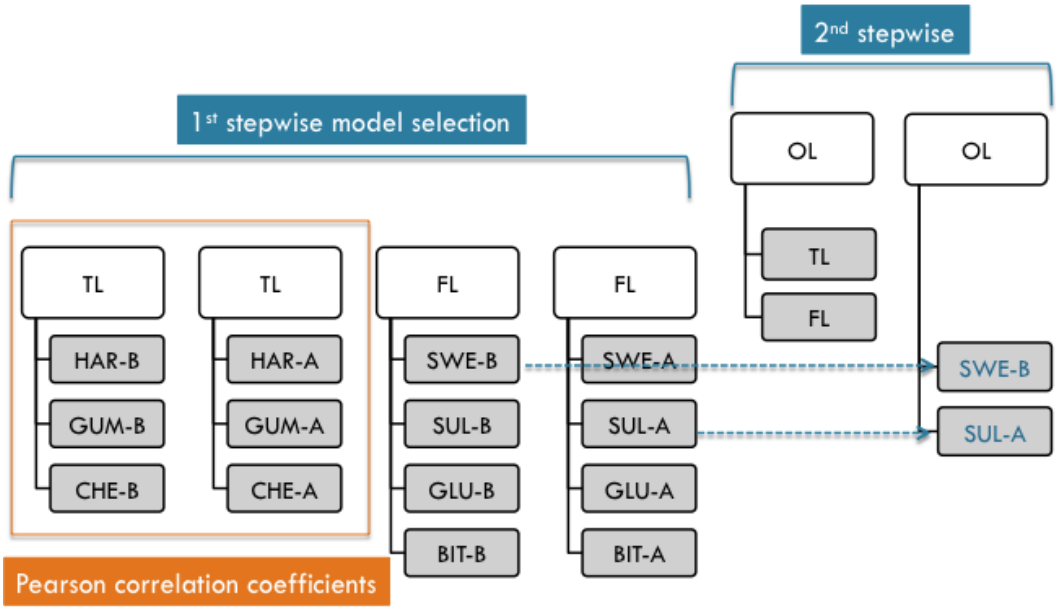


Figure 5.1 Multiple linear regression models and statistical analytical procedures

5.3 Procedures

Two rounds of stepwise model selection were conducted for the purpose of selecting correlated predictors to fit linear regression models at the confidence level of 85%. In the 1st round, linear regression models of texture likeability (TL) and flavor likeability (FL) were established. In the 2nd round, model of overall likeability (OL) was established with the significant predictors of the 1st round. Additionally, in the 2nd round, attributes of texture likeability (TL) and flavor likeability (FL) were also selected to fit linear regression model of overall likeability (OL). Linear regression analysis in this study did not take differences among treatments and sample periods into account. Pearson Correlation Coefficients were generated to verify the correlations between three textural parameters, including hardness (HAR), gumminess (GUM) and chewiness (CHE), and response texture likeability (TL).

Results and Discussion

1. Consumer sensory

1.1 Texture Likability

Texture, flavor and overall likeability of consumer preferences are shown in figure 5.2. The majority of consumer participants selected firmness as the key texture sensory attribute to the eastern oysters. However, consumer participants couldn't distinguish texture differences among three aquaculture-treated eastern oysters in terms of statistic analysis. Elliott (2010) revealed that firmness and chewiness were two important elements of the texture of the Pacific oysters. The firmness can be expressed by hardness. Therefore, the correlations between hardness and texture likeability were considered as a focus in linear regression analysis.

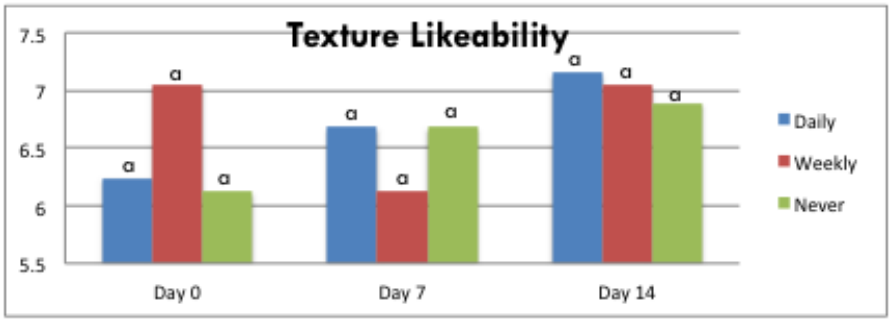


Figure 5.2 Texture likeability of consumer preference on day 0, day 7 and day 14 of storage

1.2 Flavor Likeability

For flavor likeability, consumer participants thought there were no significant differences among three treatments. The key flavor sensory contributing to the eastern oysters were saltiness and a little tone of sweetness. Saltiness, which is related to inorganic salt, has also been found as key attribute to oyster flavor in previous researches. Chen (2011) found the differences of saltiness were significant among oysters from different locations. Research of Otwell et al. (2011) also indicated that the key sensory attributes of post-harvest (PHP) oysters affecting consumer

favorable acceptance was also saltiness. However, the importance of sweetness, another key attribute found in this study, was barely reported in previous study of oysters.

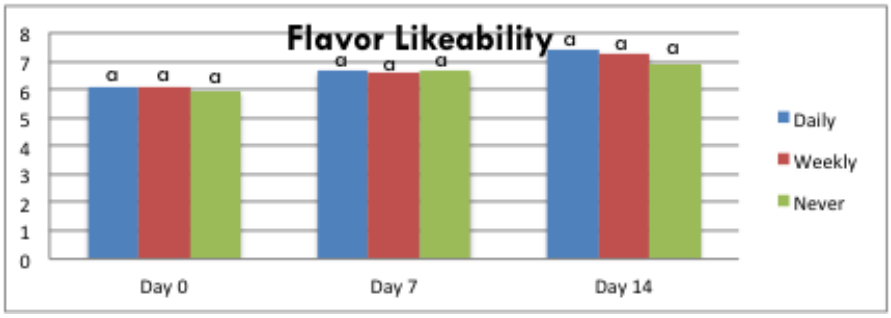


Figure 5.3 Flavor likeability of consumer preference on day 0, day 7 and day 14 of storage

1.3 Overall Likeability

For overall likeability, consumers were not able to statistically distinguish differences among three treatments, either among three sample periods, even though these differences had already been determined using instruments in chapter 3 and chapter 4. In this study, individual consumer who did eat live oysters very often were able to distinguish different kinds of oysters, but who liked but did not eat live oyster very often might not be able to distinguish different kinds of oysters easily. It leaded to no statistical difference determined by consumers. Trained descriptive sensory evaluation was performed on oyster (*Crassostrea virginica*), and found the differences among oysters from different locations (Chen 2011). Expert sensory evaluation was performed on post-harvest (PHP) oysters, and it indicated that differences existed among four validated PHP operated-oysters (high pressure, low temperature freezing, gamma irradiation and mild heat). It has been demonstrated that both trained sensory panels and expert could distinguish different kinds of oysters (Otwell et al. 2011). However, trained descriptive sensory evaluation and expert evaluation ignore consumer preferences for oysters. The consumer preferences evaluation can help to better understand the demand of oyster market in order to

improve their competitiveness. Thus it is necessary to collect data of sensory evaluation from consumer participants instead of trained panels or experts.

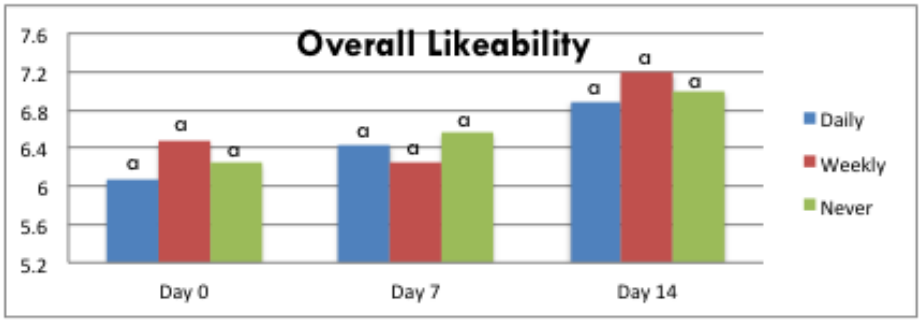


Figure 5.4 Overall likeability of consumer preference on day 0, day 7 and day 14 of storage

2. Linear Regression Model

Linear regression analysis was conducted to better understand the relationships among instrumental parameters and likeability scores. So the information of linear association could be related back to oyster quality. The correlated instrumental parameters could be considered as good indicators for oyster qualities.

2.1 Texture Likeability

Among instrumental hardness, gumminess and chewiness, no one met the confidence level of 85% and fit the linear regression model of texture likeability. Strong correlations, between instrumental hardness and chewiness, and sensory hardness and chewiness, were reported on shellfish as abalones (Sanchez-Brambila 2002). Trained descriptive sensory and classified index (hardness and chewiness) were used in the research of Sanchez-Brambila (2002). The trained panel and classified index could enhance the correlations between predictors and responses of linear regression models. However, the limitation was that the attributes of instrumental textural parameters to integrated texture preferences of consumers were still unknown. The consumer participants and integrated texture likeability in this research could work around this limitation.

Pearson Correlation Coefficient test (table 5.3) was conducted to verify the results obtained from linear regression analysis of texture likeability. It showed all the textural parameters had weak correlations with texture likeability. Therefore, it could be concluded that indeed no textural parameters significantly correlated to texture likeability.

Table 5.3 Pearson Correlation Coefficients of textural parameters with texture likeability of consumer preferences for oyster a) body muscle and b) adductor muscle

	HAR-A	**GUM-A**	**CHE-A**	**HAR-B**	**GUM-B**	**CHE-B**
TEX	-0.23377 (0.5449)	-0.29172 (0.4463)	-0.31653 (0.4066)	-0.36138 (0.3393)	-0.20714 (0.5928)	-0.26993 (0.4824)

2.2 Flavor Likeability

The sweet FAAs of body (SWE-B) and sulfurous FAAs of adductor muscle (SUL-A) had significant linear association (p value < 0.15) with flavor. Linear regression equations of flavor likeability were shown in table 5.4 (equation 1-2). For body, SWE-B was positively (r = 0.010) correlated with flavor likeability (FL) (equation 1), which meant sweetness could stimulate consumer preferences of oyster flavor. For adductor muscle, SUL-A was negatively (r = -0.025) correlated with flavor likeability (FL) (equation 2). However, the R-squares of equation (1) and equation (2) were 0.230 and 0.340, respectively. The reason why both of the R-squares were small might be the subjective judgment of consumer sensory. Another reason might be the sensations of flavor-active compounds were complicated, including interactions and synergy between them. It is difficult to obtain large R-squares of relationships between instrumental measurements and consumer preferences. The investigation of linear regression model showed concentrations of sweet FAAs and sulfurous FAAs correlated with consumer preferences and could be focused on in quality evaluation of the eastern oysters.

Table 5.4 Linear regression equations

No	Regression equations	MSE	R^2	P value
1	FL = 4.120 + 0.010 (SWE-B)	0.111	0.582	0.028
2	FL = 7.148 – 0.025 (SUL-A)	0.219	0.340	0.099
3	OL = 4.758 + 0.008 (SWE-B)	0.088	0.473	0.041
4	OL = 2.123 + 0.670 (FL)	0.026	0.817	0.0005

2.3 Overall Likeability

For overall likeability, after stepwise model selection from two variables (SWE-B and SUL-A), only SWE-B was significant to fit the linear regression model. Equation (3) illustrated SWE-B positively (r = 0.008) correlated to overall likeability (OL), which had similar trend of flavor likeability (FL). Sweet FAAs as alanine and glycine are studied abundant in both eastern oyster *(Crassostrea virginica)* and Pacific oyster *(Crassostrea giga).* The positive effect of sweet FAAs to overall likeability demonstrated that they were beneficial to oyster consumptions. Equation (3) also revealed that no textural parameter but one flavor variable was linear correlated with overall likeability (OL). The results could be verified by equation (4), which showed consumer overall likeability (OL) of oyster flavor were influenced by flavor likeability (FL) (p value = 0.0368 < 0.05), but not by texture likeability (TL) (p value = 0.6790 > 0.05). Equation (4) indicted when FL score increased per unit (one point), OL score increased 0.670 point. Therefore, it could be concluded the attribute of flavor was stronger than texture to oyster consumption.

Conclusions

For consumer sensory evaluation, no significant difference among three treatments, either no significant change during storage was found. For Linear regression analysis, no textural parameter significantly linear related to texture likeability. Sweetness of body and sulfurous flavor of adductor muscle were positively (r = 0.010) and negatively (r = -0.025) correlated to flavor likeability, and could be considered as good indicators (p < 0.15). Sweet FAAs of body

also positively ($r = 0.008$) correlated to overall likeability. The flavor attributes were more important than texture attributes to oyster consumptions.

References

Caine, W. R., J. L. Aalhus, D. R. Best, M. E. R. Dugan, and L. E. Jeremiah. (2003). "Relationship of texture profile analysis and Warner-Bratzler Shear force with sensory characteristics of beef rib steaks." Meat science 64(4): 333–39.

Chen L. "Sensory and chemical characteristics of eastern oysters (*Crassostrea virginica*)" dessertation. Virginia Polytechnic Institute and State University, 2011.

Hough, Guillermo et al. (1996). "Partial Least Squares correlations between sensory and instrumental measurements of flavor and texture for Reggianito grating cheese." Food quality and preference 7(1): 47–53.

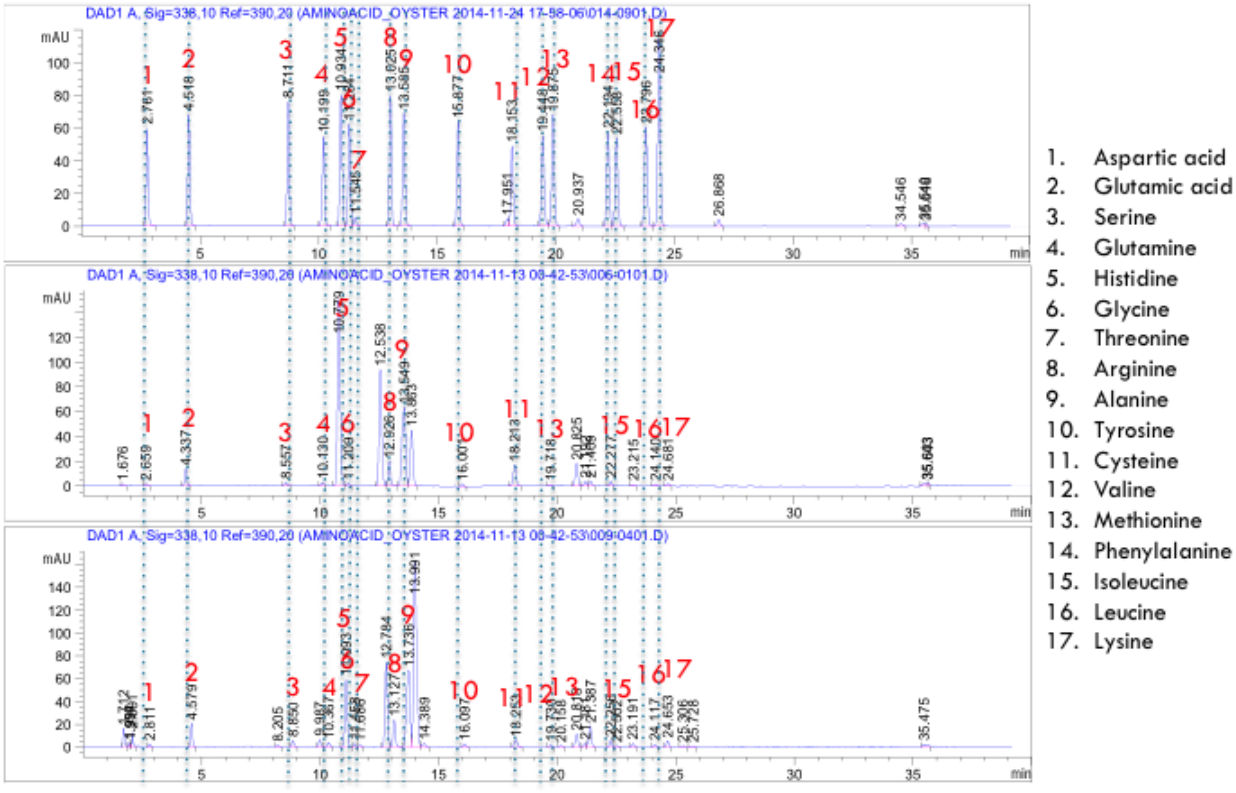
Hsu K C, Hwang J S, Chi H Y, et al. (2010). "Effect of different high pressure treatments on shucking, biochemical, physical and sensory characteristics of oysters to elaborate a traditional Taiwanese oyster omelette." Journal of the science of food and agriculture, 90(3): 530-535.

Nell J A, O'RIORDAN P J, Ogburn D M. (2006). "Consumer evaluation of diploid and triploid Pacific oysters subjected to high pressure treatment." Journal of shellfish research, 25(3): 1101-1104.

Otwell, S., Garrido, L., Garrido, V., and Sims, C. (2011, May). "Assessment studies for Post-Harvest (PHP) oysters: part 1. consumer and expert sensory assessments." in report to the Interstate Shellfish and Sanitation Conference, Food Science and Nutrition Department, University of Florida.

Sanchez-Brambila, G. Y., Lyon, B. G., Huang, Y. W., Lyon, C. E., and Gates, K. W. (2002). "Sensory characteristics and instrumental texture attributes of abalones, *Haliotis fulgens* and *Cracherodii*. " Journal of food science, 67(3): 1233-1239.

Appendix



HPLC chromatograms of FAAs of 1) standard, 2) adductor muscle, and 3) body

OYSTER SENSORY QUESTIONNAIRE

You will receive three treated-oysters randomized. **Please taste them and use the nine-point scale below to indicate how you liked or disliked for each attribute (overall liking, flavor liking, texture liking).** Please use unsalted cracker and water to clean palate before and between tasting samples.

Sample number ( )

Point	*9*	*8*	*7*	*6*	*5*	*4*	*3*	*2*	*1*
Attribute	**Like Extremely**	**Like Very Much**	**Like Moderately**	**Like Slightly**	**Neither Like Nor Dislike**	**Dislike Slightly**	**Dislike Moderately**	**Dislike Very Much**	**Dislike Extremely**
Overall liking									
Flavor liking									
Texture liking									

Sample number ( )

Point	*9*	*8*	*7*	*6*	*5*	*4*	*3*	*2*	*1*
Attribute	**Like Extremely**	**Like Very Much**	**Like Moderately**	**Like Slightly**	**Neither Like Nor Dislike**	**Dislike Slightly**	**Dislike Moderately**	**Dislike Very Much**	**Dislike Extremely**
Overall liking									
Flavor liking									
Texture liking									

Sample number ( )

Point	*9*	*8*	*7*	*6*	*5*	*4*	*3*	*2*	*1*
Attribute	**Like Extremely**	**Like Very Much**	**Like Moderately**	**Like Slightly**	**Neither Like Nor Dislike**	**Dislike Slightly**	**Dislike Moderately**	**Dislike Very Much**	**Dislike Extremely**
Overall liking									
Flavor liking									
Texture liking									

Please select the flavor of oysters (earthy, sweet, saltiness, metallic)

Please select the texture of oysters (juice, chewiness, firmness)

Name_____ Date_____

AUBURN
UNIVERSITY

COLLEGE OF AGRICULTURE

ANIMAL SCIENCES

INFORMED CONSENT
for a Research Study entitled
"Evaluation of Oysters Treated with Different Processing (Non-Thermal)"

You are invited to participate in a research study "Evaluation of oysters treated with different processing (non-thermal). The study is being conducted by *Jue Wang, research assistant*, under the direction of *Luxin Wang, Assistant Professor,* in the Auburn University Department of Animal Sciences. You were selected as a possible participant because you are not allergic to shell fish or oysters and are age 19 or older.

If you decide to participate in this research study, you will be asked to try three oysters and complete a brief questionnaire based on the evaluation of the different oysters. Your total time commitment will be approximately 5 min.

The risks associated with participating in this study are allergic to shell fish or oysters. To minimize these risks, we will ask you to provide the allergic history of yourself.
IF YOU HAVE AN ALLERGY TO OYSTERS OR SHELLFISH DO NOT PARTICIPATE IN THIS STUDY.

If you change your mind about participating, you can withdraw at any time during the study. Your participation is completely voluntary. If you choose to withdraw, your data can be withdrawn as long as it is identifiable. Your decision about whether or not to participate or to stop participating will not jeopardize your future relations with Auburn University, the Department of Animal Sciences or Luxin Wang__.

Your privacy will be protected. Any information obtained in connection with this study will remain confidential. Information obtained through your participation may be published in a professional journal.

210 UPCHURCH HALL
AUBURN, AL 36849-5415

TELEPHONE:
334-844-4160

FAX:
334-844-1519

www.auburn.edu

Participant's initials _____

AUBURN
UNIVERSITY

If you have questions about this study, *please ask them now or* contact Luxin Wang at 334-844-8146 or at lzw0022@auburn.edu. A copy of this document will be given to you to keep.

If you have questions about your rights as a research participant, you may contact the Auburn University Office of Research Compliance or the Institutional Review Board by phone (334)-844-5966 or e-mail at IRBadmin@auburn.edu or IRBChair@auburn.edu.

HAVING READ THE INFORMATION PROVIDED, YOU MUST DECIDE WHETHER OR NOT YOU WISH TO PARTICIPATE IN THIS RESEARCH STUDY. YOUR SIGNATURE INDICATES YOUR WILLINGNESS TO PARTICIPATE.

Participant's signature	Date	Principal investigator, Luxin Wang	Date

Printed Name

210 Upchurch Hall
Auburn, AL 36849-5415

Telephone:
334-844-4160

Fax:
334-844-1519

www.auburn.edu

Additional Consent Form for Descriptive Sensory Analysis

For people who want to attend descriptive sensory analysis of oysters.

By signing this form, I certify that I am not allergic to following shellfish, especially not allergic to oysters. I agree to participate in this study and will be responsible for any cost associated with medical considerment.

Shellfish:

Oyster	No		Yes		Crab	No		Yes	
Mussel	No		Yes		Octopus	No		Yes	
Clam	No		Yes		Abalone	No		Yes	
Shrimp	No		Yes		Cockle	No		Yes	
Prawn	No		Yes		Geoduck	No		Yes	
Lobster	No		Yes		Krill	No		Yes	
Crayfish	No		Yes		Scallop	No		Yes	
Cuttlefish/ Squid	No		Yes		Sea urchin	No		Yes	

If you are allergic to any oysters or any shellfish on the list, you are excluded from this study.

Sign _____ Date _____

Luxin Wang

AUBURN UNIVERSITY INSTITUTIONAL REVIEW BOARD for RESEARCH INVOLVING HUMAN SUBJECTS

REQUEST FOR EXEMPT CATEGORY RESEARCH

For Information or help completing this form, contact: **THE OFFICE OF RESEARCH COMPLIANCE**, 115 Ramsay Hall
Phone: 334-844-5966 **e-mail:** IRBAdmin@auburn.edu **Web Address:** http://www.auburn.edu/research/vpr/ohs/index.htm

Revised 2/1/2014 Submit completed form to IRBsubmit@auburn.edu or 115 Ramsay Hall, Auburn University 36849.

Form must be populated using Adobe Acrobat / Pro 9 or greater standalone program (do not fill out in browser). Hand written forms will not be accepted.

Project activities may not begin until you have received approval from the Auburn University IRB.

1. **PROJECT PERSONNEL & TRAINING**

PRINCIPAL INVESTIGATOR (PI):

Name Luxin Wang Title Assistant professor Dept./School Animal Science.
Address 210 upchurch Hall AU Email lzw0022@auburn.edu
Phone 334-844-8146 Dept. Head Wayne Greene

FACULTY ADVISOR (if applicable):

Name _____ Title _____ Dept./School _____
Address _____
Phone _____ AU Email _____

KEY PERSONNEL: List Key Personnel (other than PI and FA). Additional personnel may be listed in an attachment.

Name	Title	Institution	Responsibilities
Jue Wang	Graduate student	Auburn U.	conduct experiment

KEY PERSONNEL TRAINING: Have all Key Personnel completed CITI Human Research Training (including elective modules related to this research) within the last 3 years? ☑ YES ☐ NO
TRAINING CERTIFICATES: Please attach CITI completion certificates for all Key Personnel.

2. **PROJECT INFORMATION**

Title: Evaluation of oysters treated with different processing (Non-thermal)

Source of Funding: ☐ Investigator ☑ Internal ☐ External
List External Agency & Grant Number: _____
List any contractors, sub-contractors, or other entities associate with this project.
_____
List any other IRBs associated with this project (including those involved with reviewing, deferring, or determinations).
_____

FOR ORC OFFICE USE ONLY

DATE RECEIVED IN ORC: _____ by _____ APPROVAL # _____
DATE OF IRB REVIEW: _____ by _____ APPROVAL CATEGORY: _____
DATE OF ORC REVIEW: _____ by _____ INTERVAL FOR CONTINUING REVIEW: _____
DATE OF APPROVAL: _____ by _____
COMMENTS:

3. **PROJECT SUMMARY**

a. Does the research involve any special populations?

- ☐ YES ☑ NO Minors (under age 19)
- ☐ YES ☑ NO Pregnant women, fetuses, or any products of conception
- ☐ YES ☑ NO Prisoners or Wards
- ☐ YES ☑ NO Individuals with compromised autonomy and/or decisional capacity

b. Does the research pose more than minimal risk to participants? ☐ YES ☑ NO

Minimal risk means that the probability and magnitude of harm or discomfort anticipated in the research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests. 42 CFR 46.102(i)

c. Does the study involve any of the following?

- ☐ YES ☑ NO -Procedures subject to FDA Regulation Ex. Drugs, biological products, medical devices, etc.
- ☐ YES ☑ NO Use of school records of identifiable students or information from instructors about specific students
- ☐ YES ☑ NO Protected health or medical information when there is a direct or indirect link that could identify the participant
- ☐ YES ☑ NO Collection of sensitive aspects of the participant's own behavior, such as illegal conduct, drug use, sexual behavior or use of alcohol
- ☐ YES ☑ NO Deception of participants

If you checked "YES" to any response in Question #3 STOP. It is likely that your study does not meet the "EXEMPT" requirements. Please complete a PROTOCOL FORM for Expedited or Full Board Review.
You may contact IRB Administration for more information. (Phone: 334-844-5966 or Email: IRBAdmin@auburn.edu)

4. **PROJECT DESCRIPTION**

a. Subject Population (Describe, include age, special population characteristics, etc.)

general public (However, pregnant women and people who are allegic to shellfish or oysters will be avoided).

* Location: Poultry Science Building, Sensory lab, Room 256A.

b. Describe, step by step, all procedures and methods that will be used to consent participants.

☐ N/A (Existing data will be used)

All participants will be informed with the basic information about the source of the product, the way they are prepared and served. The potential risk of developing allergic reactions and the symptoms will also be explained. Participants will then read and sign the consent form. If they want to drop or do not want to sign the form, then they can not participate.

2 of 3

c. **Brief summary of project.** (Include the research question(s) and a brief description of the methodology, including recruitment and how data will be collected and protected.)

* First of all, only the People who are not allergic to shellfish or oysters will be hired for this study. Three samples will be randomly labeled and served to every panel. They will be asked to evaluate the overall flavor, overall texture and overall acceptance level of the product.

- Scale of 9-1 will be used.

They will rank the like the most a # of 9. extremely dislike (1).

Numbers will be averged - and student t-test will be used for data analysis.

Panels will also be asked to rank their overall likelihood of the product (oyster).

d. **Waivers.** Check any waivers that apply and describe how the project meets the criteria for the waiver.

- ☐ Waiver of Consent (Including existing de-identified data)
- ☐ Waiver of Documentation of Consent (Use of Information Letter)
- ☐ Waiver of Parental Permission (for college students)

e. **Attachments.** Please attach Informed Consents, Information Letters, data collection instrument(s), advertisements/recruiting materials, or permission letters/site authorizations as appropriate.

Signature of Investigator _____ Date 11/4/2014

Signature of Faculty Advisor _____ Date _____

Signature of Department Head _____ Date 11-4-14

SENSORY EVALUATION OF OYSTER

We would appreciate your participation!

TIME: NOV 5, 2014 WEDNESDAY 1PM-5:00PM

NOV 12, 2014 WEDNESDAY 10:00AM-5:00PM

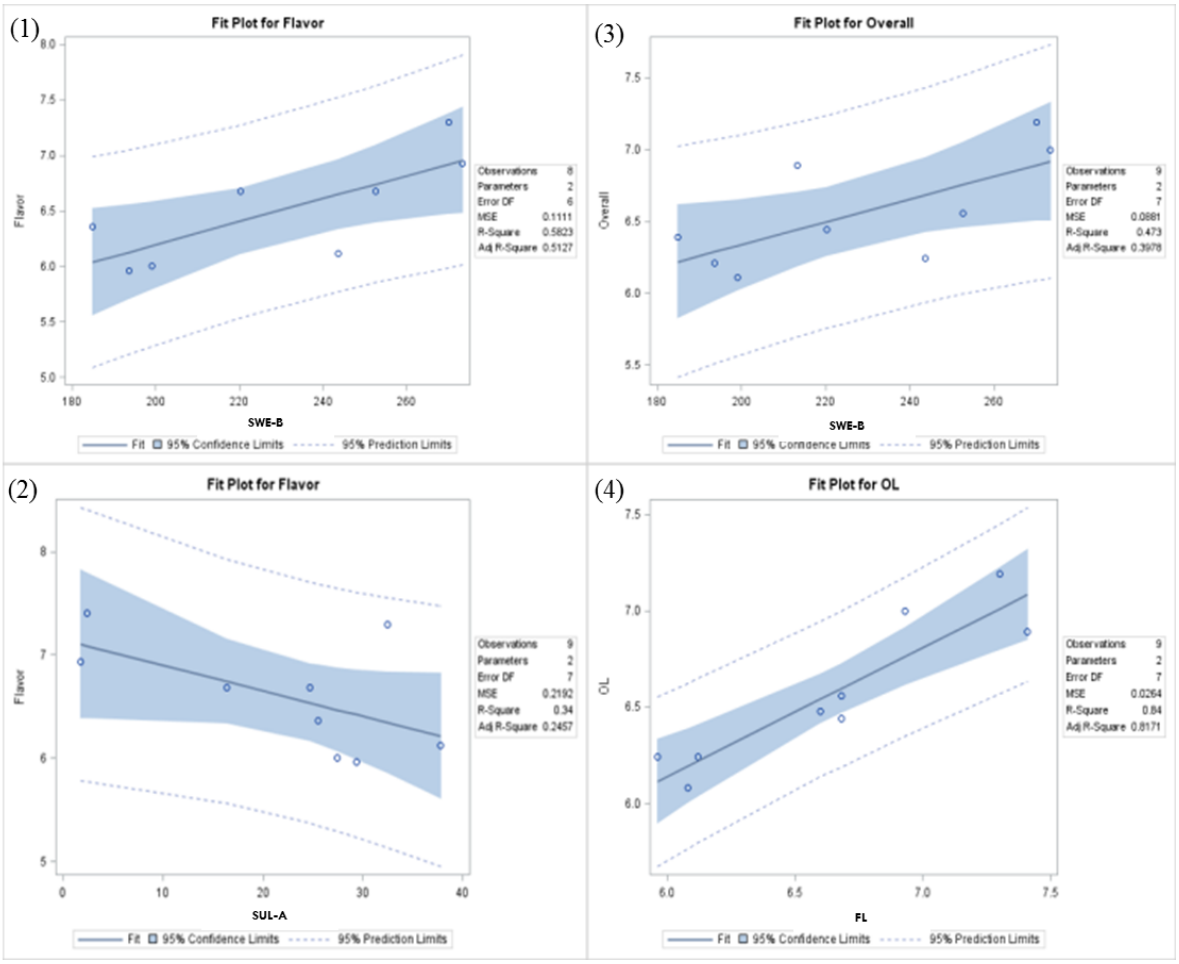
NOV 19, 2014 WEDNESDAY 10:00AM-5:00PM

LOCATION: POULTRY SCIENCE BUILDING

SENSORY LAB ROOM 256A

(Three oyster samples will be tasted and a questionnaire will be filled. Take ~5 min)

If you are allergic to certain shellfish, participation is not encouraged.
If you are allergic to oysters or raw oysters or if you are pregnant, participation is not allowed.
If you have questions related to this test or study, Please feel free to contact:
Dr. Luxin Wang at 334-844-8146 or Jue Wang at 334-329-8423



Diagnostics plots of linear regression relationships of 1) sweet flavor of oyster body and flavor likeability, 2) sulfurous flavor of adductor muscle and flavor likeability, 3) sweet flavor of oyster body and overall likeability, and 4) flavor likeability and overall likeability

* Each point in plot represented the mean (n = 3) of analytic textural parameter (or FAA concentration) versus the mean (n = 30) of sensory likeability scores for a single treatment as well as a single sample period.