

Sensory and Chemical Characteristics of Eastern Oysters
(*Crassostrea virginica*)

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Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

Master of Science in Life Science

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June 22nd, 2011
Blacksburg, VA

Keywords: Eastern oysters, Chesapeake, sensory, chemical analysis

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ABSTRACT

Eastern Oysters, or *Crassostrea virginica*, are an important dietary component in the Chesapeake region and have supported a major fishery in the Chesapeake for more than 100 years. Virginia oysters do not always receive attention in up-scale markets. It is possible that the lack of information on sensory characteristics of Chesapeake oysters may contribute to this problem. In order to differentiate Chesapeake oysters from other oysters, a descriptive sensory test (n=8) was conducted and chemical composition attributes were measured, including glycogen content, proximate analysis, sodium chloride content, and fatty acid analysis. Statistical differences were found for the attributes: volume of liquor, gray/brown and tan colors, roundness of shell, plumpness and salty taste when comparing eastern oysters from Chesapeake to oysters from Rhode Island and New Brunswick, Canada. The glycogen contents in eastern oysters followed the reproductive cycle and glycogen increased from September to December, and started to decrease by April. There were small but significant ($p < .05$) differences in the contents of moisture, ash, protein and fat of oysters from Chesapeake and other areas. Oysters from Chesapeake had higher percentages of long-chain n-3 fatty acids, which were about 4 times higher than Beau Soleil, an oyster from New Brunswick, Canada. The high percentage of long chain n-3 fatty acids in Chesapeake oysters may be valuable for marketing.

ACKNOWLEDGEMENTS

I want to express my sincere thanks Dr. O'keefe for guiding me through this project, throughout all problems and successes, and always offering positive feedback. His mentoring has helped me to grow as a food scientist and as an individual. Thank you to Dr. Jahncke for the financial support and the guidance during the whole project. Thank you to Dr. Duncan for her guidance and advice on my sensory projects. Thank you to Dr. Kauffman and Virginia Seafood Agriculture Research and Extension Center, for the work and financial support with acquiring my fresh oysters samples.

Thank you to all of the Food Science graduate students and staff, especially those who participated in my many sensory panel and were subjected to repeated raw oysters in the morning training. Special thanks to Dr. Wang, Walter Hartman and Harriet Williams for their expert assistance and guidance in running the equipment, instrumentation, in the department. Thank you to all of my friends in the department for encouraging me and providing balance in life.

Thank you to my family for supporting me through more education, especially my mom dad and my litter brother for financial support, unconditional love. Thanks to my dearest friends Liyun, Qin for the sleepover nights, continuous encouragement and great friendships. Thank you to Yu Lu for the great job you did as my undergraduate assistant. Thank you to my friends in Blacksburg Christian Fellowship for introducing the wonderful God to my life and grow with me in God. Thank you my great friends in China, Lu Han, Hui Lin, Xiaolei Zhang, thank you for the encouragement and support.

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Chapter 1: Introduction

The Eastern oyster, Atlantic oyster, or the Virginia oyster, *Crassostrea virginica*, is species of oyster that is native to the eastern seaboard and Gulf of Mexico coast of North America (Apple 2006). The eastern oyster forms extensive reefs both intertidally and subtidally on the eastern coast of Florida, including the estuaries of the Indian River lagoon (Grizzle et al. 2002, Boudreaux et al. 2006). The eastern oyster is one of the most economically and ecologically important shellfish species along the eastern seaboard. In recent years, the total U.S harvest of oysters has been 30 million pounds of meats; about 75 percent of the total is the eastern oyster. About 18 million pounds of total oyster production (all species) is by cultivation (Wallace 2001).

The area's first settlers documented the abundance of the oyster in Chesapeake Bay. *Crassostrea virginica* is an important dietary component in the Chesapeake region and has supported a major fishery in the Chesapeake since colonial times. This oyster has also been an important component of the bay ecosystem (Mackenzie and Burrell 1997). However, the decline of the wild oyster harvest from the bay over the past hundred years has placed an economic strain on Virginia and Maryland's shellfish industries. The decline was a result of oyster diseases (Davis and Barber, 1999) and poor harvesting techniques (Committee on Nonnative Oysters in the Chesapeake Bay 2004).

After realizing the seriousness of the problem, the Chesapeake 2000 Agreement was signed by Maryland, Virginia, Pennsylvania, the District of Columbia, the Chesapeake Bay Commission, and the U.S. Environmental Protection Agency. This agreement calls for 10-fold increase in the native oysters in the bay by 2010, relative to the 1994 baseline.

On Feb 8th 2011, Governor Martin O'Malley announced the number of spat or baby oysters in Maryland waters was at its highest level since 1997. Survival rates for young oysters were also improved, and more Marylanders were looking to start up or expand aquaculture businesses (Anonymous 2011).

But unfortunately, Virginia aquaculture oysters don't always get attention in up-scale markets. There is large difference in price between Virginia oysters and other high-end oysters. However, according to sensory experts on oysters, the sensory characteristics of Virginia oysters are described as: "a perfect balance of salt and sweet, are milder in complexity comparing to other northern oysters, but wonderfully plump and meaty". (McMurray 2007). Thus Chesapeake oysters have largely unrecognized sensory characteristics. In order to differentiate Chesapeake oysters from other oysters and increase name recognition, the identification of specific sensory and chemical composition attributes is needed.

Goal

The goal of this research was to use a sensory panel to compare the sensory difference of raw Chesapeake oysters with other oysters in the current market. A second goal was to compare the chemical composition attributes that may increase the recognition of Chesapeake oysters in the market.

Objectives

1. Assess qualitative and quantitative sensory characteristics of raw oysters from Chesapeake area and other areas using a trained descriptive panel.
 - Determine the qualitative sensory characteristics of raw oysters and establish a reference standard on the sensory attributes that have been determined.
 - Develop a sensory panel and train the panel on the determined attributes.
 - Determine the assessment of trained panel on the determine attributes by using 15cm unstructured line.

2. Determine and compare the chemical composition attributes between oysters from Chesapeake area and other areas.
 - Measure glycogen content of Chesapeake oysters and oysters from other areas on time basis between September and April.
 - Assess the approximate analysis of oysters which include the analysis of moisture content, ash content, protein content, fat content, sodium chloride contents.
 - Analyze the fatty acid compositions of oysters using gas chromatography (QP-5050 GC/MS with GC-17A, shimadzu, Kyoto, Japan).

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Chapter 2: Literature Review

General Classification of oysters

The word oyster is used as a common name for bivalve molluscs that live in marine or brackish habitats and have two hard protective shells. Oysters are members of the family Ostreacea, class Bivalvia, in the phylum Mollusca. Under the current taxonomic terminology, most commercially important species are classified in three major genera: *Ostrea*, *Saccostrea*, and *Crassostrea* along with number of a other minor genera (Carriker and Gaffney 1996). In North America, five species of oysters are commercially cultivated: the Eastern (*Crassostrea virginica*), Pacific (*Crassostrea gigas*), Kumamoto (*Crassostrea sikamea*), European Flat (*Ostrea edulis*) and Olympia (*Ostrea conchaphila*) (Jacobsen 2007).

General biology and ecology of *Crassostrea virginica*

Life Story

Crassostrea virginica is an alternative hermaphrodite, reproducing by shedding sperm and eggs into the water column where fertilization occurs (Fretter and Graham 1964). Reproduction of *C. virginica* is seasonal, with annual cycles and is largely influenced by temperature. Given the seasonality of their environment, oysters are adaptive and breed when conditions are optimal for development and growth of the larvae. Gametogenesis begins in the spring resulting in development and maturation of sperm and eggs and thickening of gonadal epithelium (Shumway, 1996). Spawning occurs from late May to late September, with the season shortened or lengthened from the north and south, respectively. Spawning is initiated by a rapid water temperature increase. As the spawning cycle ends, follicles shrink, amoebocytes invade the reproductive tissue, and

the quiescent state resumes (Kennedy and Breish 1981). Fertilization and partial larval development in *Ostrea* occur in the interior of the oyster's shell. This results in an unpleasant appearance and texture during spawning. In the winter months, when northern East Coast water becomes cold, oysters close their shells, lower their metabolism rate to near zero, and hibernate to save energy. By spring, their meat is thin and almost translucent. Oysters resume feeding and depositing new shell in preparation for the mating cycle to begin anew (McMurray, 2007).

The pelagic larvae resulting from spawning are planktotrophic, feeding upon phytoplankton. Larvae grow through various larval stages over a period of two or three weeks; the length of the larval period is temperature dependent. Longer larval periods expose larvae to predation. Thus, increased time spent in the water column will result in greater larval losses (Kennedy and Breish 1981). The larvae are dispersed or concentrated by water currents and wind. At the end of the larval period, oysters settle into mud and can shift around as adults; oyster larvae cement themselves to clean, hard substrates and lose their mobility (Yonge and Thompson 1976). Kenny et al. (Kenny et al., 1996) conclude that the start and extent of the period of larval recruitment are relatively consistent among years, within a given location along the geographic range of the species. The Eastern oyster, *C. virginica*, is particularly well known for the large, three-dimensional reefs that it builds as successive generations of oysters settle on each other (Anonymous 2004). On a scale smaller than the eastern oyster's natural range, some regions of an estuary may have consistently higher recruitment than others over the long term. The James River, Virginia, consistently has had later sets than elsewhere in

Chesapeake Bay, perhaps because a decline in freshwater discharge allows net upriver flow of saline, larvae-bearing water (Andrews 1980).

Crassostrea virginica is an alternative hermaphrodite, which means the species has the ability to change sex (the reported incidence of hermaphroditism is < 0.5%) during the winter when they are reproductively inactive (Coe 1943). Temperature and nutritional conditions within the oyster may influence this sex change. Rapid growth in yearling oysters was linked with presence of functional female gonad while slower growing yearling oysters were predominantly male.

Diploid and Triploid

Crassostrea virginica in the current market can be divided into diploid and triploid categories based on the chromosome numbers. In nature, most of the Eastern Oysters are diploid; polyploidy individuals (including triploid) seem to arise most commonly through the occasional failure of meiosis (Gillies 1989). If an egg with an unreduced diploid number of chromosomes is fertilized, it will become a triploid organism. Two general characteristics of all polyploids are their increased cell and nuclear size and reduced reproductive potential, but they can grow under wide range of environmental conditions (Foighil and Thiriot-Quievreux 1991). Triploid oysters are of interest and importance in aquaculture because they have less energy expenditure for gonad development, which leads to better condition and growth (Allen and Downing 1991). Oyster eggs can be artificially polyploidized by deliberately inhibiting the meiotic division of their

chromosomes; spawned eggs can be polyploidized by treatment with chemicals (usually cytochalasin B), heat or cold shock, or high pressure (Quillet and Panelay 1986).

In one study on triploid oysters produced by treating newly fertilized eggs with cytochalasin B, triploids grew linearly throughout the period of reproduction, whereas diploids grew little until spawning. Growth of diploids and triploids was parallel subsequently. Diploids showed a typical pattern of glycogen utilization, reaching lowest point prior to spawning and increasing thereafter. Glycogen levels in triploids also declined, but at a slower rate. The difference in glycogen levels may lie in the type of gametogenic tissue remaining after spawning. Because less glycogen is utilized during this normally stressful period, triploids have greater energy reserves available and may utilize these reserves for growth rather than gametogenesis (Downing 1986).

Triploid has been induced in the Eastern and also in the Pacific, European flat, and Sydney rock oysters and triploid oysters have been successfully reared (Kennedy et al. 1996). Triploidy has been induced as well in several other mollusks (Baron, Diter and Bodoy, 1989).

A sensory test conducted with 61 regular consumers of pacific oysters indicates that there was no difference in consumer acceptance for triploid and diploid oysters (Nell 2006). This finding is in agreement with previous hedonic comparisons of diploid and triploid Pacific oysters by consumer panels (Allen & Downing 1991, Maguire et al. 1994, Chao et al. 2001). Similarly, Korac et al. (1996) found similar consumer acceptance ratings for

triploid and diploid Sydney rock oysters. Barber and Mann (1991) reported that triploid Eastern oysters reached a market size of 60mm shell height or 50g whole weight 5 months earlier than diploid, out of a growing period of 17 months.

***Crassostrea virginica* in Chesapeake Bay**

The Chesapeake Bay is a long, narrow, relatively shallow estuary created more than 10,000 years ago when the Susquehanna River valley was drowned by the sea-level rise following the Wisconsin ice age (Boicourt et al. 1991). The bay extends from Cape Charles, Virginia, at its mouth, northward for more than 300km to Havre de Grace, Maryland, at its head (Boicourt et al. 1991; Seagle et al. 1999). The Eastern oyster, *Crassostrea virginica*, is an important dietary component in the Chesapeake region and has supported a major fishery in the Chesapeake since colonial times. This oyster has also been an important component of the bay ecosystem (Mackenzie and Burrell 1997). In the late 1880s, the Chesapeake Bay was the greatest oyster-producing region in the world, with an oyster harvest twice that of the rest of the world combined (Kennedy and Breish 1983). But oyster landings have declined steadily since a peak in the late 1800s. The decline has been attributed to “reduced water quality”, diseases, and over fishing (Rothschild et al. 1994).

After realizing the seriousness of the problem, the Chesapeake 2000 Agreement was signed by Maryland, Virginia, Pennsylvania, the District of Columbia, the Chesapeake Bay Commission, and the U.S. Environmental Protection Agency. This agreement calls for 10-fold increase in the native oyster in the bay by 2010, relative to the 1994 baseline.

This was to be achieved through continued improvements in bay water quality, reduced oyster fishing pressure in the selected areas, shell deposition to rebuild reef structure, and continued development of disease-resistant oyster strains (Luckenbach 2001). On Feb 8th 2011, Governor Martin O'Malley announced the number of spat or baby oysters in Maryland waters was at its highest level since 1997. Survival rates for young oysters was also improved and more Marylanders were looking to start up or expand aquaculture businesses (Anonymous, 2011).

Sensory Analysis

Descriptive Sensory Tests

Sensory evaluation is defined as “scientific discipline used to evoke, measure, analyze and interpret reactions to those characteristics of foods and materials as they are perceived by the sense of sight, smell, taste, touch and hearing” (Stone and Sidel 1993). Due to the essential role of sensory activities in product development, product cost reduction, quality control and quality assurance, more and more foods are using scientific sensory techniques to evaluate food quality. Descriptive sensory tests are the most sophisticated tools in the arsenal of sensory scientists (Lawless & Heymann 1998) and involve the detection (discrimination) and the description of both the qualitative and quantitative sensory aspects using trained panels of from 5 to 100 judges (Meilgaard, Civille & Carr 1999). Panelists must be able to describe the perceived sensory attributes of a sample which include the appearance, aroma, flavor, texture and sound properties. In addition, panelist must learn to differentiate samples quantitatively. There are several different methods that can be used in the descriptive sensory analysis, including the

Flavor Profile Method, Texture Profile Method, Quantitative Descriptive Analysis™, the Spectrum™ method, Quantitative Flavor Profiling, Free-Choice Profiling and generic descriptive analysis (Murray, Delahunty & Baxter 2001). The panelists are screened from the potential candidates based on their ability to describe the characteristics using verbal descriptors and to detect differences in sensory characters and intensities. All descriptive sensory tests require panel training. For some descriptive sensory procedures, an evaluation test is required to evaluate the success of the training. Descriptive analysis is using as the most comprehensive, flexible and useful sensory method and provides detailed information on all of a products' sensory properties. In the next millennium, it is expected that it will be used increasingly for a wider range of uses (Murray, Delahunty & Baxter 2001)

Modified Quantitative Descriptive Analysis

Quantitative Descriptive Analysis (QDA®) was developed by the Tragon Corporation to overcome the lack of statistical treatment of data which are obtained with the Flavor Profile or related methods. Currently, most of the tests are using a modified QDA procedure. Subjects for QDA are recruited from sources not directly involved in the specific project, are screened with dietary questionnaires and are familiar with the food product being studied (Sawyer, Stone, Abplanalp & Stewart 1962). The panel leader acts as a facilitator, rather than as an instructor, in order to prevent bias. Generally speaking, the panel leader will not participate in the generation of descriptive terminology. The descriptor source in QDA is non-technical, everyday language, and is produced by the panelists. Attention is given to development of consistent terminology, but panelists are

free to develop their own approach to scoring, using 15 cm line scales (Meillgaard, Civille & Carr 1999). Panelists are also required to have a certain level of practice before they can rate each attribute. The result of a successful training is that all the panelists are calibrated with respect to the relative differences between the samples. The QDA methodology is based on the principle that it is possible and meaningful to summarize, through a mean, the individual perceptions for each descriptor associated with a product. It is important that panel leaders ensure that panelists are trained with the same vocabulary and have the same understanding of the attribute intensity scales. A set of qualitative references and a sensory protocol are used to ensure that all the subjects associate the same sensory concept behind the same descriptor (Monrozier and Danzart 2001). In the product evaluation part of QDA testing, panelists are required to evaluate the randomly numbered samples in separate booths, to eliminate distraction and suggestions between panelists. The results of a QDA test are analyzed statistically. The mark made by the panelist indicating intensity on the unstructured line is converted to a numeric value by measuring from the left end of the line. The QDA report generally contains a graphic representation of the data in the form of a “spider web”, with a branch or spoke from a central point for each attribute. In order to determine the differences between the intensity of each sensory attribute, analysis of variance (ANOVA) is applied. Unlike the other methods, QDA assumes that panelists will use different parts of the scale when evaluating samples; therefore it is the relative differences among the products, not absolute differences, which provide the information (Murray, Delahunty, Baxter 2001). This method is widely used in the analysis of target products for new product development, and the results of the test can be used to track sensory changes in a product

to understand the effects of shelf life, packaging, processing variables and ingredients. QDA training takes less time than other methods such as FPM or Spectrum and has been applied in many diverse studies (Lawless & Heymann 1998).

Descriptive sensory studies of shellfish (Shrimp, Clam, and Oysters)

On average, Americans ate 15.8 pounds of fish and shellfish in 2009, a slight decline from the 2008 consumption figure of 16.0 pounds (National Marine Fisheries Service 2010). The consumption is comprised of 11.8 pounds of fresh and frozen finfish and shellfish and 3.7 pounds of canned seafood. Although shellfish are a significant part of seafood consumption patterns for Americans, very few studies have been conducted to evaluate sensory characteristics of shellfish. Most of these studies have used descriptive sensory tests to develop descriptive terms that can be used in the distinction of appearance, texture and flavor. Descriptive vocabularies are listed below that have been used in the sensory study of shellfish (Edmunds & Lillard 1979, Josephson, Lindsay & Stuiber 1985, Erisckson et al. 2007).

Table 2-1: Descriptive vocabularies in the sensory study of shellfish

Aroma	Taste		Texture
RAW OYSTERS			
fishy	fishy	dry	gritty
aromatic	bitter	earthy	chewy
fresh	sweet	oil/rich	fibrous
pungent	salty	melon-like	dry
sour	metallic		rubbery
boiled potato-like	astringent		
bland	sour		
	plant-like		
	seaweed-like		
CLAMS			
fishy	salty	sour	tough
fresh	fishy	meaty	rubbery
sea breeze	fresh		chewy
nutty or buttery	liver-like		sandy or gritty
	sharp		soft
	cheesy		chicken liver-like
SHRIMP			
fishy	sweet		juicy
sea breeze	salty		crisp
marsh smelly	sour		firm
nutty or buttery	meaty		chewy
boiled corn	fresh		soft
	bitter		tough

These sensory characteristics of shellfish are impacted by internal and external factors.

The most influential factors are species, harvest location, season, size, gender, and growth method (wild or cultured). For external factors, different storage and cooking methods have a significant effect on the sensory characteristics of shellfish.

Sensory Characteristics of Raw Oysters

Although limited scientific research has been conducted on the sensory characteristics of raw oysters, many connoisseurs are fascinated by raw oyster taste. The essayist Michel de Montaigne compared oyster flavor to violets. Eleanor Clark described oysters as a "shock

of freshness” and the French poet Leon-Paul Fargue mentioned eating raw oysters was “like kissing the lip of the sea”. In modern society, many gastronomists would view raw oysters as a delicacy. Chef Jasper White compared the sensory experience of tasting the raw oysters to high quality sturgeon caviar. He mentioned the taste stimulated the appetite, awakened the taste buds, palate, sense of smell, and imagination (White, 2011). Rowan Jacobsen, a oyster connoisseur, mentioned in his book that oyster flavor, like perfume notes, comes in three stage (Jacobson 2007). The first stage is the initial sensation of salt; then the taster senses the body of the oysters which is firm and slippery at the same time, according to his description. When chewing the oysters, the sweetness begins to be released. All flavors will be encountered during the third and final stage, which is generally known as the finish. These descriptors are also described by Patrick McMurray as the three essential elements to the taste of an oyster: salinity, texture and pure taste (McMurray 2007). He concluded that these are influenced by the water where the oysters originate. Local waters each have their own characteristics. According to his experience in raw oysters sensory evaluation, he developed a tasting wheel (showing below) which summarizes the potential sensory characteristics that raw oysters may have and help tasters to identify them (McMurray, 2007)

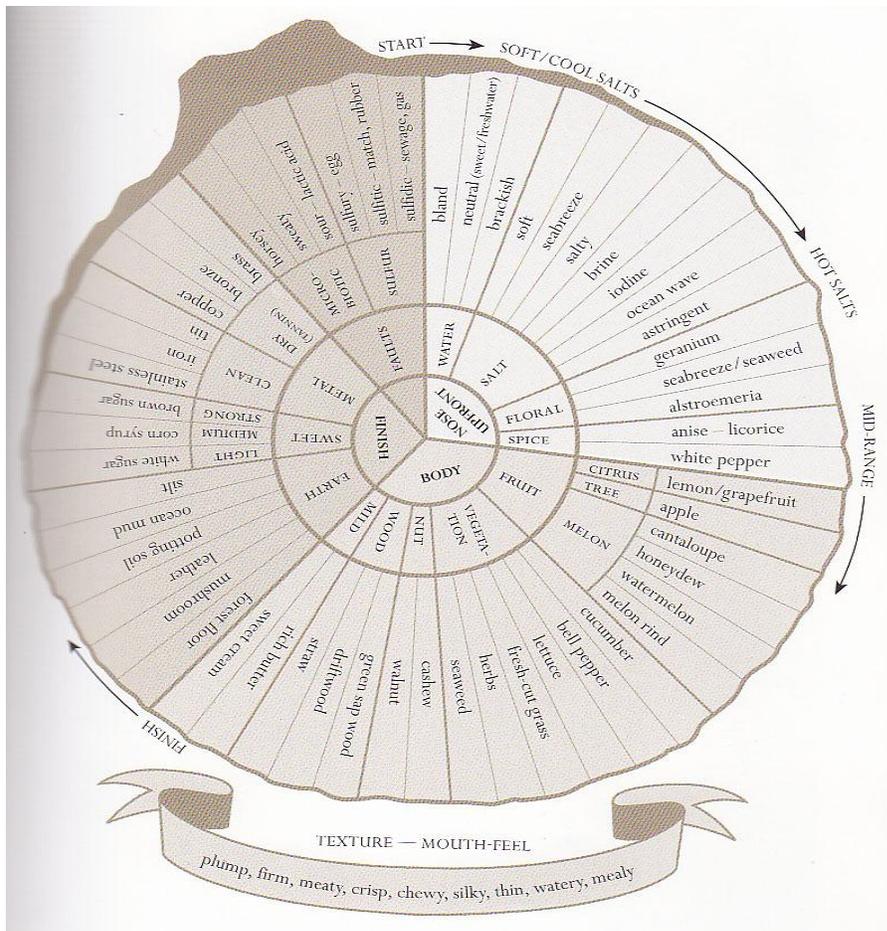


Figure 2-1: Tasting wheel of raw oysters (McMurray, 2007)
Used under fair use guidelines, 2011

McMurry described *Crassostrea virginica* as everyone’s favorite species, a perfect balance of salt and sweet. Many are grouped under regional names, but each has its own unique flavor: Malpeque, Blue Point, Maine, New England, Chesapeake Bay, Apalachicola and Gulf oyster. Chesapeake oysters are milder in complexity comparing to other northern oysters, but wonderfully plump and meaty (McMurray, 2007). Jacobsen (2007) described Chesapeake oysters as mild in flavor and soft. Oysters have a taste of salty sea and various minerals. In the northern parts of the Chesapeake bay, water salinity decreases and various flavors such as nutty, buttery, musky, algal, fungal, citrus, seaweed, black tea and grainlike turn up in Eastern oysters (Jacobsen 2007).

Nutrition Aspects of Raw Oysters

Oysters are a natural source of several minerals, including calcium, iron, zinc and selenium, which are often low in the modern American diet. They are also an excellent source of Vitamin B12. The Reference Daily Intake or Recommended Daily Intake (RDI) for Vitamin B12 is 6 µg (US Council for Responsible Nutrition 2001). Vitamin B12 deficiency can potentially cause severe and irreversible damage, especially to the brain and nervous system. At levels only slightly lower than normal, a range of symptoms such as fatigue, depression, and poor memory may be reported (Herbert, 1996.). Oysters contained on average 20 and 26 µg/100g in raw and cooked samples, respectively, which is nearly 10 times higher than crayfish and other shellfish (Nettleton & Exler, 1992). In addition, raw oysters contain omega-3 fatty acids, which can improve cardiovascular health. Consumption of raw mollusk shellfish is the main cause of illness in consumer eating seafood because of some concerns on *Vibrio vulnificus*. However, the post harvest processing (PHP) method that can eliminate the occur of vibrio in raw mollusk shellfish. Those PHP methods include: high pressure process (HHP), radiation, quick freeze storage, and mild heat when cooking (Parker et.al 1994).

Glycogen Content of Oysters

Glycogen is a non-reducing, white, amorphous, polysaccharide, which functions as a primary storage form of glucose in animals and plants. Most tissues of the body are able to store small amounts of glycogen, but the main sites of glycogen storage are the liver and skeletal muscles. Glycogen is made mainly by the liver and the muscles, but can also

be made by glycogenesis within the brain and stomach (Saladin 2007). Thus, the amount of glycogen stored in body can reflect metabolic activities, which is also seen in marine bivalves.

In many marine bivalves, glycogen storage and utilization is an important component of the annual energy budget, because glycogen is intimately tied to the reproductive cycle in bivalves. Energy stored in the form of glycogen prior to gametogenesis is utilized during periods of high metabolic demand during the production of gametes. Glycogen provides the essential substrates for lipogenesis, i.e. during vitellogenesis in eggs, and pentoses for nucleic acids. Typically, the reproductive cycle in bivalve mollusks in temperate climates begins with a period of winter dormancy, a "rest" period, followed by a period of gonad differentiation, rapid growth, and nutrient storage. When the availability of food is high in summer months, glycogen is stored, but glycogen is mobilized when gamete development starts in the autumn. These glycogen reserves may allow the synthesis of lipids in the gametes in addition to maintaining the energy requirements of gametogenesis (Darriba et al 2005).

According to Allen and Downing's (1991) research on pacific oysters (*Crassostrea gigas*), diploids and triploids showed distinctly different patterns of glycogen utilization. Glycogen content in diploids decreased steadily throughout the gametogenic period until it reached the lowest point in July. After spawning (between July and August), glycogen levels in diploids increase. Glycogen content in triploids was significantly greater than diploids in all sampling periods. The other notable feature of the glycogen content in

triploids is that, like diploids, triploids show a steady decline in glycogen content as the season progresses, but after spawning the glycogen content in triploids continue to decrease while it increased in diploids (Allen & Downing 1986). A similar pattern of glycogen utilization was observed in triploids of *Crassostrea rivularis*, but with about three times the increase in the glycogen content of diploid (Perdue & Erickson 1984). Besides of reproductive activity, species, chromosomes type, the other factors such as the available food, environmental conditions may also affect metabolic activity of marine bivalves, and this can be directly affect glycogen contents (Darriba et al 2005). The methods for determination of glycogen in tissues can be divided into two main categories. One is based on the extraction of the glycogen with trichloroacetic acid (TCA) or hot alkali. The glycogen is measured using an anthrone reaction and colorimetric procedure (van der Vies 1954). The other method category uses enzymatic procedures. The degradation of glycogen by diazyme is followed by glucose measurement with glucose oxidase (Passonneau & Lauderdale 1974). In general, the enzymatic method is easier and less time consuming, however, the high cost and limited enzyme available for this method are the two biggest disadvantages.

Recently, another innovative method for determining glycogen content has been introduced. The glycogen in the sample tissue is hydrolyzed and converted to glucose-6-phosphate, followed by quantitative determination by ion chromatography (Gao et al. 2008). More research is needed to prove the accuracy and sensitivity of this method.

Lipids in marine organism

The principle lipid class that occurs in most seafood lipids is triglycerides (triacylglycerols, TAG). Also, in nearly all seafood oils, various phospholipids (PL) always occur, usually in concentrations considerably less than TAGs. The other main classes of lipids are wax esters, diacylglyceryl ethers and hydrocarbons.

TAG are composed of one glycerol molecule esterified with three fatty acids, serving as the most concentrated form of energy storage known (37.6 kJ/g) (Lee and Patton, 1989). TAG differ from each other by the type and position in the glycerol molecule of attached the fatty acids. Lipids predominantly containing polyunsaturated fatty acid are liquid at room temperature and are called “oils”, while lipids with more saturation are more likely to become a solid in room temperature, and are called “fats”. Most fat deposits consist of TAG stored in the subcutaneous layer and contribute a protective function, especially for marine species that lived in low ambient temperature environments (Watanabe 1982).

Among the various phospholipids that may occur in the marine organisms, lecithin (phosphatidylcholine, PC) is the main phospholipids in marine invertebrate and vertebrates, while phosphatidyl ethanolamine (PE) is the second most common class. Some sponges, soft corals, and mollusks may contain more PE than PC. The proportion of PE is over 60% and even more than 80% of the total phospholipids in some of these animals (Holmer, 1989).

Wax ester in terrestrial organisms primarily serves a protection function, e.g., in the coverings of leaves of plants and the skin of animals. Uses of wax esters in marine animals include an energy storage function in some zooplankton and an aid for biosonar in whales (Lee and Patton, 1989).

Characteristics of Fatty Acid in Seafood and Oysters

The fatty acids derived from seafood oils are of three principal types: saturated, monounsaturated, and polyunsaturated. The fatty acid composition is more complicated in marine organisms than observed in terrestrial plants and animals (O'Keefe Ackman, 1987) Fatty acid composition of marine organisms is characterized by significant amounts of 20 and 22 carbon chain length, highly-unsaturated, omega 3 (n-3) fatty acids (Stansby 1982).

The dominate fatty acids identified in raw oysters (*Crassostrea gigas*) were: 16:0, 16:4n-3, 18:0, 18:1n-9, 18:3n-3, 20:4n-3, 20:5n-3 (EPA), 22:5n-3 (DPA) and 22:6n-3) DHA. These fatty acids contribute approximately 75-83% of the total (Lofstedt 2010, Soudant et al 1999).

Many factors impact the fatty acid composition, such as diet, geographic locations of catch and seasons of the year, which may be related to water temperature. The influence of microalgal diets was observed by Pennarun et al. (2003) on fatty acid composition of raw oysters (*Crassostrea gigas*). A significant difference in the contents of the fatty acid of the n-9, n-7, n-6 and n-3 classes occurred when two groups of oysters were feed two different microalgae diets (Pennarun 2003).

Another study indicates that the composition of fatty acids in oysters will have seasonal variability, which is related to seawater temperature and food supply. The fatty acids showing the greatest seasonal changes belong to the n-3 series. The fatty acid 16:4n-3

reaches its maximum value after the spawning period, followed by a decrease from July to October, being strongly correlated ($p < 0.001$) with temperature (Abad et al. 1995).

Nutrition and Human Health Aspects of Marine Oils

Marine lipids generally contain a wider range of fatty acids than vegetable oils or animal fats. The polyunsaturated fatty acids, especially n-3 fatty acids of marine lipids, decrease the risk of coronary artery disease (CAD). A high level of serum cholesterol is one of the major risk factors for CAD, the leading cause of death in many industrialized nations (Keys 1980, Myant 1981). Dietary fat has for many years been known to influence serum lipid levels. In general, saturated fats raise the level of serum cholesterol, while polyunsaturated fats lower serum cholesterol. Effects of marine oils on lowering serum cholesterol levels have been investigated both in experimental animals and in humans (Vaskovsky 1989). In addition, a high level of consumption of fish may help to explain the low incidence of cardiovascular disorders in Japanese (Kagawa et al 1982).

Besides the effects of n-3 fatty acids on heart diseases, there may be other health benefits of marine oils. Some research suggests that n-3 fatty acids help in diminishing the undesirable effects of inflammatory diseases, have beneficial effects on stroke, may reduce the incidence of breast cancer, and may help alleviate certain skin diseases (Stansby 1982).

The Analysis of Marine Fatty Acids

Fatty acid identification and quantification is most accurately done using gas chromatography (GC) using polar wall coated open tubular (WCOT) capillary columns. These columns, generally 10-60m in length and 0.25-0.32 mm in internal diameter, contain the liquid phase deposited in a thin film on the interior wall of the column (Stansby 1990).

Fat extraction

Lipids can be extracted from marine organisms through variety of methods. One of the oldest and still acceptable is soxhlet extraction. Other procedures are also used, for example the methods of Folch et al. (1957) and Bligh and Dyer (1959). The Bligh and Dyer procedure is a modification of the Folsh method and was developed specifically for solvent savings during the extraction of lipids from low-fat fish, such as cod.

It is generally recognized that soxhlet extraction with petroleum ether (PE), n-hexane or diethyl ether mainly extracts the nonpolar TAG. Polar solvent mixtures such as chloroform/methanol can be used for soxhlet extraction, where information on total lipid composition is required. One study compared four different solvents (chloroform/methanol, CM, n-hexane/isopropanol, HIP, diethyl ether, EE and methylene chloride/methanol, MM) for the soxhlet extraction of beef lipids. The soxhlet procedure employing either PE or EE extracted less than 75% of total lipid, 89% of triglycerides and 15% of polar lipids. The results of this study also demonstrated that freeze-dried samples of meat can be effectively extracted with chloroform/methanol (2:1, v/v) or

methylene chloride/methanol (2:1, v/v) in a soxhlet type apparatus (Sahasrabudhe & Smallbone 1983).

Because triglycerides, phospholipids and most free fatty acids are not volatile enough to be chromatographed directly, they must first be converted into esters prior to fatty acid analysis. Fatty acid methyl esters can be prepared either by acid- or base-catalyzed transesterification (Christie 1989).

Gas Chromatography (GC) Instrumentation

There are three critical elements of a GC: injector, column and detector. The design and operation of these components will impact separation and quantitation. A heated injection in split mode is usually used in the analysis of fatty acid methyl esters (FAME). In this type of injector, sample is vaporized in the carrier gas and flow regulated by a control valve to give a desired ratio of streams which are directed to the column or a purge vent (Freeman 1981).

Column performance is a function of the characteristics of the stationary phase coated onto the silica tubing inner wall. Suitable GC column for marine FAME analysis include flexible fused silica, wall-coated open tubular (FFS WCOT) column containing a cross-linked and bonded (to the inner column wall) polyglycol liquid phase based upon Carbowax-20M, or a crosslinked cyanopropylpolysiloxane column, due to the polarity of these phases and the characteristics of marine FAME (Stansby 1990, AOCS).

The most commonly used detector in the analysis of FAME is a flame ionization detector (FID). Since the introduction of capillary columns in the 1960s, and the introduction of inexpensive bench top mass spectrometers in the 1980s, GC/MS has developed to a degree that the MS itself, may be considered a specific GC detector (Traitler 1987). The low carrier gas flow rates, typical of capillary columns, permit a direct column connection to the ion source chamber of the mass spectrometer, eliminating the need for interfacing devices required for packed GLC columns (Stansby 1990). Generally, a packed column carrier gas flow rate would be 10-30 ml/min whereas the flow in a capillary column would be in the range of 1-3 ml/min. Thus, the MS detector is very suitable for analysis of FAME.

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Chapter 3: Sensory and Chemical Characteristics of Eastern Oysters (*Crassostrea virginica*)

ABSTRACT

A descriptive sensory test (n=8) was conducted comparing oysters from different regions of the Virginia Bay (VA lower Eastern Shore area, VA Tidewater area, Mill Creek, VA Upper Bay area Western Shore, VA Upper Bay area Eastern Shore, VA Middle Bay area Western Shore T.A.) and oysters from Rhode Island and New Brunswick. Statistical differences were found for the attributes: volume of liquor, gray/brown and tan colors, roundness of shell, plumpness and salty taste when comparing eastern oysters from Chesapeake to oysters from Rhode Island and New Brunswick, Canada. The glycogen contents in eastern oysters followed the reproductive cycle and glycogen increased from September to December, and started to decrease by April. There were small but significant ($p < .05$) differences in the contents of moisture, ash, protein and fat of oysters from Chesapeake and other areas. Oysters from Chesapeake had higher percentages of long-chain n-3 fatty acids, which were about 4 times higher than Beau Soleil, an oyster from New Brunswick, Canada.

KEYWORDS

Raw oysters, Chesapeake, Descriptive, Glycogen, Proximate Analysis, Fatty Acid Analysis

INTRODUCTION

The Eastern oyster or *Crassostrea virginica* is one of the most economically and ecologically important shellfish species along the eastern seaboard. Also an important dietary component in the Chesapeake region and has supported a major fishery in the Chesapeake since colonial times. However, the lack of information on sensory characteristics and other chemical characteristics eliminate the sales of Chesapeake oysters in high end market.

On average, Americans ate 15.8 pounds of fish and shellfish in 2009, a slight decline from the 2008 consumption figure of 16.0 pounds (National Marine Fisheries Service 2010). The consumption is comprised of 11.8 pounds of fresh and frozen finfish and shellfish and 3.7 pounds of canned seafood. Although shellfish are a significant part of seafood consumption patterns for Americans, very few studies have been conducted to evaluate sensory characteristics of shellfish.

To identify the sensory Characteristics of Eastern oysters in Chesapeake area, descriptive sensory tests were conducted in this study. Descriptive sensory tests are the most sophisticated tools in the arsenal of sensory scientists (Lawless & Heymann 1998) and involve the detection (discrimination) and the description of both the qualitative and quantitative sensory aspects using trained panels (Meilgaard, Civille & Carr 2007). Because of the limit of the training time, modified QDA methodology was applied in the sensory tests. The QDA methodology is based on the principle that it is possible and meaningful to summarize, through a mean, the individual perceptions for each descriptor

associated with a product. A set of qualitative references and a sensory protocol are used to ensure that all the subjects associate the same sensory concept behind the same descriptor (Monrozier and Danzart 2001).

To better understand the differences between Chesapeake oysters and oysters from other areas, chemical compositions analysis is also needed which include glycogen content, proximate analysis (moisture, ash, protein, fat, sodium chloride) and fatty acid analysis.

The studies supporting this objective were:

Study 1: Evaluate of sensory attributes of raw oysters on a 15cm unstructured scales by an experienced sensory panel;

Study 2: Measure glycogen content of Chesapeake oysters and oysters from other areas on time basis between September and April;

Study 3: Assess the approximate analysis of oysters which include the analysis of moisture content, ash content, protein content, fat content, sodium chloride contents;

Study 4: Analyze the fatty acid compositions of oysters using gas chromatography (QP-5050 GC/MS with GC-17A, shimadzu, Kyoto, Japan).

MATERIALS AND METHODS

Oysters Samples and Storage

All the samples of Chesapeake oysters were purchased from local seafood stores in Hampton, VA, shipped on gel pack overnight to Virginia Tech and stored at 4 °C in the shell.

Oysters that used in this research are from following locations:

Table 3-1: Oysters Samples from Chesapeake Area:

Sample Code	Harvest Location	Chromosome type	Harvest Method	Trade Name
A	VA Tidewater area	Triploid	Agriculture	Lynnhaven
B	VA lower Eastern Shore area but ocean side	Triploid	Agriculture	Sewansecott
C	VA Middle Bay area Western Shore	Triploid	Agriculture	McMinn
D	VA Middle Bay area Western Shore	Triploid	Agriculture	Purcell Seafood
E	VA Upper Bay area Eastern Shore	Triploid	Agriculture	Shore Seafood
F	VA Middle Bay area Western Shore	Triploid	Agriculture	Mobjack Bay
I	VA Lower Bay area	Diploid	Wild	

	Western Shore Bay			
J	Lower Bay area Western Shore	Diploid	Agriculture	York River, Research Oysters
K	Lower Bay area Western Shore	Triploid	Agriculture	York River, Research Oysters
L	VA Middle Bay area Western Shore	Triploid	Agriculture	Rappahannock River
M	VA Upper Bay Eastern Shore but Ocean Side	Triploid	Agriculture	Old Salts

Table 3-2: Oysters Samples from Other Areas:

Sample Code	Harvest Location	Chromosome type	Harvest Method	Trade Name
G	New Brunswick Canada	N/A	N/A	Beau Soleil
H	Rhode Island	N/A	N/A	Beaver Tail
N	Massachusetts	N/A	N/A	Island Creeks
O	Prince Edward Island Canada	N/A	N/A	Mapeques

Raw oysters were shucked and placed individually in 25mL beakers, covered with two layers of cheese cloth and placed in a freezer (-10°C) until completely frozen (3 hr). Once frozen, beakers were freeze-dried for 48 hours (Labconco Freezone 18, Kansas City,

MO). Freeze dried oysters were used in the determination of glycogen, lipid, protein and fatty acids. Moisture content was determined by the weight difference of the raw samples to the freeze dried sample. Freeze dried samples were stored in a desiccator.

Study 1: Sensory Characteristics of Eastern Oysters

Sensory characteristics of Eastern Oysters (Sample A, B, C, D, E, F, G, H) was conducted using a trained descriptive panel (n=8) using modified QDA method . Sensory studies were approved by the Virginia Tech Institutional Review Board (IRB 10-425, Approved September 15, 2010, Appendix A). Protocols for the descriptive trained panel followed those described by Meilgaard et al. (2007). Sensory training took place in the dairy laboratory of the Food Science and Technology (FST) department at Virginia Tech. Sensory testing was conducted in the sensory laboratory using individual booths. Information was collected using an unstructured 15 cm line scale on paper scorecards.

Panelist Training

Recruitment of panelists for trained descriptive panel was conducted by email and using a survey with Virginia Tech's online survey system (www.survey.vt.edu) (Appendix B, Appendix C).

Eight panelists (5 males, 3 females) age from 22-60 were selected based on their availability, willingness to participate, descriptive ability and indication that their acceptability of raw oysters was either moderate or extreme. A informed consent form describing the detail of the study and expected time of the research was given to panelists

to review and sign on the first day of training (Appendix D). Training sessions occurred once a week for one hour and lasted 9 weeks (9 hours). The training plan was divided into two parts: in the first part, Term Generation, panelists were encouraged to generate the descriptive terms for appearance, aroma, flavor, texture, and aftertaste of raw oysters under the lead of panel trainer and based on the understanding of first training handout (Appendix E), which provided basic knowledge of sensory attributes. Sensory attributes used in evaluations is showing below (Table 3-1). The second part, Scaling Training, included three stages: initial practice, small product practice and final practice. Panelists were first presented examples of products as a frame of reference (Appendix F). Once the panel had a grasp on the terminology and a general understanding of the use of each scale, a series of samples were evaluated one at a time; these samples had a range of qualitative and quantitative differences. Panelists were then presented with samples that had smaller differences (raw oysters that from closer locations) and encouraged to refine the procedures for evaluations. The final practice approached real testing. First panelists were presented 4 oysters from each location (Lynnhaven and Beau Soleil) in a plastic box with ice, separated from each other in individual cups, to evaluate appearance attributes. Panelists were asked to mark all four samples on the same scale for each appearance attribute on 15cm scales. The second part was to present one oyster from each location for panelists to evaluate odor, flavor and texture on 15cm scales. Results of the tests were discussed after every practice.

Table 3-3: Sensory Attributes determined in the first training session

	Determined Sensory Attributes
Appearance	volume of the liquor, color of the meat (pink, gray/brown, tan), roundness of the shell, plumpness
Odor	seaweed, fishy, earthy
Flavor	salty, sweet, umami
Texture	firm, chewy

Panelist Validation

The next step in training was to validate if panelists could replicate their individual performances on the thirteen attributes they learned in training. This was done by evaluating two different oysters from Beaver Tail and Mobjack, and having each panelist rate the samples on unstructured 15 cm line scales. Samples were purchased from local seafood stores in Hampton VA, shipped on ice overnight and stored at 4 °C in the shell. For the appearance attributes evaluation, each panelist was provided with a plastic box with ice coded with a random three-digit number which contained four oysters on the half shell in individual cups. One oyster was presented for aroma and taste tests. Samples were placed on white plates which were coded with random three-digit numbers. Oysters were presented to the subjects in a balanced order, following worksheet plans (see Appendix G). All oyster samples in half shell were stored on ice at 4 °C for no more than 2 hr prior to sensory testing and temperature was maintained during testing. Oysters were treated in the same manner during validation testing.

The goal of the validation test was to verify each panelist's ability to evaluate the samples for thirteen attributes listed above. Panelists following the major trends for all samples were given a pass rating. Panelists that did not follow major trends had one extra hour training for the trends they did not pass. The major trends were determined by the results of majority of the panelists.

Attribute Rating of Raw Oysters

For sensory evaluation of raw oysters, experienced panelists (n=8) assessed each product for thirteen attributes (volume of the liquor, pink, gray/brow, tan, roundness of the shell, seaweed, fishy, earthy, salty, sweet, umami, firm, chewy) on 15 cm unstructured line scales. Panelists tasted oyster samples (n=3) in the same manner as during validation, for each test. Six tests, eight different oysters in total, were evaluated in this session for six different days. Each oyster was tested twice to evaluate the consistency of the panelists' performance. After signing consent forms (Appendix H), panelists were provided with the reference frame (Appendix F), a plastic fork, a pencil, napkin, paper scorecards (Appendix I, Appendix J) and a cup of lemon water as fresher between each sample. Each cup of lemon water was made by squeezing a fresh lemon into half cup of water. Oysters were presented to the subjects in a balanced order, following worksheet plans (see Appendix K).

Statistical Analysis

Panelists' marks on the 15cm unstructured line scales were measured (cm) starting from the left side of the line. Results from all replications of the line scales were

tabulated and TWO WAY ANOVA table with Tukey's HSD compared the six formulations for each attribute at the α level of 0.05.

Study 2: Glycogen Content

Materials

A solution of 5% trichloroacetic acid (weight / volume) in water was prepared. The anthrone reagent was prepared by placing 280ml of distilled water in a 2 liter Erlenmeyer flask and 720 ml of concentrated H₂SO₄ was gently added in to the flask. After cooling the mixture to 35 °C, 500 mg of reagent grade anthrone (Fisher Scientific, St. Louis MO) was added and stirred until dissolved. Finally, 30 g of reagent grade thiourea and stirred until dissolved.

Glucose standards: 100 mg of dry glucose was dissolved in 100 ml of saturated benzoic acid solution (dissolve 0.34g benzoic acid into 100ml water). For a working standard, 5 ml of this stock standard was diluted to 100 ml with saturated benzoic acid solution. 2 ml of this solution contains 0.1 mg glucose.

Method

The glycogen content was determined by the modified anthrone method described by Roe and Daily (1966). Glucose was used as the standard.

Oysters were ground in a mortar and pestle after freeze-drying. Around 10 mg was weighted to a 15 ml screw cap centrifuge tube, and add 10ml of 5% trichloroacetic acid aq. was added. The sample was extracted overnight at 4-5 °C before centrifugation at 2500 rpm for 10 min (approximately 900xg). A 2 ml aliquot of the supernatant was pipetted into a 15 ml tube and 10ml anthrone reagent added. Concomitantly, 2 ml of a

standard solution containing 0.1 mg glucose was placed in another test tube with 10 ml anthrone reagent, and mixed thoroughly. A reagent blank was also prepared and was used to zero the spectrophotometer. All tubes were placed on a boiling water bath for 15 minutes and then removed to a cold-water bath. After the tubes reached room temperature, they were transferred to cuvetts and absorbance at 620 nm was read in Genesys model 10uv spectrophotometer (Thermo Fisher Scientific Co., Madison WI) at 620nm.

The glycogen contents were calculated by using the following equation:

$$\frac{AU}{AS} * 0.1 * 5 * 10 \text{ mg tissue} * 0.9 * 100 = \frac{\text{mg glycogen}}{\text{g tissue}}$$

AU = absorbance of unknown, AS = absorbance of standard, 0.1= mg glucose in 2ml standard solution, 5= dilution factor and 0.9= factor for converting glucose value to glycogen value.

Glycogen content was expressed as mg/g of freeze dried oysters and fresh oyster meat.

Study 3: Proximate Analysis

Moisture

The moisture content of oyster flesh was determined by using an oven method (modified AOAC, 24.003a, 1984). Approximately 2-3 g sample was weighted into a pre-dried crucible (without lid) and dried for 16-18 hr at 100 °C. Three replications of each sample were performed in the analysis. The crucible was transferred to desiccators, cooled and weighted. The loss in weight was used to calculate moisture content.

Ash

Ash content of the oyster flesh was determined by dry ashing the dried sample obtained from the moisture determination (modified AOAC 31.012, 1984). Samples were placed

in a temperature controlled furnace (Lindberg, Watertown WI) at 550 °C overnight until light gray ash resulted. Crucibles containing ash were transferred to a desiccator and weighed soon after reaching room temperature. The loss in weight was used to calculate ash content.

Fat Content

The fat content of freeze dried oysters was determined by using the Soxtec Ether Method. Thimble filter papers (Whatman # 4, Buckinghamshire, England) and extraction cups were dried at 95 ± 5 °C for 2 hours and cooled in desiccators before starting the experiment. Approximately 0.2-0.4 g samples of ground, freeze dried oyster was weighed onto a filter paper, wrapped, and placed in a thimble. Three replication of each sample were extracted by using a Soxtec fat extraction unit (models HT2 or HT6, Foss North America, Eden Prairie, MN) using petroleum ether as extraction solvent. The extraction procedure took ~3 hr. All fat extracts were collected in the extraction cups, which were dried at 95-100°C for 15 - 20 min. Cups were cooled and placed in a desiccator for 30 min. The gain in weight of extraction cup relative to sample weight was used to calculate fat content.

Protein Content

The protein content was determined using ground freeze dried oysters by measuring nitrogen content using the Kjeldahl Method (991.36, AOAC 1998). Three replications of each sample were performed. Approximately 0.2 g sample was weighted into a glass tube with a filter paper (Whatman #541, no-nitrogen). One pack of Kelmate'N Kjeldahl Digestion Mixture 200 (each pack contains 10.0 g K_2SO_4 and 0.3 g $CuSO_4$), 15 ml concentrated sulfuric acid, and 3 ml 30-35% hydrogen peroxide were added. The mixture

was digested in a block digester for about 50-60 min, cooled for 5-10 min, and 50-70 ml distilled water and 50-60 ml of 10 N sodium hydroxide were added. The glass tubes were placed into the distillation unit. A 250 ml Erlenmeyer flask containing boric acid solution was connected to act as receiver for the distillation apparatus. The collected sample was titrated with 0.20 N HCl standard.

Percentage of nitrogen and protein was calculated from the following formulas:

1. Recovery rate

$$R = \frac{(V_a * - V_b) \times 1.4007 \times N}{W * \times 18.65}$$

W* = Weight of glycine, g

18.65 = % nitrogen in glycine

Va* and Vb = volumes of the standard HCl used to titrate glycine sample and the blank.

In most cases, Vb = 0

1.4007 = Milliequivalent weight of nitrogen x 100 (%),

N = Normality of HCl. In this case, N=0.20

In the Virginia Tech meat chemistry lab, the measured R = 0.9351 if the Kjeltab is used.

And R = 0.9811 if the Kelmate'N Kjeldahl Digestion Mixture 200 pack is used.

2. Calculation:

$$\% \text{ Nitrogen} = \frac{(V_a - V_b) * 1.4007 * N}{W * R}$$

$$\% \text{ Crude Protein} = \frac{(V_a - V_b) * 1.4007 * N * 6.25}{W * R}$$

W = weight of the experimental sample, g

V_a and V_b = volumes of the standard HCl used to titrate sample and the blank. In most cases, $V_b = 0$

6.25 = protein factor for meat products (16% nitrogen).

N = Normality of HCl. In this case, N=0.20

Sodium Chloride Content

The sodium chloride content of oyster flesh was determined by using Chloride QuanTabs® (Hatch Inc.) Oysters were shucked and patted dry on paper towels. Five oysters for each oyster type were ground in a blender. Three replications of each sample were performed for salt content analysis. Approximately 10 g of ground samples were weighted into a beaker. Filtered the sample by adding 10 ml hot water and placed QuanTab strip into the filtered liquid. Three replications of each sample were performed for this analysis.

Study 4: Fatty Acid Composition Analysis

Crude fat was extracted from oyster flesh by using a Soxtec and chloroform/ methanol (1:1, v/v). Extracted fat samples were nitrogen flushed and stored at -10 °C until fatty acid analysis.

Fatty acids were converted to methyl esters and separated by gas chromatography followed an AOCS official method (Ce 1b-89, AOCS 2005). A shimadzu QP-5050 GC/MS with GC-17A (Shimadzu, Kyoto, Japan) was fitted with a SP-2560 (cross-linked and bonded *bis*-cyanopropylpolysiloxane, Supelco Sigma-Aldrich, St. Louis, MO) capillary column (100m x 0.25mm, i.d., 0.20 µm film thickness). A program using a split

ratio of 1:20 with an injector temperature of 270°C was used. Helium was used as carrier gas at 21 cm/sec linear flow velocity. Initial temperature was 140 °C and was ramped to 250 °C at 2 °C/min with a final hold time of 20 min. The total run was 80 min. Fatty acids were identified using a quadruple mass analyzer and authentic standards (Supelco 37, Chrompack 68A). The amounts of individual fatty acids were calculated based on method AOCS official method Ce 1b-89 (AOCS 2005).

RESULTS AND DISCUSSION

Study 1: Sensory Characteristics of Eastern Oysters

A total of 14 attributes were generated by the panel in training sessions including: liquor volume, meat color (pink, gray/brown, tan), roundness of the shell, plumpness, seaweed, fishy, earthy, salty, sweet, umami, firm, chewy. These attributes represent a variety of appearance, aroma, flavor and mouthfeel attributes of raw oysters that may result from harvest locations. Most of the attributes are self-explanatory, and used information provided in handouts generated in the training sessions (Appendix E). References for the sensory terms are identified in Appendix F. Oysters from six different locations in the Chesapeake bay and one each from Rhode Island and New Brunswick, Canada were tested for the above attributes by the trained panel. Oyster of each location were tested twice.

Panelists' marks on 15cm unstructured line scales were converted to 0-15 numerical values. Average scores of each attribute are shown in the following radar (spider graph) chart (Figure 3-1). Two-way analysis of variance (ANOVA) tests were conducted on each attribute with oyster location and panelists as the two factors. There were significant differences ($p < 0.05$) in the oysters examined for the following attributes: volume of liquor, gray/brown, tan, roundness of shell, plumpness, and salty. There were no significant differences for the other attributes (pink, seaweed, fishy, earthy, sweetness, umami, firm, chewy). The values of each attribute are shown on Table 3-4. The panelist factor in the ANOVA was significant for pink (appearance) and sweetness (flavor), indicating inconsistent performance between panelists. It may indicate have significant

difference($P<0.05$) in these two attributes, but due to the inconsistent performance of panelist, it cannot be detected in this test. More efficient training on the panel may solve this problem. For the other factors, the panelist factor was not significant, indicating that training was successful.

As shown in Table 3-1 and 3-2, there was noticeable difference ($P<0.05$) between the oysters originating from Virginia lower Eastern Shore area, Virginia Middle Bay area Western Shore and New Brunswick Canada on the attribute of volume of liquor. The two oysters from Virginia lower Eastern Shore area, Virginia Middle Bay area Western Shore had higher liquor than the one from New Brunswick. Liquor volume may be associated with raw oyster freshness by consumers because older oysters tend to dry out. Another attribute that may be associated with the freshness of raw oysters is plumpness. There was a significant difference ($P<0.05$) in the plumpness of VA Middle Bay area Western Shore (Chesapeake oyster) and the one from Rhode Island. This Chesapeake oyster had an average rating about 2.18 higher than Beaver Tail for plumpness. The standard of plumpness of this test is shown below. The gray brown and tan colors were higher in Chesapeake oysters than those from Rhode Island and New Brunswick.

Very few scientific studies have been conducted to evaluate sensory characteristics of raw Eastern oysters, especially Chesapeake oysters. But According to one gastronomist described the raw Virginia oysters in his book: “a perfect balance of salt and sweet, are

milder in complexity comparing to other northern oysters, but wonderfully plump and meaty”. (McMurray 2007). Our results of sensory study support the description.



Pic 3-1 Plumpness standard for raw Eastern Oyster Testing
From left to right: standard 3, 6, 11

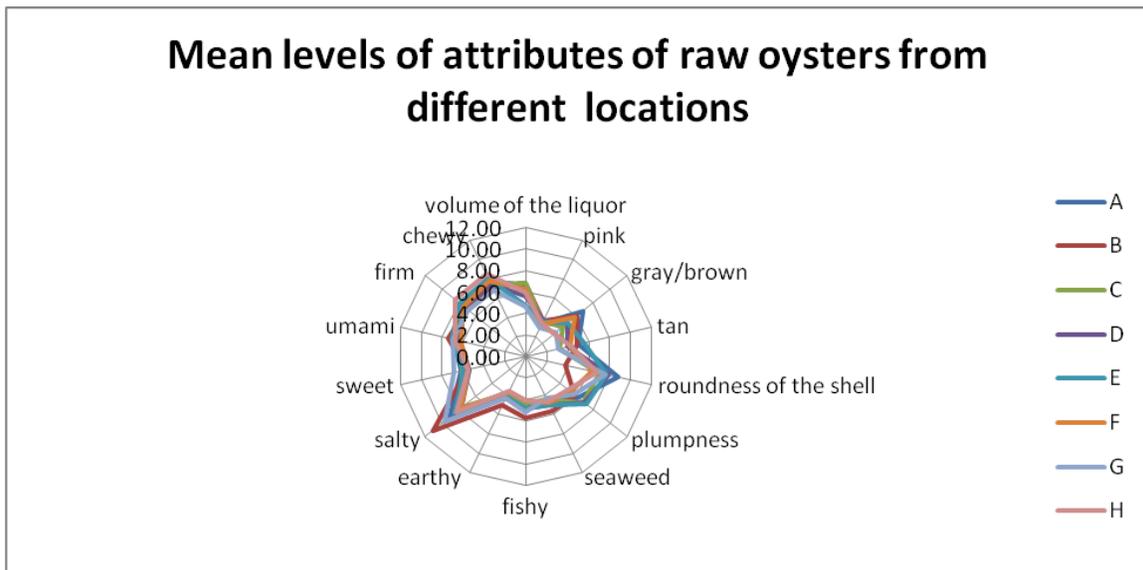


Figure 3-1: Mean levels of attributes of raw oysters from different locations as determined by experienced sensory panel (n=8 panelists) using a 15cm line scale; n=2 replications.

Table 3-4: Sensory Rating of Eastern raw oysters.

Numerical values with different letters are significantly different (p<0.05)

Sample Code	Appearance						Aroma
	liquor volume	pink	gray/brown	tan	roundness of the shell	plumpness	seaweed
A	6.55±3.0ab	3.49±2.1	6.74±2.4a	5.00±2.2a	8.80±2.1a	6.15±2.3ab	4.61±2.7
B	6.81±2.4a	3.68±2.1	6.06±2.8ab	4.85±2.2a	3.76±1.4c	5.81±2.0ab	5.66±2.3
C	6.82±2.4a	3.52±2.0	4.43±1.9bc	3.22±1.8bd	7.06±1.8ab	6.84±2.5ab	4.46±2.1
D	5.64±2.3ab	3.71±2.6	4.93±2.3abc	3.98±1.6ab	7.85±2.2ab	6.94±1.9a	5.03±2.2
E	4.78±1.9ab	3.61±1.9	4.95±2.3abc	5.50±2.3a	7.63±2.8ab	7.13±2.5a	5.01±2.6
F	6.28±2.8ab	3.28±2.0	5.84±2.6ab	4.26±2.6ab	6.31±2.1b	5.28±2.5ab	4.84±2.7
G	4.65±2.4b	3.01±1.9	3.55±1.5c	3.07±1.8bc	7.54±2.1b	5.72±2.6ab	4.36±1.4
H	5.89±2.7ab	3.48±1.6	3.34±1.4c	4.35±1.3ad	6.80±2.2b	4.95±2.0b	4.64±1.7

Sample Code			Flavor			Texture	
	fishy	earthy	Salty	sweet	umami	Firm	Chewy
A	4.78±2.5	4.01±2.2	9.18±3.0ab	6.26±1.8	6.84±2.3	7.66±2.0	7.79±2.4
B	5.71±2.5	5.04±2.9	11.08±2.7a	5.66±2.5	7.39±1.8	7.04±1.7	7.18±2.1
C	4.42±2.1	3.81±1.7	7.68±2.4b	6.04±1.4	6.58±1.3	7.45±1.5	7.46±1.9
D	4.86±1.7	4.29±2.3	7.79±2.6b	5.44±1.6	6.36±1.2	7.42±2.2	7.42±2.3
E	4.83±2.6	4.23±1.8	8.41±2.6b	5.98±1.6	7.09±1.8	7.87±1.9	8.11±2.2
F	4.09±2.5	3.68±2.3	7.73±2.8b	5.58±1.7	6.23±1.6	7.42±2.1	7.81±2.6
G	5.14±1.4	4.33±1.4	9.65±2.5ab	6.83±1.5	7.01±1.7	6.92±2.3	6.83±2.7
H	4.13±1.5	3.58±1.8	8.31±2.5b	5.51±0.9	6.79±1.2	8.43±2.1	8.50±2.5

This research also did a chemical analysis of Sodium Chloride content of fresh oysters because it is directly related to sensory attributes. The salt content of the samples ranged from 4.6% to 16% (Figure 3-2). There were significant differences ($p < 0.05$) in salt contents between samples (Table 3-5). Tukey's HSD at α level of 0.05 was $1.731E-10^5$. The result of sodium chloride analysis indicates that the oysters from Rhode Island had the highest chloride content, followed by raw oysters from VA lower Eastern Shore area, New Brunswick Canada. However the assessment of the trained panel indicated that oysters from Rhode Island and New Brunswick Canada were not statistically significant difference in salty, while VA lower Eastern Shore area was the most salty among the samples tested. There are possible explanations for these results. To eliminate panelist fatigue, only three oysters were presented in one test. Oysters from VA lower Eastern Shore area was evaluated with other two Chesapeake oysters which are lower in salt in the same test. There may have been some effects on panelists judgments due to the differences in saltiness of oysters presented. Beau Soleil and Beaver Tail oysters were tested alongside VA Tidewater area which had a high salt content. More effective panelist training may have helped eliminate the judgment bias when a sample high in one attribute is tasted alongside one with one low in that attribute. Another possible explanation is that we use Chloride QuanTabs® (Hatch Inc) to analyze the sodium chloride content. There may be other components which contain chloride in oysters that affect the sodium chloride 'salty' response, which may not have caused a change in analytical measurement of NaCl.

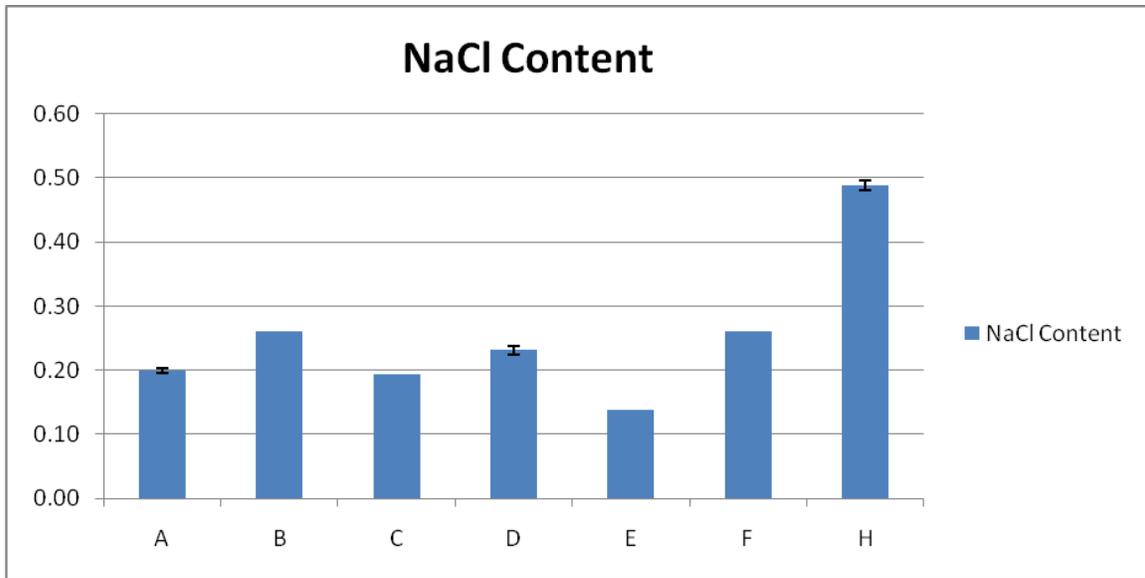


Figure 3-2 : NaCl contents of the flesh of raw oysters (g/100g wet basis)

Table 3-5: F test table of NaCl contents of the flesh of raw oysters

Source of Variation	Sum of Squares	d.f.	Variance	F	P
Between Groups:	0.0291	6	0.0049	306.9087	0.0000
Within Groups:	0.0002	14	1.42 e-5		
Total:	0.0293	20			

Study 2: Glycogen Content

The glycogen contents of oysters was measured in freeze dried oysters flesh three different times during the 2010-2011 season in September, December and April using a modified anthrone method (Roe and Daily, 1966).

The glycogen contents measured in September (Figure 3-3) varied significantly among different locations. No clear differences were seen between aquacultured or wild oysters. However, there was a statistically significant difference ($p < 0.05$) between diploid wild and triploid wild oysters in York River. Wild triploid oysters in York River had higher glycogen contents compared to diploid. In general, the glycogen contents increased from September to December and started to decline in April (Figure 3-4, Figure 3-5).

In many marine bivalves, glycogen storage and utilization is an important component of the annual energy budget, because glycogen is intimately tied to the reproductive cycle in bivalves.

As the Figure 3-6 demonstrates, Chesapeake oysters from VA lower Eastern Shore area and the one from New Brunswick Canada showed the same trend of glycogen content changes from September to April. The value of glycogen content is much more higher in December and declined when tested in April. These results agree with other reports (Allen & Downing 1986). Typically, the reproductive cycle in bivalve mollusks in temperate climates begins with a period of winter dormancy, a "rest" period, followed by a period of gonad differentiation, rapid growth, and nutrient storage (Darriba 2005). Thus, the glycogen content start to increase during the winter and spring and decrease steadily

throughout the gametogenic period, until it reaches the lowest point in July. This is just prior to spawning and glycogen increases thereafter.

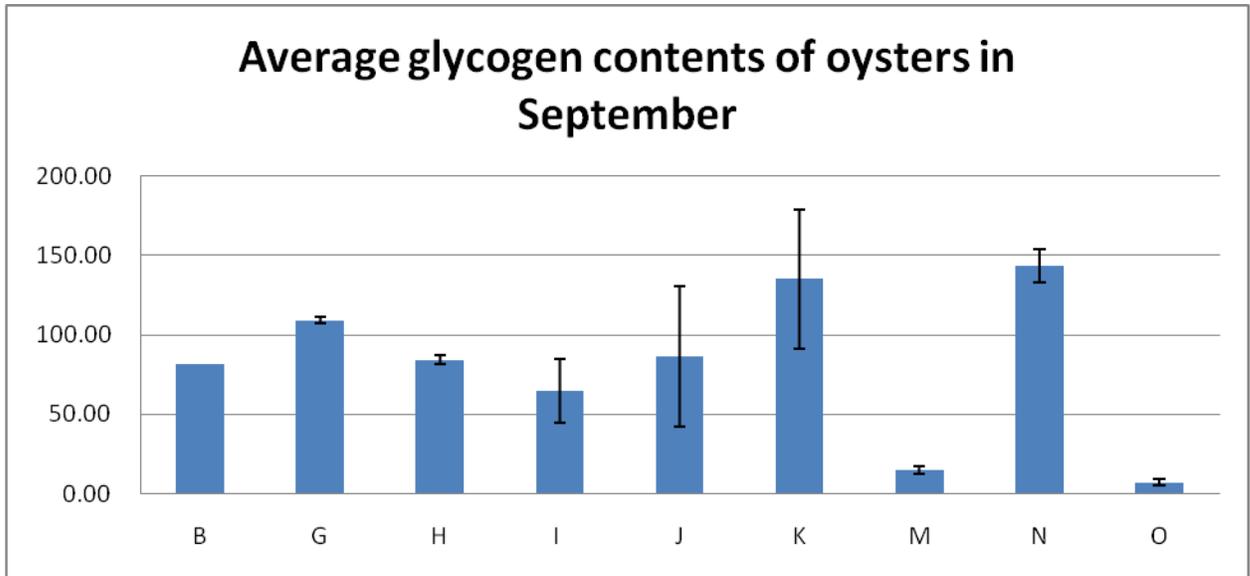


Figure 3-3: Average glycogen contents of oysters in September (mg/g dry basis)

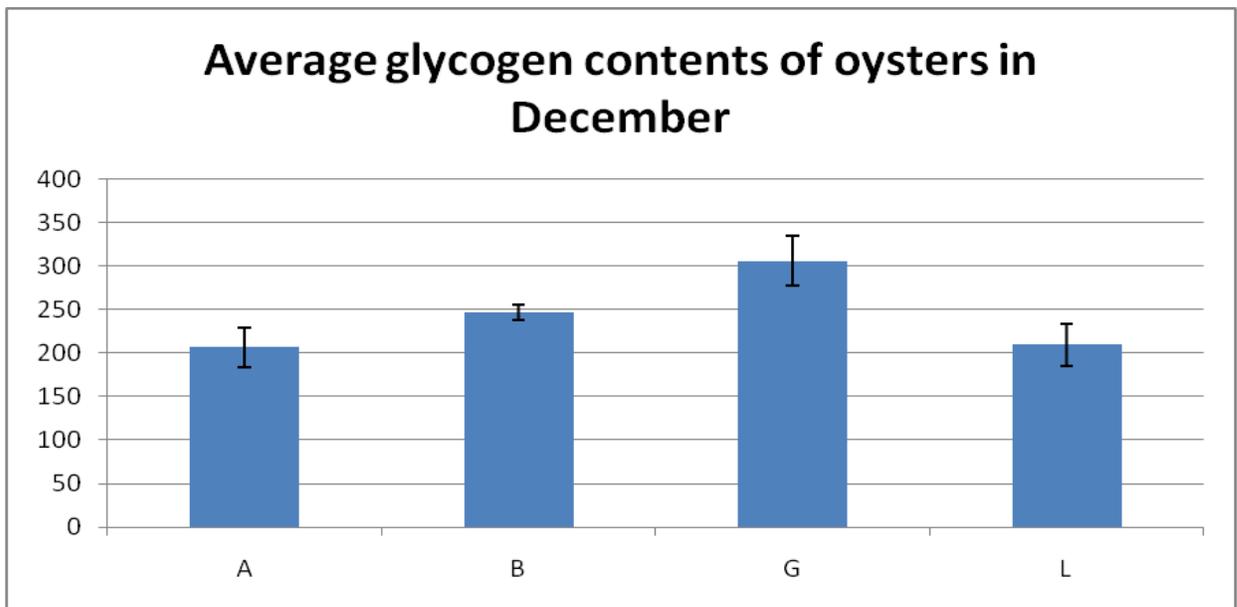


Figure 3-4: Average glycogen contents of oysters in December (mg/g dry basis)

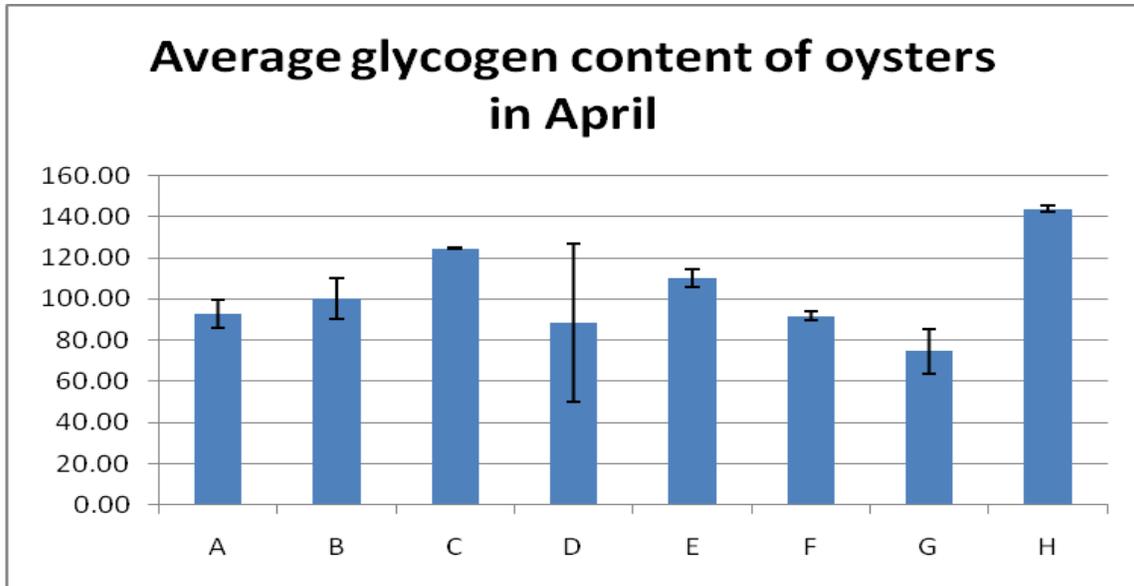


Figure 3-5: Average glycogen content of oysters in April (mg/g dry basis)

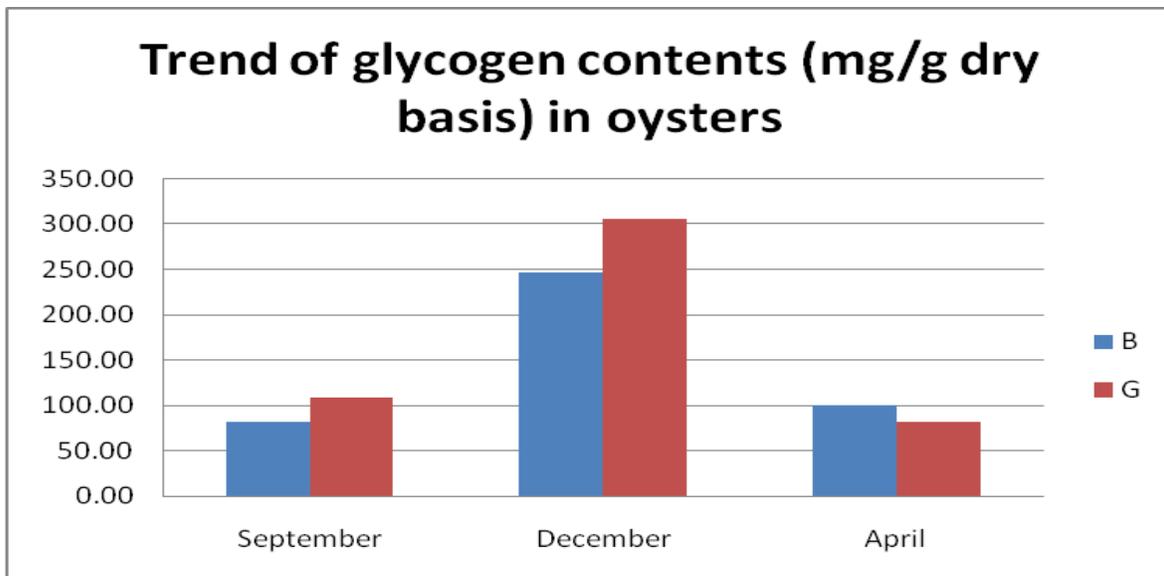


Figure 3-6: Trend of glycogen contents (mg/g dry basis) in oysters
 B is from VA lower Eastern Shore area but ocean side(Chesapeake)
 G is from New Brunswick Canada

Study 3: Proximate Analysis

Proximate analysis was performed to elucidate the differences in chemical and nutrient composition among raw oysters from VA Tidewater area, VA lower Eastern Shore area but ocean side, VA Middle Bay area Western Shore, VA Upper Bay area Eastern Shore, New Brunswick Canada, Rhode Island in March 2011.

Moisture

Moisture was major component in the raw oyster flesh. The average moisture content of the oyster tested ranged from 76.36% to 82.58% (Figure 3-7). The results were close to the moisture content of most marine animals (Kent 1985). Results of statistical analysis showed that moisture content was significantly different ($p < 0.05$) among the test samples using one-way ANOVA (Table 3-6). Therefore a Tukey's HSD compared the moisture content of test samples. According the calculation, the value HSD at α level of 0.05 is 3.5%. Generally speaking, the standard deviations of moisture content from Chesapeake are ranged from 1% to 2%, while that of the oyster from Canada and Rhode Island are 3.91, and 0.41 respectively. The moisture content of flesh may directly influence texture attributes when people taste the raw oysters and also the intensity of some flavors.

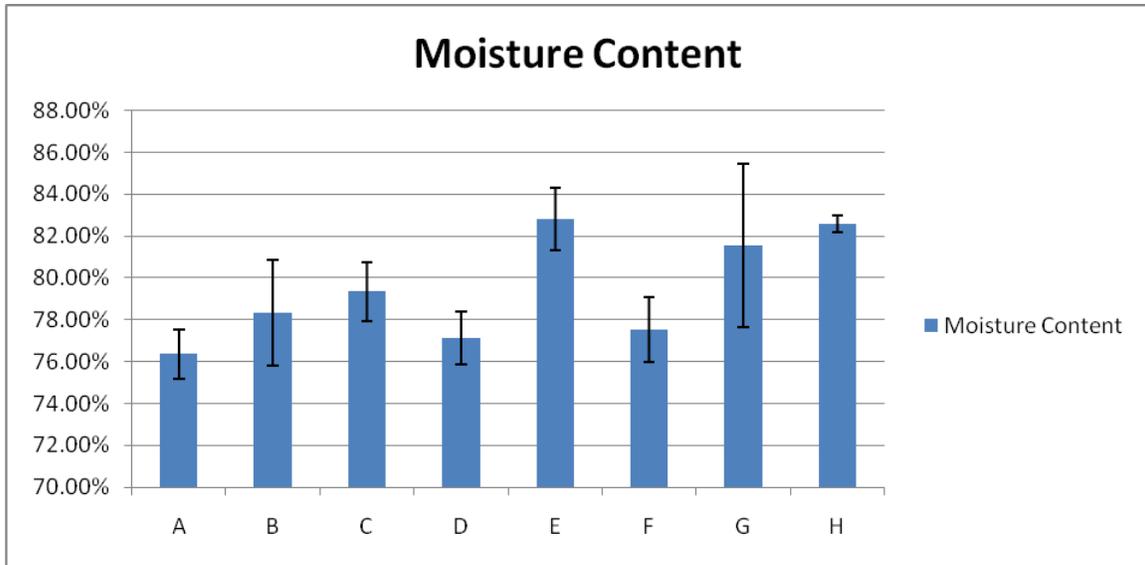


Figure 3-7 : Moisture Content of the flesh of raw oysters

Table 3-6: F test table of moisture content of the flesh of raw oysters

Source of Variation	Sum of Squares	d.f.	Variance	F	P
Between Groups:	0.0136	7	0.0019	4.9647	0.0038
Within Groups:	0.0063	16	0.0004		
Total:	0.0199	23			

Ash

The average ash content of the oyster ranged from 1% to 2% (Figure 3-8). Results of statistical analysis showed that ash content was significantly different ($p < 0.05$) among the test samples using one-way ANOVA (Table 3-7). Therefore a Tukey's HSD compared the ash content of test samples. According the calculation, the value HSD at α level of 0.05 is 0.43%.

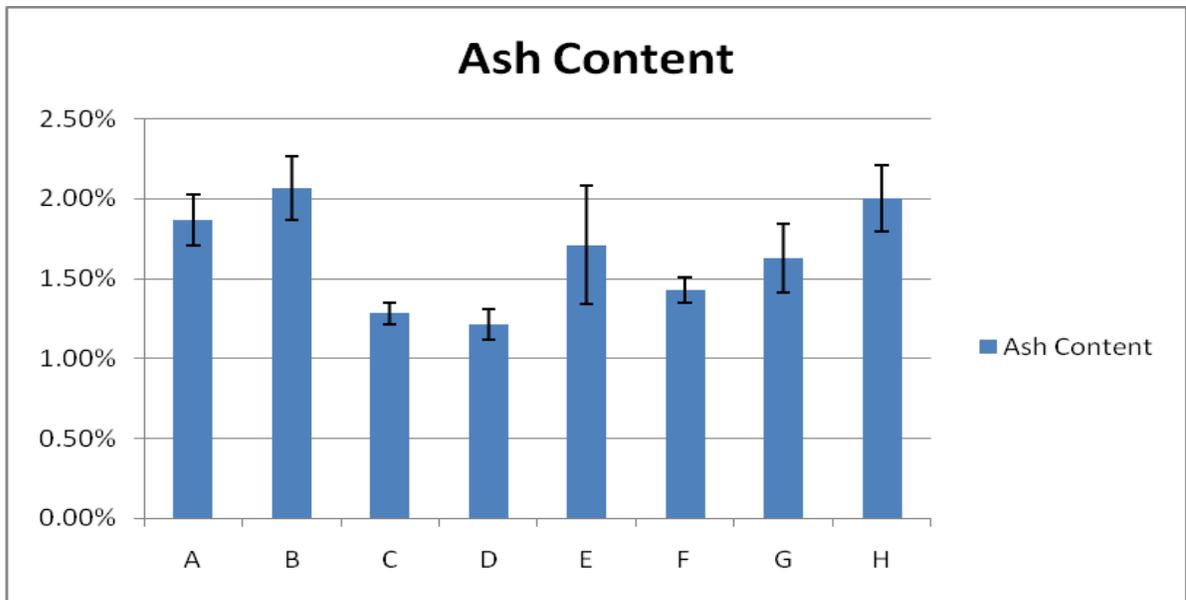


Figure 3-8: Ash Content of the flesh of raw oysters

Table 3-7: F test table of ash content of the flesh of raw oysters

Source of Variation	Sum of Squares	d.f.	Variance	F	P
Between Groups:	0.0002	7	0.000028	7.9045	0.0003
Within Groups:	0.0001	16	0.00000625		
Total:	0.0003	23			

Fat Content

The fat content was analyzed in freeze dried oysters and was converted wet basis using moisture contents. The fat content of the samples ranged from 1.26% to 4.20% (Figure 3-9). One way ANOVA showed that fat contents were significantly different ($p < 0.05$) between samples (Table 3-8), therefore a Tukey’s HSD compared the fat content of test samples. According the calculation, the value HSD at α level of 0.05 is 0.0566%.

Oysters from VA Middle Bay area Western Shore had the highest fat contents which was 4.20% while oysters VA Upper Bay area Eastern Shore which are also from Chesapeake, had the lowest one (1.26%).

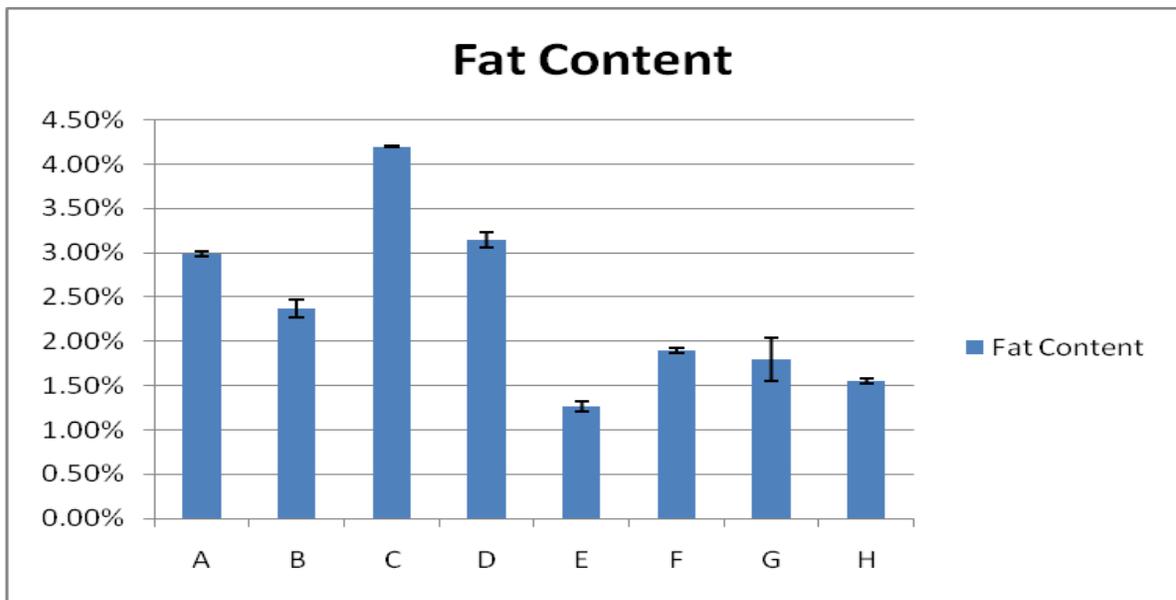


Figure 3-9: Fat contents of the flesh of raw oysters (g/100g wet basis)

Table 3-8: F test of fat contents of the flesh of raw oysters

Source of Variation	Sum of Squares	d.f.	Variance	F	P
Between Groups:	0.0020	7	0.0003	284.7224	0.0000
Within Groups:	0.0000	16	0.000000107		
Total:	0.00020	23			

Protein Content

The protein contents ranged from 7.17 % to 10.05% (Figure 3-9). Protein content was significantly different ($p < 0.05$) among the oyster samples using one way ANOVA (Table 3-6). Therefore a Tukey’s HSD compared the moisture content of test samples.

According the calculation, the value HSD at α level of 0.05 is 0.7495%.

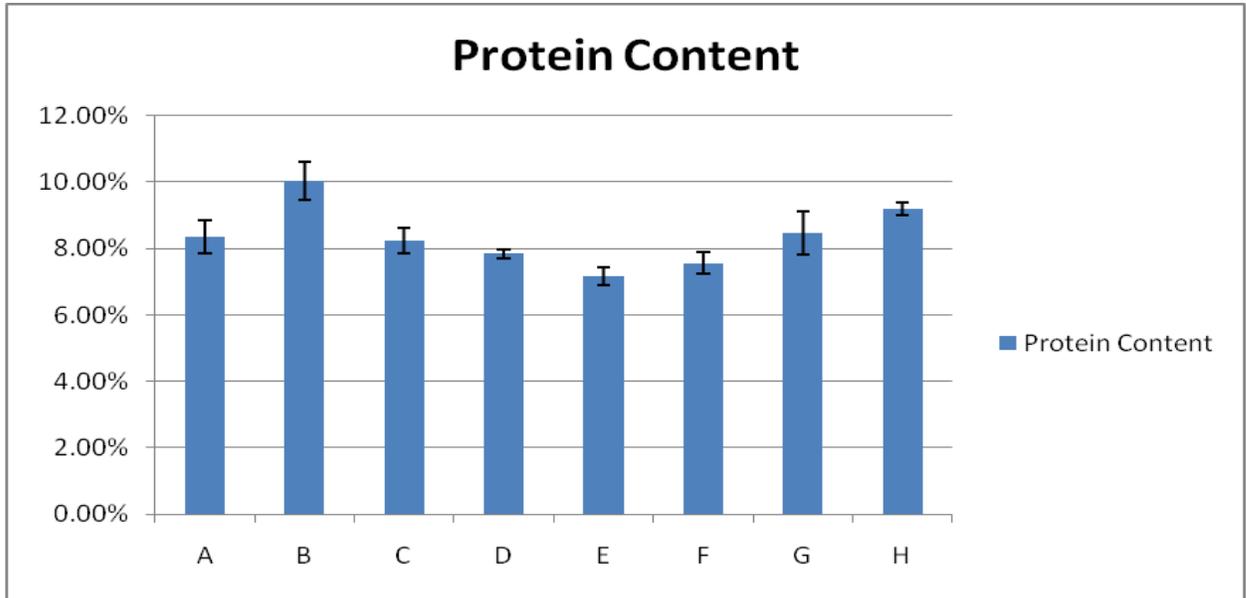


Figure 3-10: Protein contents of the flesh of raw oysters (g/100g wet basis)

Table 3-9: F test table of protein contents of the flesh of raw oysters

Source of Variation	Sum of Squares	d.f.	Variance	F	P
Between Groups:	0.0018	7	0.0003	15.2167	0.0000
Within Groups:	0.0003	16	0.00001875		
Total:	0.00020	23			

Summary

The proximate analyses of raw oysters are shown below (Table 3-8). Although there were significant differences in each component (moisture, ash, fat and protein), there was no apparent differences between Chesapeake oysters and oysters from the other locations. The USDA National Nutrient Database for Standard Reference, stated that for Mollusks, oyster, eastern, farmed, raw , the average moisture content is 86.2%, ash content is 1.5%, protein content 5.22% and total lipid content is 1.55% (USDA National Nutrient Database, 2011), which has litter bit higher value on moisture content but lower value on the fat and protein content, comparing to the sample that we tested

Table 3-10: Proximate composition of raw oysters.
Means with different letters are significantly different (p<0.05)

Sample Name	Moisture Content	Ash Content	Fat Content	Protein Content
A	76.36%±1.19% a	1.87%±0.16% a	2.99%±0.03% a	8.35%±0.48% a
B	78.32%±2.52% ac	2.07%±0.20% a	2.38%±0.10% b	10.05%±0.57% b
C	79.34%±1.43% acd	1.28%±0.07% b	4.20%±0.01% c	8.23%±0.37% a
D	77.12%±1.26% a	1.21%±0.09% b	3.15%±0.09% a	7.84%±0.13% ac
E	82.81%±1.49% b	1.71%±0.37% acd	1.26%±0.06% d	7.17%±0.26% c
F	77.53%±1.53% a	1.43%±0.08% bc	1.89%±0.02% e	7.56%±0.32% c
G	81.54%±3.91% bc	1.63%±0.22% abcd	1.79%±0.24% de	8.46%±0.64% ad
H	82.58%±0.41% bd	2.00%±0.21% acd	1.56%±0.03% de	9.19%±0.18% d

Study 4: Fatty Acid Analysis

Fatty acids were quantified as fatty acid methyl esters using a gas chromatograph mass spectrometer (Appendix L). Cod liver oil and commercial standards were used with mass spectra for FAME identification. According to the analysis, fatty acids (greater than 0.2 wt%) compositions are found to be very similar between oyster samples (Appendix M). The main fatty acids identified were 14:0, 15:0, 16:0, 16:1n-7, 17:0, 18:0, 18:1n-11, 18:1n-9, 18:1n-7, 18:2n-6, 20:1n-11, 18:3n-3, 20:1n-9, 20:1n-7, 18:4n-3, 20:4n-6, 22:2 NMID, 20:4n-3, 20:5n-3, 22:4n-6, 22:5n-3, 22:6n-3. In addition, oysters from New Brunswick Canada were not found to have detectable levels of 22:4n-6 and 20:4n-3.

Among the identified fatty acids, three contribute to the nutrition value of raw oysters- EPA (eicosapentaenoic acid , 20:5n-3), DPA (clupanodonic acid , 22:5n-3) and DHA (Docosahexaenoic acid , 22:6n-3). A great deal of research has demonstrated the health benefits of those three fatty acids on cardiovascular disease, inflammatory disease, brain development and function (Ruxton, Reed and others 2004). The American Heart Association (AHA) now recommends that patients with known coronary heart disease (CHD) consume about 1 g/d of EPA + DHA. For individuals without known CHD, the AHA recommends at least two servings/wk of (preferably oily) fish to reduce the risk of cardiovascular disease; this would equate to approximately 500 mg/d of EPA + DHA (Sands, Reid, Windsor and Harris, 2005).

The following figures (Figure 3-11, Figure 3-12, Figure 3-13) compare the contents of EPA, DPA and DHA in the oysters. As can be seen from the figures and composition tables,

the main omega-3 fatty acids in oysters were EPA and DHA, which account for about 30% of the total fat weight, while the content of DPA is only around 0.5% of the total. When comparing levels of long chain n-3 fatty acids in oysters from different locations, the one from New Brunswick, Canada had the lowest level for EPA, DPA and DHA, and levels were about one third of values for the other oysters. The weight contents of EPA, DPA and DHA of oysters from Chesapeake and other US locations were in the same range.

The weight contents of the sum of the n-3 fatty acids (Figure 3-13) in tested samples also demonstrates the same trend. The oysters from US (Chesapeake and New York State, Rhode Island) contain 34-42 % of ω 3 fatty acids in the extracted oil while the oyster from Canada only contained 10.3%.

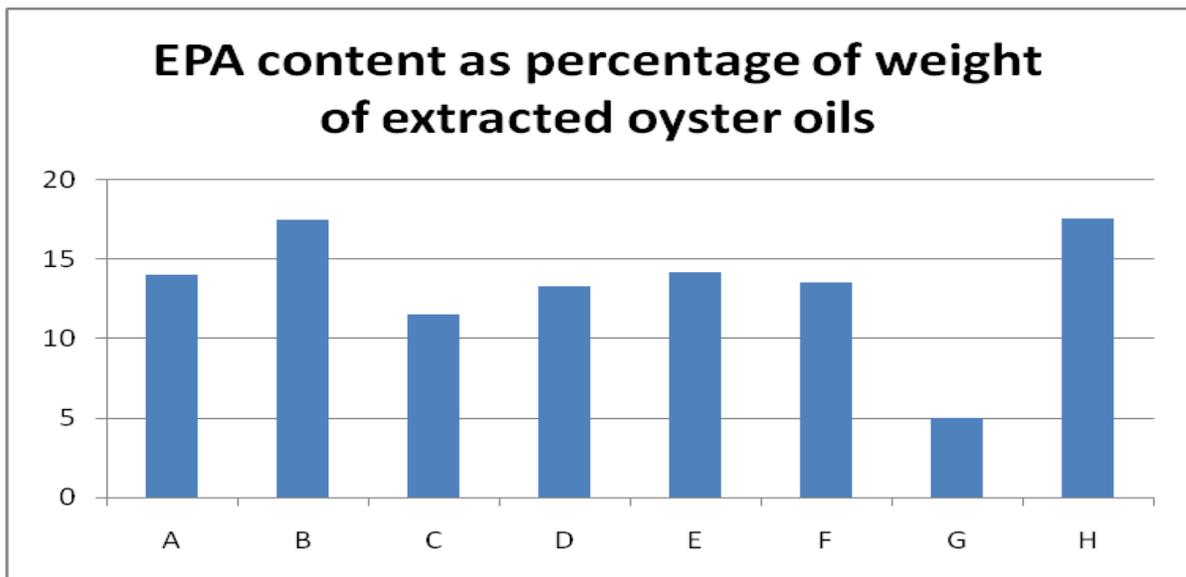


Figure 3-11: EPA content as percentage of weight of extracted oyster oils

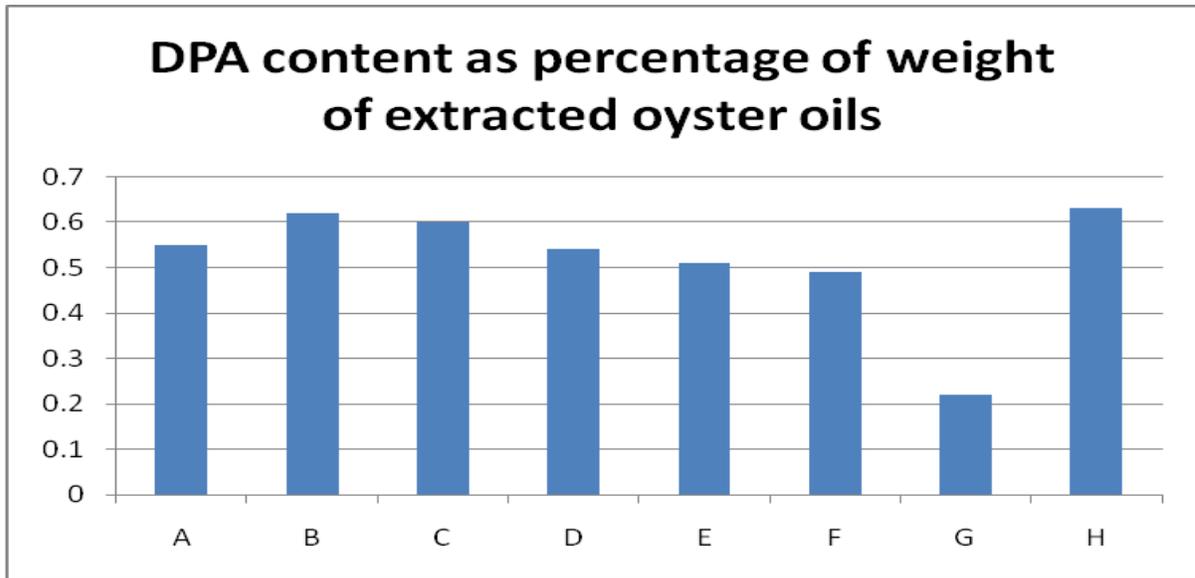


Figure 3-12: DPA content as percentage of weight of extracted oyster oils

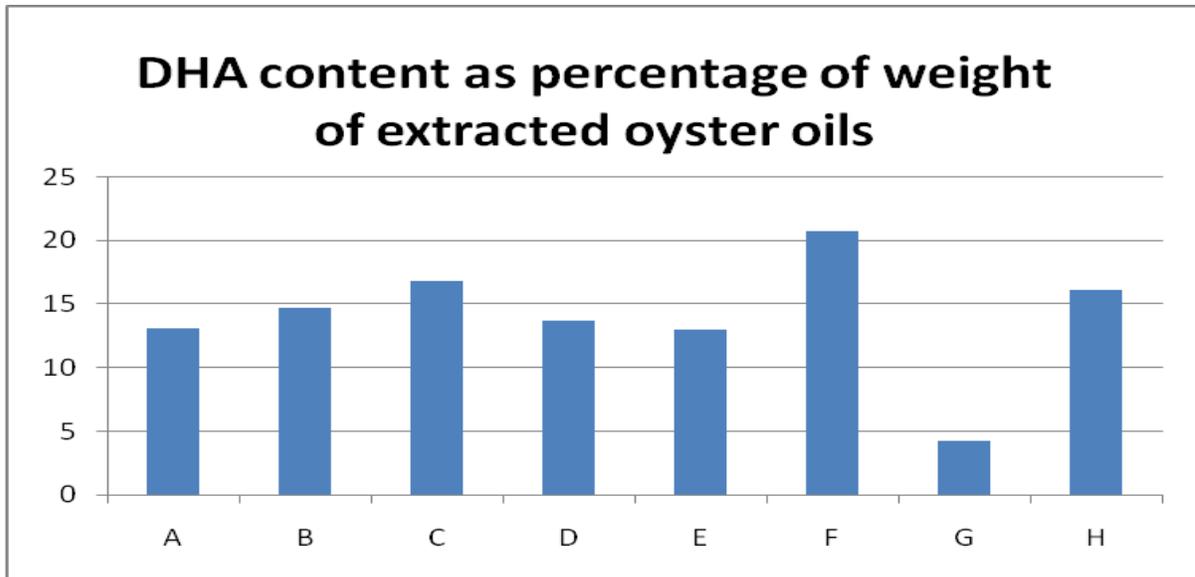


Figure 3-13: DHA content as percentage of weight of extracted oyster oils

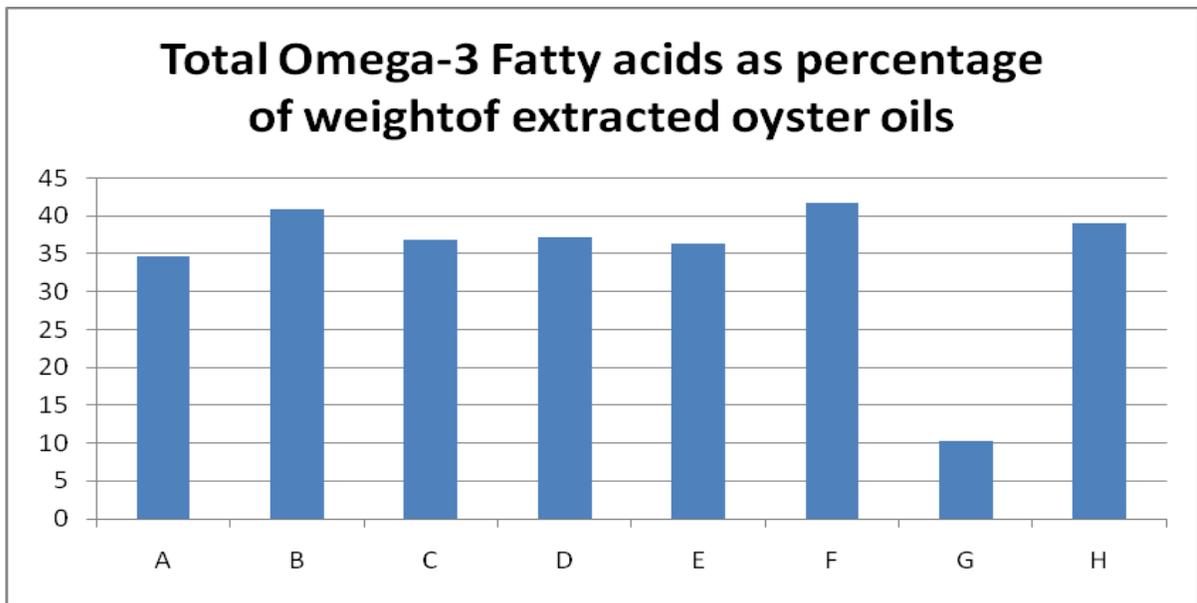


Figure 3-14: Total Omega-3 fatty acids as percentage of weight of extracted oyster oils

CONCLUSIONS

Sensory characteristics of eastern Oysters were evaluated using a trained panel. Sensory characteristics examined include volume of the liquor, color of the meat (pink, gray/brown, tan), roundness of the shell, plumpness, seaweed, fishy, earthy, salty, sweet, umami, firm, and chewy, in appearance, aroma, flavor and texture sensory attribute classes. When comparing eastern oysters from Chesapeake area to other two areas (one from Rhode Island, another from New Brunswick, Canada), statistical differences were found in the attributes of volume of liquor, gray/brown, tan, roundness of shell, plumpness and salty. The glycogen contents in the eastern oysters followed the reproductive cycle, and glycogen increased through September to wintertime and started to decrease when temperature got warmer (the tests were conducted in September, December and April). Although no relationships was found in the content of moisture, ash, protein and fat of oysters from Chesapeake and other areas, the oysters from Chesapeake had higher percentages of n-3 fatty acid, which were about 4 times higher comparing with Beau Soleil, an oyster from New Brunswick, Canada.

The characteristics of higher volume of liquor, plumpness and higher weight content of omega 3 fatty acids in Chesapeake Oysters can be used in the marketing promotion of Chesapeake Oysters.

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USDA National Nutrient Database. 2011 July 1st.

<http://www.nal.usda.gov/fnic/foodcomp/cgi-bin/list_nut_edit.pl>

Appendices
Appendix A
IRB Approval Letter



Office of Research Compliance
Institutional Review Board
2000 Kraft Drive, Suite 2000 (0497)
Blacksburg, Virginia 24060
540/231-4606 FAX 540/231-0959
E-mail irb@vt.edu
Website: www.irb.vt.edu

MEMORANDUM

DATE: September 15, 2010

TO: Michael L. Jahncke, Daniel E. Kauffman, Luman Chen, Susan E. Duncan

FROM: Virginia Tech Institutional Review Board (FWA00000572, expires June 13, 2011)

PROTOCOL TITLE: Sensory Characteristics of Eastern Oysters

IRB NUMBER: 10-425

Effective September 15, 2010, the Virginia Tech IRB Chair, Dr. David M. Moore, approved the amendment request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report promptly to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at <http://www.irb.vt.edu/pages/responsibilities.htm> (please review before the commencement of your research).

PROTOCOL INFORMATION:

Approved as: Exempt, under 45 CFR 46.101(b) category(ies) 6

Protocol Approval Date: 5/14/2010

Protocol Expiration Date: NA

Continuing Review Due Date*: NA

*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

FEDERALLY FUNDED RESEARCH REQUIREMENTS:

Per federal regulations, 45 CFR 46.103(f), the IRB is required to compare all federally funded grant proposals / work statements to the IRB protocol(s) which cover the human research activities included in the proposal / work statement before funds are released. Note that this requirement does not apply to Exempt and Interim IRB protocols, or grants for which VT is not the primary awardee.

The table on the following page indicates whether grant proposals are related to this IRB protocol, and which of the listed proposals, if any, have been compared to this IRB protocol, if required.

Invent the Future

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY
An equal opportunity, affirmative action institution

Date*	OSP Number	Sponsor	Grant Comparison Conducted?

*Date this proposal number was compared, assessed as not requiring comparison, or comparison information was revised.

If this IRB protocol is to cover any other grant proposals, please contact the IRB office (irbadmin@vt.edu) immediately.

cc: File

Appendix B
Descriptive Panel Recruiting Email

Greeting fellows!

You are invited to attend a sensory study on Chesapeake raw oysters which are pretty expensive & rarely can be found in town. I am inquiring in your interest and availability to participate in this trained panel.

The time commitment would include 4-5 times x 1 hr sessions during late January and Early March (winter time) based on every panel's time availability. Your time would be compensated with a luncheon provided by myself by the end of the training session.

We can work together to schedule times for these events.

And there are no more than minimal risks for participating in this study. All the oysters sample are freshly shipped and you are supposed to spit the raw oysters out during the sensory study.

If you are interested, Please follow the link below to fill out a selection survey.

<https://survey.vt.edu/survey/entry.jsp?id=1289511814224>

For more information, contact Luman Chen at lumanchen@gmail.com

P.S. Please make sure you can give the time contribution and come every training session (once a week) before filling out the survey. All the sensory studies will be conducted in Food Science & Technology Building. Your generosity is greatly appreciated.

Appendix C

Descriptive Panel Recruiting On line Survey

Sensory Study of Oysters

Name

Contact E-mail

Please indicate your gender

- Male
- Female

Please indicate your age group:

- 18-24
- 25-30
- 31-40
- 41-50
- 51-60
- 61-70
- 71+

What is your ethnic group?

- Hispanic, Latino, or Spanish Origin
- White/ Caucasian
- Black/ African American
- American Indian or Alaskan Native
- Asian or Pacific Islander
- Other

Do you have any of the following?

- Nasal disease
- Hypoglycemia
- Sea food allergy
- None of the above

If you have seafood allergy, please specify your allergen

How often do you consume seafood?

- More than 3 times a week
- 1-3 times a week

- 1-3 times a month
- Less than once a month

In what way you prefer to consume oysters?

- Raw
- Boil
- Deep Fry
- Other

How often do you consume raw oysters?

- More than 3 times a month
- 1-3 times a month
- Few times a year
- Never consume raw oysters before

If you consume raw oysters, please indicate your likeness of raw oysters:

- Like extremely
- Like moderately
- Like slightly
- Neither like nor dislike
- Dislike slightly
- Dislike moderately
- Dislike extremely

If you consume raw oysters, please lists few words that you use to describe the taste of oysters:

	 
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Appendix D

Consent Form for Panel Training

Virginia Polytechnic Institute and State University

Virginia Seafood Agriculture Research and Extension Center

Informed Consent for Participants in Research Projects Involving Human Subjects (Sensory Evaluation)

Title Project: Sensory Characteristics of Eastern Oysters

Principal Investigator(s): Michael L. Jahncke, Ph.D.; Professor of Food Science and Technology

Co-Investigators: Dr. Daniel Kauffman; Dr. Susan Duncan; Luman Chen.

I. Purpose of this Research/Project

This is a research study. The purpose of this study is to identify sensory characteristics of Eastern oysters from locations within the Chesapeake Bay. The results of this study will be distributed to oyster aquaculturists in Virginia and surrounding states. The results will also be used as part of a graduate student's research dissertation (Luman Chen, anticipated Master, Department of Food Science and Technology, Virginia Tech).

This consent form will give you information needed to help you decide if you would like to participate in this study. You may ask questions about the research, your participation in the study, risks, benefits or anything else this form does not make clear. When all your questions have been answered, you can then decide if you would like to participate in the study. Please read this form carefully.

II. Procedures

If you agree to participate, you will be required to evaluate several samples of oysters on the half-shell during each training/testing session. There will be 3-6 training sessions held over a period of month during the winter of 2010. Training sessions will be one-hour. You will be required to attend at least 4 of these sessions. Testing sessions will begin in March 2011. Testing sessions will require you to evaluate the flavor of several samples of raw commercial oysters on the half-shell. **You will be required to chew the oyster for a specific period of time to evaluate the flavor, at which point you will expectorate the sample.** A lexicon developed during training sessions will be used to determine characteristics in oyster samples on a paper ballot. All recorded data will be coded in a confidential way to protect your identity during testing and data analysis. Testing will be completed by March 31, 2011. No more than 20 testing sessions will be held over the

whole study. All training and testing will be held at Virginia Seafood Agriculture Research and Extension Center (VSAREC) 102 S King St Hampton, VA 23669

III. Risks

Foreseeable risks include those associated with consuming fresh raw commercial shellfish. **People with seafood/shellfish allergies are not eligible to participate.** You will be required to chew and expectorate raw oysters on the half-shell. As recommended by the Interstate Shellfish Sanitation Conference (www.issc.org), consumption of raw oysters creates a risk of serious illness to individuals with certain medical conditions. These include:

- Liver Disease
- Chronic alcohol abuse
- Cancer
- Diabetes
- Inflammatory bowel and stomach diseases
- Steroid dependency (as used for conditions such as chronic obstructive pulmonary disease, etc.)
- Achlorhydria (a condition in which the normal acidity of the stomach is reduced or absent)
- AIDS

It is highly recommended that individuals with these conditions avoid eating raw or undercooked oysters. If you have one or more of these conditions you will not be allowed to participate in this study.

Oysters will be collected from an Interstate Certified Shellfish Shippers List (ICSSL) harvester within a 3-day period before testing. Oysters that come from an ICSSL commercial harvester have been certified by regulatory authorities in the United States. The publication is distributed under the authorities of the Public Health Service Act and the Food, Drug and Cosmetic Act by the U.S. Food and Drug Administration (FDA) in conjunction with the Office of Compliance, Shellfish Safety Team. All handling of sample oysters will follow commercial HACCP plans for harvesting, packing and storing of shellfish. Additionally, you will be required to expectorate samples after flavor evaluations to reduce risks associated with consuming raw oysters and also to keep from dulling your sensory capabilities because of satiation.

IV. Benefits

Potential benefits include training in sensory evaluation techniques of local oysters. Broader benefits will be to the Virginia oyster aquaculture industry in their ability to distinguish and better market their product. No promise or guarantee of benefits has been made to encourage your participation. At the completion of the research you may contact the researchers for a summary of the research results.

V. Extent of Anonymity and Confidentiality

The results of your performance as a panelist will be kept strictly confidential except to the investigators. Individual panelists will be referred to by a code number for data analyses and for any publication of the results.

VI. Compensation

You will be compensated with a snack for participating in this study.

VII. Freedom to Withdraw

If you agree to participate in this study, you are free to withdraw from the study at any time without penalty. There may be reasons under which the investigator may determine you should not participate in this study. If you have allergies to egg or any cake products, or are under the age of 18, you are asked to refrain from participating.

VII. Subject's Responsibilities

I voluntarily agree to participate in this study. I have the following responsibilities:

1. Attend all the training sessions of sensory study of Eastern Oysters
2. Evaluate the flavor of several samples of raw commercial oysters on the half-shell.
You will be required to chew the oyster for a specific period of time to evaluate the flavor, at which point you will expectorate the sample.

IX. Subject's Permission

I have read the Consent Form and conditions of this project. I have had all my questions answered. I hereby acknowledge the above and give my voluntary consent:

_____ Date _____

Subject Signature

Subject Printed Name

Appendix E
First Training Handout

Session 1: Basic Knowledge of sensory evaluation techniques and term generation

1. Determination of sensory evaluation:

Scientific discipline used to evoke measure, analyze and interpret reactions to those characteristics of foods and materials as they perceived by the senses of sight, smell, taste, touch and hearing. (Stone and Sidel, 1993)

Descriptive sensory test: Detection, discrimination, description of qualitative (sensory variable) and quantitative (intensity) aspect of a product by highly trained panelist.

In this sensory test, we will be evaluated the sensory variables that generated in the training session in 15 cm scales.

2. Sensory Attributes:

Appearance	Color, size and shape, surface texture, clarity...
Odor/ Aroma	
Taste	Five basic taste: salt, sour, bitter, sweet, umami
Texture	Reaction to stress, measured as mechanical properties (eg. hardness, cohesiveness, chewiness...) Tactile feel properties, measured as geometrical properties(eg. grainy, flaky, gritty...)

3. Term Generation:

Appearance:

Name of variables	Definition	Related food Reference
Volume of liquor	Quantity of oyster liquor in the shell	
Translucency of liquor	How clear/ translucent or how opaque the oyster is	
Plumpness	How well rounded and full form the oyster meat is(from flaccid to very plump)	

Odor:

Name of variables	Definition	Related food Reference
Seaweed	Related to seaweed	
Earthy	Refers to the characteristics to damp soil, and wet plants	
Fishy	Refers to a fishy aroma	
Metallic	Relating to the characteristics of a metal	
Grass	Refers to grass aroma	

Taste

Name of variables	Definition	Related food Reference
Salt		
Sour		
Bitter		
Sweet		
Umami	A meaty, savory, or mouth filling sensation	
Seaweed		
Fishy		
Spinach		
Astringency	Aftertaste: Astringency is the	

	drying, roughing and sometimes puckering sensation eg. red wine	

Texture

Name of variables	Definition	Related food Reference
Firmness	Consistency of how soft versus how firm in resistance the oysters flesh holds	
Chewiness	Amount of maceration required to comfortably swallow the oyster	

The variables would be evaluated in this training panel are:

Appendix F
Frame of Reference

Appearance: plumpness, volume of the liquor, color of the meat, roundness of the shell,

Odor: Seaweed, Fishy, earthy

Flavor: Salty, Sweet, Umami

Texture: Firmness, Chewiness

Standards of sensory attributes

1. Volume of liquor



A



B



C

A: Standard 1

B: Standard 6

C: Standard 11

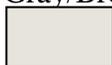
2. Color of meat

Pink



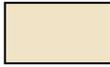
	A: Standard 5	B: Standard 7	C: Standard 11	D: Standard 15
RGB	241/232/233	229/202/215	229/167/190	206/109/137

Gray/Brown



	A: Standard 4	B: Standard 5	C: Standard 9	D: Standard 12
RGB	231/228/219	221/215/205	203/196/185	182/171/157

Tan



	A: Standard 3	B: Standard 6	C: Standard 9	D: Standard 13
RGB	240/227/198	236/194/129	216/165/81	194/137/24

3. Roundness of the shape



A



B



C



D

- A: Standard 2
- B: Standard 6
- C: Standard 11
- D: Standard 13

4. Plumpness

Pics



From left to right: standard 3, 6, 11

Aroma

Seaweed, Fishy, earthy

1. Seaweed

standard 5: 1-1 ½ cups of water for 2 to 3 standards of dried seaweed. Bring water to boil or close to boil. Break dried seaweed into 2-3 inch pieces and put in hot water. Allow to soak overnight & cool

Standard 10: leave the seaweed for 48 hours or more at refrigerated temperature after warm liquid on the soaked seaweed cools down

2. Earthy

Std 10- wet fresh soil

Fishy

Std 5- Clam Juice

Std 10- can of sardines in water

Flavor

1. Salt

Std 3 0.3% NaCl (0.725 g salt in 240 g water)

Std 8 0.53% NaCl (1.31 g salt in 248 g water)

Std 13 7% NaCl (1.675 g salt in 237.5 g water)

2. Sweet

Std 6 0.27% Splenda (1.3g sugar in 475 g of water)

Std 15 0.53% Splenda (2.5g sugar in 470.8 g of water)

3. Umami

Monosodium Glutamate (MSG)

Std 5 0.22% MSG (1g MSG in 474.4 g of water)

Std 10 0.45% MSG (2.1g MSG in 465 g of water)

Texture

1. Firmness

Std 1 Yogurt

Std 7 Canned peaches

Std 15 Almond

Chewiness

Std 1 Yogurt

Std 7 Canned peaches

Std 14 Raisin

Appendix G
 Work Sheet for Training Evaluation Test
 Day I
 Appearance

Code	Sample Name
629	Beaver Tails
504	Mobjacks

Judge No.	Order of samples	
1	629	504
2	504	629
3	629	504
4	504	629
5	629	504
6	504	629
7	629	504
8	504	629

Flavor & Texture

Code	Sample Name
903	Beaver Tails
258	Mobjacks

Judge No.	Order of samples	
1	903	258
2	258	903
3	903	258
4	258	903
5	903	258
6	258	903
7	903	258
8	258	903

Day II
Work Sheet for Training Evaluation Test
Appearance

Code	Sample Name
658	Beaver Tails
322	Mobjacks

Judge No.	Order of samples	
1	658	322
2	322	658
3	658	322
4	322	658
5	658	322
6	322	658
7	658	322
8	322	658

Flavor & Texture

Code	Sample Name
760	Barcat
092	Mobjacks

Judge No.	Order of samples	
1	760	092
2	092	760
3	760	092
4	092	760
5	760	092
6	092	760
7	760	092
8	092	760

Appendix H

Consent Form for Test

Virginia Polytechnic Institute and State University

Virginia Seafood Agriculture Research and Extension Center

Informed Consent for Participants in Research Projects Involving Human Subjects (Sensory Evaluation)

Title Project: Sensory Characteristics of Eastern Oysters

Principal Investigator(s): Michael L. Jahncke, Ph.D.; Professor of Food Science and Technology

Co-Investigators: Dr. Daniel Kauffman; Dr. Susan Duncan; Luman Chen.

I. Purpose of this Research/Project

This is a research study. The purpose of this study is to identify sensory characteristics of Eastern oysters from locations within the Chesapeake Bay. The results of this study will be distributed to oyster aquaculturists in Virginia and surrounding states. The results will also be used as part of a graduate student's research dissertation (Luman Chen, anticipated Master, Department of Food Science and Technology, Virginia Tech).

This consent form will give you information needed to help you decide if you would like to participate in this study. You may ask questions about the research, your participation in the study, risks, benefits or anything else this form does not make clear. When all your questions have been answered, you can then decide if you would like to participate in the study. Please read this form carefully.

II. Procedures

If you agree to participate, you will be required to evaluate several samples of oysters on the half-shell during each training/testing session. There will be 6 training sessions held over a period of month during the winter of 2010. Training sessions will be one-hour. You will be required to attend at least 6 of these sessions. Testing sessions will begin in March 2011. Testing sessions will require you to evaluate the flavor of several samples of raw commercial oysters on the half-shell. **You will be required to chew the oyster for a specific period of time to evaluate the flavor, at which point you will expectorate the sample.** A radar graph developed during test sessions will be used to determine characteristics in oyster samples on 15cm scale. All recorded data will be coded in a confidential way to protect your identity during testing and data analysis. Testing will be completed by March 31, 2011. No more than 5 testing sessions will be held over the

whole study. All training and testing will be held at Virginia Seafood Agriculture Research and Extension Center (VSAREC) 102 S King St Hampton, VA 23669

III. Risks

Foreseeable risks include those associated with consuming fresh raw commercial shellfish. **People with seafood/shellfish allergies are not eligible to participate.** You will be required to chew and expectorate raw oysters on the half-shell. As recommended by the Interstate Shellfish Sanitation Conference (www.issc.org), consumption of raw oysters creates a risk of serious illness to individuals with certain medical conditions. These include:

- Liver Disease
- Chronic alcohol abuse
- Cancer
- Diabetes
- Inflammatory bowel and stomach diseases
- Steroid dependency (as used for conditions such as chronic obstructive pulmonary disease, etc.)
- Achlorhydria (a condition in which the normal acidity of the stomach is reduced or absent)
- AIDS

It is highly recommended that individuals with these conditions avoid eating raw or undercooked oysters. If you have one or more of these conditions you will not be allowed to participate in this study.

Oysters will be collected from an Interstate Certified Shellfish Shippers List (ICSSL) harvester within a 3-day period before testing. Oysters that come from an ICSSL commercial harvester have been certified by regulatory authorities in the United States. The publication is distributed under the authorities of the Public Health Service Act and the Food, Drug and Cosmetic Act by the U.S. Food and Drug Administration (FDA) in conjunction with the Office of Compliance, Shellfish Safety Team. All handling of sample oysters will follow commercial HACCP plans for harvesting, packing and storing of shellfish. Additionally, you will be required to expectorate samples after flavor evaluations to reduce risks associated with consuming raw oysters and also to keep from dulling your sensory capabilities because of satiation.

IV. Benefits

Potential benefits include training in sensory evaluation techniques of local oysters. Broader benefits will be to the Virginia oyster aquaculture industry in their ability to distinguish and better market their product. No promise or guarantee of benefits has been made to encourage your participation. At the completion of the research you may contact the researchers for a summary of the research results.

V. Extent of Anonymity and Confidentiality

The results of your performance as a panelist will be kept strictly confidential except to the investigators. Individual panelists will be referred to by a code number for data analyses and for any publication of the results.

VI. Compensation

You will be compensated with a snack for participating in this study.

VII. Freedom to Withdraw

If you agree to participate in this study, you are free to withdraw from the study at any time without penalty. There may be reasons under which the investigator may determine you should not participate in this study. If you have allergies to egg or any cake products, or are under the age of 18, you are asked to refrain from participating.

VII. Subject's Responsibilities

I voluntarily agree to participate in this study. I have the following responsibilities:

3. Attend all the training sessions of sensory study of Eastern Oysters
4. Evaluate the flavor of several samples of raw commercial oysters on the half-shell.
You will be required to chew the oyster for a specific period of time to evaluate the flavor, at which point you will expectorate the sample.

IX. Subject's Permission

I have read the Consent Form and conditions of this project. I have had all my questions answered. I hereby acknowledge the above and give my voluntary consent:

_____ Date _____

Subject Signature

Subject Printed Name

-----For human subject to keep-----

IRB No.: 10-425

Should I have any pertinent questions about this research or its conduct, and research subjects' rights, and whom to contact in the event of a research-related injury to the subject. I may contact:

Luman Chen, Graduate Teaching Assistant,
Investigator

(540) 818-0933; lmchen@vt.edu
(757) 727-4861;

Michael L. Jahncke
mjahncke@vt.edu

David Moore

Chair, Virginia Tech Institutional Review
moored@vt.edu

(540) 231-4991;

Board for the Protection of Human Subjects
Office of Research Compliance
1880 Pratt Drive, Suite 2006 (0497)
Blacksburg, VA 24061

Appendix I
Sensory Test Score Card for Appearance

Scaling Test

Name:

Date: March 2nd

Instructions:

1. Receive a Ziploc container with four samples in a container and note each sample code is marked on the containing cup.
2. Evaluate each sample on the following attributes of appearance
3. Mark the four samples on the same scale of each attribute.
4. When finish turn the card to the instructor.

Sample No:

Volume of liquor

Color of meat

Pink

Gray/ Brown

Tan

Roundness of Shape

Plumpness

Appendix J
Sensory Test Score Card for Aroma, Taste, Texture

Scaling Test

Name:
Judge No.:

Date: March 2nd

Instructions:

1. Receive a sample in a marked tray
2. Evaluate each sample on the following attributes
3. When finish turn the card to the instructor, rinse with lemon water and continue, we will evaluate 3 samples in total in this session

Sample No: 087

Odor

Seaweed

Fishy

Earthy

Flavor

Saltiness

Sweetness

Umami

Texture

Firmness

Chewiness

Appendix K
Work Sheet for Sensory Test I
Appearance

Code	Sample Name
496	Sample B
529	Sample A
608	Sample F

Judge No.	Order of samples		
1	496	608	529
2	529	496	608
3	608	529	496
4	496	608	529
5	529	608	496
6	608	496	529
7	496	529	608
8	529	608	496

Flavor& Texture

Code	Sample Name
553	Sample B
087	Sample A
802	Sample F

Judge No.	Order of samples		
1	553	802	087
2	087	553	802
3	802	087	553
4	553	802	087
5	087	802	553
6	802	553	087
7	553	087	802
8	087	802	553

Work Sheet for Sensory Test II
Appearance

Code	Sample Name
985	Sample C
766	Sample D
392	Sample E

Judge No.	Order of samples		
1	985	392	766
2	766	985	392
3	392	766	985
4	985	392	766
5	766	392	985
6	392	985	766
7	985	766	392
8	766	392	985

Flavor & Texture

Code	Sample Name
279	Sample C
650	Sample D
143	Sample E

Judge No.	Order of samples		
1	279	143	650
2	650	279	143
3	143	650	279
4	279	143	650
5	650	143	279
6	143	279	650
7	279	650	143
8	650	143	279

Work Sheet for Sensory Test III
Appearance

Code	Sample Name
644	Sample B
076	Sample C
113	Sample D

Judge No.	Order of samples		
1	076	644	113
2	113	076	644
3	644	113	076
4	076	644	113
5	113	644	076
6	644	076	113
7	076	113	644
8	113	644	076

Flavor & Texture

Code	Sample Name
583	Sample B
496	Sample C
228	Sample D

Judge No.	Order of samples		
1	583	228	496
2	496	583	228
3	228	496	583
4	583	228	496
5	496	228	583
6	228	583	496
7	583	496	228
8	496	228	583

Work Sheet for Sensory Test IV
Appearance

Code	Sample Name
079	Sample F
884	Sample E
660	Sample A

Judge No.	Order of samples		
1	884	079	660
2	660	884	079
3	079	660	884
4	884	079	660
5	660	079	884
6	079	884	660
7	884	660	079
8	660	079	884

Flavor & Texture

Code	Sample Name
209	Sample F
266	Sample E
116	Sample A

Judge No.	Order of samples		
1	209	116	266
2	266	209	116
3	116	266	209
4	209	116	266
5	266	116	209
6	116	209	266
7	209	266	116
8	266	116	209

Work Sheet for Sensory Test V
Appearance

Code	Sample Name
186	Sample A
800	Sample G
227	Sample H

Judge No.	Order of samples		
1	800	186	227
2	227	800	186
3	186	227	800
4	800	186	227
5	227	186	800
6	186	800	227
7	800	227	186
8	227	186	800

Flavor & Texture

Code	Sample Name
699	Sample A
273	Sample G
110	Sample H

Judge No.	Order of samples		
1	699	110	273
2	273	699	110
3	110	273	699
4	699	110	273
5	273	110	699
6	110	699	273
7	699	273	110
8	273	110	699

Work Sheet for Sensory Test VI
Appearance

Code	Sample Name
489	Sample A
776	Sample G
901	Sample H

Judge No.	Order of samples		
1	776	489	901
2	901	776	489
3	489	901	776
4	776	489	901
5	901	489	776
6	489	776	901
7	776	901	489
8	901	489	776

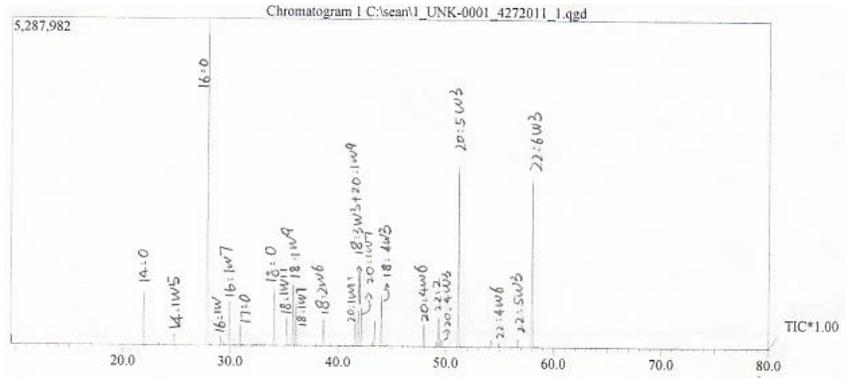
Flavor & Texture

Code	Sample Name
009	Sample A
168	Sample G
558	Sample H

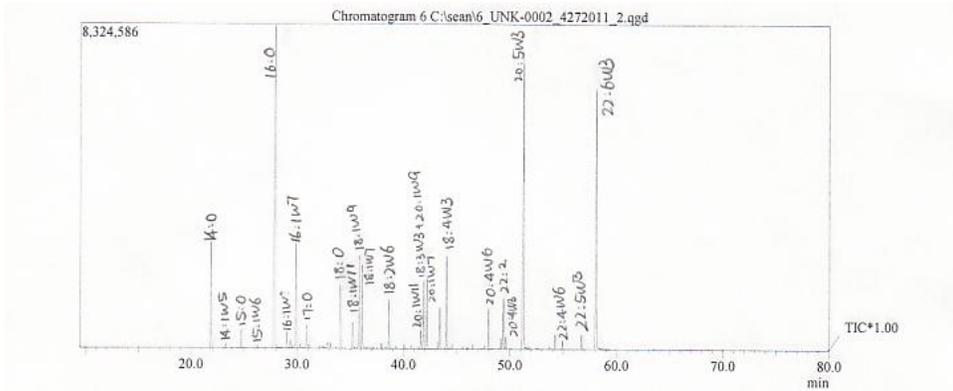
Judge No.	Order of samples		
1	009	558	168
2	168	009	558
3	558	168	009
4	009	558	168
5	168	558	009
6	558	009	168
7	009	168	558
8	168	558	009

Appendix L Chromatogram of oysters

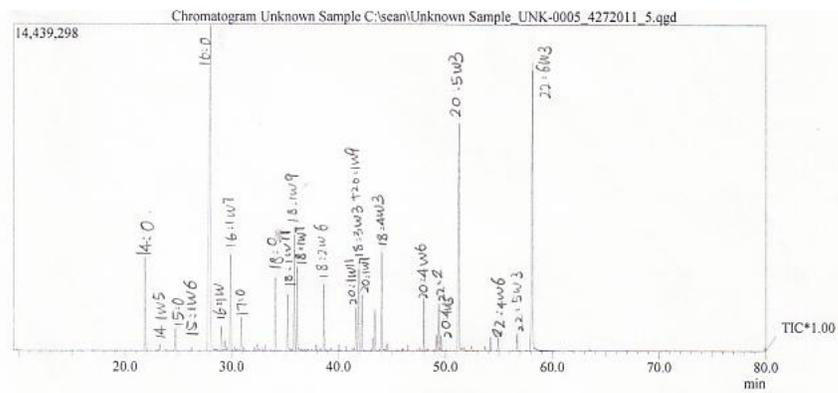
Sample A



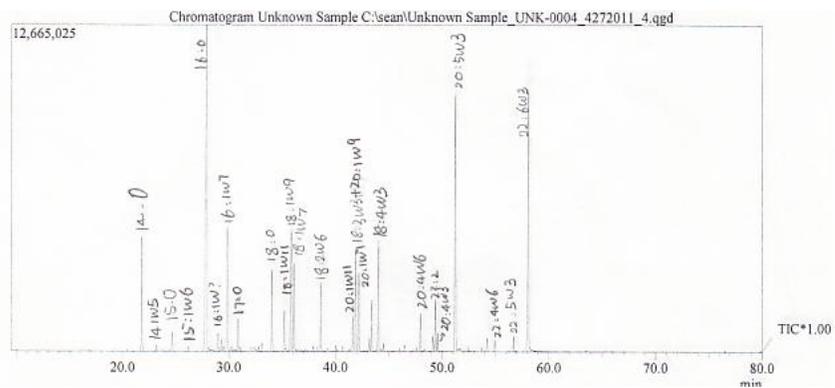
Sample B



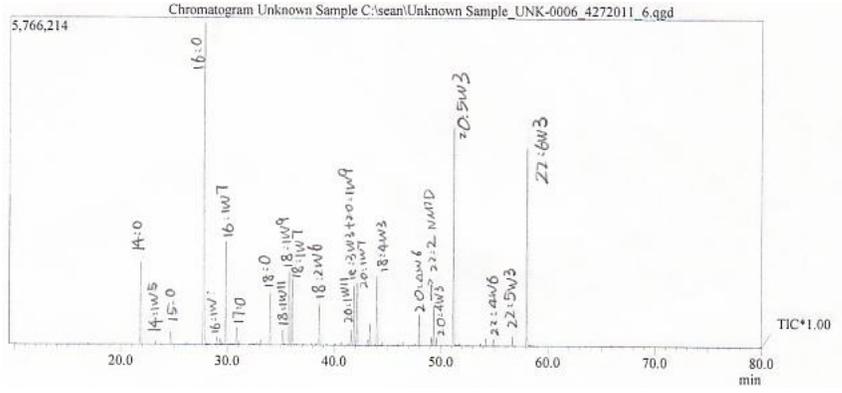
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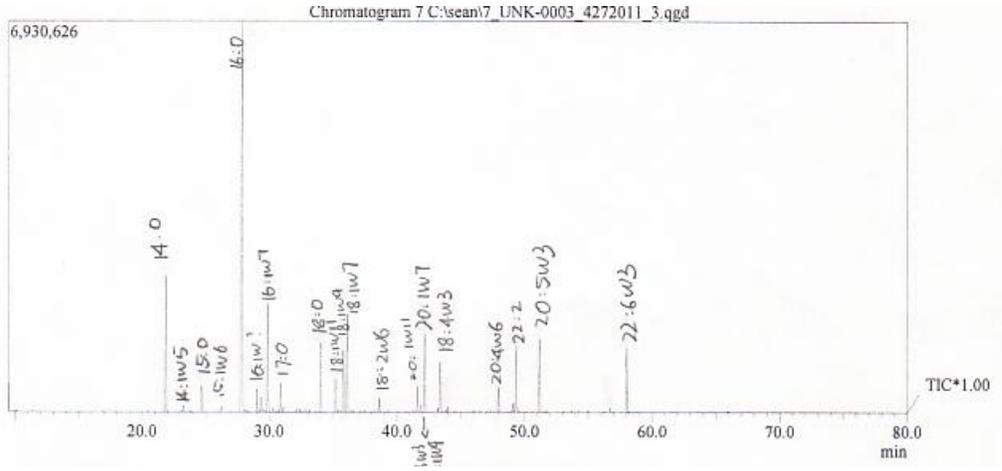
Sample D



Sample E



Sample G



Sample H

