AN ABSTRACT OF THE THESIS OF

Silvana Dinaintang Harikedua for the degree of Master of Science in Food Science and Technology presented on August 25, 2016

Title: The Application of Brine Injection Technology to Improve Quality of Pacific whiting (Merluccius productus) Fillets

Abstract approved:

__________________________________________________________________

Christina A. Mireles DeWitt

Existing research reports the importance of brine injection technology in enhancing the quality of muscle products such as poultry, beef, and pork. In fish, the utilization of brine injection technology is well-known for delivering marinade ingredients for salted or smoked fish production. Until now, there has been limited published scientific evidence on the incorporation of brine ingredients containing protease inhibitors to improve the quality of fresh or frozen fish fillets. Thus, the studies presented in this thesis focused on improving the quality of Pacific whiting (Merluccius productus) fillets through brine injection technology. Pacific whiting fillets are not considered desirable in the U.S. market because their meat is easily softened during slow cooking.

The first study explored whether multi-needle injection technology is applicable to the improvement of Pacific whiting raw fillet quality. Color, texture, rancidity, moisture and protein were determined to understand how brine treatments
affected appearance, oxidative stability and composition of the fillets. The impact of brine ingredients on myofibril protein was evaluated by gel electrophoresis. All brines were formulated with a base solution (3% sodium tripolyphosphate and 3% salt). Treatments consisted of base brine (B), base brine + 3% egg white (B_{EW}), and base brine + 0.1% xanthan gum + 3% dried potato extract (B_{PE}). Ten fillets were injected per treatment and a 10% brine uptake was targeted for all fillets. Actual brine incorporation was 12.35 ± 2.28%. The lightness (L*) value of raw fillets indicated that B_{EW} and B_{PE} were darker than C and B (P < 0.05). Breaking strength demonstrated higher variability in non-injected samples than in injected samples. Enzyme assays showed no differences between all treatments when it was tested using buffer at pH 5.5 (P = 0.06). SDS-PAGE analysis showed minor changes in myofibril proteins between injected fillets and non-injected fillets.

The second study aimed to evaluate the brine effectiveness in inhibiting protease activity in the fillet through heating. Control samples included non-injected (NI) fillets and fillets injected with water (W) only. For all other treatments, brines contained 3% (w/w) salt and sodium tripolyphosphate as a base brine (B). Additional, brine treatments with egg white were a combination of the base brine (B) with either 1, 2 or 3% egg white (B_{1EW}, B_{2EW}, B_{3EW}). For potato extract, 0.1% xanthan gum was added to the base brine to aid in its suspension (B_{XG}). Potato extract was added to B_{XG} at similar levels as egg white (B_{1PE}, B_{2PE}, B_{3PE}). Previously frozen butterfly fillets were tempered to 2.0 ± 1.0 °C, injected with a selected treatment, vacuum packaged and
immediately cooked in either one of two different heating conditions. Vacuum packed fillets were either submerged in water at 90 °C for 20 min to achieve a final internal temperature 62.8 °C (145 °F) or submerged in a 60 °C water bath for 30 min and then immediately transferred to a 90 °C water bath for 20 minutes. The pH, moisture, crude protein, total extractable protein, total non-extractable protein, texture profile analysis, and SDS-PAGE electrophoretic pattern in cooked fillets were determined. For TPA measurements, an uninjected cod sample was used as a reference sample. Compositional analysis determined the cooked NI fillet was less moist than all injected samples ($P < 0.05$), suggesting TPA attributes would be higher than injected samples, which was confirmed. Protein patterns from SDS-PAGE electrophoresis indicated that the myosin bands were slightly diminished when fillets were heated at 60 °C prior to cooking at 90 °C for all treatments, except in fillets containing egg white or potato extract ingredients, suggesting these treatments provided some protection to myofibrillar structure. Fillets cooked using the challenge heat treatment were significantly ($P < 0.05$) softer than those cooked using the *sous vide* for all TPA attributes.

Additionally, future research considerations that would benefit the applicability of the injection process to fillet quality improvement should include determining the appropriate fish size to target for the injection process, the shelf life of injected fillets during frozen or refrigerated storage, and consumer acceptance of the injected fillets.
The Application of Brine Injection Technology to Improve Quality of Pacific Whiting (Merluccius productus) Fillets

by
Silvana Dinaintang Harikedua

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

Silvana Dinaintang Harikedua, Author
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### Introduction

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1 GENERAL INTRODUCTION

Pacific whiting (Merluccius productus), also known as Pacific hake, is one of the Pacific Northwest’s the most abundant species. This species is found in the territorial waters off Pacific coast and in the inland waters of the United States and Canada (Helser and others 2006; Zhou and Li-Chan 2009; Nelson and others 1985). In 2014, the total catch of this species in the United States was 264,137 metric tons (Taylor and others 2015). Despite the fact that this species is abundantly available, its susceptibility to be infected by myxosporean parasites (Kudoa spp.) results in high levels of endogenous protease activity (Patashnik and others 1982; Nelson and others 1985; Kudo and others 1987; An and others 1994; Zhou and Li-Chan 2009). This quality issue significantly impacts its commercial value and limits the prospective market for Pacific whiting.

Pacific whiting were considered underutilized fish prior to 1990. Since 1991, Pacific whiting mince has been utilized for surimi production. As the demand for raw material for the surimi industry increased, on-shore whiting fisheries started altering their post-catch handling practices to meet the surimi seafood processor requirements. Peters and others (1995) showed that processing Pacific whiting into surimi within a 24h period after catch resulted in higher quality of surimi product. Reductions in time from harvest to processing helped to mitigate the impact of protease activity on quality of Pacific whiting. It also subsequently allowed for the utilization of Pacific whiting for frozen H/G (headed and gutted) products, which are exported to European countries. Nevertheless, the effort has still not been sufficient
enough to make whiting more acceptable to U. S. consumers, particularly in fillet form. Therefore, further innovation is needed.

The technology that might facilitate adding value to Pacific whiting fillets is marinade injection. Over the past few decades, the use of a multi-needle injectors for delivery of various ingredients to improve the quality of fish products has been explored by many seafood scientists. The injector has been used as a means to improve the quality of salted cod with sodium bi-carbonate (Asli and Møkøre 2012), to rehydrate a salt-cured cod product (Bjorkevoll and others 2004), to increase the yields of a salted cod product (Thorarinsdottir and others 2010), and to improve the appearance and reduce the curing flavor of heavy salted cod (Jonsdottir and others 2011). A considerable amount of research has also demonstrated that multi-needle injection can be utilized to effectively distribute salt throughout the fish during the processing of smoked salmon (Birkeland and others 2003, 2007, 2008; Rora and others 2004; Akse and others 2008; Almli and Hersleth 2012), cod (Akse and others 2008) and rainbow trout (Jittinandana and others 2002). However, only a few studies have assessed the use of multi-needle injection to incorporate ingredients into chilled or frozen fish. Thorarinsdottir and others (2004) examined the effects of added salt, phosphates, and proteins on the physicochemical characteristics of frozen cod fillets and Kin and others (2010) evaluated use of various phosphate to physicochemical quality catfish fillets.

Quality of fish products can be affected by extrinsic and intrinsic factors including species, the catching method, handling and processing, post-mortem
condition, and stage of enzymatic or microbial autolysis. The deterioration process in fish will result in changes in color, odor, and texture. As stated earlier, one main cause of Pacific whiting quality degradation is infection by Myxosporean parasites, namely *Kudoa paniformis* and *Kudoa thyrsites* (Kabata and Whitaker 1985). Higher amounts of cathepsin L-like protease activity were found in Pacific whiting tissue infected by *K. paniformis* (An and others 1994; Seymour and others 1994). The enzyme cathepsin L can disrupt fish muscle protein during post-mortem storage, which affects the subsequent processing of whiting (An and others 1994; Seymour and others 1994; Samanarayaka and others 2007). In the last two decades, a considerable amount of literature has been published on the use of protease inhibitors to inhibit protease activity in various fish species. Many of these studies were conducted to incorporate food grade ingredients into mince products for surimi production. Nonetheless, to the best of the author’s knowledge, there are only two studies that have investigated the incorporation of protease inhibitors through injection. Both studies were conducted using Arrowtooth flounder. In 1990, Babbit reported the potency of 6 groups of ingredients used to inhibit muscle softening of flounder. However, this author did not explain the experimental design in much detail. Therefore, many statements were questionable. On the other hand, Kang and Lanier (2005) provided evidence of the effectiveness of recombinant cystatin produced by *Escherichia coli* as a protease inhibitor in flounder fillets. So far, however, their method has only shown the benefit of injecting protease inhibitors in fish myofibrils. This indicates a need to understand the effect of blended ingredients on the ability to improve fish quality. The term
"blended ingredients" herein is defined in this paper as a mixture of brine ingredients and protease inhibitor. Salt and phosphate are common marinade ingredients in the injection process of meat and poultry (Wynveen and others 2001, Claus and others 2010; Lowder and others 2011, Roldan and others 2014). Literature on the application of salt and phosphate in seafood is extremely limited when compared to land animals, however a few researchers have reported its effects in cod and catfish (Thorarinsdottir and others 2004; Kin and others 2010). Protease inhibitors chosen for investigation where egg white and potato extract because they have been utilized to control protease activity in surimi seafood made from Pacific whiting (Morrissey and others 1993; Weerasinghe and others 1996).

Based on the above information, there are some issues that still need to be addressed. First, Pacific whiting fillets have limited presence in the U.S market due to the inconsistent texture quality. Second, the scientific literature on the use of brine injection to improve fish quality texture through incorporation of protease-inhibiting ingredients is extremely limited, though the few studies (Babbit 1990, Kang and Lanier 2005) that do exist suggest the approach could be feasible. Third, there are no scientific publications on using multi-needle injection to improve texture of Pacific whiting. Therefore, our research goal is to evaluate the effect of protease inhibitor concentration incorporated through multi-needle injection on the physicochemical parameters of raw and cooked Pacific whiting fillets. Data provided in the thesis is based on two studies. The first study focused on raw fillets while the other study focused on cooked fillets. Additionally, this overall study is intended to provide
evidence that injection technology can be utilized to improve the cooked texture of Pacific whiting fillets through incorporation of a brine containing protease inhibiting ingredients for optimal utilization and better commercialization of Pacific whiting.
References


2 LITERATURE REVIEW

“ADDING VALUE TO PACIFIC WHITING BY USING MARINADE INJECTION TECHNOLOGY”

This chapter of the thesis will be focused on understanding the aspects of injection technology that add value to Pacific whiting. There are six sections in this chapter. Each section will refer to previous studies and the essential facts that should be emphasized for a fish injection project.

2.1 Pacific whiting

2.1.1 Biology of Pacific whiting

Pacific whiting, also known as Pacific hake, are identified as a semi-pelagic schooling species (Taylor and others 2015). This paper will use the term Pacific whiting since Pacific hake is considered as an old term. Pacific whiting are abundantly available and broadly scattered off the coasts of California, Oregon, Washington and British Columbia with a range from 25° N. to 55° N. latitude (Stauffer 1985; Taylor and others 2015). The migratory pattern of this fish has been fully documented by many marine biologists. Data regarding their migratory patterns was originally generated from Russian (previously Soviet Union) fishing information. The migratory patterns of Pacific whiting are primarily dictated by seasonal changes (i.e. Winter to Spring, etc). Pacific whiting spawn during winter in California and Mexico. The juveniles can be found from southern to central California, but reside primarily off the central California coast. After spawning, adult Pacific whiting move northward to feed during
the summer from northern California to Queen Charlotte Islands in British Columbia, Canada (Bailey and others 1982). Large schools of spawned fish first appear off the coast of San Francisco. These fish then migrate north. Typically, by the third week of April, adult Pacific whiting can be found off the coast of both Oregon and Washington. By late May, Pacific whiting can be captured in areas surrounding Vancouver Island. It has been suggested that larger fish can migrate faster up to the north during spring and summer (Bailey and others 1982).

From the point of view of inshore-offshore distribution within the water column, both season and time of day are factors that influence Pacific whiting distribution. During spring, Pacific whiting caught in Oregon, Washington, and California are found over the continental slope but not over the shelf (Ermakov 1974). In the beginning of June, Pacific whiting schools will continue to move over the shelf to depths of 90 m or less. By July and early August the schools are scattered, some of them will continue to reside over the continental slope until the schools begin their spawning migration to the west of the shore. Then, by early September most of the Pacific whiting begin to move to the south. Most of the schools migrate to the south by fall. Additionally, Ermakov (1974) depicted their vertical distribution as diurnal migrations. Diurnal by definition is the time of day from morning to noon where fish are most active. It should be noted that the diurnal migrations of Pacific whiting occur concomitantly with the activity of euphausiids, their primary food (Methot and Dorn 1995). The schools of whiting can be seen massively concentrated between 100 and 250 m. Whiting of similar size are commonly seen moving gradually in random
directions during the daytime. As common to diurnal species, after sunset and especially at night, they will have different resting places. Pacific whiting can be seen rising toward the surface and can be found to a depth of about 20 m from the surface from 10:00 pm to 3:00 am. However, when the sun comes up a school of fish will rapidly swim to the bottom of the ocean. The fishing activity of Pacific whiting occurs every year during April to October with mid-water trawls. Methot and Dorn (1995) stated that most of the fishing activity occurred over a depth of 100–500 m.

In the recent stock assessment report of Pacific whiting in the U. S. and Canadian waters, Grandin and others (2016) reported that the stock with the largest population is found between northern California to northern British Columbia (coastal stocks). Meanwhile, smaller populations can be found in the major inlets of the Northeast Pacific Ocean, including the Strait of Georgia, Puget Sound, and the Gulf of California (inshore stock). In their assessment, they only include the coastal stock and exclude the inshore stock. The term south stock will be referred to the sub population found off the northern Californian coast. Meanwhile, the north stock will be attributed for the subpopulation found around northern British Columbia, Canada. The various patterns of weather from variations in the equatorial Pacific oceans temperature also affect the migration pattern and distribution of whiting populations. During warm ocean conditions (El Nino) there is a sharpened phenomenon of northward transport so that a greater percentage of the stock is observed in Canadian waters (Dorn 1995; Agostini and others 2006; Taylor and others 2015). On the other hand, in colder water conditions (La Nina) there is a reverse phenomenon where the
south stock’s distribution is larger than the north stock’s (Taylor and others 2015). Thereby, a lesser percentage of population is seen in the Canadian waters.

From a commercial point of view, fish with higher yield will be the best raw material for further processing. Therefore, adult whiting are supposed to be the target of the commercial fishing industry. The size of Pacific whiting can be affected by age, sex, and genetic differences. Methot and Dorn (1995) stated that Pacific whiting can obtain a size of about 26 cm during their second summer (age +1) and 34 cm during their third summer (age+2). Additionally, they reported that Pacific whiting make a meaningful contribution to the U.S. fisheries by age 3 when their average size is 39 cm. For Pacific whiting, the female is larger and heavier than their male counterpart. Dark (1975) found that the average maximum weight and length for male and female are 1 kg and 56 cm and 1.33 kg and 61 cm, respectively. He also stated that at a similar age, adult Pacific whiting males were about 1 inch shorter than their female counterpart. According to Dorn (1991), the sex ratio of Pacific whiting is near 50:50 in U.S. waters during summer. However, since larger fish can migrate faster and longer northward, Canadian waters are dominated by the larger females in the summer (Smith and others 1990). McFarlane and Beamish (1985) found that the female size at 50% maturity (age 3 years) was 37 cm in the inshore stock. In addition, the mature female from the coastal stock has a length of about 40 cm (MacGregor 1971). Size differences in the stocks at similar maturity stages suggests genetic differences amongst the stock, which was first suggested by Vrooman and Paloma (1977). Recent
evidence confirms genetic differences indeed exist between the south and the north stocks (Iwamoto and others 2004; King and others 2012).

2.1.2 Quality Issue of Pacific whiting

Over the past three decades, a number of studies have documented numerous factors that contribute to the deterioration process of Pacific whiting. Two predominant factors are the effect of exogenous (via a Myxosporean parasite) and endogenous sources of proteolytic enzymes. Those two factors synergistically and detrimentally influence the final texture of cooked Pacific whiting fillets and concomitantly shorten the refrigerated shelf life of uncooked fillets.

The researchers whom pioneered studies into determining the source of muscle degradation of infected Pacific whiting are Patashnik and others (1982) and Tsuyuki and others (1982). They stated that the muscle deterioration of infected Pacific whiting was due to an enzyme induced proteolysis. Similarly, Kabata and Whitaker (1985) claimed that Kudoa thyrsitis and Kudoa paniformis were the predominant parasites found in Pacific whiting flesh. They also reported that K. thyrsitis is less damaging to Pacific whiting flesh than K. paniformis (Kabata and Whitaker 1985).

Up until now, to the best of the author knowledge, the life cycle of neither Kudoa thyrsitis nor Kudoa paniformis have not been described in the literature. However, we might postulate how these Myxosporean could infect Pacific whiting by observing the life cycle of the fish itself. Two possible sources of parasite contamination are food and environment. Diamant (1997) successfully demonstrated
the fish-to-fish transmission of marine Myxosporean by using *Myxidium leei* and sea bream (*Sparus aurata* L) as a model. There are three types of parasite route and source: 1) when healthy fish are cultured with diseased fish; 2) when healthy fish is exposed to a water source from a disease fish tank; and 3) when healthy fish digest excretions from infected fish (Diamant 1997). They also emphasized that their model can be used for the development of other marine Myxosporeans life cycles pattern. Therefore, it could be hypothesized that direct fish-to-fish transmission of Myxosporea might occur in Pacific whiting, as well. However, further study should be conducted to ascertain this assumption.

In 1987, Kudo and co-workers extensively measured the pseudocyst intensity in Pacific whiting fillets and correlated pseudocyst intensity to the ultimate cooked texture of fillets. Their data analysis showed that 1) cooked texture quality were more intently associated with white pseudocysts than black pseudocysts; 2) Pacific whiting caught from northern fishing areas had lower white pseudocyst counts than from southern fishing areas. Therefore, fish caught from southern fishing areas showed more soft abnormal texture; 3) there was no evidence that sex of the fish impacted the sensory quality of Pacific whiting.

Furthermore, Kudo and others (1987) highlighted a few facts which are also confirmed and support the findings of Kabata and Whitaker (1985). As previously mentioned, Kabata and Whitaker (1985) stated that *K. paniformis* is more damaging to flesh than *K. thyrsitis*. Likewise, Kudo and others (1987) reported that *K. paniformis* and mixed infections correlated well with sensory texture while *K. thyrsitis* infections
poorly correlated. Both studies showed that anterior areas behind the head are most likely infected by *Kudoa* while the posterior portions are less likely infected. Kabata and Whitaker (1985) concluded that the larger the fish, the greater the proportion of abnormal textures. In the same vein, Kudo and others (1987) reported a weak positive correlation exist between fish length and textural quality of cooked fillets.

In the 90’s, numerous studies were conducted to identify the main proteolytic enzyme that was responsible for the muscular degradation in Pacific whiting, either in fillets or mince. An and others (1994) reported that myosin degradation during conventional heating of both fillets and surimi gels was caused by Cathepsin L. In raw fillets, An and others (1994) showed that cathepsin B was the predominantly active protease. In addition, it was found that the washing process in surimi production can removed cathepsin B and H, but not cathepsin L (An and others 1994). The proteolytic enzyme cathepsin L mainly hydrolyzes myosin heavy chain (MHC) as a primary substrate and to a lesser degree can hydrolyze actin and myosin light chain with longer (2 h) incubation. Also, the purified cathepsin L could hydrolyze native and heat-denatured collagen (An and others 1994). Furthermore, Seymour and others (1994) purified and characterized the protease from the sarcoplasmic fluid of Pacific whiting (*Merluccius productus*) with ion exchange chromatography using DEAE Bio-Gel A gel (an anion exchanger). The result showed that the purified protease from the sarcoplasmic fluid is a cathepsin L. It has a molecular weight 28800 Dalton. The enzyme showed highest activity toward Z-Phe-Arg-NMec, a specific substrate for
lysosomal cysteine protease, and it can be activated by thiol-activating agents such as dithiotreitol (DTT) and EDTA.

The recent studies by Samaranayaka and others (2007) and Zhou and Li-Chan (2009) highlighted a relationship between Kudoa spore counts, proteolytic activity and mince cooked texture quality. Samaranayaka and others (2007) provided evidence that fish captured near Vancouver Island, Canada were primarily infected by *K. paniformis*. The highest activity of proteolytic enzyme was observed within pH 5.25–5.50 and at 52–55°C. They concluded that significant (*P* < 0.05) correlations were found among fish mince, *K. paniformis* spore counts, endogenous proteolytic activity, cooked texture when measured as maximum compression force (g), and the change in free amino groups during autolysis. Changes in texture during cooking was significantly and negatively correlated with the proteolytic activity of fish mince (*r* = 0.620, *P* = 0.031), and with the free amino content during autolysis (*r* = 0.874, *P* = 0.005) over the whole range of *K. paniformis* spore counts study.

In contrast, Zhou and Li-Chan (2009) showed that soft texture (maximum force <150 g) was observed in fish with $10^4$–$10^6$ *Kudoa thyrsites* spores g$^{-1}$ mince as opposed to $10^5$–$10^8$ *K. paniformis* spores g$^{-1}$ mince. Their result suggested that *K. thyrsites* may have a greater impact than *K. paniformis* on Pacific whiting quality. They demonstrated that texture was significantly (*P* < 0.05) negatively correlated with spore counts as well as protease activity. Also, heating treatment, freeze-thaw cycle, and storage period can affect the texture quality of Pacific whiting. Softer texture was observed on the fish which were pre-incubated for 15 min at 52 °C. Then, it re-cooked
at temperature 90 °C for 5 min until fish reach internal temperature at 73 °C. However, fish storage for 6 mo or longer at -25 °C or fish which were subjected to 6 freeze/thaw cycles did not show soft texture quality. This phenomenon can be caused by protein denaturation and aggregation during an opposing toughening effect attributed to protein denaturation and aggregation during extended or destructive frozen storage conditions.

In an attempt to mitigate the existence of parasites in Pacific whiting on the production line, Kudo and others (1987) suggested Pacific whiting fillets be sorted by visual culling. Their results demonstrated that it is possible to take out the white and/or black pseudocyst from the Pacific whiting fillets, however, they acknowledge that it was time-consuming to examine the white pseudocysts since it is hard to differentiate them form Pacific whiting flesh. It is indicated that “visual culling” is an unproductive process that could be applied to the processing line. Additionally, with respect to the public health consequences of parasite infection of Pacific whiting, myxosporean parasites have never been reported to cause illness in humans (Nagahisa and others 1983).

Collectively, the above mentioned studies described outline the critical role of parasites and proteolytic enzymes in causing post-mortem “myoliquefaction”, a condition where fish flesh became softer and cannot be sold commercially. Therefore, a collaborative study from fisheries and seafood scientist along with the industry practitioners is still needed to overcome the quality issue of Pacific whiting.
2.2 Fish Muscle Protein Properties

By definition, locomotion is movement through the environment which is dictated by the morphology and physiology of the animal. There are three types of animal locomotion: fly, swim and walk. Each locomotion type influences the quality of the animal that is commonly used for human consumption. For instance, two main animal protein sources for human consumption, land (cow, pig, lamb, chicken or turkey) and aquatic animals (fish, crustacean, or shellfish) have different muscular structure. Since aquatic animals live in water, they lack of supporting locomotion that land animals have. This differing characteristic depends on the muscle protein composition. For the purpose of clarification, it is necessary to differentiate “muscle” from “meat”. Hultin (1984) suggested that “muscle” indicates the organ in the living animal which is responsible for locomotion, while “meat” refers to condition of muscle after animal dies and goes through several biochemical changes. Muscle and meat will be used in this paper by these definitions.

There are 3 types of muscles in animals: smooth (involuntary) muscle such as stomach walls, cardiac muscle (heart muscle), and striated muscle (voluntary). In general, striated muscle is the muscle type that has been most commonly consumed by humans (Venugopal and Shahidi 1996). There are two types of striated muscle, dark and white. The striated muscle of fish is different from terrestrial animals. Higher amounts of dark muscle can be found in groups of fish swimming near the surface of the water (pelagic fish), while little or no dark muscle is found in fish that live at the bottom of the ocean (demersal fish). Dark muscle uses aerobic metabolism and is
utilized for routine activities such as foraging, migration, or continuous swimming motions. White muscle uses anaerobic metabolic pathways for rapid energy bursts, especially for escaping from predators (Johnston 1999).

The arrangement of muscle fiber in fish also differs than terrestrial animals. Fish muscle fiber has a “W”-shaped segments, called myotomes (Foegeding and Lanier 1996). Each myotome has one forward and two backward curves. Myotomes are a collection of muscle fiber parallel to the long axis of the fish (Venugopal and Shahidi 1996). Thin connective tissue membranes, called myocommata, divided fish muscles into segments (Venugopal and Shahidi 1996). The individual muscle fiber (myofibril) is surrounded by sarcolemma, which is a membrane cell that contains thin collagenous fibrils. The collagenous fibrils join myocommata at the myotome-myocommata junction (Venugopal and Shahidi 1996). Sarcomere is a small segment of the myofibril, consisted of thick and thin filaments (Hultin 1984). The structural unit of sarcomere also has alternate arrangements of anisotropic (A) and isotropic (I) bands bordered by Z-lines (Venugopal and Shahidi 1996). A band or birefringent band has a dark structure when viewed by polarized light while I-band reveal lighter bands. The Z-lines is a dark line at the center of the I-band, while the H-zone is a light area at the center of each A-band, and the M-line is located at the center of H-zone (Hultin 1984). During the muscle contraction, the thick filaments and thin filaments overlap and slide over one another through the acto-myosin sliding mechanism (Venugopal and Shahidi 1996).
There is a consensus among muscle scientists that muscle protein can be categorized into three parts based on their solubility in the aqueous solution, i.e. myofibrillar, sarcoplasmic, and stroma proteins. Myofibrillar protein cannot be solubilized by solutions of low ionic strength, but can be with relatively high ionic strength. Sarcoplasmic protein, or soluble protein, can be separated from the muscle in water or a dilute salt solution. Stroma protein, or insoluble protein, are not soluble in high or low ionic strength salt solutions (Hultin 1984; Venugopal and Shahidi 1996). This section reviews the structure and properties of each muscle protein. How the muscle protein changes during storage and processing will be discussed in a later section (see section 2.3).

2.2.1. Myofibrillar Protein

A great deal of research on muscle food has focused on the functional properties of myofibrillar protein and the changes during post-mortem storage and processing. Myofibrillar protein constitutes about 55–60% of the total muscle protein (Asghar and others 1985; Skaara and Regenstein 1990). Hultin (1984) claimed that the myofibrillar protein is directly associated with muscle contraction and the conversion of chemical energy to mechanical energy. Based on physiological function in the muscle, the myofibrillar can then be categorized into two subgroups: 1) contractile proteins or 2) regulatory proteins (Skaara and Regenstein 1990).

The contractile protein can be found in the sarcomere, particularly in the thick and thin filaments of the muscle fiber (Hultin 1990; Venugopal and Shahidi 1996) and
it consists of myosin and actin (Suzuki 1981). Myosin and actin are directly involved in
the muscle contraction-relaxation cycle (Skaara and Regenstein 1990). Myosin is
located in thick filaments while actin is in thin filaments. The total amount of myosin
in myofibrillar protein has been reported differently by few researchers. Suzuki (1981)
indicated that myosin comprised 50-58% of the myofibrillar proteins while Yates and
others (1983) suggested that it constitutes approximately 45%. Meanwhile, actin is
known to make up 15–30% of total myofibril protein (Hultin 1984).

The myosin molecule is comprised of two identical polypeptide chains which
are intertwined to each other with molecular weight around 470 kD (Hultin 1984). The
molecule consists of a globular head and a tail section. The globular head,
approximately 19 nm long, contains two pairs of light chains connected to one end of
the tail portion. The light chain has a molecular weight range from 16 to 28 kD (Hultin
1984; Venugopal and Shahidi 1996). The tail portion contains heavy chains and
resembles a thread (2 nm × 160 nm). The two heavy chain with a molecular weight of
200 and 240 kD are connected non-covalently with the two pairs of light chains. Thus,
each myosin molecule is composed of four light chains (Bailey 1982; Hultin 1984;
Venugopal and Shahidi 1996). The myosin molecules in muscle govern the ATPase
activity, which is coordinated by the presence of Ca$^{2+}$ and Mg$^{2+}$ (Suzuki 1981).
Although there are slight differences in amino acids, the myosin of all vertebrates is
similar (Skaara and Regenstein 1990). However, fish myosin is more unstable and
sensitive to chemical changes such as denaturation, coagulation, and degradation
than mammalian myosin (Suzuki 1981; Venugopal and Shahidi 1996).
The regulatory proteins of myofibril include tropomyosin, troponin, and actinin (Suzuki 1981). These proteins are indirectly involved in the contraction-relaxation cycle of muscle and they participate mostly in cross-bridge formation (Maruyama and others 1970 as cited by Skaara and Regenstein 1990). Obinata and others (1981) divided these proteins into two subgroups, the major and minor regulatory proteins. The major regulatory proteins consist of troponin and tropomyosin, while the minor regulatory proteins consist of small protein such as α-, β-, γ-actinin, C-, F-, H-, I-, M-, and X-protein, etc. (Skaara and Regenstein 1990). These minor regulatory proteins can be found in the ultrastructure filaments of M-line, A-band, Z-disk, and the I-band. Together, tropomyosin and troponin account for 10% of the muscle proteins of which their main regulatory function is to transmit the Ca$^{2+}$ ion to the actomyosin cycle (Skaara and Regenstein 1990). However, the content of these proteins in meat is very low and does not impact food processing.

2.2.2. Sarcoplasmic Protein

Almost every paper that has been written on the changes of fish protein during postmortem storage or processing includes a section relating to sarcoplasmic proteins in fish. However, a comprehensive review on this topic has not been presented. Previous studies showed that during post-mortem storage, the sarcoplasmic protein are much more stable than the myofibrillar proteins (Nakagawa and others 1996; Verrez-Bagnis and others 2002; Delbarre-Ladrat and others 2003; Munasinghe and others 2005). Sarcoplasmic protein consists of low molecular weight components such as albumins, myoglobin, hemoglobin, and the enzymes of the glycolytic pathway,
lysosomes, various proteases and peptides (Haard and others 1994). Pelagic fish usually have higher quantities of sarcoplasmic proteins than demersal fish, with sarcoplasmic proteins comprising 20–25% from the total fish muscle proteins (Haard and others 1994).

Two types of sarcoplasmic protein that play particularly important roles on fish quality during post-mortem storage are myoglobin (Mb) and hemoglobin (Hb), which are the globular proteins found in the muscle and blood, respectively. Both proteins have iron in their prosthetic group. The concentration of iron ranges from 0.3 to 0.35% (Venugopal and Shahidi 1996). The iron atom in the heme group can be in the reduced (ferrous/Fe$^{2+}$) or oxidized (ferric/Fe$^{3+}$) form (Suman and Joseph 2013). Hemoglobin is composed of four chains of two different types of polypeptides (tetrameric heme protein), while myoglobin contains only one polypeptide chain (monomeric heme protein) (Venugopal and Shahidi 1996; Thiansilakul and others 2012). Each polypeptide chain has 140 to 160 amino acid residues (Venugopal and Shahidi 1996). The amounts of Mb and Hb vary by muscle type and species (Chaijan and others 2004; Masood and Benjakul 2011). For example, the concentration of Mb and Hb in dark muscle of chub mackerel (Scomber japonicas) are 390 mg and 580 mg per 100 g, respectively, while the concentration of Mb and Hb together in white muscles is 10 mg per 100 g (Sikorski 1990). Additionally, Thiansilakul and others (2012) also reported that the dark muscle of bighead carp (Hypophtalmichthys nobilis) contains 61% Mb and 39% Hb of the total heme protein.
Livingston and Brown (1981) pointed out that hemoglobin can simply vanish during handling and storing of fish, while myoglobin is retained by the muscle intracellular structure. This explains why myoglobin has been the most discussed and studied pigment in seafood and other muscle food products over the past two decades. The amino acid composition of fish myoglobin protein is different than that of terrestrial animals. The primary difference is that fish myoglobin has a cysteine residue which is not present in mammalian species (Venugopal and Shahidi 1996). Also, the number of amino acid residues found in teleost species (~147) is less than the number found in their mammalian counterparts (~154) (Hasan and others 2012). That is why the myoglobin in meat from land animals (i.e. beef and pork) differ from fish. However, it should be noted that the concentration of myoglobin will depend on species, breed, sex and age, training and nature of nutrition, muscular activity, oxygen availability, blood circulation, muscle type, and handling and processing (Livingston and Brown 1981; Postnikova and others 1999).

2.2.3. **Stroma Protein**

It has been suggested that levels of stroma protein, collagen and elastin in fish exist in smaller amounts than in land animals. Several studies in different fish species showed the average concentration of stroma protein is 3% in haddock (Reay and Kuchel 1936), 3% in cod (Dryer 1949), and 6–21% in farm raised giant catfish (Pangasianodon gigas) (Chaijan and others 2010). Moreover, data from several sources have indicated that in anterior body parts of the fish, the amount of collagen
varies with species, ranging from 1.6–12.4% of the total muscle protein (Sato and others 1986; Hatae and others 1986 as cited by Kimura and others 1988).

It has been suggested that collagen is closely related to the firmness of raw fish meat; the higher the collagen, the firmer the raw meat. Collagen in fish provides a weak network of connective tissue as compared to the collagen in their terrestrial counterparts. However, the smaller amount of collagen in fish makes fish meat flakier and much tender than the meat of warm-blooded animals when cooked. To date, 27 different types of collagen have been identified. However, type I collagen is the most widely occurring collagen in connective tissue (Gomez-Guillen and others 2011). Collagen molecules are made up by three $\alpha$-chains twisted together into a collagen triple helix, which is primarily stabilized by intra and inter chain hydrogen bonding (Asghar and Henrikson 1982 as cited by Gomez-Guillen and others 2011). It was concluded that collagen mostly consists of a repeating Gly-X-Y-sequence, where X is mostly proline and Y is mostly hydroxyproline.

An earlier study of fish collagen was conducted by Kimura and others (1988). They characterize the muscle type I collagen of five teleosts (eel, mackerel, saury, carp and chum salmon) with respect to its thermal stability and subunit composition. Additionally, the stability of muscle collagen can be explained by the degree of proline hydroxylation in the muscle collagen (Berg and Prockop 1973 as cited by Kimura and others 1988).
2.3 Functional Alterations to Muscle Protein

Handling and processing muscle food significantly changes muscle protein. In this section we will review the various effects of storage temperature and heating treatments on muscle contractile protein (myofibrillar). There are many methods of analysis that can be used for measuring muscle protein changes. In this section we briefly discuss 5 of them: ATPase, protein hydrophobicity, total sulfhydryl, total carbonyl content, and electrophoresis. Understanding of the principle of each method helps clarify the changes in muscle protein during storage or thermal processing.

Ochiai and Chow (2000) examined the use of ATPase to measure product quality. ATPase activity is regulated within the muscle by Ca\(^{2+}\). The structural component of the muscle, myosin, plays an important role in ATPase activity. Ca\(^{2+}\)-ATPase activity is a good parameter to evaluate the quality or the degree of deterioration of muscle protein in food. Meanwhile, Mg\(^{2+}\)-ATPase activity of acto-myosin (or actin-activated Mg\(^{2+}\)-ATPase of myosin) is a direct measurement of muscle contraction. In the presence of actin, myosin is activated by both Mg\(^{2+}\) and Ca\(^{2+}\). However, in the absence of actin, myosin is activated by Ca\(^{2+}\), but inhibited by Mg\(^{2+}\). K\(^{+}\) ions or EDTA can be used to pull out the divalent cations Mg\(^{2+}\) and Ca\(^{2+}\) from myosin. Therefore, in the presence of K\(^{+}\) or EDTA, ATPase is also activated.

Mackie (1993) asserted that the measurement of protein hydrophobicity can be recorded by determining the fluorescence of compounds such as cis-parinaric acid and sodium-8-analino-1-napthalene sulfate. The increased of hydrophobic in the interior of the protein molecules indicated that protein is undergoing denaturation.
In this analysis the increase in fluorescence is an indication of a hydrophobic environment.

Furthermore, protein oxidation can be measured by the formation of carbonyl groups and sulphydryl groups (Stadtman 1990). The –SH groups (sulfhydryl) are oxidized to –S–S– bonds (disulfide), which may generate the formation of either intra- or inter protein disulfide crosslinking or the formation of mixed disulfide conjugates with glutathione, cysteine or other low molecular weight (Mackie 1993; Xiong 2000). To a limited degree, –S–S– bond formation occurs during protein denaturation. Thus, the decrease in total sulfhydryl means more stages of protein oxidation have taken place (Stadtman 1990). Also, protein free radicals can react with oxygen radical species to form peroxy radicals, subsequently forming protein hydroperoxides that then break apart into carbonyls (Decker 1993; Xiong 2000). Therefore, the increase in total carbonyl content implies further protein oxidation.

Electrophoresis can be utilized to measure protein modification. One of the most common methods is using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). SDS solutions disrupt protein non-covalent bonds. Solubility in SDS is an effective indicator of the formation of covalent crosslinks (Mackie 1993). Also, SDS-PAGE patterns help to assess the muscle protein changes caused by proteases (An and others 1994), the incorporation of protease ingredients to prevent proteolytic activity (Weerasinghe and others 1996), and the purification of the enzyme from muscle protein (Seymour and others 1994).
2.3.1 Storage Temperature

Three common household and commercial storage conditions for seafood products are iced, refrigerated and frozen. Many enzymes degrade nutritional compounds such as protein and lipid, the two major compounds in seafood besides water, during post-mortem storage. The action of endogenous enzymes, either from the fish or the fish microbiota can be detrimental to seafood products since they affect texture, color, and flavor (odor and taste) and concomitantly shorten the shelf life. Haard (2000) listed various enzymes that affect seafood quality and divided them into sections based on the quality index. For instance, the sections include enzymes that affect nutritional value (glunolactone oxidase and thiaminase), color (polyphenol oxidase, enzymes involved with carotenoid synthesis), texture (amylose-like enzyme, TMAO demethylase, and proteinases), and flavor (various antioxidant enzymes, inosine hydrolase and inosine phosphorylase, various enzymes involved with pyruvate metabolism, and urease). Such enzymes mentioned above cannot be fully inhibited at storage temperature (iced, refrigerate or frozen storage). That is why suitable storage temperature are critical in maintaining quality of seafood products. This section will review how specific storage conditions change the functionality of fish muscle protein.

2.3.1.1 Iced

The most common and convenient way to distribute and store seafood is by using ice. There are many types of ice that can be used such as flake ice, nano (slurry) ice, packed ice, and dry ice. The majority of literature has exclusively studied the effect
iced storage on the shelf life of various fishes, including Atlantic salmon (*Salmo salar*) (Hultmann and Rustad 2004), arrowtooth flounder (*Atheresthes stomias*) (Keys 2014), cod (*Gadus morhua*) (Bonilla and others 2007), lizardfish (*Saurida tumbil*) (Benjakul and others 2003), Pacific whiting (*Merluccius productus*) (Benjakul and others 1997), hake (*Merluccius hubbsi* Marini) (Roura and Crupkin 1995), rainbow trout (*Oncorhynchus mykiss*) (Ozogul and Ozogul 2004; Rezaei and Hosseine 2008), sardine (*Sardinella gibbsa*) (Chaijan and others 2005), and skipjack tuna (*Katsuwonus pelamis* L) (Zhang and others 2015).

Roura and Crupkin (1995) examined the muscle protein of hake (*Merluccius hubbsi* Marini) before and after spawning, during iced storage. Their results revealed that Ca$^{2+}$ sensitivity of myofibrils in post-spawning hake was 60% more than that of myofibrils in pre-spawning hake. SDS-PAGE profiles indicated that pre-spawning hake myofibrils demonstrated a moderately denatured myosin heavy chain (MHC). Post-spawning fish had triple the enzymatic of pre-spawning fish at day 0. During 10 days of ice storage, no further degradation of myofibrils was detected in fish caught before or after spawning.

Furthermore, Benjakul and others (1997) observed changes from the Myofibril ATPase during iced storage of Pacific whiting fillets. At day 8 of storage muscle protein degradation and denaturation was obviously seen. Myosin heavy chain (MHC) was degraded by 45%, but no noticeable changes were seen in actin, as confirmed by SDS-PAGE pattern. ATPase measurements indicated no changes in actomyosin Ca$^{2+}$, Mg$^{2+}$, or Mg$^{2+}$-Ca$^{2+}$-ATPase activities, but Mg$^{2+}$-EGTA-ATPase activity increased with a loss of
Ca$^{2+}$-sensitivity. Surface hydrophobicity of actomyosin accumulated significantly within 2 days then remained stable over the storage period. In contrast, the total sulfhydryl content remained steady for 2 days of storage then steadily decreased.

2.3.1.2 Refrigeration

Refrigeration at 4 °C has been used by consumers to store fish before processing. Fish muscle undergo many structural changes during refrigeration. Roy and others (2012) measured the changes of structure and ultrastructure of cultured Pacific Bluefin tuna muscle slices during chilled storage. They indicated that the changes of the fish muscle slices were due to the loss of myofiber to myofiber adhesion, detachment of the sarcolemma, increase of the inter-myofibrillar spaces, and adjustment of hexagonal arrangement of thick and thin contractile myofilaments in myofibrils. Oxidation of lipids or proteins can happen during refrigerated storage. A recent study by Tokur and Polat (2010) demonstrated that the composition of myofibril and sarcoplasmic protein of gray mullet (*Liza ramada*) changes due to proteolysis during refrigerated storage at 4 °C. Interestingly, they concluded that there is a possible connection between protein oxidation and degradation of thin-lipped mullet myofibril proteins. The proteolysis process might be activated by the oxygen radicals during post-mortem storage.

In addition, Caballero and others (2009) provided evidence that muscle degradation steadily alters the freshness stage of fish during post mortem storage. The activation of proteinases mainly led to degradation of myofibrillar protein. In their
study, structural changes were correlated with the textural properties of sea bream (*Sparus aurata*) muscle during 14 days of post mortem cold storage through the immunohistochemical detection of muscle filament proteins (desmin, actin and dystrophin) and endoproteases (μ-calpain, m-calpain). Their results demonstrated that after 4 days of storage, calpain activity remained unchanged but dystrophin disappeared. Meanwhile, actin and desmin remained stable until 14 days of storage. There is a link between the reduction of textural hardness value and the detachment of myofibers and myocommata, as well as to the disappearance of dystrophin. It is apparent that refrigerated storage did not affect actin and desmin even though the appearance and texture of the muscle deteriorated.

2.3.1.3 Frozen

Numerous studies have attempted to explain the effect of frozen storage on fish muscle protein. Frozen storage is the leading preservation method for seafood products. However, temperature abuse or fluctuation in the freezer may occur during transportation, storage, and market display, therefore affecting the quality and shelf life of products (Gormley and others 2002; Olafsdottir and others 2006). Moreover, freeze-thaw (FT) cycles contribute to the denaturation of fish protein, leading to textural changes (Yoon and Lee 1990; Benjakul and Bauer 2000, 2001; Lee and Park 2016).

Benjakul and Bauer (2000) studied the effects of freeze-thaw (FT) cycles on the physicochemical and enzymatic changes of cod muscle proteins. They observed the
loss of Ca\(^{2+}\)-ATPase and Mg\(^{2+}\)-ATPase after one FT cycle. However, Mg\(^{2+}\)-EGTA-ATPase activity increased simultaneously with the decrease in Ca\(^{2+}\)-sensitivity. They demonstrated that the frequency of FT cycles resulted in the decrease of surface sulfhydryl groups and protein solubility. The number of FT cycles did not affect the surface hydrophobicity. Also, no cross-linked proteins were detected while monitoring the existence of formaldehyde. Additionally, Benjakul and Bauer (2001) reported the effect of FT cycles on physicochemical and enzymatic properties of catfish (*Silurus glanis* Linne). They indicated that the autolytic degradation of catfish muscle proteins, after subjected to FT cycles, was increased after 3 days of chilled storage. Nevertheless, no evidence of degradation products were detected in the samples with varied FT cycles. The increase in degradation products revealed the activity of proteolytic enzyme due to the FT process. They concluded that FT cycles caused the destabilization of the muscle structure, re-distribution of proteinase in fish muscle, and acceleration of lipid oxidation. Furthermore, they suggested that the alteration of native conformation of muscle proteins would make them more sensitive to proteinase.

### 2.3.2 Thermal Processing

Tiwari and O’Donell (2012) classified the thermal processing of meat and meat products into 2 different methods i.e. conventional and novel thermal processing. Conventional thermal processing includes dry heat methods (roasting, frying and smoking) and moist heat methods (boiling and steaming). Novel thermal processing consists of microwaving, dielectric heating, infrared processing, and *sous vide*
processing. To a certain extent, these processing methods could also be applied to seafood products. To date, the research of muscle protein alterations has tended to focus on the changes in meat protein during thermal processing but less documentation in available on various cooking techniques (boiling, frying, microwave heating, etc.). It should be noted that cooking conditions (temperature, time, type of meat cut, meat characteristics, etc.) will affect the characteristics of final products; however, the effect of heating on muscle protein will cause similar phenomena. Therefore, in this section we will discuss the common effects on muscle protein during and after thermal processing.

Thermal processing of meat results in weight loss and changes in water-holding capacity, texture, muscle fiber shrinkage, color, and flavor (Walsh and others 2010). Factors contributing to the physicochemical changes during thermal processing are the denaturation of protein, moisture loss, muscle characteristics, heating method, and time/temperature transformation during cooking (Christensen and others 2000; Mora and others 2011).

Tornberg (2005) indicated that cooking affects the texture of meat as a result of muscle protein denaturation. Many thermographic studies suggested three stages of protein denaturation of meat, which are (1) myosin (rod and light chain) denaturation between 40–60°C, (2) sarcoplasmic protein and collagen denaturation between 60–70°C, and (3) actin denaturation around 80°C (Stabursvik and Martens 1980; Wright and Wilding 1984, Deng and others 2000). On the other hand, the study by Ofstad and others (1993) highlighted that changes in fish muscle structure
happened at temperatures <40 °C. This view is supported by Mendez and Abuin (2012) who asserted that during the early stages of cooking (30 °C – 50 °C), peptide chains uncoiled as a consequence of partial denaturation of sarcoplasmic proteins. This process resulted in higher moisture loss, decreased water-holding capacity and decreased texture forming ability (Ofstad and others 1993; Lund and others 2011). At increased temperatures (50 °C – 70 °C), the denatured and coagulated protein developed stable cross-linkages (Mendez and Abuin 2012). Furthermore, protein oxidation happens during the heating process. Heating processes break hydrogen bonds, resulting in the unfolding of the protein (Mendez and Abuin 2012). Heating can induce formation of amino acid derivatives including carbonyls, alter protein hydrophobicity, decrease protein solubility due to polymerization, and modify the sensitivity of protein substrate to proteolytic activity (Xiong 2000; Lund and others 2011).

2.4 Enhancement Ingredients and Its Effect to Fish Fillets

This section focusses on the 5 ingredients that were used in this study: salt, phosphate, egg white, potato extract, and xanthan gum. The review discusses functional properties of each ingredient as well as how they impact fish quality.

2.4.1 Salt

There are three primary functions of salt in seafood product. First, it serves as preservation agent. Two of the most commonly ways to preserve seafood product, salted and fermented fish, is done through the use of high concentration of salts.
Several studies have revealed that, as a preservative agent, salt can reduce water activity ($a_w$) and ensures microbial stability in dried-salted fish products (Lupin and others 1981; McMeekin and others 1987; Turan and others 2006) and fermented fish product (Um and others 1996; Anhouvi and others 2006; Harikedua and others 2012). Moreover, Albarracin and others (2011) also noted that the actions of salt to decrease water activity ($a_w$) in food helps decelerate or interfere with microorganism physiology. Thus, prolong the shelf life of fish product.

The second function of salt is to serve as a functional ingredient in seafood processing because it can influence the water holding capacity (WHC) of contractile muscle proteins. The interaction between salt and proteins, or salt with lipids, contributes to chemical and biochemical processes in food (Albarracin and others 2011). For example, a light salting method (5% salt concentration by immersion or injection) can act as a cryoprotectant to fish muscle during freezing (Thorarinsdottir and others 2002). Furthermore, numerous studies have also attempted to explain how salt can influence the water holding capacity (WHC) in poultry, meat, and seafood products during processing and thereby increased yield of the product (Shults and Wierbicki 1973; Offer and Trinick 1983; Thorarinsdottir and others 2004). It is suggested that WHC increases with the incorporation of salt concentration of up to about 3% (salting-in process). Thorarinsdottir and others (2002) showed that light salting method in fish, up to 2% brine uptake contain of commercial grade salt by immersion or injection, increased the moisture content in seafood and reduced drip. The mechanism of salt to increased water holding capacity was introduced by Hamm
Salt contain of sodium and chloride ion. Sodium is a cation ($\text{Na}^+$) and chloride ($\text{Cl}^-$) is an anion. The mechanism of water holding capacity involve the interaction between charged amino acid in myofibril protein and the presence of salt, mainly chloride ($\text{Cl}^-$) ion. In general, fish contain 80% of water. Water in muscle can be divided into 3 main part which is bound, immobilized, and free water. When fish die, the rigor mortis can affect the actomyosin linkage in between muscle and water. When we added salt, the structure of protein can alter due to the electrostatic repulsion.

Kin and others (2010) demonstrated that mixes of sodium chloride and various types of phosphate (sodium tripolyphosphate, sodium monophosphate, poly and pyrophosphates, potassium + sodium polyphosphates) impacted the quality of catfish fillets. Similarly, Thorarinsdottir and others (2010) explored the efficacy of injecting a saturated brine (25% NaCl) or a mixture of 22.5% NaCl and 2.5% phosphate in cod. The main goal of their study was to compare the different pre-salting steps and brine concentration before the dry salting step for heavy-salted cod production. The addition of phosphate is known to increase water retention and therefore increase the weight in salted products. However, the results showed that after rehydration and cooking there were no significant weight differences between fillets injected with only salt or the combination of salt and phosphate.

On the other hand, when the salt concentration reaches levels above 10%, denaturation of proteins leads to decreased WHC of the muscle (salting-out process). This result is supported by Gallart-Jornet and others (2007) where they determined
the effect of various brine concentration (4%, 10%, 15%, 18% and 25% NaCl w/w) on Atlantic salmon fillets. The study concluded that the weight gain of the fillets decreased as the brine concentration increased.

Lastly, salt can also influence the sensory perception and consumer acceptance of a product. Salt masks the bitterness in food, while also enhances the flavor of food since it can react with lipid and protein leading to biochemical and enzymatic reaction (Albarracin and others 2011). Almli and Herslet (2012) demonstrated that if food does not contain sufficient salt, several flavor (i.e. umami, sweet, etc.) cannot be detected by our retronasal olfactory system. In other words, the addition of salt in food processing would not only give salty sensation, but also enhances other flavors in the food product. In addition, Galvis-Sanchez and others (2011) noted the important function of salt, in the form of sodium and chloride ions, in human physiology, which is to help regulate circulatory and digestive systems of human. Sodium ions maintain the pressure and the volume of the blood, while chloride ions maintain the acid-base balance necessary for the formation of hydrochloric acid in the stomach, which is essential for digestive process (Galvis-Sanchez and others 2011).

2.4.2 Phosphates

Data from several sources have identified that the effectiveness of phosphate on water retention of meat products depends on the interaction between phosphate and various ions contained in the specific food product itself. Thorarinsdottir and
others (2004) indicated that the activity of phosphate is due to the effects on pH and ionic strength and specific interactions of phosphate anions with divalent cations and myofibrillar proteins. Similarly, other researchers have also highlighted the importance of the concentration of magnesium, calcium, and chloride ions ($\text{Mg}^{2+}$, $\text{Ca}^{2+}$, $\text{Cl}^-$, respectively), temperature, and pH to influence the effectiveness of phosphate’s interaction with muscle proteins in meat (Offer and Knight 1988; Chang and Regenstein 1997). The combination of phosphates and sodium chloride (NaCl) increase moisture retention in meat more than when only one ingredient was used (Thorarinsdottir and others 2004). This means that adding both phosphate and salt to meat and seafood products is synergistic and can increase the water holding capacity (WHC) during processing more than either ingredient does alone.

The different types of phosphate also determine how effective this ingredient is in regulating water retention of meat products. Killinc and others (2009) studied the effects of phosphates treatment on the quality of frozen-thawed fish species. The objective was to evaluate the quality attributes of frozen-thawed fish species treated with sodium monophosphate (SMP), sodium diphosphate (SDP), sodium triphosphate (STP), and also to improve the quality of frozen-thawed fish species. The study concluded that the treatment of dipping frozen-thawed saithe ($\textit{Pollachius virens}$) and sea bass ($\textit{Dicentrarchus labrax}$) in 5% phosphate solutions (SMP, SDP, or STP) made the fillets much softer, less chewable, and highly cohesive. In addition to that, they also claimed that treatment of 5% STP solution was the most effective approach to reduce bacterial loads, when compared with other phosphate groups. In the same
vein, Kin and others (2010) demonstrated that the enhancement of an agglomerated blend of sodium phosphate through multi-needle injection improved yields and quality of channel catfish (*Ictalurus punctatus*) fillets when compared to the non-injected product. Therefore, it was suggested that phosphate treatment can serve as an alternative way to improve the quality of refrigerated fish and frozen-thawed fish.

### 2.4.3 Egg White

Much of the available literatures on egg white as functional ingredients to fish product deal with the incorporation of egg white to fish mince or surimi production (Chang-Lee and others 1989; Hamann and others 1990; Morrissey and others 1993). Previous studies have identified and characterize the active components of egg white as proteinase inhibitors (Nagase and others 1983; Stevens 1991; Weerasinghe and others 1996). Their studies revealed that ovomucoid and ovoinhibitor are present in egg white as inhibitors for serine protease, cystatin for cysteine protease, and ovostatin acted as alpha-2-macroglobulin, a higher molecular weight protease inhibitor.

One particular study focused on the use of egg white on fish fillets was conducted by Yetim and Ockerman (1994) with catfish as the raw material. Their study was with a combination with tumbling process. The tumbling technique is an operation using physical forces to accelerate curing procedures and improve the quality of products. It was concluded that the addition of egg white (EW) would be an effective practice to improve functional properties of fish muscle, such that an acceptable restructured fish product can be obtained. The electrophoretic pattern of
these treatments was not noticeably different from fresh (zero time) tissue, which might be due to minimal proteolysis occurring during the tumbling process. The ability of EW to bind moisture results in the increase of moisture in a product.

Aside from increasing moisture content, EW also helps lower both lipid and secondary lipid oxidation product, thiobarbituric acid (TBA) values of the food product. In their study, Yetim and Ockerman (1994) showed that TBA values were significantly lower in EW added product compared to control (no added EW). This might be due to the fact that the lipid content in EW added products were reduced, since significant correlation between lipid content and TBA numbers was observed.

2.4.4 Potato Extracts

Another well-known protease inhibitor from plants is potato extract (PE). Plant protease inhibitors can be found in storage tissues, such as in tubers and seeds, in the form of small protein (De Leo and others 2002). The application of potato extract as protease inhibitor in fish processing/surimi production has been extensively studied over the past two decades. A seminal study in this area is the work of Morrissey and others (1993). They concluded that increased potato extract (1, 2, 3, and 4%) concentration to surimi enhanced the strain value of pacific whiting surimi. PE increased the strain value of pacific whiting surimi 43% from 1.4 (1%PE) to 2.0 (3% PE), but decreased the value to 1.9 (4%PE) for fish cooked at 90°C. Meanwhile, for fish that was cooked at 60/90°C, the strain value increased 35% from 0.9 (1%PE) to 1.4 (4%PE). For the autolysis study in fish mince, at 1%PE, there was 70% proteolytic inhibition, while the addition of 2%, 3%, and 4% PE showed more than 80% inhibition.
On the other hand, the proteolytic inhibition of 1% PE in surimi paste are 53%, whilst the inhibition of 2%, 3%, and 4% of PE are around 70%, 75%, and 78%, respectively (Morrissey and others 1993).

In recent years, the application of potato to fish meat has been advanced to not only to inhibit proteolytic activity, but also to act as a source of natural antioxidant. In their case study, Farvin and others (2012) studied the effect of potato peel extract in chill stored minced horse mackerel (*Trachurus trachurus*). They used the potato peel which was dried in hot air oven at 45 °C for 72 h and powdered. The peel was then extracted using 5% ethanol and water. These extracts then underwent lyophilization (freeze-dry). The extract was stored at -80 °C before analysis. They concluded that adding 2.4 and 4.8 g of ethanol extracted potato peel per kg of minced horse mackerel provided the best protection towards lipid and protein oxidation. There was no evidence to suggest that the extracts reduced protein solubility or loss of sulphydryl groups. Protein aggregation, as monitored by losses in solubility, did not diminish through the addition of the extracts. Thus, the results of this study suggested that ethanol extracts of potato peel can be employed as a natural antioxidant to prevent lipid and protein oxidation of fish fillets/mince in the chill stored mackerel. Likewise, Hidalgo and Kinsella (1989) stated that the formation of protein carbonyls compounds have been reported to be the second sign of protein oxidation due to proteins interacting with secondary lipid oxidation products.
2.4.5 Xanthan Gum

Gums are commonly used in many food industries since it has the ability to form gel and to thicken food product. Furthermore, gum can also be utilized to improve restructured products because it is readily available, cheap, and add benefit to human health by providing fibers without adding more calories (Ramirez and others 2011). Most gums are compatible with fish muscle protein, which means that it can improve yield and WHC, without giving negative effect on color and texture (Da Ponte and others 1984; Ramirez and others 2011).

Xanthan gum is a polysaccharide produced through an aerobic fermentation of *Xanthomonas campestris* (BeMiller and Huber 2008; Morris and Harding 2009; Rodriguez-Hernandez and Tecante 1999). Xanthan consists of a non-linear anionic chain, exists in solution in a rigid, ordered, chain conformation (Rodriguez-Hernandez and Tecante 1999; Morris and others 2014). Xanthan gum is soluble in both hot and cold water as well as in acidic systems (BeMiller and Huber 2008). It is also able to form highly viscous solution at low concentrations, it has excellent compatibility with salt, and its stability is not affected by temperature or pH (BeMiller and Huber 2008). Because of all of these features, xanthan gum is an attractive ingredient to be considered for incorporation in seafood product processing.

Da Ponte and others (1984) examined the stability of frozen stored minced fillets of whiting (various anionic and neutral hydrocolloids) after addition of xanthan gum and demonstrated that it could slow down the formation of dimethylamine (DMA) and fatty acids (FA). At the same time this method also increased the WHC of
raw material and cooked product, but decreased the cook drip loss. The whiteness of minced fish cakes was also improved. They also suggested that the combination of locus bean gum and xanthan gum (1:1) presented the highest values of WHC. This combination is well known for its formation of strong and thermoreversible gels. This result was in agreement with the finding of Ramirez and others (2002) who demonstrated that locust bean and xanthan gum at a 0.25/0.75 ratio improved the mechanical properties of surimi gels from silver carp (*Hypophthalmichthys molitrix*).

### 2.5 Quality Attributes of Fish Fillets

The quality of seafood products is an important attribute that can characterize or distinguish one product from another. The quality of seafood products is mostly determined by color, texture, and flavor (odor and taste). Therefore, the importance of texture and color as quality traits of injected fish fillets will be reviewed in this section.

#### 2.5.1 Color

Color is one of the quality traits in fish products that should be taken into account by the processor before selling. Kristinsson and others (2006) stated that there are necessary actions for the seafood processor to preserve fresh color of seafood during processing, transport, storage, and retail display, while assuring quality and safety. The appearance of seafood has been strongly correlated to consumer buying behavior. Gupta and others (2007) stated that pigmentation is one of the most valuable quality attributes of the fish for consumer acceptability. The
changes in fish color can reflect the fish deterioration process. Moreover, McCaig (2002) pointed out that other qualities of food such as freshness, maturity, variety and desirability are closely related with color. It is also a significant grading factor for most food products.

In general, the color of fish is complicated and can be affected by fish species, harvest season, chemical composition (water, lipid, and protein content), freshness level, muscle type (dark vs. white muscle), and type and quantity of heme proteins (Kristinsson and others 2007). Among those factors, type and quantity of heme proteins mostly determines the color in fish (Kristinsson and others 2007). Two heme proteins that are responsible for fish color are myoglobin (pigment in muscle) and hemoglobin (pigment in blood). Additionally, Gupta and others (2007) claimed that carotenoids are also responsible for pigmentation in fish. They suggested that various types of carotenoids are typically responsible for fish color. For example, the most dominant carotenoid, astaxanthin, prevails in red fish and gives salmon flesh its characteristic color. Tunaxanthin, a yellow pigment, can be found in scombrina, carangina, and percina fish and is abundant in yellow fish, like yellowtail (Seriola quinqueradiata). Lutein, the greenish-yellow pigment, is found in freshwater fish as well as marine fish. They also concluded that carotenoids in fish exist in small amounts and their proportions are different in various body parts. Additionally, the different percentage of carotenoids in fish exist due to physiological and/or dietary conditions.

It has conclusively been shown that during the handling and storage of fish, a number of biochemical, chemical and microbiological changes occur, leading to
discoloration (No and Sotrebakken 1991; Chaijan and others 2005; Sohn and others 2005). No and Storebakken (1991) studied the effects of frozen storage (3 and 6 mo at \(-20\,^{\circ}\text{C}\) and \(-80\,^{\circ}\text{C}\)) on the stability of carotenoids and color (CIELAB) in vacuum-packed fillets of rainbow trout. Their results showed that frozen storage for 6 months at \(-20\,^{\circ}\text{C}\) or \(-80\,^{\circ}\text{C}\) did not impair the stability of carotenoids in trout flesh (up to 5% loss). However, they observed different Lab color value in the neck, back and tail parts. This is probably because of the way the different carotenoid concentrations were deposited throughout the body of the fish. Frozen storage intensified L* (lightness), a* (redness) and b* (yellowness), and degraded H (\(\ell_{\text{ab}}\)) (hue) values. Furthermore, heating treatment can also change the color of fish. Nakamura and others (2011) identified 4 steps that influence changes in fish muscle during grilling: (1) protein denaturation, (2) water evaporation, (3) a browning reaction, and (4) a carbonization reaction. Their results are in agreement with Matsuda and others (2013).

A great deal of studies have examined the natural color or discoloration of fish using various color measurement devices and systems. The Minolta Chromameter, Hunter Lab colorimeter, and Dr. Lange colorimeters are common instruments for the measurement of color (Leon and others 2006). The advantage of using colorimeters is that they are rapid, simple and accurate. The accuracy is high due to the process of calibration before the measurement with standard tiles (Oliveira and Balaban 2006). Several studies on fish mince and fish fillets using L*, a*, b* color measurement have been carried out to investigate color of salmon, cod, yellowtail, rainbow trout, sardine and mackerel muscles (No and Storebakken 1991; Schubring 2003; Sohn and others
2005; Chaijan and others 2005; Erikson and Missimi 2008). To date, it seems that colorimeters with the L*, a*, and b* measurement system (CIELAB) are the most commonly used. It is suggested that the L*a*b* color space gives the most uniform distribution of colors. Likewise, CIE L*a*b* gives uniform perception by human eyes (Leon and others 2006). Recently, Wu and others (2012) evaluated the feasibility of a hyperspectral imaging system in the LW-NIR spectral region (964 to 1631 nm) for rapid measurement of color in intact salmon fillets. Their study demonstrated that this method was rapid, contact-free, and gives consistent evaluation. They asserted that this method can be used as a reliable and alternative method for measuring the color of salmon fillets for the food industry.

Adding ingredients through the injection process can affect the color of fish muscle. Until now, Kin and others (2010) has shown that adding various phosphate treatments to catfish muscle showed no difference on CIE a* (redness) values, but give significant differences in CIE L* (lightness) and b* (yellowness) values. They suggested that the lower CIE L* values indicated that the fillets were darker in color after being treated with phosphate through multi-needle injection. The fillets were darker since more light was being absorbed by the fillets rather than scattered. On the other hand, when light is scattered as opposed to absorbed, meat color appears lighter. The darker color is also associated with the water holding capacity (WHC) (Bauermeister and McKee 2005). For the CIE a* (redness) values, Kin and others (2010) results were in agreement with Lu (2008) who stated that the CIE a* values were not affected (P > 0.05) by phosphate treatments because catfish fillets have
minimal pigmentation. Therefore, slight changes in moisture content and/or yield after the enhancement would have fewer effects on red color. Moreover, the differences in color values of CIE L* and b* also indicated that a more open protein structure causes changes in light reflection of fish meat.

2.5.2 Texture

Commonly, fish and other seafood products are considered a good source of protein. The protein in fish products has advantages from nutritional and textural aspects. In the last two decades, there have been only three review papers that summarize and overview the textural quality of fish (Hyldig and Nielsen 2001; Coppes and others 2008; Cheng and others 2014). They emphasized of the challenges in measurement of fish and other seafood products. All of the papers suggested that there is no specific method that can be applied universally to measure fish fillet texture. Hyldig and Nielsen (2001) pointed out that the researcher should be mindful in picking the method according to the goal of their study. Cheng and others (2014) asserted that texture measurements by instruments are more objective and reliable. They noted that the measurement of texture by instruments can provide information about texture alterations during specific conditions of processing, such as freezing, chilling, salting, and smoking. Similarly, Coppes and others (2002) noted that a better overall understanding of seafood texture characteristics can be obtained by correlating instrumental and sensory test. Furthermore, a number of authors have reported different programs and instruments that can be used to measure the textural quality of fish fillets as can be seen in Table 2.1. Much of the current literature pays particular
attention to the texture of fish during storage (either refrigerated or frozen), and on the different cooked products.
<table>
<thead>
<tr>
<th>Fish name</th>
<th>Species</th>
<th>Instrument</th>
<th>Probe</th>
<th>Program</th>
<th>Test Properties</th>
<th>Conditions</th>
<th>Sample Preparations</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atlantic Cod</td>
<td>(Gadus morhua)</td>
<td>Texture analyzer, TA-XTplus</td>
<td>Flat-end cylinder d:12.5 mm</td>
<td>Trigger force; 10.0 g, speed 1.0 mm/s until 30% compression of the sample</td>
<td>TPA</td>
<td>Raw/Cooked in different heat temperature</td>
<td>Stored in ice prior to analysis (0 – 1 °C)</td>
<td>Skipnes and others (2007)</td>
</tr>
<tr>
<td>Atlantic Cod</td>
<td>(Gadus morhua)</td>
<td>Texture analyzer, TA-XTplus</td>
<td>Flat end cylinder d:0.5 inc of type P/0.5 AOAC for gelatine</td>
<td>Speed 1.00 mm/s until 80% compression of the sample</td>
<td>TPA</td>
<td>Raw/Cooked in different heat temperature (Fillet)</td>
<td>Stored in ice prior to analysis (0 – 1 °C)</td>
<td>Skipnes and others (2011)</td>
</tr>
<tr>
<td>Atlantic Cod</td>
<td>(Gadus morhua)</td>
<td>Texture analyzer, TA-XTplus</td>
<td>a 12.5-mm flat-end cylinder (type P/0.5)</td>
<td>Speed (1 mm/s). Height compression was 12-mm.</td>
<td>TPA</td>
<td>Frozen (Fillet)</td>
<td>The fillets were slowly thawed at +2 °C until the core temperature reached 0 °C. The fillets were thereafter acclimated to 20 °C before being analyzed.</td>
<td>Møkøre and Lillehol (2007)</td>
</tr>
<tr>
<td>Atlantic Cod and Atlantic Salmon</td>
<td>(Salmo salar)</td>
<td>Texture analyzer, TA-XT2</td>
<td>Flat-ended cylindrical plunger (12 mm diameter).</td>
<td>Speed of 1.00 mm s⁻¹ into the fillets until it had reached 60% of the sample height. The holding time between the compressions was 5 s.</td>
<td>TPA</td>
<td>Raw (Fillet)</td>
<td>Stored in ice on closed plastic tanks prior to analysis (4 ± 1 °C)</td>
<td>Gallart-Jornet and others (2007)</td>
</tr>
<tr>
<td>Atlantic Salmon</td>
<td>(Salmo salar)</td>
<td>Texture analyzer, TA-XT2</td>
<td>Cylindrical plunger d:12.5 mm</td>
<td>Speed of 1.00 mm s⁻¹ into the fillets until it had reached 60% of the sample height</td>
<td>Compression</td>
<td>Raw (Fillet)</td>
<td></td>
<td>Einen and Thomassen (1998)</td>
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<tr>
<td>Fish Name Species</td>
<td>Instrument</td>
<td>Probe</td>
<td>Program</td>
<td>Test Properties</td>
<td>Conditions</td>
<td>Sample Preparations</td>
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<tr>
<td>Atlantic Salmon (Salmo salar)</td>
<td>Texture analyzer, TA-XT2</td>
<td>Blade thickness 0.75 mm and width 70 mm</td>
<td>Speed 2.00 mm/s</td>
<td>Shear</td>
<td>Compression</td>
<td>Vacuum packaged and stored at 2 °C</td>
<td>Casas and others (2006)</td>
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<tr>
<td>Atlantic Salmon (Salmo salar)</td>
<td>Texture analyzer, TA-XT2</td>
<td>Spherical d:15 mm</td>
<td>Speed 2.00 mm/s</td>
<td>Compression</td>
<td>Compression</td>
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<tr>
<td>Atlantic Salmon (Salmo salar)</td>
<td>Texture analyzer, TA-XT2</td>
<td>Cylindrical d:10 mm</td>
<td>Speed 2.00 mm/s until 30% compression of the sample</td>
<td>Compression</td>
<td>Compression</td>
<td></td>
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<tr>
<td>Atlantic Salmon (Salmo salar)</td>
<td>Texture analyzer, TA-XT2</td>
<td>Cylindrical d:20 mm</td>
<td></td>
<td></td>
<td>Compression</td>
<td>Raw (Fillet)</td>
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<tr>
<td>Atlantic Salmon (Salmo salar)</td>
<td>Warner-Bratzler knife</td>
<td>A spherical probe d: 25 mm</td>
<td>Speed 5.00 mm/s and 2.00 mm/s. Compression force 20%, 30%, and 40% mm compression</td>
<td>TPA</td>
<td>Raw (Fillet)</td>
<td>Stored at different temperatures before analysis (0, 4, 10 and 20 °C)</td>
<td>Vevland and Torrisen (1999)</td>
<td></td>
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<tr>
<td>Atlantic Salmon (Salmo salar)</td>
<td>Texture analyzer, TA-XT2</td>
<td>Blade (knife-edge, 60°) of thickness of 3.0 mm and width 70 mm</td>
<td>Pressing the blade through the muscle vertical to the muscle fibers</td>
<td>Shear force</td>
<td>Raw (Fillet)</td>
<td></td>
<td>Sigurgisladottir and others (2000)</td>
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<tr>
<td>Herring (Clupea harengus L.)</td>
<td>Texture analyzer, TA-XT2</td>
<td>Stainless steel cylinder d:11 mm, height 6 mm</td>
<td>Speed 1 mm/s until compression 60% of the sample</td>
<td>Compression</td>
<td>Raw (Fillet)</td>
<td>Stored fillets on a plastic sheet on ice in a polystyrene box between 5 – 7 °C.</td>
<td>Nielsen and others (2005)</td>
<td></td>
</tr>
<tr>
<td>Arctic charr (Salvelinus alpinus)</td>
<td>Texture analyzer, TA-XT2</td>
<td>Aluminium compression plate with a 100 mm diameter</td>
<td>Speed 0.8 mm/s and the strain to 80%; 60 s apart.</td>
<td>TPA</td>
<td>Raw (Right Fillet)</td>
<td>Skin was removed, cut fillet into four square pieces (2.5 × 2.5 cm) from above the lateral line. The raw flesh samples were stored on ice. Other pieces were cooked in an aluminum box in a steam oven at 100 °C for 7 min.</td>
<td>Gines and others (2004)</td>
<td></td>
</tr>
<tr>
<td>Fish name</td>
<td>Species</td>
<td>Instrument</td>
<td>Probe</td>
<td>Program</td>
<td>Test Properties</td>
<td>Conditions</td>
<td>Sample Preparations</td>
<td>Reference</td>
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<tr>
<td>Sea bass fillets</td>
<td><em>Dicentrarchus labrax</em></td>
<td>Texture analyzer, TA-XT2</td>
<td>a flat-ended cylindrical plunger (7.5mm diameter)</td>
<td>Speed of 0.8 mm/s until it reached 50% of sample height, TPA</td>
<td>Raw and Smoked</td>
<td>4×2×2</td>
<td>Cold storage at 4 °C for 42 d Samples were obtained by cutting out parallelepiped pieces (4×3 cm²) from the dorsal part of the fillet</td>
<td>Fuentes and others (2012)</td>
</tr>
<tr>
<td>Cat fish</td>
<td><em>Ictalurus punctatus</em></td>
<td>Texture analyzer, TA-XT2</td>
<td>Warner Bratzler Speed of 10 mm/min, 90° angle inverted knife.</td>
<td>Warner Bratzler Speed of 2 mm/s</td>
<td>Shear force</td>
<td>Raw</td>
<td>Stored at 4 °C for 14 days. Seven rectangular shaped samples (4×2×2 cm). Each sample was cut perpendicular to the longitudinal orientation of the muscle fibers.</td>
<td>Rawdkuen and others (2010)</td>
</tr>
<tr>
<td>Cat fish</td>
<td><em>Ictalurus punctatus</em></td>
<td>Instron Universal Testing Machine</td>
<td>Kramer shear compression cell (CS-2)</td>
<td>Speed 100 mm/s</td>
<td>Shear force</td>
<td>Cooked</td>
<td>25 g square piece was removed from the center of cooked fillet and placed in the Kramer cell and sheared once.</td>
<td>Kin and others 2010</td>
</tr>
<tr>
<td>Pacific whiting</td>
<td><em>Merluccius productus</em></td>
<td>Instron Universal Testing Machine</td>
<td>Kramer shear attachments (4 blades)</td>
<td>Speed 1 mm/s</td>
<td>Shear press</td>
<td>Raw</td>
<td>Frozen Pacific whiting was tempered at ambient temperature for approximately 2 hours or until semi frozen. Shear press reading were taken on 15 g of muscle tissue removed from the dorsal portion. Fish size over 27 cm were used in all analysis.</td>
<td>Kudo and others (1987)</td>
</tr>
<tr>
<td>Herring</td>
<td></td>
<td>Instron Universal Testing Machine</td>
<td>Kramer Shear Cell (4 blades)</td>
<td>-</td>
<td>Shear press</td>
<td>Canned</td>
<td>-</td>
<td>Bilinski and others (1977)</td>
</tr>
<tr>
<td>Fish name Species</td>
<td>Instrument</td>
<td>Probe</td>
<td>Program</td>
<td>Test Properties</td>
<td>Conditions</td>
<td>Sample Preparations</td>
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<tr>
<td>Haddock and Red Hake</td>
<td>Instron Universal Testing Machine</td>
<td>Kramer Shear Cell (4 blades)</td>
<td>Speed 10cm/min</td>
<td>Shear press</td>
<td>Cooked</td>
<td>Samples were cooked by baking in a 230 °C oven for 12 min. After cooking, the fillets were cut into approximately 1 cm³ pieces and 50-g samples placed in sealed plastic containers and cooled to 22 + 2 °C.</td>
<td>Gill and others (1979)</td>
<td></td>
</tr>
<tr>
<td>Atlantic Cod</td>
<td>Instron Universal Testing Machine</td>
<td>Kramer Shear Cell (4 blades)</td>
<td>-</td>
<td>Shear press</td>
<td>Cooked</td>
<td>-</td>
<td>Botta and others (1987); LeBlanc and others (1988)</td>
<td></td>
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<tr>
<td>Skipjack (Katsuwonus pelamis); Flyingfish; Common horse mackerel (Trachurus japonicus); Plaice (Limanda yokahamae); Channel rock fish (Sebastolobus macrochir)</td>
<td>Texturometer (Zenken Co. Model GTX-2, General Foods type)</td>
<td>Aluminium plunger d:13mm</td>
<td>1.5 mm clearance, 1 Volt, speed 750 mm/min</td>
<td>Firmness</td>
<td>Cooked</td>
<td>Sample size (2.5 cm thick, 4.5 cm wide and 9-11 cm long), 100 g per piece, sealed in a polypropylene bag, evacuated and cooked for 10 min in boiling water (96 °C).</td>
<td>Hatae (1984)</td>
<td></td>
</tr>
</tbody>
</table>
2.6 Injection Technology

Meat injection technology was first invented in the 18th century by a meat processor in the United Kingdom. Advancements in technology have equipped the brine injector machine with adjustable pressure and belt speed (or needle speed, depending on manufacturer), variety of brine delivery mechanisms (i.e. either on downstroke and/or upstroke needle), larger assortment of needle designs, and more manageable cleaning procedures. This section reviews the application of various brines to improve fish products, factors contributing to the success of injection, and the advantages and disadvantages of injection technology.

2.6.1 Application and Equipment

A multi-needle injection system can incorporate brine into product faster, more uniformly, and more efficiently than traditional methods such as soaking or vacuum tumbling. Birkeland and others (2003) suggested that the increase in popularity from using injection for salting in smoked-salmon processing was due to the fact that this process was faster than wet soaking or dry salting processes. This view is also supported by Karlsdottir (2009), who stated that the aim of brine injection was to obtain a homogenous distribution of brine in the muscle in a short time and to minimize the variation in injection percentage due to the size or thickness of the product.

The rate of brine uptake and distribution of brine in the fillets depend on the method, species, fillet size, thickness, and a number of intrinsic muscle factors (e.g.
chemical composition, muscle structure and rigor condition) (Wang and others 2000; Jittinandana and others 2002; Birkeland and others 2003). Freixenet (1993) held the view that the parameters which determine the quality of the injection process and that have a direct impact on the quality of finished product are: 1) The accuracy of injection on each piece; 2) Brine drip loss after the injection; and 3) Brine distribution. Moreover, he also pointed out that the selection of needles is important in selecting the method for proper injection. Needles can be selected based on the brine viscosity and the amount of brine injected per product. Additionally, he explained that the brine flow rate influences the amount of brine injected into the muscle tissue per injection and, consequently, impacts the total amount of brine injected.

Several attempts have been made to incorporate various ingredients into various fish meats through the use of an injection machine with the overall goal of improving the quality and shelf life of the fish product. The use of multi-needle injection has been investigated in fish of different species and sizes such as catfish (Kin and others 2010), cod (Thorarondosttir and others 2010; Jonsdottir and others 2011; Bjorkevoll and others 2011; Asli and Møkøre 2012), and salmon (Birkeland and others 2003, 2007; Rora and others 2004; Akse and others 2008; Almli and Hersleth 2012).

Meanwhile, research using multi-needle injection on fish has primarily focused on topics such as: the delivery of salt and phosphate to change the protein functionality (increased water holding capacity (WHC)) and to improve the texture stability of fillets during storage and cooking (Kin and others 2010), the delivery of salt and sodium bicarbonate to improve liquid retention and sensory attributes of fish
(Asli and Møkøre 2012); the distribution of salt and phosphate to improve the color and reduce the intense curing flavor of heavily salted cod (Jonsdottir and others 2011); the incorporation of water as a means to rehydrate a salt-cured cod product (Bjorkevoll and others 2014); the effect of rigor stage of fish on effectiveness of brines delivered by multi-needle injection (Birkeland and others 2003, 2007; Rora and others 2004); consumer preference of injected fillets and the descriptive sensory profile of injected fillets (Almlø and Hersleth 2012).

2.6.2 Benefits and Drawbacks of Injection

There are two advantages of modern injection systems: 1) they are continuous, as opposed to batch; 2) they can deliver a homogenous distribution of brine in the muscle over a short time frame. Several studies thus far have linked the injector design factors with the quality of fish fillets. Birkeland and others (2003) investigated the effects of needle speed, injection pressure, injection direction (down/up), and the number of repeated injections in pre-rigor Atlantic salmon (Salmo salar) fillets intended for cold smoking. In this study, they used a needle array of 3×19 and the repeated injection was achieved by passing product through the machine twice. The time interval between the first and second injections were not specified, so it must be assumed that the product was immediately passed through the injector a second time as soon as it emerged from the first injection pass. The results indicated that product yield after smoking was influenced by the injection pressure and the number of repeated injections (once or twice), but no difference was observed when needle speed or brine injection directions were changed. It was also shown that
duplicating the injection process can increase yield up to 5.3% (w/w). Higher injection pressure (improvement from 1.5 to 3.0 bar) increased the likelihood of gaping in smoked salmon fillets by 18%.

Furthermore, Birkeland and others (2007) also tested the effects of injector parameters (i.e. injection volume, brine temperature, and brine concentration) on pre- and post-rigor salmon fillets intended for the cold smoking process by measuring weight changes, fillet contraction, muscle gaping and salt content. In this study, they carried out the injection process using two different injectors: Guenther Brine Injector PIF 21/57 (Guenther Maschinenbau GmbH, Dieburg, Germany) with 3×19 needles array and Fomaco Brine Injector FGM 16/64F (Fomaco Food Machinery Co. A/S, Koge, Denmark) with 4×16 needles array. The results showed that there was a significant correlation ($P < 0.01$) between the injection volume, weight gain, and salt content. Fillet contraction was simply measured by relating raw fillet length to injected fillet length, with the latter measured at 5 min., 30 min., 12 h, or 24 h, depending on experiment. The weight gain increased significantly even more with the repeated injection process than a single injection process. Lower brine concentration (12% vs 25% NaCl) resulted in less fillet contraction and gaping, reduced salt content, and higher weight gain in the fillet, as would be expected. It was also observed that contraction seemed to occur immediately upon brine injection. Temperature (-1 °C, 4 °C, and 10 °C) did not affect any of the parameters measured. However, authors did note that there was a trend in higher temperatures resulting in higher final weight gain. Higher percentage of brine uptake was observed after 30 min. equilibration time
with increased brine injection volume \((P < 0.01)\), with brine uptake remained consistent up to 12 h. For muscle gaping score in pre-rigor fillets, with 0 indicating no gap, 1 for some gap, and 2 for severe gap, the score increased with higher amount of brine injection volumes per needle stroke (0.2, 0.3, 0.4, 0.6, and 1.0 L brine/needle stroke) for both single and double injections. Therefore, the volumes of brine injection per needle stroke can act as a tool to target and standardize the amount of brine delivery to the fillets when different injectors are used. This study also demonstrated that post-rigor injected fillets had higher weight gain, higher salt content, more muscle gaping, and lower fillet contraction when compared to the pre-rigor injected fillets.

Despite the aforementioned benefits, several limitations of the injection technology still exist, including risks of microbial contamination, damage of the muscle structure, and changes in the meat appearance. Metallic materials in the equipment used for food processing that come into contact with food need to be harmless to human health and not unacceptably change the food composition or the sensory characteristics of the final product. One metallic material that is commonly used in food processing equipment is stainless steel. The North Star Ice Equipment Corporation noted 7 advantages of stainless steel in food processing equipment: worldwide acceptance, legislation and standards, hygiene, corrosion resistance, durability, flavor protection, and recycling. Although the injector needles are made from stainless steel material, which is generally accepted as the superior material for food processing equipment, there is still a chance for microbial cross-contamination
either from the brine or the meat itself during the injection process. Furthermore, the multi-needle injector would also deposit brine during the needle stroke through the meat (Freixanet 1993). Higher pressure during injection can then damage the meat structure, break the meat fibers, and create brine pockets in the needle holes after the stroke (Freixanet 1993). Additionally, after multiple needles have passed through the meat, it is possible for the needle to leave a hole in the meat and for the brine to change the surface color of the injected fillets.
References


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Tsuyuki, H. (1982). Relationship between acid and neutral protease activities and the incidence of soft cooked texture in the muscle tissue of Pacific hake (Merluccius productus) infected with Kudoa paniformis and/or K. thyrsitis, and held for varying times under different pre-freeze chilled storage conditions.


3 PRELIMINARY INVESTIGATIONS INTO THE ABILITY OF BRINE INJECTION TECHNOLOGY TO IMPROVE THE QUALITY OF PACIFIC WHITING (*Merluccius productus*) FILLETS

3.1 Abstract

There is considerable research demonstrating the ability of multi-needle injection technology to deliver marinades that improve the quality of whole muscle products from poultry, beef, and pork. In fish, most multi-needle injection technology has focused on high sodium applications in order to promote efficiencies in salting, drying and smoking processes. However, there is limited published scientific data on the incorporation of low sodium marinades (i.e. brines) into whole muscle fish for purposes of quality improvement. The purpose of this study was to determine whether multi-needle injection technology could be utilized to improve the quality of Pacific whiting fillets. The fillets from Pacific whiting are not considered acceptable for the U.S. market because they soften too much during cooking. This softening is caused by both endogenous and exogenous proteases. An exploratory study was designed to determine if any benefit in terms of either reduced protease activity and/or improved texture in the raw fillet could be achieved by incorporating a brine containing ingredients that have previously been identified to inhibit the protease activity in Pacific whiting. Parameters such as color, rancidity, moisture and protein were measured to understand how brine treatments impacted appearance, oxidative stability and composition of the fillets. Gel electrophoresis was utilized to understand how individual proteins in the myofiber where impacted by brine incorporation and
hopefully protease inhibition. All brines investigated contained 3% sodium tripolyphosphate and 3% salt as a base. Treatments included the base brine (B), base brine + 3% egg white (B_{EW}), and base brine + 3% dried potato extract (B_{PE}). In the B_{PE} brine, 0.1% xanthan gum had to be added in order to keep the dried potato extract dispersed. Ten fillets were injected per treatment and a 10% brine uptake was targeted for all fillets. Actual brine incorporation was 12.35 ± 2.28%. The lightness (L*) value of cooked fillets was significantly different among treatments ($P < 0.05$). B_{EW} and B_{PE} are darker than C and B. Breaking strength was more variable in non-injected samples than injected samples. There were significant differences in proteolytic activity ($P = 0.06$). However, SDS-PAGE analysis indicated that minor changes took place in the degradation of myofibrillar proteins between injected fillets and non-injected fillets. Data from this study was utilized to develop more in depth studies on the application of multi-needle brine injection to improve quality of Pacific whiting fillets.

**Keywords** Pacific whiting, *Merluccius productus*, brine injection, protease inhibitor, cathepsin L, texture

### 3.2 Introduction

Pacific whiting (*Merluccius productus*) is one of the Pacific Northwest's most abundant species. In 2014, the total catch of this species in the United States was 264,137 metric tons (Taylor and others 2015). Despite the fact that this species is
available in abundant quantity, it has textural issues that significantly impact its commercial value.

Previous approaches to enhancing the commercial value of Pacific whiting have focused on improving handling and storage (Morrisey and others 1996). Prior to 1990, Pacific whiting were harvested for on-shore processing using bottom trawlers on 3–4 day fishing trips. This resulted in Pacific whiting having very soft flesh and made the commodity virtually unusable. Research demonstrated that Myxosporean parasites infected Pacific whiting limiting its utilization (Kabata and Whitaker 1985). It was subsequently learned that the soft texture is caused by protease of the Myxosporean parasites *Kudoa* (Kudo and others 1987). To be successful, the onshore whiting fishery needed to alter its post-catch handling practices. Previous research has demonstrated that time and temperature are the most critical parameters in maintaining good raw material for higher quality processed product (Peters and others 1995). Based on laboratory study, Peters and others (1995) suggested that the maximum time to storage Pacific whiting on ice is 2 days for an acceptable final surimi product. Moreover, for high grade surimi, fish should be processed into surimi within 24h of capture. That is why current handling practices suggest that Pacific whiting be processed after capture by seafood processors. Consequently, the textural degradation of Pacific whiting during post mortem storage is minimized. Improvements in handling practices, however, have not been sufficient to make Pacific whiting fillets acceptable to the U.S. consumers. This is because one of the proteases responsible for softening, cathepsin L, has a high temperature of activation.
(55 °C) and significant softening therefore occurs during the cooking process. Thereby, further innovation is needed to control Pacific whiting fillet softening during both storage and cooking.

There is a growing amount of literature on the ability of multi-needle injection technology to improve the quality of whole muscle beef and poultry products. Multi-needle injection technology is therefore proposed as a means to improve the quality of a whole muscle fish product, such as Pacific whiting fillets. Two ingredients that successfully inhibit protease activity in a minced Pacific whiting product are dried egg white and dried potato extract (Morrissey and others 1993; Weerasinghe and others 1996). Therefore, the main objective of this study was to evaluate whether these ingredients when incorporated in a brine and subsequently injected into a whole muscle product could be distributed sufficiently and at a high enough concentration to inhibit protease activity and improve the texture of Pacific whiting fillets.

3.3 Material and Methods

3.3.1 Raw Material

Pacific whiting (Merluccius productus) were obtained from Pacific Seafood (Astoria, OR, USA). All product was stored on in a seawater/ice slurry on the vessel less than 24h and obtained as the product was being off-loaded to the processing plant. Fish were transported immediately to the OSU Seafood Laboratory in an insulated container in ice (transportation time <10 min), eviscerated and cut into butterfly fillets, placed in vacuum packed bags in one layer and immediately frozen at
-30 °C until injection. Vacuum refined granulated salt (Morton Salt Inc., Chicago, IL), sodium tripolyphosphate (Nutrifos-088, Integra Chemical, Kent, WA), dried egg white (P-110, Henningsen Food, Omaha, NE), dried potato extract (NP-3 Potato extracts, packed by Pacific Blends Ltd, Port Coquitlam, BC, Canada), and xanthan gum (packed by Pacific Blends Ltd, Port Coquitlam, BC, Canada) were used for brine preparations.

3.3.2 Injection

Previously frozen butterfly fillets (n=10/treatment) were used for injection. Fillets were thawed at room temperature (23 °C) for 45 min before injections process. All fillets were patted dry with paper towel prior to weighing process so the purge water from thawing was not accounted for during injection. Initial weight was recorded ($w_0$). Fillets were placed skin-side down and injected at speed 5 at 0.5 bar using an IMAX 350 Injector (Wolf-Tec Inc., Kingston, NY) with 50 hypodermic style needles. The brine uptake target was a 10% weight increase relative to the original weight. The base treatment brine, B, consisted of 3% salt and a 3% sodium polyphosphate granular blend. The treatment brine $B_{EW}$ contained the base brine with 3% dried egg white, and the treatment brine $B_{PE}$ contained the base brine with 3% dried potato extract and 0.1% xanthan gum. Xanthan gum was added to $B_{PE}$ to aid in suspension of the dried potato extract. Non-injected fillets (C) were evaluated as well. Fillets were allowed to equilibrate for 30 min in 2–3 °C and then re-weighed ($w_1$). The determination of brine uptake was calculated: $(w_1-w_0)/w_0 \times 100\%$. Fillets were subsequently vacuum packaged individually, and stored at -18 °C after treatment. For color analysis, four butterfly fillets were subsequently thawed overnight (8–12 hours)
in a refrigerator, color was measured and then fillets were re-frozen. Chemical analysis, 8 butterfly fillets were thawed and re-frozen and then thawed again for homogenization. In addition, 2 butterfly fillets were thawed and then homogenized. For texture analysis, 4 butterfly fillets were thawed and re-frozen and then thawed again and re-frozen. Color analysis was conducted on the whole fillet. Chemical analysis was conducted on either half of a fillet from a fish (n=7) or from the entire butterfly fillet (n=3). For texture analysis was conducted on half of a fillet from a fish (n=4) per treatment. Each Fillet assigned to the chemical evaluation group was homogenized by dipping in liquid nitrogen and blending with a Waring blender (Waring Commercial, Torrington, Connecticut, USA) as previously described by Parsons and others (2011).

### 3.3.3 Color Analysis

Color was determined in duplicate on the dorsal (nearest to the head), middle, and caudal (nearest to the tail) sections of the fillet using a Minolta chromameter CM 700d (Osaka, Japan) calibrated to a standard white plate. Measurements on each section Lightness (L*), redness (a*), and yellowness (b*) values were recorded from the device. The illuminant was D65 with 10° observer. Only the Specular Component Excluded (SCE) value, or fish color as perceived by human eyes was evaluated.

### 3.3.4 Chemical Analysis
3.3.4.1 Moisture and Protein Content Characterization

The moisture content was determined by AOAC method 950.46 (AOAC 2005). Samples were weighed to 3.0 ± 0.050g then dried at 105 °C overnight (8–12 hours) until constant weight were obtained. The total crude protein content was determined by Kjeldahl method according to AOAC method 981.10 (AOAC, 2005). Moisture and protein were determined in each fish of the treatments (n=10).

3.3.4.2 Thiobarbituric Acid Reactive Substance Analysis (TBARs)

TBARs (Thiobarbituric Acid Reactive Substance) was used to measure secondary products of lipid oxidation. The principle is that one molecule of malondialdehyde (MDA) will react with two molecules of thiobarbituric acid and produce a pink color that can be measured at 535nm (Buege and Aust 1978). The evaluation was conducted as described by Cerruto-Noya and others (2009) with slight modification. A 5 g sample was homogenized with 15 mL of deionized water for 60s (Kinematica CH-6010, Kriens, Lucerne, Switzerland). After centrifugation for 10 min at 3000 xg at 4°C (J6-M1, Beckman Instruments Inc., Fullerton, California, USA), 2 mL of the supernatant was mixed with 4 mL of 20 mM thiobarbituric acid and 15% trichloroacetic acid solution (TCA) and 100 µL of butyhydroxyanisol (BHA). Tubes were then heated at 100°C for 15 min. The pink color that formed was measured using a UV–VIS spectrophotometer (UV-2401PC, Shimadzu, Kyoto, Japan) at 531 nm. The results were expressed as mg MDA/kg of sample. TBARs determinations for each sample were performed in duplicate.
3.3.4.3 Protease Analysis

All ten fish fillets from each treatment were measured for protease activity. Cathepsin L protease activity was measured as described by An and others (1994) with slight modifications. 12 g powdered fish were weighed into 50 mL centrifuge tube then 8 mL of 0.1% Brij was added to tube. Samples was homogenized using Polytron homogenizer (Kinematica CH-6010, Kriens, Lucerne, Switzerland) for 1 min at maximum speed then centrifuged 20 min at 17000 xg at 4 °C (Avanti J-25, Beckman Instruments Inc., Fullerton, California, USA). Supernatant was collected for further analysis (enzyme and protein assays). For enzyme assay, Zhe-Phe-Arg-7-amido-4-methylcoumarin (Z-Phe-Arg-NMec) was used as the substrate. The intensity determination was measured using spectrofluorescence read at excitation 370 nm and emission at 460 nm (Perkin Elmer LS-50B, Waltham, MA). The fluorescence intensity after the reaction can be quantified and defined as unit enzyme activity (Barret and Kirshke 1981). The reaction was measured using two different testing conditions. First, the buffer activator at pH 5.5 was used as the optimum conditions for Cathepsin L. In the second experiment, deionized (DI) water was used in place of the buffer to mimic the pH treatment of each fish. Protease activity was measured in duplicate from each treated fillet.

3.3.4.4 Bradford Protein Assay

Protein was assayed (n=10/treatment) in duplicate using the Quick Start™ Bradford 1× Dye Reagent Protein Assay #5000201 (Bio-Rad, Hercules, CA).
Supernatant from the sample extraction described for the protease assay or SDS-PAGE analysis was diluted with deionized water (dd H₂O) at a ratio of 0.1:3.4 mL and then tested for protein content. The supernatant was diluted using dd H₂O to reduce the concentration of interfering compounds from the extraction buffers, i.e. SDS or Brij that can interfere with protein determination. The absorbance was measured at 595 nm using spectrophotometer UV VIS 2401 PC (Shimadzu, Kyoto, Japan).

3.3.4.5 SDS-PAGE Analysis

Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) was conducted as outlined by Laemmli (1970). Briefly, a 0.4 ± 0.01g sample (n=2/treatment) was mixed with 10 mL extraction buffer (10 mM sodium phosphate, 2% SDS, pH 7.0). Samples were homogenized for 1 min at max speed (Polytron CH-6010, Kinematica, Bohemia, NY) then centrifuged 20 min at 17000 xg at 4 °C (Avanti J-25, Beckman Instruments Inc., Fullerton, California, USA). The supernatants were collected, protein content measured (see 3.3.4.5), adjusted to 4 mg/mL using 5% SDS and 15 µL was loaded onto 10.5–14% Tris-HCl criterion precast gel (Bio-Rad, Hercules, CA). Precision Plus Protein™ All Blue Protein Standards #1610373 (Bio-Rad, Hercules, CA) was used as the reference standard. Completed electrophoresis gels were washed with distilled water three times for 5 min. Gels were then placed in 50 mL of 0.125% (w/v) Coomassie dye (Brilliant Blue Coomassie G-250, Bio-Rad) and gently shaken on an orbital shaker at 52 °C for 60 min. The gels were then de-stained using 100 mL of 50% (v/v) methanol and 10% (v/v) glacial acetic acid for 1 h at 52 °C on an orbital shaker (3528-5 Orbit Enviro-Shaker, Lab-Line Instrument Inc., Melrose Park, IL). After
1 h, the gels were placed into 100 mL of 20% methanol (v/v) and 10% (v/v) glacial acetic acid for another hour, after which the gels were placed in 100 mL of distilled water for 60 min. Molecular weights were determined by comparing the relative band mobility of a molecular standard with Gel Doc XR System (Bio-Rad, Hercules, CA).

### 3.3.5 Texture Analysis

In a cold room (2 °C), the dorsal section of fillets selected for texture analysis (n=4/treatment) were partially thawed for about 2h (-1.0 °C internal temperature) and cut into one piece of 5.0 × 2.5 cm cubes. Cube thickness varied (1.5–2.5 cm) based on location and treatment. Cubes were allowed to fully thaw (~2 h, end point temperature 0 °C internal temperature). Cubes were kept on ice immediately prior to TPA analysis. Texture attributes were measured using Texture Analyzer TA-XT2 (Stable Micro Systems Ltd, Surrey, UK) using a Warner-Bratzler shear (3 mm, 73° V cut) (Veland and Torrissen 1999). The blade moved down with a constant speed 2.00 mm/s, with 0.2 N trigger force (Lee and Park 2016). Trigger force is an initial resistance of a value before the blade moved down to cut samples. The breaking force value was recorded from Warner-Bratzler shear measurement in g unit.

### 3.3.6 Statistical Analysis

All results, except color, were analyzed using One-Way ANOVA in SigmaPlot with treatment as the main factors (Sigma-Plot, San Jose, CA). For color Two-Way ANOVA was performed with the different parts of the fillet as a second factor. The effect of treatment on all parameters was considered to be significant at $P < 0.05$. The
least significance difference (LSD) was used to determine if significant differences occurred among treatments.

3.4 Results and Discussions

3.4.1 Enhancement and sample preparation

Enhancement was conducted on previously frozen fillets to mimic the most likely commercial application. Currently, primary processors either headed and gutted and shipped whiting frozen to one or two secondary processors. In addition, fish being frozen and thawed multiple times as they go from one processing plant to the next is not uncommon. This was done in order to mimic a multiple freeze-thaw scenario and insure that all treatments were handled equitably so comparisons across treatments could be made. As this was study was intended originally to be a preliminary investigation, some samples were not strictly handled in similar manners. Two fillets for each treatment did not go through 3 freeze-thaw cycles prior to homogenization, they only experienced two freeze-that cycles. However, these fillets were utilized only for chemical analysis and therefore should not have impacted overall results. A statistical evaluation subsequently verified this assumption.

The injected fillets weights were targeted to increase 10%, relative to green weight, following brine injection. However, actual brine incorporation was $12.35 \pm 2.2\%$, which was higher than the original target. Significant differences between treatments ($P = 0.03$) were observed. $B_{EW}$ had lower brine uptake ($10.5 \pm 1.6\%$) than $B$ ($12.4 \pm 1.7\%$) and $B_{PE}$ ($12.4 \pm 1.2\%$), but there was no statistical evidence that $B$
differed compared to BPE. This result suggested that mixing either 3% dried egg white
or dried potato extract to 3% salt and 3% sodium phosphate resulted in well-mixed
brine that could be steadily circulated into injector systems. The lower value of BEW
indicated that egg white acted differently than potato extracts in the brine. A possible
explanation of this result might be that the viscosity of BEW is higher than B or BPE, in
which increased viscosity resulted in lower brine deliverance through the needle
during the injection process. This phenomenon was anticipated; dried egg white in
food processing can act as emulsifying, foaming, or thickening agent. Nevertheless,
Campbell and others (2003) noted that spray-dried egg white can improve functional
properties of food due to its emulsifying ability and water binding capacity.

3.4.2 Color

On average, the CIE L* values of C were significantly higher than BEW or BPE (P
= 0.032), but showed no difference compared to B (Appendix B). The changes of light
reflection from the C treatment fillets might be associated with a more open protein
structure in the fillets (Damodaran 2008). Kin and others (2010) reported CIE L*
values of catfish fillets that were injected with agglomerated phosphates were lower
than non-injected fillets. They explained enhanced water holding caused by the brine
containing phosphates decreased lightness because there is less liquid on the surface
to cause light scattering.

The darker color of BEW or BPE fillets was more likely to be affected by the
addition of either egg white or potato extract. Moisture content and brine uptake data
support this conclusion because B had higher or equivalent brine uptake and moisture content, yet lighter color.

Evaluation of color on different sections of the fillet (Appendix B) indicated that for B injected fillets, the middle was significantly different than the anterior \( (P = 0.009) \) while other treatments showed no differences between sections. There is a trend that the middle part of injected fillets had lower CIE L* value than other parts of the fillets (Table 3.2), which may be due to the needles delivering more brine to the middle part of the fillets.

Raw fillets injected with \( B_{PE} \) had higher CIE a* (redness) values than \( B \) \( (P < 0.001) \), but were not different from C or \( B_{EW} \). The CIE a* values were in the range of -1.44 to 4.34. The low a* values in Pacific whiting can be attributed to its lack of pigmentation. Kristinsson and others (2007) noted that the type and quantity of heme proteins mostly determined color in fish. Two heme proteins that are responsible for fish color are myoglobin (pigment in muscle) and hemoglobin (pigment in blood). Since Pacific whiting is categorized as white fish, lower CIE a* values are expected. For the CIE b* (yellowness), raw fillets injected with \( B_{EW} \) had higher b* (yellowness) values than \( B \) \( (P < 0.001) \), but were not significantly different than C or \( B_{PE} \). Again, the color changes in CIE b* are likely due to the ingredients delivered through the injection process.
3.4.3 Fillet chemical characterization

3.4.3.1 Moisture and Protein Content

Moisture analysis differed between treatments ($P < 0.001$) (Table 3.1). Fillets treated only with salt and phosphate (B) had higher moisture content than other treatments. Additionally, there were no significant differences between C, $B_{EW}$, and $B_{PE}$. For fillets treated with protease inhibiting ingredients, these results suggest that there was a possibility for protein-protein interaction between myofibrillar protein and either egg white or potato extract that interfered with moisture retention. Protein content of non-injected fillets was higher than the injected fillets ($P < 0.001$) (Table 3.1). Fillets treated with B had lower protein content than other treatments. There were no significant differences between $B_{EW}$ and $B_{PE}$, but B is different from $B_{EW}$ ($P < 0.001$) and $B_{PE}$ ($P = 0.027$). The finding suggested that adding brine reduced the protein content in the fillets as would be expected since moisture content increased (Table 3.1). This confirms the association between moisture and protein content in injected cod ($Gadus morhua$) fillets as previously reported by Thorarinsdottir and others (2003).

3.4.3.2 TBARs

TBARs values of injected fillets ranged from 0.23—0.55 mg/kg tissue (data not shown in tabular form). There were no significant differences between injected samples and non-injected samples ($P = 0.08$). At TBARS value between 12—20 µmol/kg
tissues (0.86–1.44 mg/kg tissue), rancidity was detected in surface tissue from mackerel fillets, as indicated by sensory evaluation (Richards and others 1998). Thus, it can be suggested that no rancidity was observed on those Pacific whiting fillets during the time they were frozen for this study. Furthermore, Nelson and others (1985) indicated that on average the fat content of Pacific whiting fillets from coastal waters of Washington, Oregon and California are 1.6 ± 0.74%. This evidence also suggested that Pacific whiting has lower lipid content in the fillets. In addition, brine ingredients did not seem to contribute to oxidation. Salt is likely promote oxidation at an ionic strength 0.6 M (3.5%) but slightly inhibited lipid oxidation at ionic strength 0.1 M (0.6%) on beef heart surimi-like material measured at pH 6.0 during refrigerated storage at 6 °C (Srinivasan and Xiong 1996). Additionally, phosphate has been demonstrated to inhibit oxidation in restructured meat products during frozen storage (Akamittah and others 1990). The phosphate delivered in the base brine, in combination with a salt content <0.1 M appears to not have contributed to significant oxidation in the injected fillets.

3.4.3.3 Proteolytic Activity

The cathepsin degradation study of Pacific whiting fillets indicated that cathepsin B was the most active cysteine protease with the highest activity at 20 °C whilst cathepsin L showed highest activity at 55 °C (An and others 1994). Despite the fact that cathepsin B is more prominent in Pacific whiting fillets, in this particular study we only measured cathepsin L activity because we wanted to further characterize the
impacts of the ingredients on cooked fillets. An and others (1994) suggested that cathepsin L most likely affects firmness in the fillet during cooking. Furthermore, Turk and others (2012) suggested that cathepsins located outside of lysosomes could be irreversibly inactivated at neutral pH very quickly. Thus, it was hypothesized that maintaining physiological pH of fish muscle during post mortem storage using the brine ingredients selected for this study might prevent the activation of lysosomal cathepsins. Phosphate can also act as a buffer to maintain pH above optimal cathepsin B (3.5–6.0) and L (5.0–6.0) activity. Furthermore, egg white and potato extract also contain competitive inhibitors that inhibit cysteine proteases such as cathepsin L.

The cathepsin L activity of all treatments is illustrated in Figure 3.2. At pH 5.5, the results indicated that cathepsin L activity in injected fillets, on average, was lower than in non-injected fillets at $P = 0.06$. The results also suggested that there was a significant difference at the 90% confidence level (CL) but not at 95%. In addition, fillets injected with $B_{PE}$ had higher enzymatic inhibition than either $B_{EW}$ or $B$. When water was used as a replacement for buffer, cathepsin L activity of all treatments decreased by 50%. There were also no significant differences among treatments ($P = 0.63$). However, the cathepsin L activity of non-injected fillets (0.7 ± 0.2) was numerically higher than all brine injected fillets (0.35 ± 0.14). All brine treated samples had lower cathepsin L activities probably due to the neutral pH from the brine. This finding supported Turk and others’ (2012) hypothesis that stated that cathepsins located outside of lysosomes could be irreversibly inactivated at neutral pH very quickly.
3.4.3.4 Electrophoretic Pattern

The proteolytic degradation pattern of muscle protein analyzed by SDS-PAGE (Figure 3.3) indicated that the bands of myosin heavy chain (MHC), actin, troponin and tropomyosin appeared in both non-injected and injected fillets. It has been demonstrated that among the Pacific whiting proteins, MHC was the most extensively hydrolyzed, followed by troponin-T and β-tropomyosin (Benjakul and others 1997). However, the finding of this study depicted MHC, troponin-T and β-tropomyosin in similar pattern, with the disappearance of the band below 20 kDa on every lane except for B2, B EW2 and B PE2 (Figure 3.3). This study suggested that brine incorporation through a multiple needle injector might distribute protease inhibitor uniformly within fish muscle, preventing cathepsin L activity in Pacific whiting muscle.

3.4.4 Texture

Figure 3.1 presents the firmness of Pacific whiting raw fillets expressed as force (g) necessary to compress samples to 40%. There was no significant difference in breaking force values among the injected raw fillets. This phenomenon could be related to the amount of salt and phosphate in fish muscle. The incorporation of salt and phosphate alters myofibrillar structure and is known to improve water holding ability in muscle systems. When salt penetrate to fish muscle through multi needle injection, swelling and hydration occured. The negatively charged chloride ion (Cl⁻) preferentially binds to the positively charged amino acids from the myofibril (Hamm
and Deatherage 1960). Electrostatic repulsion between the remaining negative charged amino acid side chains increases, allowing more hydration in the muscle (Hamm and Deatherage 1960). Moreover, sodium tripolyphosphate is highly alkaline (pH=9.8). The incorporation of sodium tripolyphosphate will also increase the pH of fish fillet. As the pH increases, the protein is shifted further away from its isoelectric point, further enhancing electrostatic repulsion and hydration in the muscle. Since non-injected products had less moisture, it was not surprising that they had higher breaking force than injected products. However, there was no difference between treatments ($P = 0.36$). Interestingly, there was more variability in the breaking force values of non-injected fillets than injected fillets (Figure 3.1). This suggests that fillet firmness was more uniform and consistent in injected fillets than in non-injected fillets. These results are supported by those observed by Kin and others (2010). They showed that all phosphate treatments had lower shear force value ($P < 0.05$) when compared to the non-injected treatment, but no differences ($P > 0.05$) existed among phosphate treatments.

### 3.5 Conclusion

Color was impacted by brine treatment, however, ingredient injected fillets were not significantly different in lightness when compared to the non-injected treatment. Injection technology can be utilized to incorporate protease inhibitor ingredients (egg white and potato extract) at levels sufficient to reduce protease activity in the fillet. This was further supported by the muscle protein patterns of Pacific whiting analyzed by SDS-PAGE being more likely to be intact in the injected
fillets than in the non-injected fillets. All brines produced similar results with respect to raw fillet texture hardness. Results suggest feasibility to further explore the application of protease inhibitors by determining their effect in a cooked product.
Table 3.1 Main effect least square of Moisture and Protein Content of Pacific whiting injected fillets

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Moisture (%)</th>
<th>Protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>82.2 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.7 ± 0.8&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>84.1 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.7 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>BEW</td>
<td>82.6 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.9 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>B&lt;sub&gt;PE&lt;/sub&gt;</td>
<td>82.8 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.4 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup> Means within a column and main effect with different superscripts are significantly different (\( P < 0.05 \ ± \ \text{standard deviation, N=10} \)). C = Control, non injected fillet; B = Brine, consist of 3% salt and 3% sodium tripolyphosphate (STTP); B<sub>EW</sub> = Brine plus 3% egg white; B<sub>PE</sub> = Brine plus 3% potato extract and 0.1% xanthan gum.
Table 3.2  Main effect of least square of CIE color measurement of Pacific whiting fillets, in middle fillet sections

<table>
<thead>
<tr>
<th>Treatment</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>33.7 ± 8.7\textsuperscript{y}</td>
<td>1.37 ± 1.3\textsuperscript{xy}</td>
<td>3.10 ± 2.1\textsuperscript{y}</td>
</tr>
<tr>
<td>B</td>
<td>24.9 ± 5.6\textsuperscript{xy}</td>
<td>0.14 ± 0.8 \textsuperscript{x}</td>
<td>0.25 ± 1.6 \textsuperscript{x}</td>
</tr>
<tr>
<td>B\textsubscript{EW}</td>
<td>22.8 ± 5.0\textsuperscript{x}</td>
<td>1.2 ± 1.6 \textsuperscript{xy}</td>
<td>2.8 ± 2.7 \textsuperscript{y}</td>
</tr>
<tr>
<td>B\textsubscript{PE}</td>
<td>23.7 ± 2.0\textsuperscript{x}</td>
<td>2.3 ± 0.3 \textsuperscript{y}</td>
<td>3.6 ± 0.3 \textsuperscript{y}</td>
</tr>
</tbody>
</table>

\textsuperscript{x,y} Means within a column and main effect with different superscripts are significantly different between treatments \((P < 0.05, \text{ mean± standard deviation, n=4})\). C = Control, non injected fillet; B = Brine, consist of 3% salt and 3% sodium tripolyphosphate (STTP); B\textsubscript{EW} = Brine plus 3% egg white; \textsubscript{BPE} B = Brine plus 3% potato extract and 0.1% xanthan gum.
Figure 3.1  Texture measurement of thawed Pacific whiting injected fillets (P > 0.05). C = Control, non injected fillet; B = Brine, consist of 3% salt and 3% sodium tripolyphosphate (STTP); BEW = Brine plus 3% egg white; BPE = Brine plus 3% potato extract and 0.1% xanthan gum.
Figure 3.2 Protease activity of Pacific whiting raw injected fillets (Means for each treatment at pH 5.5 (p=0.06) and pH 7.0 (p=0.63). C = Control, non injected fillet; B = Brine, consist of 3% salt and 3% sodium tripolyphosphate (STTP); BEW = Brine plus 3% egg white; BPE = Brine plus 3% potato extract and 0.1% xanthan gum. * showed significant difference with other treatments (P < 0.1) at similar pH.
Figure 3.3  SDS-PAGE patterns of Pacific whiting raw injected fillets (R=reference standard protein Kaleidoscope Precision Blue (Biorad, Hercules, CA), C = Control, non injected fillet; B = Brine, consist of 3% salt and 3% sodium tripolyphosphate (STTP); B_{EW} = Brine plus 3% egg white; B_{PE} = Brine plus 3% potato extract and 0.1% xanthan gum.

MHC=Myosin Heavy Chain

AC=Actin

TM=Tropomyosin

TNT=Troponin T
References


4 DETERMINING APPROPRIATE BRINE CONCENTRATIONS TO IMPROVE THE QUALITY OF PACIFIC WHITING (*Merluccius productus*) TEXTURE BASED ON COOKED FILLETS PROPERTIES

4.1 Abstract

Pacific whiting fillets were injected with different brine treatments to evaluate their effectiveness in inhibiting protease activity in the fillet through cooking. Control samples included non-injected fillets and fillets injected with water only. All brines were formulated with 3% (w/w) salt and sodium tripolyphosphate. Brines were also tested with 1, 2, or 3% (w/w) dried egg white (EW) or 0.1% (w/w) xanthan gum (XG) and 1, 2, or 3% (w/w) dried potato extract (PE). Injected and non-injected fillets were poached in heat-sealed barrier packaging at 90 °C until the internal temperature reached 62.8 °C, where it was held for 20 min. In addition, protease inhibitor ingredient activity was challenged by first pre-heating at 60 °C for 30 min and then heated to 90 °C. The pH, moisture, crude protein, total extractable protein, total non-extractable protein, texture profile, and electrophoretic pattern in cooked fillets were evaluated. Electrophoretic evaluations demonstrated that the myosin bands slightly disappeared when fillets were pre-incubated at 60 °C prior to the 90 °C cooking period for all treatments, except in those containing protease inhibitory ingredients. The integrity of myosin bands noticeably increased with increasing EW concentration, but not in the PE treatment.

**Keywords** Pacific whiting, *Merluccius productus*, brine injection, heating, cooked fillets, protease inhibitor, cathepsin L, texture
4.2 Introduction

Over the past several decades, the injection of brines into fresh meat to achieve functional improvements in quality had been extensively studied in beef, lamb, pork, turkey and chicken (Wynveen and others 2001; Claus and others 2010; Lowder and others 2011; Roldan and others 2014). The literature on brine injection for fish is primarily focused on its application for processed foods including smoked (Almli and Hersleth 2012) or salted cod (Thorarinsdottir and others 2011). Brine can maintain tenderness and juiciness of meat products even after heating treatment (Robbins and others 2003) and is being used to replace more traditional, slower, and less controllable soaking processes. Ingredients for brine injection in fresh meat typically consist of water, salt, phosphates and other functional or flavor ingredients (Robbins and others 2003). Several studies have suggested that incorporation of marinated ingredients to meat and poultry products can be done more effectively through multi-needle injection (Detienne and others 2003; Alvarado and Sams 2004). Kin and others (2010) verified the effectiveness of various phosphates to maximize the yield of catfish fillets and improve the water holding capacity of fish fillets through multi-needle injection.

Although incorporation of brine into fish has been well studied, there is limited research on the quality of Pacific whiting injected fillets. Pacific whiting has textural quality issues due to high cathepsin L protease activity (An and others 1994; Seymour and others 1994). Fillets from Pacific whiting can experience significant softening during the cooking process not only because of high level of protease, but also
because the protease maximum for activity occurs at high temperatures (60 °C). Pacific whiting fillets, therefore, are not typically commercially available in the U.S. It is hypothesized that incorporation of brine ingredients with protease inhibitory activity will improve fillets quality by inhibiting the action of natural endogenous and exogenous protease in whiting through cooking. The effectiveness of protease inhibitors has been demonstrated in processed seafood products such as surimi (Morrissey and others 1993; Weerasinghe and others 1996). There is a very limited literature on a similar approach being used with fillets since injection systems were originally developed to incorporate brines into large products such as hams and loins. Recent advancements, however, have significantly improved the system to the point where injection in small delicate fillets is now feasible. Kang and Lanier (2005) attempted a similar approach to improving the quality of arrowtooth flounder (Atheresthes stomia). Like Pacific whiting, arrowtooth flounder contains significant protease activity. They concluded that arrowtooth flounder fillets injected with recombinant cystatin had a better texture than fillets marinated with same ingredients. They also stated that there was not a noticeable effect on the injected fillet color regardless of cook treatment.

Dried egg white and dried potato extract are GRAS ingredients which have been successfully demonstrated to inhibit protease activity in pacific whiting mince (Morrissey and others 1993). Preliminary results in our laboratory demonstrated that potato extract can effectively reduce cathepsin L activity in the raw fillet at an equivalent concentration as egg white (private measurement). However,
effectiveness in the raw fillet may not translate to the cooked fillet. Texture profile analysis (TPA) was used as a quality parameter in this study since it might indicate the sensory quality of injected fillets.

The objective of this study was to determine the level of potato extract and egg white needed to improve the quality of cooked Pacific whiting fillets. Two heating protocols were utilized to evaluate the impact of temperature and were based on a previous study by Kang and Lanier (2005). The first heating treatment mimicked a sous vide style of preparation, the second was used to challenge the effectiveness of the protease inhibitor in preventing fillet softening during cooking. The aim was to accelerate whiting meat degradation at an optimum temperature of cathepsin L activity (55–60 °C).

4.3 Material and Methods

4.3.1 Raw Materials

Pacific whiting (Merluccius productus), <24 hours of postharvest, were obtained from a local processor in July 2015. They were 27–30 cm in length, average weight 235 ± 40 g. Fish were transported to the OSU Seafood Laboratory in an insulated container in ice (transportation time <15 min). Fish were eviscerated, butterflied and immediately frozen at -18 °C. Vacuum refined granulated salt (Morton Salt Inc., Chicago, IL), sodium tripolyphosphate (Nutrifos-088, Integra Chemical, Kent, WA), dried egg white (P-110, Henningsen Food, Omaha, NE), dried potato extract
(NP3, packed by Pacific Blends Ltd, Port Coquitlam, BC, Canada) and xanthan gum (Pacific Blends Ltd, Port Coquitlam, BC, Canada) were used for preparation of brines.

### 4.3.2 Fish Injections

Prior to injection, butterflied fillets were thawed for 10–14h in a cold room (2.0 ± 1.0 °C). The final internal temperature of the butterflied fillet ranged from 0–1 °C. All injections were conducted in a cold room 3.0 ± 1.0 °C. Ten treatments were evaluated (Table 4.1): 1. Non-injected (NI), no injection; 2. Injected with Water (W); 3. Brine 3% salt and 3% sodium tripolyphosphate (w/w) (B); 4. Brine plus 1% Egg White (B_{1EW}), 5. Brine plus 2% egg white (B_{2EW}); 6. Brine plus 3% egg white (B_{3EW}); 7. Brine plus 0.1 xanthan gum (B_{XG}); 8. Brine plus 1% potato extract and 0.1% xanthan gum (B_{1PE}); 9. Brine plus 2% potato starch and 0.1% xanthan gum (B_{2PE}); 10. Brine plus 3% potato starch and 0.1 xanthan gum (B_{3PE}). Brine target uptake was 10% (w/w) of initial weight. Fish were injected using a multichannel pipette fitted with 22-gauge hypodermic needles (Eppendorf, Hauppauge, NY, USA). Butterfly fillet weight was obtained immediately prior to injection (w_0). In addition, weight was re-measured after an equilibration period of 30 min following injection (w_1). Brine uptake was calculated \((w_1-w_0)/w_0 \times 100\) (Table 4.1). Following brine equilibration, butterfly fillets were halved and each half was placed individually in a non-air permeable bag (Oxygen transmission rate 24h at 23 °C is 63 cc/sq.m, Summit Packaging, Auburn, WA, USA), vacuum with a 0.9 bar (setting at 7) and sealed for 15 s (setting at 7) (Reiser, Canton, MA) and stored on ice for ±30 min prior to being randomly assigned for cooking in one of two different heating protocols.
4.3.3 Heating Conditions

Heating protocols were adapted from Kang and Lanier (2005). Preliminary studies were conducted to verify time to reach 62.8 °C for protocol 1 and time to hold at 60 °C for protocol 2 (Appendix C). Internal temperature was measured using a k-type thermocouple and measurements were taken every 5 sec. Individually packaged fillet halves were cooked in their package. For heating protocol 1, one packaged fillet (-2 ± 1 °C) was submerged in approximately 20 L at 90 °C using a recirculating water bath (WB1120 Lindberg Blue M, Waltham, MA) for 20 min with the lid cover on. The targeted internal temperature was 62.8 °C (145 °F), the safe minimum internal temperature as suggested by USDA. For heating protocol 2, one packaged fillet (-2 ± 1 °C) was submerged in approximately 16 L at 60 °C in a non-recirculating water bath (Precision shaking waterbath model 25, Thermo Scientific Inc., Waltham, MA) for 30 min (lid cover on) and then immediately transferred to a recirculating water bath at 90 °C for 20 min. The aim was to accelerate whiting meat degradation at an optimum temperature of cathepsin L (55–60 °C). Following heat treatment, fillets were submerged in an ice slurry for 15 min (average temperature of ice slurry was -1.0 °C) then immediately frozen (-18 °C) and stored at -18 °C until further analysis.

4.3.4 Chemical Analysis

4.3.4.1 pH, Moisture Content, and Protein Content

Cooked fish pH was measured using a pH meter (Accumet® Excel XL15, Fisher Scientific, Pittsburg, PA). One gram of cooked sample was homogenized with 10 mL
of deionized water (Sheard and others 1999). The moisture content was determined by AOAC methods 950.46 (AOAC 2005). Samples were weighed 3.0 ± 0.05 g then dried at 105 °C overnight (12–18 hours) until the constant weight were obtained. The total crude protein content was determined by Kjeldahl method according to AOAC method 981.10 (AOAC 2005).

4.3.4.2 Total Extractable Protein Content and Total Non-Extractable Protein Content

Samples were weighed 0.6 ± 0.02 g into centrifuge tubes. Samples were extracted with 7.5 mL buffer contain 8 M Urea, 2% SDS (sodium dodecyl sulphate), 2% β-mercaptoethanol, and 20 mM Tris-HCl as outlined by Pongviratchai (2002). The total extractable protein content was measured using Quick Start™ Bradford Protein Assay Kit 1 #5000201 (Bio-Rad, Hercules, CA) using bovine serum albumin (BSA) as a standard (Quick Start Bovine Serum Albumin Standard #5000206 Bio-Rad, Hercules, CA). Supernatant from the sample extraction described above was diluted with deionized water (dd H₂O) at a ratio of 0.1:3.4 mL and then tested for protein content. The supernatant was diluted using dd H₂O to lessen the concentration of interfering compounds from the extraction buffers, i.e. Urea, SDS, β-mercaptoethanol, and Tris-HCl that would interfere with protein determination. The absorbance was measured at 595 nm using spectrophotometer UV-VIS 2401 PC (Shimadzu, Kyoto, Japan). Total Non-Extractable Protein content was calculated by subtracting the total extractable protein content from total protein content based on Kjeldahl analysis.
4.3.4.3 Electrophoretic Pattern

The supernatant from total extractable protein procedure was then further analyzed with Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) as outlined by Laemmli (1970) using 10% TGX criterion precast gel (Bio-Rad, Hercules, CA). The protein content of samples was adjusted to 4 mg/mL using 5% SDS and loaded 15 µL into the well. Kaleidoscope precision plus protein™ all blue standard #1610732 (Bio-Rad, Hercules, CA) was used as a protein standard. Completed electrophoresis gels were washed with distilled water for 5 minutes three times. Gels were placed in 50 mL of 0.125% (w/v) Coomassie dye (Brilliant Blue Coomassie G-250, Bio-Rad) and gently shaken on an orbital shaker heated to 52 °C for 60 min. The gels were then destained using 100 mL of 50% (v/v) methanol and 10% (v/v) glacial acetic acid for 1 h while being heated to 52 °C on an orbital shaker (3528-5 Orbit Enviro-Shaker, Lab-Line Instrument Inc., Melrose Park, IL). After 1 h, the gels were placed into 100 mL of 10% methanol (v/v) and 10% (v/v) glacial acetic acid for another hour, after which the gels were placed in 100 mL of distilled water for 60 min. Molecular weights were determined by comparing the relative band mobility of a molecular standard with Gel Doc XR System (Bio-Rad, Hercules, CA).

4.3.5 Textural Measurement

Cooked fillets were thawed in a cold room (3 ± 1 °C) for about 2h. Partially thawed cooked fillets (average temperature around -1.1 °C) were cut (Figure 4) into approximately 2.5 × 2.5cm (L x W) cubes. Thickness of the cubes roughly varied from 1.5–2.5 cm due to natural variations in fillet structure and brine treatment. Skin was
subsequently removed from cubes. Cubes (n=3/fillet) were equilibrated in a cold room (3 ± 1 °C) before measurement for about 12h (Kang and Lanier 2005) A 2-cycle compression test was conducted using a cylindrical stainless steel probe of 3-inch diameter. Texture Profile Analysis was applied using TA-XT2 Texture Analyzer (Stable Microsystems, Godalming, Surrey, UK). The analyzer was calibrated to the load cell 1 kg and the height of the probe was adjusted to 15 cm. The test conditions were two consecutive cycles of 30% compression with 5s between cycles. The crosshead moved at the constant speed of 1 mm/s (Skipnes and others 2008; program details in Appendix D). Force by time data from each test was used to calculate mean values for the TPA parameters. The values for hardness, cohesiveness, springiness, adhesiveness, chewiness, and resilience were determined from samples. Pacific cod fillets (*Gadus macrocephalus*) were used as a reference sample and procured frozen from a local grocery store in Astoria, OR (Safeway, Inc.).

### 4.3.6 Statistical Analysis

The two factorial complete randomized block design with three replications was used in this experiment. Blocking was the day of the injection process. The first factor was the brine compositions and the second factor was heating conditions. In total, 40 fish were used. Two-way analysis of variance (ANOVA) was conducted to understand the effects of added ingredients and heating conditions to fish fillets. Multiple comparisons of the mean according to the least significance difference method (LSD) were analyzed using SigmaPlot 13 (San Jose, CA, 2015). Treatments were significantly different if the p value was less than 0.05. Correlation analysis were
conducted to determine whether there is a correlation between each attribute of TPA to physicochemical measurements of those fillets.

4.4 Results and Discussions

4.4.1 Enhancement

Previously frozen fillets were injected as this form of the product would be utilized at a commercial facility. Currently, Pacific whiting is delivered fresh to a primary processor where it is headed, gutted, frozen and shipped to a secondary processor for value-added processing. The base brine was formulated to contain 3% salt and 3% sodium tripolyphosphate based on reported literature (Bigelow and Lee 2007; Almli and Hershelth 2012), preliminary studies and discussions with phosphate suppliers. Sodium tripolyphosphate (STPP) is the most common form of phosphate used in commercial fish products (private communications). Preliminary investigations looked at brine uptake and flavor of fish injected with brines containing 3.6% and 4.5% STPP. However, flavor of the subsequently cooked product was clearly impacted by the higher levels of salt and STPP. The levels selected for this study were levels that did not clearly impact flavor yet still supported sufficient brine uptake.

The injected fillets were targeted for a 10% increase over green weight. The highest solution uptake was \( B_{2PE} \) (11.4 ± 1.4%) while the lowest was \( B_{XG} \) (9.0 ± 2.3%) (Table 4.1). No differences existed between treatments (Table 4.1, \( P = 0.225 \)). Additionally, the results present a good basis for treatment comparisons as suggested by Lowder and others (2011).
4.4.2 Chemical Characterization

4.4.2.1 pH measurement

The initial pH of the fillets before injection was 6.65 (data not shown in tabular form). Table 4.1 shows the initial pH of each brine solution. The mean pH value was 8.33 (range 8.16 – 8.50). For the cooked fillet, no interactions (treatment × heating) were found in mean pH value \( (P = 0.971, \text{Table 4.2}) \). Kin and others (2010) observed that the pH of raw catfish fillets injected with sodium tripolyphosphate (STPP) and salt was numerically higher (6.48) than the pH of non-injected fillets (6.40). Oz and others (2007) observed that upon cooking the pH values of the rainbow and brown trout increased. However, there were no differences in pH \( (P = 0.224) \). Similarly, Kin and others (2010) reported that the samples containing no salt and STPP were numerically lower than all other brines.

4.4.2.2 Moisture Content

There was no treatment × heating interaction \( (P = 0.904) \) for moisture content (Table 4.2). However, the main effect of treatment or heat conditions was significant \( (P < 0.001) \). Non-injected fish (NI) had lower moisture content than injected fillets under both heating conditions. A similar observation was made for W with the exception of B_{3PE} being similar to W. The results support the idea that injection of brine containing salt and phosphate can increase water holding capacity (WHC) of fish muscle (Thorarinsdottir and others 2003). Similarly, Thorarinsdottir and others (2004)
reported that the addition of salt and/or phosphate and/or protein (soy protein/fish protein) resulted in higher water contents of the fillets than in control fillets.

Fish that were treated at 60 °C for 30 min, then 90 °C for 20 min had a lower moisture content compared to fish that were only heated at 90 °C for 20 min (Table 4.2). As previously discussed, this was expected for two reasons. First, the 60 °C treatment is a longer heat treatment. Second, this condition also promotes cathepsin L activity which should result in degradation of protein (An and others 1994) and thereby reduce the muscle’s capacity to retain water. Ofstad and others (1993) suggested that heating of cod muscle results in expulsion of water induced by denaturation of myosin and shrinkage of myofibrils. Also, particular amino acid side chains in myofibrillar protein would oxidized during cooking. The oxidation process can create intramolecular crosslinking reducing its ability to interact with water. Liu and others (2009) stated that strongly oxidized myofibrillar proteins are responsible for high water losses during cooking. Consequently, the heating process decreased immobilized and free water in meat systems, which drew protein substrates closer to the proteolytic enzymes. It seems that exposing the fish fillet to longer heating periods might cause enzymes to hydrolyze muscle protein, weakening the protein-protein interaction and resulting in higher water loss.

4.4.2.3 Protein Content

This study did not detect any evidence for two way (treatment × heating) interaction in crude protein content values. Protein content of NI and W were higher than the injected fillets (P < 0.001) (Table 4.2). Fillets treated with B had lower protein
content than other treatments. The finding suggested that added brine diluted the protein content in the fillets. In contrast to moisture content, fish treated at 60 °C for 30 min, then at 90 °C for 20 min have a higher protein content compared to fish that were only heated at 90 °C for 20 min (Table 4.2), as expected.

4.4.2.4 Total Extractable Protein (TEP) and Total Non-Extractable Protein (TNEP)

TEP and TNEP of the cooked fillet was conducted to understand whether or not brine treatment impacted the extractability of the myofibrillar protein. TEP is an indicator of protein solubility while TNEP is an indirect measurement of intermolecular networking. A high TEP value suggests higher proteolytic activity whereas a high TNEP value suggests enhanced intra- and inter- crosslinking of myofibrillar protein. Lin and others (2000) observed that the decreased protein solubility on soy protein meat analog might be due to either the establishment of a new chemical bond between the protein (i.e. non-disulfide covalent) and the soy ingredient which cannot be disrupted by solvents or the formation of high molecular weight polymers that are resistant to solubilization. Since brine treatments in this study also contained ingredients that could either promote or interfere with myofibrillar crosslinking, it was hypothesized that TEP and TNEP would highlight any changes that might occur. However, there was no significant difference in the interaction (treatment × heating) for TEP \((P = 0.799)\) nor TNEP \((P = 0.833; \text{Table 4.2})\) content. Additionally, the main effect of treatment or heat conditions was not significant \((P > 0.05)\).
Despite the fact that heating conditions did not significantly affect TEP content, data from Table 4.2 does suggest the injected products result in numerically lower TEP and higher TNEP. Similarly, pre-incubated fish at 60 °C for 30 min and 90 °C for 20 min resulted in higher TEP content than fish heated at 90 °C for 20 min. Sante-Lhoteullier and others (2007) suggested that oxidation and thermal denaturation of proteins can induce structural changes leading to an increase of protein surface hydrophobicity in myofibrillar proteins. Also, thermal denaturation induces the oxidation of myofibril protein, which in turn results in various amino acids modifications such as the formation of disulfide, dityrosine, and other intermolecular bridges that promote aggregation and polymerization (Zhang and others 2013). It can be suggested that protein-protein interactions accomplished in a shorter heating time might lead to the formation of aggregates resistant to proteolysis. Meanwhile, subjecting whiting fillets to conditions that favored cathepsin L degradation reduced protein crosslinking and polymerization.

4.4.2.5 Electrophoretic Pattern

Figure 4.3 compares the SDS-PAGE pattern of all samples. From the sizes and intensities of myosin heavy chain (MHC) bands on the SDS-PAGE gels, myosin degradation occurred extensively when fillets were cooked initially at 60 °C for 30 min. This is in agreement with protein content result which suggests that weaker protein networking can be created during the longer heating period even when total protein was higher in quantity (Table 4.2). Furthermore, from the MHC bands, it is apparent
that added protease inhibitor to brine resulted in more intense bands when comparing to fillets injected with water (Figure 4.2). Taken together, these results suggest that there is an association between brine treatment and the inhibition of myofibril breakdown by cathepsin L during cooking. Although, the hardness value of those samples (Table 4.3) did not well-correlated to the MHC density on SDS-PAGE bands. These results support those observed in the study by Kang and Lanier (2005).

4.4.3 Texture Profile Analysis (TPA)

There were no interactions (treatment × heating) for all TPA attributes such as hardness, springiness, cohesiveness, resilience, chewiness and adhesiveness (Table 4.3). Nevertheless, the main effect of treatment was significant for most of the TPA attributes ($P < 0.05$), except for adhesiveness ($P = 0.735$). Furthermore, the main effect of heating condition was significant for all TPA attributes. Table 4.3 indicated that treatment and combination of time and temperature of heating alters the texture profile of whiting fillets. All fillets that were initially heated at 60 °C for 30 min had lower mean value on all TPA attributes compare to fillet cooked at 90 °C for 20 min ($P < 0.05$). As expected cathepsin L, a heat-induced protease, plays an important role in the textural degradation of whiting fillets (Morrissey and others 1993; Seymour and others 1994). Also, the lower TPA attributes might be due to the extended heating process. Ofstad and others (1996) found decreasing WHC with increasing holding time of heating in cod. Likewise, Skipnes and others (2008) demonstrated that WHC is shown to decrease with increasing temperature. Although we did not measure WHC in this study, the measurement of moisture content could indirectly point out how the
fillets behave during the heating process. It can thus be suggested that increased holding time and temperature during heating process will reduce the moisture content of the fillets and consequently changes the texture profile of the fillets.

Szczesniak (2002) classified texture properties into a primary and secondary properties. Hardness, cohesiveness, springiness, and adhesiveness are associated with primary properties whilst chewiness and gumminess are part of secondary properties. However, Texture Technologies Corp treated resilience as a primary parameter since it is likely similar to springiness and adhesiveness as a secondary parameter since there is a suitable method to measure adhesion than with a TPA test (Texture Tech 2015).

Since the 90 °C cook protocol followed normal sous vide technique, it most closely represents how the consumer would perceive the texture attributes of treated fillets. In general, NI fillets had higher values than injected fillets. This is not unexpected as they are also from a compositional standpoint significantly different from injected fillets. This makes comparisons between injected and non-injected fillets difficult. As a result, the remainder of this part of the discussion will focus on differences found amongst the injected treatments and will compare treatment effects within cook treatment. For the 90 °C cook protocol, none of the TPA attributes in fillets injected with egg whitewere impacted by level of ingredient (Table 4.3a). The base brine, B, was essentially the control brine for egg white treatment. Treatments B₂EW cohesiveness, B₂EW and B₃EW resilience, and B₂EW chewiness were all significantly higher than B. For samples injected with potato extract only the springiness attribute
was impacted by level of ingredient. However, since \( B_{1PE} \) springiness was not different from \( B_{3PE} \), it is not conclusive that level of ingredient is truly important for springiness of cooked potato extract injected fillets. When compared to its control brine, \( B_{XG} \), only \( B_{3PE} \) resilience was higher. As a result, texture profile analysis of the 90 °C cook treatment does not clearly demonstrate that \( W \), \( B \), or \( B_{XG} \) injection treatments are inferior in texture to those containing protease ingredients. However, those brines containing egg white as a protease inhibiting ingredient do appear to have numerically higher values than all other treatments.

The intent of the 60 °C cook protocol was to stimulate cathepsin L activity in order to elucidate whether or not any protease inhibitory activity was promoted by the ingredients added to the brines. As with the 90 °C cook, level of ingredient within type did not impact TPA attributes for the 60 °C cook, except for egg white chewiness. For this attribute \( B_{3EW} \) was different than \( B_{2EW} \), but not \( B_{1EW} \). When comparing the ingredient treatment brines with their base brine treatment (\( B \) and \( B_{XG} \)), no conclusive evidence found that \( B \) was inferior to \( B \) plus egg white. This was not the case for \( B_{XG} \).

For potato extract, \( B_{3PE} \) hardness, \( B_{1PE} \) springiness, all levels of \( B_{PE} \) cohesiveness, and \( B_{3PE} \) resilience were significantly higher than \( B_{XG} \).

By comparing the 90 °C and 60 °C cook separately, it is difficult to come to conclusions on the actual benefit of ingredients added to the brines. It does appear as if the fillets injected with the \( B_{XG} \) brine are benefited by the addition of potato extract, but they do not appear to be any better in texture when compared to the \( B \) injected fillets. However, electrophoresis patterns from the TEP suggest that protease
activity is being inhibited. Therefore, in order to more clearly see the differences and, hence, resistance of injected fillets to protease activity as measured by TPA, spider graphs were constructed overlaying both the 90 °C and 60/90 °C cook (Figure 4.2). Only cohesiveness, springiness, and chewiness are shown.

Cod was included as a reference sample and demonstrates how texture in a fish with minimum protease issues responds to the cook treatments in this study. With cod, no significance differences were found between cohesiveness, springiness, and chewiness of cod fillets between two heating treatment (t-test $P > 0.05$). In contrast, the non-injected control Pacific whiting fillet, however, was clearly negatively impacted by the 60 °C heating protocol. This supports the utilization of the 60 °C protocol to demonstrate the action of potease on Pacific whiting fillets and the determination of whether or not a protease inhibiting ingredient can resist that action. Resistance to the degradative effects of the 60 °C protocol can be seen for $B_{3EW}$ and $B_{2PE}$ for the cohesiveness and springiness spider graph. For chewiness, $B_{3EW}$ and $B_{3PE}$ demonstrated a similar trend. An observation of note in all 3 selected spider graphs is the extent to which $B_{XG}$ is impacted by cooking protocol. This is especially apparent in the chewiness spider graph were $B_{XG}$ is exceedingly low when compared to all other treatments. This suggests that although xanthan gum is easily solubilized and effectively suspends the potato extract ingredient in the brine, it acts antagonistically against fillet texture even at very low concentrations in the fillet ($\sim 0.01\%$). Therefore, further study should be conducted to find the alternative replacement of xanthan gum as a suspension agent in the brine with potato extract.
4.4.4 Relationship between Texture Profile Analysis and Chemical Analysis

The correlation between all texture profile analysis and chemical analysis (moisture, TEP, and TNEP) is given in Table 4.2. All bolded values in Table 4.4 are significant correlations at $P < 0.05$. Springiness, cohesiveness, chewiness, and resilience have a significant positive correlation to each other. Hardness have a positive correlation to cohesiveness, chewiness, and resilience but showed no correlations with springiness. Hardness, cohesiveness and chewiness attributes have a significant negative correlation to moisture content. Meanwhile, there is no evidence that TEP and TNEP positively correlated to any TPA attributes ($P > 0.05$). Additionally, the result also showed that TEP has a significant negative correlation with moisture and TNEP, as expected. These combinations of findings provide some support for the conceptual premise of texture attributes to chemical properties. This finding, while preliminary, suggests that injected fillets were more chewable, cohesive and springy than the non-injected fillets due to its protein and moisture content.

4.5 Conclusions

Heating conditions justified the effectiveness of incorporation protease inhibitor with salt and phosphates to improve the textural quality of whiting fillets. Adding ingredients at all levels were able to protect the integrity of the myofibrillar structure as observed with SDS-PAGE, but not with TEP or TNEP data. Textural data was not convincing that addition of protease inhibitor at the 3% level is sufficient to justify its use over that of 1 or 2% ($P > 0.05$). Therefore, the results obtained from this
study suggested that incorporation at least 1% egg white and 1% potato extracts can be used for further quality characterization in the larger scale of injection study.
Table 4.1  Treatments Designation, Characteristics of Solution, and Brine Uptake (%) of Injected Pacific whiting (*Merluccius productus*) Fillet

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Brine Composition and Concentration</th>
<th>pH in solution</th>
<th>Brine Uptake (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>NI</td>
<td>None/non-injected</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>W</td>
<td>Water</td>
<td>7.00</td>
<td>9.3 ± 1.2</td>
</tr>
<tr>
<td>B</td>
<td>3% salt (S) + 3% sodium phosphate (SP)</td>
<td>8.50</td>
<td>9.4 ± 0.6</td>
</tr>
<tr>
<td>B1EW</td>
<td>3% S + 3% SP + 1% Dried Egg White (EW)</td>
<td>8.39</td>
<td>9.3 ± 1.6</td>
</tr>
<tr>
<td>B2EW</td>
<td>3% S + 3% SP + 2% Dried Egg White (EW)</td>
<td>8.37</td>
<td>9.8 ± 0.8</td>
</tr>
<tr>
<td>B3EW</td>
<td>3% S + 3% SP + 3% Dried Egg White (EW)</td>
<td>8.34</td>
<td>9.7 ± 1.2</td>
</tr>
<tr>
<td>BXG</td>
<td>3% S + 3% SP +0.1% Xanthan Gum (XG)</td>
<td>8.42</td>
<td>9.0 ± 2.3</td>
</tr>
<tr>
<td>B1PE</td>
<td>3% S + 3% SP + 0.1% XG + 1% Dried Potato Extract (PE)</td>
<td>8.28</td>
<td>10.6 ± 1.0</td>
</tr>
<tr>
<td>B2PE</td>
<td>3% S + 3% SP + 0.1% XG + 2% Dried Potato Extract (PE)</td>
<td>8.23</td>
<td>11.4 ± 1.4</td>
</tr>
<tr>
<td>B3PE</td>
<td>3% S + 3% STPP + 0.1% XG + 3% Dried Potato Extract (PE)</td>
<td>8.16</td>
<td>10.8 ± 1.3</td>
</tr>
</tbody>
</table>

*(Mean ± Standard Deviation)*
Table 4.2  Main effect least square means of pH, moisture, total protein, total extractable protein (TEP) content, and total non-extractable protein (TNEP) content of Injected Pacific whiting (*Merluccius productus*) Cooked Fillet

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH</th>
<th>Moisture (%)</th>
<th>Total Protein (mg/g)</th>
<th>Total Extractable Protein (TEP) (mg/g)</th>
<th>Total Non-Extractable Protein (TNEP) (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NI</td>
<td>7.22</td>
<td>78.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>183.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>136.8</td>
<td>46.9</td>
</tr>
<tr>
<td>W</td>
<td>7.21</td>
<td>80.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>185.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>126.3</td>
<td>59.1</td>
</tr>
<tr>
<td>B</td>
<td>7.31</td>
<td>82.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>157.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>110.8</td>
<td>46.3</td>
</tr>
<tr>
<td>B&lt;sub&gt;1EW&lt;/sub&gt;</td>
<td>7.29</td>
<td>81.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>167.9&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>112.9</td>
<td>54.9</td>
</tr>
<tr>
<td>B&lt;sub&gt;2EW&lt;/sub&gt;</td>
<td>7.39</td>
<td>81.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>166.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>107.8</td>
<td>58.5</td>
</tr>
<tr>
<td>B&lt;sub&gt;3EW&lt;/sub&gt;</td>
<td>7.34</td>
<td>81.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>165.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>113.9</td>
<td>51.6</td>
</tr>
<tr>
<td>B&lt;sub&gt;XG&lt;/sub&gt;</td>
<td>7.33</td>
<td>81.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>161.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>110.7</td>
<td>50.8</td>
</tr>
<tr>
<td>B&lt;sub&gt;1PE&lt;/sub&gt;</td>
<td>7.28</td>
<td>82.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>160.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>113.7</td>
<td>46.9</td>
</tr>
<tr>
<td>B&lt;sub&gt;2PE&lt;/sub&gt;</td>
<td>7.35</td>
<td>81.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>162.8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>109.1</td>
<td>53.7</td>
</tr>
<tr>
<td>B&lt;sub&gt;3PE&lt;/sub&gt;</td>
<td>7.28</td>
<td>81.3&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>174.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>121.8</td>
<td>52.6</td>
</tr>
<tr>
<td>SEM</td>
<td>0.05</td>
<td>0.3</td>
<td>4.7</td>
<td>6.9</td>
<td>7.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Heating</th>
<th>pH</th>
<th>Moisture (%)</th>
<th>Total Protein (mg/g)</th>
<th>Total Extractable Protein (TEP) (mg/g)</th>
<th>Total Non-Extractable Protein (TNEP) (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60°C + 90°C</td>
<td>7.28</td>
<td>80.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>172.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>119.4</td>
<td>53.1</td>
</tr>
<tr>
<td>90°C</td>
<td>7.32</td>
<td>81.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>164.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>113.4</td>
<td>51.3</td>
</tr>
<tr>
<td>SEM</td>
<td>0.02</td>
<td>0.1</td>
<td>2.1</td>
<td>3.1</td>
<td>3.2</td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup> Means within a column and main effect with different superscript are significantly different ($P < 0.05$)
Table 4.3  Main effect least square means of pH, moisture, total protein, total extractable protein (TEP) content, and total non-extractable protein (TNEP) content of Injected Pacific whiting (*Merluccius productus*) Cooked Fillet

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Primary TPA Parameters</th>
<th>Secondary TPA Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hardness (g)</td>
<td>Springiness</td>
</tr>
<tr>
<td>NI</td>
<td>842.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.683&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>W</td>
<td>520.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.709&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>501.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.625&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>B&lt;sub&gt;1EW&lt;/sub&gt;</td>
<td>670.5&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.637&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>B&lt;sub&gt;2EW&lt;/sub&gt;</td>
<td>684.7&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.687&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>B&lt;sub&gt;3EW&lt;/sub&gt;</td>
<td>743.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.678&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>B&lt;sub&gt;1XG&lt;/sub&gt;</td>
<td>385.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.569&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>B&lt;sub&gt;1PE&lt;/sub&gt;</td>
<td>446.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.629&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>B&lt;sub&gt;2PE&lt;/sub&gt;</td>
<td>408.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.576&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>B&lt;sub&gt;3PE&lt;/sub&gt;</td>
<td>514.7&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.646&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM</td>
<td>68.7</td>
<td>0.02</td>
</tr>
<tr>
<td>Heating</td>
<td></td>
<td></td>
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<tr>
<td>60°C + 90°C</td>
<td>526.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.616&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>90°C</td>
<td>616.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.672&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM</td>
<td>30.7</td>
<td>0.01</td>
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</table>

<sup>a,b,c</sup> Means within a column and main effect with different superscript are significantly different (*P* < 0.05)
Table 4.4  Main effect least square means of texture profile analysis (TPA) of Injected Pacific whiting (*Merluccius productus*) Cooked Fillet when heated at 90°C

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Primary TPA Parameters</th>
<th>Secondary TPA Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hardness (g)</td>
<td>Springiness</td>
</tr>
<tr>
<td>Non-Injected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>875.1\textsuperscript{b}</td>
<td>0.73\textsuperscript{cd}</td>
</tr>
<tr>
<td>Injected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W</td>
<td>505.8\textsuperscript{a}</td>
<td>0.69\textsuperscript{bcd}</td>
</tr>
<tr>
<td>B</td>
<td>542.1\textsuperscript{a}</td>
<td>0.66\textsuperscript{acd}</td>
</tr>
<tr>
<td>B1EW</td>
<td>706.9\textsuperscript{ab}</td>
<td>0.65\textsuperscript{acd}</td>
</tr>
<tr>
<td>B2EW</td>
<td>790.6\textsuperscript{ab}</td>
<td>0.74\textsuperscript{d}</td>
</tr>
<tr>
<td>B3EW</td>
<td>738.3\textsuperscript{ab}</td>
<td>0.69\textsuperscript{bc}</td>
</tr>
<tr>
<td>BXG</td>
<td>507.4\textsuperscript{a}</td>
<td>0.62\textsuperscript{ab}</td>
</tr>
<tr>
<td>B1PE</td>
<td>530.0\textsuperscript{a}</td>
<td>0.64\textsuperscript{ac}</td>
</tr>
<tr>
<td>B2PE</td>
<td>498.6\textsuperscript{a}</td>
<td>0.59\textsuperscript{a}</td>
</tr>
<tr>
<td>B3PE</td>
<td>471.9\textsuperscript{a}</td>
<td>0.69\textsuperscript{bcd}</td>
</tr>
</tbody>
</table>

\textsuperscript{a,b,c,d} Means within a column and main effect with different superscript are significantly different \((P < 0.05)\)
Table 4.5  Main effect least square means of texture profile analysis (TPA) of Injected Pacific whiting (*Merluccius productus*) Cooked Fillet when pre-incubated at 60°C then continue heated at 90°C

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Primary TPA Parameters</th>
<th>Secondary TPA Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hardness (g)</td>
<td>Springiness</td>
</tr>
<tr>
<td>Non-Injected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NI</td>
<td>810^e</td>
<td>0.64^bcd</td>
</tr>
<tr>
<td>Injected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W</td>
<td>534^abcd</td>
<td>0.72^d</td>
</tr>
<tr>
<td>B</td>
<td>460^abc</td>
<td>0.63^bc</td>
</tr>
<tr>
<td>B1EW</td>
<td>634^cde</td>
<td>0.62^b</td>
</tr>
<tr>
<td>B2EW</td>
<td>579^bcde</td>
<td>0.64^bc</td>
</tr>
<tr>
<td>B3EW</td>
<td>749^de</td>
<td>0.66^cd</td>
</tr>
<tr>
<td>BXG</td>
<td>264^a</td>
<td>0.52^a</td>
</tr>
<tr>
<td>B1PE</td>
<td>363^abc</td>
<td>0.61^bc</td>
</tr>
<tr>
<td>B2PE</td>
<td>317^ab</td>
<td>0.56^ab</td>
</tr>
<tr>
<td>B3PE</td>
<td>558^bcde</td>
<td>0.60^abc</td>
</tr>
</tbody>
</table>

^a,b,c,d Means within a column and main effect with different superscript are significantly different (P < 0.05)
Table 4.6  Correlation between chemical properties (moisture, TEP, and TNEP) and TPA attributes ofInjected Pacific whiting (*Merluccius productus*) Fillets Cooked at 90°C where bolded values are significant at \( P < 0.05 \)

<table>
<thead>
<tr>
<th></th>
<th>Moisture</th>
<th>TEP</th>
<th>TNEP</th>
<th>Hardness</th>
<th>Springiness</th>
<th>Cohesiveness</th>
<th>Resilience</th>
<th>Chewiness</th>
<th>Adhesiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TEP</td>
<td>-0.44</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNEP</td>
<td>0.17</td>
<td>-0.71</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hardness</td>
<td>-0.49</td>
<td>0.06</td>
<td>0.09</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Springiness</td>
<td>-0.15</td>
<td>-0.21</td>
<td>0.25</td>
<td>0.39</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cohesiveness</td>
<td>-0.41</td>
<td>-0.04</td>
<td>0.11</td>
<td>0.64</td>
<td>0.74</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resilience</td>
<td>-0.31</td>
<td>-0.07</td>
<td>0.12</td>
<td>0.73</td>
<td>0.64</td>
<td>0.84</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chewiness</td>
<td>-0.47</td>
<td>-0.02</td>
<td>0.14</td>
<td>0.95</td>
<td>0.61</td>
<td>0.79</td>
<td>0.81</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Adhesiveness</td>
<td>-0.08</td>
<td>-0.09</td>
<td>0.04</td>
<td>0.12</td>
<td>0.28</td>
<td>0.31</td>
<td>0.55</td>
<td>0.16</td>
<td>1.00</td>
</tr>
</tbody>
</table>
Figure 4.1 Sample Portion for Analysis
Figure 4.2  Texture Profile Analysis (TPA) Attributes of Cooked Fillets, Pacific cod (Gadus macrocephalus) and Pacific whiting (Merluccius productus) (a) Cohesiveness, (b) Springiness, and (c) Chewiness
Figure 4.3  Electrophoretic Pattern of Pacific whiting Injected Cooked Fillet. Abbreviation with apostrophe (') for fillets pre-heated at 60 °C.
References


5 GENERAL CONCLUSION

The presented study was undertaken to assess the effect of ingredients added to improve the quality of Pacific whiting fillets. Addition of salt and sodium phosphate into fillets is a very common method used to increase the water binding properties and quality of muscle food products. Furthermore, it is hypothesized that the addition of protease-inhibiting ingredients to the brine is not only advantageous for enzyme inhibition, but also for maintaining the muscle integrity and textural quality of the fish.

As mentioned earlier, Babbit (1990) was a pioneer investigator in fish injection studies. He demonstrated the use of protease inhibitors suppresses the muscle softening of arrowtooth flounder. However, Babbit (1990) study did not provide information on the concentrations of protease-inhibiting ingredients used. Admittedly, this current injection study on Pacific whiting made several contributions to the previous literature. Our study in raw fillets demonstrated that the addition of enzyme–inhibiting ingredients (either egg white or potato extract) at similar concentration can reduce protease activity. Brine injected fillets either EW or PE demonstrated less variability in texture breaking strength compared to the non-injected fillets. The SDS-PAGE analysis results confirmed that fillets injected with brine mixed with protease inhibitor showed weaker band below 37 kDa than the non-injected fillets.

In a cooked fillet, our model study suggested that 1% egg white and 1% potato extracts could be utilized for a pilot plant study of fish injection. This finding is supported by TPA attributes and chemical data (Protein, TEP, and TNEP). The
approach of using a model study to select and match brine concentration for further injection studies provides additional evidence that this method can be utilized to explore different species as raw materials for fish injection or to understand the impact of different ingredients added to the muscle of Pacific whiting.

Although our injection study has successfully indicated the feasibility of incorporating functional ingredients using brine injection technology, several limitations to this study still need to be acknowledged. First, the current study was unable to analyze the implications of added ingredients to the protein functionality parameter such as the surface hydrophobicity or the total sulfhydryl content, except for the SDS-PAGE analysis. Second, the current study did not evaluate factors related to the shelf life of injected fillet product either during frozen or refrigerated storage.

In terms of directions for future research, there are four aspects that will be considered as a fruitful area for further investigations. First, further work should be conducted to know the appropriate fish size for injection. Marine biologists proved that as the age of the fish increases, there is a higher chance for the muscle to get infected by parasites. Fish muscle is more delicate compared to poultry, beef or pork. Injection processes incorporate brine to muscle using multiple needles. Needle penetration would disrupt muscle of fish. Larger fish seems to be able to withstand needle penetration. That is why it is proposed to use fish which are longer than 20 cm and weight more than 500 g. Second, since xanthan gum interferes the functionality of potato extracts to inhibit protease in whiting, there is a need to explore the use of other gum or the interaction between xanthan gum and other gum to assist the
dispersion of potato extracts in a cold brine. Preliminary result showed the capability of xanthan gum and locus bean gum (1:1 ratio) or locus bean gum and kappa carrageenan (0.25: 0.75 ratio) to replace xanthan gum function in the brine. Three, another possible area of future research would be to investigate the shelf life of injected fillets during frozen or refrigerated storage. Lastly and most importantly, it would be interesting to assess the consumer acceptance of the injected fillets and compare their perception to the commercial cod fillets in the market.

All in all, the findings of this study have a number of important implications for the future applications. From economic and marketing perspectives, it is likely that adding either egg white or potato starch to fish will result in different consumer segments if they are commercially sold. Nowadays, the consumer market is divided into different food tribes. Fish injected with potato extract powder instead of egg white would appeal to people who are allergic to eggs. However, fish injected with egg white could be sold to the majority of consumers.


Haard NF, Simpson BK, Pan BS. 1994. Sarcoplasmic proteins and other nitrogenous compounds. In Seafood proteins (pp. 13-39). Springer US.


Tsuyuki, H. (1982). Relationship between acid and neutral protease activities and the incidence of soft cooked texture in the muscle tissue of Pacific hake (*Merluccius productus*) infected with Kudoo paniformis and/or K. thyrsitis, and held for varying times under different pre-freeze chilled storage conditions.


## APPENDIX A  Pre-Mixing Brine Data Trial

### Table 1. Part 1 of mixing trial at 25 °C

<table>
<thead>
<tr>
<th>Time (Min)</th>
<th>3% EW</th>
<th>3% PE</th>
<th>3%XG</th>
<th>3% STPP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Foam (mm)</td>
<td>In Solution?</td>
<td>Foam (mm)</td>
<td>In Solution?</td>
</tr>
<tr>
<td>0</td>
<td>6</td>
<td>Y</td>
<td>2</td>
<td>Y</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>Y</td>
<td>1</td>
<td>N</td>
</tr>
<tr>
<td>20</td>
<td>4</td>
<td>Y</td>
<td>0</td>
<td>N</td>
</tr>
<tr>
<td>30 - 90</td>
<td>4</td>
<td>Y</td>
<td>0</td>
<td>N</td>
</tr>
</tbody>
</table>

**Notes:**
- Yellow color, high foam, 0.5 mm sedimentation > 40 min
- Quick separation, 10 mm separation > 10 min, brown color, low foam
- No foam, too thick for injector
- No color, watery consistency

EW = egg white; PE = potato extract; XG = xanthan gum; STPP = sodium tripolyphosphate

### Table 2. Part 2 of mixing trial at 25 °C.

<table>
<thead>
<tr>
<th>Time (Min)</th>
<th>1% XN, 3% PE</th>
<th>1% STPP, 3% PE</th>
<th>1% XN, 3% EW</th>
<th>1% STPP, 3% EW</th>
<th>1% EW, 3% PE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Foam (mm)</td>
<td>In Solution?</td>
<td>Foam (mm)</td>
<td>In Solution?</td>
<td>Foam (mm)</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>Y</td>
<td>1</td>
<td>Y</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>Y</td>
<td>0</td>
<td>N</td>
<td>2</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>Y</td>
<td>0</td>
<td>N</td>
<td>2</td>
</tr>
<tr>
<td>30 - 90</td>
<td>0</td>
<td>Y</td>
<td>0</td>
<td>N</td>
<td>2</td>
</tr>
</tbody>
</table>

**Notes:**
- Brown color, too thick for injector
- 10 mm separation > 10 min, brown color
- No foam, brown color, too thick for injector
- Yellow color, acceptable consistency
- Brown color

EW = egg white; PE = potato extract; XG = xanthan gum; STPP = sodium tripolyphosphate
Table 3. Part 1 of mixing trial at 4 °C.

<table>
<thead>
<tr>
<th>Time (Min)</th>
<th>3%EW</th>
<th>3%PE</th>
<th>3%XG</th>
<th>3%STPP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Foam (mm)</td>
<td>Solution?</td>
<td>Foam (mm)</td>
<td>Solution?</td>
</tr>
<tr>
<td>0</td>
<td>8</td>
<td>Y</td>
<td>0</td>
<td>Y</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>Y</td>
<td>0</td>
<td>N</td>
</tr>
<tr>
<td>20</td>
<td>7</td>
<td>Y</td>
<td>0</td>
<td>N</td>
</tr>
<tr>
<td>30 - 90</td>
<td>7</td>
<td>Y</td>
<td>0</td>
<td>N</td>
</tr>
</tbody>
</table>

EW = egg white; PE = potato extract; XG = xanthan gum; STPP = sodium tripolyphosphate

Table 4. Part 1 of mixing trial at 4 °C

<table>
<thead>
<tr>
<th>Time (Min)</th>
<th>1% XG, 3% PE</th>
<th>1% STPP, 3% PE</th>
<th>1% XG, 3% EW</th>
<th>1% STPP, 3% EW</th>
<th>1% EW, 3% PE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Foam (mm)</td>
<td>Solution?</td>
<td>Foam (mm)</td>
<td>Solution?</td>
<td>Foam (mm)</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>Y</td>
<td>0</td>
<td>Y</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>Y</td>
<td>0</td>
<td>Y</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>Y</td>
<td>0</td>
<td>N</td>
<td>0</td>
</tr>
<tr>
<td>30 - 90</td>
<td>0</td>
<td>Y</td>
<td>0</td>
<td>N</td>
<td>0</td>
</tr>
</tbody>
</table>

EW = egg white; PE = potato extract; XG = xanthan gum; STPP = sodium tripolyphosphate
APPENDIX B  Color Data of Pacific Whiting Fillets

Table 1  Main effect of least square of CIE color measurement of non-injected Pacific whiting fillets, in different fillet sections

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Section</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>Anterior</td>
<td>34.7±10.5</td>
<td>2.58±2.1</td>
<td>3.72±3.1</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>33.7±8.7</td>
<td>1.37±1.3</td>
<td>3.10±2.1</td>
</tr>
<tr>
<td></td>
<td>Posterior</td>
<td>35.3±3.1</td>
<td>3.18±1.07</td>
<td>5.3±1.7</td>
</tr>
</tbody>
</table>

Table 2  Main effect of least square of CIE color measurement of brine treatment of injected Pacific whiting fillets, in different fillet sections

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Section</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>Anterior</td>
<td>38.8±8.8</td>
<td>-0.74±0.5</td>
<td>-1.35±1.1</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>24.9±5.6</td>
<td>0.14±0.8</td>
<td>0.25±1.6</td>
</tr>
<tr>
<td></td>
<td>Posterior</td>
<td>31.8±5.9</td>
<td>1.53±0.8</td>
<td>3.35±1.7</td>
</tr>
</tbody>
</table>

*xy Means within a column and main effect with different superscripts are significantly different between different body parts (p<0.05 mean± standard deviation, n=4)

Table 3  Main effect of least square of CIE color measurement of brine with egg white treatment of injected Pacific whiting fillets, in different fillet sections

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Section</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>B_{EW}</td>
<td>Anterior</td>
<td>29.4±3.7</td>
<td>0.53±0.8</td>
<td>1.18±1.1</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>22.8±5.0</td>
<td>1.2±1.6</td>
<td>2.8±2.7</td>
</tr>
<tr>
<td></td>
<td>Posterior</td>
<td>28.6±7.5</td>
<td>1.4±0.5</td>
<td>4.0±0.6</td>
</tr>
</tbody>
</table>

*xy Means within a column and main effect with different superscripts are significantly different between different body parts (p<0.05 mean± standard deviation, n=4)

Table 4  Main effect of least square of CIE color measurement of brine with potato extract treatment of injected Pacific whiting fillets, in different fillet sections

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Section</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>B_{PE}</td>
<td>Anterior</td>
<td>32.0±5.8</td>
<td>0.56±0.4</td>
<td>0.78±1.7</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>23.7±2.0</td>
<td>2.3±0.3</td>
<td>3.6±0.3</td>
</tr>
<tr>
<td></td>
<td>Posterior</td>
<td>27.0±5.5</td>
<td>2.2±0.7</td>
<td>3.7±1.4</td>
</tr>
</tbody>
</table>

*xy Means within a column and main effect with different superscripts are significantly different between different body parts (p<0.05 mean± standard deviation, n=4)
APPENDIX C  Cooking Temperature Profile of Pacific Whiting Fillets

Figure 1. Temperature profile of Pacific whiting heated at 90°C for 20 min

Figure 2. Temperature profile of Pacific whiting heated at 60°C for 30 min
### APPENDIX D  TPA Program

#### T.A. Setting

<table>
<thead>
<tr>
<th>Caption</th>
<th>Value (Unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Test Speed</td>
<td>1.00 (mm/s)</td>
</tr>
<tr>
<td>Test Speed</td>
<td>1.00 (mm/s)</td>
</tr>
<tr>
<td>Post-Test Speed</td>
<td>10.00 (mm/s)</td>
</tr>
<tr>
<td>Target Mode</td>
<td>Strain</td>
</tr>
<tr>
<td>Strain</td>
<td>30%</td>
</tr>
<tr>
<td>Time</td>
<td>5.00 s</td>
</tr>
<tr>
<td>Trigger Type</td>
<td>Auto (Force)</td>
</tr>
<tr>
<td>Trigger Force</td>
<td>10.0 (g)</td>
</tr>
<tr>
<td>Tare Mode</td>
<td>On</td>
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</tbody>
</table>

#### Test Configuration

<table>
<thead>
<tr>
<th>Caption</th>
<th>Value (Unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample width (w)</td>
<td>2.5 (cm)</td>
</tr>
<tr>
<td>Sample length (l)</td>
<td>2.5 (cm)</td>
</tr>
<tr>
<td>Strain height (h)</td>
<td>Measured at runtime (mm)</td>
</tr>
<tr>
<td>Stress Area</td>
<td>4.560.370 (mm²)</td>
</tr>
<tr>
<td>Sample weight</td>
<td>-</td>
</tr>
<tr>
<td>Temperature</td>
<td>-</td>
</tr>
</tbody>
</table>
# Cod Fillets Characteristics

<table>
<thead>
<tr>
<th>Fillets Characteristics:</th>
<th>Heating Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90 °C</td>
</tr>
<tr>
<td>Chemical Data:</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.47 ± 0.08</td>
</tr>
<tr>
<td>Moisture</td>
<td>79.2 ± 1.0 (%)</td>
</tr>
<tr>
<td>Crude Protein</td>
<td>202.2 ± 1.2 (mg/g)</td>
</tr>
<tr>
<td>Total Extractable Protein</td>
<td>120.9 ± 11.0 (mg/g)</td>
</tr>
<tr>
<td>Total Non Extractable Protein</td>
<td>81.2 ± 18.4 (mg/g)</td>
</tr>
<tr>
<td>Texture Profile Analysis:</td>
<td></td>
</tr>
<tr>
<td>Hardness</td>
<td>769 ± 352</td>
</tr>
<tr>
<td>Springiness</td>
<td>0.586 ± 0.185</td>
</tr>
<tr>
<td>Cohesiveness</td>
<td>0.686 ± 0.122</td>
</tr>
<tr>
<td>Chewiness</td>
<td>326.5 ± 237.0</td>
</tr>
<tr>
<td>Adhesiveness</td>
<td>-13.2 ± 9.5</td>
</tr>
<tr>
<td>Resilience</td>
<td>0.318 ± 0.058</td>
</tr>
</tbody>
</table>
**APPENDIX F Additional Information for the Ingredients**

**Table 1. Sodium Phosphate**

<table>
<thead>
<tr>
<th>Properties</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH, 1% solution @25°C</td>
<td>9.8</td>
</tr>
<tr>
<td>Arsenic</td>
<td>≤ 3.0 ppm</td>
</tr>
<tr>
<td>Fluoride</td>
<td>≤ 50 ppm</td>
</tr>
<tr>
<td>Lead</td>
<td>≤ 2 ppm</td>
</tr>
<tr>
<td>Retained on 16 mesh</td>
<td>≤ 2.5%</td>
</tr>
<tr>
<td>Thru 100 mesh</td>
<td>≤ 30%</td>
</tr>
<tr>
<td>Bulk Density, lbs/cu.ft</td>
<td>36.2</td>
</tr>
<tr>
<td>Bulk density, g/cc</td>
<td>0.58</td>
</tr>
</tbody>
</table>

Source: Integra Chemical, Kent, WA (private communications)

**Table 2. Potato Extracts**

<table>
<thead>
<tr>
<th>Properties</th>
<th>Specification per 100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories (kcal)</td>
<td>365.52</td>
</tr>
<tr>
<td>Calories from Fat (kcal)</td>
<td>6.48</td>
</tr>
<tr>
<td>Calories from SatFat (kcal)</td>
<td>1.17</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>7.89</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>81.87</td>
</tr>
<tr>
<td>Dietary Fiber (g)</td>
<td>6.81</td>
</tr>
<tr>
<td>Total Sugars (g)</td>
<td>3.48</td>
</tr>
<tr>
<td>Other Carbs (g)</td>
<td>71.58</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>0.72</td>
</tr>
<tr>
<td>Saturated Fat (g)</td>
<td>0.13</td>
</tr>
<tr>
<td>Mono Fat (g)</td>
<td>0.01</td>
</tr>
<tr>
<td>Water (g)</td>
<td>7.50</td>
</tr>
<tr>
<td>Vitamin A - IU (IU)</td>
<td>10.56</td>
</tr>
<tr>
<td>Vitamin B1 - Thiamin (mg)</td>
<td>0.43</td>
</tr>
<tr>
<td>Vitamin B2 - Riboflavin (mg)</td>
<td>0.24</td>
</tr>
<tr>
<td>Vitamin B3 - Niacin (mg)</td>
<td>4.57</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>35.41</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>39.35</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>1.05</td>
</tr>
<tr>
<td>Phosphorus (mg)</td>
<td>40.00</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>674.77</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>64.30</td>
</tr>
</tbody>
</table>

Source: Pacific Blends Ltd, Port Coquitlam, BC, Canada (private communications)
Table 3. Xanthan Gum

<table>
<thead>
<tr>
<th>Properties</th>
<th>Specification per 100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories (kcal)</td>
<td>328</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>5.0</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>77.0</td>
</tr>
<tr>
<td>Dietary Fiber (g)</td>
<td>77.0</td>
</tr>
<tr>
<td>Total Sugars (g)</td>
<td>77.0</td>
</tr>
<tr>
<td>Water (g)</td>
<td>9.0</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>16.0</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>1.0</td>
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<tr>
<td>Magnesium (mg)</td>
<td>30.0</td>
</tr>
<tr>
<td>Phosphorus (mg)</td>
<td>166.0</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>3530.0</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>757.00</td>
</tr>
</tbody>
</table>

Source: Pacific Blends Ltd, Port Coquitlam, BC, Canada (private communications)