Sensory and Physical Assessment of Microbiologically Safe Culinary Processes for Fish and Shellfish

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ABSTRACT

Numerous food-borne illnesses are associated with fish and shellfish annually due to consumers choosing to eat seafood raw or undercooked and consumers not properly handling and preparing seafood. The 2009 FDA Food Code suggests intact fish and shellfish should be cooked to an internal temperature of 63°C to target *Salmonella* spp. Selected fish and shellfish were cooked to 64°C ± 1 and 74°C ± 1 and evaluated for consumer acceptability, characteristics of doneness at temperature endpoints, and physical changes of texture (TPA, KSC, and WB), color, and cook loss. Temperature endpoints represented the recommended internal temperature and ten degrees higher to increase lethality of *Salmonella* spp. Differences ($p < 0.05$) in texture were determined among the methods and products tested. However, consumer acceptability ($n = 50$) for fish and shellfish products (salmon: baked, poached; tilapia: baked, pan-fried; and shrimp: boiled) cooked to 64°C ± 1 and 74°C ± 1 were liked equally ($p > 0.05$), with mean hedonic scores falling between 6 (like slightly) and 7 (like moderately). A trained descriptive panel ($n = 7$) reviewed visual and non-oral texture indicators of doneness to distinguish 64°C ± 1 and 74°C ± 1. Firmness and shape of shrimp, separation between muscle flakes and fillet edge color of baked tilapia, and firmness and edge color of the fillet for baked salmon were identified as indicators to determine doneness. Overall, 74°C could be recommended as the internal temperature for cooking fish/shellfish such as salmon, tilapia, and shrimp without diminishing eating quality or acceptability.

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Chapter 1

Introduction

Fish and shellfish are consumed annually in large volumes in the United States (U.S.) and numerous food-borne related illness cases, outbreaks, and deaths associated with fish and shellfish consumption have been documented. Food-borne illnesses connected to fish and shellfish can be reduced through improving consumer knowledge on safe food handling and preparation. However, fish and particularly shellfish are commonly chosen to be consumed raw or undercooked even when risks are known.

The U.S. was ranked as the third largest consumer of seafood in the world behind China and Japan, according to Food and Agriculture Organization's (FAO) calculations based on imports, exports, and countries' landings from 2005 - 2007. In 2009, the U.S. imported $13.1 billion of edible fishery products totaling 5.2 billion pounds. Almost a third (29%) of the imported edible product was shrimp followed by tuna (NMFS 2009). From 2001 – 2009, shrimp has been the number one seafood product consumed per-capita (lbs) in the U.S. (Table 1-1) (NFI 2011). In 2009, the U.S. per-capita consumption of edible fish and shellfish was 15.8 lbs with fresh and frozen fishery products accounting for 11.8 lbs. Of the 11.8 lbs of fresh and frozen seafood consumed per-capita, fin fish and shellfish consumption was 6.2 lbs and 5.6 lbs respectively. Canned fishery products accounted for 3.7 lbs per-capita and cured fishery product consumption was 0.3 lbs per capita in 2009 (NMSF 2009).

Table 1-1: Top Ten Seafood Species Consumed Per-Capita in the U.S. in 2009

Rank	Species	Lbs
1	Shrimp	4.100
2	Canned Tuna	2.500
3	Salmon	2.040
4	Pollock	1.454
5	Tilapia	1.208
6	Catfish	0.849
7	Crab	0.594
8	Cod	0.419
9	Clams	0.413
10	Pangasius	0.356

NFI (2011)

The Center for Disease Control and Prevention (CDC) 2011 estimates "each year 1 out of 6 Americans (48 million people) gets sick, 128,000 are hospitalized, and 3,000 die from food-borne diseases" (2011). Public health officials working with the CDC compared the amount of outbreaks per commodity item and identified fin fish as being associated with the most outbreaks in 2007 (CDC 2010). Among pathogen-commodity pairs, *Vibrio parahaemolyticus* in mollusks was one of the most outbreak-related cases for 2006 (CDC 2009). In 2007, numerous multistate *Salmonella* outbreaks associated with various food products occurred including an outbreak involving 44 reported illnesses associated with raw tuna (CDC 2010).

The majority of food-borne illnesses are linked to consumers not practicing recommended food-handling guidelines such as proper refrigeration, separating raw and cooked food and proper storage of leftovers (Knabel 1995). Improper cooking by consumers also can result in food-borne illness. Consumers often follow cooking guidelines provided by cookbooks and package information that indicate visual cues to determine doneness or provide timed cooking instructions with visual indicators. To ensure fish and shellfish products are cooked properly to prevent food-borne illness the appropriate internal temperature must be reached. However, it also must be considered that consumers may choose to eat seafood products raw or only lightly cooked on occasion (NACMCF 2008).

To reduce the incident of food-borne illness consumers must be educated in how to properly cook and handle various food products. The Food and Drug Administration (FDA) Food Code (2009) provides information and guidelines to the industry and all levels of the government on food safety to assist them with updating rules and regulations. For example, the 2009 FDA Food Code recommends cooking parameters for raw fish and shellfish, comminuted fish and shellfish, and stuffed fish and shellfish products (Table 1-2). These cooking guidelines were developed with *Salmonella* spp. as the target pathogen due to the large number of associated outbreaks. However, the 2009 FDA Food Code temperature guidelines are not based on inactivation studies of *Salmonella* spp. in fish and shellfish, because only limited research has been conducted on the thermal inactivation of *Salmonella* spp. in seafood. The FDA Food Code's recommended endpoint temperatures were based on the expectation that the raw fish or shellfish being prepared would have low levels of microbial contamination, proper handling and cooking techniques would occur, that the fish/shellfish were consumed immediately after preparation, and leftovers refrigerated and consumed within a day or two (NACMCF 2008).

Table 1-2: 2009 FDA Food Code's Recommended Cooking Guidelines for Fish and Shellfish for the Destruction of *Salmonella* spp.

Product	Cooking Guidelines
Raw Fish and Shellfish	63°C (145°F) or above for 15 seconds
Comminuted Fish and Shellfish	68°C (155°F) or above for 15 seconds
Stuffed Fish and Shellfish	74°C (165°F) or above for 15 seconds

FDA (2009)

The FDA and National Marine Fisheries Service desired to determine safe cooking parameters for seafood and posed several questions on the subject. The National Advisory Committee on Microbiological Criteria for Foods (NACMCF 2008), responding to their request, made numerous conclusions including:

- proper handling and storage of seafood products from harvesting to consumer's home is necessary;
- once the product has been purchased by the consumer proper storage, cooking and storing leftovers is key for eliminating food safety hazards;
- cookbook guidelines are generic and may not consider size or species of fillets being prepared; and
- no single internal endpoint temperature can be used for all seafood products to produce optimal eating quality and food safety.

The NACMCF report (NACMCF 2008) identified gaps and challenges in the knowledge of seafood preparation including:

- consumers must be made aware of appropriate cooking procedures and heating sources;
- consumers need to be educated and made aware of the dangers of consuming raw and undercooked seafood, specifically dangers to at-risk individuals (young, old, pregnant, and immunocomprimised); and
- consumers need to understand the importance of proper food handling and sanitation.

This project was designed to provide a better understanding of how internal endpoint temperatures, including the 2009 FDA Food Code's recommended temperature, affect popular types of fish and shellfish (Atlantic salmon, tilapia, shrimp, oysters). Sensory and physical assessments were conducted to evaluate the effect of endpoint temperature as well as culinary preparation methods (baking, broiling, poaching, pan-frying, and boiling) on popular fish and shellfish products (Atlantic salmon, tilapia, shrimp and oysters). Finally, consumers were surveyed to gain insight into consumer consumption habits and safety concerns related to fish and shellfish.

Goal

The goal of this research is to provide guidance on properly preparing fish and shellfish to consumers, food service professionals and industry through exploring physical changes and acceptability of popular fish and shellfish products prepared by common culinary processes to designated microbiologically safe internal temperature endpoints.

Objectives

1. Determine physical characteristics of two fish and two shellfish products cooked to three endpoints (visual, 64°C ± 1 (147°F) and 74°C ± 1 (165°F)) using popular consumer cooking methods (Table 1-3).
    - Review and evaluate visual guidelines for doneness described for fish/shellfish products and/or culinary methods in cookbooks on there ability to prepare a microbiologically safe product.
    - Assess qualitative visual characterization and quantitative non-oral texture characteristics of the products at two endpoints (64°C ± 1 (147°F) and 74°C ± 1 (165°F)) using a trained descriptive panel (n = 7) (Meilgaard and others 2007) with the Sensory Information Management System (SIMS, Sensory Computer Systems, LLC, Morristown, NJ).
    - Measure color using a tristimulus colorimeter (Minolta Colorimeter Model CR-300, Minolta Corporation, Japan) and Adobe® Photoshop® CS5 Extended (Version 12.0 x 32).
    - Quantify texture characteristics using a texture analyzer (TA-XTplus Texture Analyzer, Texture Technologies, Scarsdale, NY).
2. Assess consumer sensory preference for fish and shellfish products prepared by different culinary processes (Table 1-3) to two different thermal endpoints.
    - Determine consumer (n = 50) preference for fish and shellfish products prepared to two different thermal endpoints (64°C ± 1 (147°F) and 74°C ± 1 (165°F)) using the 9-point hedonic scale for overall acceptability (1 = dislike extremely, 9 = like extremely) (Meilgaard and others 2007) for each culinary process (Table 1-3).

- Administer a demographic survey including age, gender, frequency of seafood/fish consumption, type of seafood/fish consumed, experience in preparing seafood and seafood safety concerns

Table 1-3: Culinary Processes Applied to Fish and Shellfish Relative to Research Objectives

Fish and Shellfish Products	**Culinary Processes for Evaluation**					
	Baked	Broiled	Poached	Pan-Fried	Boiled	Sautéed
Atlantic Salmon	X[1,2,3,4]	X[1,2]	X[1,2,3]			
Tilapia	X[1,2,3,4]	X[1,2]		X[1,2,3]		
Shrimp		X[1,2]			X[1,2,3,4]	X[1]
Eastern Oysters		X[1]		X[1]		

[1]Cooked to visual doneness recommended by cookbooks
[2]Physically assessed for color and texture
[3]Evaluated for consumer acceptability using 9 point hedonic scale
[4]Reviewed by trained descriptive panel for visual and non-oral texture indicators of doneness

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Chapter 2

Literature Review

Many people enjoy consuming seafood and often prepare it at home and/or purchase it at a restaurant. For the entirety of this thesis the use of “seafood” shall be considered to include edible fish and shellfish, from both fresh water and saltwater and either farmed or wild. The large incidence of food-borne illnesses associated with seafood products suggests that seafood is not being handled and cooked properly. The FDA Food Code (2009) recommends internal temperature guidelines for fish and shellfish to target the destruction of *Salmonella* spp. The required internal temperature guidelines provided for seafood can affect eating quality and physical changes of appearance and structure relative to individual seafood products and culinary processes applied to them. This review discusses the cooking guidelines commonly provided to consumers, and the current knowledge of seafood microbiology and related seafood-borne illness due to pathogens, and descriptions of the physical attributes of texture and color and sensory evaluation as affected by thermal processing.

Microbiology of Seafood and Potential Sources of Contamination

Live, healthy fish are considered to have sterile internal flesh with naturally occurring microflora on the outside of their skin and gills and within their intestinal tract (Mayer and Ward, 1991). Numerous microorganisms natural occur in the water from which seafood is harvested and some can be pathogenic; for example, V*ibrio* spp. are commonly found in estuaries. The introduction of new pathogenic microorganisms into marine environments can result from poor sanitation practices and natural wildlife. Therefore, when seafood is harvested, proper handling, storage, processing, and sanitation must occur to keep microorganisms from multiplying. Pathogenic bacteria such as *E. coli*, *Campylobacter*, *Staphylococus aureus* and *Salmonella* spp., which do not naturally occur in marine environments, can be introduced to harvested seafood due to improper handling (Table 2-1) (Wekell and others 1994; Galavis-Silva and others 2009).

Among the total fish consumed in the United States of America (U.S.) in 2009, 84% was imported (NMFS 2009). Many of the countries that export to the U.S. are developing countries that lack proper internal control systems and several have difficulty meeting quality and safety standards due to insufficient monetary resources to develop quality control, train employees and purchase necessary equipment. Additionally, many of the exporting countries are located in tropical areas that often have increased toxin and bacteria hazards. Various products imported into the U.S. are from aquaculture production and additional research on ways to improve aquaculture sanitation could be beneficial to decrease bacterial contamination (Allshouse and others 2003). Currently, the aquaculture industry uses preventative agents including antibiotics, vaccines, chemotherapeutics and probiotics to inhibit pathogens (Galavis-Silva and others 2009). However, a survey of imported aquatic products, conducted by the FDA, found *Salmonella* spp. to be antibiotic resistant (Zhao and others 2006).

Table 2-1 Pathogens Commonly Associated with Seafood-borne Diseases

Bacterial Pathogen	Naturally Present in Aquatic Environment?
Salmonella spp.	No
Shigella spp.	No
Pathogenic *E. coli*	No
Campylobacter spp.	No
Vibrio cholerae	Yes
Vibrio parahaemolyticus	Yes
Vibrio vulnificus	Yes
Aeromonas spp.	Yes
Plesiomonas spp.	No
Yersinia entercolitica	No
Clostridium botulinum	Yes
Listeria monocytogenes	Yes
Staphlococcus aureus	No

Modified from: Venugopal (2006) and Galavis-Silva and others (2009)

Incidence of *Salmonella*-Related Food-borne Illnesses in the U.S.

According to FoodNet Surveillance preliminary data for 2009, approximately 40% of the laboratory-confirmed cases of all food-borne infections were caused by *Salmonella* spp. In addition, pathogens associated with laboratory-confirmed cases were reported by incidence per 100,000 population with *Salmonella* spp. associated with the highest incidence (15.19/100,000 population) followed by *Campylobacter* spp. (13.02/100,000 population), and *Shigella spp.* (3.99/100,000 population). Among all age groups, *Salmonella* spp. was found to have the highest incidence (72.93/100,000 population) among children < 4 years old in 2009 (CDC 2010a).

Finalized data by the CDC for the surveillance of food-borne disease outbreaks in 2007 identified *Salmonella* spp. as the agent responsible for 5 of the 18 reported deaths (2010b).

Among food-borne outbreaks associated with a single laboratory-confirmed etiology in 2007, *Salmonella* spp. was the second most common agent (CDC 2010b). Surveillance of food-borne illness is limited due to several factors including under reporting, improper diagnoses, illnesses caused by pathogens that have not been identified, and by the ability of many food-borne pathogens to be spread via water or human contact (Mead and others 1999).

Incidence of *Salmonella* spp. in Fish and Shellfish

Between 1993 –1997, the CDC reported 2,751 food-borne disease outbreaks of which 6.8% were related to the consumption of seafood (Olsen and others 2000). An increase of 0.5% in food-borne disease outbreaks associated with the consumption of fin fish and shellfish occurred from 1998 – 2002. Of the outbreak cases during 1998 – 2002 with vehicle transmission by fin fish and shellfish, 1.2% was known to be caused by *Salmonella* spp. (Lynch and others 2006).

Over a 9 year period (1990 to 1998) the FDA tested for the presence of *Salmonella* spp. on imported and domestic seafood including fish (fresh, saltwater), shellfish, crustaceans, other aquatic products and ready-to-eat products containing seafood as the primary ingredient. The FDA determined the incidence of *Salmonella* spp. for imported and domestic seafood to be 7.2% and 1.3% respectively. Of imported seafood sampled, fin fish/skin fish had the highest incidence of *Salmonella* spp. (12.2%) followed by other aquatic creatures (10.9%) and raw crustaceans including shrimp, lobster, prawns, crayfish and langostinos (8.5%) (Heinitz and others 2000).

The FDA inspects imported seafood, rejects seafood shipments that are adulterated and inspects domestic seafood importing facilities as well as foreign processing facilities exporting to the U.S. Analysis of FDA detention data from 2001 for imported seafood products was conducted by Allshouse and others (2003). Among fish and fishery products *Salmonella* spp. was found to be the most frequent contaminate. Additionally, *Salmonella* spp. accounted for 34% of all the adulteration violations. Shrimp and prawns, both farm-raised and wild caught, accounted for over half of the violations for *Salmonella* spp., which was anticipated with the large volume of shrimp imported (Allshouse and others 2003).

Major *Salmonella* spp. outbreaks associated with fish and fish products have occurred outside the U.S. In Japan in 1988, 330 children became infected with salmonellosis from eating cuttlefish containing *S. champaign*. Over a decade later in 1999, two additional outbreaks occurred in Japan. The first resulted in over 400 cases associated from eating dried squid produced at a processing plant with a contaminated water source. The second outbreak was linked to cuttlefish chips contaminated with *S. chester* and *S. oranienburg*, which resulted in over 1,500 cases of salmonellosis (D'Aoust and others 2007).

Thermal Inactivation of *Salmonella* spp. in Fish and Shellfish

Salmonella species are resilient and can adapt to various environmental conditions. They can survive at freezing temperature as well as elevated temperatures (54°C). Several factors can increase the heat resistance of *Salmonella* spp. including a decrease in water activity and the availability of nutritionally rich media. Additionally, exposure of *Salmonella* spp. to sub-lethal temperatures can greatly increase heat resistance. They are facultative anaerobic gram-negative bacteria, which have the ability to grow in a pH range of 4.5 to 9.5 and are typically inhibited by 3-4% sodium chloride (D'Aoust and others 2007; Montville and Matthews 2005). Infectious doses of *Salmonella* spp. have been found to vary from as little as 10^0-10^1 to large amounts such as 10^6-10^7. Many of the foods associated with low infectious doses are high in fat such as chocolate and cheese (Montville and Matthews 2005).

Only a limited number of studies on the thermal inactivation of *Salmonella* spp in fish and shellfish have been conducted (Wan Norhana and others 2010). In order to reduce the bacterial load, specifically *Salmonella* spp, to below infection dose levels, cooking or processing to a minimum temperature for a given time is required. Thermal death time is considered to be amount of time required to kill a particular microorganism at a specific temperature. The decimal reduction time (*D* value) is the amount of time (minutes) at a constant temperature to decrease the number of viable cells by 1 log reduction (90%) (Montville and Matthews 2005). Research to determine the thermal death time at 60°C for *Salmonella typhimurium* in oyster meat has been conducted. The study was able to establish a 5 log reduction (99.999%) at 60°C, while retaining the desired sensory characteristics related to appearance, texture and aroma (Plaza and Gabriel 2008). Using the FDA's current cooking guidelines for intact (63°C for 15 sec) and homogenized (68°C for 15 sec) seafood, Brookmire (2010) tested for the log reduction of *Salmonella* spp. achieved in shrimp and salmon. The findings concluded that the desired three log reduction was achieved by the recommended time-temperature combinations for homogenized products and intact shrimp; however, the intact salmon fillet only demonstrated a two log reduction (Brookmire 2010).

Cooking Fish and Shellfish

Fish must be cooked enough to inactivate pathogens while still maintaining the optimal eating quality of the fish, yet fish tissue is more fragile than beef or pork (NACMCF 2008). Overcooking fish can lead to decreased flavor, dryness, toughness and excessive flakiness (Brown 2007). Under-cooked fish may appear tender and moist, but can lead to food safety concerns such as causing food-borne illness. Microorganisms are most commonly located on the surface of the seafood due to improper handling, but can migrate into a fillet if it exhibits gaping or if the shellfish is not fully intact (Wekell and others 1994; Galavis-Silva and others 2009). While seafood is being cooked to a designated internal temperature, the outside of the seafood is reaching temperatures well above the internal temperature. Therefore, the microorganisms located on the surface will be subjected to a higher heat treatment than those at the thickest internal section of the seafood.

To determine when fish is done cooking, the general rules for cookbooks are that the fish should be opaque in color, flake easily with a fork, and/or the flesh separates from the bone (Brown 2007). The Culinary Institute of America (CIA) (2006) recommends an internal temperature of 63°C (145°F) or until opaque. For shrimp, the color will appear opaque and pink when done. Oysters in their shell will open slightly, but must be cooked for at least 5 minutes from when the shell opens or to 63°C (145°F) (Brown 2007). For shucked oysters, doneness is indicated by the edges curling (Southern Living 1984). However, it must also be considered that consumers may choose to prepare and consume raw or lightly cooked seafood products on occasion (NACMCF 2008). Additionally, consumers may form their own perception of what the seafood product should look like when done cooking (thermal endpoint) based on previous experiences and/or knowledge. Several methods of preparation including steaming, baking, broiling, and poaching can be used for fish and shellfish (Table 2-2), which may each yield obvious or subtle variations in visual cues and texture at the targeted thermal endpoint.

Table 2-2: Methods of Preparation for Selected Fish and Shellfish

Common Name	Common Methods of Preparation
Atlantic Salmon	Baked, broiled, barbecued, fried, steamed or poached
Tilapia	Baked, broiled, fried, poached or steamed
Shrimp	Simmered, baked, broiled, fried, or oven finished; cocktail
Eastern Oysters	Steamed, baked, broiled, fried, sautéed or used in a variety of dishes

Modified from Brown (2007)

Texture

According to Hyldig and Nielsen (2001), producers, processors, and consumers consider the texture of fish to be one of the most important quality parameters. Texture is a difficult attribute to define and several definitions are given throughout scientific literature (Hyldig and Nielsen 2001). For example, Peleg (1987) states that "The characteristics of perceived "texture" are determined by different physical and physicochemical properties of the food and by the unique and complex features of the human sensory systems." Meilgaard and others (2007) define texture as "a sensory manifestation of the structure or inner makeup of products in terms of their:

- Reaction to stress, measured as mechanical properties (such as hardness/firmness, adhesiveness, cohesiveness, gumminess, springiness/resilience, viscosity) by the kinesthetic sense in the muscle of the hand, fingers, tongue, jaw, or lips.
- Tactile feel properties, measured as geometrical particles (grainy, gritty, crystalline, flaky) or moisture properties (wetness, oiliness, moistness, dryness) by the tactile nerves in the surface of the skin of the hand, lips, or tongue."

The texture of fish is affected by several factors such as species, age, diet, and size. Texture also is affected by both postmortem factors including rigor mortis, pH, and glycolysis and external factors such as storage temperatures, cooking treatments, and the addition of table salt (Hyldig and Nielsen 2001). Casas and others (2006) found significant textural differences among three points (tail, back and belly) along raw Atlantic salmon fillets (Figure 2-1). Their results conclude the tail portion of a raw salmon fillet to be firmer than the belly and back portions. Thus, when studying the texture of fish it is important to consider what section of the fillet is being sampled (Casas and others 2006). Sigurgisladottir and others (1999) found that texture varied within raw Atlantic salmon fillets, with both shear force and hardness increasing from the head of the fillet through the tail. They determined the area of the fillet below the dorsal fin to be the most reliable for studying texture. Kong and others (2007) found similar findings in raw Pacific pink salmon with firmness increasing from head to tail. Additionally, they found that cooking Pacific pink salmon caused no noticeable differences in firmness throughout the outer, middle and inner layers (Kong and others 2007) (Figure 2-2).

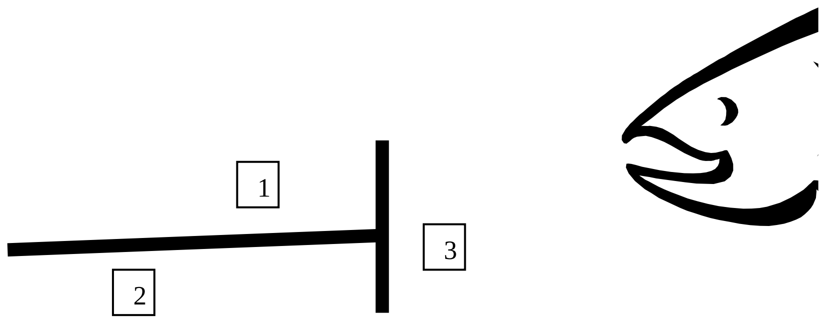

Figure 2-1: Sites of Salmon Fillet Used for Raw Textural Measurements: (1) Back; (2) Belly; (3) Tail Regions. Modified from Casas and others 2006. Image source Microsoft® Word® clip art (April 5, 2011) and adapted by Renée Felice.

Figure 2-2: Sampling Layers for Measuring Texture of Raw and Cooked Pacific Pink Salmon: (White) Inner, (Gray) Middle and (Black) Outer Layer. Modified from Kong and others 2007. Image source Microsoft® Word® clip art (April 5, 2011) and adapted by Renée Felice

Instrumental Texture Analysis

When choosing a texture method for evaluating a seafood product it is important to consider the material and purpose of the study. The seafood product's size, structure and fragility influence selection of texture test methods. A clear understanding of the purpose of the study is necessary for selecting the appropriate test method. For example, identifying properties to be measured, the speed of the sampling, destruction or nondestruction of the sample must be considered before choosing a test method (Sanchez-Alonso and others 2010). Several methods are available and often differ in the properties they measure. Kramer shear cell (KSC) test measurements involve a combination of compression, shearing, and extrusion; similar forces are created between the molars during chewing (Dunajski 1979). The Warner-Bratzler (WB) blade test performs a cutting motion similar to a guillotine, which involves both compression and shearing, comparable to the front incisors cutting through food. Texture profile analysis (TPA) test is a series of two compressions designed to mimic chewing (Sanchez-Alonso and others 2010). Instrumental texture analysis can be used to mimic both oral and non-oral texture evaluation. The above methods described are used to imitate oral evaluation. The settings for a compression test, such as TPA can be adjusted to allow researchers to achieve oral and non-oral texture evaluation. For example, a compression test with only 30% strain would results in none to limited destruction and mimic applying pressure with an index finger ("finger test"), compared to 75% compression which would imitate with oral compression between molars (Veland and Torrissen 1999).

Instrumental Texture Analysis of Fin Fish

The majority of studies that measure texture instrumentally study the texture of raw seafood, while few studies focus on the texture of cooked seafood. Methods used for raw fin fish include texture profile analysis (TPA) and the Warner-Bratzler (WB) blade, which have not typically been applied to cooked seafood due to the change in the structure of the fish when cooked (Dunajski 1979). Heating dissolves the connective tissue that holds the structure of the fish together, causing the fish to flake easily apart and making it difficult to handle (Hyldig and Nielsen 2001).

Dunajski (1979) recommended using Kramer Shear Cell (KSC) compression test with the thin blade version of the cell (CS-2) and a large sample size to measure the texture of cooked fish. Additionally, homogeneity and randomness of the sample to the KSC blades can be increased by dismembering the muscle blocks (myotomes), allowing a smaller sample weight to be used (Dunajski 1979). Kong and others (2007) measured the texture of cooked salmon using a multiple thin blade attachment, which was similar in design to the KSC; they allowed their samples to cool to room temperature and placed the cooked fibers of the sample perpendicular to the blades for the test. The multiple thin blade design developed provided for greater sensitivity and the use of a smaller sample size compared to KSC (Kong and others 2007). Morkore and others studied the texture of raw and baked farmed Atlantic salmon using a flat-ended cylinder compression test and Warner-Bratzler. Baked fillets were cut into 4 x 4 x 4 cubes and measured using the WB knife once the fillets cooled to room temperature (20°C). The baked samples were not evaluated with the flat-ended cylinder due to the myotomes slipping under compression (Morkore and others 2009). Hatae and others (1984) studied cooked fish muscle of five species by measuring firmness with a 13 mm aluminum plunger attachment, cohesiveness using the same aluminum plunger with two consecutive biting tests, and penetration with a pointed needle loaded with 8.5g. Botta (1995) reported punching and shearing by puncture tests and KSC to be the general popular methods for determining the texture of cooked seafood compared to analyzing raw fillets for springiness and hardness.

Instrumental Texture Analysis of Shellfish and Mollusks

Limited studies on the texture of cooked shellfish and mollusks are available. Of the studies found, the most common test methods used for cooked product included compression tests and shearing using a WB blade. Meinert and others (1999) measured the texture of restructured shrimp, which had been battered and deep fried. Using a Stevens LFRA Texture Analyzer with a shear plunger attachment of 2.8 mm thick, 30 mm high, and 60 mm long, the plunger traveled 75% of the samples height and performed two penetrations per shrimp sample (Meinert and others 1999). Niamnuy and others (2007) studied the hardness of the thickest segment of boiled, deshelled shrimp using a compression probe to a depth of 70% deformation at a speed of 50 mm/min and found the hardness increased with the length of time shrimp were boiled. The toughness of the boiled shrimp was also measured using the WB at a crosshead speed of 50 mm/min. Schubring (2009) measured tenderness of cooked shrimp using a modified WB shear cell, cutting across the length of the shrimp tail. Plaza and Gabriel (2008) measured the softness of raw and heated oysters and found significant differences ($p < 0.05$) using a penetrometer with a large needle attachment, measuring the millimeters traveled by the needle over 10 sec.

Color

The color of raw seafood products is an indicator of quality for consumers, who have certain expectations of the color that particular seafood products should possess (Robb 2001). According to Hunter and Harold (1987), the human eye can discriminate and distinguish very small differences between colors when compared next to each other. However, humans have a poor color memory and the human eye can not make quantitative measurements of color (Hunter and Harold 1987). Viewing conditions including the light source or colors in the object's surroundings, can highly affect the perceived color of an object (Skrede and Storebakken 1986).

Methods for Measuring the Color of Fish and Shellfish

Several instrumental methods are available for measuring color of seafood products. Colorimeters, chroma meters, and computer vision systems (CVS) or machine vision systems (MV) are common instrumental methods for measuring color. Most of these tools measure in CIE L^*, a^*, and b^* values (Kong and others 2007; Erikson and Misimi 2008). In the CIE L^*, a^*, and b^* value system, L^* measures lightness on a 100 point scale, a^* measures redness ($+a^*$) to greenness ($-a^*$) and b^* measures yellowness ($+b^*$) to blueness ($-b^*$) (Hallier and others 2008; Skrede and Storebakken 1986).

Many colorimeter and chroma meters are portable and provide relatively quick results; however, they analyze a small portion of an object and many replications across the surface of an object must be conducted to estimate the color (Kong and others 2007; Erikson and Misimi 2008). Therefore, colorimeters and chroma meters are best suited for samples with uniform color distribution.

To analyze the whole area of a sample CVS or MV are recommended and can be used for both raw and cooked products. The CVS method utilized for detecting the color of raw Atlantic salmon fillets as described by Erikson and Misimi (2008), was comprised of the use of a light box with two fluorescent tubes as its light source and a digital camera mounted at the top of the box to capture a photo of the entire fillet situated inside the box. The CVS methods had several advantages including fast results that were both nondestructive and contact free, along with the capability to evaluate the color of an entire fillet using computer software (Erikson and Misimi 2008). The MV method described by Balaban (2008) allows the researcher to understand the nonhomogeneity of colors within a sample. Typically, a MV system allows images of the sample to be analyzed by individual pixels by the computer software. Color blocks are often used for nonuniform samples as a way to reduce the number of possible colors that can be detected. There are more than 16 million colors using the RGB (red, green, and blue) color model and color blocks are designed to reduce the potential number of colors that can be identified. On average the more color blocks utilized, the more nonhomogeneous the sample. The computer software calculates the number of pixels that fall within the range of a given color block and calculate the percentage of that color based on the total amount of pixels in the sample. A final RGB color reading is determined for the overall sample, which can be converted into CIE *L**, *a** and *b** values (Balaban 2008).

As discussed previously, a machine vision (MV) system can be used to determine the color of nonhomogeneous objects without destroying the sample or having to measure several sections of the sample; however the method is costly (Balaban 2008). For seafood products with inconsistent color, such as shrimp, a ground homogenized sample may be measured for color. A portion of the seafood product can be chosen for sampling purposes or the whole seafood product can be used. Once the seafood product sample is homogenized, it should be placed in a measuring cell and a colorimeter can be used to measure the color, with several measurements taken over the area of the cell. It is important to note that homogenizing the sample can alter both the light scattering properties of the original sample as well as the overall visual characteristics (Botta 1995). Schubring (2009) performed color analysis on shrimp, following the previously described protocol, using comminuted shrimp muscle and a colorimeter. Niamnuy and others (2007) also measured the color of shrimp using a colorimeter; however, they sampled a whole shrimp by taking several measurements along the length of the shrimp and averaging the CIE *L**, *a**, and *b** values.

For raw (or cooked) Atlantic salmon, a Roche *SalmonFan*™ color wheel can be used; it contains 15 color cards ranging in color from gray-pink to deep red (Kong and others 2007; Erikson and Misimi 2008). The amount of pigments in a raw fish also can be used to determine the color, such as the levels of carotenoid pigments in salmon. Spectrophotometry and high pressure liquid chromatography (HPLC) are two methods that can be used to measure the level of pigments (Robb 2001).

Factors Affecting the Color of Atlantic Salmon and Shrimp

According to Botta (1995), the flesh color of seafood, within their respective species, can be affected by the location, method and season of catching as well as handling and storage methods. Raw Atlantic salmon flesh has a characteristically deep reddish-orange color, which is due to astaxanthin and cantaxanthin pigment content (Erikson and Misimi 2008). Astaxanthin produces the pink-red pigment naturally occurring in wild Atlantic salmon and can also be found in algae, yeast, crustaceans, and plants including paprika, pimentos and red peppers. The amount of astaxanthin varies by species of salmon and between wild versus farm-raised. For example, wild Atlantic salmon contains approximately 3 – 11 ppm compared to farm-raised Atlantic salmon which usually contains 4 – 6 ppm of astaxanthin. Cantaxanthin is an orange-reddish pigment found in small amounts in wild salmon and is naturally occurring in algae, crustaceans and chanterelle mushrooms (Hardy 2005; Nickell and Springate 2001). Astaxanthin and cantaxanthin are both carotenoid pigments that can be added to the feed of farm-raised Atlantic salmon to provide its characteristic red-orange color (Hardy 2005). Paler color perception can occur with high fat content in the fish or in specific regions of the fish such as the belly flap (Christiansen and others 1995; Aursand and others 1994). Heating causes the protein in the fish to denature and coagulate causing the translucency of the fish to disappear and tilapia becomes white and salmon becomes a pale shade of red-orange (Charley and Weaver 1998).

Several types of pigments are found within crustaceans including hemoglobins, hemocyanins, flavins, cytochromes, carotenoids, melanins, ommochromes, and rhodopsin (Latscha 1989). However, carotenoids are responsible for the visual color associated with crusteaceans. Carotenoids can not be synthesized by crustaceans and must be supplied by the diet. Astaxanthin found in salmon contributes to the red-pink color exhibited in cooked shrimp and farm-raised shrimp are fed diets containing astanxanthin and cantaxanthin (Kim 2010). In shrimp and other invertebrates, carotenoids bind with proteins to form a more stable complex referred to as carotenoproteins, often producing a color that differs from the original pigment. Cooking cause the carotenoprotein to denature, releasing the carotenoid (ie. astaxanthin) and producing the red color commonly associated with cooked crustaceans (Kalogeropoulos and

Chiou 2010). The change in color between the raw and cooked shellfish is a visual cue of doneness commonly referred to in cookbooks and culinary textbooks (Brown 2007; Rombauer, and others 1997; Southern Living 1984)

Sensory Analysis

The sensory quality of fish and shellfish can be influenced by several factors including species, age, diet, and handling from harvest to final processing to consumer. During sensory evaluation of seafood attributes of appearance, odor, flavor and texture are considered. Human subjects evaluate samples using their senses of sight, smell, hearing, taste, and touch. Both objective and subjective sensory tests can be performed. Objective sensory evaluation includes discriminative tests (triangle test, simple difference test) and descriptive sensory tests (quantitative descriptive method (QDA®), The Spectrum™ method). Subjective tests measure the consumers (untrained panelists) response to a sample such as how much they like/dislike a sample or their preference of one sample over another (Hyldig 2010; Meilgaard and others 2007).

Descriptive Sensory Test Methods

Common descriptive analysis methods include the quantitative descriptive method (QDA®), The Spectrum™ method, the Texture Profile method, the Flavor Profile method and modified versions of each are often used (Meilgaard and others 2007). Many studies have used trained descriptive panels to evaluate sensory characteristics of cooked fish and shellfish. Trained descriptive panels have qualitative and quantitative components. The qualitative portion involves panelists describing attributes and characteristics of the product forming sensory descriptors or terms. Panelists come to an agreement of terms and their definitions as they relate to appearance, aroma/odor, flavor, and texture of the product (Meilgaard and others 2007; Hyldig 2010).

Edmund and Lillard (1979) conducted an 18-person trained panel to determine descriptive terms associated with the aroma, taste and texture of oysters, clams, and shrimp. Descriptive terms selected by panelists to describe the texture of oysters included gritty, chewy, fibrous, dry and rubbery. The quantitative component is used to measure the intensity or degree of which each descriptive term is present. For example, a 15 cm unstructured line scale with reference points for the extremes of each attribute is commonly used for QDA® (Meilgaard and others 2007). Edmund and Lillard (1979) chose to have panelists indicate whether a descriptive term never applied, sometimes applied, or usually applied.

Ostrander and others (1976) used a trained descriptive panel (n = 8) to determine descriptive characteristics of odor, texture, moisture, flavor, color, fiber structure and brownness along the lateral line of baked pen-reared salmon and trout. The researchers wanted to determine if the previously mentioned characteristics varied due to water source (fresh, salt) between salmon species. Fillets were baked at 205°C (400°F) for 20 – 30 min in a rotary hearth oven. Panelists received each sample individually while still hot (served within 10 min of being removed from oven). Each characteristic had its own 9, 7 or 5 point scale with designated descriptions associated at each odd number throughout the scale. For example, texture was expressed as a 9 point scale with 1 = "mushy gelatinous", 3 = "slightly mushy", 5 = "slightly soft, fibers barely hold together", 7 = "moderately firm, flakes with slight pressure", and 9 = "firm, flakes with pressure". Organoleptic characteristics of each sample were evaluated under red light to mask visual color differences between samples. Visual characteristics related to color, fiber structure and lateral line were evaluated under daylight (Ostrander and others 1976).

Warm and others (2000) conducted a trained descriptive panel (n = 9) to develop sensory vocabulary including descriptive terms for odor, appearance, taste and texture that could be used for evaluating five fish species including: cod, saithe, rainbow trout, herring, and flounder. Sample preparation included heating each sample in its own porcelain bowl with lid in a convection oven; no internal temperature or oven temperature was provided. An unstructured line scale (15 cm) was used to evaluate each sample (Warm and others 2000).

Sensory Evaluation Methods for Determining Non-Oral Texture Characteristics

Non-oral texture measurements, such as flakiness and firmness, are frequently used as indicators of doneness in fish and shellfish. Instrumental methods for evaluating the texture of seafood can be manipulated to mimic non-oral texture evaluation as previously discussed. Limited studies have used non-oral texture measurements for evaluating food products and currently no studies have been identified for seafood. Studies found using non-oral texture methods were in the area of dairy research. Drake and others (1998) studied non-oral and oral texture evaluation of cheeses using a trained panel (n = 11). Terms evaluated non-orally by hand included rubbery/springy, firmness, brittleness/crumbliness, sticky and slipperiness of film. To determine firmness by hand panelists were instructed to “Press your thumb all the way through the unworked sample. Firmness is the force required to compress the sample completely” (Drake and others 1998). The results indicated that both oral and non-oral methods could be used to discriminate the texture of cheeses (Drake and others 1998). Pereira and Singh (2003) examined acid milk gels for oral and non-oral texture characteristics. Measuring non-oral firmness was described as “force required to compress gel, before fracture and permanent structural damage, using the middle finger.” Results indicated that both oral and non-oral methods were capable of discriminating between gels and the interrelationship between single non-oral and oral attributes was high ($p < 0.01$) based on correlation analysis (Pereira and Singh 2003).

Consumer Sensory Testing Methods

Consumer sensory panels consisting of untrained panelists can be utilized for evaluating seafood based on acceptance, preference or discrimination between products. Understanding the purpose of your study in necessary in determining which test to conduct. Acceptance tests using the 9 point hedonic scale can determine overall liking of seafood products. Preference tests force panelists to choose one sample over another, but do not provide information on how well each product is liked. Finally, discrimination test are used to verify if differences can be detected between samples, either overall differences or specific attribute differences (Meilgaard and others 2007).

Several studies conducting sensory evaluation of fish and shellfish include both a trained descriptive panel and a consumer panel. As previously discussed, Ostrander and others (1976) conducted a trained descriptive panel for salmon and trout. Significant differences in characteristics identified by the trained panel were tested by consumers. The consumer panel ($n = 150$) included the collection of demographic data, the evaluation of appearance characteristics of raw salmon and trout and evaluation of baked salmon and trout in a preference test using a scale with five faces to indicate like to disinterest to dislike (Ostrander and others 1976). Durance and Collins (1991) studied the quality of late-run chum salmon (sexually mature) after being retorted in a can compared to a retortable pouch. Typically, late-run chum salmon is not consumed by humans due to a decrease in sensory quality related to late-run flavor characterized as burnt, sour, and muddy and texture. A trained panel evaluated texture and flavor differences in addition to overall acceptability separately. Panelists were asked to evaluate overall acceptability using their previous knowledge and experience with canned salmon. Overall acceptability was found to be higher for the retortable pouch samples, with late-run flavor having the most influence on overall acceptability (Durance and Collins 1991).

Limited studies on determining consumer acceptability of fish and shellfish at various internal endpoints and/or cooking methods are available. Charley (1951) studied the effects of internal temperature and oven temperature on baked salmon. A panel of four judges evaluated flakiness, moistness, tenderness, flavor and overall desirability of each sample. First, judges evaluated baked salmon fillets with an internal temperature of 75°C that had been cooked at four different oven temperatures including: 177°C (350°F), 204°C (400°F), 232°C (450°F), and 260°C (500°F). The judges determined no difference between the palatability of the salmon at the various oven temperatures. Secondly, the judges evaluated for the previously described characteristics for four samples of baked salmon cooked at 204°C (400°F) to different internal endpoints including: 70°C (158°F), 75°C (167°F), 80°C (176°F), and 85°C (185°F). No difference in palatability was detected between 75°C and 85°C. Salmon baked to 70°C ranked the lowest in overall desirability and was considered undercooked and salmon baked to 85°C ranked high in flavor, but lower in moisture compared to 70°C and 75°C (Charley 1951).

Conclusion

A large amount of fish and shellfish is consumed yearly in the U.S. and a high level of food-borne illness has been related to seafood consumption. Improper food handling behaviors and limited generic guidelines to assist consumers and food service professionals in cooking seafood increase the risk of food-borne illness occurring. Educating consumers on safe food handling practices and proper cooking of fish and shellfish is needed. Improved guidelines for determining doneness of specific seafood products related to internal temperature and visual and non-oral textural characteristics would help reduce related food-borne illnesses.

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Chapter 3

Relationship between Internal Temperature and Physical Properties of Texture and Color for Fish and Shellfish

Abstract

To prevent food-borne illness, it is recommended fish and shellfish be cooked to 63°C for 15 seconds. Physical characteristics of fish and shellfish prepared by culinary processes to this minimum internal temperature are unknown (FDA 2009). Consumers consider texture and color to be indicators of quality and doneness for cooked fish and shellfish. The delicate nature of cooked fish and shellfish and the often non-homogenous color prevent challenges for evaluating these physical properties. Atlantic salmon (baked, poached, broiled), tilapia (baked, pan-fried, broiled), shrimp (boiled, broiled) and oysters (broiled) were studied at two internal endpoint temperatures (64°C ± 1 and 74°C ± 1). Texture was analyzed using Texture Profile Analysis (30% compression), Kramer Shear Cell and Warner-Bratzler blade. As internal temperature increased, a significant ($p < 0.05$) increase in hardness measured by TPA was determined for baked salmon, broiled tilapia, pan-fried tilapia and boiled shrimp. Cook loss (%) was calculated; a positive correlation between cook loss (%) and internal temperature was identified. Non-oral texture attributes of firmness, flakiness and springiness were evaluated by a trained descriptive panel ($n = 7$) for baked salmon, baked tilapia and boiled shrimp. High degrees of flakiness for baked tilapia and firmness for baked salmon were associated with safe internal temperature. Color was measured using a colorimeter and Adobe Photoshop. Quantifying physical attributes of texture and color for fish and shellfish prepared by culinary processes to a safe internal temperature range (64°C - 74°C) will aid in the development of specific descriptions of doneness to supplement cookbooks, online recipe websites and cooking instructions located on fish and shellfish product packaging.

Introduction

Limited studies have evaluated physical attributes including texture and color of fish and shellfish prepared by different culinary processes. Many challenges have been found to be associated with measuring the texture of cooked seafood (fish and shellfish). For example, when heating, the connective tissue is gelatinized and dissolves creating a delicate structure susceptible to falling apart under light compression or movement (Hyldig and Nielsen 2001). Methods commonly used for raw seafood texture analyses were chosen because they have been used for evaluating raw red meat. However, the delicate structure of cooked fish and shellfish is substantially different then red meat and must be taken into consideration (Dunajski 1979). Before choosing a method it is important to determine the purpose of the study, which can narrow possible method options. For example, Texture Profile Analysis (TPA) is a double compression test designed to mimic chewing (Veland and Torrissen 1999). Kramer Shear Cell (KSC) measurements involve a combination of compression, shearing, and extrusion; similar forces are created between the molars during chewing (Dunajski 1979). The Warner-Bratzler (WB) blade performs a cutting motion similar to a guillotine, which involves both compression and shearing, comparable to the front incisors cutting through food (Sanchez-Alonso and others 2010).

Texture can be measured by destructive and non-destructive tests and can mimic oral and non-oral texture evaluations. Non-destructive methods are commonly used to correlate with non-oral texture evaluations. For example, a compression test (ie TPA) with 30% strain would result in none to limited destruction and mimic applying pressure with an index finger (“finger test”) (Veland and Torrissen 1999). Evaluation of cooked products for doneness requires non-oral texture methods with minimum destruction. Generic cooking guidelines suggest “firm” as an indicator of doneness, which may lead consumers to apply pressure with their fingers to test for firmness of the product. Additionally, it is recommended to consumers to apply pressure with a fork to determine if fish is flaky and muscle segments separate easily from each other (Brown 2007; CIA 2006; Southern Living 1984; Ramette and Sternberg 1998; Schiel 1994; Rombauer, Becker and Becker 1997). The FDA Food Code (2009) recommends heating fish and shellfish to an internal temperature of 63°C for 15 min for determining doneness, which would require a meat thermometer and cause minimal destruction to the product.

Consumers consider the color of raw fish and shellfish as an indicator of quality (Robb 2001). Multiple methods for measuring the color of seafood are available. Colorimeters are portable and provide relatively quick results for products with uniform color. However, they analyze a small portion of an object and many replications across the surface of an object must be conducted to estimate the color (Kong and others 2007; Erikson and Misimi 2008). Many seafood products are non-homogeneous in color (ie shrimp) and methods for evaluating color are more costly and time consuming. Computer Vision Systems (CVS) and Machine Vision Systems (MV) are non-destructive methods for measuring the color across a whole sample uniform or non-uniform in color. The methods use computer software to analyze photographs of the sample (Erikson and Misimi 2008; Balaban 2008).

The primary research objective of this project was to determine physical characteristics of two fish and two shellfish products cooked to two endpoints (64°C ± 1 (147°F) and 74°C ± 1 (165°F)) using popular consumer cooking methods. Two studies supporting this objective were conducted.

Study 1 Objective: To measure texture, color and cook loss for two seafood and two fish products prepared using popular consumer cooking methods (Table 1) to two internal endpoints (64°C ± 1 (147°F) and 74°C ± 1 (165°F)).

Study 2 Objective: To evaluate non-oral texture characteristics of doneness for baked Atlantic salmon, baked tilapia, and boiled shrimp prepared to two internal temperature endpoints (64°C ± 1 (147°F) and 74°C ± 1 (165°F)) using descriptive sensory analysis.

Materials and Methods

Fish and Shellfish Samples and Storage

All fish and shrimp were purchased frozen from BJ’s Wholesale Club, transported on ice in coolers, and stored at -20°C (Table 3-1). Jars of shucked raw oysters in a liquid mixture of water and oyster liquor were purchased from a local seafood store in Hampton, VA, shipped on ice overnight and stored at 4°C. These specific fish and shellfish products were chosen to mimic what an average consumer would have available to purchase and prepare.

Table 3-1: Product Information for Fish and Shellfish Used in This Study

Product	Product Description	Brand	Product Information
Atlantic salmon	Farm-raised, skinless, boneless, frozen, individually vacuum packed	Orca Bay Orca Bay Seafoods, Inc. Renton, WA	Net Wt. 2 lbs, about 8 fillets per package
Tilapia	Farm-raised, skinless, boneless, frozen, individually vacuum packed	Berkley & Jensen BJWC Natick, MA	Net Wt 2.25 lbs, about 6 fillets per package
Shrimp	Extra jumbo, individually frozen, uncooked, de-veined, de-headed, shell on	Berkley & Jensen BJWC Natick, MA	Net Wt 2 lbs, 21-25 per pound
Oysters	Grade: Selects, shucked, wild caught, fresh, raw, packed in jar with a liquid mixture of water and oyster liquor	Bevans Oyster Co., Inc Kinsale, VA	16 fl. oz. (1 pint), about 25 oysters per jar

Characterization of Fish and Shellfish Composition

Raw Atlantic salmon, tilapia, shrimp and oysters were analyzed for fat, protein and moisture content. Sampling material included 2 tail and 2 center-cut fillets of Atlantic salmon, 3 tilapia fillets, 10 shrimp and 7 oysters. All samples were analyzed raw and reduced to a homogenous sample using a meat grinder, except for oysters which were cut in half.

For fat analysis the ground raw product (4 g of salmon, 6 g of tilapia or shrimp) or half of an oyster (5 – 8 g) was added to Whatman® filter paper (90 mm diameter), folded and placed in thimble. Thimbles were placed in freezer (-10°C) until completely frozen (3 – 5 hr). Once frozen, thimbles were freeze-dried for 48 hours. Moisture content was determined by the weight difference of the raw sample to the freeze dried sample. Fat analysis was conducted using ether extraction of freeze-dried samples (modified AOAC 991.36, 1998). Protein was determined using ground raw product or half an oyster by measuring nitrogen content using the Kjeldahl Method (modified AOAC 981.10, 1998). Three replications of each product were performed per method except for oysters which had four replicates for protein analysis.

Internal Temperature Measurement

Internal temperature (IT) was measured at the thickest section of each fish and shellfish product during cooking or when removed from heating (ie boiling, poaching). The temperature was recorded only after it remained constant for 10 sec. A data logger (ECD® Series 5000, Model 5100 LoggerLab) was used to track internal temperatures of fish and shellfish. Hypodermic needle thermocouples (Omega Engineering, Inc. probe model HYP-2: standard (21 gage) hypodermic needle 38 mm (1.5 in) long, type T (Copper-Constantan) element, and 1.2 m (4 ft) PFA-insulated lead wires) were used to track IT. A variety of thermocouple probes were evaluated and tested during preliminary studies. The probes selected were chosen for their durability and ability to be used for all fish and shellfish products tested.

Study 1: Physical Assessment of Texture, Cook Loss and Color

Sample Preparation

Two fish and two shellfish products were prepared using popular consumer cooking methods (Table 3-2) to two internal endpoints (64°C ± 1 and 74°C ± 1). All fish and shellfish samples were thawed and stored under refrigerated temperatures. Before cooking, the fish and shellfish were rinsed with cold water and patted dry with paper towels to remove excess moisture. For each sample the raw weight (g) was recorded and the thickest section (mm) of the product was measured using a caliper (Appendix A). Cooking protocols were established for all culinary methods tested based on popular cookbooks and culinary textbooks (Table3-3). Once cooked to the appropriate endpoint with a hold time of at least 15 sec at the endpoint, the internal temperature was recorded, the sample was patted with paper towels and the weight was measured. Next, each sample was photographed and the color was measured. Finally, samples were cooled to room temperature (22 – 24°C (72 – 75°F)) to prevent the effect of temperature variation among samples on texture analyses.

Table 3-2: Culinary Processes for Fish and Shellfish Products

Fish and Shellfish Products	**Culinary Processes for Evaluation**
Atlantic Salmon	Poach, Bake and Broil
Tilapia	Broil, Pan-Fry (no breading) and Bake
Shrimp	Boil and Broil
Oysters	Broil

Table 3-3 Detailed Instructions for Preparing Fish and Shellfish by Individual Culinary Processes

Culinary Process	Products	Culinary Process Description
Baking[1]	Atlantic salmon & tilapia	• Oven was preheated to 375°F • Fillets were placed in a glass baking dish lightly coated with cooking spray • Baking dishes were placed on the middle rack of the oven
Poaching[1]	Atlantic salmon	• Poaching ingredients: 1/2 tbsp butter, 1/3 cup celery, 1/3 cup carrots, 1/3 cup onion, ½ white wine and 5 cups water (1200 mL) • Sauté veggies with butter for 5 min then add water and wine • Water was heated to between 160 – 180°F (never allowed to boil)
Pan-Frying[2]	tilapia	• Tilapia was added to an electric skillet pre-heated to 350°F coated with a thin layer of vegetable oil • The product was allowed to cook on one side to a golden brown color before being flipped. The second side will be cooked to a brown color and until the internal temperature reaches the desired endpoints. • No breading was used
Boiling[1]	shrimp	• Water was brought to a low boil with gentle bubbles barely breaking the surface, around 180°F - 185°F • Shrimp were added to the water once it reached the desired temperature • After cooking and the internal temperature was measured the shrimp were submerged in a ice water bath

Note: A minimum hold time of 15 sec at the endpoint (64°C ±1 or 74°C ±1) was experienced.
[1]Brown 2007
[2]CIA 2006

Cook loss

Moisture in fish and shellfish lost during cooking was calculated as cook loss. Cook loss (%) was determined using the weight of the raw sample (g) and the weight of the cooked sample (g).

$$\text{Cook Loss (\%)} = \frac{\text{Weight Before (g)} - \text{Weight After (g)}}{\text{Weight Before (g)}} \times 100$$

Photo Documentation

Photo documentation for each sample was taken after the internal temperature and weight of the cooked sample was measured. The sample was photographed under north daylight at several angles using a Canon PowerShot SX110 IS Digital Camera (ISO 400, manual setting, 1/125, whiteness balance – Fluorescent H, macro setting, F 4.0).

Color Assessment

Color was measured using the portable tristimulus colorimeter (Minolta Colorimeter Model CR-300, Minolta Corporation, Japan) on the surface of the seafood/fish product in four locations for oysters, eight locations for shrimp, 13 locations for tilapia, and 10-12 locations for Atlantic salmon fillets (see Appendix B for location diagrams). Adobe® Photoshop® (CS5 Extended, Version 12.0 x 32) was used to measure the overall color of the samples from pictures captured during photo documentation. The top portion of the sample was outlined using the magnetic lasso. The selected area was then filtered using blur and average settings to average the color of each individual pixel within the selected area. Using the dropper tool, the color was measuring for the area with CIE *L*, a*, and b** values displayed in the histogram. The color analysis using Adobe® Photoshop® is similar to the computer vision system (CVS) discussed in the literature review except for the computer software used and the set-up to capture the picture.

Texture Assessment

Based on a literature review and preliminary results, texture methods were determined for each product including Texture Profile Analysis (TPA), Kramer Shear Cell (KSC) and Warner-Bratzler blade (WB). Each texture method quantifies different texture attributes such as firmness, toughness, chewiness and springiness. The texture of Atlantic salmon and tilapia was evaluated using TPA and KSC, while shrimp and oyster texture was measured using TPA and WB (Table 3-4). Test settings and sample location and/or preparation were standardized for each product and texture method (Table 3-5 and Table 3-6).

Table 3-4: Texture Analysis Methods Applied and Attributes Measured for Fish and Seafood for Study 1

Products	Characteristic Being Measured	Method of Measurement	Attachment / Probe
Atlantic Salmon & Tilapia	Firmness	KSC	5 Blade KSC
	Hardness, Springiness, Chewiness, Cohesiveness & Resilience	TPA	TA-23 - rounded end stainless steel, ½” diameter, ¼” radius
Shrimp & Oysters	Toughness / Tenderness	WB	Warner-Bratzler Blade
	Hardness, Springiness, Chewiness, Cohesiveness & Resilience	TPA	TA-23 - rounded end stainless steel, ½” diameter, ¼” radius

Note: Kramer Shear Cell (KSC), Texture Profile Analysis (TPA) and Warner-Bratzler Blade (WB)

Table 3-5: Texture Analysis Test Settings and Sampling for Fish

Product	Texture Profile Analysis		Kramer Shear Cell	
	Setting	Sample Location	Setting	Sample Preparation
Atlantic Salmon	• Test and post test speeds: 1 mm/s • 30% strain/ compression	• Fillet was kept intact; • Measurements were taken at the thickest section of fillet • Measured at two spots	• Test speed: 60 mm/s • Blades traveled through sample and reappeared through bottom of the cell	• Sampled at thickest section of fillet; • Recorded dimensions of section • Flaked sample into individual muscle segments (myotomes) • Measured two samples of flakes weighing 23±1g each • Spread one sample evenly along the bottom of the cell covering all slits
Tilapia	• Test and post test speeds: 1 mm/s • 30% strain/ compression	• Fillet was kept intact; • Measurements were taken at the thickest section of fillet • Measured at two spots	• Test speed: 60 mm/s • Blades traveled through sample and reappeared through bottom of the cell	• At the thicket section of the fillet, a rectangular sample of 45mm x 65 mm was cut • Measured dimensions and weight of sample • Positioned sample in the center of the cell

Note: See Appendix C for diagrams of sampling location and/or sample preparation

Table 3-6: Texture Analysis Test Settings and Sampling for Shellfish

<table>
<tr><th rowspan="2">Product</th><th colspan="2">Texture Profile Analysis</th><th colspan="2">Warner-Bratzer</th></tr>
<tr><th>Setting</th><th>Sample Location</th><th>Setting</th><th>Sample Location</th></tr>
<tr><td>Shrimp</td><td>• Test and post test speeds: 1 mm/s
• 30% strain/ compression</td><td>• Kept shrimp intact;
• Positioned shrimp so it appeared as a question mark (?)
• Measured at the second segment of the shrimp</td><td>• Test speed: 50mm/min
• The blade traveled completely through the sample</td><td>• Trimmed shrimp down to have only segments 1, 2 and 3 remaining;
• Positioned shrimp on its side with the blade over the second segment;
• The deveined part of the shrimp was on the right</td></tr>
<tr><td>Oyster</td><td>• Test and post test speeds: 1 mm/s
• 30% strain/ compression</td><td>• Kept oyster intact;
• Measured at the thickest section of the belly (adducter muscle)</td><td>• Test speed: 50mm/min
• The blade traveled completely through the sample</td><td>• Kept oyster intact;
• Centered the blade over the belly of the oyster (adducter muscle)</td></tr>
</table>

Note: See Appendix C for diagrams of sampling locations and/or sample preparation

Sampling

For fish samples, 12 replications (6 reps at 64°C ± 1 and 6 reps at 74°C ± 1) were used per texture method evaluated, per product, and per culinary process. Atlantic salmon fillets purchased had various cuts of fillets that were categorized as center-cuts and tail portions. Equal amounts of center-cut and tail portions were tested (ie 3 reps of center-cuts and 3 reps of tails were tested at 64°C). For oysters, 10 replications (5 reps at 64°C ± 1 and 5 reps at 74°C ± 1) were evaluated per texture method and per culinary process. For shrimp, 16 replications (8 at 64°C ± 1and 8 at 74°C ± 1) were evaluated per texture method and per culinary process.

Study 2: Non-Oral Texture Characterization

Non-oral texture characterizations for baked Atlantic salmon, baked tilapia, and boiled shrimp at two internal endpoints (64°C ± 1 and 74°C ± 1) was conducted using a trained descriptive panel (n = 7). The product and culinary method combinations were chosen because they are popular among consumers. The Virginia Tech Institutional Review Board approved the sensory study (IRB 10-152, Approved March 3, 2010, Appendix D). Protocols for descriptive trained panel followed those described by Meilgaard and others (2007). Sensory training for the descriptive panel took place in the sensory laboratory kitchen of the Food Science and Technology (FST) department at Virginia Tech. Sensory testing was conducted in individual booths in the sensory laboratory. Information was collected using an unstructured 15.2 cm (6 inch) line scale on paper scorecards. Recruitment of panelists for trained descriptive panel was conducted by email and using a survey with Virginia Tech's online survey system (www.survey.vt.edu) (Appendix E).

Panelist Training

Seven panelists (4 males, 3 females) were selected based on availability, willingness to participate, and indication that they cook and consume fish and shellfish at least 1-2 times a year. A consent form outlining the details of the study and expectations of participants was given to panelists to review and sign on the first day of training (Appendix F). Training sessions occurred twice a week for 1 hour and lasted 4.5 weeks (9 hours). Three non-oral texture characteristics were pre-determined including firmness, flakiness, and springiness. During the first week of training panelists were instructed on how to evaluate for the different attributes and practice applying them to reference samples (Figure 3-7). Reference samples used for training were chosen based on their ability to express the attributes at levels within the range or at the extremes of those exhibited by fish and shellfish products. Panelists were asked to evaluate references samples and rank them in order; for example from most firm to least firm. The Texture Profile Analysis (TPA) methods described in Study 1 was applied to the individual reference samples and compared to the texture data from Study 1 for the particular products (baked salmon, baked tilapia and boiled shrimp) to ensure they were at the extremes of the standard deviation exhibited by the individual products.

Using the texture data, reference anchors for each product were determined (Table 3-8). Individual training days for each product were included to allow panelists to familiarize themselves with each product and practice using the reference samples and line scale. For baked salmon training, panelists were presented three salmon samples one at a time and asked to evaluate them individually. After the evaluation was completed, panelists shared how they scored the sample and why. Samples provided to the panelists included cook times of 15, 20 and 25 min. Four fillets were cooked at each time point, three for the panelist to evaluate and one for texture measurement using TPA. Once samples were removed from heat, the internal temperatures were recorded, then immediately given to the panelist to guarantee that the product was evaluated hot. Evaluation of the product once promptly removed from heating source was done to mimic typical consumer behavior for doneness evaluation. Baked tilapia was presented after 10, 15, and 20 min (4 fillets were prepared – 1 for texture, 3 for evaluation) and boiled shrimp after 1, 2, and 3 min of cooking (8 shrimp were prepared – 1 for texture, 7 for evaluation).

Table 3-7: Instructions for Evaluating Non-Oral Texture Attributes of Firmness, Flakiness and Springiness for Fish and Shellfish

Attribute	Products	Guidelines for Measuring Attribute
Firmness	Atlantic salmon, Tilapia & Shrimp	• Using the index finger of your dominant hand apply pressure to the thickest section of the fillet (salmon and tilapia) or the second segment (shrimp), so that the pad of your finger indents and resistance from the product is detected. Note for tilapia: avoid areas with separation among muscle segments.
Flakiness	Atlantic salmon & Tilapia	• Using a fork, apply slight pressure with the back tips of the fork's prongs and pull toward you, observe the ease at which the muscle segments separate to individual segments. Observe flakiness along the edge near the thickest portion of the salmon fillet or at the thickest portion of the tilapia fillet
Springiness	Shrimp	• Amount of Recovery - Using the index finger of your dominant hand apply pressure to the second segment section of the shrimp, so that the pad of your finger indents and resistance from the product is detected. Remove pressure form finger and observe the products ability to return (amount) to its' original structure/form • Rate of recovery - Using the index finger of your dominant hand apply pressure to the second segment section of the shrimp, so that the pad of your finger indents and resistance from the product is detected. Remove pressure from finger and observe the rate (speed) at which the product returns to its' original structure/form

Table 3-8: Reference Samples to Demonstrate Degrees of Firmness, Flakiness and Springiness Associated with Fish and Shellfish to Panelists

Non Oral Texture Characteristics			Products		
			Baked Atlantic Salmon	Baked Tilapia	Boiled Shrimp
Firmness		Soft	Kraft® Velveeta® Cheese[1]	Kraft® Velveeta® Cheese[1]	Kraft® Velveeta® Cheese[1]
		Firm	Medium Cheddar Cheese[1]	Peanut Butter Fudge	Medium Cheddar Cheese[1]
Flakiness		Slightly Flaky	Imitation Crabmeat[1]	Imitation Crabmeat[1]	N/A
		Flaky	StarKist® Canned Tuna[1]	StarKist® Canned Tuna[1]	N/A
Springiness	Amount of Recovery	None	N/A	N/A	Little Debbie® Cosmic Brownie[2]
		Springy	N/A	N/A	Hotdog[3]
	Rate of Recovery	Slow	N/A	N/A	Marshmallow[4]
		Fast	N/A	N/A	Hotdog[3]

[1]Sample was at room temperature (24°C (75°F)) for 1 hr; [2]Rainbow colored chocolate chip pieces were removed; [3]Hotdogs were boiled for 4 min and given to the panelist hot and used immediately; [4]Standard size marshmallow positioned horizontally on its side; See Appendix G for complete reference product information

Panelist Validation

Panelist validation testing consisted of evaluating nine samples (three of each product/method combination) over two days. Samples and cook times evaluated included: baked salmon at 15, 20 and 25 min, baked tilapia at 10, 15 and 20 min and boiled shrimp at 1, 2 and 3 min. Each panelist was provided with the reference samples respective to the product being evaluated and a guide with verbal and pictorial instructions for evaluating the characteristics (see Appendix H). Samples were coded with a random three-digit number and presented on white plates (paper for salmon and shrimp; Styrofoam for tilapia). Silverware forks were provided for evaluating flakiness. Panelists made a dash along the 15.2 cm (6 inch) unstructured line to indicate their perception of each attribute relative to the reference samples.

The goal of the validation test was to verify each panelist’s ability to evaluate the samples for firmness, flakiness, and springiness. Using the results of Study 1and the researcher’s familiarity with handling the products, expectations for samples were developed. For baked salmon it was expected that firmness and flakiness would increase with internal temperature. For baked tilapia, firmness should decrease with and increase in internal temperature (or possibly remain the same between 15 and 20 min) and flakiness should increase with internal temperature. Finally, for boiled shrimp firmness should increase with an increase in internal temperature. The amount and rate of recovery was expected to increase with an increase in internal temperature and be similar to the hotdog reference for the amount and rate of recovery. Panelists following the expected trends for all samples were passed. Panelists not following trends were reviewed with for the trends they varied from.

Non-Oral Texture Assessment

Non-oral texture assessment included evaluation of baked salmon, baked tilapia, and boiled shrimp at two internal endpoints (64±1°C and 74±1°C) (see Appendix I for scorecards). Panelists were scheduled for one hour time block for individual testing. Each panelist was presented with the two samples of salmon, followed by two samples of shrimp and finally two tilapia samples. Panelists were provided with the appropriate reference samples, the guide for evaluating the samples, a silverware fork, a pencil, napkin, and paper scorecards. Samples were coded with a random three-digit number and presented on white plates (paper for salmon and shrimp; Styrofoam for tilapia). Each sample was cooked to the appropriate endpoint, the internal temperature was recorded and immediately the sample was passed to the panelists for evaluation. The mark on the unstructured 15.2 cm (6 inch) line was measured and the mean value and standard deviation for every attribute of each sample was calculated (Appendix J). Baked salmon fillets are often inconsistent in size, shape and thickness. Before testing, matched pairs of fillets were selected to reduce variability between the fillets not associated with cooking.

Statistical Analysis

Study 1: The endpoints per preparation methods per product per texture measurement were evaluated by one-way analysis of variance (ANOVA). Average CIE L^*, a^*, and b^* values, hue and chroma values were calculated for each product, method and endpoint temperature combinations. Average color values were compared within product groups using a paired t-test.

Study 2: Panelists' values along the 15.2 cm unstructured line scales were measured (cm) starting from the left side of the line. A comparison of means using a paired t-test was conducted to determine statistical significant differences between the two endpoints.

Results and Discussion

Study 1: Physical Assessment of Texture, Cook Loss and Color

Texture of Fish

The unique structure of cooked fish creates challenges for measuring texture. Compression tests (ie 75% strain) applied to cooked fish often result in muscle segments (myotomes) sliding and separating apart (Borderias and others 1983). WB requires a sample size small enough to fit underneath the guillotine-like blade and to have muscle fiber arranged perpendicular to the blade. Cutting the fish, particularly the salmon is difficult and changes the structural integrity of the fillet. Cutting the salmon before cooking into the appropriate sample size would not represent how a consumer would prepare the fish at home. The challenges associated with fish were found through literature review and preliminary studies using selected methods including TPA, KSC, and WB. Based on preliminary findings and restrictions from the individual samples, TPA and KSC were chosen for evaluating Atlantic salmon and tilapia (see Appendix K for preliminary research). TPA and KSC were considered manageable, reproducible and able to assess a variety of textural attributes. Due to the parameters measured by each method, results from TPA and KSC can not be compared directly.

Based on TPA results for Atlantic salmon and tilapia, statistical differences ($p < 0.05$) between the two endpoints for baked salmon, broiled tilapia, and pan-fried tilapia were determined (Figure 3-1A). Increased coagulated protein, shrinkage of the muscle fibers, and loss of moisture at 74°C ± 1 (Figure 3-5) is responsible for the difference in firmness between the two endpoints for baked salmon. Coagulated sarcoplasmic protein forms on the surface and between the myotomes of the salmon due to the muscle fibers contracting during heating, pushing the protein from the muscle fibers. The presence of the coagulated protein has been reported to contribute to firmness due to decreased slippage or separation of the myotomes (Hatae and others 1990). Broiled tilapia and pan-fried tilapia visually had more browning, crust formation (Figure 3-9(2)), and increased moisture loss at 74°C ± 1 (Figure 3-5) compared to baked tilapia, which contributed to greater shearing and compressing of the sample.

Chewiness (N*mm) and firmness (N) determined by TPA were found to be larger for all salmon samples compared to all tilapia samples (Figures 3-1A and 3-2). A difference in firmness between salmon and tilapia was expected. Salmon is referred to as a fatty fish with firm raw texture and comprised of larger myotomes, compared to tilapia, a lean fish with a moderate raw texture and smaller myotomes (Brown 2007). Physical characterization of fat, protein and moisture of the salmon and tilapia studied revealed salmon to have a higher amount of fat compared to tilapia (13.5%, 2.6% respectively) and a slightly higher amount of protein (19.4%, 17.5% respectively). Tilapia was found to have higher moisture content (79.2%), compared to salmon (65.6%) (see Appendix L, Table L-1). Dunajski (1979) reports fat and water content contribute to the sensorial quality of fish texture suggesting tenderness increases as fat content increases for fatty fish and moisture content has a positive relationship with tenderness for lean fish. Tilapia (baked, broiled, pan-fried) was found to be more tender compared to salmon for all culinary processes and had a higher moisture content.

Using KSC, each culinary process for tilapia was found to have significant statistical differences ($p < 0.05$) between the two internal endpoints for max force, but no differences were found between any of the salmon samples (Figure 3-1B). As previously mentioned browning, crust formation, and increased moisture loss at 74°C ± 1 are responsible for the differences in max force required between the endpoints. It is also important to note that sample preparation methods differed between tilapia and salmon. A section of the thickest part (standardized size) was evaluated for tilapia, while salmon myotomes were flaked apart and arranged in a single layer across the bottom of the cell (standardized sample weight). Tilapia’s myotomes are small and do not produce solid flakes as salmon does, individual muscle segments would have fallen through slits of the cell. The difference in KSC sampling may not allow for direct comparison between max force required for salmon and tilapia samples.

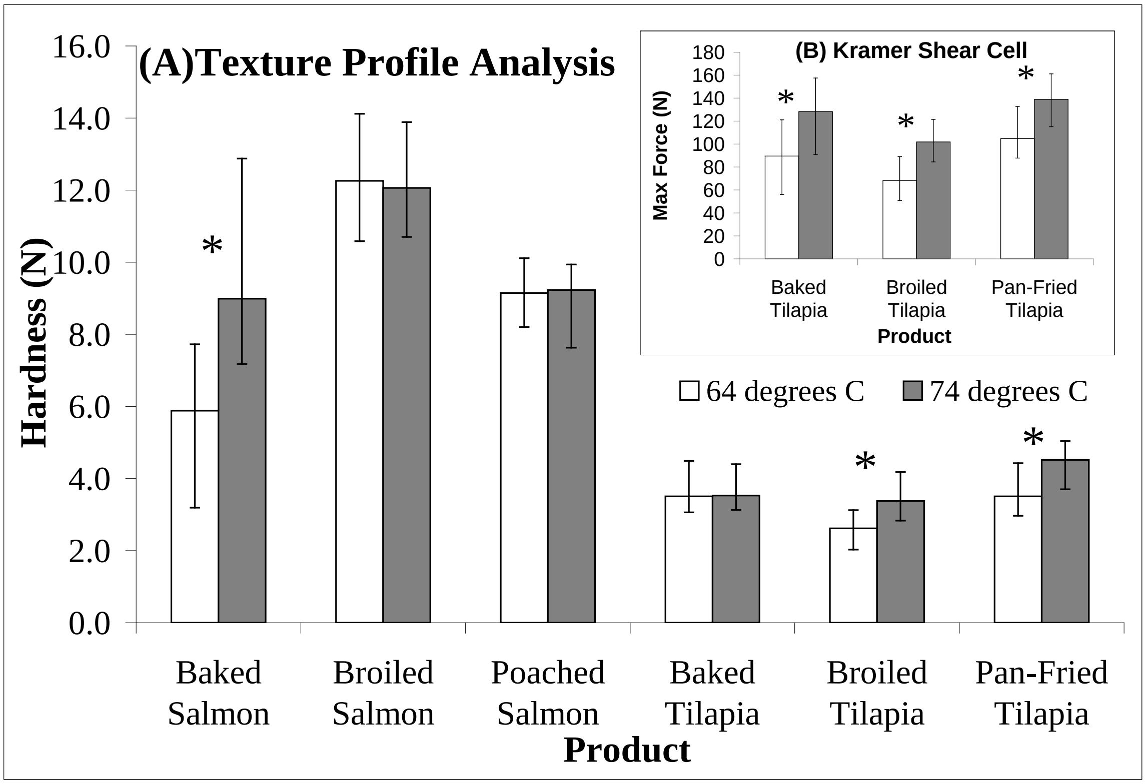

Figure 3-1: (A) Hardness as Determined by Texture Profile Analysis (30% compression) for Cooked (64°C ± 1 and 74°C ± 1 endpoints) Atlantic Salmon and Tilapia; and (B) Max Force (Hardness) as Determined by Kramer Shear Cell for Tilapia. (*) indicates significant statistical difference ($p < 0.05$) between internal temperature endpoints within a culinary process and seafood product combination.

Texture of Shellfish

Researchers observed the texture of shellfish to differ greatly from the flaky structure of fish exhibiting a rubbery and springy texture that remains intact after cooking. Shrimp were able to be cut to create the desired sample size and area, however the semisolid structure of the inside of the oyster made it challenging to cut or reduce its size. All methods for the oysters had to allow the oyster to remain intact. Compression test and the WB blade were common methods used for shellfish throughout a review of the literature. The amount of product required to cover the bottom of the KSC was a restricting factor and involved evaluating the entire shrimp as opposed to only the second segment.

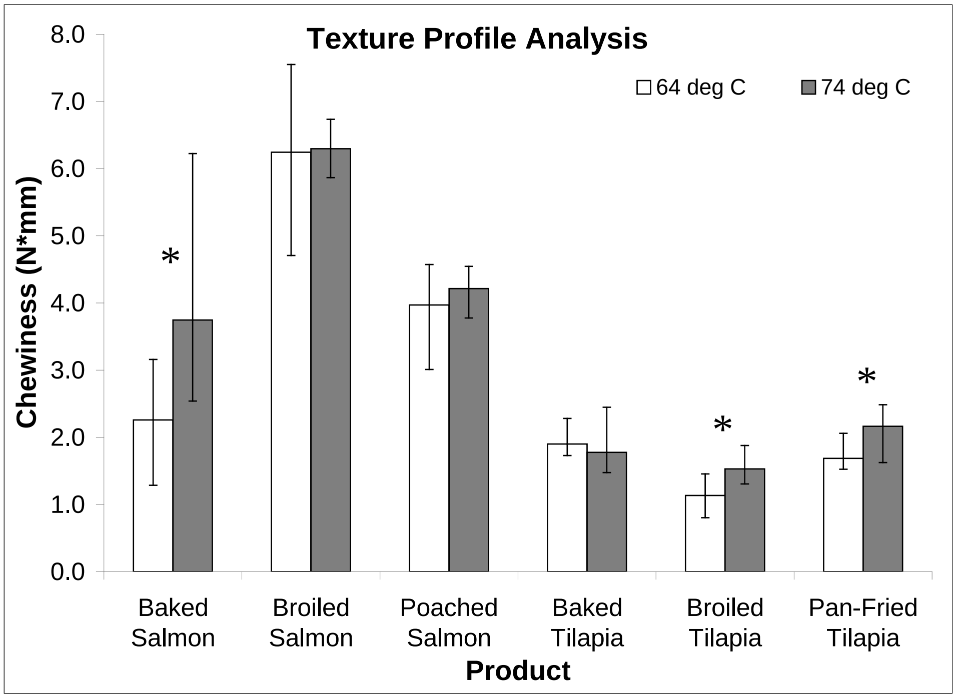

Figure 3-2: Chewiness as Determined by Texture Profile Analysis (30% compression) for Cooked (64°C ± 1 and 74°C ± 1 endpoints) Atlantic Salmon and Tilapia Prepared By Various Culinary Methods. (*) indicates significant statistical difference ($p < 0.05$) between internal temperature endpoints within a culinary process and seafood product combination.

Evaluation of shrimp texture at the second segment for this study was chosen based on a review of the literature. Mizuta and others (1999) determined penetration resistance (firmness) at the second segment of prawns when studying how heat processing affects the relationship of histological changes in collagen to texture development. A change in penetration resistance was seen as the processing temperature increased from 30°C to 90°C ($p < 0.05$) (Mizuta and others 1999). Niamnuy and others (2007) studied the quality changes including texture for shrimp boiled in salt solutions. Texture was evaluated at the second segment using 70% compression and WB. For the compression test, the second segment of the shrimp was reduced to a square (8 x 8 mm^2) sample. During WB test the shrimp remained intact and the blade was positioned over the second segment (Niamnuy and others 2007). Niamnuy and others (2007) were able to keep shrimp samples intact when using WB; however researchers were unable to for this study because the shrimp were tightly curled and the tail or head portion of the shrimp impeded the blade. Shrimp were reduced to segments one, two, and three and the blade was positioned over the center of segment two. The shrimp were allowed to remain intact for TPA (30% compression) due to the probes ½” diameter size and rounded end with ¼” radius being of similar size to the second segment area. Niamnuy and others found hardness measured by TPA to increase as boiling time increased. Toughness determined by WB increased initially with time, but after 3 min it decreased from gelatinization of collagen at temperatures of 80°C or above and the presence of salt (Niamnuy and others 2007).

For shrimp and oysters, significant statistical differences ($p < 0.05$) were seen between the two internal endpoints for oysters using WB (Figure 3-3), but for shrimp using TPA (Figure 3-4). The mean max force (toughness) determined by WB was higher for shrimp (boiled and broiled) than oysters (Figure 3-3). Hardness (N), chewiness (N*mm) and springiness (%) determined by TPA showed increases with increased temperature for both shrimp and oysters (Appendix M, Figures M-1 and M-2). Overall, shrimp was found to be chewier than oysters, regardless of cooking method used with boiled shrimp exhibiting a chewier texture ($p < 0.05$) at 74°C ± 1. Springiness increased as the internal temperature endpoint increased ($p < 0.05$) for boiled shrimp (Appendix M, Figure M-2).

During heating myofibrillar proteins denature and coagulate, collagen shrinks, and loss of moisture occurs resulting in a compact muscle structure and firmer texture (Benjakul and others 2008; Mizuta and others 1999; Niamnuy and others 2007). Benjakul and others (2008) studied changes in physical properties and microstructure of black tiger shrimp and white shrimp resulting from processing. Both species exhibited shrinkage of the sarcomere after being cooked for 3 min at 100°C followed by draining for 5 min at 4°C. Shear force measured by WB increased ($p < 0.05$) for both species as heating time increased (Benjakul and others (2008).

Oysters are commonly consumed raw or slightly cooked because of the increase sensory attributes associated with them including color, texture and flavor; therefore physical and compositional changes occurring in oysters due to heating are less commonly studied. It is expected that an increase in firmness is due to protein denaturation, muscle fiber shrinkage, and moisture loss as with other fish and shellfish (Figure 3-6). There is interest in using high pressure processing for reducing microbial load, extending shelf-life, and maintaining quality associated with raw oysters. Cruz-Romero and others (2008) examined changes in high pressure processed oysters during chilled storage relate to microbiology and physiochemical quality. Microbial growth was delayed during storage, but cutting strength increased as the high-pressure processing temperature received increased (Cruz-Romero and others 2008).

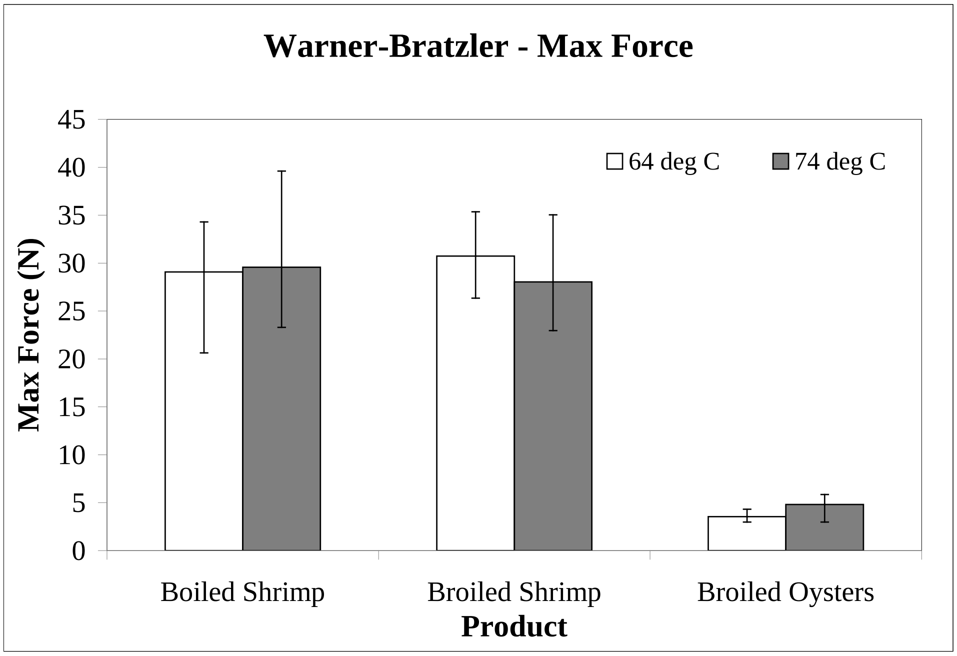

Figure 3-3: Max Force (Hardness) as Determined by Warner-Bratzler for Cooked (64°C ± 1 and 74°C ± 1 endpoints) Shrimp and Oysters. (*) indicates significant statistical difference ($p < 0.05$) between internal temperature endpoints within a culinary process and seafood product combination.

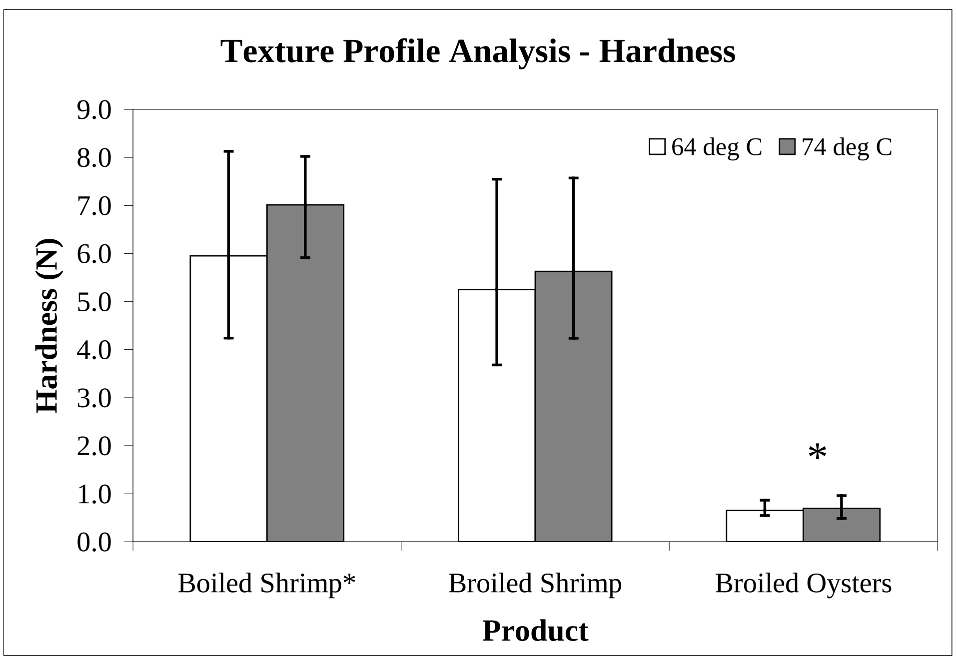

Figure 3-4: Hardness as Determined by Texture Profile Analysis (30% compression) for Cooked (64°C ± 1 and 74°C ± 1 endpoints) Shrimp and Oysters. (*) indicates significant statistical difference ($p < 0.05$) between internal temperature endpoints within a culinary process and seafood product combination.

Cook Loss

Cook loss was statistically different ($p < 0.05$) between the two internal endpoints (64°C ± 1 and 74°C ± 1), increasing as the internal endpoint increased for each product and preparation combination (Figures 3-5 & 3-6). For salmon, broiling to 74°C ± 1 resulted in 19.5% cook loss compared to 6.6% for baked salmon at 64°C ± 1 (Figure 3-5). Kong and others (2007) experienced 14 – 22% cook loss along the length of Pacific pink salmon (Oncorhynchus gorbuscha) fillets heated at 121°C for 20 min in a oil bath with glycerol heating medium. Broiled oysters experienced the most cook loss, losing almost 51.3% when heated to 74°C ± 1 (Figure 3-6). Cook loss was greatest for 74°C ± 1 samples compared to 64°C ± 1 samples because increased heat-induced protein denaturation, which correlates to decreased water holding capacity (Kong and others 2007). Additionally, heat causes shrinkage and contractions of the muscle fibers which results in water being pushed from the product (Tornberg 2005). The texture of fish and shellfish is affected by the amount of cook loss, which can vary due to product, culinary process, and internal temperature. Seafood producers are concerned with cook loss because loss of water from the product increases the amount of product needed to meet the net weight requirements. Minimizing cook loss is essential for producers to maximize profits.

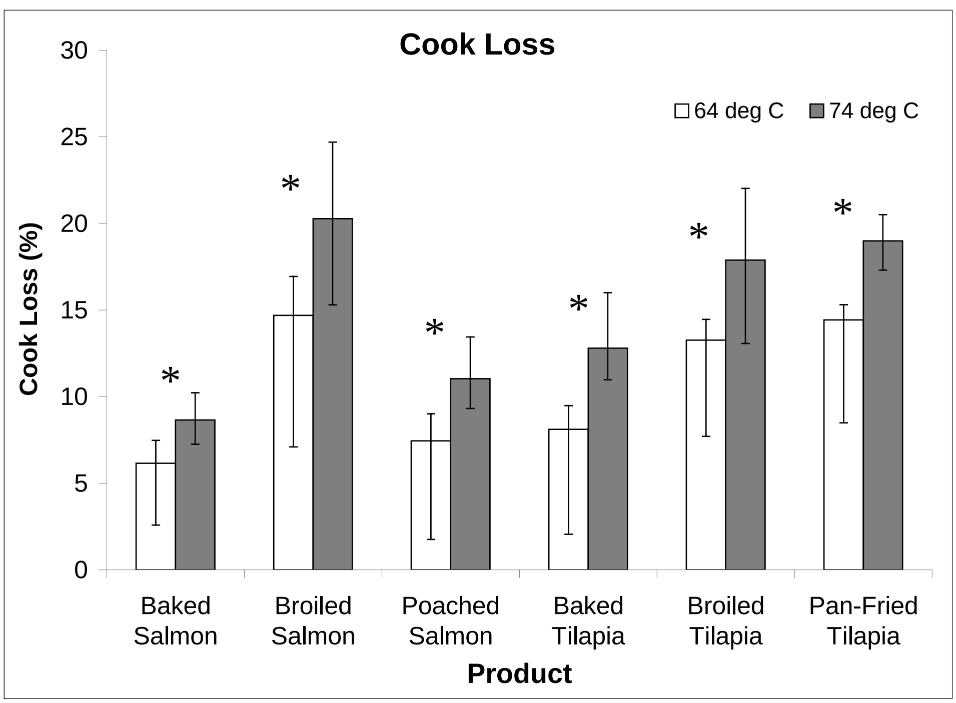

Figure 3-5: Cook Loss for Cooked (64°C ± 1 and 74°C ± 1 endpoints) Atlantic Salmon and Tilapia Prepared By Various Culinary Methods. (*) indicates significant statistical difference ($p < 0.05$) between internal temperature endpoints within a culinary process and seafood product

combination. Chart includes cook loss determined from TPA and KSC replications for each product.

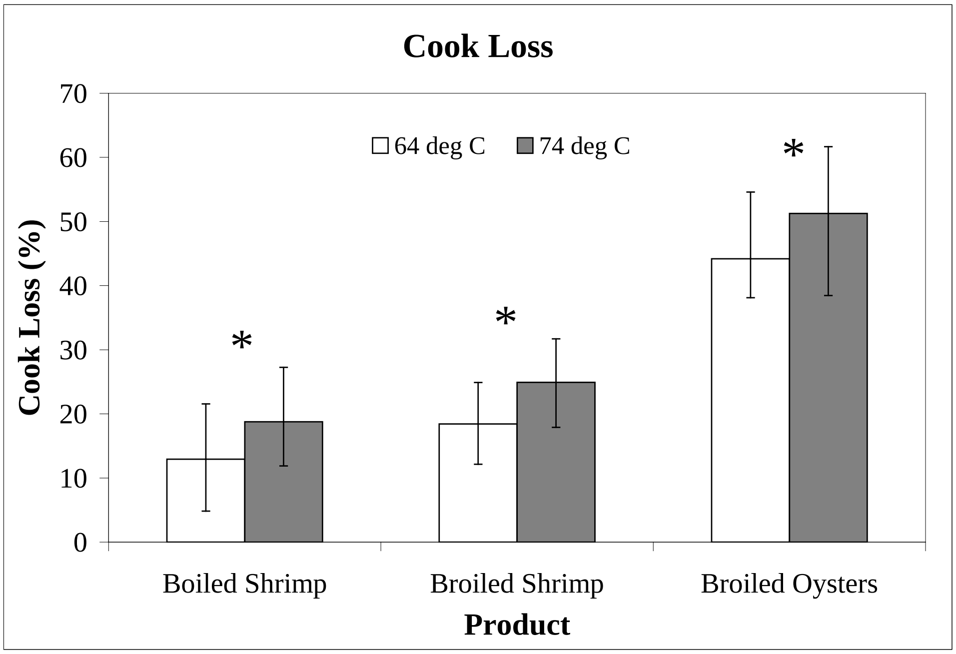

Figure 3-6: Cook Loss for Cooked (64°C ± 1 and 74°C ± 1 endpoints) Shrimp and Oysters Prepared By Various Culinary Methods. (*) indicates significant statistical difference ($p < 0.05$) between internal temperature endpoints within a culinary process and seafood product combination. Chart includes cook loss data determined by TPA and WB replications.

Methodology Review

The majority of studies that measure texture instrumentally study the texture of raw fish/shellfish, while limited studies focus on the texture of cooked fish/shellfish prepared by culinary processes. Based on the results of Study 1 and execution of the texture methods used (KSC, TPA and WB), several advantages and disadvantages were discerned (Table 3-9).

Color

The expectation of the color and overall appearance of a fish or shellfish product is based on consumers' past experiences and the culinary method applied. Color differences were apparent between cooking methods of the same fish product (Figure 3-7). Additionally, differences in color between the two internal endpoints varied among products and culinary processes applied (Figures 3-8 & 3-9). Many of the culinary methods in combination with the internal temperatures resulted in products exhibiting non-homogeneous color. Therefore, the difference of color values (L^*, a^*, b^*, hue and chroma) between 64°C ± 1 and 74°C ± 1 measured by colorimeter will be compared to those determined through Adobe® Photoshop® analysis.

Table 3-9: Advantages and Disadvantages of Various Methods for Evaluating the Texture of Cooked Fish and Shellfish

Texture Method	Advantages	Disadvantages
Texture Profile Analysis (30% Compression)	• Mimics non-oral texture evaluation ("finger test")[1,2] • Non-destructive • Determines five different texture parameters • Cooked fillets remain intact with minimal sliding of muscle segments under compression[3] • Experiment III concluded sensitivity for salmon and shrimp	• Does not correspond well with oral sensory evaluation[2] • Small (localized) area for measurement when using the stainless steel, 3inch tall rounded end probe of 1/2" diameter with 1/4" radius end as was chosen for Experiment III
Kramer Shear Cell (5 blade)	• Mimics mastication (shearing, compression, extrusion) between molars teeth[3,4] • Has been found to correlates with oral texture evaluation[5,6] • Experiment III found sensitivity to texture changes in tilapia	• Destructive • Difficult to clean the blades thus extra time is required[3] • Muscle fibers must be oriented perpendicular to blades (recommended)[3]
Warner-Bratzler	• Mimics biting using front incisors [3] • Can be used with oysters (high sensitivity for oysters determined from Experiment III)	• Destructive • Consistent sample size and dimensions for cooked fish is difficult to achieve due to flaking and segments sliding apart • Muscle fibers must be oriented perpendicular to blade (recommended) [3] • Requires often cleaning[3] • Small (localized) area for measurement[3]

[1] Veland and Torrissen (1999)
[2] Szczesniak (1987)
[3] Sanchez-Alonso and others (2010)
[4] Dunajski (1979)
[5] Keith and others (1979)
[6] Karl and Schreiber (1985)

In the CIE $L*$, $a*$, and $b*$ value system, $L*$ measures lightness on a 100 point scale, $a*$ measures redness ($+a*$) to greenness ($-a*$) and $b*$ measures yellowness ($+b*$) to blueness ($-b*$) (Hallier and others 2008; Skrede and Storebakken 1986). Hue ($H°_{ab} = \tan^{-1}(b*/a*)$) represents the relationship between the red hue ($H°_{ab} = 0$) to yellow hue ($H°_{ab} = 90$). Chroma ($C^*_{ab} = (a^{*2} + b^{*2})^{1/2}$ representing the clarity and saturation or intensity of a color, as the chroma value increase the color intensity and clarity increases (Christiansen and others 1995).

The mean and standard deviation for the color values (L^*, a^*, b^*, hue and chroma) were calculated for each product at 64°C ± 1 and 74°C ± 1 (Appendix N). The difference in each of the color values was calculated between 64°C ± 1 and 74°C ± 1 to allow for comparison between the color evaluation methods and between endpoints (Appendix N). For baked, poached, and broiled salmon no differences ($p > 0.05$) were found among the color values by Photoshop® analysis or colorimeter for poached salmon. Broiled salmon was found to have significant difference ($p < 0.05$) for all color values between the two internal temperature endpoints; L^* decreased between 64°C ± 1 and 74°C ± 1, indicating darkening of the fillet (Appendix N, Table N-1). Large differences (Δ) between internal endpoints for color values were determined by Photoshop®. Baked salmon had significant difference between 64°C ± 1 and 74°C ± 1 for b^*, hue and chroma; b^* increase from 64°C ± 1 to 74°C ± 1 indicating yellow-golden color development (Appendix N, Table N-1).

Hue and chroma values were not calculated for tilapia because a^* is often zero and can not be used in the formula for hue and chroma. Baked, broiled and pan-fried tilapia had no difference ($p < 0.05$) in color values determined by Photoshop® between internal endpoints. Statistical differences ($p < 0.05$) between all color values at 64°C ± 1 and 74°C ± 1 for baked and pan-fried tilapia were determined by colorimeter. However difference between baked tilapia samples may not be noticeable to consumers if not placed side by side (Figure 3-8; Appendix N, Table N-2). Broiled tilapia at 74°C ± 1 has a smaller L^* value and a larger b^* value compared to 64°C ± 1, which coincides with the product darkening and developing more yellow-golden color at 74°C ± 1 (Figure 3-9; Appendix N, Table N-2).

The color of the shrimp from the second segment to the tail (sixth segment, no tail shell remaining) was measured using the colorimeter and Adobe® Photoshop®. No differences in color values between the endpoints for boiled or broiled shrimp were determined using Photoshop®. For boiled shrimp, significant differences in all color values except for L^* between

64°C ± 1 and 74°C ± 1 were determined using colorimeter. The only difference between endpoints for broiled shrimp was hue measured by colorimeter, which increased with increasing temperature suggesting an increase in yellowness (Appendix N, Table N-3).

Evaluation of broiled oysters using Photoshop® revealed no differences in color values between 64°C ± 1 and 74°C ± 1. Broiled oysters at 74°C ± 1 has a smaller L^* value and a larger b^* value compared to 64°C ± 1, which coincides with the product darkening and developing more yellow-golden color at 74°C ± 1 (Appendix N, Table N-4).

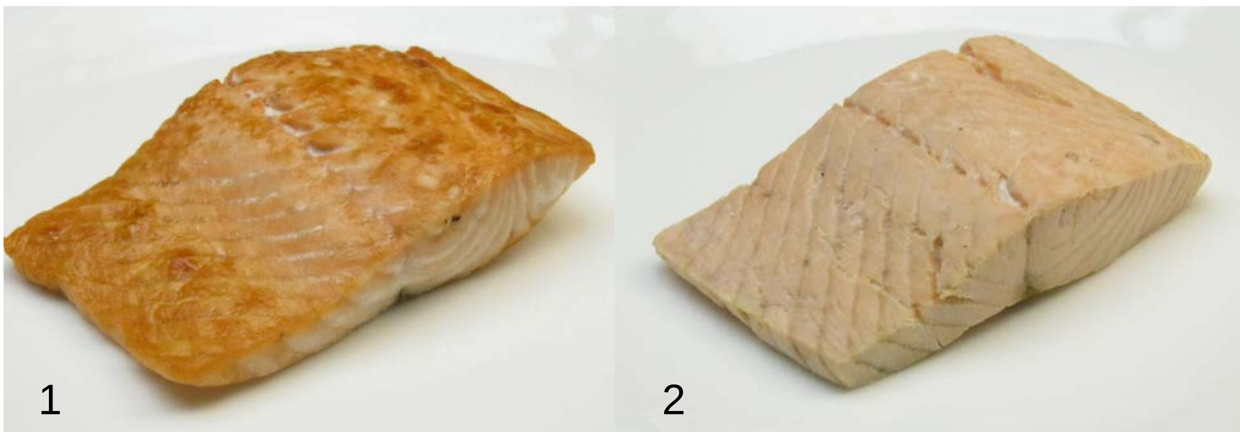

Figure 3-7: Atlantic Salmon Prepared to an Internal Temperature Endpoint of 64°C ± 1 Using Broiling and Poaching Culinary Methods: Color and Visual Differences
(1) Broiled Salmon: L^* 65.69, a^* 16.67, b^* 39.96, Hue 65.80 and Chroma 40.65
(2) Poached Salmon: L^* 76.76, a^* 10.66, b^* 22.06, Hue 64.25 and Chroma 24.53.
Note: L^* (lightness, 0 = black, 100 = white), a^* (red – green), b^* (yellow – blue), Hue (relationship of yellowness and redness), and Chroma (intensity of the color).

The obvious variety of color and physical characteristics among seafood products, internal temperatures, and culinary methods supports the need for detailed descriptive characteristics of doneness. The outside of fish and shellfish reaches temperatures above that of the internal temperature, which can contribute to browning. Additionally, browning can be influenced by the culinary process applied. Browning does not occur with wet heating methods such as poaching and boiling, but often occurs with dry heating methods such as baking and broiling. Colorimeter data found more statistical differences between endpoints then Photoshop®. The analysis of color with Photoshop® (3 replications per product per internal temperature randomly selected) was less powerful than the colorimeter analysis (5 – 9 replications per product per internal temperature). Overall variability seen between the values measured by the colorimeter versus Adobe® Photoshop® are created by the lighting used from the colorimeter compared to the north daylight used in photo documentation of the products. Lighting influences the color perceived (Hunter and Harold 1987). The lighting, positioning of the sample, positioning of the camera, and camera settings were standardize to allow photographs taken on various days to be consistent and comparable.

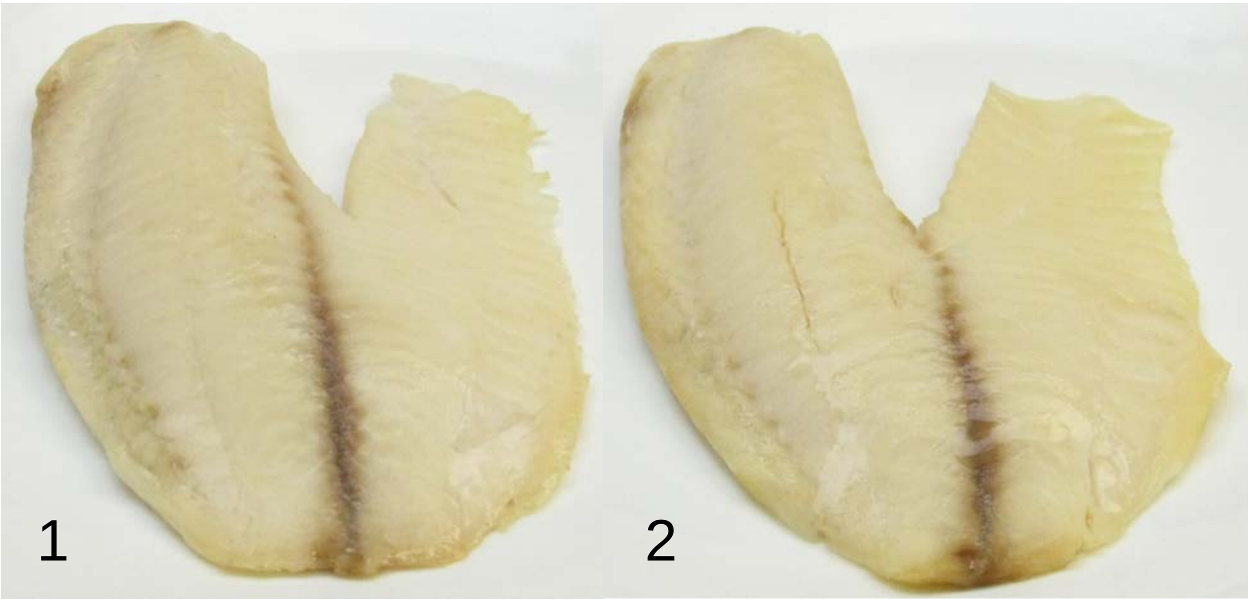

Figure 3-8: Baked Tilapia Cooked to Two Internal Endpoints: Color and Visual Differences
(1) 64°C ± 1: L^* 78.38, a^* -2.37, b^* 10.51, Hue 75.94 and Chroma 10.84
(2) 74°C ± 1: L^* 79.46, a^* -2.14, b^* 11.74, Hue 79.14 and Chroma 11.97
Visual color difference between the two internal temperature endpoints is difficult to determine for baked tilapia, however L^* is considered statistically different ($p < 0.05$) between the ITs.

Challenges

The literature review identified several aspects to consider when measuring the texture of seafood. Several studies have measured the texture along the length of raw salmon fillets and found that firmness increases from head to tail (Sigurgisladttir and others 1999; Kong and other 2007). The tail of a raw salmon fillet is lean with low lipid content and composed of a large amount of muscle fibers small in diameter, both of which have been noted to be associated with firmness (Katikou and others 2001; Love 1970). Kong and others (2007) heated salmon fillets to see if the same effects occurred with cooked samples. Overall force required to shear a cooked tail region required less force than the central portion of the salmon (Kong and others 2007). The tail portion of the salmon is composed of red muscle (slow muscle), which contains muscle fibers with a smaller diameter than those found in white muscle (fast muscle) (Love 1970). Fiber diameter and density have been linked to the tenderness in salmon fillets (Dunajski 1979). A smaller difference in texture was experienced between red and white muscle after cooking and is attributed to muscle fibers shrinking due to protein denaturation caused by cooking (Kong and others 2007). The known difference in firmness among the fillet and the variety of cuts sold within the purchased bags of Atlantic salmon fillets directed the researcher to testing even amounts of center-cuts and tail filets. For consumers, fish and shellfish will be consumed within minutes of removal from heating element and the texture experienced would be different then that measured in Study 1 at room temperature (75°F). The texture was measured after allowing the samples to cool to room temperature to prevent temperature variability and improve consistency between measurements.

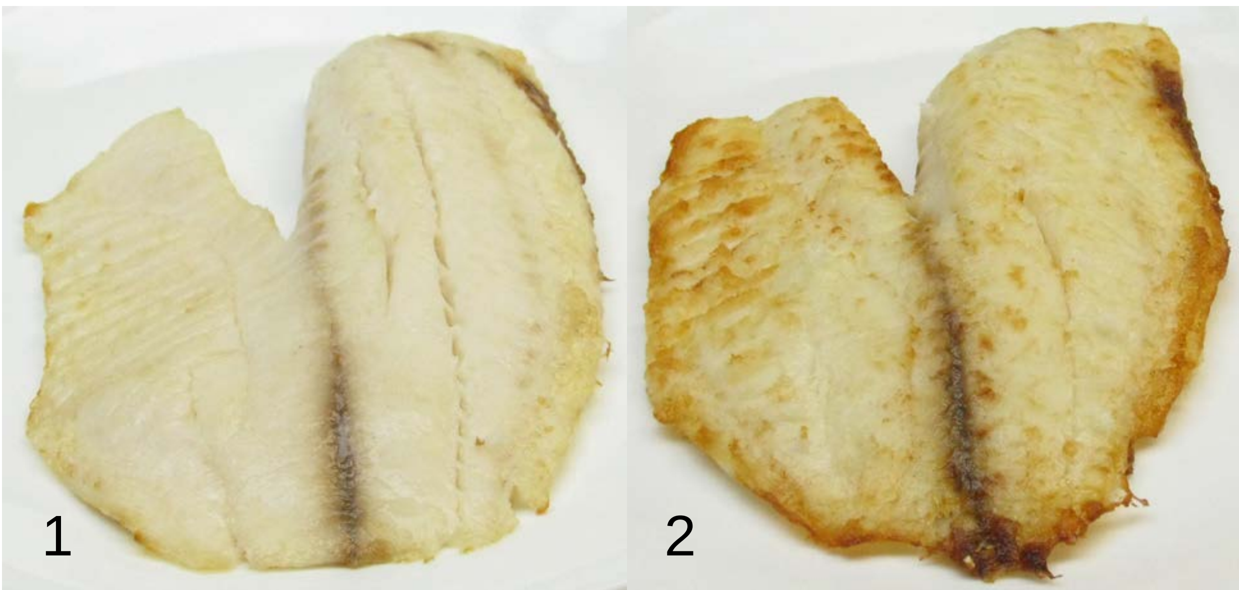

Figure 3-9: Broiled Tilapia Cooked to Two Internal Endpoints: Color and Visual Differences
(1) 64 °C ± 1: L^* 79.81, a^* -2.10, b^* 14.20, Hue 80.52 and Chroma 14.40
(2) 74 °C ± 1: L^* 76.42, a^* -1.55, b^* 19.36, Hue 82.67 and Chroma 19.70
Unlike baked Tilapia, visual differences in color can be seen for broiled tilapia at the two internal temperature endpoints. L^* and b^* values were significantly different ($p < 0.05$) for the two endpoints.

Reaching the desired endpoints of 64±1°C and 74±1°C for samples often required a rest period or calculation based on weight or thickness (Appendix O). A limitation of the thermocouple probes included not being to be immersed in water. During preliminary testing a factor was determined for poached salmon based on thickness (mm) and for boiled shrimp by weight (g) (Appendix O). All other products required a resting period to allow the internal temperature to rise to the desired range of internal temperature with product/method combination ranging in rest period needed Appendix O). Proper determination of the internal temperature of oysters was complicated by their un-solidified ventral structure (belly or adductor muscle).

Study 2: Non-Oral Texture Characterization

Non-oral texture characteristics for baked salmon, baked tilapia, and boiled shrimp can be used to supplement current generic cooking guidelines for the respective products. The trained descriptive panel (n = 7) identified possible characteristics to distinguish between 64°C ± 1 and 74°C ± 1 and how to determine if the product has reach the minimum safe temperature. The mean scores and standard deviations for each product and endpoint were calculated (Table 3-10). Replications were not conducted for individual panelists.

Table 3-10: Mean Scores for Non-Oral Texture Characterization of Fish and Shellfish by a Trained Descriptive Panel (n = 7)

Product and IT (°C ± 1)		**Firmness**	**Flakiness**	**Springiness**	
				Amt of Recovery	**Rate of Recovery**
		Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
Baked Salmon	64	9.48 ± 3.06	8.12 ± 2.15	N/A	N/A
	74	10.4 ± 1.74	9.34 ± 2.53		
Baked Tilapia	64	5.62 ± 2.98	11.5 ± 2.36	N/A	N/A
	74	3.96 ± 2.36	11.9 ± 2.23		
Boiled Shrimp	64	9.27 ± 2.14	N/A	11.19 ± 1.77	10.58 ± 1.47
	74	10.3 ± 1.89		10.45 ± 3.36	11.04 ± 1.72

Non-oral texture assessment by the trained panel did not identify differences ($p > 0.05$) in firmness or flakiness in salmon baked to two endpoints (Table 3-10). Six of the seven panelists reported either no change or an increased firmness with an increase in internal temperature. The single panelist not in agreement scored the samples opposite of the panel majority. Five of the seven panelists scored the 74°C ± 1 sample flakier than the 64°C ± 1 or discerned no difference. For baked salmon at a safe internal temperature range (64°C - 74°C) the firmness is slightly less firm then medium cheddar cheese (at 24°C (75°F) for 1 hr) and relatively flaky, but not as much as canned tuna (at 24°C (75°F)), which were both used in the training sessions as reference standards.

For baked tilapia no differences in firmness or flakiness ($p > 0.05$) was determined between the two endpoints (Table 3-10). Six of the seven panelists found the firmness to decrease or not change with an increase in internal temperature. For baked tilapia at the safe internal temperature range (64°C-74°C) the firmness is similar to Velveeta® cheese (at 24°C (75°F) for 1 hr) and flakiness resembles canned tuna.

Firmness of boiled shrimp at 74°C ± 1 had an overall higher mean score, which was expected; however it was not statistically different ($p > 0.05$) from 64°C ± 1 (Table 3-10). All of the panelists found either an increase in firmness or no change as the internal temperature increased. The amount and rate of recovery for 64°C ± 1 and 74°C ± 1 were not significantly different. The amount of recovery was high, identifying both endpoints as springy in nature. The rate of recovery was similar to a hot dog, thus recovery time was fast. Boiled shrimp at the safe internal temperature range (64°C - 74°C) has a firmness similar to medium cheddar cheese (at 24°C (75°F) for 1 hr) and springiness resembling a hotdog (boiled for 4 min) in both amount and rate of recovery.

Determining a Difference Between Below Safe Internal Temperature and at Safe Internal Temperature

During training, the validation test showed the average firmness rating of baked salmon to be lower than that the safe internal temperature range (64°C – 74°C) indicating firmness similar to Velveeta® cheese may not be safe to consume (Figure 3-10). Flakiness validation results did not indicate the typical flakiness observed at below safe internal temperature range due to the thickness of the samples used. The researcher observed flakiness of the samples, but little flakiness should have been apparent. The thickness of the 15 min samples caused the outside of the sample to appear cook and flake easily, while the center was below the safe internal temperature. Baked tilapia under the minimum safe temperature is firm, more similar to peanut butter fudge than Velveeta® cheese (at 24°C (75°F) for 1 hr) (Figure 3-11). The flakiness of undercooked baked tilapia is significantly reduced exhibiting a slightly flaky characteristic similar to imitation crab meat (at 24°C (75°F) for 1 hr) (Figure 3-12). Training validation testing for boiled shrimp found little difference in amount and rate of recovery for all cooking times and endpoints tested. The firmness for the shrimp at 1 min (Below Safe IT (41°C)) was slightly less firm then those at the safe internal temperature range (64°C - 74°C) (Figure 3-13). Although no significant differences between the two internal temperature assessed by the trained panel were determined, validation test results indicate significant differences between below the minimum safe internal temperature and at the safe internal temperature may be distinguishable for non-oral texture attributes.

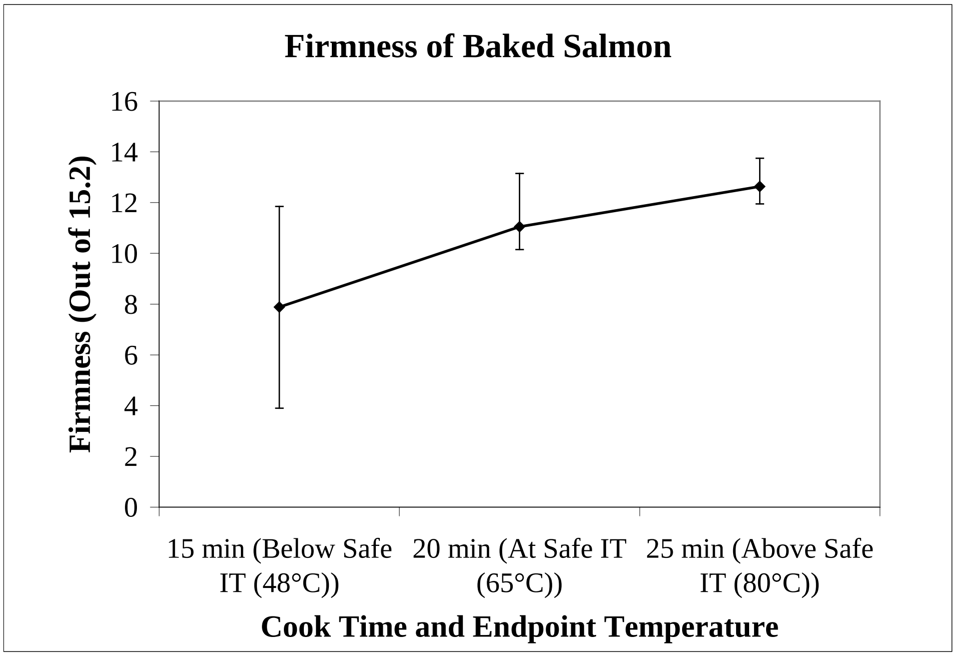

Figure 3-10: Firmness as Determined by Validation Test for Baked Salmon by Trained Descriptive Panel (n = 7). A positive correlation between firmness and internal temperature was identified.

Correlations between Texture Analyzer and Trained Panelists

The hardness values determined by TPA in Study 1 could potentially correlate with the firmness values determined by the trained descriptive panel. The TPA test in Study 1 utilized a 30% compression to mimic a consumer using pressure applied by their fingers to determine firmness of the products. Springiness determined by TPA could also be compared with amount and rate of recovery determined by panelists. The TPA method used in Study 1 was employed to measure the firmness or springiness of reference samples to determine how they compared to the average measurements for baked salmon, baked tilapia and boiled shrimp at 64°C ± 1 and 74°C ± 1 in Study 1. The goal was to identify reference samples that exhibit firmness and springiness characteristics at the extremes of the standard deviations for each product (Table 3-11). It was taken into consideration that the reference samples used are typically stored refrigerated and that during training and evaluation they would sit at room temperature for up to an hour. To minimize variation of texture among reference samples, the samples were allowed to come to room temperature (Table 3-12).

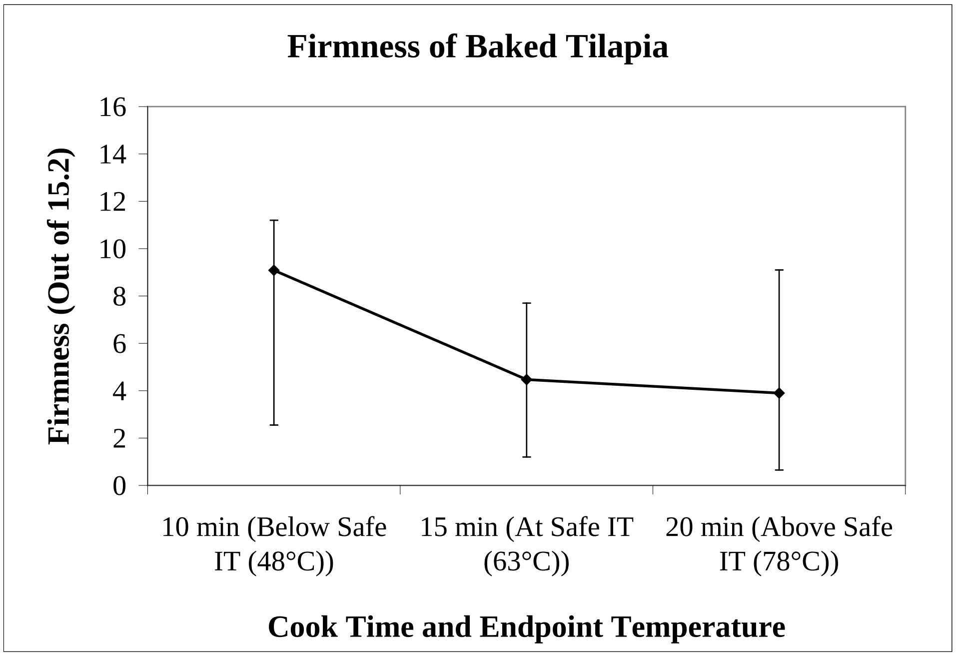

Figure 3-11: Firmness as Determined by Validation Test for Baked Tilapia by Trained Descriptive Panel (n = 7). A negative correlation between firmness and internal temperature was identified.

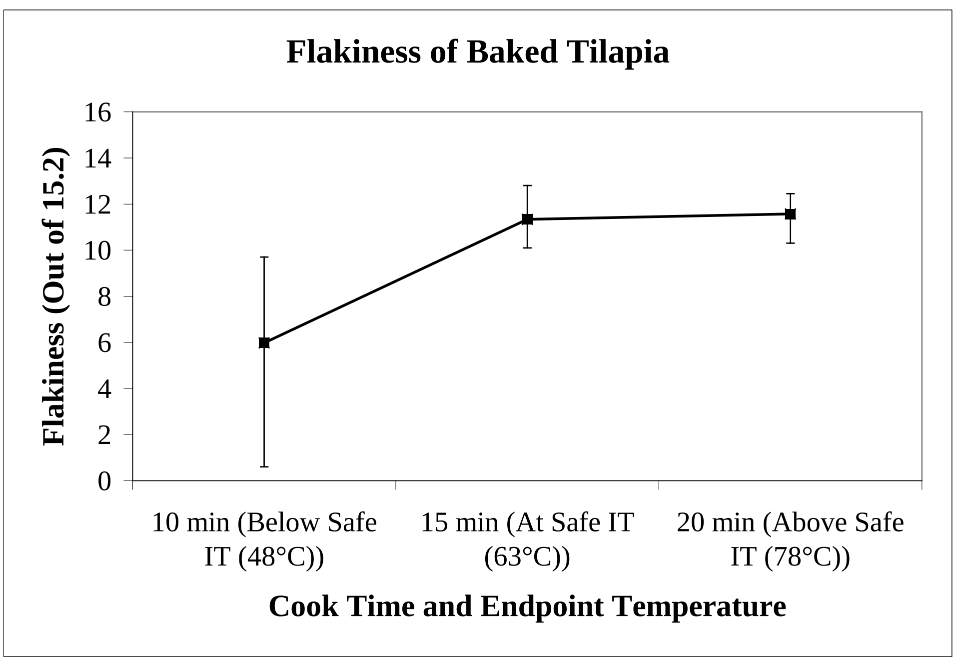

Figure 3-12: Flakiness as Determined by Validation Test for Baked Tilapia by Trained Descriptive Panel (n = 7). The difference in flakiness between below safe IT and at safe IT was larger than between at safe IT and above safe IT.

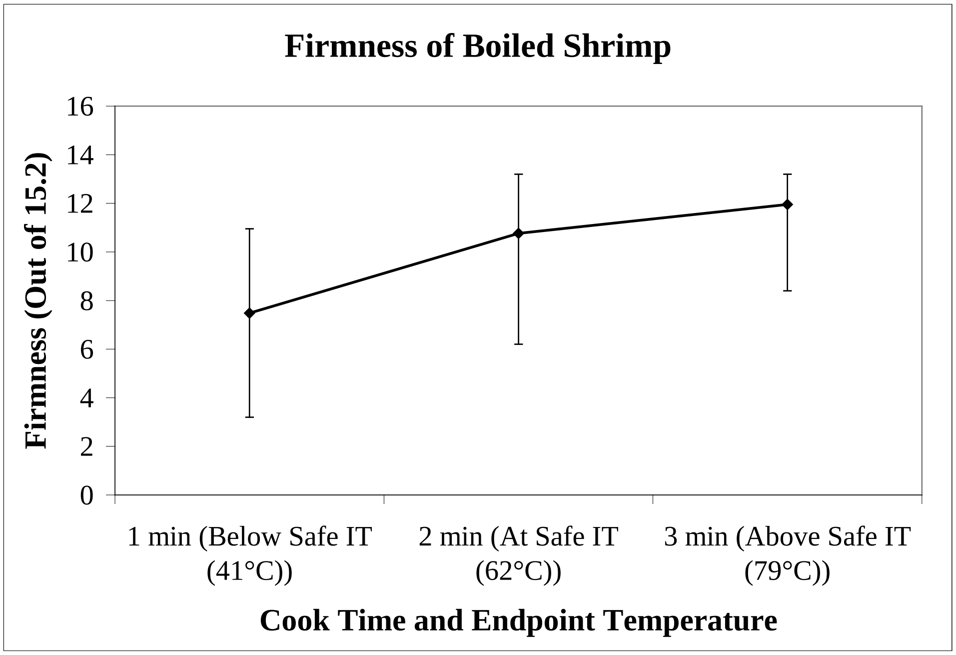

Figure 3-13: Firmness as Determined by Validation Test for Boiled Shrimp by Trained Descriptive Panel (n = 7). A positive correlation between firmness and internal temperature was identified.

Table 3-11: Range of Potential Firmness and Springiness Experienced by Fish and Shellfish at Room Temperature as Determined by TPA (30% Compression) in Study 1

Product	Firmness Range (N)	Springiness Range
Baked Salmon	4 – 11	N/A
Baked Tilapia	3 – 4	N/A
Boiled Shrimp	5 - 8	0.85 – 0.92

Note: Estimates were rounded to the closest integer

Table 3-12: TPA Measurements of Hardness and Springiness for Reference Samples Used in Trained Panel

Product	Firmness (N)
Velveeta® Cheese	1.4
Peanut Butter Fudge	8.5
Medium Cheddar Cheese	16
Product	**Springiness**
Brownie	0.36
Marshmallow	0.94
Hot Dog	0.96

Note: Measurements taken at room temperature (72°F), except for hotdog which was measured hot because it was used for only one sample and was required to demonstrate optimal characteristics of springiness.

Conclusions

Heating raw fish and shellfish causes physical changes associated with texture, color and cook loss to occur. The degree to which these physical changes happen varies depending on the seafood product, culinary process and internal endpoint temperature. Understanding the physical changes that occur based on culinary method and endpoint temperature can offer guidance for overall acceptability of the products in consumer testing. Evaluation of color and non-oral texture characteristics by a trained panel can assist with the development of descriptions of doneness relative to a safe internal temperature range (64 - 74°C), seafood product and culinary process. Detailed descriptions can be used to replace generic guidelines currently published in cookbooks, to increase consumer safety when preparing food at home or in a food service facility.

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Chapter 4

Evaluation of Culinary Processes and Internal Temperature on Consumer Acceptability and Descriptive Characteristics of Fish and Shellfish

Abstract

Cookbooks commonly recommend visual guidelines using terminology such as “firm”, “opaque”, and “flakes easily with a fork” for determining doneness of fish and shellfish. Generic cookbook guidelines were tested for success in achieving a minimum safe internal temperature (63°C) of four fish and shellfish products (Atlantic salmon, tilapia, shrimp, and oysters) prepared by popular culinary methods tested. Many samples exhibited doneness characteristics but were below the minimum safe internal temperature of 63°C. A trained descriptive panel (n = 7) determined visual characteristics of doneness for commonly consumed products including baked Atlantic salmon, baked tilapia, and boiled shrimp prepared to two internal temperature endpoints (64°C ± 1 (147°F) and 74°C ± 1 (165°F)). Specific attributes for each product were identified including coagulated protein (white film) for baked salmon, separation between muscle flakes for baked tilapia and the overall shape for boiled shrimp. To determine the eating quality associated with the recommended minimum internal temperature (63°C), a consumer acceptability test was conducted using a 9-point hedonic scale. Consumer acceptance (n = 50) of Atlantic salmon (baked, poached), tilapia (baked, pan-fried) and shrimp (boiled) prepared to two internal temperature endpoints (64°C ± 1 (147°F) and 74°C ± 1 (165°F)) was tested. No statistical difference ($p>0.05$) between the two endpoints for each respective product and preparation combinations was found. Therefore, a higher minimum internal temperature could be recommended for the tested products without compromising eating quality and overall acceptability.

Introduction

A search of popular cookbooks including *The Joy of Cooking*, cookbooks specifically focused on fish and shellfish recipes, and culinary textbooks yielded visual characteristic guidelines for determining doneness of fish and shellfish. Visual descriptors for fish included “firm”, “opaque in color”, and “flakes easily with a fork” (Brown 2007; CIA 2006; Southern Living 1984; Ramette and Sternberg 1998; Schiel 1994; Rombauer, Becker and Becker 1997). For shrimp, the color appears “opaque” and “pink” when done. Oysters in their shell will “open slightly”, but must be cooked for at least 5 minutes from when the shell opens or to 63°C (145°F) (Brown 2007). For shucked oysters, doneness is indicated by “edges curling” (Southern Living 1984). Although several different cooking methods were used within these cookbooks, very few offered unique doneness characteristics associated with individual methods; however timed cooking per piece/side or per inch of thickness was provided occasionally. While numerous recipes are typically provided for a specific type of fish, generic terms of “firmness”, “opaque” and “flakiness” are provided to indicate doneness for all varieties of fish. Internal temperature measured with a thermometer is listed as a method for determining doneness of fish in *Joy of Cooking* (1997) and *Culinary Institute of America* (2006) textbook. *Joy of Cooking* reports “All fish is cooked through at 137°F” (58°C). The cookbook further recommends 135°F (57°C) as being at a level of doneness to exhibit desired eating characteristics to satisfy everyone and 120°F (49°C) for fish that are often preferred undercooked such as tuna (1997). The *Culinary Institute of America* (2006) textbook recommends an internal temperature of 63°C (145°F) for determining the doneness of fish, which reflects the recommendation of the FDA. A minimal internal temperature of 63°C for 15 sec is recommended for intact fish by the FDA Food Code and is provided as guidance to consumers, food service professionals and industry (2009).

The FDA Food Code’s minimal internal recommendation for intact fish and shellfish was determined based on reducing *Salmonella* spp., but no mention of the eating quality it renders is provided (2009). Numerous varieties of fish and shellfish are consumed and these products vary in structure, composition, size, shape and how they are prepared and consumed. A generic recommendation may be unsuitable to achieve the desired eating quality for all products and culinary preparation methods. Individuals have their own expectations on how specific fish and shellfish should appear, and the taste and the texture they should exhibit when cooked. A minimum temperature of 63°C may be too high, not high enough or on target with consumer's expectations of fish and shellfish eating quality.

The risk of contracting a food-borne illness is associated with consuming undercooked fish and shellfish. The Center for Science in the Public Interest (CSPI) reviewed data from the Centers for Disease Control and Prevention (CDC) over a 10 year period from 1998 – 2007. CSPI identify food-borne illness (outbreaks and cases) related to specific foods; seafood was associated with the most outbreaks totaling 838 outbreaks involving 7,298 cases (2009). Three types of syndromes are associated with the ingestion of *Salmonella* spp. including typhoid fever, enteric fever and gasteroenteritis syndrome (food poisoning). Food poisoning is the most common in the U.S. and symptoms include vomiting, headache, diarrhea, nausea, abdominal pain, chills and fever (Doyle and Cliver 1990). Undercooked fish or shellfish, which can occur in a home or food service preparation and serving environment, may be the cause of a single case or an outbreak of food-borne illness. The effects on each individual, in terms of comfort, health costs, and lost income from missed work days, and the home or food service establishment, such as loss of reputation, clientele, and business revenue, may be preventable with more education and improved detection methods for assessing the endpoint of cooking fish and shellfish. Providing improved fish and shellfish product descriptions of doneness and minimum internal temperature recommendations can help reduce the chance of undercooking fish. The new guidelines will improve the confidence of cooks and chefs, at home and in restaurants, so that they know they are providing a safe product with desirable eating quality.

The primary research objective of this project was to assess visual characteristics of doneness and consumer sensory preference for fish and shellfish products prepared by different culinary processes to two different thermal endpoints (64°C ± 1 (147°F) and 74°C ± 1 (165°F)). Three studies supporting this objective were conducted.

Study 1: Determination of the internal temperature of fish and shellfish (Atlantic salmon, tilapia, shrimp, oysters) prepared by standard culinary methods (baking, pan-frying, broiling, boiling, poaching, sautéing) using visual guidelines provided in cookbooks.

Study 2: Description of visual characteristics of doneness for baked Atlantic salmon, baked tilapia, and boiled shrimp prepared to two internal temperature endpoints (64°C ± 1 (147°F) and 74°C ± 1 (165°F)).

Study 3: Consumer acceptance of Atlantic salmon (baked, poached), tilapia (baked, pan-fried) and shrimp (boiled) prepared to two internal temperature endpoints (64°C ± 1 (147°F) and 74°C ± 1 (165°F)).

Materials and Methods

Fish and Shellfish Samples and Storage

Please refer to Chapter 3

Characterization of Fish and Shellfish Composition

Please refer to Chapter 3

Internal Temperature Measurement

Please refer to Chapter 3

Study 1: Determining the IT of fish and shellfish using cookbook guidelines

Cooking instructions for various culinary methods and visual indicators of doneness for fish and shellfish were collected from cookbooks and textbooks (Table 4-1 and 4-2). Four fish and shellfish products were studied with two to three culinary methods each (Table 4-3). Fish and shellfish samples were thawed and stored under refrigerated temperatures. Before cooking, the fish and shellfish were rinsed with cold water and patted dry with paper towels. Raw weight and thickness of each sample was recorded (see Appendix A). Three replications were completed for each fish/shellfish product and culinary method combination. Each product and preparation method combination was prepared on one individual sample at a time. Once the product exhibited the visual and non-oral textural indicators of doneness noted, the product was removed from the heating element and the internal temperature was measured using a thermocouple (Table 4-2). Oven, griddle and water temperatures were monitored and total cooking time was recorded. After recording the internal temperature, the sample was photographed immediately under north daylight conditions (Canon PowerShot SX110 IS Digital Camera, ISO 400, manual setting, 1/125, whiteness balance – Fluorescent H, macro setting, F 4.0). The time required to cool each product to 50°C (122°F), 35°C (95°F) and room temperature ~27-30°C (80-86°F) was monitored.

Table 4-1: Culinary Processes Developed From Culinary Textbooks for Fish and Shellfish

Culinary Processes	Products	Culinary Process Description
Baking[1]	Atlantic salmon & tilapia	• Oven was preheated to 375°F • Fillet was placed in a glass baking dish lightly coated with cooking spray • Baking dishes were placed on the middle rack of the oven
Broiling[1]	Atlantic salmon, tilapia, shrimp & oysters	• Oven was set to broil (electric stove was used) • Fish/shellfish was placed on broiler pans coated with cooking spray • Broiler pans were placed on the 2nd shelf from the top of the oven • Oven door was left ajar 3-4 inches • Tilapia was coated with melted butter because it is a lean fish
Poaching[1]	Atlantic salmon	• Poaching ingredients: 1 tbsp butter, 1/3 cup celery, 1/3 cup carrots, 1/3 cup onion, ½ white wine and 4 cups water • Sauté veggies with butter for 5 min then add water and wine • Water was heated to between 160 – 180°F (never allowed to boil)
Pan-Frying[2]	Tilapia & oysters	• Oysters were breaded by first being dredged in flour, followed by an egg wash (whole eggs and water), and finally dipped in bread crumbs. • The product was added to an electric skillet with vegetable oil pre-heated to 350°F (the oil should come about half-way up the side of the product). • The product was allowed to cook on one side to a golden brown color before being flipped. The second side was cooked to a golden brown color then promptly removed from oil. • For Tilapia, no breading was used and the electric skillet was coated with only a thin layer of oil
Boiling[1]	shrimp	• Water was brought to a low boil with gentle bubbles barely breaking the surface, around 180°F - 185°F • Shrimp were added to the water once it reached the desired temperature
Sautéing[1]	shrimp	• Shrimp were heated in an electric skillet with ½ tbsp of butter at 350°F • Shrimp were moved and turned with a spatula constantly until the desired end point was reached

[1] Brown (2007)
[2] CIA (2006)

Table 4-2: Visual and Non-Oral Texture Characteristics of Doneness for Fish and Shellfish as Described in Cookbooks and Culinary Textbooks

Product	Culinary Process	Visual Doneness Characteristics
Salmon	Poached	Firm, opaque and just begins to flake[1]; Flakes easily with a fork[2]; It should look rosy, not deeply salmon-colored[4]; Still moist and separates easily into segments[6]
	Baked	
	Broiled	
Tilapia	Broiled	Firm, opaque in color, separating from the bone, flakes easily without falling apart[5]; Still moist and separates easily into segments[6]
	Pan-fried	
	Baked	
Shrimp	Sautéed	Will look opaque and pink[5]; Flesh becomes pearly opaque[6]
	Boiled	
	Broiled	
Oysters	Pan-fried	If breaded – 3 min on each side or until golden brown[3]
	Sautéed / Broiled	Until edges of oysters begin to curl[3]

[1] Ramette and Sternberg (1998)
[2] Schiel (1994)
[3] Southern Living (1984)
[4] Rombauer and others (1997)
[5] Brown (2007)
[6] CIA (2006)

Table 4-3: Culinary Processes Applied to Respective Fish and Shellfish Products

Fish and Shellfish Products	Culinary Process
Atlantic Salmon	Poached, Baked & Broiled
Tilapia	Pan-Fried, Baked & Broiled
Shrimp	Sautéed, Boiled & Broiled
Oysters	Pan-Fried (breaded) & Broiled

Study 2: Descriptive Visual Indicators of Doneness Determined by a Trained Panel

Visual characterizations for baked Atlantic salmon, baked tilapia, and boiled shrimp at two internal endpoints (64°C ± 1 and 74°C ± 1) were conducted using a trained descriptive panel (n = 7). The Virginia Tech Institutional Review Board approved the sensory study (IRB 10-152, Approved March 3, 2010, Appendix D). Protocols for consumer acceptability testing and descriptive trained panels followed those described by Meilgaard and others (2007). Sensory training for the descriptive panel took place in the classroom at the Food Science and Technology (FST) department at Virginia Tech. Sensory testing was conducted in individual booths in the sensory laboratory of the FST department. Information was collected with paper scorecards and samples were displayed electronically using touch screen computers with sensory software (Sensory Information Management System (SIMS) 2000, Version 6, Morristown, NJ).

Recruitment for panelists was conducted using Virginia Tech's online survey system (www.survey.vt.edu) (Appendix E).

Panelist Training

Seven panelists (4 males, 3 females; age range 21-70) were selected based on availability, willingness to participate, and indication that they cook and consume fish and shellfish at least 1-2 times a year. A consent form outlining the details of the study and expectations of participants was given to panelists to review and sign on the first day of training (Appendix F). Training sessions occurred twice a week for 1 hour and lasted 4 weeks (8 hrs total). Panelists were presented with photographs and asked to describe visual characteristics of baked Atlantic salmon, baked tilapia and boiled shrimp. The fish/shellfish and culinary combinations tested were chosen because they were popular among consumers and to help establish a foundation of possible visual indicators for each product. Fish sample training included photographs of each product raw and after being baked at 375°F for 5, 10, 15, 20 and 25 min. Internal temperatures associated with the timed cooking increments were presented to panelists. Similarly, shrimp training included photographs which indicated internal temperatures of the shrimp while raw and after being heated in boiling water for 15 s, 30 s, 45 s, 1 min, 1.5 min, 2 min, 2.5 min, and 3 min. Panelists were presented with each photograph and asked to record visual characteristics they discerned. Once individual characterizations were written down, the group shared observations and discussed. The descriptions applied to each photograph were compiled and repeating characteristics/attributes were noted. From the descriptive terminology applied most frequently, the panel developed qualitative descriptive terms to associate with each respective fish and shellfish product (Appendix P).

Panelist Validation

Validation testing consisted of six photos for each product/method combination. Each product consisted of three different samples varying in cooking time and, thus, internal temperature including: baked Atlantic salmon and baked tilapia at 10, 15, and 20 min and boiled shrimp at 1, 2, and 3 min. Each sample was represented in duplicate by showing the product from two angles, thus six photos were presented per product/method combination. Photographs were selected by the researcher to illustrate attributes of importance at various levels of visual evidence. The goal of the validation test was to determine the panel's ability to apply the qualitative descriptive terms properly and consistently. Validation testing used SIMS to present photographs on computer monitors with answers recorded on paper scorecards (Appendix Q). Sample photographs were identified with 3-digit codes. Three to four specific attributes that exhibited changes with endpoint temperature were identified by the researcher (Appendix R). To successfully verify the panel performance on each validation test, at least 5 of the 7 panelists needed to correctly characterize the predetermined attributes. Panelists went through additional training on characteristics they unsuccessfully described in the first round of validation testing and were re-tested (Appendix Q). Discussion among the panel after the first round of validation testing led to changes on the scorecard and rephrasing of qualitative terms. For baked salmon, "Indicate whether or not separation is occurring along the central line" replaced "Indicate visibility of the central line" and "Indicate the indentation appearance along the central line." The options for appearance characteristics associated with the outer edges of baked tilapia changed from "rounded", "crispy" and "curling" to "none", "crispy" and "curling". The validation re-test consisted of the same set of pictures that were re-coded with a new random 3-digit code; presentation order was randomized. Panelists were required to correctly identify all of the re-test attributes for each cooking time increment at least one of the two times presented.

Visual Characteristic Assessment

The visual characterization test used SIMS to present photographs on computer monitors with answers recorded on paper scorecards (Appendix S). The scorecard questions reflected those of the validation test, changes that were made during the validation re-test and several questions were removed due to limited changes in the characteristic between 64°C ± 1 and 74°C ± 1 (Table 4-4). Questions removed for fish included: color intensity of color/hue and lines between muscle segments for baked salmon and baked tilapia and color vibrancy of baked salmon. Questions removed for shrimp included: color intensity, undertone color, surface shine, and visibility of segments. Options to describe the qualitative descriptive terms were also refined. The visual characterization test was administered in a darkened room to allow optimal visual assessment of the pictures on the computer monitor. Panelists were allowed only a minimum amount of light provided through the hatch door needed to view the scorecards. The visual characterization test consisted of six randomized pictures of different individual samples.

For each product (baked Atlantic salmon, baked tilapia, and boiled shrimp) three pictures that had an IT of 64°C ± 1 and three pictures at 74°C ± 1 were presented individually and electronically on a touch-screen computer using SIMS (see Appendix T for outline of training sessions). Using Adobe® Photoshop® (CS5 Extended, Version 12.0 x 32) the test pictures used for validation testing and final assessment were analyzed for average CIE L^* a^* b^* color values for the overall color of the sample, the outer edges of the salmon and tilapia fillets, and the remaining head portion of the shrimp (head was removed).

A total of 21 responses were provided among the seven panelists for three photographs of product (triplicates) at 64°C ± 1 and at 74°C ± 1; the three product photographs shown at each endpoint represented three independent samples (not images of the same sample/photo from different angles). A decision tree was established for determining the strength (weak, moderate, strong) of the trend related to the qualitative term (Figure 4-1). The decision tree is applicable to terms that have only two qualitative choices available and panelists are only allowed to choose one term. However, for some of these terms, there was one right answer determined by the researchers per replication. Each replication was analyzed to see if the majority (five of the seven) of the panelists gave the correct answer. Next, a comparison between the internal temperature endpoints was conducted to determine if panelists who didn't perceive the attribute at 64°C ± 1 were able to at 74°C ± 1. For qualitative terms with three options and terms that allowed panelists to select more than one term, a generalization was made on the overall trend. The information gathered from these terms will be used in the second phase of the research to gain more clarity on their ability to distinguish doneness.

Study 3 – Consumer Acceptance for Fish and Shellfish Prepared to Two Endpoints

Consumer panels (n = 50) were conducted to determine overall acceptability, using a 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely), for five product and culinary method combinations cooked to two internal endpoints 64°C ± 1 (147°F) and 74°C ± 1 (165°F)) (Table 4-5). The broiling method was not tested because the thermocouples were not able to withstand broiling conditions for long periods of time, the inconsistency with reaching the appropriate internal endpoints, and the lower frequency of use among consumers compared to other culinary processes.

Table 4-4: Visual Qualitative Descriptors for Fish and Shellfish Determined By Trained Panel and Used for Visual Assessment Test

Products	Visual Qualitative Descriptor Term	Definition of Term	Qualitative Descriptor Choices
All Products	Color/Hue	Dominating primary color family associated with the product (red, orange, yellow, green, blue, violet, white and black)	Salmon/Shrimp: Red, Orange Tilapia: White & Yellow
Baked Salmon Only	Overtone Color	Color that develops over the primary color/hue	None, Yellow, & Golden
	Surface Shine	Amount of light reflected from the product's surface	Matte or Glossy
	Definition of the lines between the muscle flakes	Visibility of the connective tissue (lines) between the myocommata (appear as striations)	Not Defined or Defined
	Separation along central line	Separation along lateral line	No Separation or Separation
	Existence of white film coverage	Appearance and amount of coagulated protein	Absent (none to small spots) or Present (small spots, with some large white areas)
	Appearance associated with outer edges of fillet	Appearance of the outer perimeter / border of the fillet	Round or Sharp
	Color associated with outer edges of fillet	Color of the outer perimeter / border of the fillet	None, Yellow, & Golden
Baked Tilapia Only	Color Vibrancy	Chroma/saturation of the color/hue	Dull or Vibrant
	Overtone Color	Color that develops over the primary color/hue	None, Yellow, & Golden
	Surface Shine	Amount of light reflected from the product's surface	Matte or Glossy
	Definition of the lines between the muscle flakes	Visibility of the connective tissue (lines) between the myocommata (appear as striations)	Not Defined or Defined
	Degree of separation occurring between individual muscle flakes	Separation between flakes (myotomes) and along vertical connective tissue lines (myocommata)	No Separation or Separation
	Appearance characteristics associated with the outer edges of the fillet	Appearance of the outer perimeter / border of the fillet. Crispy (brown, dry, crunchy/crumbly looking) & Curling (edges are noticeably curving up or down (not laying flat))	None , Crispy & Curling
	Color associated with the outer edges of the fillet	Color of the outer perimeter / border of the fillet	None, Yellow, & Golden
Boiled Shrimp Only	Color Intensity	Strength of the color/hue (lightness to darkness)	Light or Dark
	Color Vibrancy	Chroma/saturation of the color/hue	Dull or Vibrant
	Color/Hue associated with flesh	Color associated with the head of the shrimp	Translucent or White
	Overall shape of the shrimp	Silhouette or figure of shrimp	Moderately Coiled or Tightly Coiled

Decision Tree for Visual Qualitative Test Responses

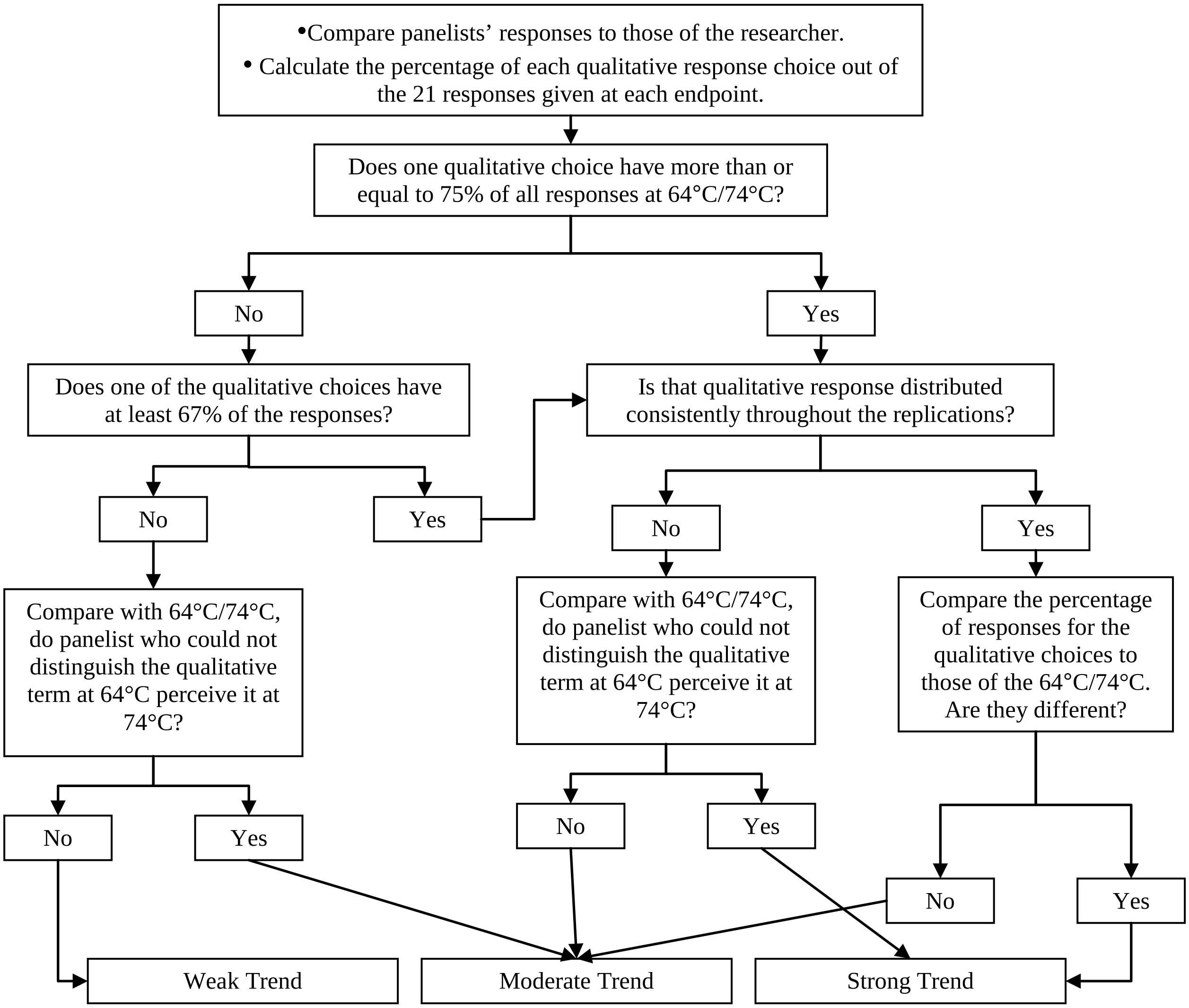

Figure 4-1: Decision Tree for Determining Overall Strength of Trends for Qualitative Visual Terms with Two Qualitative Choices

The Virginia Tech Institutional Review Board approved the sensory study (IRB 10-152, Approved March 3, 2010, Appendix D). Protocols for consumer acceptability testing followed those described by Meilgaard and others (2007). Sensory testing was conducted in individual booths in the sensory laboratory of the Food Science and Technology (FST) department at Virginia Tech. Information was collected with paper questionnaires and electronically using touch screen computers with sensory software (Sensory Information Management System (SIMS) 2000, Version 6, Morristown, NJ). Recruitment for panelists was conducted using Virginia Tech's online survey system (www.survey.vt.edu) (Appendix U). The survey system also was used for panelists to complete a demographic questionnaire; if not completed online prior to attending the panel, panelists were given a paper copy to complete and data was subsequently entered into the on-line system by the researchers (Appendix V). Before every consumer acceptability test, panelists were provided with consent form detailing the study and expectations of participants to review and sign (Appendix W).

Table 4-5: Fish and Shellfish Products Each Evaluated at Two Internal Temperature Endpoints (64°C & 74°C) for Overall Acceptability by a Consumer Sensory Panel (n = 50)

Fish and Shellfish Products	Culinary Processes for Evaluation
Atlantic Salmon	Baked and Poached
Tilapia	Baked and Pan-Fried (no breading)
Shrimp	Boiled

Preparation and Sampling

Cooking protocols established in Study 1 were followed with minor changes including: (1) poaching water amount was increased to 5 cups (1200 ml); and (2) after boiling, the internal temperature was measured and the shrimp were submerged in an ice water bath (Table 4-1). The commercially purchased farm-raised Atlantic salmon product had various cuts of fillets that were categorized as center-cuts and tail portions. Equal amounts of center-cut and tail portions were tested among panelists, with each panelist either receiving two samples from center-cut fillets or two from tail fillets. Atlantic salmon sampling occurred at the thickest portion of the fillet along the lateral line. The area was trimmed with a knife and dark muscle along the lateral line on the skin side of the fillet was removed. Each salmon fillet was flaked by hand into 6 – 8 pieces to be used as samples (Figure 4-2). For tilapia, the thickest portion of each fillet was cut into 6 – 8 equal square pieces using a knife (Figure 4-3). Each panelist received one whole peeled shrimp that was de-veined, de-headed and had only its tail remaining. Sampling areas were selected to mirror the location of texture analysis described previously in Chapter 3.

Sample Presentation

Samples were presented individually on solid white plastic plates (4” diameter), coded with a random 3-digit code; presentation order was randomized. Tilapia and salmon samples were served at approximately 55°C except for boiled shrimp which was served cold at approximately 7°C. Tilapia and salmon samples were placed in resealable polyethylene bags, placed inside reusable Whirl-Pak® bags weighed down with binder clips, and submerged in a water bath set to 57°C to maintain temperature until presented to panelists. Samples remaining after 45 min were discarded. Boiled shrimp were cooled in an ice water bath after cooking, patted dry with paper towels once removed, placed in resealable polyethylene bags and refrigerated until served. A preliminary study was conducted to ensure fillets and shrimp exhibited the same textural characteristics after a 30 min hold period in the water bath or refrigerator, no statistical changes were found. Instructions and overall acceptability questions were displayed and answered electronically using touch screen computers with sensory software SIMS (Appendix X).

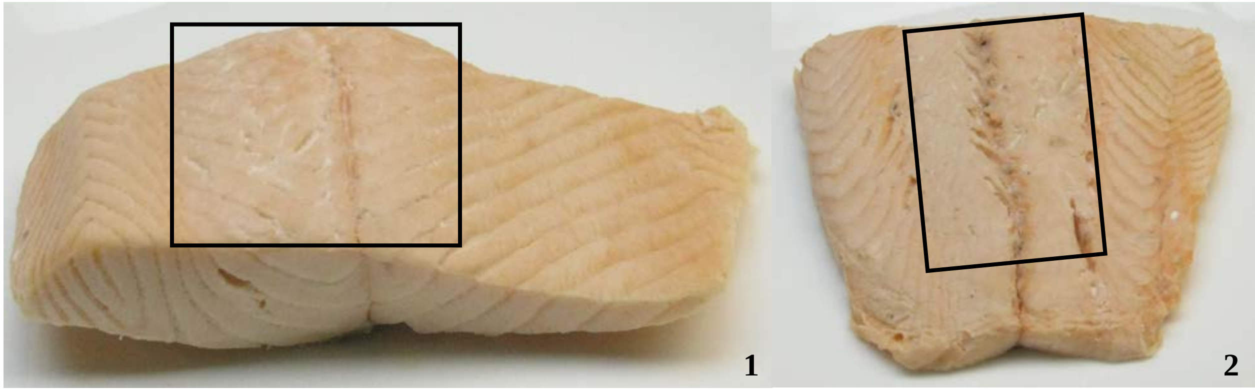

Figure 4-2: Consumer Acceptability Test Sampling Locations for Atlantic Salmon: (1) Center-Cut Fillet and (2) Tail Portion

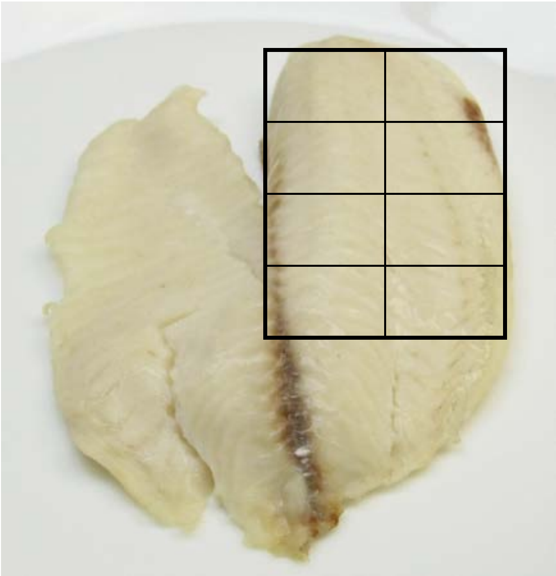

Figure 4-3: Consumer Acceptability Test Sampling Location for Tilapia

Demographic Questionnaire

Demographic information, including purchasing and consumption habits of fish/seafood and food safety concerns related to fish/seafood, was collected (20 questions). Panelists had the opportunity to participate in five different consumer panels; of the 107 panelists 76 participated in two or more panels. Virginia Tech's online survey system (www.survey.vt.edu) was used for panelists to complete a demographic questionnaire, if not completed online prior to attending the panel, panelists were given a paper copy to complete and data was subsequently entered into the on-line system by the researchers. A total of 107 different participants (68 females and 39 males) participated in the five consumer panels and each completed the questionnaire only once (Appendix V). Panelist questionnaire information was coded by a number assigned by the survey system starting with one, once a panelist completed the questionnaire online or their answers were manually entered into the systems by the researchers. If the questionnaire was filled out by the same person more than once, their first questionnaire answers were accepted and the others were removed from analysis.

Statistical Analysis

Study 1: The mean and standard deviation was calculated for the internal temperatures measured for each fish/shellfish product and culinary process combination (Table 3-3) prepared to visual doneness.

Study 2: The percent of responses in agreement were calculated, with each of the panelist responses per replication considered as an individual observation, therefore a total of 21 observations were made per attribute. A decision tree was used to determine the trend strength for attributes with two qualitative option choices. See *Visual Characteristics Assessment* for additional details. JMP 8 Statistical Software (SAS, Cary, NC) was used to conduct a one-way analysis of variance (ANOVA) to analyze color data collected from the validation and visual assessment pictures and Tukey's HSD Test to determined statistical differences ($p < 0.05$) between endpoints.

Study 3: The mean acceptability scores for 64°C ± 1 and 74°C ± 1 samples within a fish/shellfish and culinary process combination were calculated and compared for significant difference ($p < 0.05$) using Statistical Analysis Systems' (SAS) Tukey's / Studentized Range (HSD) Test. For questionnaire responses to multiple choice questions and "check all that apply" questions, the number of responses per answer choice was tallied and reported as a percentage of all responses. Answers to written questions were categorized into broad topics that encompassed popular replies from respondents. The frequency of responses for category were tallied and reported as a percentage of total responses.

Results and Discussion

Study 1: Determining the IT of fish and shellfish using cookbook guidelines

Two of the three of the cooking methods for Atlantic salmon (baked, poached) and tilapia (baked, broiled) appeared fully cooked (visual endpoint cues based on cookbook guidelines) but had internal temperatures lower than the minimum safe temperature of 63°C (Table 4-6). The undercooking of both salmon and tilapia is most likely due to the uneven thickness of the fillets. For shrimp, sautéing was the only method of the three preparation methods that was found to have a visual endpoint that did not yield the minimum safe internal temperature (63°C). Sautéing requires high heat and constant movement of the shrimp. The shrimp are subjected to brief moments of direct heat contact and limited heat penetration, causing the outside to appear done before the center is. For both methods of oysters (broiling, pan-frying), the visual endpoints were well above the safe temperature for intact (63°C) and comminuted fish (68°C). The endpoint for breaded pan-fried oysters was based on browning of the breading, which resulted in a safe internal temperature well above the minimum required, thus the researchers concluded further testing was not necessary. Breaded pan-fried oysters at 64°C and 74°C would most likely appear undercooked and not meet to consumers' visual expectations. Consumers using cookbook recommended visual cues for doneness may be at risk for undercooking select fish and shellfish depending on culinary method, resulting in food-borne illness. Overall, baking appears to have the greatest risk of undercooking based on visual indicators of doneness compared to all other culinary processes.

The time required to cool replications to 50°C (122°F) and room temperature (~27-30°C (80-86°F)) varied by product and cooking method. An estimated time for the salmon and tilapia to cool is 60 min, while oysters and shrimp needed around 20 min to reach room temperature. The information regarding cooling of individual products was used for estimating the time for samples to cool to room temperature before texture could be measured as discussed in the previous chapter.

Table 4-6: Internal Temperature[1] of Fish (Salmon, Tilapia) and Shellfish (Shrimp, Oysters) at Cooking Endpoints Based on Visual Doneness Characteristics

Product	Cooking Method	Mean Final IT (°C)	Standard Deviation
Salmon	**Baked**	**60.5**	0.80
	Broiled	67.7	1.82
	Poached	**62.5**	2.40
Tilapia	**Baked**	**59.4**	1.14
	Broiled	**61.0**	5.70
	Pan-fried (not breaded)	66.4	3.27
Shrimp	Boiled	65.4	4.26
	Broiled	69.9	3.12
	Sautéed	**60.1**	5.45
Oysters	Broiled	77.9	8.84
	Pan-fried (breaded)	78.0	3.71

Note: Methods in bolded type for each product have a visual endpoint below the minimum safe temperature of 63°C. [1]Products were removed from heating source prior to IT measurement.

Study 2: Descriptive Visual Characteristics Determined by Trained Panel

Baked Salmon

Baked salmon was identified as an “orange” color/hue for 64°C ± 1 (weak trend) and 74°C ± 1 (moderate trend) with an overtone color expressed as a “yellow-golden” color for 64°C ± 1 and 74°C ± 1 (Table 4-7). The color associated with the outer edges of the fillet was described as “golden” for 74°C ± 1, but generally as “yellow-golden” for 64°C ± 1 (Table 4-7). Adobe® Photoshop® color analysis of the pictures for the final visual assessment found differences ($p < 0.05$) for b^* and chroma in outer edge color between the two endpoints (Appendix Y, Table Y-1). Definition occurring between individual muscle flakes was characterized as “defined” for both endpoints (strong trends) (Table 4-7). Based on validation test results during the training phase, definition between individual muscle flakes was seen in fillets cooked to internal temperatures of 44°C and 59°C; therefore this attribute is not capable of distinguishing between cooked and undercooked (Appendix Z). Separation along the central line was correctly identified for each individual fillet by at least five of the seven panelists and occurred in one of the three fillets at 64°C ± 1 and two of the three fillets at 74°C ± 1 (Table 4-7). The chance of separation along the lateral line appears to increase as internal temperature increases due to the muscle fibers above and below the lateral line shrinking and contracting to become more compact (Kong and others 2007; Hatae and others 1990). The presence of white film was detected by panelist for both 64°C ± 1 and 74°C ± 1 which was expected (Table 4-7). According to visual training validation test results, white film was “absent” at 44°C and “present” at 59°C, indicating the presence and amount of white film could be useful in differentiating between cooked and undercooked (Appendix Z). The term white film was given to the coagulated sarcoplasmic protein that forms on the surface of the salmon due to the muscle fibers contracting during heating, pushing the protein from the muscle fibers (Charley 1951; Hatae and others 1990). A minimum amount of coagulated protein has not been quantified for differentiating between undercooked and above 63°C; therefore the presence of white film will have to be coupled with other indicators. Overall, several new descriptive terms for baked salmon were identified and their ability to distinguish between uncooked and cooked needs to be further explored. Separation along central line and the color associated with the outer edges of the fillet, were identified as being able to differentiate between 64°C ± 1 and 74°C ± 1 and are recommended for further study to determine their ability to distinguish undercooked from 63°C.

Baked Tilapia

The overall color/hue discerned was “white” for 64°C ± 1 (strong trend) and 74°C ± 1 (moderate trend) and the vibrancy associated with both internal temperatures was described as “dull” (64°C ± 1 (weak trend), 74°C ± 1 (strong trend)) (Table 4-8). The overtone color and the color associated with the outer edges of the fillet indicated 64°C ± 1 to be “none” to “yellow” and 74°C ± 1 to be “yellow” to “golden”. The Adobe® Photoshop® analysis of the pictures used for the visual assessment determined all edge color values were statistically significantly different ($p < 0.05$) between the two endpoints, supporting the difference noted by the panelists (Appendix Y, Table Y-1). Pictures used for the validation test during found edge color for samples with an internal temperature below or around 63°C were typically statistically different ($p < 0.05$) from samples cooked to above 63°C (Appendix Y, Table Y-2). The surface shine for 74°C ± 1 was described as “glossy” (moderate trend), while 64°C ± 1 had no definite discernment (weak trend) (Table 4-8). The increase in glossy appearance may be due to the muscle fibers contracting during heating causing moisture to be pushed out of the product, in addition to the known higher degree of cook loss at 74°C ± 1 as discussed in Chapter 3 (Tornberg 2005).

Table 4-7: Qualitative Visual Characterization Test Responses from a Trained Panel (n = 7) for Baked Atlantic Salmon Cooked to Two Internal Endpoints

Questions	Baked Salmon	
	64°C	74°C
1. Indicate the dominating color/hue[1]	Red: 38% Orange: 62%	Red: 14% Orange: 76%
2. Indicate the overtone color present[3]	None: 5% Yellow: 43% Golden: 52%	None: 10% Yellow: 33% Golden: 57%
3. Indicate the term that best describes the surface shine[1]	Matte: 57% Glossy: 43%	Matte: 57% Glossy: 43%
4. Indicate the definition of the lines between muscle flakes[1]	Not Defined: 14% Defined: 86%*	Not Defined: 19% Defined: 81%*
5. Indicate whether or not separation is occurring along the central line[2]	Separation along the central line was correctly identified for each individual fillet by at least 6 of the 7 panelists (86%) and occurred in 1 of the 3 fillets at 64°C	Separation along the central line was correctly identified for each individual fillet by at least 5 of the 7 panelists (71%) occurred in 2 of the 3 fillets at 74°C
6. Indicate the existence of white film coverage[2]	Absent: 5% Present: 95%	Absent: 0% Present: 100%
7. Indicate the appearance associated with the outer edges of the fillet[1]	Sharp: 14% Round: 86%*	Sharp: 10% Round: 90%*
8. Indicate the color associated with the outer edges of the fillet[3]	None: 5% Yellow: 43% Golden: 52%	None: 5% Yellow: 14% Golden: 81%

[1]Decision tree was used to evaluate the question and identify weak, moderate and strong trends
[2]A correct answer was assigned to the question by the researcher
[3]Generalizations of the answers were made from the question
*Indicates strong trend

Separation between muscle flakes was correctly identified for each individual fillet at 74°C ± 1 by at least five of the seven panelists and occurred in all three of the fillets at 74°C ± 1. Less confidence in identifying separation between muscle flakes was detected for fillets at 64°C ± 1, with only one of the fillets being correctly characterized by six of the seven panelists (Table 4-8). According to the validation test results established during training, the sample at 52°C was described as exhibiting no separation between muscle flakes; therefore the attribute has potential for being able to discriminate between cooked and undercooked (Appendix Z). Separation between muscle flakes is occurring due to the gelatinization of connective tissue during heating which results in the muscle flakes no longer being held together (Dunajski 1979). Fillets at 64°C ± 1 commonly were described as having no distinguished edge characteristics, compared to 74°C ± 1 fillets, which demonstrated "crispiness" and "curling" on two of the three fillets (Table 4-8).

Varied combinations of edge characteristics were applied to fillets by panelists, which could indicate difficulty of discernment and/or confusion among panelists on the definition for each term.

Overall, new visual indicators were identified for determining the doneness of baked tilapia and a few were identified for assisting with discerning between 64°C ± 1 and 74°C ± 1 and are recommended for further study including: overtone color, outer edge color, separation between muscle flakes and outer edge characteristics. Visual characteristics able to distinguish between 64°C ± 1 and 74°C ± 1 may be useful in distinguishing between undercooked and 63°C. Many of the attributes for baked tilapia used need better clarification of terminology used such as in the case of outer edge characteristics.

Table 4-8: Qualitative Visual Characterization Test Responses from a Trained Panel (n = 7) for Baked Tilapia Cooked to Two Internal Endpoints

Questions	Baked Tilapia	
	64°C	**74°C**
1. Indicate the dominating color/hue[1]	White: 90%[*] Yellow: 10%	White: 67% Yellow: 33%
2. Indicate the vibrancy of the color/hue[1]	Dull: 62% Vibrant: 38%	Dull: 81%[*] Vibrant: 19%
3. Indicate the overtone color present[2]	None: 43% Yellow: 43% Golden: 14%	None: 10% Yellow: 48% Golden: 43%
4. Indicate the term that best describes the surface shine[1]	Matte: 48% Glossy: 52%	Matte: 33% Glossy: 67%
5. Indicate whether or not the lines between muscle flakes are defined[1]	Not Defined: 52% Defined: 48%	Not Defined: 52% Defined: 48%
6. Indicate whether or not separation is occurring between individual muscle flakes[2]	Separation occurred in 2 of the 3 fillets at 64°C; Only 1 fillet was correctly identified by 6 of the 7 panelists	No Separation: 10% Separation: 90%
7. Indicate appearance characteristics associated with the outer edges of the fillet: (check all that apply)[3]	None: 62% Crispy: 14% Curling: 24%	None: 24% Crispy: 57% Curling: 57%
8. Indicate the color associated with the outer edges of the fillet[3]	None: 38% Yellow: 43% Golden: 19%	None: 0% Yellow: 38% Golden: 62%

[1]Decision tree was used to evaluate the question and identify weak, moderate and strong trends
[2]A correct answer was assigned to the question by the researcher
[3]Generalizations of the answers were made from the question
[*]Indicates strong trend

Boiled Shrimp

The color/hue associated with the surface of the boiled shrimp at 74°C ± 1 was characterized as “red” (moderate trend), compared to 64°C ± 1 samples where the color was unclear (weak trend) (Table 4-9). The color intensity of shrimp at 64°C ± 1 was described as “light” (strong trend) compared to 74°C ± 1, which was indistinguishable (weak trend) (Table 4-9). The vibrancy of the color/hue is a characteristic that differs distinctly between the two endpoints; shrimp at 64°C ± 1 was described as “dull” (strong trend) and 74°C ± 1 was described as vibrant (strong trend) (Table 4-9). The flesh color/hue for boiled shrimp was described as “white” (strong trend) for both 64°C ± 1 and 74°C ± 1 (Table 4-9). The shrimp should appear white at the endpoints due to the heat denaturing the protein and causing it to coagulate loosing its translucency (Charley and Weaver 1998). Adobe® Photoshop® analysis calculated the overall color for segments 1 – 5 and the remaining flesh from the head portion of the shrimp (head was removed); differences between endpoints were identified for the final visual assessment. The overall color for segments 1 – 5 had differences ($p < 0.05$) for *a**, *b** and chroma and overall color of the remaining head portion was statistically different ($p < 0.05$) for hue (Appendix Y, Table Y-3). The chroma value is higher at 74°C ± 1 indicating a great intensity of color that reflects the panelist interpretation the 74°C ± 1 being “vibrant” and 64°C ± 1 being described as “dull” and light. Pictures for the validation test during training found that samples with an internal temperature below or around 63°C were typically statistically different ($p < 0.05$) from samples cooked to above 63°C (Appendix Y, Table Y-4). The shape of the boiled shrimp at 64°C± 1 was correctly identified for each of the three samples, of which one was “moderately coiled” and two were “tightly coiled”. For 74°C ± 1 samples, the shape exhibited was “tightly coiled”, but only two of the three samples were correctly identified by at least five of the seven panelists, indicating there may have been confusion between discerning “moderately coiled” and “tightly coiled” (Table 4-9). The curling or change in shape of the shrimp during heating is caused by the muscle fibers shrinking (Benjakul and others 2008; Mizuta and others 1999). The results of the visual training valuation test indicated that panelists described 43°C sample as “moderately coiled”; therefore shape may be able to distinguish between undercooked and 74°C ± 1 (Appendix Z). Overall, vibrancy was found to be distinctly different between the two endpoints for boiled shrimp. Color/hue of the shrimp and color intensity provided some differentiation between the endpoints, but not a clear distinction.

Table 4-9: Qualitative Visual Characterization Test Responses from a Trained Panel (n = 7) for Boiled Shrimp Cooked to Two Internal Endpoints

Questions	Boiled Shrimp	
	64°C	74°C
1. Indicate the dominating color/hue associated with the shrimp (do NOT consider the tail/shell portion)[1]	Red: 57% Orange: 43%	Red: 76% Orange: 24%
2. Indicate the color intensity associated with the color/hue identified in Question 1[1]	Light: 100%* Dark: 0%	Light: 57% Dark: 43%
3. Indicate the vibrancy of the color/hue and color intensity identified in Question 1 and 2[1]	Dull: 86%* Vibrant: 14%	Dull: 24% Vibrant: 76%*
4. Indicate the color/hue associated with the flesh of the shrimp's head[2]	Translucent: 10% White: 90%	Translucent: 24% White: 76%
5. Indicate the overall shape of the shrimp[2]	1 shrimp was moderately coiled and 2 were tightly coiled, all were correctly identified by 6 of the 7 panelists	All 3 shrimp exhibited a tightly coiled shape, but only 2 of the 3 samples were correctly identified by at least 5 of the 7 panelists

[1]Decision tree was used to evaluate the question and identify weak, moderate and strong trends
[2]A correct answer was assigned to the question by the researcher
[3]Generalizations of the answers were made from the question
*Indicates strong trend

Many of the original attributes identified for boiled shrimp during training were only detectable between raw and cooking times of 15 s, 30 s, 45 sec and 1 min (43°C). Limited visual characteristics of the shrimp could be discerned between 1 min (43°C), 2 min (62°C) and 3 min (69°C) of boiling. During training, varying shades of white were noticeable for the flesh of the shrimp. When not presented side-by-side, panelist could not discern differences in shades of white. A laminated, detachable doneness chart inside a cookbook with pictures of shrimp flesh color gradients and shapes (degrees of curling) for shrimp would allow people to easily compare and determine doneness. Non-oral texture characteristic of firmness previously discussed in Chapter 3 is critical for determining doneness of boiled shrimp in addition to vibrancy and a moderately coiled to tightly coiled shape.

Study 3 – Consumer Acceptance for Fish and Shellfish Prepared to Two Endpoints

Understanding what seafood products and culinary methods are popular among consumers and the concerns regarding seafood safety consumers have can direct research studies and educational programs to assist with providing consumers useful knowledge. The majority of panelists were students, faculty and staff at Virginia Tech due to the testing location at the University (full questionnaire data in Appendix AA). According to the fish and seafood questionnaire most panelists (78%) indicated they routinely ate fish/seafood (1 – 2 times a month or more) (Table 4-10). Over half (55%) often cooked fish/seafood (12 – 24 times a year or more) (Table 4-10).

The majority (88%) purchase raw seafood/fish, thus proper cooking instructions and handling guidelines are required to prevent against food-borne illness (Table 4-10). Participants were asked to write in the required minimum safe internal temperature (°F) for cooking fish/seafood and only 16% correctly wrote in 145°F (63°C) exactly; however 41% responded 146°F or above with answers ranging from 150°F – 250°F (Table 4-10). Most (74%) indicated they use visual indicators to detect doneness, suggesting improved visual guidelines could easily be implemented by consumers (Figure 4-4). Anderson and others (2004) videotaped consumers (n = 99) preparing a meal at their home and compared consumers' cooking and food handling behaviors to those recommended by the Partnership of Food Safety Education's campaign called Fight BAC! Among the participants, 30 owned a food thermometer, but only five consumers used a thermometer to check for doneness during the study. Almost half of the participants did not know the recommended internal temperature for chicken (n = 43) and ground beef (n = 44). When checking for doneness of chicken breast, meatloaf, or halibut, participants most often "cut with a knife" (n = 40), "poked with utensil" (n = 36), and used "visual" cues (n = 13). The internal temperature of each participants sample was measured by researchers and the chicken breast was found to be most frequently undercooked (Anderson and others 2004).

Two-thirds (67%) indicated that they knowingly eat raw or undercooked fish/seafood (Table 4-10). Byrd-Bredbenner and others (2008) surveyed young adults (n = 4,343) currently enrolled in higher education on risky eating behaviors. The study found 29% consumed sushi and 11% consumed raw oysters, clams, or mussels. Greater consumption of risky foods was reported for males and whites compared to women or nonwhites respectively (Byrd-Brebenner 2008). No difference related to gender was determined among participants who answered "Yes" to knowingly consuming raw or undercooked fish/seafood; however non-whites reported "Yes" (71%) more frequently that whites (65%). Klontz and others (1995) conducted a telephone survey (n = 1,620) to evaluate food consumption and food preparation behaviors related to an increased risk for food-borne illness. Consumption of raw clams and oysters (17%) and raw sushi or ceviche (8%) was reported with respondents from coastal regions (27.4%) more often eating raw fish and shellfish than those living in inland regions (19.9%) (Klontz and others 1995). Consumers may choose to eat sushi (raw fin fish) or raw/undercooked oysters for several reasons including: perceived high quality, overall eating experience, or cultural tradition (Klontz and others1995). Atanassova and others (2007) tested the microbiological quality of sushi in Germany purchased frozen from supermarkets and fresh from sushi bars. Aerobic mesophilic bacteria, *Escherichia coli* and *Staphylococcus aureus* were present at higher levels in fresh sushi samples compared to frozen industrially processed samples purchased from supermarkets (Atanassova and others 2007). Decreasing the risk of food-borne illness from with eating raw oysters is difficult. Naturally present in marine environments, *Vibrio vulnificus* is most commonly responsible for food-borne illness associated with raw oyster consumption (Valley and others 2008). High concentrations of *V. vulnificus* in marine environments have been found during the summer months (La Valley and others 2008). Frequent raw oyster eaters can not distinguish safe oysters from ones contaminated by *V. vulnificus* (HHS 2009).

Participants associate a variety of microorganisms, bodily illness, and mercury as health risks when consuming raw/undercooked seafood (including fish) (Figure 4-5). The responses suggest the participants were aware that microorganisms may be present in raw/undercooked fish and shellfish and that physical illness (i.e. vomiting, nausea, upset stomach) can result from consumption. Seafood is a good source of protein, omega-3 fatty acids and other nutrients. Health risks beyond food-borne illness have been caused by contaminants such as inorganic compounds, metals, and persistent organic pollutants have been associated with fish and shellfish. Women who are pregnant, nursing or may become pregnant need to be cautious of consuming seafood due to the presence of methylmercury, which has been found to cause damage to the development of the central nervous system (Nesheim and others 2007). Hicks and others (2008) conducted an internet survey (n = 1062) to assess seafood consumption and consumer knowledge and perception of seafood. Respondents considered seafood contaminants (62%) as an important or very important factor to their purchase decision. Additionally, respondents reported that they currently get information regarding seafood from the media (63%) and considered media (30%) the best way to get information about seafood. The media is responsible for creating many of the concerns we have regarding seafood quality (Anderson and Anderson 1991).

Almost half (48%) of participants are concerned that when cooking seafood (including fish) the product is undercooked and not safe to eat (Table 4-10). As determined in Study 1, current generic cues for doneness can not guarantee reaching the minimum safe internal temperature. Only a small portion (16%) of participants correctly indicated the minimum safe temperature for fish and shellfish, suggesting temperature is not currently being used when evaluating seafood for doneness. Therefore, the development of specific visual and non-oral texture characteristics for individual fish and shellfish can help improve consumer confidence in determining doneness. Updating cookbooks, online recipe sources and cooking instructions raw seafood packaged with the visual and non-oral texture cues will increase potential for reaching a safe internal temperature and maintain eating quality.

Table 4-10: Consumer Sensory Panel Seafood Questionnaire Data

	Panelists (n = 107)
Frequency of Seafood Consumption	
Never	0%
1 – 2 times a year	8%
3 – 11 times a year	14%
1 – 2 times a month	18%
3 – 4 times a month	41%
More than once a week	19%
Frequency of Cooking Seafood	
Never	9%
1 – 2 times a year	9%
3 – 11 times a year	26%
12 – 24 times a year	19%
3 – 4 times a month	27%
More than once a week	9%
Purchase Raw Seafood	
Yes	88%
No	12%
Internal Temperature for Cooked Seafood (°F)	
Correctly indicated 145°F	16%
Indicated above 145°F	41%
Indicated below 145°F	18%
Indicated that they did not know	26%
Concerns When Cooking Seafood	
I do not have any concerns	20%
I am concerned the product is undercooked and not safe to eat	48%
I am concerned the product is overcooked	31%
I am concerned the product was not harvested from safe waters	31%
Other	7%

Note: See Appendix AA for full questionnaire results

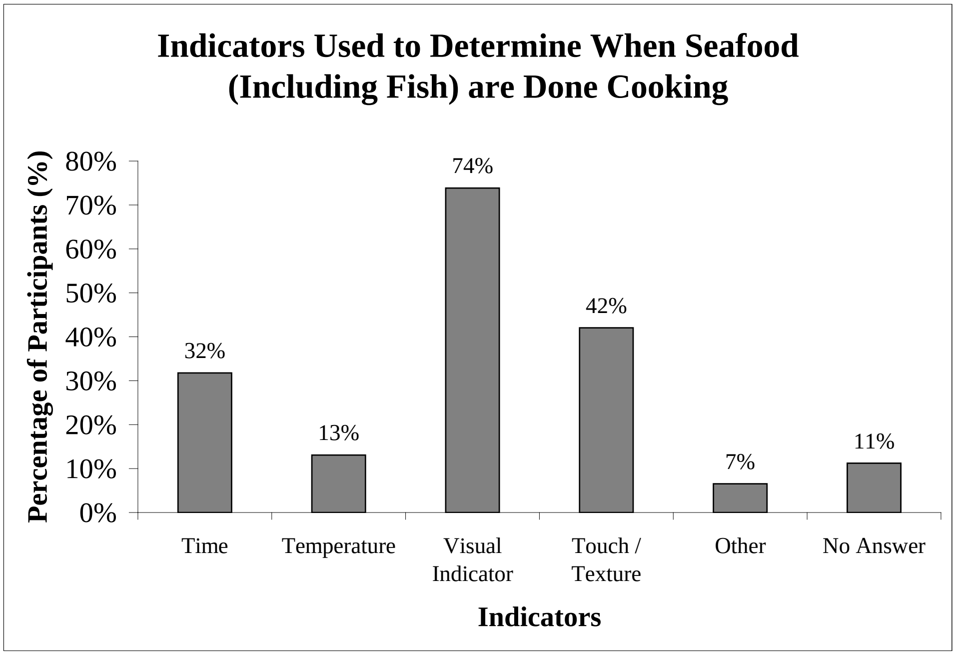

Figure 4-4: Indicators Used to Determine When Seafood (Including Fish) are Done Cooking. (n = 107) Participants wrote in visual indicators they used and were asked to reference the seafood products they used them for.

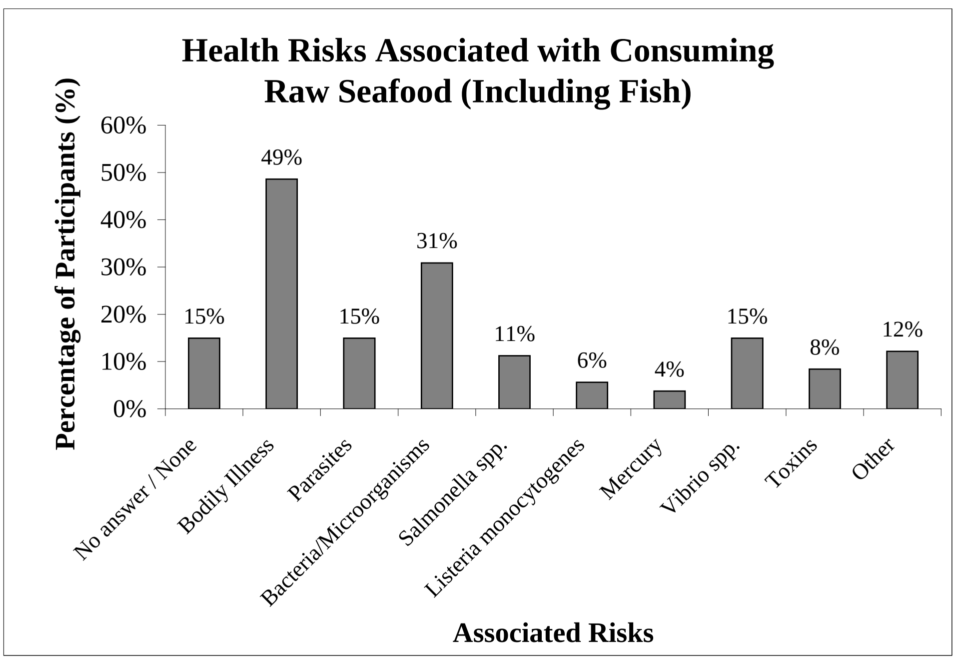

Figure 4-5: Health Risks Associated with Consuming Raw Seafood (Including Fish). (n = 107) Respondents were asked to write in risks they associated with consuming raw/undercooked seafood. Responses were grouped into categories based on repeated responses. The “other” category included: death, viruses (norovirus), disease, tapeworms/worms and histamine.

The 74°C ± 1 (165°F) endpoint had an overall higher, but not statistically different ($p > 0.05$), mean hedonic score for all products except poached salmon, with average acceptability scores between 6 (like slightly) and 7 (like moderately) (Table 4-11). Therefore, 74°C ± 1 (165°F) could be recommended as the internal temperature endpoint when cooking fish/shellfish specifically Atlantic salmon, tilapia, and shrimp. An internal endpoint temperature of 74°C ± 1 (165°F) would not diminishing eating quality and acceptability (see Appendix BB for overall acceptability histograms). Acceptability of Atlantic salmon center-cut fillets and tail fillets showed no statistical ($p > 0.05$) difference at each respective endpoint.

Table 4-11: Consumer overall acceptability mean hedonic scores

Product	n	64°C	±	SD	74°C	±	SD
Baked Salmon	50	6.5	±	1.8	6.8	±	1.8
Poached Salmon	47	6.9	±	1.3	6.6	±	1.7
Baked Tilapia	76	6.3	±	1.7	6.7	±	1.5
Pan-Fried Tilapia	49	6.5	±	1.4	6.8	±	1.5
Boiled Shrimp	52	6.6	±	1.5	7.0	±	1.2

Note: For baked salmon and poached salmon center-cut fillets or tail fillets were divided evenly among the panelists, with each panelist receiving only one type of fillet.

Conclusions

Current visual guidelines for determining doneness provided in cookbooks can not guarantee safe internal temperature for all types of fish and shellfish prepared by common culinary methods. Detailed visual descriptors of doneness relative to individual types of fish and shellfish as well as culinary methods would increase potential for reaching a safe endpoint. The trained descriptive panel identified key attributes to help determine safe products including: separation along central line and the color associated with the outer edges of the fillet for baked Atlantic salmon, overtone color, outer edge color, separation between muscle flakes and outer edge characteristics for baked tilapia and vibrancy of color and shape for boiled shrimp. Consumers need to be educated on proper cooking and handling of fish and shellfish as well as the dangers associated with consuming raw or undercooked fish and shellfish. Based on consumer acceptability and indicators to identify visual endpoint, 74°C (165°F) could be recommended as the internal temperature endpoint when cooking fish/shellfish specifically Atlantic salmon, tilapia, and shrimp without diminishing eating quality or acceptability.

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Chapter 5

Summary and Conclusions

To help prevent against food-borne illness improved cooking guidelines including recommended internal temperature and visual and non-oral texture guidelines specific to seafood product and/or culinary process are needed. Consumers frequently purchase raw seafood, thus safe food-handling and cooking behaviors ware required. Many questionnaire participants indicated they use visual and texture/touch indicators or follow instructions provided either on the package or a recipe to determine doneness, suggesting improved descriptors would be applied by consumers in the home.

Although differences in texture, color, and cook loss were identified between 64°C ± 1 and 74°C ± 1, consumers did not appear to detect theses differences. Consumer acceptability scores for all product and culinary method combinations had no difference between 64°C ± 1 and 74°C ± 1. A higher mean score was associated with all 74°C ± 1 samples except for poached salmon. The trained descriptive panel identified visual and non-oral texture indicators that can distinguish between 64°C ± 1 and 74°C ± 1. A review of the validation testing results suggests some attributes may be useful to distinguish between cooked and undercooked. Overall, 74°C (165°F) could be recommended as the internal temperature endpoint when cooking fish/shellfish specifically Atlantic salmon, tilapia, and shrimp without diminishing eating quality or acceptability. Not only were the samples at 74°C ± 1 equally preferred to samples at 64°C ± 1, visual and non-oral texture indicators were distinguished to determine doneness at 74°C ± 1.

Appendices

Appendix A: Physical Measurements

Table A-1: Physical Measurements of Raw Fish and Shellfish

Product		Raw Weight (g)				
		Mean	**±**	**SD**	**Median**	**Range**
Atlantic Salmon	Center-Cuts	139.8	±	17.9	132.6	70.2
	Tails	138.9	±	22.1	130.0	77.9
	Combined	139.3	±	20.0	132.1	80.1
Tilapia		153.1	±	15.5	145.7	55.9
Shrimp		17.5	±	0.9	17.5	4.5
Oysters		8.48	±	2.83	7.53	11.44
Product		**Thickness (mm)**				
		Mean	**±**	**SD**	**Median**	**Range**
Atlantic Salmon	Center-Cuts	20.6	±	1.9	20.3	8.3
	Tails	19.2	±	2.7	18.8	9.8
	Combined	19.9	±	2.4	19.9	10.6
Tilapia		18.2	±	1.7	17.9	6.9
Shrimp		13.8	±	0.6	13.7	2.2
Oysters		8.63	±	1.17	8.48	4.37

Note: Measurements were combined from the replications used in Chapter 3, Study 1: Physical Assessment of Texture, Cook Loss and Color

Appendix B: Color Assessment Locations

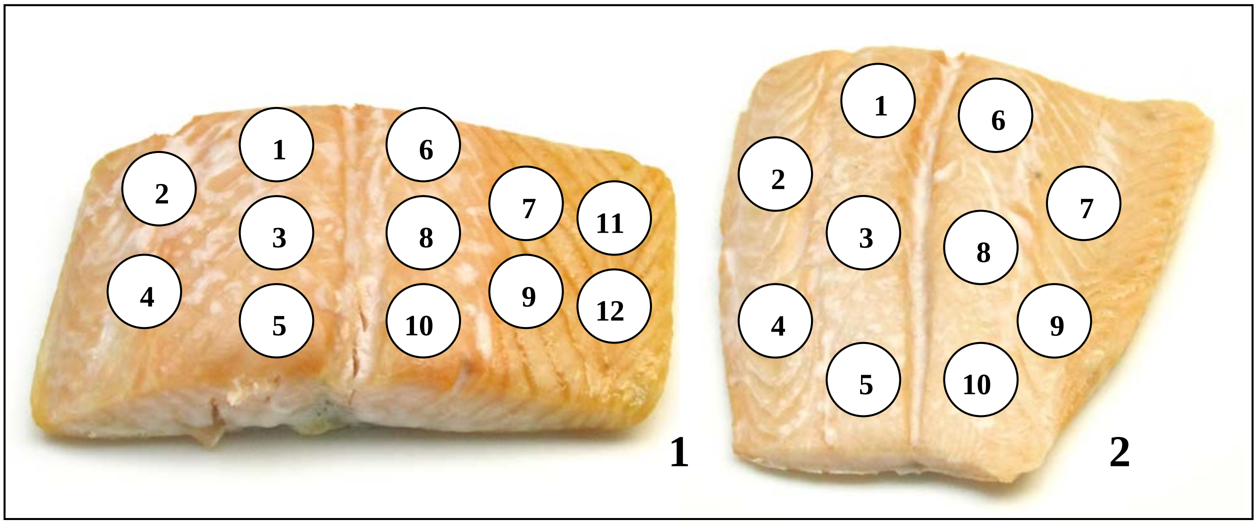


Figure B-1: Colorimeter Measurement Locations for Atlantic Salmon (1) Center-Cut Fillet and (2) Tail Fillet

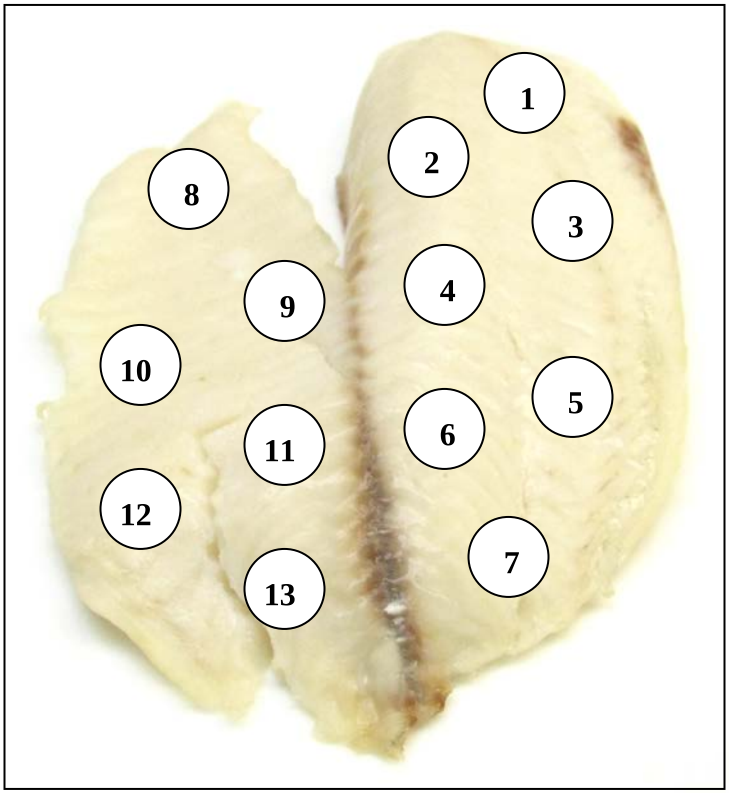


Figure B-2: Colorimeter Locations for Tilapia Fillets

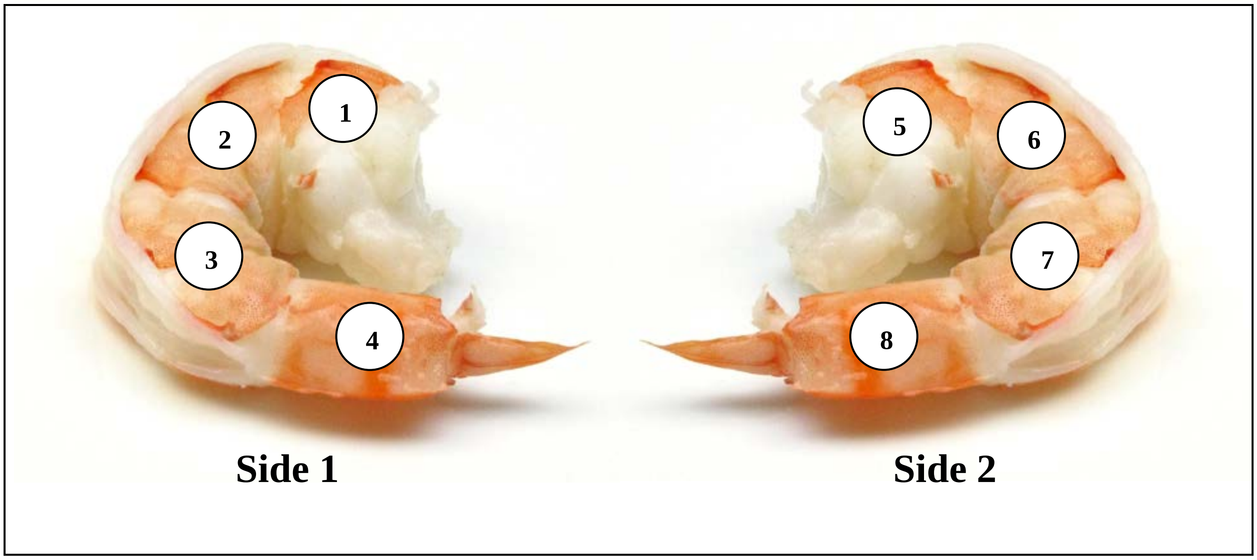


Figure B-3: Colorimeter Measurement Locations for Shrimp

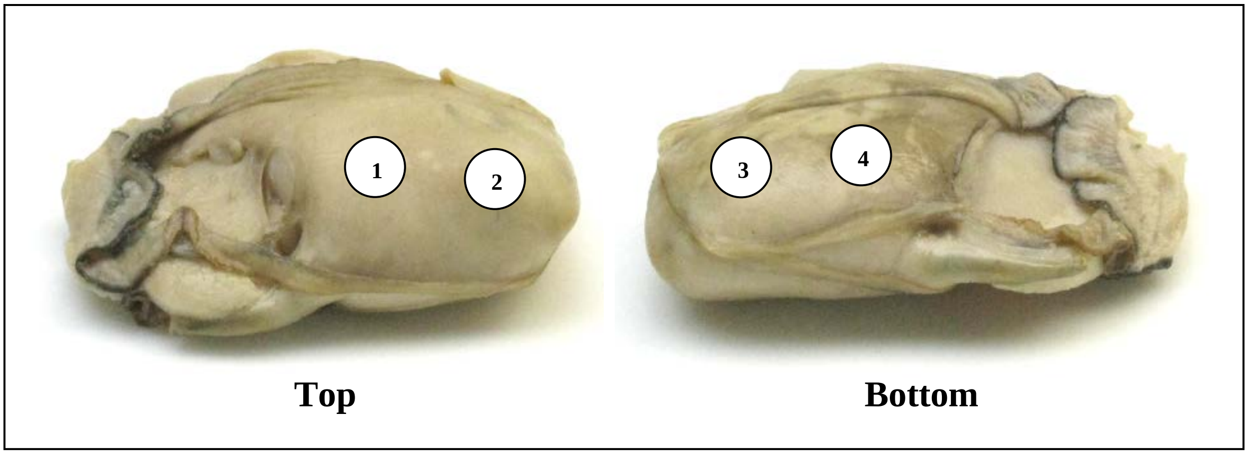


Figure B-4: Colorimeter Measurement Locations for Oysters

Appendix C: Texture Analysis Sampling Locations

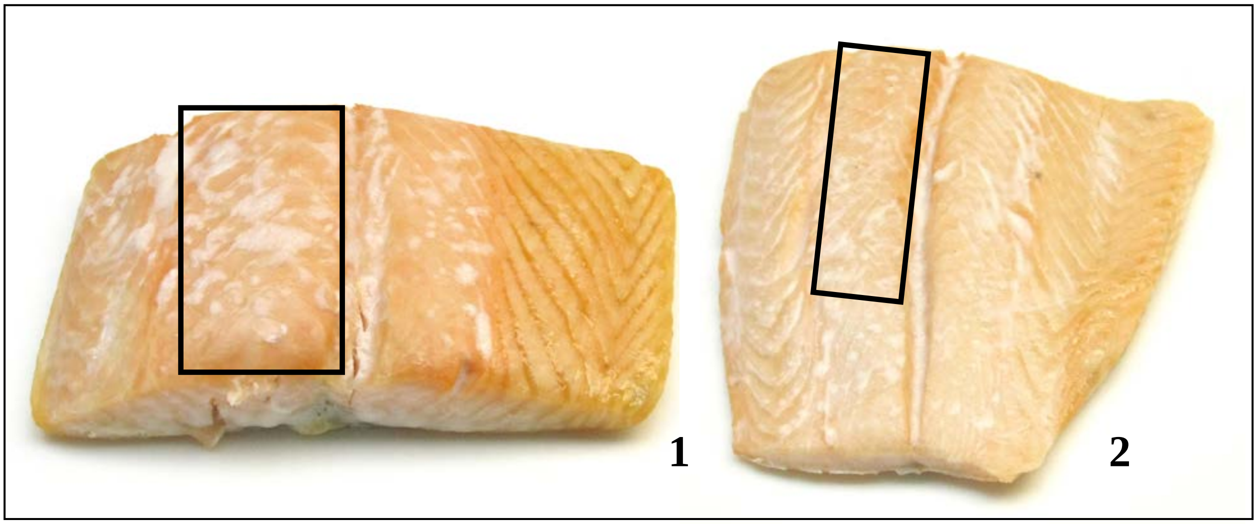


Figure C-1: Sampling Location for Kramer Shear Cell Texture Analysis of Atlantic Salmon (1) Center-Cut Fillet and (2) Tail Fillet

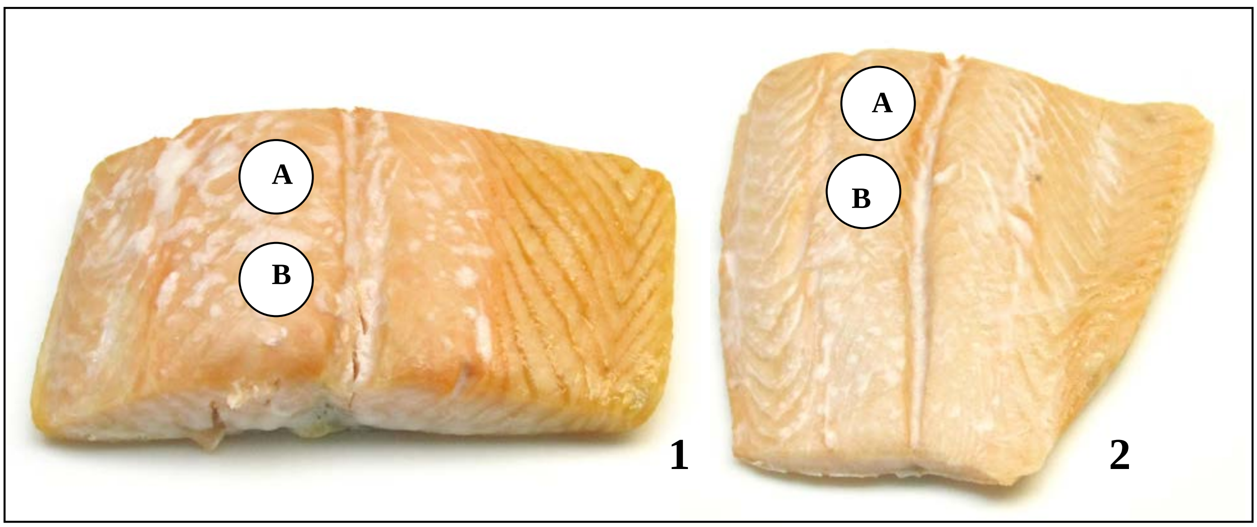


Figure C-2: Sampling Location for Texture Profile Analysis of Atlantic Salmon (1) Center-Cut Fillet and (2) Tail Fillet

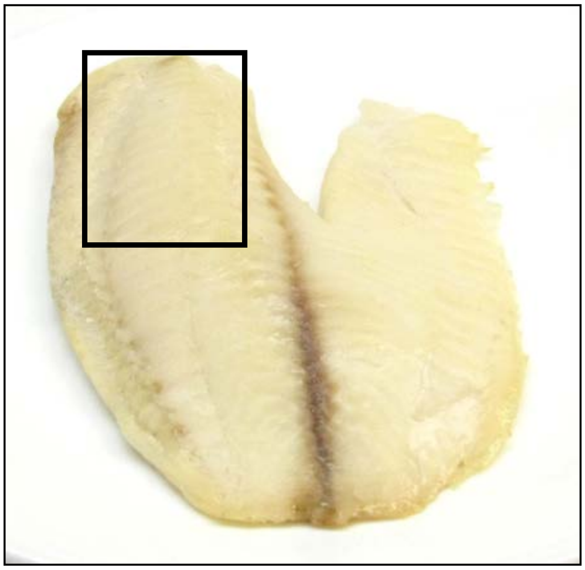


Figure C-3: Sampling Location for Kramer Shear Cell Texture Analysis of Tilapia
Note: A rectangle 45mm x 65 mm was cut from the thickest portion of the fillet.

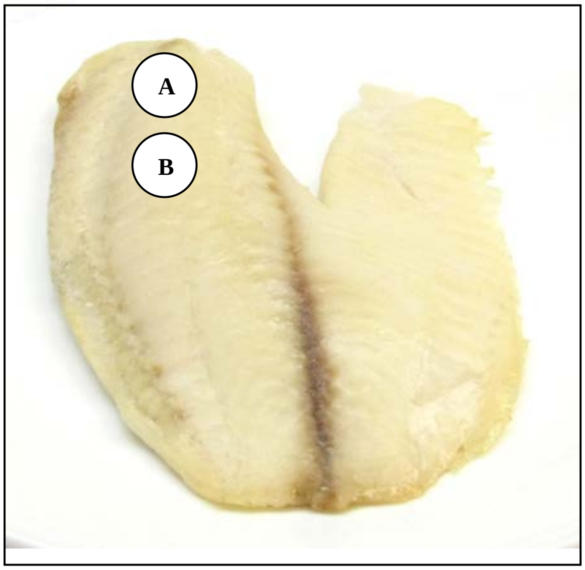


Figure C-4: Sampling Location of Tilapia for Texture Profile Analysis

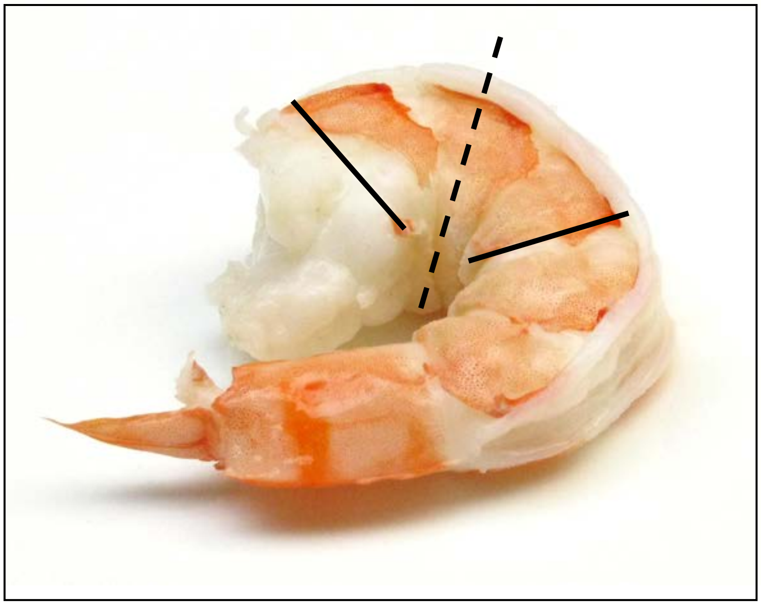


Figure C-5: Sampling Location and Preparation for Warner-Bratzler Blade for Shrimp
Note: Shrimp cut at solid black lines leaving segments one, two and three. The Warner-Bratzler blade was positioned over the second segment as indicated by the dashed line.

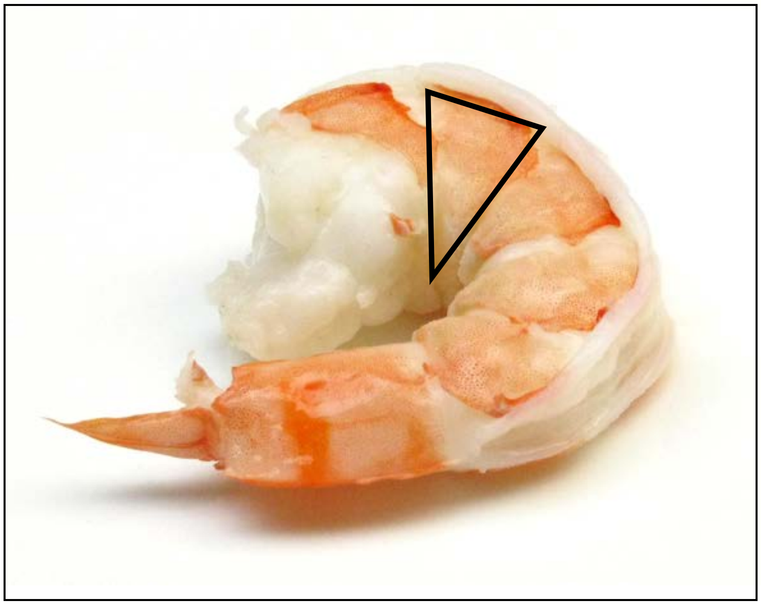


Figure C-6: Sampling Location for Texture Profile Analysis for Shrimp
Note: Shrimp remained intact and measurement was taken at the second segments outlined by the black triangle.

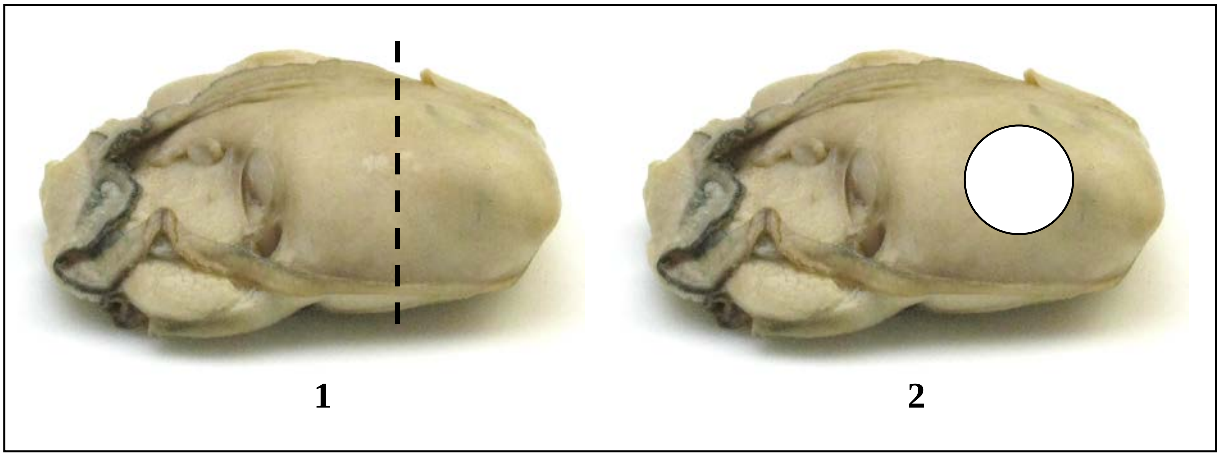


Figure C-7: Sampling Locations of Oysters for (1) Warner-Bratzler Blade and (2) Texture Profile Analysis Note: (1) Warner-Bratzler blade was positioned over the center of the oyster's adductor muscle (belly) as indicated by dashed line. (2) One measurement for Texture Profile Analysis was performed at the center of the oyster's adductor muscle (belly) as indicated by white circle.

Appendix D: Institutional Review Board Approval Letter

VirginiaTech

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

MEMORANDUM

DATE: April 1, 2011

TO: Susan E. Duncan, Renee Felice, Kim M. Waterman

FROM: Virginia Tech Institutional Review Board (FWA00000572, expires October 26, 2013)

PROTOCOL TITLE: Sensory and Physical Assessment of Microbiologically Safe Culinary Processes for Fish and Seafood

IRB NUMBER: 10-152

Effective April 1, 2011, the Virginia Tech IRB Chair, Dr. David M. Moore, approved the amendment request for the above-mentioned research protocol.

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

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

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

PROTOCOL INFORMATION:
Approved as: **Expedited, under 45 CFR 46.110 category(ies) 7**
Protocol Approval Date: **3/3/2011 (protocol's initial approval date: 3/3/2010)**
Protocol Expiration Date: **3/2/2012**
Continuing Review Due Date*: **2/17/2012**
*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

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

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

Invent the Future

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY

An equal opportunity, affirmative action institution

Appendix E – Trained Descriptive Panel Recruitment Survey

Fish & Shellfish Trained Panel

What is your name? (Please include first and last)

What is your email address?

How often do you consume fish and/or shellfish?

- Never
- 1-2 times a year
- 3-11 times a year
- 1-2 times a month
- 3-4 times a month
- More than once a week

How often do you cook fish and/or shellfish?

- Never
- 1-2 times a year
- 3-11 times a year
- 1-2 times a month
- 3-4 times a month
- More than once a week

What types of fish and/or shellfish do you consume? Check all that apply

- [ ] Fatty fish such as Salmon
- [ ] Lean fish such as Tilapia
- [ ] Shrimp
- [ ] Crab
- [ ] Lobster
- [ ] Oysters
- [ ] Mussels
- [ ] Scallops

- [ ] Clams
- [ ] Prawns
- [ ] Crawfish

other: ____________

How is the fish and/or shellfish you consume prepared? Check all that apply

- [ ] Baked
- [ ] Broiled
- [ ] Blackened
- [ ] Sautéed
- [ ] Grilled
- [ ] Breaded
- [ ] Deep fried
- [ ] Boiled
- [ ] Steamed
- [ ] Raw

other: ____________

What days and times are you available throughout January, February and March? Check all that apply

- [ ] Monday - morning (8:00AM - 10:00AM)
- [ ] Monday - mid-day (10:00AM - 2:00PM)
- [ ] Monday - afternoon (2:00PM - 4:00PM)
- [ ] Monday - evening (4:00PM - 7:00PM)
- [ ] Tuesday - morning (8:00AM - 10:00AM)
- [ ] Tuesday - mid-day (10:00AM - 2:00PM)
- [ ] Tuesday - afternoon (2:00PM - 4:00PM)
- [ ] Tuesday - Monday - evening (4:00PM - 7:00PM)
- [ ] Wednesday - morning (8:00AM - 10:00AM)
- [ ] Wednesday - mid-day (10:00AM - 2:00PM)
- [ ] Wednesday - afternoon (2:00PM - 4:00PM)
- [ ] Wednesday - evening (4:00PM - 7:00PM)
- [ ] Thursday - morning (8:00AM - 10:00AM)

- [ ] Thursday - mid-day (10:00AM - 2:00PM)
- [ ] Thursday - afternoon (2:00PM - 4:00PM)
- [ ] Thursday - evening (4:00PM - 7:00PM)
- [ ] Friday - morning (8:00AM - 10:00AM)
- [ ] Friday - mid-day (10:00AM - 2:00PM)
- [ ] Friday - afternoon (2:00PM - 4:00PM)
- [ ] Friday - evening (4:00PM - 7:00PM)

Thank you for your willing participation! An email will be sent to you shortly with more information regarding the panel including the days and times the panel will be held on each week. Thank You!

Appendix F: Trained Descriptive Panel Consent Form

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY
Informed Consent for Participants
in Research Projects Involving Human Subjects

Title of Project: Sensory Assessment of Microbiologically Safe Culinary Processes for Fish and Seafood

Investigator(s): Dr. Susan Duncan, PhD. and Renee Felice

I. Purpose of this Research/Project

You are invited to participate in a trained panel to determine visual and non-oral textural indicators of doneness for fish and shellfish.

II. Procedures

The trained panel will meet twice a week for 10 weeks, with each session lasting no more than 1 hour. During the sessions you will be asked to view photographs of fish and shellfish and actual fish and shellfish. As part of the panel you will work with the other panelists to describe visual and non-oral textural characteristics of salmon, tilapia, shrimp and oysters. Some sessions will involve group discussions, while others will be individual testing. You will not be orally evaluating any products.

III. Risks

There are no more than minimal risks for participating in this study. If you have a fish and/or shellfish allergies or an aversion to fish or shellfish please inform the investigator.

IV. Benefits

Your participation in the study will provide better visual and non-oral textural indicators of doneness for fish and shellfish to supplement current guidelines provided by cookbooks. If you would like a summary of the research results, please contact the researcher at a later time.

V. Extent of Anonymity and Confidentiality

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

VI. Compensation

You will be compensated with luncheon at the end of the panel.

VII. Freedom to Withdraw

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

VIII. Subject's Responsibilities

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

1. To attend all scheduled training and test sessions.

2. To participate with the other panelist in group discussion of visual and non-oral textural characteristics.

3. Participate in validation testing and description testing of various fish and shellfish products.

IX. Subject's Permission

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

____ Date__________
Subject Signature

____ Date__________
Subject Printed Name

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

Renee Felice (910) 547-6339, felicer@vt.edu
Graduate Research Assistant, Investigator

Susan Duncan, Faculty/ Investigator (540) 231-8675; duncans@vt.edu

David Moore (540) 231-4991; moored@vt.edu
Chair, Virginia Tech Institutional Review
Board for the Protection of Human Subjects
Office of Research Compliance
2000 Kraft Dr., Suite 2000 (0497)
Blacksburg, VA 24061

Appendix G: Trained Descriptive Panel Reference Sample Information

Table G-1: Product Information for Reference Samples Used in Non-Oral Texture Training for Trained Descriptive Panel (n = 7)

Product Brand	Product Name	Product Description	Presentation as Reference	Uses
Kraft®	Velveeta® Cheese	32 oz. block	Cut into 1” x 1” x 1” cubes, left at room temperature for 1 hour before being used by panelists; always position with outer edge to touch (not a surface that has been cut)	• Baked Salmon: Firmness (soft) • Baked Tilapia: Firmness (soft) • Boiled shrimp: Firmness (soft)
Kroger®	Medium Cheddar Cheese	8 oz. block	Cut into 1” x 1” x 1” cubes, left at room temperature for 1 hour before being used by panelists; always position with outer edge to touch (not a surface that has been cut)	• Baked Salmon: Firmness (firm) • Boiled shrimp: Firmness (firm)
Deskins’ Candies	Peanut Butter Fudge	Purchased from bakery section of grocery store, pre-cut, in sturdy plastic resealable container	Cut into 1” x 1” x 1” cubes, left at room temperature for 1 hour before being used by panelists; always position with outer edge to touch (not a surface that has been cut)	• Baked Tilapia: Firmness (firm)
Little Debbie®	Cosmic Brownies	Individually wrapped brownies with rainbow chocolate chip candy	Wrapping was removed and chips were removed	• Boiled shrimp: Amount of recovery (none)
Kroger®	Hot Dogs	Regular (10 pack)	Hot dogs were boiled for 4 min and given to panelists while hot	• Boiled shrimp: Amount of Recovery (full) and Rate of Recovery (fast)
Kroger®	Marshmallow	Standard size	Marshmallows were positioned horizontally	• Boiled shrimp: Rate of Recovery (slow)
Star Kist®	Canned Tuna	Canned chunk light tuna in water	Open can, drained water sat at room temperature covered with plastic wrap until used	• Baked Salmon: Flakiness (flaky) • Baked Tilapia: Flakiness (flaky)
transOCEAN®	Crab Classic Flake Style	Crab flavored seafood made from surimi and arranged like flakes	Individual flakes were left covered with plastic wrap at room temperature for 1 hour before being used by panelists	• Baked Salmon: Flakiness (slightly flaky) • Baked Tilapia: Flakiness (slightly flaky)

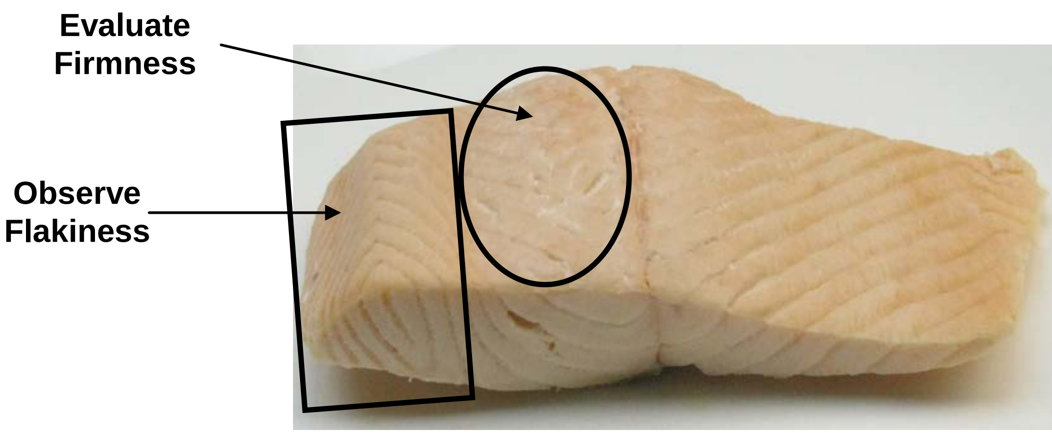
Appendix H: Trained Panelist's Guide for Evaluating Non-Oral Texture

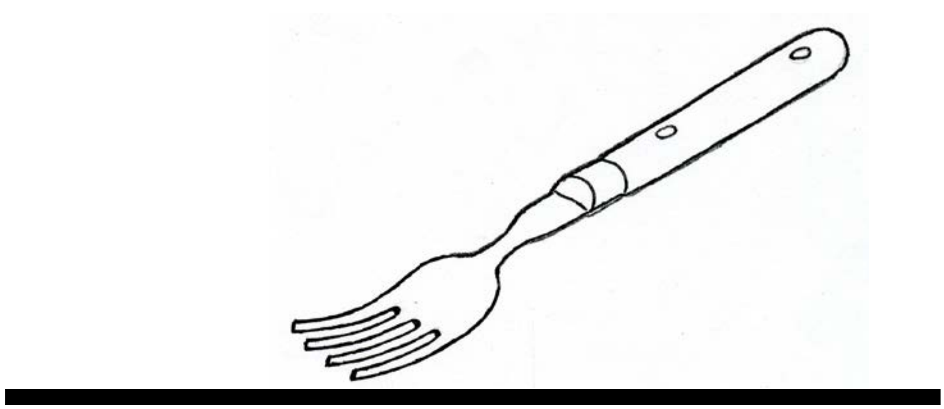
Non-Oral Texture Evaluation Guidelines (page 1 of 3)

Salmon

- Firmness – Using the index finger of your dominant hand apply pressure to the thickest section of the salmon fillet so that the pad of your finger indents and resistance from the product is detected

- Flakiness – Using a fork, apply slight pressure with the back tips of the fork's prongs and pull toward you, observe the ease at which the muscle segments separate to individual segments. Observe flakiness along the edge near the thickest portion of the fillet





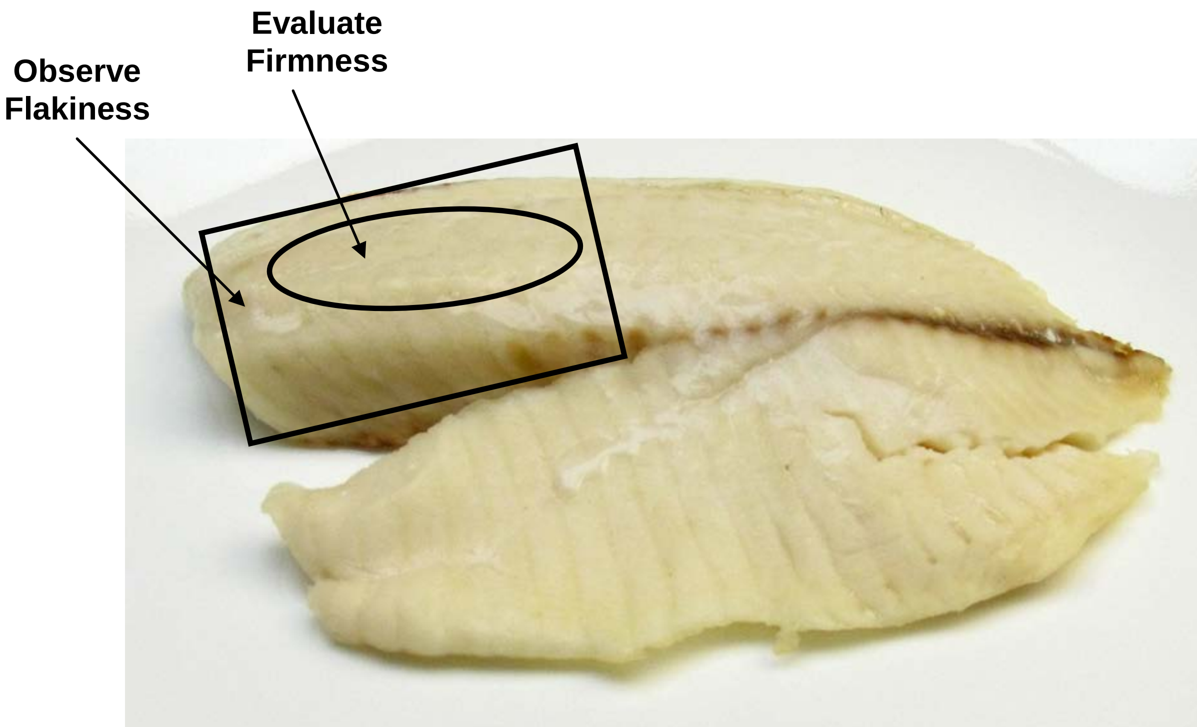
When testing for flakiness (in salmon or tilapia) apply pressure with the back tips of the fork's prongs

Non-Oral Texture Evaluation Guidelines (page 2 of 3)

Tilapia

- Firmness – Using the index finger of your dominant hand apply pressure to the thickest section of the tilapia fillet (avoid areas with separation among muscle segments), so that the pad of your finger indents and resistance from the product is detected

- Flakiness - Using a fork, apply slight pressure with the back tips of the fork's prongs and pull toward you, observe the ease at which the muscle segments separate to individual segments. Observe flakiness at the thickest portion of the fillet (see diagram on previous page)



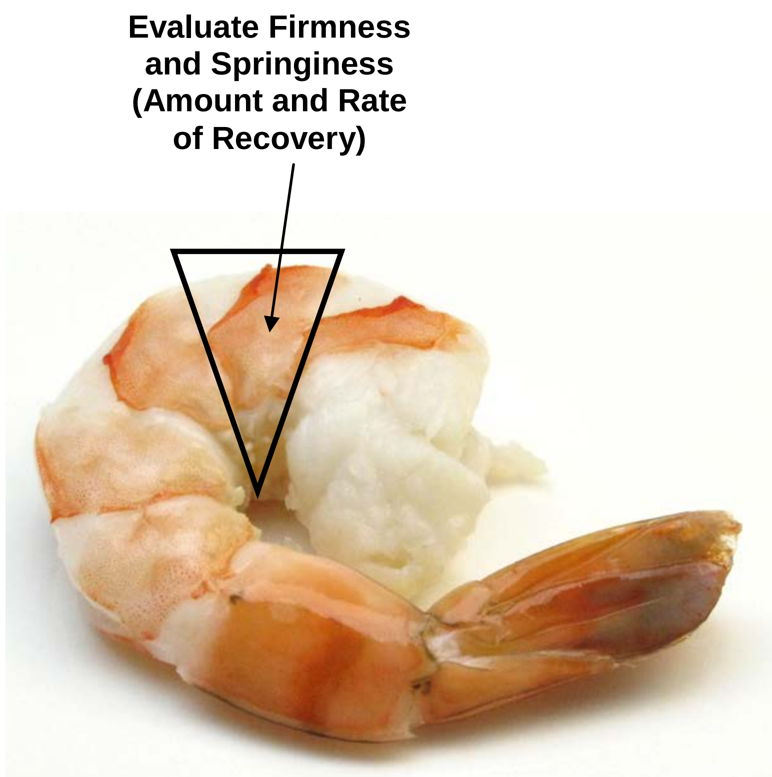
Non-Oral Texture Evaluation Guidelines (page 3 of 3)

Shrimp

- Firmness – Using the index finger of your dominant hand apply pressure to the second segment section of the shrimp, so that the pad of your finger indents and resistance from the product is detected

- Springiness:
    - Amount of Recovery - Using the index finger of your dominant hand apply pressure to the second segment section of the shrimp, so that the pad of your finger indents and resistance from the product is detected. Remove pressure form finger and observe the products ability to return (amount) to its' original structure/form

    - Rate of recovery - Using the index finger of your dominant hand apply pressure to the second segment section of the shrimp, so that the pad of your finger indents and resistance from the product is detected. Remove pressure from finger and observe the rate (speed) at which the product returns to its' original structure/form



Appendix I: Non-Oral Texture Assessment Scorecards

Non-Oral Texture Test
Baked Salmon

Panelist #__________________

Sample #__________________

Instructions: Please evaluate the sample for firmness and flakiness using the guidelines provided. Mark a vertical dash along the line indicating the relationship of the attribute relative to the references.

Firmness

Evaluate the sample for firmness:

Soft (Velveeta) — Firm (Medium Cheddar Cheese)

Flakiness

Evaluate the sample for flakiness (individual flakes are produced):

Slightly Flaky (Imitation Crab) — Flaky (Canned Tuna)

Non-Oral Texture Test
Baked Tilapia

Panelist #__________________

Sample #__________________

Instructions: Please evaluate the sample for firmness and flakiness using the guidelines provided. Mark a vertical dash along the line indicating the relationship of the attribute relative to the references.

Firmness

Evaluate the sample for firmness:

Soft (Velveeta)	Firm (Peanut Butter Fudge)

Flakiness

Evaluate the sample for flakiness (individual flakes are produced):

Slightly Flaky (Imitation Crab)	Flaky (Canned Tuna)

Non-Oral Texture Test
Boiled Shrimp

Panelist #__________________

Sample #__________________

Instructions: Please evaluate the sample for firmness and springiness using the guidelines provided. Mark a vertical dash along the line indicating the relationship of the attribute relative to the references.

Firmness

Evaluate the sample for firmness:

Soft (Velveeta) —————————————— Firm (Medium Cheddar Cheese)

Springiness

Evaluate the sample for springiness (amount of recovery):

None (Brownie) —————————————— Springy (Hot Dog)

Evaluate the sample for the rate (speed) of recovery:

Slow (Marshmallow) —————————————— Fast (Hot Dog)

Appendix J: Overview of Non-Oral Texture Training Sessions for Trained Panel

Week 1:

- Begin non-oral texture training by teaching panelist how to evaluate for firmness, flakiness and springiness.
- Teach panelists how to use 15 cm unstructured line scale.

Week 2:

- Practice applying non-oral texture evaluation to potential reference samples and differentiating between degrees of each attribute.
- Practice evaluating baked salmon for firmness and flakiness with references available.
- Decided on reference anchors for firmness and flakiness for baked salmon.

Week 3:

- Practice evaluating baked tilapia for firmness and flakiness with references available.
- Decided on reference anchors for firmness and flakiness for baked tilapia.
- Practice evaluating boiled shrimp for firmness and springiness with references available.
- Decided on reference anchors for firmness and springiness for boiled shrimp.

Week 4:

- Validation testing for non-oral texture characteristics

Week 5:

- Non-oral texture testing

Appendix K: Preliminary Texture Study to Determine Texture Methods for Cooked Fish and Shellfish

Table K-1: Kramer Shear Cell Results for Atlantic Salmon Cooked to an Internal Endpoint of 64°C

Cooking Method	Max Force (N)			Total Force (N*mm)		
	X_1	±	SD	X_1	±	SD
Baked	73.67 [B]	±	7.35	150.15 [B]	±	15.64
Broiled	97.42 [A]	±	12.98	192.02 [A]	±	29.49
Poached	70.50 [B]	±	9.48	144.46 [B]	±	10.97

Note: Values not connected by the same letter are statistically different from each other ($p < 0.05$).
Three replications were performed in duplicate for each cooking method performed

Table K-2: Texture Profile Analysis Results for Atlantic Salmon Cooked to an Internal Endpoint of 64°C

Cooking Method	Hardness (N)			Springiness (%)			Cohesiveness (%)			Chewiness			Resilience (%)		
	X_1	±	SD	X_1	±	SD	X_1	±	SD	X_1	±	SD	X_1	±	SD
Baked	9.05 [A]	±	0.76	127.60 [A]	±	24.79	51.35 [A]	±	4.76	5.73 [A]	±	0.82	78.72 [A]	±	50.98
Broiled	9.10 [A]	±	0.63	117.20 [A]	±	8.82	54.70 [A]	±	0.60	5.72 [A]	±	0.28	63.77 [A]	±	21.55
Poached	8.96 [A]	±	0.88	125.15 [A]	±	8.09	51.49 [A]	±	0.94	5.67 [A]	±	0.27	79.70 [A]	±	24.28

Note: Values not connected by the same letter are statistically different from each other ($p < 0.05$).
Three replications were performed in duplicate for each cooking method performed

Table K-3: Warner-Bratzler Results for Shrimp Cooked to an Internal Endpoint of 64°C

Cooking Methods	Mean Max Force (N)			Total Shear Force (mJ)		
	X_1	±	SD	X_1	±	SD
Broiled	23.71 [A]	±	1.27	343.43 [A]	±	17.95
Boiled	21.02 [A]	±	0.62	297.74 [A]	±	21.14
Sautéed	23.45 [A]	±	1.26	331.99 [A]	±	24.25

Note: Values not connected by the same letter are statistically different from each other ($p < 0.05$). Replications had a final IT of 65°C ± 2

Table K-4: Texture Profile Analysis Results for Shrimp Cooked to an Internal Endpoint of 64°C

	Hardness (N)			Springiness (%)			Cohesiveness (%)			Chewiness			Resilience (%)		
Cooking Methods	X_1	±	SD	X_1	±	SD	X_1	±	SD	X_1	±	SD	X_1	±	SD
Broiled	11.62 [A]	±	0.80	99.99 [A]	±	0.01	72.26 [A]	±	0.48	8.39 [A]	±	0.57	40.93 [A]	±	0.61
Boiled	10.01 [A]	±	0.64	99.99 [A]	±	0.01	76.00 [B]	±	0.82	7.60 [A]	±	0.49	45.38 [B]	±	1.00
Sautéed	7.65 [B]	±	0.58	100.00 [A]	±	0.01	74.22 [A,B]	±	0.97	5.68 [B]	±	0.44	41.34 [A]	±	1.16

Note: Values not connected by the same letter are statistically different from each other ($p < 0.05$). Replications had a final IT of 65°C ± 2

Appendix L: Physical Characterization of Fish and Shellfish

Table L-1: Fat, Protein, and Moisture of Raw Seafood for Physical Assessment

Product	Fat (%)			Protein (%)			Moisture (%)		
	Mean	**±**	**SD**	**Mean**	**±**	**SD**	**Mean**	**±**	**SD**
Atlantic Salmon	13.5	±	0.3	19.4	±	0.2	65.6	±	0.3
Tilapia	2.6	±	0.2	17.5	±	0.3	79.2	±	0.4
Shrimp	0.4	±	0.1	17.5	±	0.1	80.4	±	0.2
Oysters	1.4	±	0.3	8.9	±	1.2	85.4	±	0.03

Note: Data consists of product evaluated in June 2010 and July 2010 (Oysters) for physical assessment (texture, color and cook loss). (n = 3)

Table L-2: Fat, Protein, and Moisture of Raw Seafood for Sensory Assessment

Product	Fat (%)			Protein (%)			Moisture (%)		
	Mean	**±**	**SD**	**Mean**	**±**	**SD**	**Mean**	**±**	**SD**
Atlantic Salmon	4.8	±	0.4	20.1	±	0.04	89.7	±	0.4
Tilapia	1.0	±	0.1	17.8	±	0.3	84.9	±	0.5
Shrimp	0.2	±	0.005	19.7	±	0.1	85.0	±	0.8

Note: Data consists of product evaluated in October 2010 for sensory assessment including consumer acceptability panels and trained descriptive panel assessments. (n = 3)

Appendix M: Additional Texture Analysis Results for Shellfish

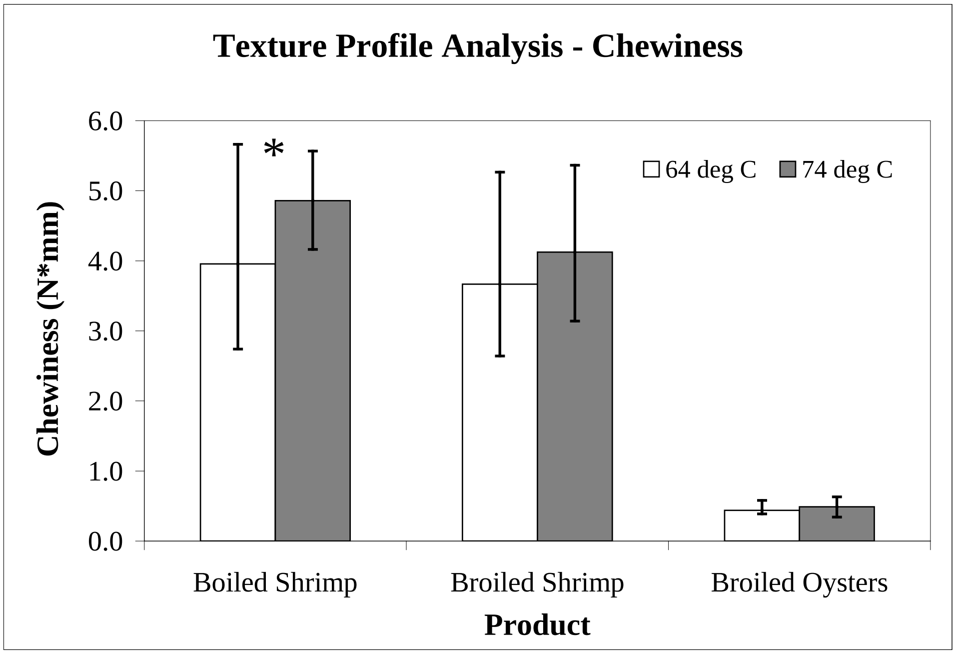


Figure M-1: Chewiness as Determined by Texture Profile Analysis (30% compression) for Cooked (64°C ± 1 and 74°C ± 1 endpoints) Shrimp and Oysters Prepared By Various Culinary Methods. (*) indicates significant statistical difference ($p < 0.05$) between internal temperature endpoints within a culinary process and seafood product combination.

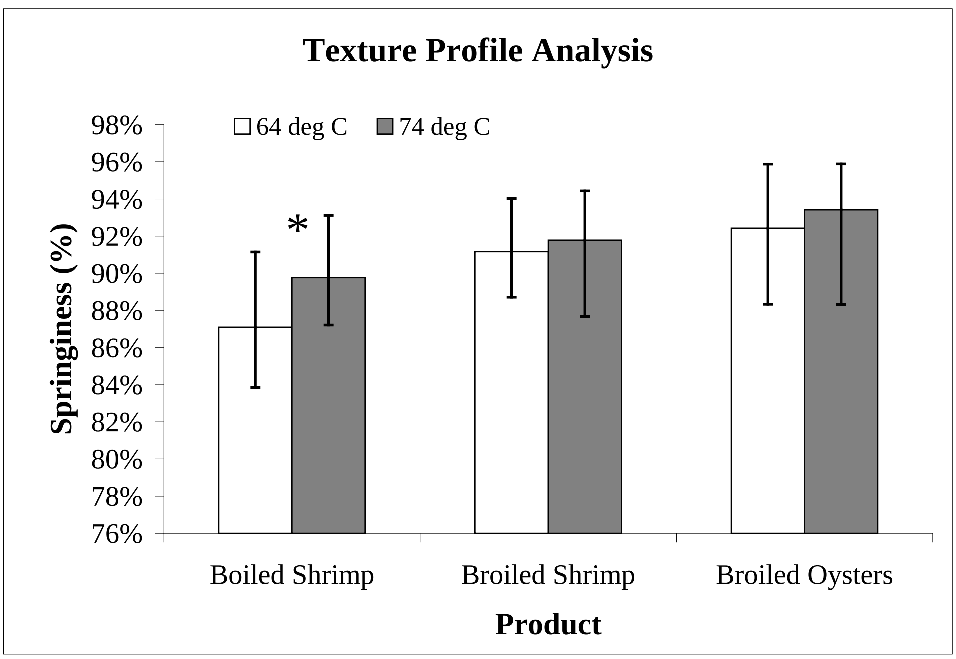


Figure M-2: Springiness as Determined by Texture Profile Analysis (30% compression) for Cooked (64° ± 1 and 74°C ± 1 endpoints) Shrimp and Oysters Prepared By Various Culinary Methods. (*) indicates significant statistical difference ($p < 0.05$) between internal temperature endpoints within a culinary process and seafood product combination.

Appendix N: Colorimeter and Adobe® Photoshop® Color Data

Table N-1: CIE *L*a*b Values for Salmon (Baked, Broiled, Poached) Determined by Colorimeter and Adobe® Photoshop®**

Product	Evaluation Method	IT (°C)	*L**				*a**				*b**				Hue				Chroma			
			Mean	±	SD	Δ	Mean	±	SD	Δ	Mean	±	SD	Δ	Mean	±	SD	Δ	Mean	±	SD	Δ
Baked Salmon	Colorimeter	64	74.5^{A}	±	3.1	0.1	11.1^{A}	±	2.3	0.1	25.1^{B}	±	4.8	2.1	66.1^{B}	±	2.7	1.8	27.5^{B}	±	5.2	1.9
		74	74.6^{A}	±	3.7		11.0^{A}	±	2.5		27.2^{A}	±	5.4		67.9^{A}	±	3.1		29.4^{A}	±	5.7	
	Adobe® Photoshop®	64	71^{A}	±	2	1	8^{A}	±	1	0	32^{A}	±	3	3	76^{A}	±	1	1	33^{A}	±	3	3
		74	72^{A}	±	4		8^{A}	±	2		35^{A}	±	6		77^{A}	±	1		36^{A}	±	6	
Broiled Salmon	Colorimeter	64	65.7^{A}	±	7.6	5.8	16.7^{B}	±	3.6	2.4	37.0^{B}	±	4.6	2.0	65.8^{A}	±	4.1	2.0	40.6^{B}	±	5.1	3.0
		74	59.9^{B}	±	9.5		19.1^{A}	±	3.2		39.0^{A}	±	5.2		63.8^{B}	±	4.6		43.6^{A}	±	5.2	
	Adobe® Photoshop®	64	62^{A}	±	4	12	17^{A}	±	4	6	47^{A}	±	4	0	70^{A}	±	3	6	50^{A}	±	5	2
		74	50^{A}	±	8		23^{A}	±	3		47^{A}	±	5		64^{A}	±	5		52^{A}	±	3	
Poached Salmon	Colorimeter	64	76.8^{A}	±	2.9	0.6	10.7^{A}	±	2.0	0.2	22.1^{B}	±	3.3	0.8	64.2^{A}	±	2.6	0.4	24.5^{A}	±	3.6	0.8
		74	77.4^{A}	±	2.8		10.9^{A}	±	1.8		22.9^{A}	±	2.9		64.6^{A}	±	2.3		25.3^{A}	±	3.2	
	Adobe® Photoshop®	64	74^{A}	±	2	0	7^{A}	±	2	0	25^{A}	±	4	1	74^{A}	±	1	1	26^{A}	±	4	1
		74	74^{A}	±	1		7^{A}	±	1		26^{A}	±	2		75^{A}	±	2		27^{A}	±	2	

Note: Photoshop only reports values as whole numbers. Values connected by the same letter within a column within, evaluation method, and product are not statistically different ($p < 0.05$).

Table N-2: CIE *L*a*b Values for Tilapia Determined by Colorimeter and Adobe® Photoshop®**

Product	Evaluation Method	IT (°C)	*L**				*a**				*b**			
			Mean	±	SD	Δ	Mean	±	SD	Δ	Mean	±	SD	Δ
Baked Tilapia	Colorimeter	64	78.4^{B}	±	2.7	1.1	-2.4^{B}	±	0.6	0.3	10.5^{B}	±	2.6	1.2
		74	79.5^{A}	±	2.7		-2.1^{A}	±	0.6		11.7^{A}	±	2.3	
	Adobe® Photoshop®	64	77^{A}	±	6	3	-1^{A}	±	1	0	18^{A}	±	2	3
		74	80^{A}	±	2		-1^{A}	±	1		21^{A}	±	1	
Broiled Tilapia	Colorimeter	64	79.9^{A}	±	2.8	3.5	-2.1^{A}	±	0.8	0.5	14.1^{B}	±	3.6	5.3
		74	76.4^{B}	±	4.6		-1.6^{A}	±	4.2		19.4^{A}	±	5.5	
	Adobe® Photoshop®	64	78^{A}	±	1	2	0^{A}	±	0	1	25^{A}	±	3	5
		74	76^{A}	±	1		1^{A}	±	1		30^{A}	±	2	
Pan-Fried Tilapia	Colorimeter	64	78.5^{A}	±	3.9	2.2	-0.6^{B}	±	1.6	1.3	16.0^{B}	±	5.7	4.1
		74	76.3^{B}	±	5.8		0.7^{A}	±	3.1		20.1^{A}	±	7.0	
	Adobe® Photoshop®	64	79^{A}	±	3	4	0^{A}	±	1	1	24^{A}	±	4	6
		74	75^{A}	±	1		1^{A}	±	1		30^{A}	±	3	

Note: Photoshop only reports values as whole numbers. Values connected by the same letter within a column within, evaluation method, and product are not statistically different ($p < 0.05$).

Table N-3: CIE *L*a*b Values for Shrimp Determined by Colorimeter and Adobe® Photoshop®**

Product	Evaluation Method	IT (°C)	*L**				*a**				*b**				Hue				Chroma			
			Mean	±	**SD**	Δ	**Mean**	±	**SD**	Δ	**Mean**	±	**SD**	Δ	**Mean**	±	**SD**	Δ	**Mean**	±	**SD**	Δ
Boiled Shrimp	Colorimeter	64	67.0^{A}	±	2.7	0.2	15.9^{B}	±	5.1	1.8	24.5^{B}	±	5.7	1.9	57.5^{A}	±	3.0	1.1	29.3^{B}	±	7.5	2.5
		74	67.2^{A}	±	3.0		17.7^{A}	±	4.7		26.4^{A}	±	5.2		56.5^{B}	±	2.8		31.8^{A}	±	6.8	
	Adobe® Photoshop®	64	65^{A}	±	1	1	21^{A}	±	1	0	37^{A}	±	2	0	60^{A}	±	1	0	42^{A}	±	2	1
		74	66^{A}	±	2		21^{A}	±	2		37^{A}	±	1		60^{A}	±	2		43^{A}	±	1	
Broiled Shrimp	Colorimeter	64	64.3^{A}	±	3.4	0.2	17.1^{A}	±	4.9	1.1	30.8^{A}	±	4.6	0.7	61.4^{B}	±	4.3	2.2	35.3^{A}	±	6.1	0.1
		74	64.5^{A}	±	3.4		15.9^{A}	±	4.8		31.5^{A}	±	5.0		63.6^{A}	±	4.7		35.4^{A}	±	6.3	
	Adobe® Photoshop®	64	62^{A}	±	4	0	23^{A}	±	3	0	45^{A}	±	3	0	63^{A}	±	1	0	51^{A}	±	4	1
		74	62^{A}	±	2		23^{A}	±	2		45^{A}	±	2		63^{A}	±	1		50^{A}	±	2	

Note: Photoshop only reports values as whole numbers. Values connected by the same letter within a column within and evaluation methods are not statistically different ($p < 0.05$). Only segments two through six were analyzed for color.

Table N-4: CIE *L*a*b Values for Broiled Oysters Determined by Colorimeter and Adobe® Photoshop®**

Evaluation Method	IT (°C)	*L**				*a**				*b**			
		Mean	±	**SD**	Δ	**Mean**	±	**SD**	Δ	**Mean**	±	**SD**	Δ
Colorimeter	64	69.1^{A}	±	2.9	4.6	2.3^{A}	±	0.8	0.5	19.0^{B}	±	1.6	1.9
	74	64.6^{B}	±	3.6		2.8^{A}	±	1.3		20.9^{A}	±	1.2	
Adobe® Photoshop®	64	56^{A}	±	1	1	1^{A}	±	1	0	23^{A}	±	2	3
	74	54^{A}	±	4		1^{A}	±	1		26^{A}	±	1	

Note: Photoshop only reports values as whole numbers. Values connected by the same letter within a column within and evaluation methods are not statistically different ($p < 0.05$).

Appendix O: Guidelines for Determining Internal Temperatures of 64°C ± 1 and 74°C ± 1

Table O-1: Cooking Guidelines for Achieving 64°C ± 1 and 74°C ± 1 Internal Temperatures for Fish and Shellfish

Seafood Product	Culinary Method	Cooking Guidelines
Salmon	Baked	64°C: Remove when IT reaches 61°C 74°C: Remove when IT reaches 73°C Record IT once it stabilizes (estimated as up to 2 min)
	Broiled	64°C: Remove when IT reaches 55-56°C 74°C: Remove when IT reaches 63°C Record IT once it stabilizes (estimated as up to 2 min)
	Poached	Measure thickest section of fillet (mm) and water must be 79-82°C (175-180°F) 64°C: 28 mm/sec (25 mm/sec for thin tail fillets) 74°C: 33 mm/sec Record IT once it stabilizes (estimated as up to 2 min)
Tilapia	Baked	64°C: Remove when IT reaches 63°C 74°C: Remove when IT reaches 73°C Record IT once it stabilizes (estimated as up to 1 min)
	Broiled	64°C: Remove when IT reaches 55-56°C 74°C: Remove when IT reaches 63-64°C Record IT once it stabilizes (estimated as up to 2 min)
	Pan-Fried	64°C: Remove when IT reaches 59-60°C 74°C: Remove when IT reaches 71-72°C Record IT once it stabilizes (estimated as up to 30 sec)
Shrimp	Boiled	Weigh raw shrimp (g) and water must be 85°C (185°F) 64°C: 7.94 sec/g 74°C: 9.9 sec/g or 11.2 sec/g Record IT once reaches safe IT and immediately submerse in ice water
	Broiled	64°C: Remove when IT reaches 57°C 74°C: Remove when IT reaches 69°C Record IT once it stabilizes (estimated as up to 30 sec)
Oysters	Broiled	Tried using thermocouples, but they would not stay in the oyster or in the center. Tried developing a sec/g or mm/sec, but nothing provided consistent results. Finally, went by the time after liquid was purging from oyster 15 sec (64°C) 30-40 sec (74°C)

Appendix P: Visual Terms Determined by Trained Descriptive Panel

Table P-1: Visual Qualitative Descriptors for Fish and Shellfish Determined By Trained Panel and Used for the Validation Test

Products	Visual Qualitative Descriptor Term	Definition of Term	Possible Descriptor Choices
All Products	Color/Hue	Dominating primary color family associated with the product (red, orange, yellow, green, blue, violet, white and black)	Salmon/Shrimp: Red, Orange & Yellow Tilapia: White & Yellow
	Color Intensity	Strength of the color/hue (lightness to darkness)	Light or Dark
	Color Vibrancy	Chroma/saturation of the color/hue	Dull or Vibrant
	Overtone Color	Color that develops over the primary color/hue	None, Yellow, Golden & Brown
	Surface Shine	Amount of light reflected from the product's surface	Matte or Glossy
Baked Salmon Only	Definition of the lines between the muscle flakes	Visibility of the connective tissue (lines) between the myocommata (appear as striations)	Not Defined or Defined
	Color intensity of the lines between the muscle flakes	Color of the connective tissue / myocommata (lines) between the myotomes (appear as striations)	Light or Dark
	Visibility of the central line	Visibility of lateral line	Not Visible or Visible
	Indentation appearance along central line	Indentation (groove) along lateral line	Not Indentation or Indentation
	Existence of white film coverage	Appearance and amount of coagulated protein	Absent (none to small spots) or Present (small spots, with some large white areas)
	Appearance associated with outer edges of fillet	Appearance of the outer perimeter / border of the fillet	Round or Sharp
	Color associated with outer edges of fillet	Color of the outer perimeter / border of the fillet	None, Yellow, Golden & Brown
Baked Tilapia Only	Definition of the lines between the muscle flakes	Visibility of the connective tissue (lines) between the myocommata (appear as striations)	Not Defined or Defined
	Color intensity of the lines between the muscle flakes	Color of the connective tissue / myocommata (lines) between the myotomes (appear as striations)	Light or Dark
	Degree of separation occurring between individual muscle flakes	Separation between flakes (myotomes) and along vertical connective tissue lines (myocommata)	No Separation or Separation
	Appearance characteristics associated with the outer edges of the fillet	Appearance of the outer perimeter / border of the fillet. Rounded (curved, not rigid or sharp), Crispy (brown, dry, crunchy/crumbly looking) & Curling (edges are noticeably curving up or down (not laying flat))	Rounded , Crispy & Curling
	Color associated with the outer edges of the fillet	Color of the outer perimeter / border of the fillet	None, Yellow, Golden & Brown
Boiled Shrimp Only	Undertone Color	Color remaining under primary color/hue	None or Gray
	Color/Hue associated with flesh	Color associated with the head of the shrimp	Translucent, Opaque, Pearly White & White (cooked rice)
	Visibility of individual muscle segments	Ability to distinguish distinct segments	Not Defined or Defined
	Overall shape of the shrimp	Silhouette or figure of shrimp	Hooked Shaped (J), Moderately Coiled, and Tightly Coiled

Appendix Q: Visual Validation Test and Re-Test Scorecards

Visual Validation Test Scorecard

Sample #:______________ **Panelists #:______________**

<u>Baked Tilapia</u> (10 Questions)

1. Indicate the dominating color/hue (check one):
   _____White
   _____Yellow

2. Indicate the color intensity associated with the color/hue identified in Question 1:
   _____Light
   _____Dark

3. Indicate the vibrancy of the color/hue and color intensity identified in Question 1 and 2:
   _____Vibrant
   _____Dull

4. Indicate the overtone color present (check one):
   _____None
   _____Yellow
   _____Golden
   _____Brown

5. Indicate the term that best described the surface shine:
   _____Glossy
   _____Matte

6. Indicate the definition of the lines between muscle flakes:
   _____Not defined
   _____Defined

7. Indicate the color intensity of the lines between muscle flakes:
   _____Light
   _____Dark

8. Indicate the degree of separation occurring between individual muscle flakes:
   _____No separation between muscle flakes
   _____Separation between muscle flakes

9. Indicate appearance characteristics associated with the outer edges of the fillet: (check all that apply)
   _____Rounded
   _____Crispy
   _____Curling

10. Indicate the color associated with the outer edges of the fillet (check one):
    _____None
    _____Yellow
    _____Brown
    _____Golden

Visual Validation Test Scorecard

Sample #:______________ **Panelists #:______________**

<u>Baked Salmon</u> (12 Questions)

1. Indicate the dominating color/hue (check one):
    _____Red
    _____Orange
    _____Yellow

2. Indicate the color intensity associated with the color/hue identified in Question 1:
    _____Light
    _____Dark

3. Indicate the vibrancy of the color/hue and color intensity identified in Question 1 and 2:
    _____Vibrant
    _____Dull

4. Indicate the overtone color present (check one):
    _____None
    _____Yellow
    _____Golden
    _____Brown

5. Indicate the term that best describes the surface shine:
    _____Glossy
    _____Matte

6. Indicate the definition of the lines between muscle flakes:
    _____Not defined
    _____Defined

7. Indicate the color intensity of the lines between muscle flakes:
    _____Light
    _____Dark

8. Indicate the visibility of the central line:
    _____Not Visible
    _____Visible

9. Indicate the indentation appearance along the central line:
    _____No Indentation
    _____Indentation

10. Indicate the existence of white film coverage:
    _____Absent (none to small spots)
    _____Present (small spots, with some large white areas)

11. Indicate the appearance associated with the outer edges of the fillet:
    _____Round
    _____Sharp

12. Indicate the color associated with the outer edges of the fillet (check one):
    _____None
    _____Yellow
    _____Golden
    _____Brown

Visual Validation Test Scorecard

Sample #:______________ **Panelists #:______________**

Boiled Shrimp (9 Questions)

1. Indicate the dominating color/hue (check one):
   _____Red
   _____Orange
   _____Yellow

2. Indicate the color intensity associated with the color/hue identified in Question 1:
   _____Light
   _____Dark

3. Indicate the vibrancy of the color/hue and color intensity identified in Question 1 and 2:
   _____Vibrant
   _____Dull

4. Indicate the overtone color present (check one):
   _____None
   _____Yellow
   _____Golden
   _____Brown

5. Indicate the undertone color present (check one):
   _____None
   _____Gray

6. Indicate the color/hue associated with the flesh:
   _____Translucent
   _____Opaque
   _____Pearly white
   _____White (cooked rice)

7. Indicate the term that best described the surface shine:
   _____Glossy
   _____Matte

8. Indicate the visibility of the individual muscle segments:
   _____Not defined
   _____Defined

9. Indicate the overall shape of the shrimp (check one):
   _____Hook shaped (J)
   _____Moderately coiled
   _____Tightly coiled

Visual Validation Re-Test Scorecard

Sample #:______________ **Panelists #:______________**

Baked Tilapia

1. Indicate whether or not separation is occurring between individual muscle flakes:
   _____No separation between muscle flakes
   _____Separation between muscle flakes

2. Indicate appearance characteristics associated with the outer edges of the fillet: **(check all that apply)**
   _____None
   _____Crispy
   _____Curling

Sample #:______________

Baked Tilapia

1. Indicate whether or not separation is occurring between individual muscle flakes:
   _____No separation between muscle flakes
   _____Separation between muscle flakes

2. Indicate appearance characteristics associated with the outer edges of the fillet: **(check all that apply)**
   _____None
   _____Crispy
   _____Curling

Sample #:______________

Baked Tilapia

1. Indicate whether or not separation is occurring between individual muscle flakes:
   _____No separation between muscle flakes
   _____Separation between muscle flakes

2. Indicate appearance characteristics associated with the outer edges of the fillet: **(check all that apply)**
   _____None
   _____Crispy
   _____Curling

Sample #:______________

Baked Tilapia

1. Indicate whether or not separation is occurring between individual muscle flakes:
   _____No separation between muscle flakes
   _____Separation between muscle flakes

2. Indicate appearance characteristics associated with the outer edges of the fillet: **(check all that apply)**
   _____None
   _____Crispy
   _____Curling

Continue to Next Page

Visual Validation Re-Test Scorecard

Sample #:______________ **Panelists #:______________**

Baked Tilapia

1. Indicate whether or not separation is occurring between individual muscle flakes:
    _____No separation between muscle flakes
    _____Separation between muscle flakes

2. Indicate appearance characteristics associated with the outer edges of the fillet: **(check all that apply)**
    _____None
    _____Crispy
    _____Curling

Sample #:______________

Baked Tilapia

1. Indicate whether or not separation is occurring between individual muscle flakes:
    _____No separation between muscle flakes
    _____Separation between muscle flakes

2. Indicate appearance characteristics associated with the outer edges of the fillet: **(check all that apply)**
    _____None
    _____Crispy
    _____Curling

Visual Validation Re-Test Scorecard

Sample #:______________ **Panelists #:______________**

Baked Salmon

1. Indicate whether or not separation is occurring along the central line:
   _____No Separation
   _____Separation

Sample #:______________

Baked Salmon

1. Indicate whether or not separation is occurring along the central line:
   _____No Separation
   _____Separation

Sample #:______________

Baked Salmon

1. Indicate whether or not separation is occurring along the central line:
   _____No Separation
   _____Separation

Sample #:______________

Baked Salmon

1. Indicate whether or not separation is occurring along the central line:
   _____No Separation
   _____Separation

Sample #:______________

Baked Salmon

1. Indicate whether or not separation is occurring along the central line:
   _____No Separation
   _____Separation

Sample #:______________

Baked Salmon

1. Indicate whether or not separation is occurring along the central line:
   _____No Separation
   _____Separation

Visual Validation Re-Test Scorecard

Sample #:______________ **Panelists #:______________**

Boiled Shrimp

1. Indicate the overall shape of the shrimp (check one):
_____Hook shaped (J)
_____Moderately coiled
_____Tightly coiled

Sample #:______________

Boiled Shrimp

1. Indicate the overall shape of the shrimp (check one):
_____Hook shaped (J)
_____Moderately coiled
_____Tightly coiled

Sample #:______________

Boiled Shrimp

1. Indicate the overall shape of the shrimp (check one):
_____Hook shaped (J)
_____Moderately coiled
_____Tightly coiled

Sample #:______________

Boiled Shrimp

1. Indicate the overall shape of the shrimp (check one):
_____Hook shaped (J)
_____Moderately coiled
_____Tightly coiled

Sample #:______________

Boiled Shrimp

1. Indicate the overall shape of the shrimp (check one):
_____Hook shaped (J)
_____Moderately coiled
_____Tightly coiled

Sample #:______________

Boiled Shrimp

1. Indicate the overall shape of the shrimp (check one):
_____Hook shaped (J)
_____Moderately coiled
_____Tightly coiled

Appendix R: Predetermined Visual Characteristics Required for Validation Test

Table R-1: Predetermined Visual Characteristics of Baked Salmon, Baked Tilapia and Boiled Shrimp for Validation Test

Product	10 min (Fish) and 1 min (Shrimp)	15 min (Fish) and 2 min (Shrimp)	20 min (Fish) and 3 min (Shrimp)
Baked Salmon	• Definition of lines between muscle flakes • Indentation of Central Line • White film (coagulated protein) coverage	• Visibility of central line • Indentation of Central Line • White film (coagulated protein) coverage	• Visibility of central line • Indentation of Central Line • White film (coagulated protein) coverage • Edge appearance
Baked Tilapia	• Color/hue • Vibrancy of color/hue • Degree of separation between muscle segments	• Color/hue • Degree of separation between muscle segments • Outer edge appearance	• Overtone color • Degree of separation between muscle segments • Outer edge appearance
Boiled Shrimp	• Shape • Visibility of individual segments • Undertone color	• Shape • Visibility of individual segments • Undertone color	• Shape • Visibility of individual segments • Undertone color

Note: For baked salmon and baked tilpaia: below safe IT (10 min), near safe IT (15 min) and above safe IT (20 min) and for boiled shrimp below safe IT (1 min), near safe IT (3 min) and above safe IT (3 min); Refer to Table # for definitions of individual characteristics.

Appendix S: Visual Test Scorecards for Trained Descriptive Panel

Visual Test Scorecard

Sample #:______________ **Panelists #:______________**

Baked Tilapia

1. Indicate the dominating color/hue (check one):
    _____White
    _____Yellow

2. Indicate the vibrancy of the color/hue identified in Question 1:
    _____Vibrant
    _____Dull

3. Indicate the overtone color present (check one):
    _____None
    _____Yellow
    _____Golden

4. Indicate the term that best described the surface shine:
    _____Glossy
    _____Matte

5. Indicate whether or not the lines between muscle flakes are defined:
    _____Not defined
    _____Defined

6. Indicate whether or not separation is occurring between individual muscle flakes:
    _____No separation between muscle flakes
    _____Separation between muscle flakes

7. Indicate appearance characteristics associated with the outer edges of the fillet: **(check all that apply)**
    _____None
    _____Crispy
    _____Curling

8. Indicate the color associated with the outer edges of the fillet (check one):
    _____None
    _____Yellow
    _____Golden

Visual Test Scorecard

Sample #:______________ **Panelists #:______________**

Baked Salmon

1. Indicate the dominating color/hue (check one):
   _____Red
   _____Orange

2. Indicate the overtone color present (check one):
   _____None
   _____Yellow
   _____Golden

3. Indicate the term that best describes the surface shine:
   _____Glossy
   _____Matte

4. Indicate the definition of the lines between muscle flakes:
   _____Not defined
   _____Defined

5. Indicate whether or not separation is occurring along the central line:
   _____No Separation
   _____Separation

6. Indicate the existence of white film coverage:
   _____Absent (none to small spots)
   _____Present (small spots, with some large white areas)

7. Indicate the appearance associated with the outer edges of the fillet:
   _____Round
   _____Sharp

8. Indicate the color associated with the outer edges of the fillet (check one):
   _____None
   _____Yellow
   _____Golden

Visual Test Scorecard

Sample #:______________ **Panelists #:______________**

Boiled Shrimp

1. Indicate the dominating color/hue associated with the shrimp **(do NOT consider the tail/shell portion)**:
   _____Red
   _____Orange

2. Indicate the color intensity associated with the color/hue identified in Question 1:
   _____Light
   _____Dark

3. Indicate the vibrancy of the color/hue and color intensity identified in Question 1 and 2:
   _____Vibrant
   _____Dull

4. Indicate the color/hue associated with the flesh of the shrimp's head:
   _____Translucent
   _____White

5. Indicate the overall shape of the shrimp (check one):
   _____Moderately coiled
   _____Tightly coiled

Appendix T: Visual Trained Descriptive Panel Session Outline

Week 1:

- Familiarize panelists with fish and shellfish products (salmon, tilapia, shrimp) by showing them pictures of cooked product.
- Ask panelist to describe visual characteristics of pictures of flowers to practice applying visual descriptions.
- Ask panelist to describe visual characteristics of each of the fish and shellfish products for practice.

Week 2:

- Ask panelist to describe visual characteristics of pictures of flowers to practice applying visual descriptions.
- Show pictures of baked salmon at raw and time increments of 5, 10, 15, 20 and 25 min to demonstrate changes during cooking.
- Show each picture individually and ask panelists to write down visual characteristics. Once everyone evaluates a picture, discuss as a group.

Week 3:

- Show pictures at raw and time increments of 5, 10, 15, 20 and 25 min to demonstrate changes during cooking.
- Show pictures of boiled shrimp at raw and timed increments of 15 sec, 30 sec, 45 sec, 1 min, 1.5 min, 2 min, 2.5 min and 3 min to demonstrate changes during cooking.
- Present each picture individually and ask panelists to write down visual characteristics. Once everyone evaluates a picture, discuss as a group.

Week 4:

- Compile a list of terms commonly repeated for each product and review with panelists to determine the exact meaning of the term/attribute.
- Validation testing of baked salmon, baked tilapia, and boiled shrimp.

Week 5:

- Visual testing of baked salmon, baked tilapia, and boiled shrimp.

Appendix U: Consumer Sensory Panel Recruitment Survey

Seafood Sensory Panel Interest

What is your name? (Please include first and last name)

What is your email address?

An email will be sent to you shortly after completing this survey confirming the panels and times you have selected.

What is your preferred method of contact?

- ☐ email
- ☐ text message
- ☐ phone call

If you would like a reminder text message or phone call about upcoming panels you signed up to participate in please include your phone number below.

For which of the following panels and times would you be willing to participate? Please check all panels and times you are interested in.

Baked Tilapia on Monday, October 11th

☐ 10:30 AM ☐ 11:00 AM ☐ 11:30 AM

Baked Tilapia on Wednesday, October 13th

☐ 10:30 AM ☐ 11:00 AM ☐ 11:30 AM ☐ 12:00 PM

Pan-Fried Tilapia on Monday, October 18th

☐ 10:30 AM ☐ 11:00 AM ☐ 11:30 AM

Pan-Fried Tilapia on Wednesday, October 20th

☐ 10:30 AM ☐ 11:00 AM ☐ 11:30 AM ☐ 12:00 PM

Baked Atlantic Salmon on Monday, October 25th

☐ 10:30 AM ☐ 11:00 AM ☐ 11:30 AM

Baked Atlantic Salmon on Wednesday, October 27th

☐ 10:30 AM ☐ 11:00 AM ☐ 11:30 AM

Boiled Shrimp on Wednesday, November 3rd
☐ 10:30 AM ☐ 11:00 AM ☐ 11:30 AM ☐ 12:00 PM

Boiled Shrimp on Friday, November 5th
☐ 10:30 AM ☐ 11:00 AM ☐ 11:30 AM ☐ 12:00 PM

Poached Atlantic Salmon on Monday, November 8th
☐ 10:30 AM ☐ 11:00 AM ☐ 11:30 AM ☐ 12:00 PM

Poached Atlantic Salmon on Wednesday, November 10th
☐ 10:30 AM ☐ 11:00 AM ☐ 11:30 AM ☐ 12:00 PM

Thank you for your time and willingness to participate!

Appendix V: Seafood Questionnaire

Fish and Seafood Sensory Panels - Questionnaire

By taking this survey you are providing consent for the use of this data. Your identification and contact information will only be used to contact you in response to this project.

What is your name? (Please provide your first name and last name)

____

Enter your email address below if you would be interested in participating in a focus group discussion.

____

Demographics

How old are you?

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

What is your gender?

- Male
- Female

What is your ethnic group?

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

other: ________________________

Seafood/Fish Consumption & Purchasing Habits

How often do you consume seafood?

- Never
- 1-2 times a year
- 3-11 times a year
- 1-2 times a month
- 3-4 times a month
- More than once a week

If you answered "Never" to the question above you are done and may submit your survey now

What types of seafood (including fish) do you consume? Check all that apply.

- [ ] Fatty fish such as Salmon
- [ ] Lean fish such as Tilapia
- [ ] Shrimp
- [ ] Crab
- [ ] Lobster
- [ ] Oysters
- [ ] Mussels
- [ ] Scallops
- [ ] Clams
- [ ] Prawns
- [ ] Crawfish

other: ____

How is the seafood (including fish) you consume prepared? Check all that apply.

- [ ] Baked
- [ ] Broiled
- [ ] Blackened
- [ ] Sautéed
- [ ] Grilled
- [ ] Breaded
- [ ] Deep fried
- [ ] Boiled

- [ ] Steamed
- [ ] Raw

other: ____

How often do you cook seafood (including fish)?

- Never
- 1-2 times a year
- 3-11 times a year
- 12-24 times a year
- 3-4 times a month
- More than once a week

Do you purchase farm raised/cultivated seafood (including fish)?

- Yes
- No
- Unsure

Do you purchase raw seafood (including fish)?

- Yes
- No

Do you ever purchase or consume prepared frozen seafood (including fish) products? ex. shrimp poppers or fish patties

- Yes
- No

<u>Seafood/Fish Safety</u>

What is the appropriate internal endpoint temperature for cooking seafood (including fish) from a food safety perspective? (degrees F)
ex. Chicken = 165 degrees F

____

Do you ever knowingly eat raw or undercooked seafood? (ex. raw oysters, sushi)

- Yes
- No

What health risks do you associate with consuming raw/undercooked seafood (including fish)?

When eating seafood (including fish), are you ever concerned about whether the seafood/fish was cooked to properly prevent against foodborne illness?

- No, I am never concerned about seafood safety
- Rarely, sometimes I wonder if there is a risk
- Frequently, often I wonder if there is a risk
- Yes, I am always concerned that it is not safe to eat

If you have ever cooked seafood or fish please respond to the following questions: (If you have never cooked seafood or fish, please submit your survey now.)

Which of the following types of seafood (including fish) have you cooked? Check all that apply.

- [ ] Fatty fish such as Salmon
- [ ] Lean fish such as Tilapia
- [ ] Shrimp
- [ ] Crab
- [ ] Lobster
- [ ] Oysters
- [ ] Mussels
- [ ] Scallops
- [ ] Clams
- [ ] Prawns
- [ ] Crawfish

other: ________________________________

Which of the following methods have you used to cook your seafood/fish? Check all that apply.

- ☐ Baked
- ☐ Broiled
- ☐ Blackened
- ☐ Sautéed
- ☐ Grilled
- ☐ Breaded
- ☐ Deep fried
- ☐ Boiled
- ☐ Steamed
- ☐ Raw

other: ______________________________

Using the methods you selected above, please answer the following question:
What indicators did you use to determine your seafood/fish was done cooking? Identify the product and method used for each example.

What are your concerns when cooking seafood (including fish)? Check all that apply.

- ☐ I do not have any concerns
- ☐ I am concerned the product is undercooked and not safe to eat
- ☐ I am concerned the product is overcooked
- ☐ I am concerned the product was not harvested from safe waters

other: ________________________________

Thank you for your feedback!

Appendix W: Consent form for Consumer Acceptability Test

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY
Informed Consent for Participants
in Research Projects Involving Human Subjects

Title of Project: Sensory Assessment of Microbiologically Safe Culinary Processes for Fish and Seafood

Investigator(s): Dr. Susan Duncan, PhD. and Renee Felice

I. Purpose of this Research/Project

You are invited to participate in a sensory study to evaluate the overall liking of METHOD SEAFOOD.

II. Procedures

There will be one sensory test lasting approximately 15 min. At the beginning of the sensory test you will be asked demographic questions, questions about your seafood/fish consumption habits, and questions about your seafood/fish safety concerns. Secondly, you will be presented with two individual METHOD SEAFOOD samples. You will be asked to evaluate each sample on overall liking.

III. Risks

There are no more than minimal risks for participating in this study. If you have a fish and/or shellfish allergies or an aversion to SEAFOOD please inform the investigator.

IV. Benefits

Your participation in the study will provide a better understanding of how the overall acceptability of boiled shrimp correlates with various thermal endpoints. The results will help with establishing optimal cooking guidelines for ensuring acceptability and safety for consumers. If you would like a summary of the research results, please contact the researcher at a later time.

V. Extent of Anonymity and Confidentiality

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

VI. Compensation

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

VII. Freedom to Withdraw

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

VIII. Subject's Responsibilities

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

2. Answer questions relating to demographics, seafood/fish consumption habits, and seafood/fish safety concerns.

2. Taste and evaluate the overall liking of each of the two samples.

IX. Subject's Permission

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

____ Date__________
Subject Signature

____ Date__________
Subject Printed Name

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

Renee Felice (910) 547-6339, felicer@vt.edu
Graduate Research Assistant, Investigator

Susan Duncan, Faculty/ Investigator (540) 231-8675; duncans@vt.edu

David Moore (540) 231-4991; moored@vt.edu
Chair, Virginia Tech Institutional Review
Board for the Protection of Human Subjects
Office of Research Compliance
2000 Kraft Dr., Suite 2000 (0497)
Blacksburg, VA 24061

Consent Form Key

METHOD = Baked, poached, boiled, and pan-fried

SEAFOOD = Atlantic Salmon, Tilapia and Shrimp

Appendix X: Generic Overall Acceptability Scorecard for Consumer Panels

Acceptance Test of "METHOD SEAFOOD"

Panelist ID:________

Instructions:

1. Today you will be evaluating two samples.
2. Please rinse your mouth with water before evaluating each sample.
3. Please consider the appearance, odor, flavor and texture (mouth-feel) of the sample.
4. Once you taste the sample, please use the hedonic scale to indicate overall liking (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely).

Sample ______________ (1 of 2)

Please taste the sample and rate OVERALL Liking

Overall Liking

1	2	3	4	5	6	7	8	9
Dislike Extremely				Neither Like nor Dislike				Like Extremely

Please return the sample and wait for the second sample.

Acceptance Test of "METHOD SEAFOOOD"

Panelist ID:________

Instructions:

5. Today you will be evaluating two samples.
6. Please rinse your mouth with water before evaluating each sample.
7. Please consider the appearance, odor, flavor and texture (mouth-feel) of the sample.
8. Once you taste the sample, please use the hedonic scale to indicate overall liking (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely).

Sample ______________ (2 of 2)

Please taste the sample and rate OVERALL Liking

Overall Liking

1	2	3	4	5	6	7	8	9
Dislike Extremely				Neither Like nor Dislike				Like Extremely

Please return the sample, you are finished!
Please come around to the Sensory Kitchen for a snack!
THANK YOU!

<u>Score Card Key</u>

METHOD = Baked, poached, boiled, and pan-fried

SEAFOOD = Atlantic Salmon, Tilapia and Shrimp

Appendix Y: Adobe® Photoshop® Color Analysis of Visual Validation Test and Visual Test Pictures

Table Y-1: Mean Color Values for the Outer Edge of Fillets and Head Section of Shrimp Determined by Adobe® Photoshop® Analysis of Pictures Used in Trained Panel (n = 7) Visual Test

Product	IT (°C)	L			a			b			Hue			Chroma		
		Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD
Baked Salmon	64	69^{A}	±	4	7^{A}	±	2	41^{B}	±	6	80^{A}	±	3	41^{B}	±	6
	74	65^{A}	±	3	8^{A}	±	1	49^{A}	±	5	81^{A}	±	1	50^{A}	±	5
Baked Tilapia	64	78^{A}	±	6	-3^{B}	±	2	26^{B}	±	7	-62^{B}	±	57	26^{B}	±	7
	74	69^{B}	±	7	2^{A}	±	3	41^{A}	±	6	47^{A}	±	76	41^{A}	±	6
Boiled Shrimp	64	79^{A}	±	1	1^{A}	±	0	14^{A}	±	1	86^{A}	±	0	14^{A}	±	1
	74	79^{A}	±	2	2^{A}	±	1	13^{A}	±	3	83^{A}	±	1	13^{A}	±	3

Note: Values not connected by the same letter within a column and product are statistically different ($p < 0.05$). The mean color for shrimp reported includes the remaining head portion of the shrimp (head was removed).

Table Y-2: Mean Color Values for Outer Edge of Fillets and Remaining Head Section of Shrimp Determined by Adobe® Photoshop® Analysis of Pictured Used in Trained Panel (n = 7) Visual Validation Test

Product	Cook Time (min) & IT (°C)	L			a			b			Hue			Chroma		
		Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD
Baked Salmon	10 min & 44°C	68^{B}	±	4	14^{A}	±	2	42^{A}	±	7	72^{B}	±	3	45^{A}	±	6
	15 min & 59°C	74^{A}	±	3	12^{A}	±	2	45^{A}	±	3	75^{AB}	±	2	46^{A}	±	3
	20 min & 70°C	66^{B}	±	4	12^{A}	±	3	48^{A}	±	7	76^{A}	±	3	50^{A}	±	8
Baked Tilapia	10 min & 52°C	82^{A}	±	2	-3^{B}	±	1	19^{B}	±	6	-82^{B}	±	2	20^{B}	±	6
	15 min & 63°C	79^{A}	±	4	-2^{AB}	±	1	26^{B}	±	7	-55^{AB}	±	70	26^{B}	±	7
	20 min & 71°C	67^{B}	±	12	2^{A}	±	5	39^{A}	±	6	27^{A}	±	86	40^{A}	±	6
Boiled Shrimp	1 min & 43°C	80^{A}	±	2	0^{A}	±	0	12^{A}	±	1						
	2 min & 62°C	78^{A}	±	2	2^{A}	±	1	12^{A}	±	1						
	3 min & 69°C	80^{A}	±	3	0^{A}	±	0	12^{A}	±	2						

Note: The sample at each endpoint was presented to panelists from two different angles (duplicates). Each photo was analyzed and the average and standard deviation is reported above. Values not connected by the same letter within a column and product are statistically different ($p < 0.05$). The mean color for shrimp reported includes the remaining head portion of the shrimp (head was removed).

Table Y-3: Mean Overall Color Values Determined by Adobe® Photoshop® Analysis of Pictured Used in Trained Panel (n = 7) Visual Test

Product	IT (°C)	L			a			b			Hue			Chroma		
		Mean	**±**	**SD**	**Mean**	**±**	**SD**	**Mean**	**±**	**SD**	**Mean**	**±**	**SD**	**Mean**	**±**	**SD**
Baked Salmon	64	69^{A}	±	2	7^{A}	±	1	41^{A}	±	3	80^{A}	±	1	41^{A}	±	3
	74	65^{A}	±	3	8^{A}	±	2	49^{A}	±	4	81^{A}	±	2	50^{A}	±	4
Baked Tilapia	64	78^{A}	±	3	-1^{A}	±	1	20^{A}	±	2						
	74	80^{A}	±	1	0^{A}	±	0	23^{A}	±	3						
Boiled Shrimp	64	70^{A}	±	1	14^{B}	±	2	30^{B}	±	1	65^{A}	±	3	33^{B}	±	2
	74	67^{A}	±	2	19^{A}	±	1	34^{A}	±	1	61^{A}	±	1	39^{A}	±	1

Note: Values not connected by the same letter within a column and product are statistically different ($p < 0.05$). The overall color for shrimp included segments one through five.

Table Y-4: Mean Overall Color Values Determined by Adobe® Photoshop® Analysis of Pictured Used in Trained Panel (n = 7) Visual Validation Test

Product	Cook Time (min) & IT (°C)	L			a			b			Hue			Chroma		
		Mean	**±**	**SD**	**Mean**	**±**	**SD**	**Mean**	**±**	**SD**	**Mean**	**±**	**SD**	**Mean**	**±**	**SD**
Baked Salmon	10 min & 44°C	66^{B}	±	1	17^{A}	±	0	36^{A}	±	1	65^{B}	±	1	40^{AB}	±	1
	15 min & 59°C	72^{A}	±	1	17^{A}	±	1	41^{A}	±	1	68^{AB}	±	1	44^{A}	±	1
	20 min & 70°C	68^{AB}	±	1	13^{B}	±	0	35^{A}	±	2	69^{A}	±	1	37^{B}	±	2
Baked Tilapia	10 min & 52°C	79^{A}	±	1	0^{A}	±	0	16^{C}	±	1						
	15 min & 63°C	78^{A}	±	0	0^{A}	±	0	19^{B}	±	0						
	20 min & 71°C	77^{A}	±	1	0^{A}	±	0	22^{A}	±	0						
Boiled Shrimp	1 min & 43°C	69^{AB}	±	1	11^{B}	±	0	26^{B}	±	1	67^{A}	±	1	28^{B}	±	1
	2 min & 62°C	67^{B}	±	1	15^{A}	±	0	28^{A}	±	0	62^{B}	±	0	32^{A}	±	0
	3 min & 69°C	70^{A}	±	1	13^{B}	±	1	28^{A}	±	0	66^{A}	±	1	31^{A}	±	0

Note: The sample at each endpoint was presented to panelists from two different angles (duplicates). Each photo was analyzed and the average and standard deviation is reported above. Values not connected by the same letter within a column and product are statistically different ($p < 0.05$). The overall color for shrimp included segments one through five.

Appendix Z: Visual Validation Test Results

Table Z-1: Visual Validation Test Results From Visual Training of Trained Descriptive Panel (n = 7) Supporting Findings from Final Visual Assessment

Product	Characteristic	Rep	10 min (44°C)	15 min (59°C)	20 min (70°C)
Baked Salmon	Indicate the definition of the lines between muscle flakes	1	Not Defined: 0 Defined: 7	Not Defined: 0 Defined: 7	Not Defined: 1 Defined: 6
		2	Not Defined: 0 Defined: 7	Not Defined: 0 Defined: 7	Not Defined: 0 Defined: 7
	Indicate the existence of white film coverage	1	Absent: 7 Present: 0	Absent: 1 Present: 6	Absent: 0 Present: 7
		2	Absent: 7 Present: 0	Absent: 1 Present: 6	Absent: 0 Present: 7
Product	**Characteristic**	**Rep**	**10 min (52°C)**	**15 min (63°C)**	**20 min (71°C)**
Baked Tilapia	Indicate whether or not separation is occurring between individual muscle flakes	1	Not Separated: 6 Separated: 1	Not Separated: 2 Separated: 5	Not Separated: 2 Separated: 5
		2	Not Separated: 5 Separated: 2	Not Separated: 2 Separated: 5	Not Separated: 2 Separated: 5
Product	**Characteristic**	**Rep**	**1 min (43°C)**	**2 min (62°C)**	**3 min (69°C)**
Boiled Shrimp	Indicate the overall shape of the shrimp	1	Hooked: 1 Moderately Coiled: 6 Tightly Coiled: 0	Hooked: 0 Moderately Coiled: 0 Tightly Coiled: 7	Hooked: 0 Moderately Coiled: 0 Tightly Coiled: 7
		2	Hooked: 0 Moderately Coiled: 7 Tightly Coiled: 0	Hooked: 0 Moderately Coiled: 0 Tightly Coiled: 7	Hooked: 0 Moderately Coiled: 0 Tightly Coiled: 7

Note: To successfully verify the panel performance on each validation test, at least 5 of the 7 panelists needed to correctly characterize the predetermined attributes.

Appendix AA: Summary of Seafood Questionnaire Responses

Table AA-1: Fish and Seafood Questionnaire Data from Consumer Sensory Panels

		Panelists (n = 107)
		%
Gender		
	Male	36%
	Female	64%
Age		
	18 - 24	53%
	25 - 30	14%
	31 - 40	7%
	41 - 50	7%
	51 - 60	12%
	61 - 70	7%
	71+	0%
Ethnicity		
	Hispanic, Latino, or Spanish Origin	6%
	White	72%
	Black, African American, or Negro	4%
	American Indian or Alaskan Native	1%
	Asian or Pacific Islander	14%
	Other	3%
	No Answer	1%
Frequency consumed		
	Never	0%
	1 – 2 times a year	8%
	3 – 11 times a year	14%
	1 – 2 times a month	18%
	3 – 4 times a month	41%
	More than once a week	19%
Types of seafood (including fish) consumed		
	Fatty fish such as salmon	85%
	Lean fish such as tilapia	82%
	Shrimp	93%
	Crab	71%
	Lobster	47%
	Oysters	40%
	Mussels	37%
	Scallops	61%
	Clams	40%
	Prawns	34%
	Crawfish	30%
	Other	7%

Methods seafood (including fish) is prepared		

Baked	93%
Broiled	66%
Blackened	50%
Sautéed	58%
Grilled	87%
Breaded	60%
Deep Fried	54%
Boiled	50%
Steamed	57%
Raw	42%
Other	4%
Frequency of cooking seafood	
Never	9%
1 – 2 times a year	9%
3 – 11 times a year	26%
12 – 24 times a year	19%
3 – 4 times a month	27%
More than once a week	9%
Purchase farm raised/cultivated seafood	
Yes	55%
No	17%
Unsure	28%
Purchase raw seafood	
Yes	88%
No	12%
Purchase or consume prepared frozen seafood products	
Yes	72%
No	25%
No Answer	3%
Required internal endpoint temperature for cooked seafood from a food safety perspective (°F)	
Correctly indicated 145°F	16%
Indicated above 145°F	41%
Indicated below 145°F	18%
Indicated that they did not know	26%
Knowingly eat raw or undercooked seafood	
Yes	67%
No	32%
No Answer	1%
Health risks associate with consuming raw/undercooked seafood	
None / No Answer	9%
Bodily Illness	29%
Parasites	9%
Bacteria / Microorganisms	19%
Salmonella spp.	7%
Listeria monocytongenes	3%
Vibrio spp.	9%

Toxins	5%
Mercury	2%
Other	7%
Concerned if seafood was cooked to prevent against food-borne illness	
No, I am never concerned about seafood safety	10%
Rarely, sometimes I wonder if there is a risk	58%
Frequently, often I wonder if there is a risk	14%
Yes, I am always concerned that it is not safe to eat	18%
Frequency of seafood cooked	
Fatty fish such as salmon	77%
Lean fish such as tilapia	66%
Shrimp	70%
Crab	42%
Lobster	21%
Oysters	16%
Mussels	15%
Scallops	38%
Clams	23%
Prawns	19%
Crawfish	10%
Other	3%
Frequency of methods used to cook seafood	
Baked	73%
Broiled	43%
Blackened	21%
Sautéed	49%
Grilled	67%
Breaded	33%
Deep Fried	21%
Boiled	39%
Steamed	37%
Raw	10%
Other	4%
Indicators used to determine seafood doneness	
Time	32%
Temperature	13%
Visual indicator	74%
Touch / Texture	42%
Other	7%
No Answer	11%
Concerns when cooking seafood	
I do not have any concerns	20%
I am concerned the product is undercooked and not safe to eat	48%
I am concerned the product is overcooked	31%
I am concerned the product was not harvested from safe waters	31%
Other	7%

Appendix BB: Consumer Acceptability Histograms

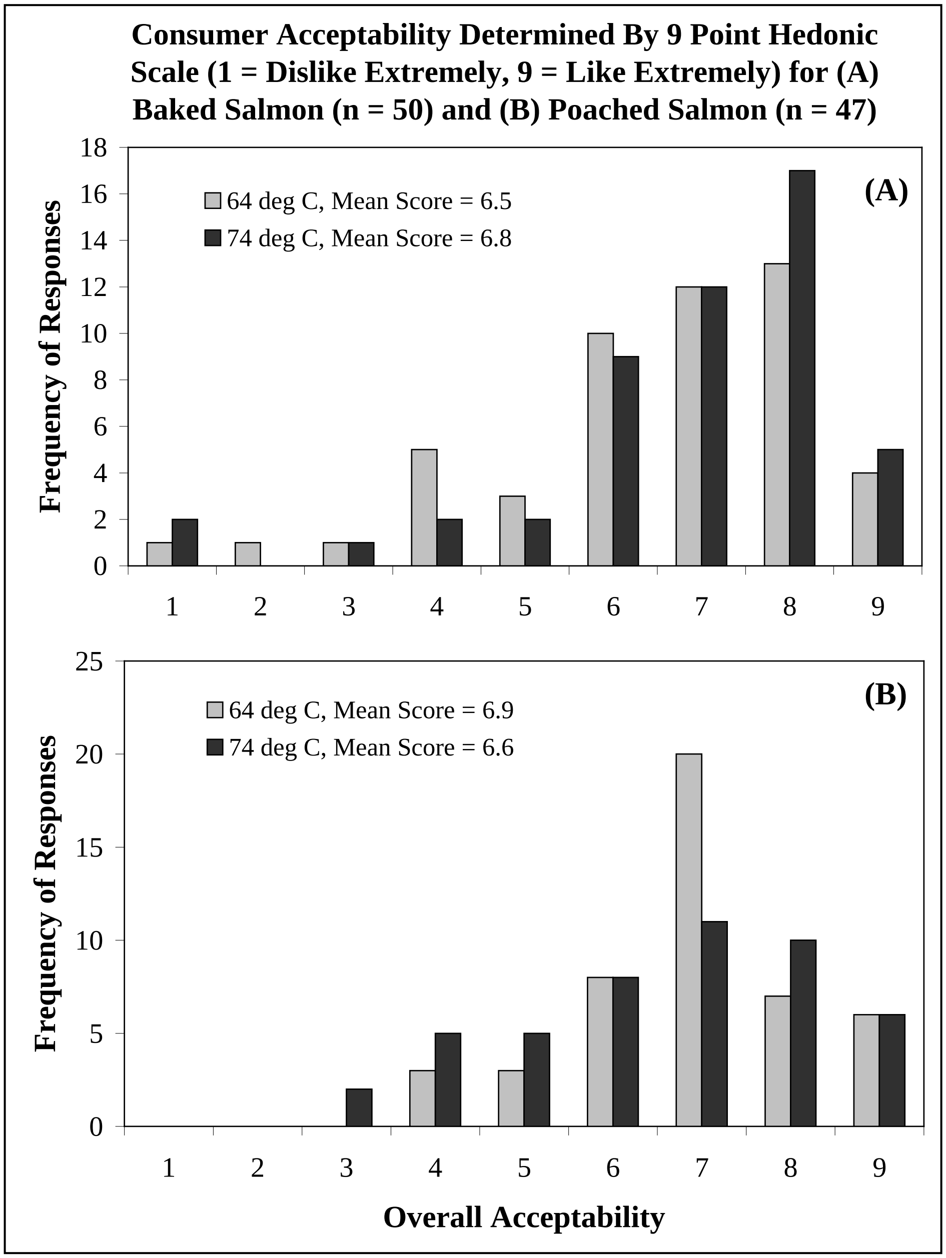


Figure BB-1: Consumer Acceptability Determined by 9 Point Hedonic Scale (1 = Dislike Extremely, 9 = Like Extremely) for (A) Baked Salmon (n = 50) and (B) Poached Salmon (n = 47)

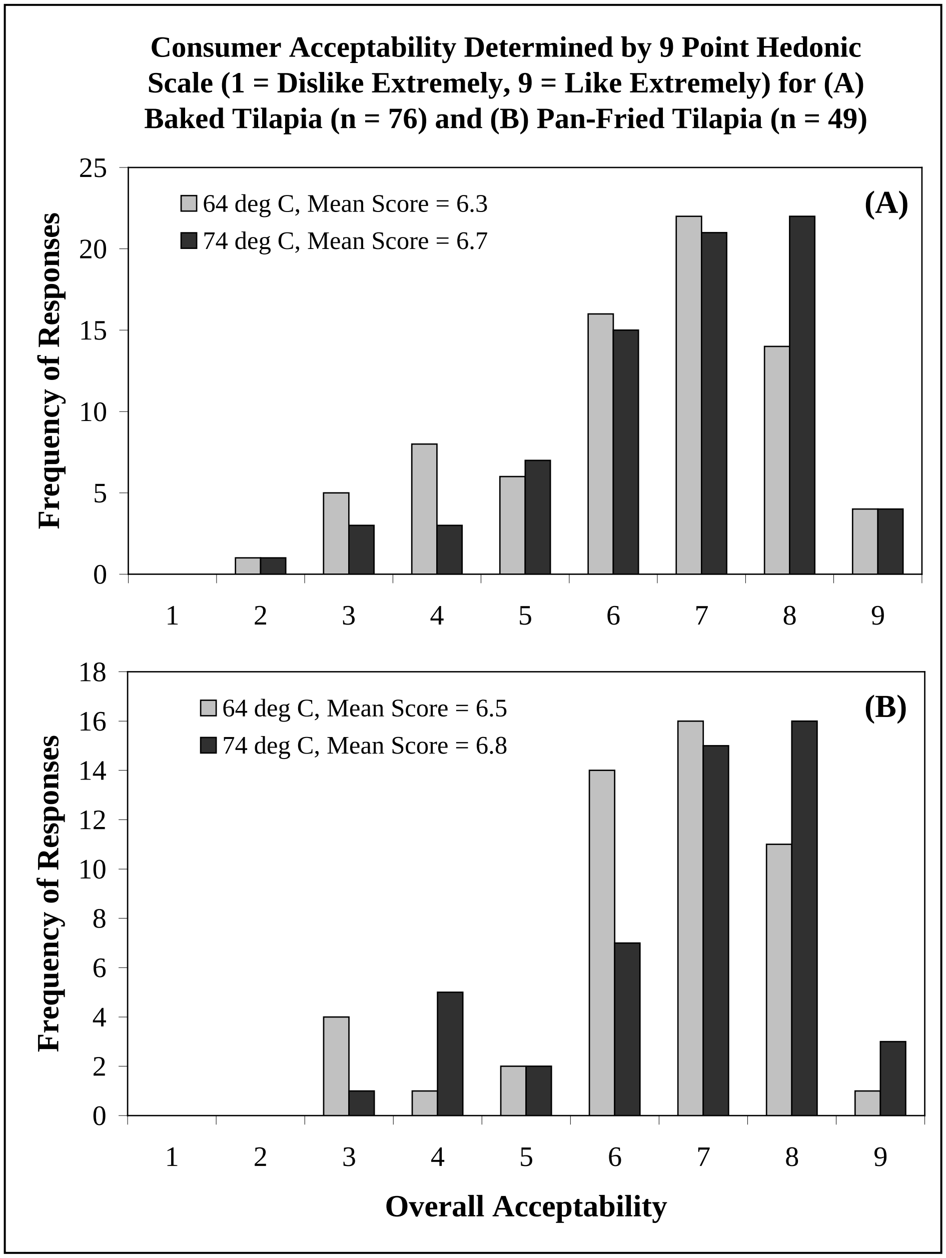


Figure BB-2: Consumer Acceptability Determined by 9 Point Hedonic Scale (1 = Dislike Extremely, 9 = Like Extremely) for (A) Baked Tilapia (n = 76) and (B) Pan-Fried Tilapia (n = 49)

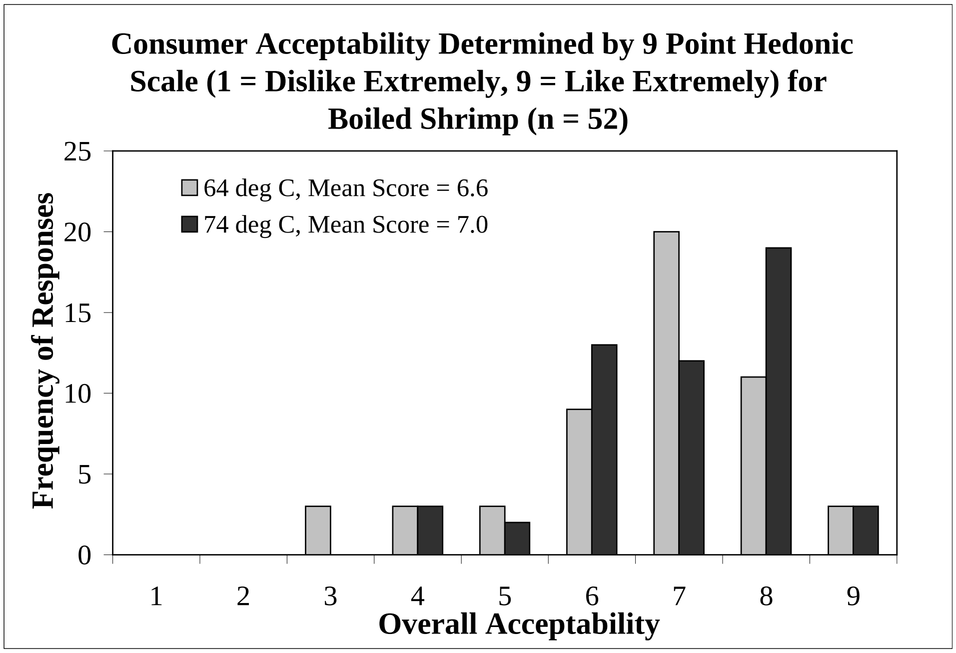


Figure BB-3: Consumer Acceptability Determined by 9 Point Hedonic Scale (1 = Dislike Extremely, 9 = Like Extremely) for Boiled Shrimp (n = 52)