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Research Article

Preventing Soft Texture Fish Fillets through Brine Injection Technology

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An exploratory study was conducted to determine if multineedle injection technology could deliver protease inhibitor ingredients into fish fillets at sufficient levels to inhibit protease activity. Pacific whiting is used as a model in this study. Fillet treatments ($n = 8$/treatment) included noninjection (C), injection of base brine containing 3% salt and 3% sodium tripolyphosphate (B), injection of base brine and 3% egg white (B_{EW}), and injection of base brine, 0.1% xanthan gum, and 3% dried potato extract (B_{PE}). Xanthan gum was used as a suspension aid. Actual brine incorporation was 12.2 ± 0.5%. Cathepsin L activity was evaluated at pH 5.5 (optimal pH) and ultimate pH. Quality measures evaluated included CIE Lab color, shear force, and lipid oxidation. Fillets injected with B_{EW} and B_{PE} were significantly lower in cathepsin L activity when measured at pH 5.5. B_{EW} and B_{PE} fillets were darker in appearance than B or C fillets. Untreated fillets (C) had higher variability in shear force value than treated fillets. There was no effect of treatment on lipid oxidation. Results suggested that injection technology can be utilized to incorporate protease inhibitor ingredients (3% EW or 3% PE) at levels sufficient to reduce cathepsin L activity in Pacific whiting fillets.

1. Introduction

A great deal of research on muscle food has focused on the myofibrillar protein changes during postmortem storage and processing. The main proteolytic system which is attributed to the weakening of myofibrillar proteins in fish is lysosomal cathepsins. A considerable number of articles have demonstrated the contribution of endogenous muscle protease enzymes to textural degradation of fish during iced, refrigerated, or frozen storage and thermal processing [1–3]. Existing studies have also examined the possible relation of those proteolytic enzymes to soft texture fish fillets on some well-known commercial species such as Atlantic salmon, Atlantic cod, rainbow trout, herring, carp, gilthead seabream, and lizard fish [1, 4–8].

One particular species that has caught the attention of seafood scientist in the US and Canada is Pacific whiting *(Merluccius productus)*. Over the past three decades, a number of studies have documented numerous factors

that contribute to the deterioration of Pacific whiting. Two predominant factors are the effect of exogenous (via a myxosporean parasite) and endogenous sources of proteolytic enzymes [9–12]. Those two factors synergistically and detrimentally influence both the raw and cooked fillets texture of Pacific whiting. The most active cysteine proteinase in raw fillets of Pacific whiting was identified as cathepsin B while the degradation of myosin in fillets and surimi gels during conventional heating was caused by cathepsin L [13]. These proteinases impact Pacific whiting's commercial value and limit the prospective market for Pacific whiting in a fillet form. As a result, Pacific whiting, because it experiences texture softening in both the uncooked and cooked fillet, can serve as an excellent model for studying how brine injection could be utilized to prevent texture softening in fish.

There is a growing amount of literature on the ability of low salt brines incorporated through multineedle injection technology to improve the quality of whole muscle beef, pork, and poultry products [14–21]. Low salt brines are typically

formulated to have less than 5% NaCl and used at levels just sufficient to impact whole muscle protein functionality without altering flavor/appearance [14, 15, 21]. Low salt brines function by reducing drip loss during retail display, reducing cook-loss, improving palatability (perceived juiciness), and extending shelf-life [17, 18, 20, 22].

To date, the majority of fish injection research has primarily focused on application of high salt brines to products such as salted cod or cold-smoked salmon [23–25]. A couple of studies have investigated low salt injection in cod and catfish [26, 27]. The latter research demonstrated that low salt injection improved uncooked fillet firmness and reduced thaw drip. The fish evaluated in that research, however, does not experience the type of texture degradation during cooking that occurs with Pacific whiting.

Previous research on minced fish products (surimi) made with Pacific whiting has demonstrated that ingredients such as dried egg white (EW) and dried potato extract (PE) can be blended into the mince to inhibit protease activity and improve cooked gel strength [9, 11]. The level of EW and PE typically incorporated ranges between 1-2% and 2-3%, respectively. In order to deliver this level of protease inhibitor the brine would need to contain 10–30% inhibitor ingredient. This, of course, is not possible because neither EW nor PE is soluble, so not only must they be suspended in the brine, but also they need to be delivered through small bore needles. Preliminary experiments demonstrated that neither of these ingredients can be suspended above 5% (w/w) and PE requires a suspension aid, such as a gum, at no less than 0.1% (see Supplementary Materials (available here)). However, work by Kang and Lanier [28] using a recombinant produced protease inhibitor injected into arrowtooth flounder suggested that in a whole muscle system, protease inhibition may be achieved at much lower levels of protease inhibitor incorporation than in a mince product.

Preliminary research suggested brine injection may be a feasible technology to prevent texture softening of fish fillets. The objective of this study, therefore, was to evaluate whether protease inhibitor ingredients such as dried egg white or dried potato extract could be distributed sufficiently and at a high enough concentration in a brine to inhibit protease activity when injected into fish fillets. As mentioned earlier, Pacific whiting was used as the model for this study since it experiences texture softening in both uncooked and cooked fillets.

2. Material and Methods

2.1. Raw Material. Pacific whiting was obtained from a local seafood processor (Astoria, OR) at time of delivery from the vessel (<24 h from capture, held in refrigerated sea water). Fish were transported immediately to the OSU Seafood Laboratory in an insulated container in ice, headed and gutted, cut into butterfly fillets, placed in individually vacuum packed bags (oxygen transmission rate 24 h at 23°C is 63 cc/sq.m, Summit Packaging, Auburn, WA, USA), vacuum packed with a 0.9 bar (setting at 7), sealed for 15 s (setting at 7)

(Reiser, Canton, MA), and immediately frozen at −30°C until injection. Vacuum refined granulated salt (Morton Salt Inc., Chicago, IL), sodium tripolyphosphate (STPP) (Nutrifos-088, Integra Chemical, Kent, WA), dried egg white (EW) (P-110, Henningsen Food, Omaha, NE), dried potato extract (PE) (NP-3, Pacific Blends Ltd, BC, Canada), and xanthan gum (XG) (Pacific Blends Ltd, BC, Canada) were used for brine preparations.

2.2. Injection and Sample Preparation. Fillets ($n = 32$) were prepared for brine injection as described in Figure 1. Fillet ($n = 8$/treatment) treatments included noninjection (C) and injection with a base brine containing 3% salt and 3% sodium tripolyphosphate (B), injection with base brine containing 3% egg white (B_{EW}), and injection with base brine containing 3% dried potato extract (B_{PE}). Xanthan gum, 0.1%, was used as a suspension aid for B_{PE}. All treated fillets were vacuum packaged, refrozen, and stored at −18°C until further evaluation. In value-added products, double-frozen fillets are a custom practice due to the extra processing requirements; however, these quality changes are not significant enough to be noticeable by consumers [29]. For evaluation of protease inhibition and lipid oxidation samples were homogenized by dipping in liquid nitrogen and blended into a frozen powder [16]. Powdered samples were stored in a Whirl-Pak® (Nasco, Fort Atkinson, WI) at −80°C until analysis.

2.3. Protease Inhibition. Cathepsin L activity ($n = 8$/treatment) was measured to evaluate protease inhibition. Evaluations were conducted as described by An et al. [13]. Samples were extracted with 1% brij and were evaluated for both cathepsin L activity and protein content. Cathepsin L activity was measured at optimum pH and temperature. Briefly, extracts were incubated with buffer (2 : 1) containing 340 mM sodium acetate, 60 mM acetic acid, 4 mM disodium EDTA, 8 mM dithiothreitol (pH 5.5), and Z-Phe-Arg-NMec as the substrate at 55°C for 10 min. The reaction was stopped with stopping reagent containing 100 mM sodium monochloroacetate, 30 mM sodium acetate, 70 mM acetic acid (pH 4.3), and liberated 7-amido-4-methylcoumarin (NMec) which was measured at excitation 370 nm and emission at 460 nm using Perkin Elmer LS-50B spectrofluorometer (Waltham, MA). In addition, extracts were also incubated at ultimate pH by replacing buffer with dd H_2O. Protein content was determined in each extract using the Quick Start™ Bradford Assay (#5000201, Bio-Rad, Hercules, CA).

2.4. Color Analysis. The CIE L^*, a^*, and b^* values of treated fillets were measured using a Minolta chromameter CM 700d (10° standard observer, illuminant D65, Osaka, Japan). Butterfly fillets ($n = 4$/treatment) were randomly selected and tempered to 2°C. Three readings at randomly selected locations in the center of fillets were averaged for each evaluation. Fillets were immediately refrozen and used for further evaluations.
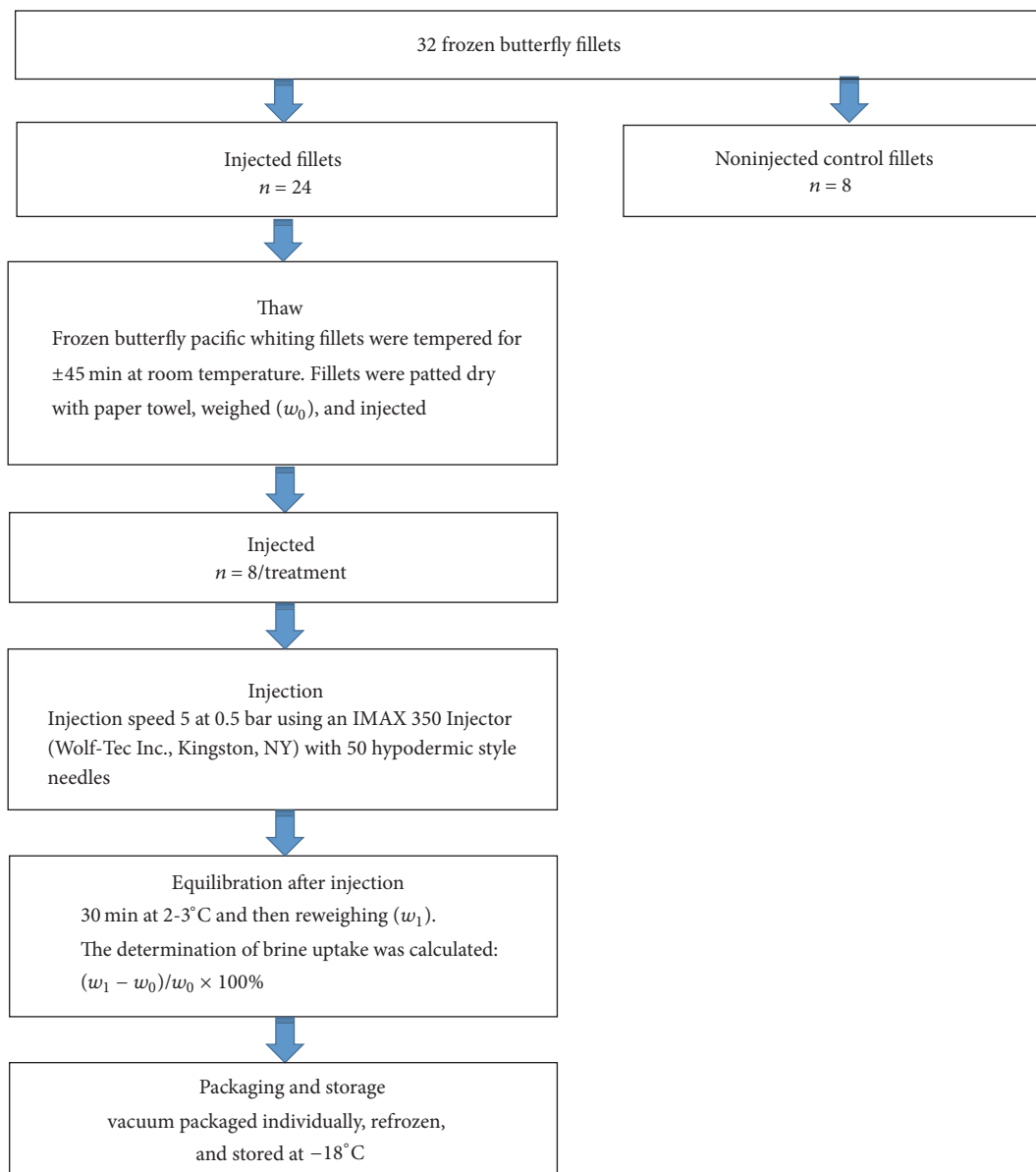
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┌─────────────────────────────────────────────────────────────────────┐
│                    32 frozen butterfly fillets                        │
└─────────────────────────────────────────────────────────────────────┘
            │                                        │
            ▼                                        ▼
┌───────────────────────────┐         ┌───────────────────────────┐
│     Injected fillets      │         │ Noninjected control fillets│
│         n = 24            │         │          n = 8             │
└───────────────────────────┘         └───────────────────────────┘
            │
            ▼
┌───────────────────────────────────────────────────────┐
│                         Thaw                          │
│  Frozen butterfly pacific whiting fillets were tempered│
│  for ±45 min at room temperature. Fillets were patted  │
│  dry with paper towel, weighed (w₀), and injected      │
└───────────────────────────────────────────────────────┘
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Thaw
Frozen butterfly pacific whiting fillets were tempered for ± 45 min at room temperature. Fillets were patted dry with paper towel, weighed (w_0), and injected

Injected
$n = 8$/treatment

Injection
Injection speed 5 at 0.5 bar using an IMAX 350 Injector (Wolf-Tec Inc., Kingston, NY) with 50 hypodermic style needles

Equilibration after injection
30 min at 2-3°C and then reweighing (w_1).
The determination of brine uptake was calculated:
$(w_1 - w_0)/w_0 \times 100\%$

Packaging and storage
vacuum packaged individually, refrozen,
and stored at $-18°$C

FIGURE 1: Brine injection protocol.

2.5. Lipid Oxidation. Fillets selected ($n = 8$/treatment) for thiobarbituric acid reactive substance (TBARs) content were utilized to measure lipid oxidation with a modified method of Buege and Aust (1978) as described by Cerruto-Noya et al. [30].

2.6. Textural Analysis. The dorsal sections of fillets ($n = 4$/treatment) were partially thawed to an internal temperature $\pm -1.0°$C. A 5.0×2.5 cm section was collected from each partially thawed fillet. Thickness varied (1.5–2.5 cm) based on fish size and treatment. Sections were allowed to fully thaw for 2 h (end point temperature 0°C) in 2-3°C cold room. Sections were held on ice (5–10 min) prior to shear force analysis using a Warner-Bratzler shear blade (3 mm, 73° V cut) attached to Texture Analyzer TA-XT2 (Stable Micro Systems Ltd, Surrey, UK). The Warner-Bratzler blade moved downwards at a constant crosshead speed of 2 mm/s to cut through the sample with 0.2 N of trigger force [31].

2.7. Statistical Analysis. All results were analyzed using One-Way ANOVA in SigmaPlot with treatment as the main factor (SigmaPlot, San Jose, CA), except for enzyme inhibition results. Two-Way ANOVA with main effects as treatment and test method pH (pH 5.5 or ultimate pH) were used to analyze cathepsin L. The least significant difference (LSD) was used to determine if significant differences occurred between treatments.

3. Results and Discussions

3.1. Injection. Previous research on injection of fish has been reported for catfish, salmon, and cod. All of these

TABLE 1: Treatment designations, pH and brine uptake (%), and color of injected Pacific whiting (*Merluccius productus*) fillet.

Treatment	Brine composition and concentration	pH in solution	Brine uptake (%)[a]	Color			TBARs (mg/kg MDA)
				L^*	a^*	b^*	
C	None	-	-	33.7 ± 8.7^y	1.37 ± 1.3^{xy}	3.10 ± 2.1^y	0.55 ± 0.21^y
B	3% salt (S) + 3% sodium tripolyphosphate (STPP)	8.50	13.8 ± 0.8^y	24.9 ± 5.6^{xy}	0.14 ± 0.8^x	0.25 ± 1.6^x	0.31 ± 0.28^x
B_{EW}	3% S + 3% STPP + 3% dried egg white (EW)	8.34	9.9 ± 0.5^x	22.8 ± 5.0^x	1.2 ± 1.6^{xy}	2.8 ± 2.7^y	0.36 ± 0.22^x
B_{PE}	3% S + 3% STPP + 0.1% xanthan gum (XG) + 3% dried potato extract (PE)	8.16	12.9 ± 0.6^y	23.7 ± 2.0^x	2.3 ± 0.3^y	3.6 ± 0.3^y	0.23 ± 0.15^x

[x,y]Means within a column with different superscripts are significantly different between treatments ($p < 0.05$, mean ± standard error, $n = 8$, except for color $n = 4$). [a]Brine uptake (%) = fillets weight after injection and equilibration for 30 min (W_1) − initial weight of fillet (W_0)/initial weight of fillet (W_0) × 100%.

fish have significantly larger, firmer, and thicker fillets than Pacific whiting. Currently, the primary processing of Pacific whiting typically involves heading and gutting and freezing. An injected product would likely only be produced at a secondary processor. As a result, this study focused on the injection of a once frozen product since this is the form most likely to be injected by a secondary processor for this particular fish. Numerous trial injections were conducted on previously frozen fillets with the base brine (B) to determine the best injection conditions and fillet form to obtain a 10% uptake in Pacific whiting fillets. Variation in brine uptake was minimized as weight of fillet increased (data not shown). As a result, butterfly fillets were selected instead of individual fillets. Reported equipment settings reflect brine injection pressure and belt speed that minimized damage to the muscle while maximizing uptake. Average brine incorporation across all injection treatments was 12.2 ± 0.5%. B_{EW} had lower brine uptake than B and B_{PE} ($p = 0.001$; Table 1). The lower value of B_{EW} could indicate that viscosity of the brine was a factor in uptake. An increased viscosity would result in lower brine deliverance through the needle during the injection process. This phenomenon was anticipated since dried egg white in food processing can act as a thickening agent [32].

3.2. Protease Inhibition.

Cathepsin L activity was measured at both the optimum pH for enzyme activity (pH 5.5) and ultimate pH (Figure 2). There was a main effect of both treatment ($p = 0.009$) and pH ($p < 0.001$), but no treatment × pH interaction ($p = 0.37$). When cathepsin L activity was measured at pH 5.5, fillets injected with B_{EW} and B_{PE} had significantly lower cathepsin L activity than in C ($p = 0.03$). This supports the hypothesis that brine injection of EW or PE, even though it is at a magnitude smaller than what is normally found in comminuted products, can inhibit cathepsin L in a whole muscle product. These results, however, represent what happens when conditions are optimized (pH 5.5) for the target enzyme activity. When fillets are cooked, pH will decrease slightly but will not be as low as pH 5.5. They will be closer to the ultimate pH of the fish. Measurement of cathepsin L activity at ultimate pH resulted in a significant decrease in cathepsin L activity when compared to pH 5.5 measurements for B and C treatments ($p < 0.05$, Figure 2), but not B_{EW} or B_{PE}. However, there were not significant differences found between treatments measured at ultimate pH ($p = 0.23$, Figure 2). Results suggest that injection of a brine with salt and phosphate alone may be sufficient to inhibit protease activity during cooking. Phosphates are well-known for their buffering capacity. When they are added to a comminuted fish product at 0.3% polyphosphate (STPP or a mixture of STPP and TSPP) the pH should be 7.2 ± 0.2 [33]. Turk et al. [34] indicated that once cathepsins are not inside the lysosomes or are extracellular, they can be quickly irreversibly deactivated at neutral pH.

3.3. Color.

Color of raw fillets were measured because there was visible difference in the color of each treatment brine. There was expectation, therefore, that brine treatment may actually change the raw fillet color. The lightness of fillets was decreased as a result of brine injection (Table 1). The CIE L^* values of C were significantly higher than B_{EW} or B_{PE} ($p = 0.03$) but showed no statistical difference compared to B ($p = 0.06$, Table 1). The decrease in lightness as a result of brine injection in muscle products is well documented [16, 21, 26, 35]. Previous study reported that CIE L^* values of catfish fillets that were injected with agglomerated phosphates were lower than noninjected fillets [26]. Decreased L^* values were attributed to phosphates from the brine enhancing water holding and therefore resulting in less liquid on the surface to cause light scattering. In addition, the protease inhibiting ingredients in and of themselves may have contributed to the lower L^* values. Brine uptake data support this possibility since B had higher or equivalent brine uptake yet lighter color (Table 1).

The redness and yellowness of fillets were impacted by brine injection. For fillets injected with salt and phosphate (B) the average a^* and b^* values were lower than noninjected controls (C). Significant reductions in color, however, were only reported for b^* values between C and B. Wide variations in a^* values of C made it difficult to discriminate significance between C and B treated fillets. Reductions in redness and yellowness would be expected as blood and fat is washed out and diluted as a result of the brine incorporation process. Fillets treated with brine containing EW or PE had a^* and b^* values that were not significantly different from the control. The color contributed by protease inhibitory ingredients appears to counteract the washing out effect observed when
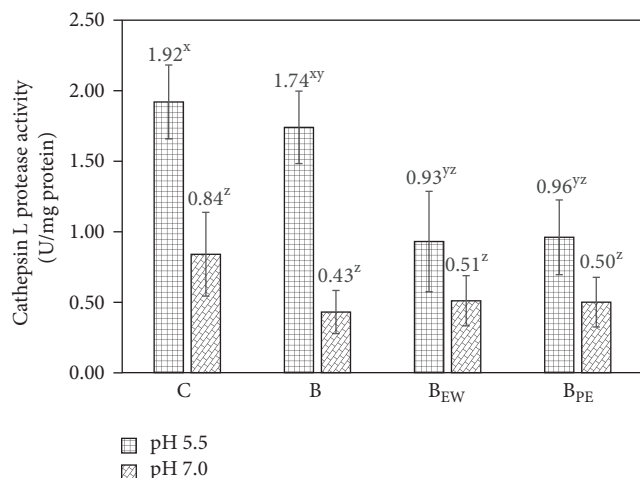
FIGURE 2: Cathepsin L protease activity of Pacific whiting raw injected fillets, means for each treatment at pH 5.5 ($p = 0.03$) and pH 7.0 ($p = 0.23$). x, y, and z showed significant difference with other treatments ($p < 0.05$) at similar pH (mean ± standard error mean, $n = 8$). C = control, noninjected fillet; B = brine, 3% salt + 3% sodium tripolyphosphate (sttp); B_{EW} = brine + 3% dried egg white; B_{PE} = brine + 0.1% xanthan gum + 3% dried potato extract.
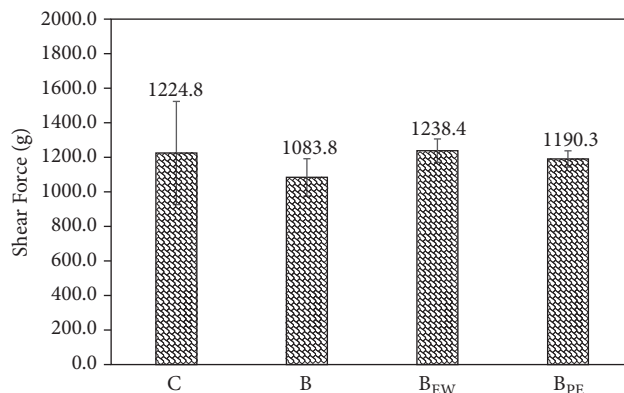

FIGURE 3: Texture measurement of thawed Pacific whiting injected fillets ($p > 0.05$). C = control, noninjected fillet; B = brine, 3% salt + 3% sodium tripolyphosphate (STTP); B_{EW} = brine + 3% dried egg white; B_{PE} = brine + 0.1% xanthan gum + 3% dried potato extract.

only salt and phosphate was in the brine. Average a^* and b^* values were highest for the PE treated samples. Data suggests that consumers may be able to perceive differences in the appearance of raw injected products. It is difficult to say, however, without a consumer study whether these differences would influence purchasing choices. Results suggest that if treatments are able to stabilize fillet texture, injected product may be more suited to either food service distribution or development as a value-added battered, breaded, or marinated fillets for the retail market.

3.4. Texture. Previous research has demonstrated that injection of a brine can enhance the firmness of raw catfish fillets [26]. A firmer and plumper appearing fillet would be more attractive to a consumer. However, catfish fillets are much thicker and firmer textured than Pacific whiting fillets. Texture, therefore, was measured to determine if raw fillet texture from thinner more delicate fillets could also be improved through brine incorporation. Unlike whole muscle meat products, there is not a universally accepted method for measurement of texture in fish fillets. Texture was measured using shear force since that technique measures the force required to cut the product. Results did not demonstrate that firmness of fillets was improved by brine injection as measured by shear force (Figure 3). In retrospect, perhaps use of a technique involving compression would have provided more insight into raw fillet texture. An interesting observation that did occur with the shear force texture data was the difference in variances amongst the treatments. For texture, there was more variability in the breaking force values of noninjected fillets than injected fillets. This suggests that fillet firmness was more uniform and consistent in injected fillets than in noninjected fillets. These results are supported by those observed by Kin et al. [26].

3.5. Lipid Oxidation. Lipid oxidation was measured to determine if the salt in the brines would act to promote oxidation. TBARs values of B and B_{PE} were significantly lower than C and B_{EW} ($p = 0.03$, Table 1). However, TBARs value of all fillets ranged from 0.23 to 0.55 mg/kg tissue. Previous results with mackerel fillets suggest sensory detection of rancidity occurs with a TBARs value between 0.86 and 1.44 mg/kg tissues [36]. Thus, it can be suggested that no rancidity was observed on those PW fillets during the time they were frozen for this study.

4. Conclusion

Color was impacted by brine treatment and suggests commercial products may have to be value-added battered, breaded, or marinated in order to be acceptable for the retail market. Texture results suggest that brine treatments reduce variation in texture of the raw fillet, which shows potential for being able to produce a product that is uniform in its performance to the consumer. These findings indicated that injection treatment could impact the sensory quality of fish fillets, albeit there is no sensory evaluation conducted in this study. Also, there was a strong trend in protease inhibition upon brine incorporation, although there were not significant differences in cathepsin L activity at the ultimate pH between treatments. Brine (3% salt and 3% sodium tripolyphosphate) mixed with either 3% egg white or 3% potato extract is sufficient for reducing protease activity in Pacific whiting fillets. In addition to protease inhibition, studies on comminuted products have demonstrated that both egg white and potato extract through either protein or starch can enhance gel networking. We would expect some degree of enhancement in texture of an injected product containing these ingredients and even possibly some synergies with the protease inhibition. Further studies are also needed to understand the effect of brine injection on ultimate sensory properties of the cooked product.

Additional Points

Practical Applications. Addition of a brine consisting of 3% salt, 3% STPP, and 3% dried egg white or 3% dried potato extract could potentially prevent softening in Pacific whiting fillets. Treated fillets should have more uniform texture than untreated fillets.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

Supplementary Materials

Figure (a): brine containing 3% salt, 3% STTP, and 3% egg white; (b) brine containing 3% salt, 3% STTP, 3% potato extract, and 1% xanthan gum. All brine was mixed using chilled water, kept at walk-in cooler (4°C), and photographed every 10 min. 5% EW or PE cannot be mixed well in a beaker. Additionally, 0.1% xanthan gum was used in the study instead of 1% xanthan gum since it is the smallest amount that helps PE to be well-solubilized. (*Supplementary Materials*)

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