QUALITY CHANGES IN CHUM SALMON (*Oncorhynchus keta*) IKURA AFFECTED BY SALT, THERMAL PASTEURIZATION, AND STORAGE TIME

By

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis of Chen Liu find it satisfactory and recommend that it be accepted.

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QUALITY CHANGES IN CHUM SALMON (*Oncorhynchus keta*) IKURA AFFECTED BY SALT, THERMAL PASTEURIZATION, AND STORAGE TIME

Abstract

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Salmon caviar or ikura is a popular food produced from eggs of Pacific salmon principally chum salmon (*Oncorhynchus keta*) and pink salmon (*O. gorbuscha*). Salmon caviar should have 3.5-5% water phase salt (WPS) to inhibit the growth of *Listeria monocytogenes*, and for products that are pasteurized, the recommended pasteurization protocols (6 log reduction of *L. monocytogenes*) are equivalent to a heat treatment of 2 minutes at 70ºC. However, thermal processing may lead to undesirable texture changes, so a kinetic study of textural change was conducted to determine at what point changes occur so that improved pasteurization protocols could be developed.

Besides thermal processing, brining also influences the eggs’ quality and factors affecting protein properties. Protein denaturation temperature (PDT) is affected by pH, and water activity in ikura at different WPS. In this study, PDT at: 0, 3.23%, 3.76%, 3.91%, 4.10%, 4.23%, 5.24% and 7.17% WPS were determined by differential scanning calorimetry. The PDT correlated with textural changes in unsalted roes heated at 70ºC for different times (1, 6, 11, 15, 19, 23, 27 and 31 min). The PDT increased significantly (92 to 96.5ºC) (*P*<0.05) with an increase in WPS from
0 to 7.17% which followed a polynomial trend ($R^2=0.99$), but pH did not appear to be affected. Compression force of the unsalted roes decreased linearly ($R^2=0.89$) with increasing heating time.

In addition to measuring textural change, oxygen permeability of packaging was examined as part of this study. Two films with different oxygen transmission rates (OTR, 40 and 62 cc/(m²·day) ) were used as packaging materials for ikura of 5.24% WPS, a common WPS for commercial ikura, over a 60-day-long shelf-life study. During storage, ikura exhibited significant changes ($P<0.05$) in pH, water activity and firmness of texture; however no significant differences in pH and water activity were observed between ikura packaged with the two film types ($P>0.05$) until the second month. The CIE L*a*b* color parameters of ikura in different films did not significantly change during storage ($P>0.05$). The TBARs (thiobarbituric acid reactive substance content as malondialdehyde) in both types of ikura packaging in the different films slightly increased ($P>0.05$) until the last 10 days of storage, at which point the oxidation of the ikura in the higher OTR was substantially higher.

These results indicate that with an appropriate increase in WPS, it could be possible to pasteurize ikura at a higher temperature for a shorter time, possibly reducing the undesirable softening that occurs to salmon caviar following pasteurization. Also, packaging ikura in materials with lower oxygen transmission rate could reduce the onset and rate of lipid oxidation during storage.
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CHAPTER 1. INTRODUCTION

1.1 Justification and Objectives

Caviar products are very popular foods with a growing market around the world. They are brined before they go to market, including in forms of whole egg skeins but more commonly as singled out eggs from the egg skeins. Most of the caviar products are sold ready-to-eat but some of them are sold after pasteurization. Compared to other food products, caviar has a relatively short shelf life due to its high lipid content. Most caviar products are sold to restaurants and retailers in glass jars or metal tins. Glass jars are common packaging because it allows consumers to see the product and because the container is impervious to oxygen. Glass is costly, and flexible packaging alternatives are being sought particularly for single service and food service applications.

Refrigerated storage is currently the only available means to preserve and extend the shelf life of caviar products. However, refrigeration is not adequate to assure a pathogen-free product with a long shelf life. Pasteurization is commonly used in the food industry as a method of reducing the risk of foodborne illness. Caviar products are difficult to pasteurize because of their sensitivity to heat treatment. High temperatures will irreversibly denature proteins in caviar and completely change the characteristics of food and drastically reduce its quality. The pasteurization temperature is regularly recommended not to exceed 70 °C. Water bath treatment is commonly used in the food industry for heating caviar. Also, Fatalian and others (2006) noted that the shelf life of sturgeon caviar increased following pasteurization at different temperature levels of storage (Table 1-1). There is clearly a need to develop improved pasteurization processes for caviar products if they are to be stored for
any period of time. The preference of consumers for caviars with lower salt contents (malasol) increases the importance of pasteurization since salt content in caviar is not sufficiently high to inhibit the growth of *Listeria monocytogenes*.

Salmon caviar, also known as ikura, was used for this research. The first objective of this study was to determine the thermal denaturation properties of the proteins in ikura of different formulations, in this case salt concentrations, and determine a range of treatment temperatures that would be suitable for caviar pasteurization. A second objective was to find an alternative to packaging with glass and to examine film packaging for caviar that has different oxygen transmission rates and monitor the quality by conducting a shelf-life study. To determine whether it is possible to develop a suitable thermal pasteurization process for ikura, two specific experimental objectives were included:

1) To determine the optimum pasteurization temperature by evaluating the effect of salt on the protein denaturation

2) To determine the optimum pasteurization time through studies of kinetics of ikura texture changes

### 1.2 Organization of Thesis

CHAPTER 2 is the literature review of this study.

CHAPTER 3 describes the work on finding a suitable thermal pasteurization process for ikura.

CHAPTER 4 describes the work on the shelf-life study of ikura.

CHAPTER 5 includes conclusions and future recommendations.
1.3 References


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Table 1-1. The shelf-life for both un-pasteurized and pasteurized sturgeon caviar (Fatalian and others 2006)

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<tr>
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<th>Un-Pasteurized</th>
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<td>10 to 15</td>
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<td>0.15-0.2</td>
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<td>0 to 2</td>
<td>6-8</td>
<td>3-4</td>
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<tr>
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CHAPTER 2. LITERATURE REVIEW

2.1 Salmon Caviar

Caviars are fish roe products; traditional foods with high value. These are very popular foods and have a growing domestic and international market. Caviars are salt-cured preserved eggs of a fish or invertebrate, and are commonly made from individual eggs that have been singled out by screening or otherwise separated from the supporting connective tissue. The most widely recognized caviar is sturgeon caviar. In the U.S., only sturgeon caviar can be labeled as “caviar”. Caviar from other fish or aquatic animal species must be identified with a qualifying term including the common name of the fish used. For example, salmon caviar or ikura must be labeled as “salmon caviar”.

“Red” or salmon caviars, or “ikura” in Japan are also very popular in the world market. Comparing to sturgeon roe, salmon roe has a much larger size (4-7 mm in diameter). Chum salmon (Oncorhynchus keta) and pink salmon (O. gorbuscha) are the major sources of salmon caviar production. Ikura production of chum salmon can reach as high as 3000 MT per year. Other Pacific salmons including coho (O. kisutch), sockeye or red (O. nerka), and king or Chinook (O. tshawytscha) are also the sources of salmon caviar. Beside Pacific salmons, Atlantic salmon (Salmo salar), Arctic char (Salvelinus alpinus) and Pacific masu or cherry salmon (O. masou) restricted to small regions of Japan and Korea (Sternin and Dore 1993) are also used for caviar production. However, the value for salmon flesh is low compared to the caviar since female fish have mobilized energy stores, both fat and protein, to produce the eggs. Laws prohibit “roe stripping”, a process of harvesting the roe and not the rest of the carcass.
Salmon eggs are a major source of income for Alaskan harvesters and processors. Most of the salmon roes harvested in Alaska are exported to Japan, Korea, and some countries in Europe. China is a growing market. Some salmon roe is processed into ikura in local facilities, but much of the fresh roe is packed in bulk, frozen and exported with a relatively small, but rapidly increasing amount consumed domestically in the United States.

Farmed salmon is most commonly harvested before reaching sexual maturity, but due to the periodic gluts of salmon on the world market, some Atlantic salmon culturists in Norway (Bledsoe and others 2003), Chile and the USA are holding fish to sexual maturity and harvesting roe commercially.

The popularity of sujiko, once the predominant salmon roe product in Japan, is decreasing. Sujiko is highly salted roe skein. Different from sujiko which was prepared from whole roe skeins, ikura was salted in individual eggs style. However the popularity of ikura and “green” or untreated salmon roe is increasing. In Europe and North America, ikura is commonly consumed in Japanese dishes or with cream or white sauces in pasta dishes, as a garnish on seafood salads, fish or poultry entrees, and served as a condiment with egg dishes.

2.2 The Chemical Components of Salmon Caviar

There are many factors that affect the chemical composition of caviar. Those factors include the species, the condition and maturity of the eggs, the harvest areas and even different harvest period. Caviar composition also differs slightly due to the variation among eggs
within a single skein with the eggs in anterior end of the skein being more mature than the ones close to the tail of the fish.

Like poultry eggs, fish roe have high concentrations of lipid and protein. In general fish roe products are high in protein (16 to 30%). Crude lipid content can vary from 5 to 20% with an average value for salmon roe of around 10%. For chum salmon (O. keta), the untreated roe consists of 50 to 60% of moisture, 27 to 35% of protein, 12 to 20% crude lipid and 1.5 to 1.7% total ash. After the roe is brined, the percentage of moisture decreases, the protein content and lipid content increase because water is removed during the brining and curing process, the ash content increases because of the added salt during the brining process. The distribution of macronutrients within individual eggs is not uniform. A waxy low-density lipid fraction and oil droplets constitutes the remaining volume. The volume of the oil droplet in chum salmon comprises up to 10% of the volume of the individual egg (Huang and others 2001). The lipids in mature roe could make up about 13.5% of the wet weight of the egg (Ishii and others 1988). The protein quality of fish roe is high for either methionine/cysteine or tryptophan/tyrosine being the limiting amino acid (Eun and others 1994; Iwasaki and Harada 1985; Kaitaranta 1980; Lu and others 1979).

The caloric content of caviar ranges from 320 to 370 calories/100 g and can vary with the quality and fat content (Bledsoe and others 2003). Salmon caviar products are highly digestible and have been used in Russia to help patients recovering from surgery and treat rickets in children. Fish roe are also a rich source of vitamins. Salmon eggs contain 50 to 3000 IU/g vitamin A, 5 to 25 IU/g vitamin D, 10 to 80 IU/100g of the vitamins B1, B2, and
B12 and 10 to 30 μg/100 g vitamin C (Eun and others 1994). The mineral nutrients in caviar include calcium, iron, magnesium, manganese, phosphorus, potassium, copper, and zinc (Iwasaki and Harada 1985).

2.3 Bacterial Contamination Risk: *Listeria monocytogenes* in Caviar

Literiosis, caused by *Listeria monocytogenes*, leads to about 28% of the deaths resulting from foodborne illness (Buchanan and Lindqvist 2000). The Centers for Disease Control and Prevention (CDC) have estimated that up to 2,500 cases of listeriosis, resulting in 500 deaths (Mead and others 1999) each year in the United States. The U.S. Food and Drug Administration (FDA) and the U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) established a policy under which ready-to-eat foods contaminated with *L. monocytogenes* at a detectable level are deemed adulterated. According to the “zero tolerance” policy, which assumes that all *L. monocytogenes* strains are pathogenic, the presence of the organism at a detectable level (0.04 CFU/g) in a ready-to-eat (RTE) food renders that product adulterated (Smoot and Pierson 1997). Foodborne listeriosis is rare; however its associated mortality rate is high. Elliot and Kvenberg (2000) estimated that the number of listeriosis cases in the U.S. between 1986 and 1993 ranged from 1092 to 1962 cases every year among which 248 to 481 were death cases.

*L. monocytogenes* is ubiquitous. High risk populations which include infants, pregnant women and immunocompromised people are at serious threat of listeriosis (McAuliffe and others 1999). *L. monocytogenes* is a recalcitrant organism with the ability to survive at refrigeration temperatures, high salt concentrations, and acidic environments. *L.*
*L. monocytogenes* is able to grow in a wide temperature range from 2 to 45°C and can survive at refrigeration temperature (2 to 4°C). There are several mechanisms in the bacterial cell which are responsible for the unique ability of *L. monocytogenes* to survive in adverse conditions (Gandhi and Chikindas 2007). When exposed to colder temperatures, *L. monocytogenes* can change the lipid membrane composition to help maintain the membrane fluidity. Membrane fluidity is essential for solute transfer across membrane and normal enzymatic action in the cell. Other mechanisms include the production of cold shock proteins, cold acclimatization proteins and proteases which are capable of degrading unusual polypeptides produced as a result of cold shock. *L. monocytogenes* also produces glycine, betaine and carnitine which act as cryoprotectants. *L. monocytogenes* has the ability to survive in acidic conditions which it encounters in foods and host GI tract. Induction of proteins such as ATP synthetase and GroEL, use of glutamate decarboxylase system, and pH homeostasis are responsible for acid adaptation. Mechanisms which are responsible for acid adaptation also provide cross resistance against heat shock as well as osmotic and alcohol stress. This phenomenon is particularly alarming to the food industry as many foods normally undergo acidic treatments in processing.

*L. monocytogenes* can be present anywhere in food processing facilities. Food products can be contaminated with *L. monocytogenes* in several different ways. The bacterium might be present on animal carcasses, on food processing surfaces, in raw materials used for food processing, due to cross contamination and contamination by human contact. For fish, *Listeria* spp are commonly present in the estuarine environment. Even if adequate sanitary measures were taken at the processing plant, cross contamination is still a hazard.
Occurrence of *L. monocytogenes* in raw meats and RTE seafood products can reach high as 96% (Vignolo and others 1996) and 78% (Guilbaud and others 2008), respectively. Intrinsic factors of these foods such as pH, water activity and nutritional content are suitable for growth of *L. monocytogenes*. *L. monocytogenes* is difficult to control since it can be reintroduced into the processing environment and poses a risk for post processing contamination (Farber and Peterkin 1991). Since *L. monocytogenes* is a psychrotrophic pathogen, refrigerated storage alone is inadequate for controlling the growth of *L. monocytogenes*. This poses a threat to the meat and seafood industries.

High value aquatic food products have been target of increased regulatory scrutiny (Bledsoe and others 2003). The FDA (2011) has emphasized improving process control and a precise determination of the destruction of the target microbe, generally a 6-D process for *L. monocytogenes* in aquatic food products. However, this is not possible for many cured products such as cold smoked salmon and most roes since there is no pasteurization process, making cleanliness and control of growth by proper refrigeration the only preventative measures available to reduce listeriosis risk. Gaze and others (1989) claimed that to achieve a six log reduction of *L. monocytogenes*, the slowest heating point in a food product should be held at 70 °C for 2 min (D values at 70 °C for *L. monocytogenes* ranged from 0.14 to 0.27 min).

### 2.4 Water Phase Salt
Water phase salt (WPS) is a term which means the amount of salt compared to the amount of moisture (water) in the fresh fish (Hilderbrand 1992). The salt content and storage temperature of caviar are key preventive controls for producing a safe caviar product. In general, salt content alone may not be high enough to inhibit the germination of *C. botulinum* spores. *C. botulinum* type E can grow at 4.5 to 6% salt and at temperatures of 3.3 °C. Water phase salt concentrations of 10% will inhibit *C. botulinum* germination for low acid foods held at room temperature. According to the hazard guidance of the FDA (Food and Administration 2011), a minimum water phase salt level of 3.5% with refrigerated storage is needed for fisheries products including caviar, to inhibit the growth and toxin formation by *C. botulinum* type E and non-proteolytic type B and F. However, studies indicated that water phase salt levels of 2.5 to 5.0% have no inhibitory effect on *Listeria monocytogenes* (Peterson and others 1993; Shin and Rasco 2007) in products of this type.

### 2.5 Processing Salmon Roes into Caviar

Roe products are to be made from wholesome, undamaged eggs. They should have a proper color and glossiness, texture, a desirable mouth feel, and their characteristic flavor such as limited fishy, bitter, or oxidized flavor. Different species lead to different preferred mouth feel. For ikura or salmon roe, a distinct fracture or “pop” when the egg is broken with the teeth or palate, a smooth, honey-like mouth feel is desired (Bledsoe and others 2003).

Often hundreds or thousands of individual eggs are enveloped within ovarian membranes. These skeins of eggs can be processed into roe products, or the individual eggs can be recovered separately and then processed. Fish roes are generally processed into three products: whole ovaries (such as sujiko), individual eggs (caviar), and pate or pastes or other
products such as dried roes, although this is not a common preparation for salmon. Technically, caviar should only be used to describe fish eggs that are separated from the connective tissue of the ovaries and then salted and cured.

Roe, regardless of the type, must be an optimal level of maturity to produce caviar. Otherwise, it will taste bitter or not take up salt uniformly. Over mature roe may be soft, lose its firmness and elasticity and will not form a plump, full egg after brining. Over mature salmon roes will develop tough, rubbery outer shells. Also, the mouth feel for caviar prepared from over mature roe is not desirable because the flavor and consistency of the lipid and protein change with maturity.

The specific process for making ikura is separation of eggs from the skein material. This process is also called screening. Screening is normally a manual, laborious, and a time-consuming process. Also enzyme-based processes could also be used for removing the connective tissue that surrounds the eggs, which decrease the labor. Protease-based processes have been widely touted for ikura production, however these processes must be carefully controlled. Otherwise, the eggs will begin to become soft and dissolve.

Fish eggs can be mechanically or enzymatically separated from the egg skein then salted, or whole skeins of fish eggs can be salted. For ikura, the separated eggs are agitated in brine (for saturated brine the egg/brine ratio is usually 1:3 or less) (Bledsoe and others 2003). The eggs are brined, generally 2 to 6 min between temperatures of 8 to 12°C or less, until the desired salt content of the final ikura is reached. The shell of the egg becomes fairly firm
and an appropriate salt content (3.0 to 3.8% salinity) is reached. The best process control for ikura manufacture is achieved using saturated brine. After the eggs are brined, they are permitted to cure for 8h or more at approximately 10°C on inclined perforated plastic baskets or trays. This allows excess surface and cellular fluid to be released from the eggs and drained off. Also, the curing permits the salt concentration within the egg to equilibrate and the eggs sheath to harden slightly.

Salmon caviar usually contains 3.0 to 4% sodium chloride, although some claim that higher salt contents in the range of 4 to 6% are required for high-grade ikura (Craig and Powrie 1988). Salt uptake of the egg varies between species of salmon and is also dependent on the condition and degree of maturity of the eggs. Roe quality also affects salt uptake rate. Salt uptake for average quality roe will be significantly faster during the first minute of brining (saturated brine, approximately 20°C). After this point, both the average and high-quality roe exhibit similar salt uptake rates. For the higher-quality roe, the salt concentration in the roe after 20 min brining is approximately 6.0%, and much higher for the average quality roe, over 9.0% (Figure 2-1) (Huang and others 2001). Salt uptake rate is also faster in frozen roe and in enzyme treated roe, compared to fresh roe, assuming that they are of the same quality.

2.6 Protein Denaturation in Caviar

Denaturation of proteins involves the disruption and possible destruction of both the secondary and tertiary structures. Since denaturation reactions are not strong enough to break the peptide bonds, the primary structure remains the same after a denaturation process. Denaturation can be brought about in various ways. Proteins are denatured by treatment
with alkaline or acid, oxidizing or reducing agents, and certain organic solvents. Heat can be used to disrupt hydrogen bonds and non-polar hydrophobic interactions.

Salted fish roe products are often ready-to-eat. However, roe are heat labile and can only be heated to a temperature of 70°C without the eggs becoming dull or loosing color (Sternin and Dore 1993; Bledsoe and others 2003; Al-Holy and others 2004). Irreversible protein denaturation occurs between 70 to 80°C. Protein denaturation will bring negative effects including losses in texture and nutritive value.

2.7 Differential Scanning Calorimetry

Differential scanning calorimetry or DSC is a thermoanalytical technique in which phase transition can be determined. The difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature. Both the sample and reference are kept at nearly the same temperature throughout the experiment. Generally, the temperature program for a DSC analysis is designed such that the sample holder temperature increases linearly as a function of time. The reference sample should have a well-defined heat capacity over the range of temperatures to be scanned.

The basic principle of this technique is that DSC can measure the amount of heat absorbed or released during phase transitions by observing the difference in heat flow between the test sample and a reference with well-characterized thermal properties. DSC may also be used to observe more subtle physical changes, such as glass transitions. It is widely used in industrial settings as a quality control instrument due to its applicability in evaluating
sample purity and for studying polymer curing (Pungor and Horvai 1994).

2.8 Measurement of Lipid Oxidation in Caviar

With few exceptions, the predominant lipid components in fish roe are triglycerides and phospholipids. In fish with a total lipid content of 10 to 15%, the majority of the lipid (70%) is polar lipid (Tocher and Sargent 1984). For fish with a high level of polar lipids, the phospholipids may serve as an energy source in addition to triglycerides, being more easily mobilized in the eggs. The requirement for alternative sources of energy for the egg is influenced by different incubation periods for individual fish species that are characteristic of the species. A high relative proportion of neutral lipids are often used to define roe ripeness (Body 1989; Ishii and others 1988; Kaitaranta and Ackman 1981). For chum salmon roe (O. keta), the lipid fraction consists primarily of triacylglycerols (63%), phospholipids (30%), sterols (4.2%), and sterol esters (0.7) with measurable levels of furan fatty acids in the phospholipids fraction (0.6% of total phospholipids) and steryl ester fraction (3.8% of total) (Ishii and others 1988). The cholesterol content of roe is about one-fourth of that of chicken eggs. The cholesterol content of vertebrate fish roe ranges from 300 to 500 mg/100g. Salmon roes contain significant amounts of cholesterol although the amount appears to be high variable. The fatty acid composition of the neutral lipid fraction in fish roe contain about 24% saturated fatty acid (mainly 16:0) and a lower ω-3 fatty acid content (37%) with a corresponding increase in monoenes (mainly 16:1 and 18:1) to 35%. The (n-3)/(n-6) ratio averaged 10:6. The fatty acid composition of the polar lipid fraction for fish roe averaged 29% saturated fatty acids (mainly 16:0), 19% monoenes (mainly 18:1), and about 50% polyunsaturated fatty acids, of which 94% are ω-3 isomers (mainly 20:5 and
The (n-3)/(n-6) ratio averaged 14:6 (Tocher and Sargent 1984).

An important quality issue with fish roes is lipid oxidation due to the high concentration of lipids in addition to the relatively high degree of unsaturation of these lipids. Lipid oxidation in foods constitutes a complex chain of reactions initiated enzymatically or chemically that first yields to primary products (peroxides), which once exposed to extended oxidation conditions, gives rise to secondary oxidation products, including aldehydes, ketones, epoxides, hydroxyl compounds, and finally oligomers and condensation polymers. Most of these secondary oxidation products produce undesirable sensorial and physiological effects (Kanner 2007; Márquez-Ruiz and others 2007). Therefore, control of lipid oxidation is a fundamental issue. Also these compounds show a wide variety of physicochemical properties, differing mainly in volatility, polarity and molecular weight. For fish, thiobarbituric acid reactive species (TBARs) are commonly measured, with the standard used being malondialdehyde.

Malondialdehyde (MDA) is one of the most abundant aldehydes generated during secondary lipid oxidation. The most widely employed method for determination of MDA is the spectrophotometric determination of the red fluorescent MDA-thiobarbituric acid (MDA-TBA) complex. MDA tends to form at low pH and high temperature, producing a red chromophore, which offers a maximum absorbance peak at 532 nm. Several variations of MDA-TBA method exist, with different procedures currently performed in food analysis: direct heating of the sample (Biggs and Bryant 1953; Turner and others 1954; Schwartz and Watts 1957; Yu and Sinnhuber 1957; Sinnhuber and Yu 1958; Younathan and Watts 1960;
Tsoukalas and Grosch 1977; Uchiyama and Mihara 1978; Ohkawa and others 1979; Williams and others 1983; Pokorný and others 1985), sample distillation (Sidwell and others 1955; Tarladgis and others 1960; Keskinel and others 1964; Rhee and Watts 1966; Witte and others 1970; Shamberger and others 1977; Gokalp and others 1978; Huang and Greene 1978; Chen and Waimaleongora-Ek 1981; Gokalp and others 1983; Negbenebor and Chen 1985; King and Earl 1988), lipid extraction with organic solvents or aqueous acid extraction (Witte and others 1970; Fioriti and others 1974; Vyncke 1975; Siu and Draper 1978; Newburg and Concon 1980; Hung and Slinger 1981; Kosugi and Kikugawa 1985; Pikul and others 1989), followed by acid reaction with TBA. The general procedure commonly consists of homogenization and centrifugation of the test sample in an acidic medium (usually trichloroacetic acid) and afterward reaction with TBA at a high temperature (around 90 to 100°C). However there is quite a lot of variability in the reaction conditions, such as heating time (Berasategi and others 2012; Jongberg and others 2011; Jung and others 2011; Peiretti and others 2012) and trichloroacetic solution concentrations (Leygonie and others 2011; Maqsood and others 2012) which can affect results.

Traditional spectrophotometric TBA tests have been criticized for some reasons like the fact that TBA is not selective for MDA, since it also reacts with many other compounds, such as other aldehydes, carbohydrates, amino acids and nucleic acids (Salih and others 1987), interfering in the TBA assay and resulting in considerable overestimation, as well as variability in the results. There is a risk of underestimating the response since malondialdehyde can, under in vivo conditions, form linear or cyclical Schiff bases, or even cross-linked bonds, with lysine and arginine from proteins. Therefore, poor quantification
sensitivity and poor molecular specificity and selectivity can be attributed to this method. Furthermore, the high temperatures (95 to 100ºC), the extended incubation times and the strong acidic conditions commonly required for the reaction of MDA with TBA may cause peroxidation artifacts even in the presence of added antioxidants. Malondialdehyde, which is mainly formed from 1, 4-pentadiene groups such as in linolenic acid oxidation, is not generated in other oxidized lipids (especially when only one double bond is present, *i.e.*, oleic acid). Consequently, it is often a minor secondary oxidation product in many foods but generally an adequate method for fish. Despite the mentioned limitations, conventional spectrophotometric MDA-TBA methods are preferred because of their simplicity (Barriuso and others 2013). In fact, it has been recently suggested that it is a more accurate and sensitive parameter in assessment of oxidative deterioration than p-anisidine test and hexanal determination (Nuchi and others 2009; Pignoli and others 2009).
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Figure 2-1. Salt uptake rate of high (run A) and average (run B) quality chum salmon roe (Bledsoe and others 2003).
CHAPTER 3. THERMAL PROPERTIES OF IKURA (O. keta)  
AFFECTED BY SALT CONCENTRATION AND HEATING TIME

3.1 Abstract

The protein denaturation temperature as determined by differential scanning calorimetry (DSC) of chum salmon (Oncorhynchus keta) ikura prepared at different salt contents (0 to approx. 7.5 % water phase salt (WPS)) increased by 4 ºC as the salt content increased. This indicates that either some heat labile proteins are lost during the brining and curing processes associated with ikura manufacture or that the presence of added salt increases the stability of the roe protein, increasing the feasibility of developing a successful thermal pasteurization process for the product.

3.2 Introduction

Ready to eat foods require preventive controls to increase the product safety and one of the most feasible processes are thermal ones since it is well understood how bacteria are inactivated by heat. The FDA (2011) recommends 6-D processes for target microbes, and in the case of ikura, this would be L. monocytogenes and potentially Clostridium botulinum type E although the salt content is generally low enough so that botulism would not be a significant risk for this foods (Shin and Rasco, 2007). Gaze and others (1989) proposed a 70 ºC for 2 min (D values at 70 ºC for L. monocytogenes ranged from 0.14 to 0.27 min). Al-Holy and others (2004) noted that fish roes could be treated at 70 ºC with minimal loss in quality as long as the temperature is closely monitored and controlled, with the potential for using volumetric heating, improving quality since the come-up time is faster. Fish roes are heat labile and can only be heated to a temperature of 70 ºC without the eggs becoming dull
or loose color (Sternin and Dore 1993; Bledsoe and others 2003; Al-Holy and others 2004). Fatalian and others (2006) noted that the shelf life of sturgeon caviar increased following pasteurization at different temperatures during storage (Table 1-1). Irreversible protein denaturation occurs between 70 to 80ºC. Protein denaturation will result in loss in texture and appearance.

Adding varying levels of salt to salmon roe may affect protein thermal stability and potentially pH and water activity. Therefore, the objectives of this study were to determine the relation between the protein denaturation temperature for ikura and differing salt concentration and determine the highest pasteurization treatment temperature and longest heating time that could be used without leading to substantial protein denaturation.

### 3.3 Materials and Methods

#### 3.3.1 Preparation and Processing of Fresh Salmon Roes

Fresh chum salmon roe (*Oncorhynchus keta*) were harvested from Puget Sound, an inlet in Washington State. The eggs had been separated from the egg skeins mechanically and kept refrigerated until tested within two days of receipt at the laboratory. The eggs were transferred into a plastic container into which saturated salt solution at 20°C was added till the egg/brine ratio reached 1:5 (w/v). The mix was agitated by using a plastic spatula for a specified time (minutes) (Figure 3-1) until the desired water phase salt content was reached as predicted from earlier reports for salt uptake rate for salmon roe (Huang and others 2001). Then the eggs were transferred into a plastic basket, allowing the excess brine to drain off from it. After that, the basket was wrapped with aluminum foil and
placed at 4°C for 8 hours for curing.

3.3.2 The Measurement of Water Phase Salt Content

Moisture Content: 3-5 grams of brined eggs ($m_{sample}$) were weighed into an aluminum tray and dried in an oven of 105°C for 10 hours. The weight difference ($\Delta m$) before and after drying was recorded. The moisture content was calculated as,

$$\text{Moisture\%} = \frac{\Delta m}{m_{sample}} \times 100\%$$

Salt Content: A modification of the Volhard’s Method was used. The eggs were homogenized by using a hand mixer. Then, 1.50 grams of homogenate was transferred into a 250-ml flask, followed by adding 8 mL of 0.1 N silver nitrite solution ($V_{AgNO3}$). The solution was allowed to sit for 10 min to allow for silver chloride precipitation. During this time the flask was shaken slightly. Then 5 ml of 85% nitrite acid was added and the flask was heated at 285°C for 5 minutes. Then 1.5 mL of ferric indicator (ferric ammonium sulfate solution, 8g of NH$_4$Fe(SO$_4$)$_2$·12H$_2$O to 20 mL of distilled water with a few drops of concentrated nitric acid) was added after the flask was cooled to room temperature. Then the solution in flask was made up to 200 mL by adding deionized water and titrated with 0.1 N ammonium thiocyanate. The color of the solution turned from yellow to orange due to the ferric thiocyanate complex indicating the end point. The usage of ammonium thiocyanate ($\Delta V_{titrant}$) was measured and %salt calculated as shown here:

$$\text{Salt\%} = \left[0.1 \times V_{AgNO3} - 0.1 \times \Delta V_{titrant}\right] \times \frac{58.44 \times 0.1}{m_{homogenate}} \times 100\%$$
The water phase salt activity was calculated as follows:

\[
\text{WPS\%} = \frac{\text{Salt\%}}{\text{Salt\%} + \text{Moisture\%}} \times 100\%
\]

3.3.3 pH Measurement

About 1.0 ± 0.1g samples were transferred from each pouch to a 50 mL plastic tube, followed by 9 mL of deionized water then homogenized at 10,000 rpm for 1 min. Then the pH for each sample was measured by using pH meter (Model FiveEasy™, Mettler-Toledo International Inc., OH, USA). The data was recorded when the number remained consistent to 0.02 pH units. Each sample was tested in duplicate.

3.3.4 Water Activity Measurement

To determine water activity (\(a_w\)), all samples were measured in an Aqualab Model 3TE (Decagon Devices Inc., Pullman, WA, USA) in duplicate at room temperature.

3.3.5 The Determination of Protein Denaturation Temperature

A differential scanning calorimeter (DSC, Q2000, TA Instruments, New Castle, DE) was used to analyze the thermal transitions in homogenized roe that occurred during the process of thermal denaturation of the proteins in this material. The thermal analysis method was a modification of the methods of Syamaladevi and others (2010). The calorimeter was calibrated by checking standard temperatures and enthalpies of fusion for indium and sapphire. An empty sealed aluminum pan was used as a reference in each test. Nitrogen gas at a flow rate of 50 mL/min was used as the purge gas to prevent water from
condensing around the caviar sample. Twelve to eighteen milligrams of salmon caviar was sealed into each aluminum pan and cooled from 25 °C to -60 °C at 10 °C/min and formation of glassy state in salmon caviar was observed. Then samples were equilibrated for 1 min. The caviar was then heated over a range from -60 °C to 120 °C looking for thermal transitions consistent with protein denaturation, and then salmon caviar was heated back to 25 °C at a rate of 10 °C/min.

DSC thermograms with the heat flow (W/g) versus temperature relationship were used to analyze the thermal transitions in salmon caviar during heating and cooling. TA Instruments Universal analysis software was used to analyze the onset points of the protein denaturation. Duplicate samples of caviar were used to determine the protein denaturation temperatures at each salt concentration (Table 3-1).

### 3.3.6 Determination of a Pasteurization Process for Salmon Caviar

Fresh chum salmon roe (*Oncorhynchus keta*) harvested from Puget Sound, Seattle, WA were used for this study. Unsalted eggs were hermetically sealed into cylindrical aluminum cells having a 35 mm inner diameter, 6 mm inner height and 2 mm wall thickness, an O-ring made of rubber was included between the lids for a hermetic seal. Each cell contained 100±10 eggs. To minimize the influence of slow heat transfer in the kinetics studies, a specially made cell with same dimensions but with a rubber gland in the top lid was used to measure temperature change during heating and then to calculate the come up time and improve heating uniformity. A 0.1 mm diameter copper-constantan thermocouple (Type-T) was inserted through the rubber gland to measure the temperature
at the geometrical center of the sample. The sealed cells were heated in a water bath (Model ISOTEMP 215, Thermo Fisher Scientific Inc., MA, USA) at 70 °C. (Figure 3-2). The come up time, which is the time for the sample center temperature to reach within 1°C of the target temperature (70 °C), was about 5 min. For the kinetics studies, the cells with roe were heated in the water bath separately for 1, 6, 11, 15, 19, 23, 27, 31 min after the come up time (5 min), then immediately placed in ice water upon when removal from the water bath. The temperature of the samples decreased to 20 °C within 20 seconds; therefore the quality change during cooling step was neglected.

3.3.7 The Measurement of Roe Texture

The firmness of the eggs were measured by a Texture Analyzer (Model TA-XT2, Stable Micro Systems Ltd., UK) with a 25-kg load cell and connected to a computer with Texture Expert Exceed software. For each measurement, 25 eggs were placed onto a customized stainless plate with a cell having an inner diameter of 51.78 mm and inner height of 6 mm (Figure 3-3). The eggs were placed in the center of the cell to avoid having the eggs touch the edge of the cell after cooling to room temperature. A plastic cylindrical probe of a 25.4 mm diameter (Figure 3-3) compressed the eggs by 2.0 mm at a pretest, test and posttest speed of 1.0, 1.0 and 10.0 mm/s, respectively. The compression force as the firmness value was measured as the peak height in the force-time profile.

3.3.8 Statistical Analysis

For physical properties, pair wise comparisons equivalent to Fisher’s LSD were conducted using Statistical Analysis Software (Version 9.2, SAS Institute, Cary, NC).
pH, water activities and protein denaturation temperatures, each mean ± standard deviation was the average from 2 replicate experiments. For kinetics study of texture, ANOVA was conducted assuming completely random design; each mean ± deviation for compression forces was the average of 4 replicates per treatment (n=4).

3.4 Results and Discussion

3.4.1 DSC Study

Ikura with different levels of water phase salt activities were obtained after salting the fresh roes for different times. Due to the difference in quality of the fresh salmon roe from earlier reports, the salt uptake rate did not follow the same trend as Figure 3-1 with longer brining time needed. Table 3-1 shows the tested WPS for the final products corresponding to their brining time and their properties of pH, water activities and protein denaturation temperatures as shown in thermograms (Figure 3-4 to 3-11). The upward peaks in the diagrams indicate when the glass state in samples started to form. The corresponding downward peaks indicate the melting process for the frozen sample. Protein denaturation after 90 °C was shown by a sudden drop on the curve. Protein denaturation temperatures were at inflexion points as marked. The protein denaturation temperatures increased significantly (92 to 96.5°C) (P<0.05) with an increase in WPS (0 to 7.17%) following a polynomial trend (R²=0.99), shown as Figure 3-12.

In studies with eggs, ovalbumin started to denature at lower temperature with a higher level of salt concentration and the temperature peak shifted higher (Hitachi High-Tech Science Corporation 1986). However Thorarinsdottir and others (2002) found that the
salting process significantly decreased the protein denaturation temperatures of cod fillets showing that the type of protein, in this case actinomyosin for fish muscle compared to alumin proteins in eggs behave differently during thermal denaturation in the presence of salt. Lipovitellins make up 70% of the proteins in egg yolk (Stadelman and others 1995); ovalbumin is the major protein in egg white which is about 54% (Powrie 1976). Based on the research of Bircan and Barringer (2002), ovalbumin was the protein responsible for protein denaturation when temperature rose because hydrophobic interactions increased the dielectric constant of the protein. However, myosin and actin are the main protein in meat. The addition of sodium chloride, above the isoelectric point of proteins (pl), to meat system will cause the protein swelling and water holding capacity will be increased. Above the maximum swelling, higher concentration of salt will make the myofibrillar proteins lose water through the salting-out process, which will help the protein denature due to stronger protein-protein bonds and shrinkage of the muscle (Thorarinsdottir and others 2002).

3.4.2 pH and Water Activity of Ikura at Different WPS

pH did not appear to be affected with an increase of WPS. Water activity is also defined as the escaping tendency of water in a system divided by the escaping tendency of pure water with no radius of curvature. The water activities for ikura showed a significant drop ($P<0.05$) as WPS increased due to the salt uptake as anticipated. Salt, as one of dissolved species, can interact with water through dipole-dipole, ionic interactions, altering the hydrogen bonding property of water, thereby reduce the escaping tendency of water in a food.
3.4.3 Kinetics Study of Texture

Compression force (N) of the unsalted roes decreased linearly ($R^2=0.89$) with increasing heating time (Figure 3-13).

Loss of strength and integrity of egg membranes along with the thermal denaturation of egg proteins is the primary mechanism leading to a loss in firmness (Bircan and Barringer 2002).
3.5 References


Figure 3-2. Salt uptake rate of high (run A) and average (run B) quality chum salmon roe (Bledsoe and others 2003).
Figure 3-2. Thermometer (Model HH21A, OMEGA Engineering Inc., USA) used for the kinetic study
Figure 3-3. Stainless plate and plastic probe used for texture measurement
Figure 3-4. DSC thermograms of fresh salmon roe (0 WPS) with repeated measurement
Figure 3-5. DSC thermograms of ikura (3.23% WPS) with repeated measurement
Figure 3-6. DSC thermograms of ikura (3.76% WPS) with repeated measurement
Figure 3-7. DSC thermograms of ikura (3.91% WPS) with repeated measurement
Figure 3. DSC thermograms of ikura (4.10% WPS) with repeated measurement
Figure 3-9. DSC thermograms of ikura (4.23% WPS) with repeated measurement
Figure 3-10. DSC thermograms of ikura (5.24% WPS) with repeated measurement
Figure 3-11. DSC thermograms of ikura (7.17% WPS) with repeated measurement
Figure 3-12. Change in protein denaturation temperature (PDT) of ikura at different water phase salt concentrations (WPS) (n=2)

\[ y = 0.1037x^2 - 0.0976x + 91.967 \]

\[ R^2 = 0.99 \]
Figure 3-13. Change in texture of unsalted salmon roes at different heating times at 70 ºC (n=4)
Table 3-1. Properties of ikura with different WPS \(^a\) obtained after different brining times (n=2)

<table>
<thead>
<tr>
<th>t (^b) (min)</th>
<th>WPS (%)</th>
<th>pH ± SD</th>
<th>(a_w) ± SD</th>
<th>(T_{PD}) (^c) ± SD (ºC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>5.60 ± 0.02</td>
<td>0.995 ± 0.001</td>
<td>92.01 ± 0.11</td>
</tr>
<tr>
<td>0.33</td>
<td>3.23</td>
<td>5.67 ± 0.04</td>
<td>0.973 ± 0.002</td>
<td>92.48 ± 0.25</td>
</tr>
<tr>
<td>0.5</td>
<td>3.76</td>
<td>5.63 ± 0.02</td>
<td>0.973 ± 0.002</td>
<td>93.06 ± 0.14</td>
</tr>
<tr>
<td>1.5</td>
<td>3.91</td>
<td>5.60 ± 0.05</td>
<td>0.973 ± 0.001</td>
<td>93.18 ± 0.10</td>
</tr>
<tr>
<td>2.0</td>
<td>4.10</td>
<td>5.67 ± 0.03</td>
<td>0.971 ± 0.002</td>
<td>93.34 ± 0.29</td>
</tr>
<tr>
<td>4.0</td>
<td>4.23</td>
<td>5.65 ± 0.01</td>
<td>0.959 ± 0.001</td>
<td>93.50 ± 0.11</td>
</tr>
<tr>
<td>7.5</td>
<td>5.24</td>
<td>5.50 ± 0.02</td>
<td>0.922 ± 0.002</td>
<td>94.48 ± 0.23</td>
</tr>
<tr>
<td>12.0</td>
<td>7.17</td>
<td>5.67 ± 0.03</td>
<td>0.845 ± 0.003</td>
<td>96.52 ± 0.21</td>
</tr>
</tbody>
</table>

\(^a\)WPS is the tested water phase salt activity for each sample
\(^b\)t is the brining time for each sample
\(^c\)\(T_{PD}\) is the mean value of the protein denaturation temperature for each sample (n=2)
CHAPTER 4. QUALITY ATTRIBUTES OF PASTEURIZED CHUM SALMON (O. keta) IKURA DURING REFRIGERATED STORAGE

4.1 Abstract
The quality of pasteurized (6-D process for *Listeria monocytogenes*, 70 °C 2 min) chum salmon ikura (0 or 5.24% water phase salt) over a 60 day refrigerated storage period was evaluated. Changes to color, texture, oxidation for ikura stored in flexible plastic pouches of two different oxygen transmission rates (OTR) 62 cc/(m2•day) or 40 cc/(m2•day) at 23 °C and 0% RH; were measured during storage. Texture became softer over the 60 days storage but the type of film did not appear to affect this. Oxidation was substantially higher after 50 days of refrigerated storage in the ikura stored in the higher OTR film indicating that a lower OTR film would be important if ikura is to be stored under refrigeration for longer than two months.

4.2 Introduction
Ikura has a short shelf life for its high water activity and lipid content. Like poultry eggs, fish roe have high concentrations of lipid and protein. In general fish roe products are high in protein (16 to 30%). Crude lipid content can vary from 5 to 20% with an average value for salmon roe of around 10%. For chum salmon (O. keta), the untreated roe consists of 50 to 60% of moisture, 27 to 35% of protein, 12 to 20 % crude lipid and 1.5 to 1.7% total ash. The volume of the oil droplet in chum salmon comprises up to 10% of the volume of the individual egg (Huang and others 2001). The lipids in mature roe could make up about 13.5% of the wet weight of the egg (Ishii and others 1988). The high content of lipids makes caviar go bad very easily due to the lipid oxidation. Commercial ikura products are commonly
packed using glass jars as the packaging material, which may have a high ultraviolet transmission which can initiate the formation of oxygen free radicals. Fortunately, glass is air proof and therefore the lipid oxidation is slow because of the relatively low concentration of molecular oxygen. However, glass has a higher cost than other packaging materials such as polymer films. Some polymer films have every low oxygen transmission rate which may also shield products from oxygen so that lipid oxidation is inhibited. Besides lipid oxidation, other quality changes such as change in pH, water activity, color and texture can be affected by oxidation in ikura kept under refrigerated storage. Films with different oxygen transmission rate were used as packaging for ikura in this study. The quality parameters for both were monitored and compared between each other. The objective of this study was to find an alternative to glass and to examine film packaging for caviar that had different oxygen transmission rates and monitor the quality by conducting a shelf-life study.

4.3 Materials and Methods

4.3.1 Brining Roes

The freshly received eggs were salted using the previously described method. The salting time was 7 min with the caviar reaching a water phase salt activity of 5.24%.

4.3.2 Packaging

Instead of glass jars for the commercial products in the market, plastic pouches were used for packaging. The pouches were made of two different plastic films: Film A from Shields Bag and Printing, Yakima, WA, consisted of five layers (LDPE/tie/Nylon/tie/LDPE; thickness = 90 µm) and an oxygen transmission rate of 62
cc/(m²·day) at 23 °C and 0% RH; Film B from Sealed Air-Cryovac, Elmwood Park, NJ, was consisted of nine layers (mLLDPE/LLDPE/LLDPE/tie/Nylon/tie/LLDPE/LLDPE/mLLDPE; thickness = 110 µm) with an OTR of 40 cc/(m²·day) under the same test conditions. Both of the films were cut into 10cm × 9cm pieces and two pieces of same film were hot sealed with the hydrophobic sides out and one opening by using a vacuum sealer (Model Koch Easy Pack, Koch Equipment LLC., Kansas City, MO, USA). The sealed parts were about 0.5 cm from the edges; therefore the actual size of each pouch was 9cm × 8cm. A total of 14 pouches (n=2) were made. Each pouch contained 28.0 ± 0.1g eggs which is similar to the amount commercially packaged into glass jars. The last opening of each pouch was vacuum sealed at vacuum level 1, at which the eggs could well remain their shape, and temperature level 4, at which the films were sealed appropriately. Then the eggs were gently spread in a uniform layer inside the package following sealing (Figure 4-1).

4.3.3 Sample Pasteurization and Storage

To minimize the influence of slow heat transfer, a special pouch with a rubber gland in the geometrical center of the pouch was prepared. The rubber gland was connected via two stainless screw threaded components (Figure 4-2). The gaps were filled with glue for hermetic sealing. Come up time was measured through the previously described method by using a thermocouple. The come up time for both pouches of two films at 70 °C were all about 8 min, somewhat longer than in the custom made kinetic cell. All the prepared pouches with samples were heated in a 70 °C water bath for 4 min after the come up time. A 4 min heating period was 2x longer than that needed for a 6-log reduction time for
Listeria monocytogenes (Gaze and others 1989) which should be sufficient to inactivate Listeria monocytogenes. All the pouches were stored in a 4 °C refrigerator for up to 60 days after immediate cooling following removal from the water bath. Tests for quality parameters were conducted on Day 0, 10, 20, 29, 37, 50, and 60.

4.3.4 pH Measurement

About 1.0 ± 0.1g samples were transferred from each pouch to a 50 mL plastic tube, followed by 9 mL of deionized water then homogenized at 10,000 rpm for 1 min. Then the pH for each sample was measured by using pH meter (Model FiveEasy™, Mettler-Toledo International Inc., OH, USA). The data was recorded when the number started to change by 0.02. Each sample was tested in duplicate.

4.3.5 Water Activity Measurement

To determine water activity (a<sub>w</sub>), all samples were measured in duplicate in an Aqualab instrument (Model 3TE, Decagon Devices Inc., Pullman, WA, USA) at room temperature.

4.3.6 Color Change Monitoring

A computer vision system (CVS) described by Zhang and others (2014) was used to capture color images of the ikura samples for color and area analyses. The CVS included 3 parts (Figure 4-3): a lighting system; a Canon EOS 60D Digital camera (Canon Inc., Melville, NY), with 18.0 megapixel solution and 18-135 mm EF-S Lens; and a desktop computer with image-processing software. The lighting systems consisted of a Copystand (Model 910-20, Bencher, Inc., Antioch, IL, USA) for the sample and the camera and an
ALZO 300 Table Top Studio with 24” Riser Platform and 2 “Cool Lites” (Akces Media LLC, CT, USA). Samples were placed in a white tray on the center of a black platform. The lights were mounted on both sides of platform, 100 cm above and at a 45° to the sample. The camera was mounted downward at a height of 50 cm above the sample. The lights were turned on at least 15 min before the pictures were taken for consistent light intensity.

The color parameters (CIE L*a*b*) were derived using Adobe Photoshop CS4 software (Version 11.0, Adobe System Inc., CA, USA). The color capturing procedures involved using a magic wand tool to select sample area, and a histogram tool to indicate the average lightness, redness and yellowness (referred as L, a, b in Photoshop) of the selected area. In CIE L*a*b*, L* ranges from 0 to 100, a* and b* ranges from -127 to +128; in Photoshop, these values indicated by histogram tool are encoded from 0 to 255. To convert these data to standard scaling values, the following formulas (Briones and Aguilera 2005) were used:

\[
\begin{align*}
L^* &= \frac{L}{2.5} \\
a^* &= \frac{240a}{255} - 120 \\
b^* &= \frac{240b}{255} - 120
\end{align*}
\]

4.3.7 Lipid Oxidation Measurements

A spectrophotometric TBARs method was used to measure lipid oxidation in the samples during the storage. The protocol of this method involved preparation of a standard curve
using malondialdehyde (MDA) as a standard followed by measurement of ikura oxidation during the storage period.

To make the standard MDA curve, 250 μL of each standard MDA solution with different concentration (0, 0.9, 1.8, 3.6, 7.2 mg/mL) was transferred to a 15 mL test tube followed by 2.5 ml of 2-thiobarbituric acid buffer (a mix of 3 g/L TBA and 50 g/L trichloroacetic acid with a ratio of 1:1). Test tubes were subsequently incubated for 60 min in 90 °C water bath and then cooled to room temperature in ice water. The solution from each tube was transferred to a 1.5 centrifuge tube (Model C-3217, BioExpress, UT, USA) and centrifuged in a VWR Galaxy 14D Centrifuge (VWR International LLC., PA, USA) at 3,800 rpm for 10 min. The absorbance of the supernatant after filtration was measured at a wavelength of 532 nm (Model Ultrospec 4000, Pharmacia Biotech, Cambridge, England).

To extract the TBARs reactive substances from the ikura sample, 2.5 g samples was mixed with 10mL of trichloroacetic acid (TCA, 50 g/L) in a 50 mL test tube. The mixture was homogenized at 17,500 rpm for 60 seconds using an Omni Mixer homogenizer (Omni International, GA, USA).The homogenate was afterward transferred to 1.5 mL centrifuge tube and centrifuged at 13000 rpm for 10 min. The supernatant was filtered. Filtrate (250 μL) was transferred to 15 mL test tube followed by 2.5 mL of 2-thiobarbituric acid buffer and 20 μL butylated hydroxyanisole (BHT, 10 g/L). TCA (250 μL) with the same amount of buffer and BHT was prepared as a reference. Test tubes were incubated and cooled as described earlier. The absorbance of supernatants obtained
after centrifugation and filtration and were measured against the reference at 532 nm. TBARs value (MDA concentration) was expressed as mg MDA/kg sample. It can be calculated as:

$$\text{TBARs Value} = \frac{c_{MDA} \times 0.001}{0.25 \times 1000 \times 10^{-6}} = \frac{c_{MDA}}{0.25} \text{mg/kg}$$

$c_{MDA}$: The MDA concentration calculated through the formula of the trend line of the standard MDA curve, µg/mL

4.3.8 Texture Measurement

The eggs were taken out of the refrigerator at least 15 min before starting texture measurements so that the impact of temperature differences on the texture could be neglected. The procedure of the test was as described previously in Chapter 3. Replicate measurements ($n=4$) were conducted for each sample.

4.3.9 Statistical Analysis

For physical properties, pair wise comparisons equivalent to Fisher’s LSD were conducted using Statistical Analysis Software (Version 9.2, SAS Institute, Cary, NC). For pH and water activity, each mean ± standard deviation was the average from 2 replicate experiments.

For shelf-life studies, ANOVA was conducted assuming completely random design; mean values were compared using pair wise comparison by SAS. For color parameters, each mean ± standard deviation was directly calculated by the Photoshop software; for lipid oxidation test, each mean ± deviation was the average of two replicates from 2 replicate
experiments (n=2). For texture measurement, each mean ± deviation for compression forces was the average of 4 replicates from 2 replicate experiments (n=4).

4.4 Results and Discussion

4.4.1 pH and Water Activity

Table 4-1 shows properties of pH and water activities of ikura (5.24% WPS) during the 60 days storage for the shelf life study. No significant difference in either pH or water activities were observed between the samples from the pouches with oxygen transmission rates on the same day during the first month (P>0.05). However, pH and water activity of the samples from low OTR pouches were significantly lower after 30 days (P<0.05). pH decreased by about 0.2 units during storage for ikura in both pouch materials over the 60 days storage period (P<0.05), which is statistically significant but would not likely have an impact on quality; shown as Figure 4-4. The water activities of samples were statistically different having decreases compared to Day 0 (P<0.05) but still remained at a high level (Figure 4-5).

4.4.2 Color

Table 4-2 shows the color parameters of ikura (5.24% WPS) during the storage period for the shelf life study. The color parameters (CIE L*, a* and b*) of the samples from both pouches changed following the same trend during the storage period without significant difference observed between them (P > 0.05) (Figure 4-6). Neither of the samples from two different pouches exhibited color loss during the storage period (P > 0.05).
Kong and others (2007) observed color loss in their research of salmon fillets in the first 10 min of heating. The color loss resulted from the protein denaturation (hemoglobin and myoglobin) and oxidation of carotenoids. For this study with salmon roes, the color also comes from the carotenoids, but the treatment temperatures used were substantially lower. Carotenoid are commonly bound to lipoprotein as seen in the egg yolk (Brooks and others 1997; Shahidi and Brown 1998), which can increase stability.

4.4.3 Lipid Oxidation

The average TBARs concentrations for ikura are presented in Table 4-3. Standard curve is shown in Figure 4-7. TBARs in ikura in the two films showed a significant increase in MDA concentration with the time ($P<0.05$) with differences becoming apparent around day 29 for ikura stored in pouches made of film A with the higher OTR (62 cc·m$^{-2}$·day$^{-1}$) than for the samples in pouches of film B (OTR=40 cc·m$^{-2}$·day$^{-1}$) (Figure 4-8).

During the tests, an interfering yellow chromagen ($\lambda_{max}$ 450-460 nm) that overlapped with the pink peak ($\lambda_{max}$ 530-537 nm) was observed as seen by previous researches (Wertheim and others 1956; Sinnhuber and Yu 1977; Crackel and others 1988). Crackel and others (1988) found that the interference became less significant as TBA numbers increased. Wertheim and others (1956) noted the yellow chromagen was formed by the reaction of TBA with sugars (galactose, maltose, dextrose, fructose, sucrose). Asakawa and others (1975) claim that addition of sodium sulfite to the TBA reaction mixture prevents production of the yellow chromagen and enhances development of the pink chromagen and this interference could have affected the reliability of results in this study.
since levels of TBARs were low.

Also Labuza and others (1970) found the rate of lipid oxidation increases as the decrease of the water activity due to the effect of an increased concentration of metal catalysts in food system.

### 4.4.4 Texture

Table 4-4 shows the firmness of salmon caviar, expressed as compression force, for the shelf life study. The ikura became softer during the storage ($P<0.05$) with no significant difference between the pouches materials observed (Figure 4-9). The texture loss resulted from the loss of membrane integrity from the heating process and enzymatic protein denaturation (Bircan and Barringer 2002; Shenouda 1980). Shenouda (1980) also mentioned that the free radicals from lipid oxidation would cause protein damage by forming protein-free radicals, which could initiate other reactions leading to protein aggregates.
4.5 References


Labuza T, Tannenbaum S, Karel M. 1970. Water content and stability of low-moisture and
intermediate-moisture foods. Food Technology.


Figure 4-1. Ikura packaged in pouches
Figure 4-2. Ikura in a specialized pouch for pasteurization
Figure 4-3. Components of the computer vision system for color analysis
Figure 4-4. Changes of pH in ikura (5.24% WPS) during 60 days storage (n=2)
Figure 4. Changes of water activity of ikura (5.24% WPS) during 60 days storage at 4 °C (n=2)
Figure 4-6. Changes of color parameters $L^*$ (a), $a^*$ (b) and $b^*$ (c) in ikura (5.24% WPS) during 60 days storage at 4 °C (n=2)
Figure 5. Standard curve (MDA) for TBARs measurement

$y = 0.0824x - 0.0072$

$R^2 = 1.00$
Figure 4-8. Results of TBARs tests of ikura (5.24% WPS) during 60 days storage at 4 ℃: the changes of absorbance at 532 nm (a) and MDA concentrations (b) (n=2)
Figure 6. Changes in texture of ikura (5.24% WPS) during 60 days storage at 4 ºC (n=4)
Table 4-1. The properties of pH and water activities for ikura (5.24% WPS) during 60 days storage at 4 °C (n=2)

<table>
<thead>
<tr>
<th>Day</th>
<th>pH ± SD</th>
<th>a_w ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Film A</td>
<td>Film B</td>
</tr>
<tr>
<td>0</td>
<td>5.53 ± 0.04 A, a</td>
<td>5.58 ± 0.03 A, a</td>
</tr>
<tr>
<td>10</td>
<td>5.44 ± 0.05 AB, a</td>
<td>5.41 ± 0.13 BC, a</td>
</tr>
<tr>
<td>20</td>
<td>5.42 ± 0.15 ABc, a</td>
<td>5.52 ± 0.12 AB, a</td>
</tr>
<tr>
<td>29</td>
<td>5.46 ± 0.13 AB, a</td>
<td>5.44 ± 0.05 ABC, a</td>
</tr>
<tr>
<td>37</td>
<td>5.32 ± 0.02 BC, a</td>
<td>5.39 ± 0.03 BC, b</td>
</tr>
<tr>
<td>50</td>
<td>5.28 ± 0.02 BC, a</td>
<td>5.34 ± 0.02 C, b</td>
</tr>
<tr>
<td>60</td>
<td>5.24 ± 0.01 C, a</td>
<td>5.29 ± 0.03 C, b</td>
</tr>
</tbody>
</table>

1 Average ± standard deviation (SD) of two measurements where are statistical analysis associated with these measurements, values followed by different capital letters in the same column and different lowercase letters in the same row for the same treatment are statistically different (P < 0.05)
2 Samples in pouch made of Film A with higher oxygen transmission rate (OTR)
3 Samples in pouch made of Film B with lower OTR
Table 2-2. The color parameters for ikura (5.24% WPS) during 60 days storage at 4 °C

<table>
<thead>
<tr>
<th>Day</th>
<th>L* ± SD</th>
<th>a* ± SD</th>
<th>b* ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Film A</td>
<td>Film B</td>
<td>Film A</td>
</tr>
<tr>
<td>0</td>
<td>56.2 ± 6.0 a</td>
<td>56.2 ± 6.0 a</td>
<td>61.7 ± 8.9 a</td>
</tr>
<tr>
<td>10</td>
<td>55.9 ± 6.7 a</td>
<td>56.0 ± 6.9 a</td>
<td>61.0 ± 8.0 a</td>
</tr>
<tr>
<td>20</td>
<td>56.8 ± 6.9 a</td>
<td>57.1 ± 7.8 a</td>
<td>61.4 ± 10.1 a</td>
</tr>
<tr>
<td>29</td>
<td>57.8 ± 5.9 a</td>
<td>57.8 ± 6.6 a</td>
<td>64.4 ± 9.7 a</td>
</tr>
<tr>
<td>37</td>
<td>56.7 ± 7.2 a</td>
<td>56.4 ± 7.0 a</td>
<td>62.3 ± 10.3 a</td>
</tr>
<tr>
<td>50</td>
<td>58.2 ± 7.0 a</td>
<td>57.4 ± 6.4 a</td>
<td>62.9 ± 10.7 a</td>
</tr>
<tr>
<td>60</td>
<td>57.3 ± 7.1 a</td>
<td>57.9 ± 6.6 a</td>
<td>63.2 ± 10.0 a</td>
</tr>
</tbody>
</table>

1 Average ± standard deviation (SD) standard scaled from the original data collected by Photoshop Software, values followed by different capital letters in the same column and different lowercase letters in the same row for the same treatment are statistically different (P < 0.05)
2 Samples in pouch made of Film A with higher oxygen transmission rate (OTR)
3 Samples in pouch made of Film B with lower OTR
Table 4-3. The results of TBARs tests for ikura (5.24% WPS) during 60 days storage at 4 °C (n=2)

<table>
<thead>
<tr>
<th>Day</th>
<th>Absorbance (532nm) ± SD</th>
<th>TBARs value ± SD (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Film A</td>
<td>Film B</td>
</tr>
<tr>
<td>0</td>
<td>0.009 ± 0.001 E. a</td>
<td>0.010 ± 0.000 E. a</td>
</tr>
<tr>
<td>10</td>
<td>0.013 ± 0.001 E. a</td>
<td>0.011 ± 0.000 E. a</td>
</tr>
<tr>
<td>20</td>
<td>0.011 ± 0.001 E. a</td>
<td>0.011 ± 0.001 E. a</td>
</tr>
<tr>
<td>29</td>
<td>0.021 ± 0.001 D. a</td>
<td>0.018 ± 0.001 D. b</td>
</tr>
<tr>
<td>37</td>
<td>0.033 ± 0.002 C. a</td>
<td>0.021 ± 0.001 C. b</td>
</tr>
<tr>
<td>50</td>
<td>0.043 ± 0.001 B. a</td>
<td>0.025 ± 0.001 B. b</td>
</tr>
<tr>
<td>60</td>
<td>0.283 ± 0.039 A. a</td>
<td>0.097 ± 0.004 A. b</td>
</tr>
</tbody>
</table>

1Average ± standard deviation (SD) of two measurements, values followed by different capital letters in the same column and different lowercase letters in the same row for the same treatment are statistically different (P < 0.05)

2Samples in pouch made of Film A with higher oxygen transmission rate (OTR)

3Samples in pouch made of Film B with lower OTR
Table 4-4. The texture (as compression force) for ikura (5.24% WPS) during 60 days storage at 4 °C (n=4)

<table>
<thead>
<tr>
<th>Day</th>
<th>Compression force ± SD (^1) (N)</th>
<th>Film A (^2)</th>
<th>Film B (^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.76 ± 1.15 (^{A,a})</td>
<td>3.76 ± 1.15 (^{A,a})</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>2.78 ± 0.78 (^{AB,a})</td>
<td>3.02 ± 0.54 (^{AB,a})</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>2.35 ± 0.89 (^{B,a})</td>
<td>2.50 ± 0.70 (^{BC,a})</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>2.39 ± 1.50 (^{B,a})</td>
<td>2.35 ± 0.71 (^{BC,a})</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>2.11 ± 0.59 (^{B,a})</td>
<td>1.80 ± 0.35 (^{C,a})</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>2.05 ± 0.53 (^{B,a})</td>
<td>2.13 ± 0.90 (^{BC,a})</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>1.87 ± 0.28 (^{B,a})</td>
<td>1.91 ± 0.33 (^{C,a})</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Average ± standard deviation (SD) of four measurements, values followed by different capital letters in the same column and different lowercase letters in the same row for the same treatment are statistically different \((P < 0.05)\)

\(^2\) Samples in pouch made of Film A with higher oxygen transmission rate (OTR)

\(^3\) Samples in pouch made of Film B with lower OTR
CHAPTER 5. CONCLUSIONS AND FUTURE WORK

The study indicates that an appropriate increase in water phase salt could make pasteurization at higher temperature for a shorter time possible reducing the undesirable softening that occurs to salmon caviar following pasteurization using the current longer time processes. Also, use of the packaging materials with lower oxygen transmission rate could slow lipid oxidation of film packaged salmon caviar during storage.

For ikura, pasteurization temperature of 70 °C for 4 min after come-up time should be sufficient for a 6-log reduction of Listeria monocytogenes without affecting its quality.

In the third chapter, the range of protein denaturation temperature for ikura has been determined. Future research is needed to confirm if increasing the heating temperature and shortening the heating time can minimize quality change of this product, particularly softening. Instead of thermal pasteurization, antimicrobial packaging for caviar products may help reduce the risk from L. monocytogenes or other pathogenic microorganisms without changing product quality. Since the shelf life study was just focusing on the changes of chemical properties, more research that focuses on sensory evaluation of ikura during storage is recommended.