AN ABSTRACT OF THE THESIS OF

Dustin R. Keys for the degree of Master of Science in Food Science and Technology presented on January 13, 2015

Title: Cooling Characterization and Practical Utilization of Sub-micron Slurry Ice for the Chilling of Fresh Seafood

Abstract approved:

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Consumption of seafood products continues to grow each year, but post-harvest losses and inedible seafood waste remain as high as 25%. Ice-based systems are the most common form of preservation to increase shelf life, preserve product quality, and enhance product safety. Slurry ice is a type of chilling media, consisting of a biphasic system of ice crystals suspended in water, which has been demonstrated to preserve product quality over traditional forms of chilling due to the smaller ice crystals providing a greater rate of heat transfer. Sub-micron slurry ice (S-MIS) is a type of slurry ice with crystals 400-700 nm in diameter, as compared to the 100-900 μm crystal diameter of other slurry ice systems. The purpose of this research was to characterize the cooling process of S-MIS compared to chilled seawater (CSW) and to practically challenge the chilling system by conducting a shelf-life study using an underutilized marine resource known for quality complications.

The first phase was to determine proper handling practices of S-MIS and to compare S-MIS ice against a form of chilling currently in practice in the seafood industry (CSW). Different ice-to-fish rations were examined (1:1, 1.5:1, and 2:1) and whether the S-MIS should be allowed to drain, effectively making the slurry ice a single-phase system. Single fish were also stored in 10:1 ice-to-fish ratios to determine a hypothetical 'best-case' chilling scenario. Results indicated that product chilling must be considered in two phases: initial chilling and sustained temperature management. Removing the liquid phase of S-MIS increased the product storage time over non-drained S-MIS and CSW; and each handling technique of S-MIS cooled the product faster, to a lower temperature, and for a longer period of time than CSW.

Based on the results of the first phase, it was hypothesized that S-MIS would improve the shelf-life of arrowtooth flounder over CSW. Fish were chilled for 48 h in either S-MIS or CSW, then held for up to 15 d at 4 °C. Chemical, microbiological, and odor decomposition methods were used to determine quality after the 48 h storage in the chilling media and every fifth day of storage at 4 °C. Results indicated that arrowtooth flounder degradation is more complex than expected because *K*-values indicated spoilage at d 0, while the d 0 fillets were deemed acceptable by every other test. In this scenario, S-MIS did not improve shelf-life of arrowtooth flounder. It is recommended that further studies should focus on storage of fish in ice directly after catch and/or the utilization of fish with better-known quality degradation.

Cooling Characterization and Practical Utilization of Sub-micron Slurry Ice for the Chilling of Fresh Seafood

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Dustin R. Keys, Author

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CONTRIBUTIONS OF AUTHORS

Dr. Christina A. Mireles DeWitt was involved in the design, results interpretation, and editing of each chapter. Dr. Austin C. Lowder aided in study design, data gathering, statistical analysis, and editing of chapter 3. Dr. Lowder also assisted in study design and editing of chapter 4. Chern Lin Koh assisted in study design and gathered data for chapter 4. Jae Heilig, Daria van de Grift, and Jacquelyn Keys provided lab assistance for chapter 4.

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CHAPTER 1

INTRODUCTION

Fish is one of the most important sources of protein around the world and the consumption of seafood continues to grow annually (FAO 2012). This is due in part to availability of significant sources of protein in many parts of the world, but also because of the nutritious constituents of seafood. Different types of seafood may contain high levels of polyunsaturated fatty acids, low amounts of saturated fat, lipid soluble vitamins, various microelements, and high levels of essential amino acids (Kulawik and others 2013, Medina and others 2009). The human population continues to increase each year and consumer increasing amounts of, which indicates that there will be less fish per capita in successive generations unless catch rates increase or waste is reduced. The per capita fish supply has been increasing from 9.9 kg live weight equivalent in the 1960s to 18.4 kg in 2009, but post-harvest losses and inedible waste remain at higher than 25% of total catch mass (Cakli and others 2007, FAO 2012). While this number cannot be reduced to 0% because of substances such as bone and chitin, even a 1% worldwide reduction in post-harvest losses would save millions of dollars and thousands of pounds of seafood per year.

To reduce post-harvest loss, fish should be chilled as quickly as possible after harvest and kept on ice to retard deteriorative processes. (Huidobro and others 2002, Liston 1992). Fish sold as fresh command higher prices at market than frozen fish and extending the shelf life of fresh fish will increase profitability (Duun and Rustad 2008b). Ice is the most common temperature-management technique in both fresh and frozen markets, while utilization of new types of ice may increase shelf life of seafood in either market. Therefore, it is likely that continued improvements and refinement of chilling technology will be needed to reduce post-harvest loss.

The purpose of this project was to determine if a type of slurry ice containing ice crystals 400-700 nm in diameter (sub-micron slurry ice) could improve the chilling rate of Pacific whiting (*Merluccius productus*), a product commonly held in chilled seawater up to 48 h between off-loading from the boat and processing. Once it was determined that sub-micron slurry ice could chill product quicker, at a lower ultimate temperature, and for a longer period of time than chilled seawater; a shelf-life study was conducted to determine if this increased chilling rate could improve quality in arrowtooth flounder (*Atheresthes stomias*). Arrowtooth flounder is a fish that has high-volume yet low-value a due to quality issues, and is therefore an underutilized marine resource.

CHAPTER 2

LITERATURE REVIEW

2.1 Species Utilized

In the United States and many other countries, gadoid fish of the genus *Merluccius* are an important percentage of fish consumption (Rodriguez and others 2004). Pacific whiting (*Merluccius productus*), also known as Pacific hake, is currently the largest domestic fishery stock of groundfish in the United States south of Alaska. Although the fish does not command a high price per pound, the fishery has a high volume and may be used extensively in surimi. Until the 1990s, whiting was not successfully utilized because a myxosporidean parasite that commonly infests the fish muscle causes the whiting to produce increased amounts of protease enzymes (Mazorra-Manzano and others 2012). Addition of protease inhibitors to surimi, as well as decreased bycatch in the fishery and the implementation of share-based fishing operations, has resulted in increased stability and economic viability of the fishery (Sylvia and others 2008). Protease inhibitors are not added until the fish are processed into surimi, so a reduction in temperature is critical to reduce the proteolytic activity (Castillo-Yanez and others 2004).

Arrowtooth flounder (*Atheresthes stomias*) functions as a key organism in the ecology of waters off the West Coast of the United States and Alaska. While arrowtooth flounder is important as an apex predator of fish and invertebrates and is a significant prey of pollock (*Theragra chalcogramma*), but it has been underutilized for human consumption due to proteolytic enzymatic degradation by a heat-stable

cysteine protease and bycatch issues with halibut and rockfish (Stark 2008, Zador and others 2011). Some of the quality issues have been mitigated through the use of protease inhibitors, so that arrowtooth flounder is currently utilized in surimi. However, the poor quality of arrowtooth flesh and low price has led to many arrowtooth thrown overboard and arrowtooth continues to be an underutilized species in the seafood industry. Subsequently, very little research has been conducted on arrowtooth flounder except for some papers concerning use of arrowtooth flounder in surimi.

2.2 Perishability

Fresh fish is considered to be one of the most perishable types of food regularly consumed and has a relatively short shelf life when compared to other foods (Fey and Regenstein 1982). Perishability is a complex idea in seafood because there are no unique freshness or spoilage indicators for all types of seafood and the shelf-life may vary from haul to haul (Dalgaard and others 1997). While there are relatively few species of thermoregulatory animals that are consumed, such as beef or pork, there are tens of thousands of species of aquatic organisms that are consumed. Many of these species may have unique spoilage organisms or autolytic processes due to the variability of aquatic animals and variety of environments in which these organisms are collected (Oehlenschlager 2009). Research suggests that high quality fish freshness only lasts several hours because spoilage from autolytic and microbial processes occurs immediately post-mortem, depending on environmental factors and without any preservation methods (Luten and others 2006). Since perishability in

seafood is so complex, many studies have been conducted to better understand post-mortem quality deterioration and seafood quality continues to be of paramount importance in research. Seafood spoilage is caused by a variety of factors including lipid oxidation, endogenous enzymatic processes, and processing techniques; however, the majority of spoilage above freezing temperatures is due to bacteria present on the fish when it is harvested (Huss 1995, Matches 1982). Since fish are poikilothermic, the microflora present at the time of catch is heavily influenced by temperature and the microorganisms in their habitat. When the fish are harvested, they come into contact with an entirely new microbiota, which reduces microbial resistance and encourages microbial rampancy.

Fish spoilage and quality may be assessed through microbial and sensory analysis, particularly odor, which specify freshness by tracking bacterial proliferation as well as physical deterioration (Boziaris 2014). Physical deterioration may include changes in odor, flavor, and texture that sensory analysts may be able to distinguish (Simpson 1997). Sensory changes are often the result of bacterial metabolic processes that form a variety of chemical end products including amines, sulfides, aldehydes, and ketones. These metabolic byproducts are often associated with unpleasant off-odors and off-flavors that sensory analysts can use to indicate fish freshness (Olafsdottir and Jonsdottir 2009). Research has shown that the best way to combat microbial activity and preserve freshness is through strict temperature control as soon as the fish is harvested (Duun and Rustad 2007, Sikorski and others 1976). While chilling does not kill all microorganisms present in fish, low temperatures reduce the rate of bacterial growth and enzymatic degradation that occur post mortem

(Venugopal 2006). Temperature control is an important step through every stage of fish processing and handling, as any degree of temperature abuse could promote spoilage and reduce quality (Digre and others 2011). As the maintenance of quality grows in demand among consumers, fishing vessels have added on-board cooling units in order to reduce the amount of fish waste per haul.

2.3 Temperature Control

2.3.1 Freezing

Aquatic food products are perishable because they contain high amounts of readily available nutrients and neutral pH levels, which allow for rapid microbial growth (Alasalvar and Quantick 1997). A large portion of harvested fish is deemed as waste each year from poor quality and high spoilage rates, yet the presence of chilling and freezing devices on boats extend the shelf life of fish and significantly lower harvest waste (Cakli and others 2007). Freezing is one of the most important preservation methods for aquatic food products because it reduces the growth rate and metabolic activity of spoilage organisms and slows enzymatic degradation. Both on- and off-board temperature control may include storage in refrigerated seawater or chemical preservatives, but the most popular method of preservation is the use of ice to quickly chill and store the fish because it is cheap, readily available, and food safe (Dessie and Dadebo 2010, Digre and others 2011, Gallart-Jornet and others 2007b). To properly retard the enzymatic and microbial activity after harvest, the fish should be chilled to temperatures between 0 and -2 °C as quickly as possible and even a

short time of temperature abuse can promote rapid decomposition and loss of quality (Venugopal 2006).

Even though the utilization of ice can hold fish at these ideal temperatures, the product may suffer loss in freshness and quality from both microbial activity and negative consequences of freezing. Endogenous and proteolytic enzymes present within the fish may degrade well below freezing temperatures because solutes are concentrated as water is frozen into ice crystals (Alizadeh and others 2007). The freezing process itself also causes physical alterations in the muscle tissue through protein denaturation, freezer burn, discoloration, and lipid oxidation (Careche and others 1998, Herrera and others 2001, Sikorski 1978). Such alterations may result in increased drip loss, textural changes such as soft muscle tissue, and loss of water holding capacity upon thawing (Dunn and Rustad 2008a). Lakshmisha and others (2008) demonstrated that time and method of freezing could have significant quality effects on fish muscle, so negative effects may be mitigated by knowing the freezing characteristics.

As the aqueous phase of the product lowers in temperature, ice crystals nucleate. This causes water diffusion from surrounding locations to balance the concentration of solutes and continued ice crystal growth. Ice crystals may continue to nucleate as well as grow larger depending on the freezing conditions. Quick freezing of the product causes the nucleation of many small intercellular ice crystals and impedes large crystal formation, while slow freezing will cause the nucleation of fewer ice crystals that are extracellular and grow to a larger size (Bahuaud and others 2008). It is these large extracellular ice crystal formations that cause texture damage,

increase the rate of oxidation, and cause greater enzyme activity. Additionally, Kaale and Eikevik (2013) determined that smaller ice crystals that exist both inter- and intracellularly cause less damage to seafood tissue and better preserve quality.

Chilling and freezing are established methods to preserve quality and reduce microbial activity in fish before they are consumed, and there are many different ways to chill or freeze aquatic food products. These methods are placed into several different categories based on temperature and type of cooling. Refrigerated storage occurs at temperatures between 4 and 0 °C, while frozen storage is from -18 to -40 °C. Superchilling is a process whereby the tissue is rapidly cooled to about -1.5 °C, so that the outer layer of tissue freezes and the core of the fillet is cooled yet not frozen (Duun and Rustad 2008a, Gaarder and others 2012, Kaale and others 2011, Magnussen and others 2008). This process lowers the temperature enough to suppress bacterial growth and enzymatic degradation, but is not low enough to cause ice crystal damage to the muscle (George 1993). Frozen storage may include the use of a conventional freezer at -18 °C, but methods such as air-blast chilling at -30 °C are also utilized to cool the product more rapidly and reduce large ice crystal formation. Recently, pressure shift freezing (PSF) has also been studied as a way to super-cool the product and keep ice-crystal size small and homogenous. Alizadeh and others (2007) found that using pressure shift freezing from 0 to -21 °C under 210 MPa followed by storage in a conventional freezer decreased damage than conventional freezing alone. Various combinations of cooling gels, chilled gases or liquids, and ice types are used to cool a product, but the most popular form of preservation is the use of direct contact with ice as a refrigerant.

2.3.2 Temperature Measurement

Whether it is a haul of fish in a ship's hold or in a tote at a processing plant, it is vital to know the temperature of the warmest fish. The warmest fish could be at the center of the batch or on the outer edges of the batch, depending on the container and environmental conditions. When a new container is being proposed to hold fish, it is important to find the location of the warmest fish to ensure acceptable quality throughout the entire batch (Johnston and others 1994). As a result, temperature measurements should be taken in as many spatial planes as possible. It is recommended that fish temperatures should be taken on the top, bottom, outer edges, and geometric center of the fish batch to prevent inadequate cooling of the product (Johnston and others 1994). The geometric center of the fish should also be the location which is measured to ensure that the temperature of the warmest location of the warmest fish is recorded (Torry Advisory Note no. 91).

The most practical instrument to measure temperature in food systems is a thermocouple wire attached to a transmitter or data recorder that is accurate to 0.5 °C. Dial-type thermometers are common because they are less expensive, but they are also less accurate and reliable than a thermocouple (Rippen and Skonberg 2012). The theory behind a thermocouple wire is two lengths of wire made of dissimilar metals are joined at the ends to form a circuit with differences in voltage. Since there are differences in voltage, there is some voltage that may be used to measure the temperature of the product. Thermocouple wires come in a variety of types (K, J, N, R, S, B, T, and E) that are distinctive due to the types of dissimilar metals that are

used. The T type of thermocouple wire, for example, is made of copper (+) and constantan (-) wires with a resolution of 0.5 °C between -40 °C and 125 °C (Childs 2001). These metals are corrosion-resistant in moisture and are often used in application involving subzero temperatures, which is ideal for temperature studies involving chilling of seafood (Park and others 1993).

2.3.3 Slurry Ice

Flake ice is the most popular among types of ice because it is relatively efficient and cheap to produce in large quantities, while keeping the fish chilled and wet. However, the study of slurry ice in the early by Chapman (1990) has gradually changed the way the seafood industry uses ice as a storage mechanism. Slurry ice is a biphasic system of microscopic spherical ice crystals distributed in saltwater that is cooled by the ice to temperatures between -0.5 and -3.0 °C (Davies 2005, Hansen and others 2009, Huidobro and others 2002, Rodriguez and others 2006). The rapidly lowered temperature quickly hinders chemical and enzymatic spoilage processes, but it is not so low that the food product freezes (Erikson and others 2011). The composition of slurry ice may vary, but there does appear to be a standard at 40% ice and 60% water in several scientific studies (Aubourg and others 2006, Campos and others 2005). The type of water may be seawater if it is readily available, or salt may be mixed with freshwater to simulate seawater. This brine salinity also varies, but generally falls within a range of 3.2% to 3.6% because that is the general range of salinity for most seawater.

Slurry ice is also known as fluid ice, slush ice, liquid ice, or flow ice because it is able to be pumped through pipes instead of having to be shoveled onto the fish. The advantages of slurry ice over other ice forms includes a faster chilling rate due to a high capacity for heat exchange, it is able to be pumped for more hygienic handling of aquatic food products, more efficient coverage of fish surfaces to prevent the formation of air pockets, reduced physical damage because the ice crystals are small and spherical, and prolonged shelf life (Huidobro and others 2001, Pineiro and others 2004). Since the ice crystals are smaller, there is more surface area to contact the surface of the product. This property allows slurry ice to more proficiently prevent oxidation from the formation of air pockets and also limits dehydration (Huidobro and others 2002, Campos and others 2005, Campos and others 2006, Cakli and others 2006b). Ice slurries exploit the latent heat of ice, therefore, they are able to transfer heat away from the product quicker than other forms of ice and single-phase fluids (Ayel and others 2003, Davies 2005, Leiper and others 2013). The high heat capacity of slurry ice also results in lower rates of mass flow and less fluctuations in temperature. As a result, the product chills quicker in slurry ice than in other forms of ice, and the lower rates of mass flow and temperature changes prolong shelf life in the final product.

Slurry ice may also be mixed with other additives, such as ozone or different salts, to inhibit microbial growth or prevent melanosis in shrimp (Huidobro and others 2002, Ortiz and others 2008). Aubourg and others (2006) demonstrated that ozone may have an antiseptic surface effect for megrim in combination with slurry ice, and the authors noted that this was similar to studies that utilized sardine and turbot. Flake

ice may also be ozonized to increase shelf life and maintain sensory quality, as demonstrated by Lu and others (2012), but there are few studies comparing ozonized flake ice to ozonized slurry ice. Aubourg and others (2009) found that farmed trout stored in ozonized slurry had a slight extension in shelf life and slightly better quality indices over slurry ice alone. However, Alvarez and others (2009) found more mixed results when using blackspot seabream. Ozonized slurry ice granted a very slight extension in shelf life, but also slightly increased oxidation of fatty acids. Therefore, it was concluded that either slurry ice or ozonized slurry ice could be used for slaughtering and storage of blackspot seabream to yield similar results.

Many authors have gathered empirical data supporting the theoretical benefits of slurry ice over traditional flake ice. Losada and others (2005) found decreased *K*-values, decreased salt content, decreased free fatty acid content, and decreased thiobarbituric acid index of horse mackerel stored in slurry ice over horse mackerel stored in traditional flake ice. Losada and others (2006) furthermore confirmed the importance of slurry ice over flake ice by holding sardines in slurry ice or flake ice for up to 8 days, and then conducting chemical analyses on cooked product. Cooked sardines that had previously been held in slurry ice led to significantly less lipid damage and inhibition of TMA-N formation than sardines previously held in traditional flake ice. Rodriguez and others (2004) and Rodriguez and others (2005) found that TVB-N and TMA were significantly lower in slurry ice and shelf life as indicated by microbiological and sensory parameters was enhanced by slurry ice over traditional flake ice for European hake and horse mackerel. Pineiro and others (2005) applied the application to farmed turbot and discovered that turbot in slurry ice

exhibited lower TMA-N and *K*-values than turbot stored in a similar ice to fish ratio of flake ice.

2.4 Freshness Indicators

2.4.1 Microbial Degradation

There are many freshness indicators that may be used to assist in determining seafood quality including microbial determination, odor evaluation, and chemical assessments. Bacterial degradation is one of the most important indicators of fish spoilage because most spoilage is caused by bacteria, and may easily be measured by plate counts. While the specific spoilage organism is often different between species of fish, gram-negative psychrophilic or psychorotophic (*Shewanella* spp., *Pseudomonas* spp.) bacteria are commonly important species in the spoilage process (Gram and others 1987, Jorgensen and others 1988). Fish are commonly rejected when the total viable counts (TVC) reaches levels of 10^6 to 10^7 colony forming units per gram (CFU/g). Although, the Food and Agriculture Organization of the United Nations notes that the European Union rejects imported fish at the level of 10^5 CFU/g for whole fish (FAO 2005). Total plate counts are often used to determine microbial spoilage by incubation at 35 °C for 24 or 48 hours because this tests allows for relatively quick results, but psychrotrophs may also be evaluated using plate count agar by incubating samples at 7-8 °C for 10 days.

Other indicators of spoilage are directly or indirectly related to microbiological concentration, such as chemical indicators of bacterial spoilage or unacceptable odor characteristics that arise as a result of bacterial degradation. Many

studies have found positive correlations between microbial plate counts and other indicators, but the strength of the correlations is highly dependent on storage conditions and species effects. For example, Kyrana and Lougovois (2002) found a high correlation between log colony forming units per gram (CFU/g) and pH (r^2 = 0.99), TMA (r^2 = 0.96), and TVBN (r^2 = 0.97) in sea bass stored in ice. Yet Aubourg and others (2007b) found more conservative correlations between CFU/g and TVBN (r^2 = 0.40) and TMA (r^2 = 0.79), while not finding a significant correlation with pH in Coho salmon stored on ice. Boskou and Debevere (2000) found that both TMA and TVB production, as well as decreased sensory acceptability, were directly caused by hydrogen sulfide-producing bacteria.

2.4.2 Sensory Evaluation

Sensory evaluation is the scientific discipline used to measure characteristics of food using the senses including taste, touch, smell, and sight. There are discriminative tests that determine if there is a difference between samples, descriptive tests that assign indicator words to certain sights or smells, and affective tests are subjective consumer tests (Abbas and others 2008). There are several sensory assessment schemes that are used for seafood products, depending on geographical location and individual preference. Some of the more popular schemes are the Torry scheme, European Union scheme, Quality Index Method (QIM), Principle Component Analysis, and Quantitative Descriptive Analysis (Dalgaard 2000, Hyldig and Green-Petersen 2004). Significant deterioration of sensory quality

occurs over time during iced or chilled storage of fish, which may have an effect on the economic viability of the product (Losada and others 2005).

Alasalvar and others (2002) correlated sensory assessment using the Tasmanian Food Research Unit Scheme to nucleotide degradation and found correlations of $r^2 \geq 98\%$ for both wild and cultured sea bass. Manju and others (2007) also demonstrated good correlations between sensory assessment and nucleotide degradation in Black Pomfret and Pearlspot during chilled storage. Morkore and others (2010) demonstrated moderate correlations between specific sensory attributes, specifically decreases in seawater ($r^2 = -0.82$) and fresh ($r^2 = -0.88$) odors with a corresponding increase in fermented ($r^2 = 0.73$) odors in salmon. Aubourg (2001) found high correlations of sensory with TMA ($r^2 \geq 93\%$), but only moderate correlations with TBA ($r^2 = 0.44\text{-}0.51$) and free fatty acids ($r^2 = 0.71\text{-}0.82$).

2.4.3 Basic Chemical Compounds (TMA/DMA/FA/TVB-N)

The most popular chemical freshness indicators include measuring the concentrations of biogenic amines, trimethylamine oxide (TMAO) degradation to trimethylamine (TMA), TMAO degradation to dimethylamine (DMA) and formaldehyde (FA), total volatile basic nitrogen (TVB-N), and ammonia (Olafsdottir and others 1997). TVB-N is widely used because it is a relatively simple process and several different methods may be used depending on laboratory capabilities. It is one of the oldest chemical fish spoilage methods and is mandatory testing in many countries (Castro and others 2012). The levels of acceptable levels of TVB-N are between 25 mg/100 g and 35 mg/100 g depending on the fish species, which was

designated by the European Union (Commission Decision 95/149/EEC). Research has also effectively demonstrated that values over 35 mg/100 g denotes a ‘spoiled’ product for any species (Gokodlu and others 1998, Gomez-Estaca and others 2007).

All of the methods include making a fish extract alkaline, the bases are volatilized, and then the bases are collected for measurement by titration (Antonacopoulos and Vyncke 1989). While the determination of TVB-N is a routine method, there are several disadvantages to using it as a sensitive indicator of freshness. The method is only sensitive enough to give a determination on whether the fish it fit for human consumption or not, it cannot be used during the early stages of degradation because of high initial values, and it is sensitive to changes in operation or equipment (Botta 1995, O’Keefe 2007).

Measurement of the concentration of trimethylamine (TMA) is often used either in place of or in conjunction with TVB-N to determine fresh freshness. Trimethylamine oxide is an osmoregulatory compound in fish that is utilized as a terminal electron receptor in bacterial anaerobic respiration and is degraded into TMA through bacterial metabolic processes (Castell and others 1971, Hansen and others 2007, Herland and others 2009, Stroem and others 1979). TMAO content varies between fish species, but generally decreases post-mortem during storage with correlating increases of TMA (Boskou and Debevere 1997, Rodriguez and others 1997). TMAO may also be degraded into dimethylamine (DMA) and formaldehyde (FA) through endogenous enzyme degradation processes during frozen storage, while TMA is often the primary degradation product during fresh or iced storage. DMA and

FA are formed during frozen storage and are highest at about -10 °C, but formation is insignificant at -26 °C or lower (Burgaard and Jorgensen 2010).

The concentrations of TMA or DMA are often measured because they are responsible for the fishy off-odor that is rapidly formed post-mortem in fish and the methods are very similar to measurement of other quality indices (Castell and others 1971, Timm and Jorgensen 2002). The formation of DMA and FA may also lead to textural issues because formaldehyde may assist in cross-linking proteins, which results in dry and fibrous fish muscle with associated water loss (Burgaard and Jorgensen 2010, Licciardello and others 1982). One of the most common methods for the measurement of TMA/TMAO, DMA, and TVB-N utilizes steam distillation, yet methods involving steam distillation often lack specificity. In recent years, however, chromatographic or spectroscopic methods have been developed for greater specificity and accuracy (Malle and Tao 1987, Malle and Poumeyrol 1989, Lundstrom and Racicot 1983, Krzymien and Elias 1990).

2.4.4 Lipid Oxidation

Lipid changes during seafood processing and storage may also have an effect on quality because lipid oxidation may damage other molecules in the product and cause rancid odors and flavors to develop (Aubourg 1999). Lipid oxidation may have direct effects of sensory parameters and consumer acceptability through negative smell (rancidity), taste (rancidity), and visual (yellow discoloration) attributes (Lauritzsen and others 1999). Therefore, lipid oxidation may be utilized in conjunction with both chemical and sensory methods to assist in determining quality

and may even be the primary indicator in spoilage. Aas and others (2010) noted that lipid oxidation that is the primary spoilage issue in salt-cured cod, even though cod is a lean fish with only 0.3-0.5% lipids within muscle. However, the predominant indices of determining quality use lipid oxidation as a secondary indicator of spoilage because lipid spoilage does not always correlate well with spoilage.

Several tests may be used to quantify lipid oxidation including hydroperoxide content (primary oxidation), the thiobarbituric acid-reactive substances test (TBARS) or anisidine value (secondary oxidation), or formation of fluorescent compounds (tertiary oxidation). Each measurement technique has specific strengths and weaknesses, and researchers need to be aware of testing limitations to determine which test is appropriate (Fernandez and others 1997). The thiobarbituric acid-reactive substances test is the most widely used test of lipid oxidation in foods because primary oxidation are difficult to quantify due to their rapid degradation to secondary products. Tertiary oxidation products are also difficult to quantify because it takes long amounts of time for secondary oxidation products to short chain fatty acids and the methods requires the use of a fluorescence spectrophotometer.

Aidos and others (2002) found that determination of secondary oxidation products were most informative when correlating fish lipid stability to temperature, and that lower temperatures exhibited slower oxidation product formation. The mechanism of action for the TBARS assay is well-known and begins with polyunsaturated fatty acid peroxidation to a free radical (Grotto and others 2009). The primary oxidation product of this reaction is a lipid hydroperoxide, but this molecule is unstable and fragments into more stable secondary products, such as

malondialdehyde (MDA). Thus, primary oxidation products are often measured if the product is in the presence of antioxidants, while the TBARS assay is used for products not in the presence of antioxidants (Antolovich and others 2002). Figure 2.1 shows the formation of MDA from lipid oxidation and subsequent formation of a chromogen when in the presence of thiobarbituric acid. This chromogen may be quantified through spectrophotometry at 532 nm wavelength.

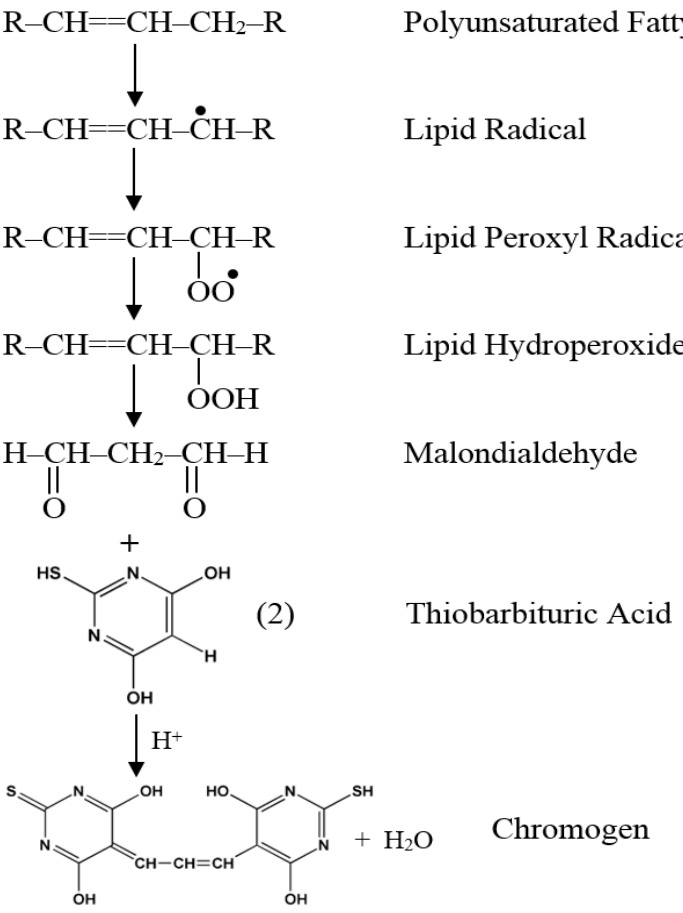

Figure 2.1 – Formation of malondialdehyde (MDA) and chromogen in the presence of thiobarbituric acid that may be viewed at 532 nm wavelength (adapted from Antolovich and others 2002).

2.4.5 Nucleotide Degradation

Another useful freshness indicator for aquatic food products is the *K*-value, which was first introduced by Saito and others (1959) to evaluate fish quality before significant bacterial degradation occurs. The *K*-value measures ATP degradation into five distinct products: adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), inosine (Ino), and hypoxanthine (Hx). The degradation of ATP occurs very quickly in fish and is an adequate indicator for fish freshness in the earliest stages of decomposition, as post-rigor fish have only trace amounts of ATP. Muscles are relaxed when the fish are killed, but ATP is irreversibly used by the muscle after death. Actin and myosin make an irreversible acto-myosin bond and the muscle enters rigor. Other ratios of the concentration of ATP and its breakdown products including the Ki, G, P, H, and Fr values are used as chemical indicators of fish freshness as well (Scherer and others 2005). However, the *K*-value remains the standard in concentration of ATP to breakdown products.

Surette and others (1988) found that both endogenous and microbial enzymes may contribute to the breakdown of ATP, which hints at the primary disadvantage to the method. *K*-value measurement cannot be a universal measure of decomposition because of the variability in catabolic pathways of ATP between different fish species and their associated microbiota. Figure 2.2 shows one common pathway of ATP degradation. Furthermore, Valle and others (1998) have demonstrated that *K*-values vary widely between different species of fish and higher *K*-values in one species of fish may not indicate as advanced spoilage as lower values in other species. Another problem with the *K*-value is that there are several species of fish that have an increase

in the *K*-value that is too fast or to slow to be used for quality control (Erikson and others 1997). It has also been proposed that different handling techniques, such as starvation prior to slaughter, may significantly affect the *K*-value (Einen and Thomassen 1998).

Cappeln and others (1999) note that decomposition of ATP may be accelerated between -0.8 °C and -5 °C, which is in the range of the storage temperature of fish in ice. It has also been documented that ATP degradation may occur at temperatures as low as -20 °C. In many different species of fish there is often a quick endogenous breakdown of ATP into ADP and IMP, followed by a slower accumulation of Ino and Hx (Simpson 1997). ADP and IMP are not associated with spoilage, while Ino and Hx are indicators of spoilage because they have a bitter flavor that becomes more pronounced as more ADP and IMP are degraded into Ino and Hx over time. There are several advantages of the *K*-value over other indicators of fish freshness. Utilization of HPLC allows for greater sensitivity, accuracy, and reproducibility than many other indicators for fish freshness including TVB-N, ammonia, and TMA/TMAO (Guillerm-Regost and others 2006, Ryder 1985, Valle and others 1998). *K*-values also include intermediate degradation products produced at separate rates depending on the metabolic pathways, which provides a greater understanding of overall quality (Boyle and others 1991).

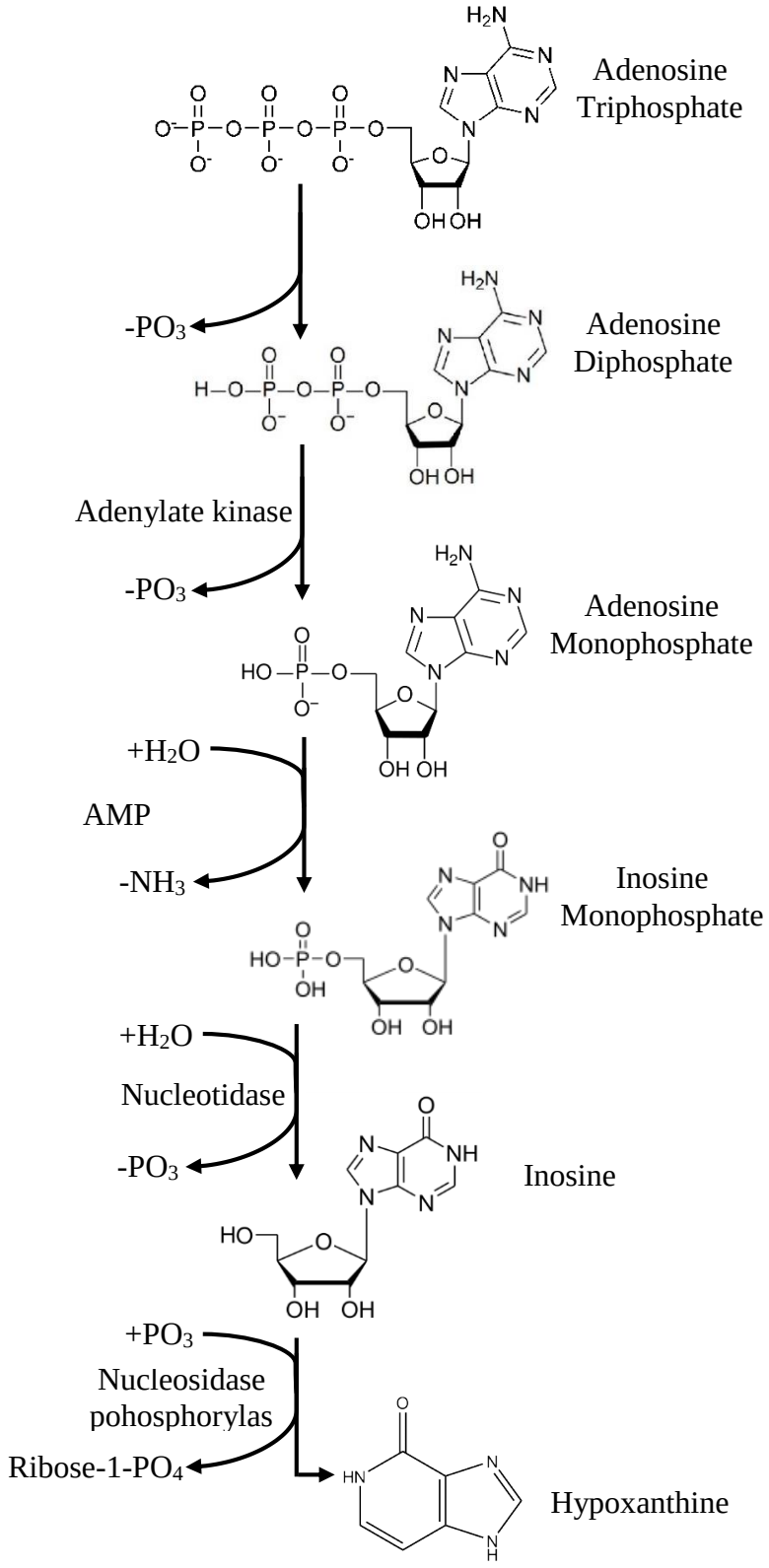

Figure 2.2: A common ATP degradation scheme for determination of the *K*-value

CHAPTER 3

CHARACTERIZATION OF PACIFIC WHITING (*MERLUCCIUS PRODUCTUS)* COOLING IN A SUB-MICRON SLURRY ICE SYSTEM COMPARED TO CHILLED SEAWATER

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3.1 Abstract

Seafood perishability is dramatically impacted by handling methods and chilling rates, which vary by chilling method. The objective of this study was to evaluate chilling rates and cooling curves of sub-micron ice slurry (S-MIS), compared to chilled seawater. Comparisons included cooling rates as impacted by ratio of S-MIS to fish (1:1, 1.5:1, 2:1); as well as handling techniques of 2:1 ice to fish ratios of S-MIS initially drained from the cooler, non-drained S-MIS, and chilled seawater. Temperature was monitored by inserting thermocouple wires into the center of whole fish and recording data every 15 s. No significant differences were observed in chilling parameters of whiting stored in continuously drained S-MIS at different ice to fish ratios ($P > 0.05$). However, the 2:1 ice to fish ratio demonstrated a significantly longer chilled product time $\leq$ °0 and < 4 °C, followed by 1.5:1 and 1:1 ($P < 0.05$). Continuously drained and initially drained S-MIS chilled the product faster, colder, and for a longer period of time than non-drained S-MIS and chilled seawater ($P < 0.05$). Non-linear regression showed significantly different cooling rates for each handling technique with continuously drained S-MIS having the quickest product chill rate and chilled seawater having the slowest ($P < 0.05$). Results indicate that draining the liquid phase of S-MIS improved chilling time over non-drained S-MIS. Non-drained S-MIS improved initial product chilling over chilled seawater, but chilled seawater chilled the product for a longer period of time.

3.2 Practical Application

Seafood is packed in ice after harvest to prevent enzymatic and microbiological degradation. Sub-micron slurry ice is a novel type of slurry ice with crystals 400-700 nm in diameter that may be used to chill seafood products, rather than conventional types of ice currently in use. This paper investigates the chilling properties of sub-micron slurry ice to assist in determining the viability of this ice as a chilling alternative for seafood products.

3.3 Introduction

The per capita fish supply has increased from 9.9 kg live weight equivalent in the 1960s to 18.4 kg in 2009, but post-harvest losses and inedible waste remain greater than 25% of total catch mass (Cakli *et al.* 2007; FAO 2012). To reduce post-harvest loss, fish should be chilled as quickly as possible after harvest and kept on ice to hinder deteriorative processes. Spoilage from microbial, oxidative, and enzymatic processes begins immediately post-mortem and is dependent on both environmental factors and preservation method (Chang *et al.* 1998). Both on- and off-board temperature control may include storage in refrigerated seawater or chemical preservatives, but the most popular method of preservation is the use of ice to quickly chill and store the fish (Aubourg *et al.* 2006; Hansen *et al.* 2009). In particular, the increasing presence of chilling and freezing devices on trawlers and in processing plants significantly improves the shelf life and quality of fish, while decreasing harvest waste from spoilage (Burgaard and Jorgensen 2010).

Slurry ice has garnered a significant amount of attention in the area of seafood preservation since the early 1990s due to the high rates of heat transfer and heat capacity (Chapman 1990; Davies 2005; Harada 1991). These are a direct result of the biphasic nature of the system, ice and water containing a freezing depressant, as well as the fact that the ice crystals are microscopic (Ayel *et al.* 2003; Campos *et al.* 2006; Egolf and Kauffeld 2005). This large heat capacity may result in reduced operating costs compared to a chilled water flow system because the required cooling flow rate may be reduced by as much as 60% for the same amount of product (Bellas *et al.* 2002; Metz and Margen 1987). Other advantages have also been attributed to slurry

ice including reduced physical product damage, chilling to colder temperatures, prolonged shelf life, reduced dehydration, and it is able to be pumped for more hygienic handling (Aubourg *et al.* 2009; Gallart-Jornet *et al.* 2007b). The sub-micron ice described in this study, termed 'S-MIS', is similar to slurry ice, yet the primary difference between S-MIS and slurry ice is that the particle size of traditional slurry ice is anywhere between 100 and 900 microns, while S-MIS is several orders of magnitude smaller at 400-700 nm. Theoretically, this should increase the rate of heat transfer because the surface area of contact between ice and product is increased.

The aim of this study was to evaluate chilling rates and cooling curves for Pacific whiting (*Merluccius productus*) stored in a sub-micron ice slurry (S-MIS) compared to chilled seawater (CSW), a traditional system of chilling seafood products for industrial applications. Comparisons included: cooling rates as impacted by ratio of S-MIS-to-fish (1:1, 1.5:1, 2:1); handling techniques of 2:1 S-MIS continuously drained, initially drained, and not drained; and handling techniques of 2:1 S-MIS vs 2:1 CSW (not drained). In this case, the terms 'drained' and 'not drained' refer to whether the ice was allowed to continuously drain from the cooler throughout the experiment or contained in the cooler for the duration of the trial. Other studies have focused on quality and shelf life comparisons of a particular species stored in either slurry ice or conventional types of chilling, but there is little information on the characterization of ice slurry cooling rates compared to conventional chilling methods. The novel nature of S-MIS, as well as the continued need for waste reduction and increased product quality, has justified the need for studying the chilling properties of S-MIS compared to a conventional chilling

method. Assessments of continuously draining the S-MIS compared to not draining the ice are justified because it is unknown if the S-MIS melt water in a closed system will perform as a sufficient cold reservoir to keep the product chilled significantly quicker or longer than if the melt water is continuously drained.

3.4 Materials and Methods

3.4.1 Raw Materials and Processing

Pacific whiting (*Merluccius productus*) were caught by trawl in northern Oregon waters, placed in refrigerated seawater in the hold for under 48 h, and brought to a local commercial processor in July and August 2013. Eighty fish per replicate were obtained from the processor, driven back to the laboratory (5 min), measured, and weighed on a floor scale (CPWplus 300, Adam Equipment, Danbury, CT).

After whiting were weighed, the fish were placed in a recirculating water bath at 18 °C to equilibrate. This temperature was selected because it is the highest temperature of fish pulled out of the ocean during the summer months in Oregon. The recirculating water bath apparatus consisted of a 378.54 L, 134.62 x 78.74 x 63.50 cm plastic stock tank (Rubbermaid, Winchester, VA) with a magnetic water pump (Pan World, Ashby, MD, Model: NH-100PX-X) and in-line water chiller (Model DS-3, Aqua Logic, San Diego, CA).

Thermocouple wires were inserted into the fish to monitor product temperature. The wires were connected to transmitters (MWTC-D, Omega Engineering, Stamford, CT) that logged data internally and transmitted data in real time every 15 s. A total of 12 out of 36 fish from each treatment replication were randomly selected for thermocouple insertion. Wires were inserted by piercing fish 2-3 mm to the right of the front of the dorsal fin with a 12 gauge stainless steel luer needle inserted 6 cm down the loin. The thermocouple wire was inserted into the hole left by the probe and a rubber band was placed on the fish snout to secure the wire.

3.4.2 Ice Treatments

Brine was hand-made in a brine tank with granulated salt and the salinity of the brine was targeted to 3.6% using a pH and electrochemistry meter (pHi 430, Beckman Coulter, Fullerton, CA). Chilled seawater (CSW) composed of 40% flake ice and 60% saltwater brine was made on-site. A NanoICE Model Conrad 2001S (NanoICE Inc, Woodinville, WA) generated a sub-micron ice slurry (S-MIS) with 40% sub-micron ice crystals and 60% brine. Evaluation of the ice crystals using a transmission electron microscope at the University of Washington Nanotechnology Center characterized a diameter of 400-700 nm per crystal (Pakhomov 2011). Brine was circulated through the NanoICE generator until the temperature reached -2.0 ± 0.1 °C, which is the temperature at which the ice is formed consistently. The output hose was inserted into the cooler to fill it with an initial target weight of S-MIS while the fish were equilibrating. The output flow (1. 75 ± 0.08 kg/min) was determined by monitoring the weight increase over one minute of fill time, in triplicate.

3.4.3 Addition of Fish to Ice

The first trial focused on determining cooling and chilling time of Pacific whiting in different ratios of S-MIS to fish (1:1, 1.5:1, 2:1). While 13.85 ± 0.90 kg of fish (32-36 individuals) were equilibrating in the water bath, ice was added to an 89 L Ice Station Zero cooler with inside dimensions of 79 x 37 x 35 cm (Moeller Marine Products, Sparta, TN). The type of handling technique utilized for this experiment was the continuously drained technique. The cooler was filled to double the target weight, so for a 1:1 ice to fish ratio, the cooler would initially be filled with ice that

was double the weight of fish added (27.70 kg). Similarly for a final ice to fish ratio of 2:1, the cooler was initially filled with ice that was four times the weight of the fish that was to be added (55.40 kg). The cooler was filled to this initial target weight of S-MIS, the liquid phase was drained from the cooler to leave only a monophasic system of ice in the cooler, the 32-36 whiting were placed on top of the ice while thermocouple transmitters began recording data, and the whiting were pushed into the ice so the chilling medium completely surrounded each fish.

Other handling techniques of S-MIS were conducted at 2:1 ice to fish ratios. These techniques included: the continuous drain described previously, initially draining the carrier liquid before then closing the drain plug to create a homogenous ice medium before fish were added to the ice (initial drain), and not draining any liquid at any point during the experiment (non-drained). CSW was not drained from the cooler and the ice to fish ratio was 2:1. All experiments were halted when 80% of the thermocouple transmitters read a product temperature of 4 ℃ or greater.

3.4.5 Single Fish Comparisons

To monitor product cooling in an idealized system, one fish was added to one 24.5 x 17.5 x 29.0 cm polystyrene cooler with 2.0 cm walls at a 10:1 ice-to-fish ratio. Whiting were treated in a similar manner as the experiment with many fish per cooler.

3.4.6 Statistical Analysis

One-way analysis of variance (ANOVA) followed by the Holm-Sidak method of multiple comparisons was performed using SigmaPlot 12.5 to examine significant differences (Systat Software Inc., Chicago, USA). Independent variables included 1:1, 1.5:1, and 2:1 Continuous Drain; 2:1 Initial Drain, 2:1 Non-Drained, and 2:1 CSW. The dependent variables were selected from the temperature data and included cooling time from 18 °C to 4 °C, cooling time from 18 °C to 0 °C, lowest product temperature, time to lowest product temperature, total product time $\leq$ 0 °C, and time < 4 °C. The same 80 fish were used several times to collect data for each different ratio of S-MIS to fish and handling techniques, and three replicates of fish were collected from the processor on three different dates in July and August 2013. 12 fish per cooler gathered temperature data. For the single fish comparisons, logarithmic non-linear regression was conducted to determine the rate of product cooling. Three coolers with fish were used for each treatment with three replicates per treatment. The significance level was set at 95% ($P < 0.05$) for all cases.

3.5 Results and Discussion

3.5.1 Fish and Ice Characteristics

The mean weight per fish was 0.41 ± 0.03 kg and the mean length was 35.43 ± 1.52 cm over all treatments with no significant differences between treatments ($P > 0.05$, data not shown). The cooler was filled with 13.85 ± 0.90 kg of fish and there were between 32 and 36 fish per cooler, depending on the weight of the individual fish in the lot. Mean brine salinity was 3.61 ± 0.05% and the mean ice generation temperature of the S-MIS machine was -2.02 ± -0.14 °C. Other studies that have utilized slurry ice machines have produced ice at a temperature of -1.5 °C with a brine salinity of 3.3% (Cakli *et al.* 2006a; Rodriguez *et al.* 2004). However, slurry ice machines often have a recommended brine salinity as dictated by the manufacturer and produce ice at a specific temperature that is not allowed to be variable. The mean pre-chill temperature for the fish was 18.01 ± 1.01 °C and the atmospheric temperature was 22.31 ± 0.64 °C with no significant differences between treatments ($P > 0.05$, data not shown).

3.5.2 Impact of S-MIS to Fish Ratio on Product Cooling

The various ratios of S-MIS to fish were investigated because there are many conflicting examples of proper ice-to-fish ratios for cooling and chilling seafood. This is true even for flake ice, which is the most common type of ice in the industry. Venugopal (2006) notes that the flake ice to fish ratio may range from 1:1 to 2:1. However, Holdsworth (1997) argues that as high as a ratio as 1 part ice to 5 parts fish may be utilized, but this statement is tempered by the author noting that the optimum

rate of cooling will not be achieved with anything less than a 1 to 1 ratio. Shawyer and Medina Pizzali (2003) divide cooling and chilling into two distinct phases and attest that as little as a 1 part ice to 4 parts fish is sufficient to chill fish to 0 °C. Yet as high as a 3:1 ice to fish ratio may be required to keep fish consistently chilled throughout the fishing excursion. There is also a difference in how ice is utilized in journal articles when compared to industry or books targeted toward practical implementation of ice as a chilling medium. Many shelf life studies that focus on the acceptability of a product in ice either combine a 1:1 ice to fish ratio with a refrigerator or chilled room (Ababouch *et al.* 1996; Alasalvar *et al.* 2001; Kyrana *et al.* 1997), while others use a 2:1 ice to fish ratio depending on study conditions (Castro *et al.* 2006).

In the current study, there were no significant differences in cooling time from 18 °C to 4 °C or in cooling time from 18 °C to 0 °C among the different ratios of continuously drained S-MIS to fish ($P > 0.05$, Table 3.1). The mean cooling time to 4 °C was 0.46 ± 0.08 hours and the mean time to 0 °C was 1.27 ± 0.32 hours over all ratios of continuously drained S-MIS to fish. There were also no significant differences observed in the lowest product temperature or the time to lowest temperature between different ratios of S-MIS to fish ($P > 0.05$, Table 3.1). The lowest mean product temperature was -0.19 ± 0.13 °C and the time to lowest product temperature was 1.91 ± 0.44 hours for all treatments of continuously drained S-MIS to fish. The results obtained from the cooling parameters in this study indicate that a 1:1 ratio of continuously drained S-MIS to fish is sufficient to chill the fish without affecting the rate of heat transfer.

Each ice-to-fish ratio was adequate to chill the fish to subzero temperatures of between 0 and -0.5 °C, which is not cold enough to be considered in the superchilled range of -1.5 to -2 °C. Superchilling lowers the product temperature 1-2 °C below the initial freezing point to freeze part of the product's water and create an internal refrigeration reservoir (Bao *et al.* 2007; Duun and Rustad 2008b). Yet studies have found that slurry ice may superchill a product with idealized very high ice to fish ratios (Pineiro *et al.* 2004; Zeng *et al.* 2005). This is significant because literature has demonstrated both benefits and disadvantages of superchilling seafood products between -1.5 °C and -3 °C, depending on the processing application and handling techniques (Bahuaud *et al.* 2008; Beaufort *et al.* 2009; Duun and Rustad 2007; Olafsdottir *et al.* 2006). The S-MIS system could possibly cool the product in the superchilled range if used at higher ice-to-fish ratios, which could have different quality effects than non-superchilled product stored in S-MIS. However, this does not occur at 2:1 ice to fish ratios or less and superchilling conditions are unlikely to be observed during commercial use.

3.5.3 Comparison of Chilled Parameters for Different S-MIS to Fish Ratios

While there were not any significant differences in product chilling among the different ratios of S-MIS to fish, there were significant differences in time that the product remained $\leq$ 0 °C. Table 3.1 shows that the 1:1 ice-to-fish ratio was $\leq$ 0 °C for a mean of 11.03 h, which was significantly shorter than both the 1.5:1 ratio at 24.80 h and the 2:1 ice-to-fish ratio at 41.55 h ($P < 0.05$). It was expected that the 2:1 ice-to-fish ratio would chill the product for roughly twice as long as the 1:1 ice-to-fish ratio,

but the results obtained were better than expected. This was likely due to the difference between the initial chilling of the product and the sustained chilling over time, suggesting that the rate of ice melt is not linear during sustained chilling over time.

One of the most relevant parameters concerning industrial application in this study was the time the Pacific whiting was cooled below 4 °C. This temperature was selected because the core temperature of aquatic food products at packing must be below 40 °F (4.4 °C) for some species of fish, according to United States HACCP guidance (Leonard 2011). Furthermore, this cooling temperature is used as a standard for best practices in the seafood industry. Table 3.1 shows that the 1:1 ice-to-fish ratio of S-MIS only cooled the product below 4 °C for a mean of 23.16 h, which was significantly shorter than the 1.5:1 ratio at 34.36 h ($P < 0.001$) and the 2:1 ratio at 47.90 h ($P < 0.001$). The 2:1 ice-to-fish ratio also kept the product below 4 °C for significantly longer than the 1.5:1 ice-to-fish ratio ($P < 0.001$). The 2:1 ice-to-fish ratio extended the cooling time < 4 °C to roughly double of the 1:1 ratio, with the 1.5:1 ratio halfway in-between. Future studies may show that a 3:1 ice-to-fish ratio triples these parameters, so that the product may be cooled below the regulatory limit of 4 °C for up to 72 h. This may be influenced more by the economics of producing more S-MIS instead of regulatory limits or quality parameters because there are examples in the literature that show no significant decreases in quality between days 1 and 5 of product stored in slurry ice at a 1:1 ratio (Rodriguez *et al.* 2006, Losada *et al.* 2005). However, this information may have noteworthy implications for industrial application, since fish kept in an industrial tote at a 1:1 ice-to-fish ratio would have to

be processed within 24 h after unloading. A 2:1 ratio, on the other hand, cools the product below 4 °C for 48 h, which may allow a processor to keep fish in totes for a longer period of time during busy seasons without refreshment.

3.5.4 Impact of Handling Technique on Product Cooling

Three different handling techniques at a 2:1 ice to fish ratio (non-drained S-MIS, continuously drained S-MIS, and initially drained S-MIS) were compared to CSW with similar parameters as the different S-MIS to fish ratios. There were no significant differences in cooling time to 4 °C for the three techniques of S-MIS ($P > 0.05$, Table 3.1). Yet the cooling time to 4 °C for product stored in CSW was significantly longer (38% longer than continuously drained, 49% longer than non-drained, and 57% longer than initially drained S-MIS) than the three handling techniques of S-MIS ($P < 0.05$). This parameter supports that fact that there is a greater rate of heat transfer when S-MIS is utilized, when compared to CSW.

Draining the carrier fluid away from the system significantly cooled the product lower than non-drained S-MIS and CSW ($P < 0.05$, Table 3.1). CSW was not able to chill the product below 0.38 °C even though the CSW contained the same amount of sodium chloride, a freezing point depressant, as S-MIS. Non-drained S-MIS cooled the product slightly lower at 0.09 °C, but continuously drained S-MIS cooled the product significantly lower at -0.23 °C ($P < 0.05$). S-MIS that was initially drained cooled the product to the lowest temperature at -0.81 °C ($P < 0.05$). The drained handling techniques of S-MIS were able to chill the product below 0 C, while the non-drained S-MIS and CSW could not. The results suggest that the product

temperature was a direct result of the ice fraction because the amount of ice in both the non-drained S-MIS and CSW was 40%. This would provide only 11.0 kg of ice to cool the fish, while the drained handling techniques contained 13.8 kg of ice at the beginning of cooling.

Over all ratios and handling techniques, the product did not experience temperatures that were sufficient to superchill fish muscle. Also, the freezing points of most seafood products fall within a range of -1.0 to -2.5 °C so the whiting muscle did not freeze at any of the ice-to-fish ratios or handling techniques (Sivertsvik *et al.* 2003). However, the capability has been demonstrated by slurry ice systems. Erikson *et al.* (2011) found that slurry ice could chill whole Atlantic salmon to between -1.0 and -2.0 °C, yet the researchers had a 9:1 ice-to-fish ratio and the slurry ice was refreshed to ensure a similar ratio throughout the storage time. Digre *et al.* (2011) conducted a reproduction of the Erikson study with cod instead of salmon, and found that the lowest core temperature of the cod was also between -1.0 and -2.0 °C. This suggests that the researchers were able to find the idealized cooling system for the slurry ice and it also demonstrates that there is a product temperature variation of at least 1 °C in this system. Campos *et al.* (2005) and Losada *et al.* (2006) also observed that slurry ice may chill sardines to between -1.0 and -1.5 °C when combined with refrigerated ambient temperatures.

3.5.5 Comparison of Chilled Parameters for Different Handling Techniques

S-MIS that was initially drained kept the product colder for a longer period of time than the other handling techniques, as determined by the time that product was

chilled $\leq$ 0 °C and time that product was chilled < 4 °C. Initially drained S-MIS exhibited the longest product chilling time $\leq$ 0 °C at 53.19 h, while continuously drained S-MIS cooled the whiting for a significantly shorter time of 41.55 h ($P <$ 0.01, Table 3.1). However, CSW and non-drained S-MIS did not have a mean lowest temperature that was at or below 0 °C.

These results were reflected in the product chilling time < 4 °C, as initially drained S-MIS significantly chilled the product < 4 °C for the longest time at 70.94 h ($P <$ 0.01, Table 3.1). Conversely, whiting stored in continuously draining S-MIS chilled the product < 4 °C for 47.90 h. Both handling techniques of S-MIS cooled the product < 4 °C significantly longer than CSW because S-MIS, as a type of slurry ice, has a higher capacity for heat exchange than the flake ice in CSW (Alvarez *et al.* 2009; Pineiro *et al.* 2005). Yet CSW cooled the product < 4 °C for a significantly longer time than whiting stored in non-drained S-MIS. This result was unexpected, but may be explained once again by the high rate of heat transfer and the ratio of ice to water in the biphasic system. The initially drained and continuously drained S-MIS systems were monophasic and were 100% ice throughout the experiment. Even though melt water drained out of the cooler in the continuously drained system, there continued to be enough ice to cool the fish for a longer period of time than non-drained S-MIS and CSW. The non-drained S-MIS was approximately 40% ice and 60% water, so there was much less ice to cool the fish. The ice was able to effectively transfer heat away from the product, as seen in the cooling parameter time to 4 °C, but the ice quickly melted away as a result.

The nature of the chilling media may also explain why CSW was able to cool the product for longer than the non-drained S-MIS. The fish are immersed in the water layer with flake ice on top in the CSW chilling system, so the ice is not in direct contact with fish. Therefore, the ice is cooling the water, which is in turn cooling the fish. In comparison, the S-MIS is in direct contact with the fish in the S-MIS system, which causes the smaller ice crystals to melt quickly as a result. This has direct implications for industry because seafood needs to be quickly chilled to preserve freshness, but fishing excursion of multiple days requires ice that will not melt before the product is brought back to the dock. Non-drained S-MIS, which is likely how this type of ice would be handled in industry, cools the product more effectively and could preserve initial freshness better than CSW. Yet it would also not be able to keep the product cool throughout a multi-day voyage unless utilized at high ice to fish ratios.

Table 3.1: Selected time/temperature parameters for different ice to fish ratios and handling techniques. S-MIS = sub-micron ice slurry

Ice Type	Cooling Time to 4 °C (Hr)	Time to 0 °C (Hr)	Lowest Temperature (°C)	Time to Lowest (Hr)	Time ≤ 0 °C (Hr)	Time < 4 °C (Hr)
1:1 Continuous Drain S-MIS	$0.42^{a} \pm 0.05$	$1.37^{a} \pm 0.33$	$-0.11^{ac} \pm 0.12$	$1.98^{a} \pm 0.38$	$11.03^{a} \pm 1.66$	$23.16^{a} \pm 2.07$
1.5:1 Continuous Drain S-MIS	$0.47^{a} \pm 0.10$	$1.25^{a} \pm 0.33$	$-0.22^{a} \pm 0.14$	$2.01^{a} \pm 0.50$	$24.80^{b} \pm 1.42$	$34.36^{b} \pm 1.75$
2:1 Continuous Drain S-MIS	$0.48^{a} \pm 0.09$	$1.20^{a} \pm 0.31$	$-0.23^{a} \pm 0.12$	$1.73^{a} \pm 0.45$	$41.55^{c} \pm 1.12$	$47.90^{c} \pm 2.87$
2:1 Initial Drain S-MIS	$0.33^{a} \pm 0.11$	$1.10^{a} \pm 0.32$	$-0.81^{b} \pm 0.40$	$2.70^{b} \pm 0.35$	$53.19^{d} \pm 2.96$	$70.94^{d} \pm 3.57$
2:1 No Drain S-MIS	$0.39^{a} \pm 0.12$	–*	$0.09^{c} \pm 0.30$	$2.36^{ab} \pm 0.47$	–*	$16.80^{e} \pm 2.51$
2:1 Chilled Seawater	$0.77^{b} \pm 0.13$	–*	$0.38^{d} \pm 0.22$	$6.25^{c} \pm 0.66$	–*	$39.87^{b} \pm 4.44$

Mean values followed by different letters in the same column are statistically significant ($P < 0.05$).

*Dashes indicate that the chilling media was not able to chill the product to 0 °C.

3.5.6 Non-linear Regression of 10:1 Ice to Fish Ratios

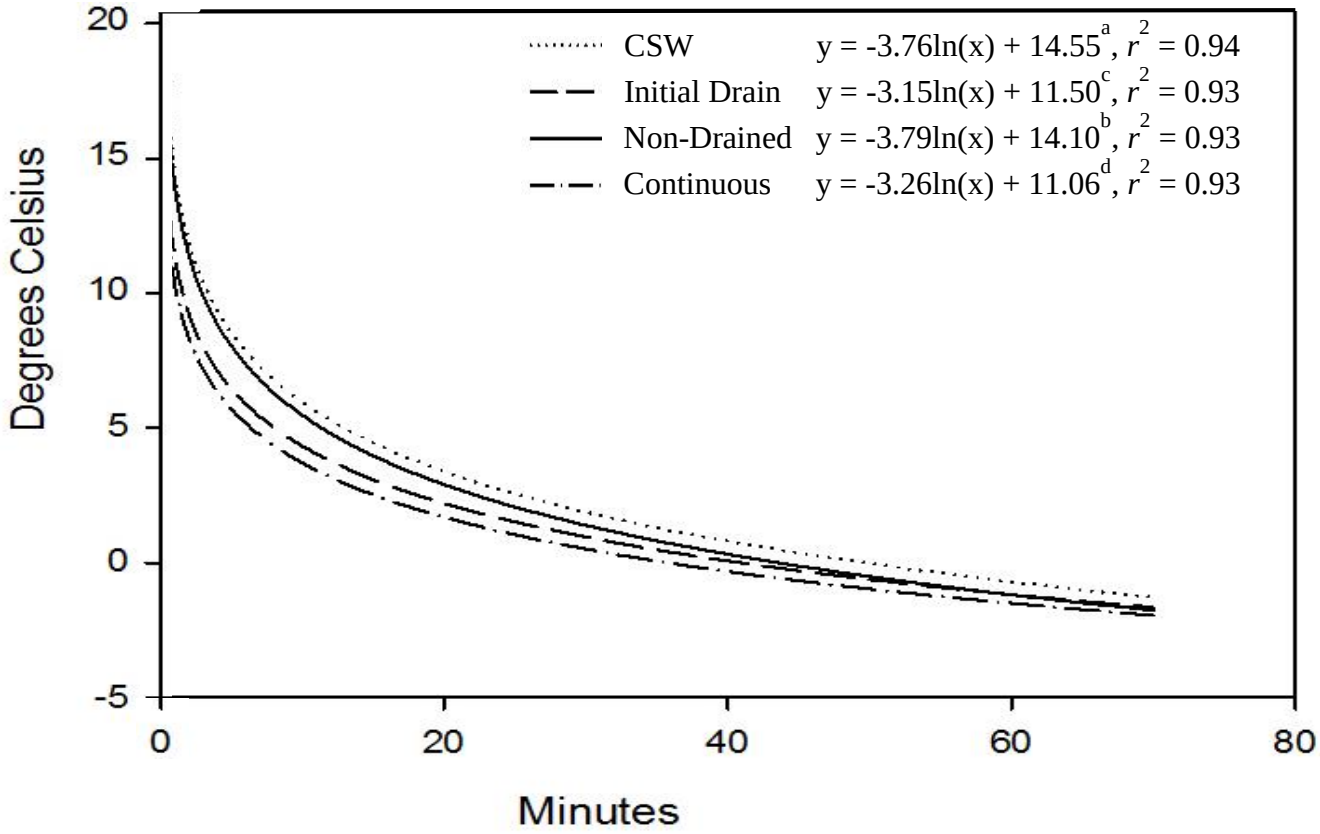
In addition to exploring the possibilities of S-MIS for industrial use, it is useful to know if there is a difference between S-MIS and CSW in cooling products in excess chilling media. This knowledge can be used to understand if S-MIS behaves differently in practical use when compared to a model system, as well as shows how changing the biphasic system of slurry ice into a monophasic system may affect chilling parameters. Non-linear regression showed the chilling curves to be logarithmic for product stored in a 10:1 ice to fish ratio for all types of chilling media, and all product cooled to an ultimate temperature within 70 min.

Whiting that was stored in continuously draining S-MIS had the quickest chilling rate at $y = -3.26\ln(x) + 11.06$ with the value (y) as temperature and (x) as minutes (Figure 3.1). This was significantly faster than whiting stored in a 10:1 ratio of initially drained S-MIS ($P < 0.05$), which was unexpected because the continuously draining S-MIS was an open system. Even though the continuously drained S-MIS was allowed more thermal energy to penetrate the system, the removal of the liquid phase allowed the S-MIS to have greater surface area with the product to chill the whiting quicker. Non-drained S-MIS had a significantly slower rate of product chilling than the other two handling techniques of S-MIS ($P < 0.05$). This demonstrates that even at 10:1 ice to fish ratios, the greater amount of ice in the drained systems causes the system to chill more efficiently.

CSW initially chilled as quickly as non-drained S-MIS yet the chilling rate slightly, but significantly, slower ($P < 0.05$). The chilling equation for CSW was $y = -3.76\ln(x) + 14.55$, while that of non-drained S-MIS was $y = -3.79\ln(x) + 14.10$. Even

though these equations were significantly different, substituting 10 minutes for x shows that there is very little difference practically. Product chilled in CSW would be at 5.89 °C within 10 minutes, while fish stored in non-drained S-MIS would be at 5.37 °C. Conversely, product stored in continuously draining S-MIS would have a temperature of 3.55 °C, which is much lower.

Figure 3.1: Non-linear logarithmic regression cooling curves of Pacific whiting (*Merluccius productus*) stored in 10:1 ice to fish ratio in different ice types. CSW = chilled seawater, initial drain = sub-micron slurry ice (S-MIS) with liquid phase initially drained from the cooler, continuous = S-MIS with liquid continuously draining from the cooler, non-drained = S-MIS with no liquid drainage.



3.6 Conclusions

Pacific whiting stored at 1:1, 1.5:1, and 2:1 ice to fish ratios in continuously drained S-MIS chilled at a similar rate and to similar ultimate temperatures. Therefore, a 1:1 ice to fish ratio was adequate to chill the fish and the rate of heat transfer was not affected, as compared to higher ice to fish ratios. However, the ice to fish ratio did have an effect on long-term storage of the product. Higher ice to fish ratios kept the fish at temperatures < 4 °C for a longer period of time, which could have practical implications on how long a boat or processor could hold fish. If a 1:1 ice to fish ratio is used, then the technician should either refresh ice or process the fish before 24 h, while a 2:1 ratio may chill product twice as long. As the technology may be prohibitively expensive for a company to install an S-MIS machine on every boat, higher ice to fish ratios may be needed to ensure temperature control throughout a multi-day excursion.

Several different handling techniques of S-MIS were investigated and compared to an industry standard, CSW, to determine which handling technique or ice chilled the product most efficiently. Each type of handling technique of S-MIS (continuously drained, initially drained, and non-drained) initially chilled the product significantly faster than CSW. Therefore, it is advised to use S-MIS instead of CSW when chilling species of seafood that require quick chilling to ensure product quality. The differences in lowest ultimate temperatures may also have an impact on product quality, depending on the species of seafood. If sub-zero temperatures may negatively affect product quality, then the handling technique of non-drained S-MIS or CSW is recommended. Removing the liquid phase of S-MIS increased the product storage

time over non-drained S-MIS and CSW. If S-MIS is utilized on boat or onshore in a tote, it is suggested to have a mechanism to drain excess liquid or refresh ice multiple times per day to keep product properly chilled.

CHAPTER 4

QUALITY OF ARROWTOOTH FLOUNDER (*ATHERESTHES STOMIAS*) AS IMPACTED BY SHORT-TERM STORAGE IN ICE SLURRIES CONTAINING SUB-MICRON ICE CRYSTALS

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4.1 Abstract

It is not uncommon for ground fish in the Pacific Northwest to be placed in totes with a saltwater brine and flake ice mixture (chilled seawater, CSW) as they are off-loaded and await processing, and this storage period can impact ground fish quality. A novel ice slurry containing sub-micron ice crystals (S-MIS) is evaluated to determine whether it can better maintain ground fish quality than CSW. Whole arrowtooth flounder (*Atheresthes stomias*) were stored for 48 h in either S-MIS or CSW, filleted and skinned, packaged in plastic bags inside waxed cardboard boxes, and stored at 3.5 °C as typical for a commercial plant. Fillets were sampled and evaluated on d 0, 5, 10 and 15. Fillets from fish stored in S-MIS had lower salt content and lower pH ($P < 0.05$). CSW treated fish were significantly lower in secondary oxidation products, as measured by TBARs ($P < 0.05$). Chilling treatment did not significantly affect fillet yield, drip loss, aerobic mesophilic plate counts (APC), total volatile basic nitrogen (TVB-N), *K*-values, or odor evaluation scores ($P > 0.05$). There was a day effect ($P < 0.05$) for every evaluation except salt-uptake and *K*-value. The *K*-value for arrowtooth flounder was above 85% at day 0 even though fish were collected immediately upon off-loading in the winter months, but this phenomenon is not unheard of in flatfish. Bottom fillets had significantly higher TBARs and drip loss ($P > 0.05$). Refrigerated shelf life of arrowtooth flounder was determined by odor evaluation to be 8 days regardless of applied chilling treatment.

4.2 Practical Application

Seafood is chilled upon harvest to ensure quality, yet the amount of fish lost to decomposition remains high because of poor initial handling practices and inadequate chilling. Therefore, there is a need to investigate alternative methods of chilling to determine if quality may be improved. Arrowtooth flounder (*Atheresthes stomias*) exhibits rapid quality deterioration, so it is a model species to determine if a slurry ice consisting of sub-micron ice crystals can be used to extend shelf-life more effectively than conventional chilling methods.

4.3 Introduction

Arrowtooth flounder (*Atheresthes stomias*) is an underutilized marine resource estimated to be the most abundant fishery resource in the North Pacific Ocean (Sathivel and others 2005). Yet consumer demand for arrowtooth is low due to undesirable qualities arrowtooth flesh exhibits when cooked, such as proteolytic enzymes causing softening of flesh (Choudhury and Gautam 2003, Oliveira and Bechtel 2006). There has been some success in processing into surimi if protease inhibitors are added, but most arrowtooth flounder is caught as by-catch and sold at low prices for fish meal (Uresti and others 2005). Quality loss in seafood is characterized by biochemical changes in fish muscle and microbiological growth (Ocaño-Higuera and others 2011, Ofstad and others 1996a). Efforts to preserve quality and extend shelf life of aquatic food products are increasing as growing consumer demand for seafood around the world has resulted in longer supply chains (Alfaro and others 2013). Ice is one of the most commonly used methods of preservation in the seafood industry to maintain product quality, due to availability and relatively low cost. Flake ice or chilled seawater, flake ice mixed with seawater, are the most common uses of ice in the seafood industry, but flake ice is not an ideal chilling medium because it is abrasive and has a poor heat transfer rate (Kauffeld and others 2010).

In recent years, ice slurries have been examined as a system to cool seafood products quicker than conventional systems. Slurry ice is a biphasic system of ice crystals in a carrier liquid containing a freezing point depressant, typically seawater, which has a hypothesized cooling capacity of four to six times higher than chilled

water (Saito 2002). Smaller ice crystals allows more ice-to-product contact to increase the heat transfer surface area and rate of product chilling (Kauffeld and others 2005, Lucas and Raoult-Wack 1998). Other advantages of slurry ice include pumpability, less damage to the product due to the spherical geometry of the ice crystals, and greater product contact (Alvarez and others 2009, Bedecarrats and others 2010). Many studies have been conducted in recent years to demonstrate the superior effects of slurry ice over conventional methods of chilling, such as flake ice and refrigerated seawater (Aubourg and others 2009, Bellas and Tassou 2005, Medina and others 2009). Slurry ice is typically made by grinding pre-made ice and mixing with seawater, but a new development is the production of slurry ice directly from seawater. This development allows the resultant ice crystal to be extremely small, 400-700 nm, which is a magnitude smaller than conventional slurry systems. Smaller ice crystal size suggests better surface contact with the product and, as a result, more efficient transfer of heat. It is hypothesized that slurry ice made with sub-micron ice crystals will be better able to maintain product quality than current chilling systems. To test this hypothesis, this research evaluates the use of sub-micron slurry ice for reception of fish at processing plants.

Fish are typically placed in flake ice or chilled seawater, up to 48 h during peak capture periods, when they are received at processing plants and await processing. This is especially true for the lower value fish, such as arrowtooth flounder. The objective of this study was to evaluate fillet quality from arrowtooth flounder stored 48 h in a slurry ice containing sub-micron ice crystals with fish stored in chilled seawater as a reference. Fillets were collected and quality was evaluated

over time by measuring development of secondary oxidation products (TBARs) and reduction of protein's ability to hold water through drip loss evaluation. Fillets were further evaluated by measuring pH and total volatile base nitrogen development, nucleotide degradation (*K*-value), growth of aerobic mesophillic bacteria, and confirmation of decomposition through sensory odor evaluation.

4.4 Materials and Methods

4.4.1 Experimental Design

A visual representation of the experimental design is provided as Figure 1. Wild, trawl-caught arrowtooth flounder were obtained during off-loading from fishing vessels on five separate trial dates between December 2013 and March 2014. Fishing excursions lasted approximately 48 h and fish were packed in flake ice. Collected fish (n = 24 per replicate) were immediately placed in 44.5 x 48.0 x 101.5 cm insulated coolers (Rubbermaid, Atlanta, GA) with excess flake ice and transported to the laboratory (10 min). Arrowtooth were weighed and measured, then randomly distributed between a cooler containing either CSW or S-MIS at a 2:1 ice-to-fish ratio (wt:wt) with n = 12 fish per cooler. Coolers were stored at ambient temperatures (21-24 °C) for 48 h.

Fish were removed from the chilling treatments after 48 h, filleted into pairs of top and bottom fillets, skinned, and packaged according to Hernandez and others (2009) with modifications. Fillets were placed in open 82 x 48 cm polyethylene plastic fresh fish liners (4 mil, Wetlock, Frontier Packaging, Tukwila, WA) and stored in 82 x 24 x 43 cm Glacier Pak™ waxed cardboard boxes (Georgia Pacific, Olympia, WA) with the top of the liner folded back over the top of the fillets. Fillets were separated by chilling treatment in the box by placing them in separate liners and boxes were stored at 3.4 ± 0.3 °C. Flake ice was packed around plastic liners in a 2:1 ice-to-fish ratio (wt:wt) and replenished every third day, while boxes contained holes for water drainage.

Three fillet pairs from each treatment were assessed after 48 h storage in chilling treatments (d 0), while the remaining nine fillet pairs from each treatment were divided between three boxes. Fillet pairs from the two chilling treatments were placed in the same box, but were separated by different plastic liners, so each box contained 6 fillet pairs total. One box was removed for sampling on d 5, 10, and 15 of storage. One fillet pair from each chilling treatment on each sampling day was collected for odor, chemical, or microbial evaluation. All fillets were evaluated for drip loss. Fillets collected for odor and chemical evaluation were vacuum packaged and stored at -80 °C until evaluation. Fillets collected for microbiological analysis were evaluated the same day they were removed from packaging.

Top and bottom fillets were individually powdered for chemical analyses. Fillets were removed from -80 °C storage, immediately cut into 1 cm^2 pieces, immersed in liquid nitrogen, and blended in a cold room using a frozen Waring® blender cup (Conair Corp, Stamford, CT). The resultant powder was placed in a 710 mL Whirl-Pak sample bag (Nasco, Fort Atkinson, WI) and stored at -80 °C prior to chemical analyses.

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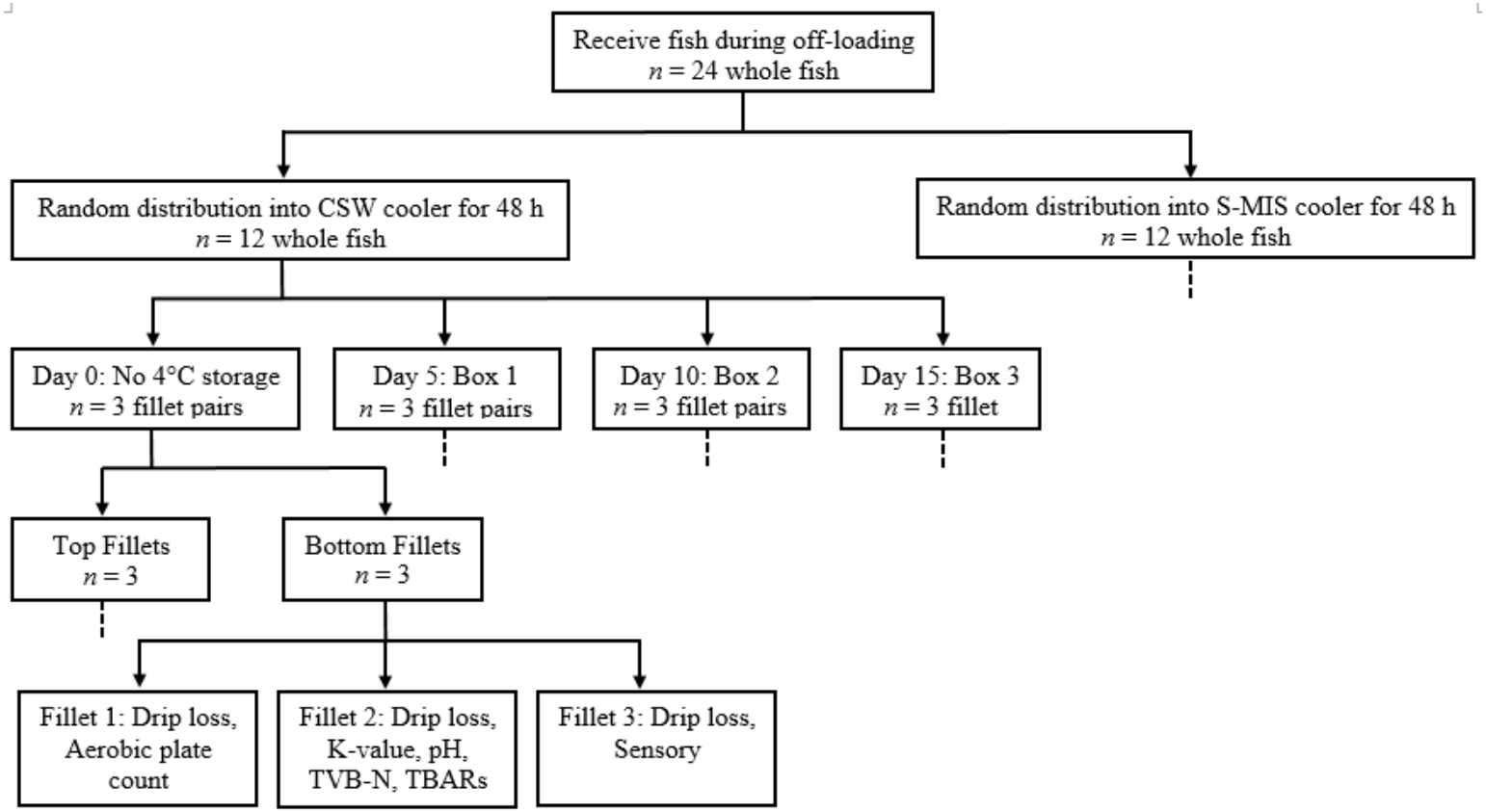

Figure 4.1: Experimental design of one treatment replicate. The study was replicated 5 times between Dec. 2013 and Mar. 2014. *n* = 24 fish were collected at the dock, stored in either chilling media for 48 h with *n* = 12 fish per cooler, filleted and skinned, 3 fillet pairs (top and bottom) from each chilling media treatment were stored in 1 of 4 different waxed cardboard boxes held at 3.5 °C for up to 15 days, and then fillets were used for either chemical, microbiological, or odor analyses on each sampling day.

4.4.2 Preparation of Chilling Media

Brine was hand-made in a brine tank and adjusted to 3.6% salt (NaCl) content using an electrochemistry meter (Model pHi 430, Beckman Coulter, Fullerton CA). Chilled seawater (CSW) was made using 40 parts flake ice and 60 parts brine (wt:wt) with a final temperature of -1.0 ºC. Sub-micron slurry ice (S-MIS) was generated from the same brine using a slurry ice machine (Conrad 2001S, NanoICE, Inc., Woodinville, WA, USA). Previous measurements have characterized ice crystals from this machine as ranging from 400-700 nm in diameter. The output flow rate of ice was 1.8 kg per min and outlet ice temperature was -2.0 °C. CSW was made in an Ice Station Zero cooler with dimensions 79.0 x 37.0 x 35.0 cm (Moeller Marine Products, Sparta, TN). S-MIS was placed into an identical cooler as it was generated from the machine.

4.4.3 Fillet Evaluations

4.4.3.1 Salt

The Volhard method of salt determination for seafood was conducted according to the Association of Official Analytical Chemists to express the percentage of salt in fish muscle (Cunniff 1995).

4.4.3.2 Drip Loss

Drip loss was defined as amount of liquid lost from the fillet muscle during the storage period. The weight of each fillet was recorded between removal from

refrigerated storage and vacuum packaging/microbial analysis. Microbial fillets were weighed on sterile cutting boards

% Drip loss = [(Initial fillet wt – Stored fillet wt) / Initial fillet wt] * 100

4.4.3.3 pH

The pH values were observed by homogenizing 3 g of powdered sample in 27 mL of deionized water using a Kinematica CH-6010 Polytron (Brinkman Instruments, Westbury, NY), followed by reading the homogenate pH by an Accumet Excel XL15 pH meter (Fisher Scientific, Hampton, NH).

4.4.3.4 Total Volatile Basic Nitrogen (TVB-N)

TVB-N was conducted according to Antonacopoulos and Vyncke (1989). Magnesium oxide (2 g) was added to 10 g of powdered sample, the samples were steam distilled using a Distillation Unit 100 (Fisher Scientific, Pittsburg, PA) into 3% boric acid containing 8 drops of Tashiro indicator, and the distillate was titrated with 0.1 N HCl. Units of volatile nitrogen were expressed as mg N per 100 g of sample.

4.4.3.5 Aerobic Plate Count (APC)

Fillets were aseptically separated into 1 cm^2 pieces and 25 g of sample was randomly obtained. Samples were mixed with 225 mL of phosphate-buffered saline and homogenized (Seward Stomacher 400, Seward, Worthing, UK) at 230 rpm for 30 s. Serial dilutions were prepared in phosphate-buffered saline. Aerobic plate counts (APC) were conducted in BBL TSA II Modified Trypticase® Soy Agar (BD, Sparks,

MD) after incubation at 37 °C for 72 h. Results were expressed as log colony forming units per gram of sample (CFU/g).

4.4.3.6 Odor Analysis

Immediately prior to odor analysis, fillets previously frozen and stored at -80 ºC were prepared for the session in an odor-free area separate from the testing area. Both preparation and testing areas were at the OSU Seafood Lab in Astoria. Preparation included thawing of packaged fish under cold, running water, removal of fillets from vacuum-packaging, and placement on odorless sterile paper. Fillets were arranged in the testing area with 3 fillet samples spaced equidistant from each other (0.5 m apart) per 1 x 3 m table, and labeled with a randomized 3-digit code. Tables were spaced 2 m apart and 6 tables were used per session. Analysis was conducted at 2:00 pm Pacific Standard Time on 5 successive days in July with 16 samples evaluated each day by 9 panelists (5 females, 4 males).

Panelists were volunteers experienced in odor evaluation of seafood from the OSU seafood lab. All volunteers had previously self-screened themselves for odor detection ability and ability to communicate descriptors using The Smell Identification Test^{TM} by Sensonics International (Haddon Heights, NJ). One week prior to analysis, panelists were calibrated to seafood descriptors according to the FDA Office of Regulatory Affairs Laboratory Manual (Mackill 2003). Calibration consisted of reviewing common descriptive smells for seafood using test references and assignment of scores along a 100 mm line determined by the identification and strength of particular odors (Morkore and Einen 2003). Panelists were subsequently

provided with examples of arrowtooth flounder fillets in increasing order of decomposition with discussion of scores and descriptors, followed by evaluation of test fillets. Individuals elected to participate based on their ability to score test fillets using the applied methodology and relate correct descriptors to odors detected. Table 4.1 provides an example of the descriptors used in seafood odor analysis with common scores associated with each descriptor, and examples of the reference odor used for each descriptor.

A standardized ballot developed during international harmonization exercises for seafood sensory standards and endorsed by the FDA and NOAA was used for evaluation (Pivarnik and others 2001, Rielly and York 1994). Ballots consisted of the randomized code, a 100 mm line scale, and an area to record the descriptive odors identified. Acceptable quality descriptors were scored between 1 and 35 mm on the scale, borderline acceptability descriptors were scored between 35 and 49 mm of the scale, and unacceptable odors were scored between 51 and 99 mm on the scale. Scores of 0, 50, or 100 are not allowed according to the methodology and fillets were deemed unacceptable with respect to quality at 50 after the mean was obtained from the scores of the panelists. Panelists were permitted unlimited time to evaluate the samples and could choose to evaluate the presented samples in any order, but it was recorded that all panelists finished within 35 min from the beginning of the test during each session.

Table 4.1 – Odor descriptors, test examples, and scores for arrowtooth flounder, according to the FDA 100 mm line scale

Quality	Term	Example	Scores
Pass Quality Characteristics	Briny	Seaweed	1 – 25
	Ocean air	Helional	1 – 25
	Fresh grassy	Fresh cut grass	1 – 25
	Cucumber	Cucumber	1 – 25
	Cooked corn	Cooked corn	10 – 35
	Metallic	Iron pill in water	10 – 35
Borderline Pass Characteristics	Stale	Cardboard	35 – 45
	Fishy	Canned sardines	40 – 49
	Oxidized	Oxidized oil	40 – 49
Reject Quality Characteristics	Sour (1)	Sour milk	51 – 65
	Sour (2)	Vinegar	51 – 65
	Yeasty	Yeast	51 – 65
	Rancid	Rancid oil	51 – 65
	Cheesy	Parmesan cheese	60 – 70
	Sulfurous	Old broccoli	65 – 75
	Ammonical	Ammonia	75 – 85
	Sickly sweet	Decomposed pork	80 – 99
	Putrid	Decomposed beef	80 – 99
	Fecal	–	80 – 99

4.4.3.7 Lipid Oxidation

Secondary lipid oxidation was determined by a modified thiobarbituric acid reactive substances (TBARs) assay described by Buege and Aust (1978) and modified by Cerruto-Noya and others (2009). Powdered sample (10 g) was homogenized for 30 s in 30 mL of deionized water, followed by centrifugation at 3000 rpm for 10 min (Model J-6M, Beckman Instruments Inc., Fullerton, CA, USA). Two mL of supernatant was extracted into a culture tube, then 4 mL of thiobarbituric/trichloroacetic acid solution and 100 µL of 10% butylated hydroxyanisole in ethanol were added. Tubes were heated in a boiling water bath for 15 min, cooled for 10 min in an ice bath, and centrifuged at the parameters described previously. Amount of malondialdehyde (MDA) was determined at 532 nm using a UV-240 UV-Vis spectrophotometer and results were expressed as mg MDA·kg^{-1} muscle (Shimadzu, Tokyo, JP). A standard curve was constructed with 1,1,3,3-tetraethoxypropane (Botsoglou and others 1994).

4.4.3.8 K-value

Nucleotide extracts were prepared according to the method of Ryder (1985) and stored at -80 °C prior to analysis. Analysis of nucleotide extracts was performed by HPLC (Shimadzu, Tokyo, JP), as described by Ozogul and others (2000). Standard curves for adenosine 5'-triphophate (ATP) and the degradation products adenosine 5'-diphosphate (ADP), adenosine 5'-monophosphate (AMP), inosine 5'-monophosphate (IMP), inosine (Ino), and hypoxanthine (Hx) were constructed in the 0 – 0.5 mM range at 0.1 mM intervals. Results for each nucleotide were expressed as

mmol·kg^{-1} muscle. The *K*-value was calculated according to Saito and others (1959) as: *K*-value (%) = (Hx + Ino) / (ATP + ADP + AMP + IMP + Ino + Hx) * 100

4.4.4 Statistical Analysis

Chemical, microbiological, and odor analysis data was subjected to three-way analysis of variance (ANOVA) followed by the Holm-Sidak method for multiple comparisons to determine significant differences between treatments using SigmaPlot 12.5 (Systat Software Inc., Chicago, USA). Independent variables included chilling media treatment, day, and top or bottom fillet. Pearson's correlations were conducted between all parameters. Linear regression of TBARs values with the dependent variable of storage day was conducted to determine rate of lipid oxidation. The significance level was set at 95% ($P < 0.05$) for all analyses.

4.5 Results and Discussion

Flounder collected from vessels were of similar size, 1.36 ± 0.32 kg and 47.0 ± 6.2 cm. Those stored in S-MIS had a fillet yield of 28.4 ± 5.4%, while those stored in CSW were not significantly different with a fillet yield of 27.7 ± 4.8% ($P > 0.05$).

4.5.1 Salt

Fillets from fish chilled in S-MIS had a significantly lower salt content, 0.25 ± 0.06%, than fillets from CSW-treated fish, 0.41 ± 0.09% ($P < 0.05$, Figure 2). There were no significant effects of storage day or fillet location (top or bottom fillet) within chilling treatments ($P > 0.05$). Previous research has demonstrated that storing fish in a salt brine may increase the ultimate salt content of fillets, which in turn leads to detrimental visual and quality defects. Results from this study are similar to those found in other studies with a moderate increase in salt content when stored in slurry ice, but detrimental salt-induced effects would more likely be observed in flounder stored in CSW because of the higher salt content (Aubourg and others 2007, Rodriguez and others 2008). Both chilling treatments used the same brine, so it was not expected that there would be differences in fillet salt uptake. Since the S-MIS system creates ice from the brine, one would expect the salt concentration of the liquid phase of S-MIS to be higher than CSW due to freeze concentration effects. Similarly, since the CSW brine is in effect "diluted" by the addition of ice, the liquid phase salt concentration should lower as ice melted.

The observed differences in salt content between chilling media treatments, however, may be attributed to the physical matrix of the chilling medium. CSW is

made with commercial flake ice and the ice tends to float on top of the brine. Since the fish tend to sink, they are primarily exposed to the liquid phase (or brine). With S-MIS, the ice does not float to the top as observed with CSW and, instead, tends to stay suspended in the brine like a gel. It is hypothesized that fillets from fish treated with the S-MIS had less salt because there was more direct contact of fish surfaces with ice, which contains no salt, during the treatment period.

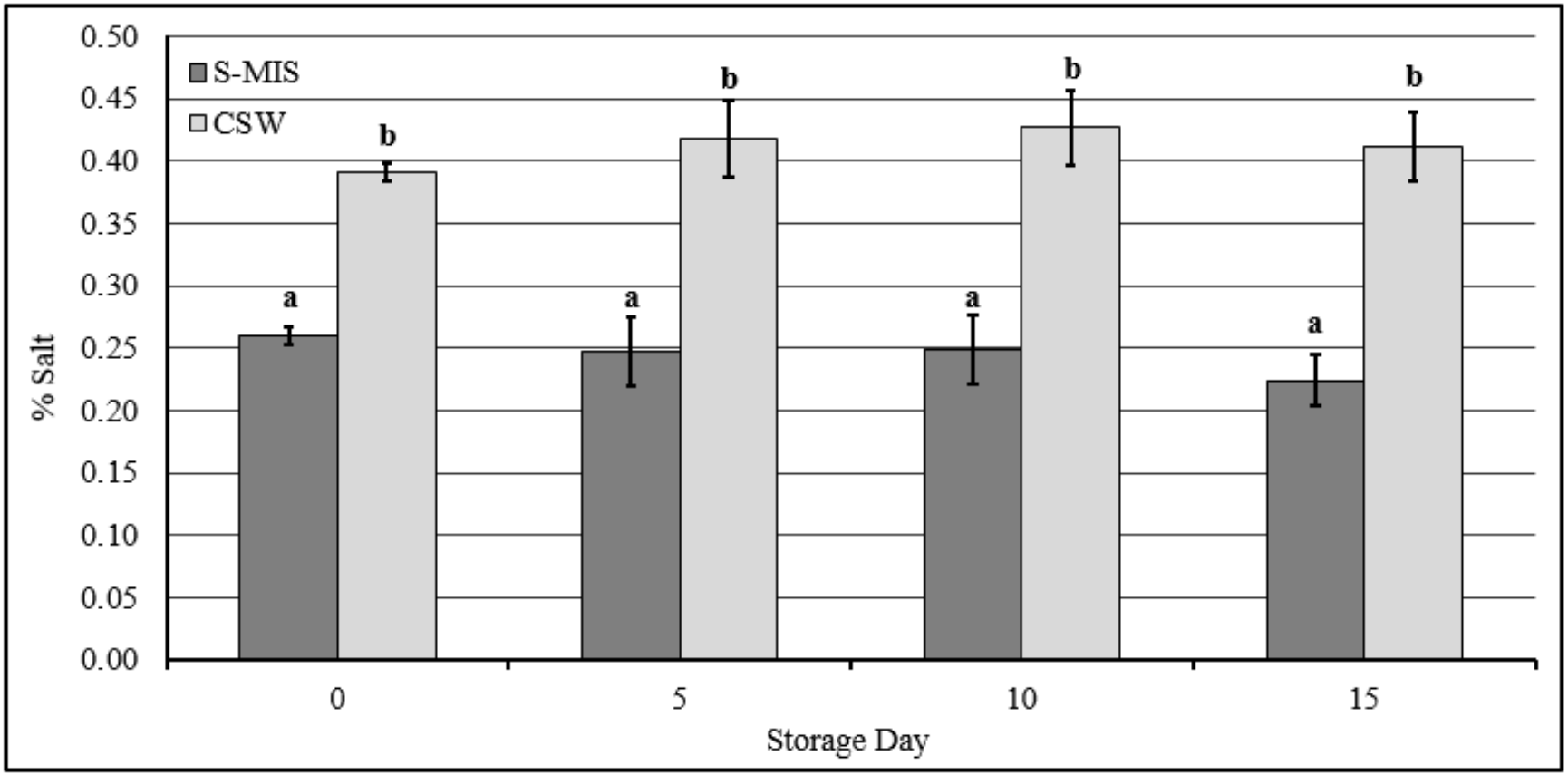

Figure 4.2 – Percent salt as determined by the Volhard method for arrowtooth flounder muscle stored at 3.5 °C for 15 days after 48 h storage in either chilled seawater (CSW) or a sub-micron ice slurry (S-MIS). Top and bottom fillet values were averaged. Mean values (n = 10) and standard deviations with different letters (a, b, c) denote significant differences ($P < 0.05$).

4.5.2 *Drip Loss*

Drip loss was not significantly influenced by chilling treatment ($P > 0.05$, Figure 3), but there were observed differences between top and bottom fillets and drip loss significantly increased during the storage period ($P < 0.05$). Bottom fillets exuded 2-3 times more drip loss than top fillets on each storage day for both chilling media treatments. Throughout the storage period, drip loss did not significantly increase until d 15 for top fillets, however, it did significantly increase each sampling day for bottom fillets ($P < 0.05$). Drip loss can be attributed to a wide variety of factors including physiological influences, slaughter method, processing conditions, and storage environments (Ofstad and others 1996b, Rora and Regost 2003). It is hypothesized in the current study that autolytic enzymes and bacterial decomposition disrupted myofibrillar structure to cause liquid loss in the extracellular matrix during the storage period (Huff-Lonergan and Lonergan 2005, Olsson and others 2007). However, this disruption was not as extensive as the damage caused by ice crystal formation in similar flatfish subjected to frozen storage, as Boyd and Southcott (1965) reported a drip loss of 8% in Dover sole after frozen storage at -10 °C. The greater amount of liquid lost in the bottom fillets may be attributed to greater myosin aggregation, which itself may be attributed to a variety of environmental, species, enzymatic, and processing factors (Attouchi and Sadok 2010, Gallart-Jornet and others 2007, Wilkinson and others 2008).

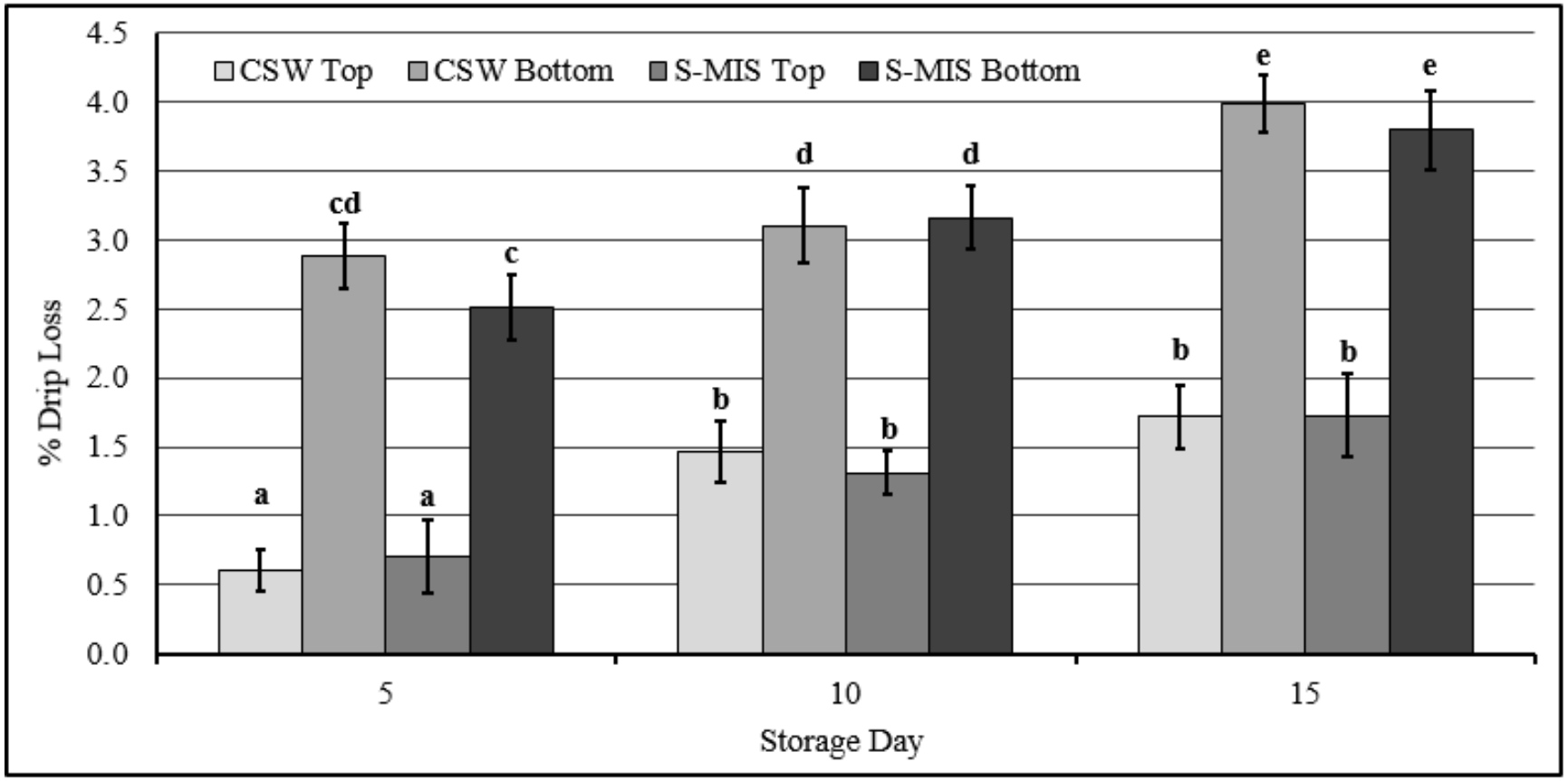

Figure 4.3 – Percent drip loss for arrowtooth flounder muscle stored at 3.5 °C for 15 days after 48 h storage in either chilled seawater (CSW) or a sub-micron ice slurry (S-MIS). Mean values (n = 5) and standard deviations with different letters (a, b, c) denote significant differences ($P < 0.05$).

4.5.3 pH

There was a chilling treatment effect of pH over refrigerated storage time ($P < 0.05$, Figure 4). Fillets from CSW-treated fish had significantly higher pH than S-MIS on d 10 and 15, which was reflected by a significant interaction between treatment and day ($P < 0.05$). The pH of CSW-treated fish increased significantly within treatment on d 10 and 15, but pH of S-MIS fillets did not significantly increase until d 15. There was not a significant fillet location effect (top or bottom fillet) of pH ($P > 0.05$).

It has been discussed that pH impacts the amount of liquid lost and muscle texture in general (Ang and Haard 1985). Olsson and others (2003a) reported that the drip loss of wild halibut with a muscle pH of 5.90 was 14.8% to 15.9%, while a follow-up experiment by Olsson and others (2003b) associated an average muscle pH of 6.54 with significantly less drip loss at 8.7%. Thus, an inverse relationship between pH and the water-holding capacity of fish muscle has been suggested (Love 1970). In the current study, there was a fillet location effect of drip loss, while there was not a fillet location effect for pH. An investigation of linear correlations between tests also showed that there was only a moderate positive correlation ($r = 0.45$) between pH and drip loss ($P < 0.05$, Table 4.2). This suggests a more complex set of factors for liquid loss than a simple relationship between pH and drip loss of fish muscle, which is similar to observations by other researchers (Herland and others 2007, Rustad 1992).

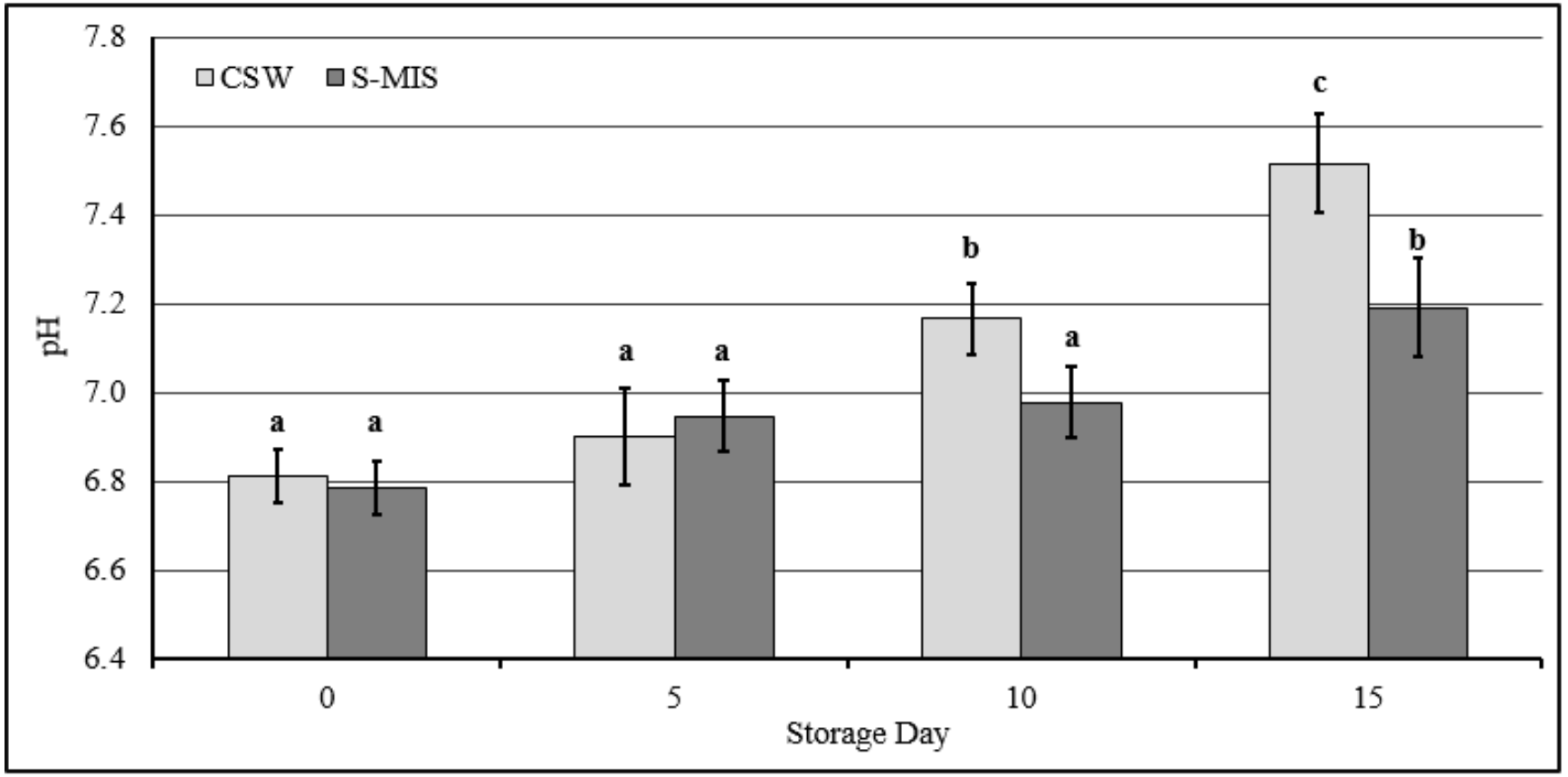

Figure 4.4 – pH values for arrowtooth flounder muscle stored at 3.5 °C for 15 days after 48 h storage in either chilled seawater (CSW) or a sub-micron ice slurry (S-MIS). Top and bottom fillet values were averaged. Mean values (n = 10) and standard deviations with different letters (a, b, c) denote significant differences ($P < 0.05$).

Table 4.2 – Linear correlation values among all parameters studied for arrowtooth flounder muscle stored at 3.5 °C for 15 days after 48 h storage in either chilled seawater (CSW) or a sub-micron ice slurry (S-MIS)

	Odor	TBARs	Micro	TVB-N	pH	% Drip loss	*K*-value	% Salt
Day	**0.90**	**0.90**	**0.97**	**0.93**	**0.72**	**0.66**	-0.15	-0.02
Odor		**0.89**	**0.96**	**0.89**	**0.72**	**0.68**	-0.02	0.03
TBARs			**0.93**	**0.89**	**0.68**	**0.75**	-0.06	-0.27
Micro				**0.93**	**0.76**	**0.69**	-0.05	-0.10
TVB-N					**0.80**	**0.55**	0.16	0.01
pH						**0.45**	-0.01	0.03
% Drip loss							-0.04	0.09
K-value								-0.18

Significant values ($P < 0.05$) are in bold

4.5.4 TVB-N

There were no significant chilling media or fillet location effects ($P > 0.05$), but TVB-N did significantly increase at each sampling day over the refrigerated storage period ($P < 0.05$, Figure 5). Initial values for TVB-N on d 0 were 9.20 ± 0.98 for CSW-treated fish and 9.66 ± 0.78 for fish stored in S-MIS, which is in accordance with values stated in the literature. Values in fresh cold-water fish are often between 10 and 15 mg N per 100 g (Perez-Villarreal and others 2008). The spoilage threshold for cold-water fish is anywhere between 25 and 35 mg N per 100 g, depending on the species of fish. For fish in the family Pleuronectidae, of which arrowtooth flounder is a member, the TVB-N threshold limit is 30 mg N per 100 g according to European Commission regulations (Commission of the European Community 1995). Fillets of both treatments reached this threshold limit between d 10 and 15 of refrigerated storage (Lakshmanan and Gopakumar 1997, Lu 2009).

Although limited for initial quality deterioration due to imprecision, TVB-N is widely used to indicate spoilage because close correlations are often observed to organoleptic score and microbiological concentration (Baixas-Nogueras and others 2001, Botta and others 1984, Malle and Poumeyrol 1989). It has also been demonstrated that the pH of fish will rise over a period of storage due to spoilage action of bacteria that produce nitrogenous metabolites, observed as TVB-N values (Gram 1992, Jorgensen and others 1988). In the current study, strong positive linear Pearson correlations were observed between TVB-N and odor decomposition score ($r = 0.89$), microbiological growth ($r = 0.93$), and pH ($r = 0.80$). Increasing values

indicate decomposition in all four analyses, as indicated in literature (Gokoglu and others 2004, Kilinc and others 2007).

Drip loss also showed a moderate positive Pearson correlation with TVB-N ($r = 0.55$). All correlations described here were significant ($P < 0.05$) and may be observed in Table 4.2. These results assist in determining direct relationships between microbial spoilage with TVB-N formation and unacceptable odor compounds, as well as the direct correlation of volatile base formation with increasing pH. Significant increases in TVB-N associated with increasing pH have been observed in several species of fish stored in either slurry or flake ice for 10 or more days, and TVB-N formation in this study is similar to those in other studies (Aubourg and others 2006, Martinsdottir and Magnusson 2001).

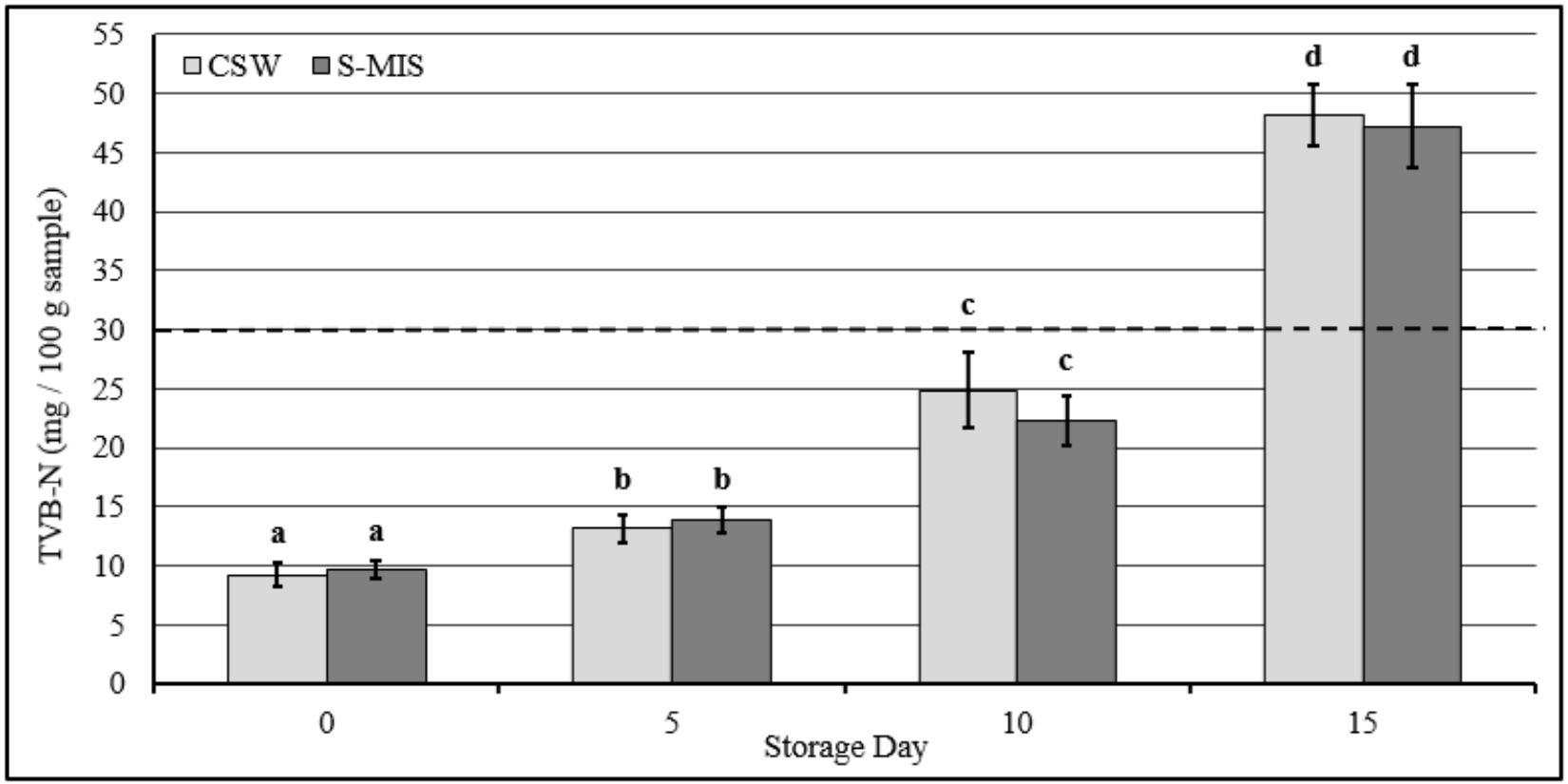

Figure 4.5 – Total volatile basic nitrogen (TVB-N) values for arrowtooth flounder muscle stored at 3.5 °C for 15 days after 48 h storage in either chilled seawater (CSW) or a sub-micron ice slurry (S-MIS). Top and bottom fillet values were averaged. Mean values (n = 10) and standard deviations with different letters (a, b, c) denote significant differences ($P < 0.05$). The dotted line indicates the point of unacceptability

4.5.5 Aerobic Plate Count

Neither chilling treatment nor fillet location impacted microbial growth ($P > 0.05$), but significant increases in bacterial counts were observed over time ($P < 0.05$, Figure 6). Fillets became unacceptable between d 10 and 15, according to the selected spoilage limit of 10^6 CFU/g determined by similar food products, as proposed by Fernandez and others (2009). Antoine and others (2002) noted that a threshold value of 30 mg N / 100 g TVB-N is often correlated with a bacterial load of 10^6 CFU/g, which was the case in the current study because both TVB-N and microbiological unacceptability limits occurred between d 10 and 15. This was also observed by a strong positively increasing Pearson correlation ($r = 0.93$) between TVB-N and microbial growth ($P < 0.05$, Table 4.2).

The arrowtooth flounder used in this experiment had less initial microbiological contamination than many other studies, but the increasing trend throughout storage periods was similar (Cox and Karahadian 1998, Lauzon and others 2009, Lu and others 2012). Aerobic plate count was used as the microbiological indicator rather than total amount of psychrotrophs because it is a more well-studied criteria and has been deemed to be an acceptable quality parameter for the determination of spoilage during refrigerated shelf life studies (Commission of the European Community 2005, Huss 1994, Sciortino and Ravikumar 1999). It has also been determined that aerobic plate count is a more useful indicator than psychrotrophic counts for comparison between products, storage length, and evidence of potential temperature abuse (ICMSF 1974).

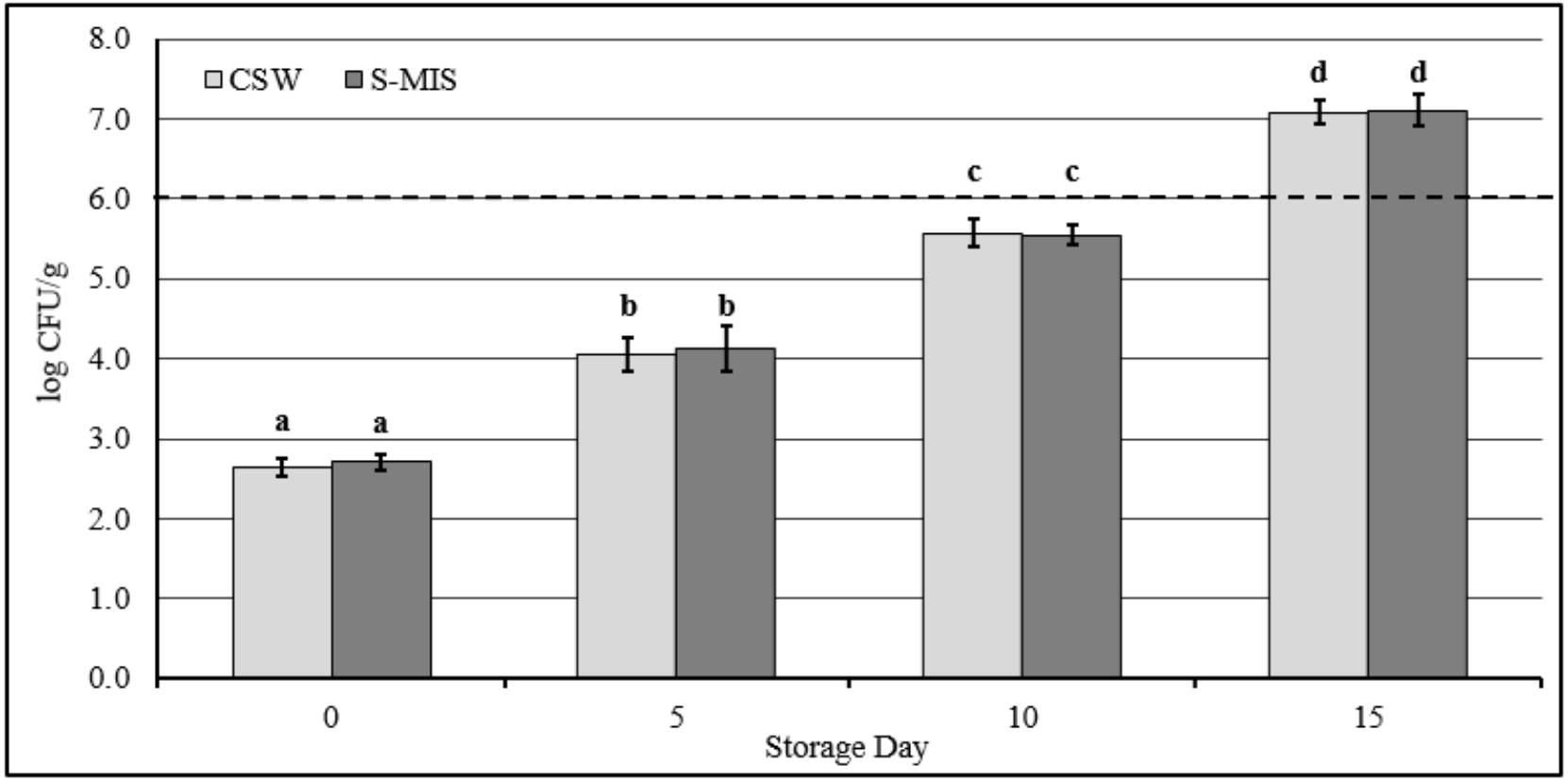

Figure 4.6 – Aerobic plate counts in log colony forming units per gram (log CFU/g) of arrowtooth flounder muscle stored at 3.5 °C for 15 days after 48 h storage in either chilled seawater (CSW) or a sub-micron ice slurry (S-MIS). Top and bottom fillet values were averaged. Mean values (n = 10) and standard deviations with different letters (a, b, c) denote significant differences ($P < 0.05$). The dotted line indicates the selected point of microbiological unacceptability.

4.5.6 Odor Analysis

Similarly to microbiological and TVB-N data, there were no significant differences between chilling media treatments or fillet location for odor decomposition values ($P > 0.05$, Figure 4.9). Panelists conferred a moderate degree of freshness by scoring 31.98 for CSW fillets and 30.16 for S-MIS fillets on storage day 0, and scores significantly increased at each sampling day ($P < 0.05$). Both S-MIS and CSW fillets reaching odor unacceptability at approximately day 8 of refrigerated storage. Panelists conferred spoilage before the selected limit for total microbes or the threshold for TVB-N concentration, but the spoilage day is more consistent between tests if 10^5 log CFU/g and 25 mg N per 100 g are used. While the limits 10^6 CFU/g and 30 mg N per 100 g were selected in this study, the absence of relevant data on arrowtooth flounder allows an opportunity to adjust these values for the species. It may be determined that the limits of 10^5 log CFU/g and 25 mg N per 100 g are better suited for arrowtooth flounder upon further investigation, as these values are used for many other species of fish (Connell 1995, Popovic and others 2010, Sen 2005).

Panelists were also asked to identify odors to justify scores. Fillets from fresh fish have initial seaweed and briny odors that degrade into off-odors and flavors, which leads to rejection of fillets by professionals and consumers (Gram and Huss 1996). Positive odor attributes noted on storage days 0 and 5 were fresh seaweed and melon, while negative odor attributes noted were stale and wet cardboard. Fillets stored for 10 days were associated with unacceptable sour and sulfur odors that were likely a direct result of microbial activity (Jorgensen and others 2000). Panelists identified the predominant odors as ammonia and fecal for fillets stored for 15 days,

which was likely a direct result of bacterial metabolic products from degradation of nitrogenous compounds. This was observed by strong increasing Pearson correlations between odor decomposition scores and microbiological growth ($r = 0.96$, $P < 0.05$), as well as between odor decomposition scores and TVB-N formation ($r = 0.89$, $P < 0.05$). Thus, microbiological growth increased the amount of volatile nitrogenous compounds through metabolic functions, as observed through TVB-N formation, which the panelists were able to identify.

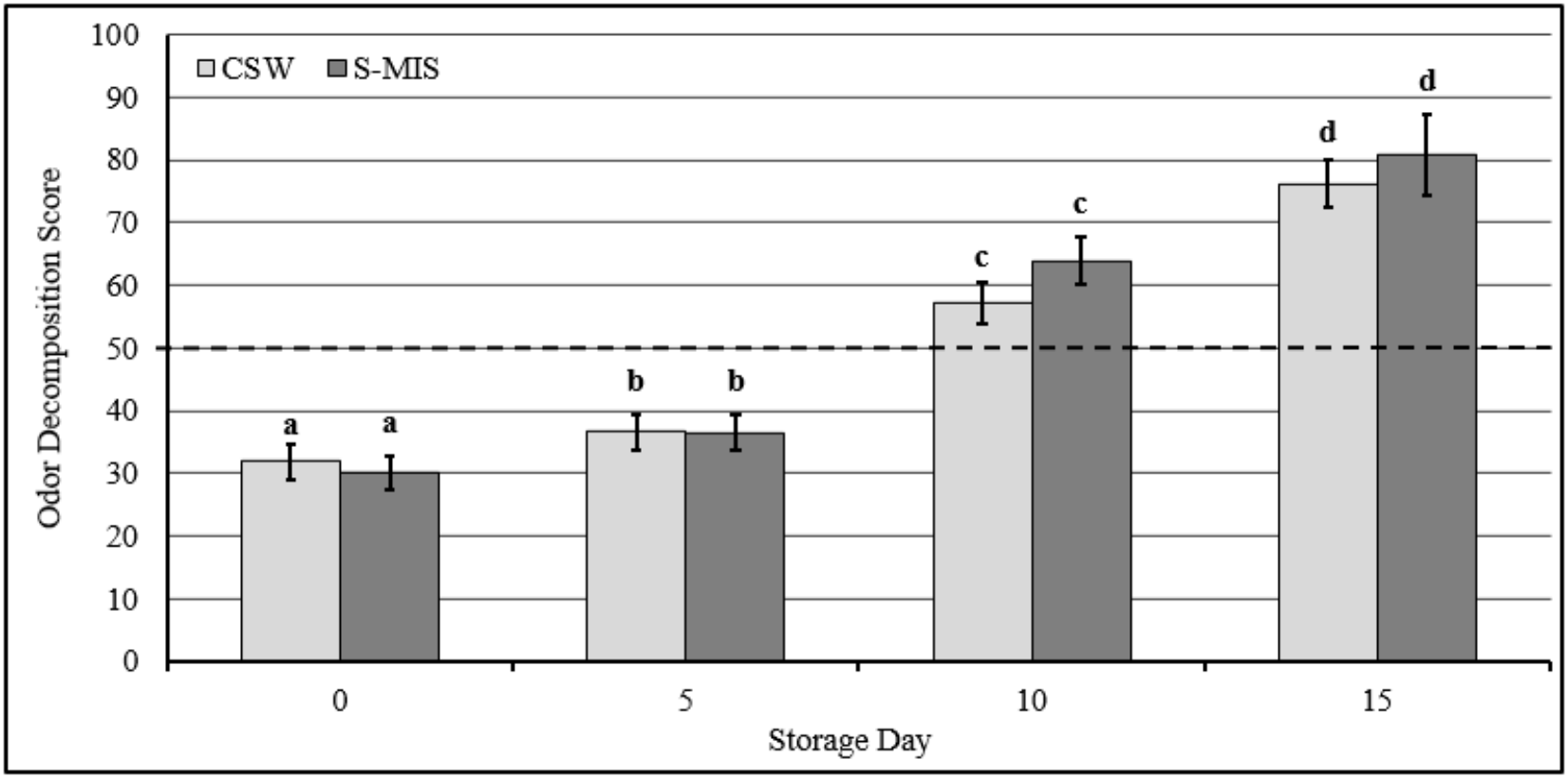

Figure 4.7 – Evolution of odor decomposition values of arrowtooth flounder muscle stored at 3.5 °C for 15 days after 48 h storage in either chilled seawater (CSW) or a sub-micron ice slurry (S-MIS). Top and bottom fillet values were averaged. Mean values (n = 10) and standard deviations with different letters (a, b, c) denote significant differences (P < 0.05). The dotted line indicates the point of unacceptability, as determined by n = 9 judges.

4.5.7 Lipid Oxidation

Fish stored in CSW for 48 hours had significantly less lipid oxidation than fish stored in S-MIS under similar conditions ($P < 0.05$, Figure 7). The trend continued for the top fillet of the fish stored in CSW at each sampling, but the bottom fillet of CSW-treated exhibited a higher rate of lipid oxidation (Figure 8). This was reflected in significantly higher TBARs values that corresponded closer to fillets of S-MIS fish in bottom fillets on d 5, 10, and 15 ($P < 0.05$, Figure 7). Storage in S-MIS caused an initially higher rate of lipid oxidation for both top and bottom fillets over fish treated in CSW, but the rate of formation was slower during refrigerated storage ($P < 0.05$, Figure 8). There were no significant differences in TBARs values or in the rate of secondary lipid oxidation between fillets of fish stored in S-MIS on similar storage days ($P > 0.05$).

Lipid oxidation remained low for all fillets with the lowest value at 0.147 mg MDA/kg muscle and the highest at 0.354 mg MDA/kg muscle, which is well below the reported threshold value of 0.85 (Dergal and others 2013). Yet the determination of lipid oxidation in low-fat seafood is justified because human odor detection threshold of oxidized fat may be as low as in the parts per billion range, low-fat fish may have higher than expected secondary lipid oxidation values, and it has been found that TBARs scores often correlate well with sensory data (Rustad 2010). Table 4.2 shows that in the current study, TBARs scores showed strong positive linear Pearson correlation with odor decomposition scores ($r = 0.89$, $P < 0.05$). Even though the values correlated strongly, TBARs scores may not be used as indicators of decomposition because values remained well below the threshold value.

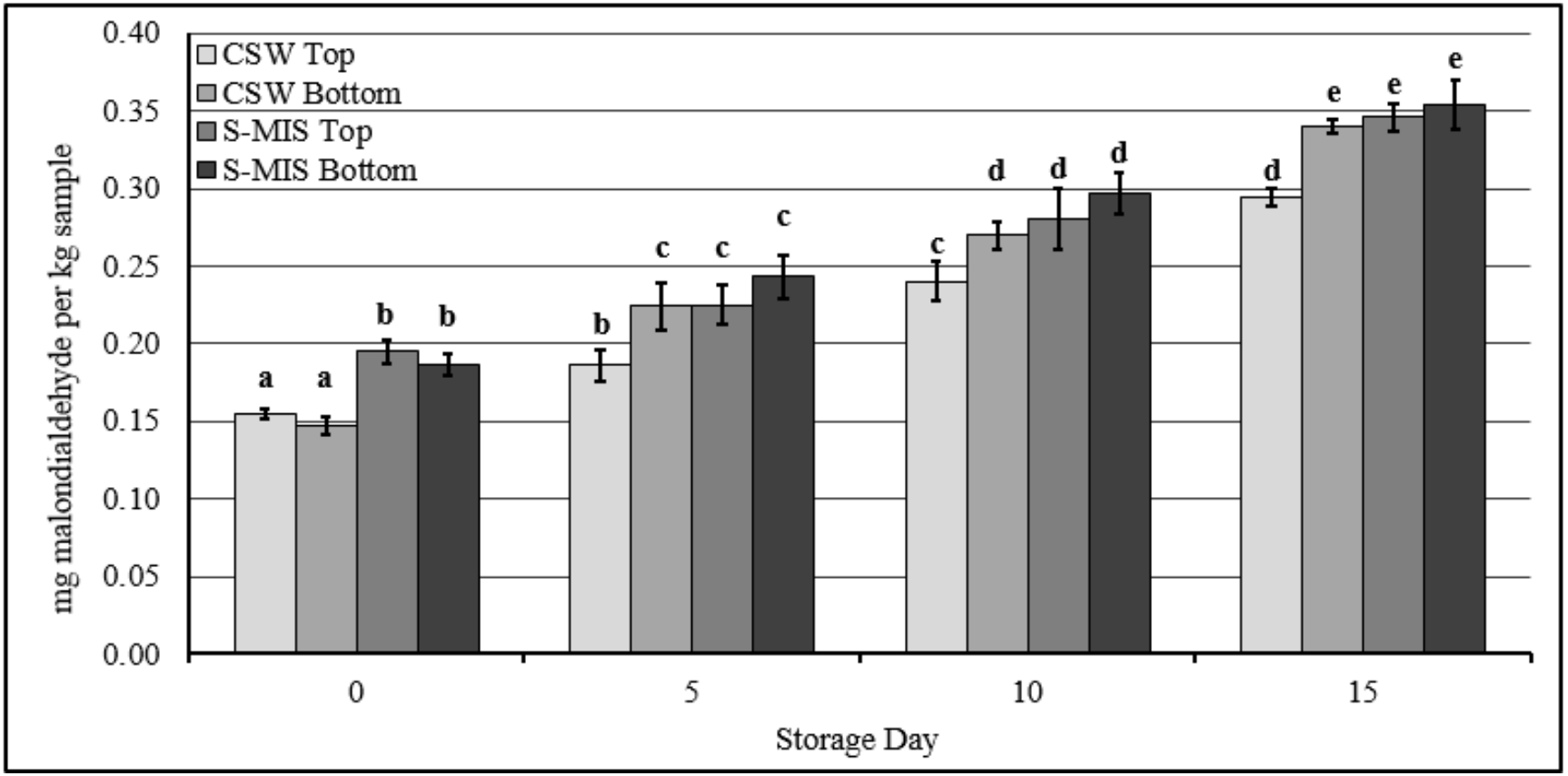

Figure 4.8 – Secondary lipid oxidation values by the TBARs assay (mg malondiadehyde/kg sample) for arrowtooth flounder muscle stored at 3.5 °C for 15 days after 48 h storage in either chilled seawater (CSW) or a sub-micron ice slurry (S-MIS). Mean values (n = 5) and standard deviations followed by different letters (a, b, c) denote significant differences ($P < 0.05$).

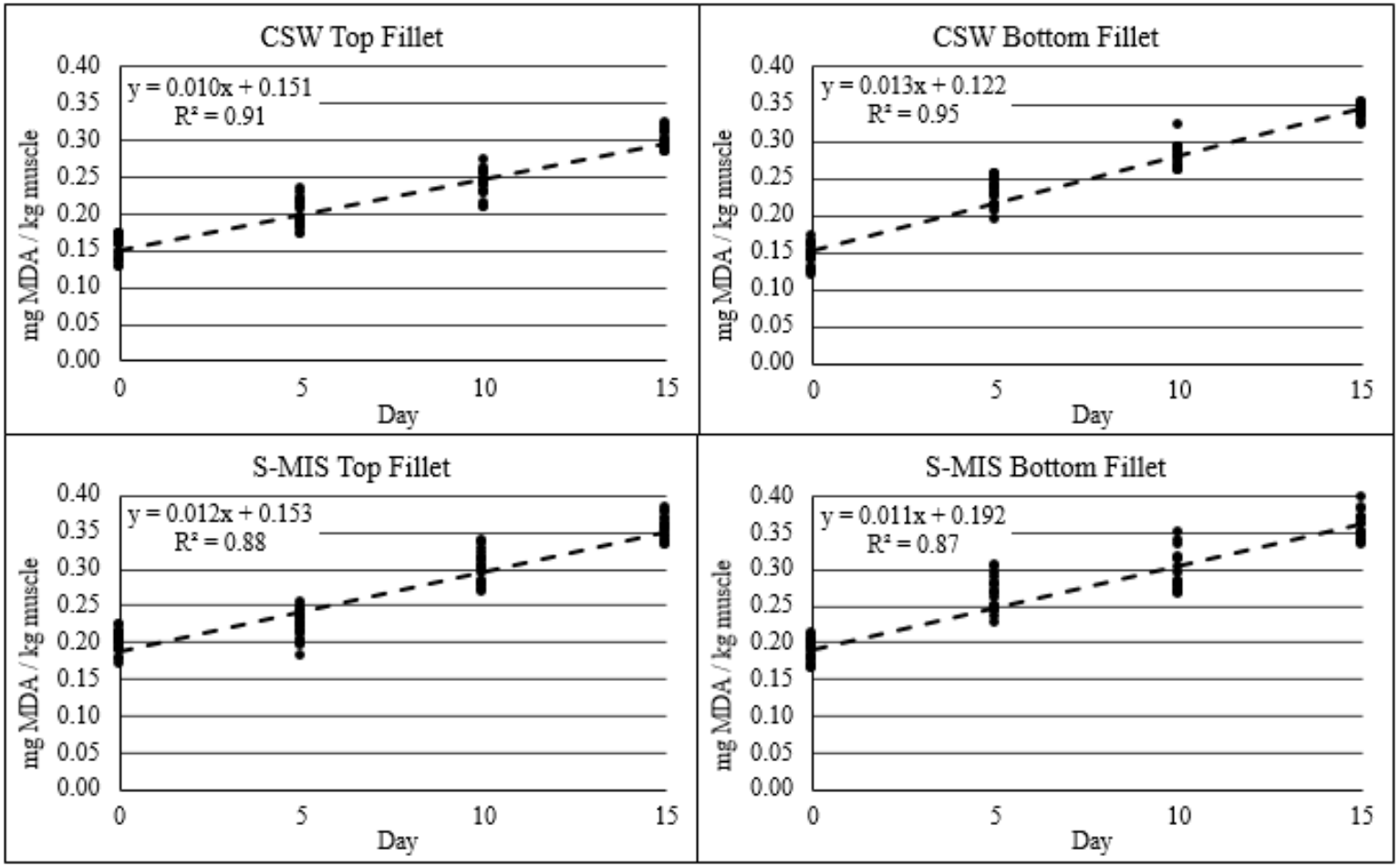

Figure 4.9 –Rate of secondary lipid oxidation by linear regression of TBARs values (mg malondialdehyde / kg muscle) for arrowtooth flounder muscle stored at 3.5 °C for 15 days after 48 h storage in either chilled seawater (CSW) or a sub-micron ice slurry (S-MIS).

4.5.8 K-value

There were no significant differences in *K*-value of arrowtooth flounder between chilling media treatment, fillet location, or storage period ($P > 0.05$, Figure A.1). *K*-values were consistently above 85% after storage in chilling media and throughout the refrigerated storage period. Arrowtooth flounder and petrale sole ($n = 5$ each) from the same catch and Pacific whiting from another catch were obtained during offloading to validate the method because a *K*-value of 85% two days after catch is very rare and suggests temperature abuse. Yet the Pacific whiting had a *K*-value of 25% and that of petrale sole was 40%, while the arrowtooth flounder caught in the same net demonstrated a *K*-value of above 85%. Therefore, the method was demonstrated to be sufficient and arrowtooth flounder did indeed experience accelerated ATP degradation.

Results indicate that autolytic enzymatic degradation of ATP occurred more rapidly than in other species of fish, as it has been observed that it often takes at least 10 days to attain a *K*-value of above 80% at refrigeration temperatures (Carrera and others 2008, Ozogul and others 2006). Comparisons to the literature suggest that the flounder may have been stressed during harvest, temperature abused on the boat, were improperly handled, and/or naturally contain high concentrations of autolytic enzymes (Luong and others 1991, Sallam 2007). For example, Greene and others (1990) reported that *K*-values of flatfish are highly variable with ranges between 10% and 80% within one day of catch, depending on species. While arrowtooth flounder were not utilized in the Greene study, the data demonstrates that advanced autolytic degradation may occur in fresh flatfish.

4.6 Conclusions

Autolytic degradation occurred quickly in arrowtooth flounder and subsequent studies will need to be conducted to determine the cause. Thus, the *K*-value could not be used as a spoilage indicator in conjunction with the other analyses, except to show that a high *K*-value does not necessarily confer spoilage. The fillets were fresh after being treated in either S-MIS or CSW, as determined by odor decomposition and microbiological analyses, and remained fresh until a refrigerated storage time of 8 days for odor decomposition analysis. However, microbiological analysis and TVB-N conferred a longer time before spoilage than odor decomposition for both S-MIS- and CSW-treated fish. TBARs values remained well below threshold values, so TBARs values do not directly relate to product unacceptability in arrowtooth flounder.

It was determined based on odor decomposition and microbiological data with support from other analyses that S-MIS did not significantly improve the shelf life of arrowtooth flounder when stored for 48 hours over fish stored in CSW after storage in conventional chilling media. It did, however, reduce ultimate salt uptake during storage, which is an important health consideration for consumers. The data from this research suggests that, for arrowtooth flounder, further investigation is warranted on the impact vessel handling practices have on the biochemical and sensory characteristics at time of catch and handling.

CHAPTER 5

GENERAL CONCLUSIONS

Several different handling techniques of sub-micron slurry ice (S-MIS) were investigated and compared to chilled seawater (CSW) to determine which handling technique or ice chilled the product most efficiently. Continuously drained, initially drained, and non-drained S-MIS significantly improved product chilling and chilled product temperature over CSW. Phase one of the project also demonstrated that Pacific whiting could be initially chilled in as low as a 1:1 ice-to-fish ratio of continuously drained S-MIS without affecting the rate of heat transfer, as well as to similar ultimate temperatures. As expected, however, high ice-to-fish ratios increased the time of successful temperature management below 4 °C.

Processors hold Pacific whiting in totes between off-loading and processing up to 48 hours during the busy season in the summer, and phase one has demonstrated that processors could hold fish at a 1:1 ratio for 24 hours and at a 2:1 ratio for 48 hours. Therefore, it is recommended that S-MIS should be drained to a single-phase system. Since ice is only 30-40% of the biphasic system while water is 60-70%, processors may not want to waste energy producing and draining water. However, the study also demonstrated that leaving the biphasic system intact at 70% water and 30% ice chilled faster than chilling media currently in use (CSW).

Phase two demonstrated that holding arrowtooth flounder in S-MIS for 48 hours cannot improve quality if a different type of chilling media were used on the

boat. Therefore, it may not be effective to use S-MIS between off-loading and processing if S-MIS was not used for the initial fish chill. Another finding from phase two is that *K*-values of arrowtooth flounder were initially 90% off the boat, although this did not correlate to a loss of freshness as determined by the other quality indices. Other flatfish (petrale sole) caught in the same haul had a *K*-value near 30%, so it is hypothesized that this advanced *K*-value is species specific rather than due to handling issues (Appendix I). Further study is warranted to understand the mechanism of this advanced nucleotide degradation and how this degradation relates to other quality indices.

CHAPTER 6

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APPENDIX I

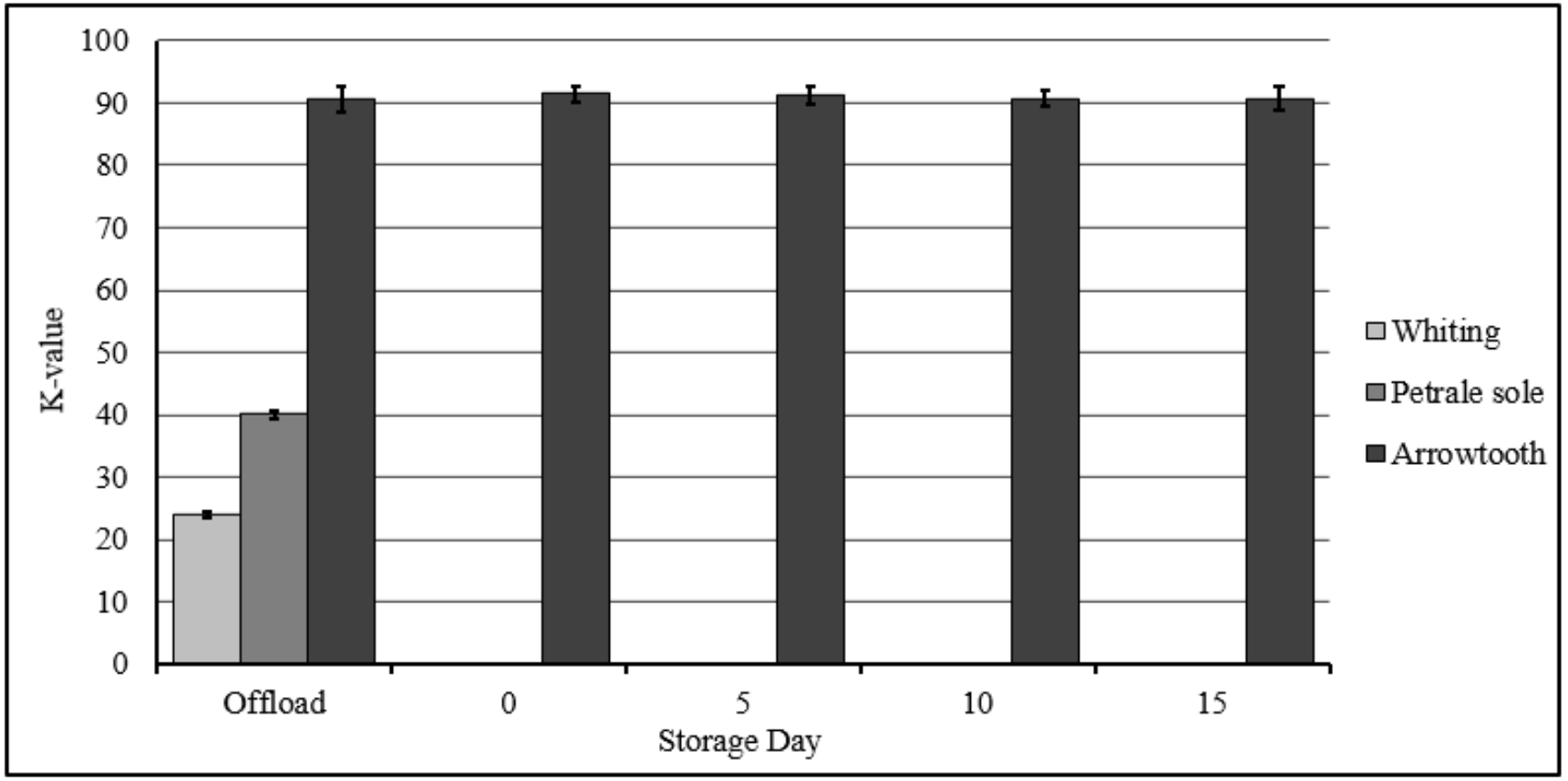

Figure A.1 – *K*-values of arrowtooth flounder muscle stored at 3.5 °C for 15 days after 48 h storage in chilling media. Offloaded petrale sole and Pacific whiting were investigated as method validation.