Food Chemistry 176 (2015) 448–454

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

# Salmon blood plasma: Effective inhibitor of protease-laden Pacific whiting surimi and salmon mince

Matthew R. Fowler [a], Jae W. Park [a,b,*]

[a] Oregon State University Seafood Laboratory, 2001 Marine Dr Rm 253, Astoria, OR 97103, United States
[b] Korea University, Dept of Food Bioscience and Technology, 145 Anam-ro, Seongbuk-gu, Seoul, South Korea

ARTICLE INFO

*Article history:*
Received 9 August 2014
Received in revised form 15 November 2014
Accepted 22 December 2014
Available online 30 December 2014

*Keywords:*
Salmon plasma
Protease inhibitor
Surimi
Pacific whiting
Autolysis

ABSTRACT

The effect of salmon plasma (SP) from Chinook salmon on proteolytic inhibition was investigated. SP was found to inhibit both cysteine and serine proteases as well as protease extracted from Pacific whiting muscle. SP was found to contain a 55 kDa cysteine protease inhibitor through SDS–PAGE inhibitor staining. Freeze dried salmon plasma (FSP) and salmon plasma concentrated by ultrafiltration (CSP) were tested for their ability to inhibit autolysis in Pacific whiting surimi and salmon mince at concentrations of 0.25%, 0.5%, 1%, and 2%. Pacific whiting surimi autolysis was inhibited by an average of 89% regardless of concentration while inhibition of salmon mince autolysis increased with concentration ($p < 0.05$). CSP performed slightly better than FSP at inhibiting salmon mince autolysis ($p < 0.05$). Serine protease inhibition decreased when SP heated above 40 °C but was stable across a broad NaCl and pH range. Cysteine protease inhibitors exhibited good temperature, NaCl, and pH stability.



## 1. Introduction

The Pacific whiting (*Merluccius productus*) fishery is the largest fishery by biomass in the state of Oregon (ODFW, 2013). Despite being an abundant resource, Pacific whiting suffers from a high concentration of endogenous proteases caused in part by infection of myxosporidian parasites (Patashnik, Groninger, Barnett, Kudo, & Koury, 1982). Pacific whiting muscle has been reported to have high levels of cathepsins B, H and L (Yongswatdigul, Hemung, & Choi, 2014). Unlike cathepsin B and H, cathepsin L is especially problematic for surimi manufacturers because it is not effectively removed by washing and it has an optimum temperature of around 60 °C (An, Weerasinghe, Seymour, & Morrissey, 1994). This protease damages myofibrillar proteins during slow heating of surimi based products causing softening of the final product, leading to an unacceptable texture. This proteolytic degradation caused by cathepsin enzymes can also lead to texture softening in salmon fillets (Dawson-Coates et al., 2003; St-Hilaire, Hill, Kent, Whitaker, & Ribble, 1997).

Blood plasma contains a variety of protease inhibitors (Travis & Salvesen, 1983), including α2-macroglobulin, a protein that inhibits several classes of proteases through a bait and trap mechanism (Barrett, 1981). In the past, surimi manufacturers used bovine blood plasma as an additive in Pacific whiting surimi in order to inhibit proteolytic degradation for slowly cooked surimi test gels, but this practice has been discontinued due Bovine Spongiform Encephalopathy. The surimi industry currently uses dried egg whites as a protease inhibitor, but this is less effective than blood plasma since egg whites contain mainly serine protease inhibitors while cathepsin L is a cysteine protease (Weerasinghe, An, & Morrissey, 1996).

Blood plasma from other sources has been investigated for protease inhibitory activity. Park reported pork plasma protein performed slightly better than beef plasma protein in slowly cooked Pacific whiting surimi (Park, 2005). Pig plasma was found to inhibit autolytic degradation and improve the gel strength of bigeye snapper surimi (Benjakul, Srivilai, & Visessanguan, 2001), and a cysteine protease inhibitor containing fraction of chicken plasma was found to inhibit proteases in both Pacific whiting and arrowtooth flounder muscle.

Fish blood from the commercial fish processing industry is not currently utilized. In 2013, 200,000 tons of salmon were processed in Alaska alone (ADR, 2014). Based on the fact that blood represents about 5% of the weight of a salmon (Halliday, 1973) and if fish are individually bled immediately following harvest, this equates to about 20 million pounds of blood entering the waste stream every year. If this blood water does not undergo costly waste water treatment, it can lead to contamination of the marine

* Corresponding author at: 2001 Marine Dr Rm 253, Astoria, OR 97103, United States. Tel.: +1 (503) 325 4531x203; fax: +1 (503) 325 2753.
*E-mail address:* jae.park@oregonstate.edu (J.W. Park).

http://dx.doi.org/10.1016/j.foodchem.2014.12.093
0308-8146/

environment, raising the biochemical oxygen demand, leading to algae bloom and other deleterious effects (Islam, Khan, & Tanaka, 2004). For economic and environmental purposes, this blood should be removed from the waste stream.

Fish blood has been found to contain protease inhibitors in previous studies. Rainbow trout plasma was found to increase gel strength in Alaska pollock surimi (Li, Lin, & Kim, 2008a) and a cysteine protease inhibitor was isolated from chum salmon plasma (Li, Lin, & Kim, 2008b). However, it is generally understood that Alaska pollock surimi except low grade does not show a significant level of texture softening protease. There have been no studies on the effect of protease inhibitors in fish blood on protease-laden Pacific whiting surimi. Pacific whiting was not utilized commercially until the introduction of beef plasma as an enzyme inhibitor in early 1990s. In addition, there have not been any studies on protease enzyme inhibition in salmon muscle. Extensive texture softening in salmon fillets due to protease degradation has been noted during routine analysis of farmed salmon in our laboratory. This issue leads to reduced quality of the product and in some cases the product must be disposed of. Adding inhibitors to salmon may lead to novel applications such as addition to salmon patties or injection into whole salmon fillets in order to prevent texture softening. The objective of this study was to investigate the ability of blood plasma obtained from Chinook salmon to inhibit proteolytic degradation in Pacific whiting surimi and salmon mince.

## 2. Materials and methods

### 2.1. Materials

Pacific whiting surimi produced at sea on May 18, 2013 without the addition of egg white was obtained from American Seafoods (Seattle, WA, USA). Chinook salmon were obtained at a local hatchery (Klaskanine Fish Hatchery (Astoria, OR, USA) during spawning season in September 2013. Pacific whiting were obtained from Da Yang Seafood (Astoria, OR, USA). Surimi, salmon, and Pacific whiting were kept at −30 °C until used. Papain (from papaya latex), trypsin (from bovine pancreas), hammarsten casein, $N_{\alpha}$-Benzoyl-DL-arginine β-naphthylamide (BANA), $N_{\alpha}$-Benzoyl-L-arginine 4-nitroanilide hydrochloride (BAPNA), and ρ-dimethylamino-cinnamaldehyde were purchased from Sigma Chemical Co (St Louis, MO, USA). Protein markers and other electrophoresis chemicals were purchased from Bio-Rad Laboratories (Hercules, CA, USA). Dry egg white (EW) was obtained from Henningsen Foods (Omaha, NE, USA). All other chemicals used were of reagent grade.

### 2.2. Collection of salmon blood and preparation of plasma

Whole blood was collected at the Klaskanine Fish Hatchery (Astoria, OR, USA) from female Chinook salmon immediately before roe collection. Blood was collected from bleeding fish into bottles containing 3.8% sodium citrate solution (as an anticoagulant), and gently mixed at a ratio of 9:1 (v:v) blood to sodium citrate solution. Blood was kept on ice and transported back to the Oregon State Seafood Laboratory (Astoria, OR, USA) where it was centrifuged for 15 min at 1500*g* at 4 °C using a Beckman J6-MI centrifuge (Beckman Coulter, Fullerton, CA, USA). The supernatant was regarded as salmon plasma (SP) and concentrated (see below) or kept at −80 °C until used.

A portion of the frozen SP was then lyophilized in a Labconco freeze drier (Kansas City, MO, USA). Lyophilization was carried out until the pressure in the chamber reached a minimum and no further decrease was noted. Freeze dried salmon plasma (FSP) was stored at −80 °C until used.

### 2.3. Salmon plasma concentration

Concentration was carried out in a 4 °C cold room. SP was concentrated using a Labscale Tangential Flow Filtration System (Millipore, Billerica, MA, USA). Plasma was re-circulated through a Pellicon XL50 Biomax 30 kDa membrane (Millipore, Billerica, MA, USA) at a feed pressure of 30 psi and a retentate pressure of 10 psi until the permeate flow was very low. The system was then cleaned using 0.1 N sodium hydroxide warmed to 45 °C and flushed with distilled water. The process was repeated once more to further concentrate the plasma.

### 2.4. Trypsin inhibition assay

Trypsin inhibition was determined according to the method of Smith, Hitchcock, Twaalfhoven, and Megen (1980) with some modification. Four different inhibitor solutions (SP, CSP, FSP, and EW) were diluted to varying concentrations with distilled water. 150 μL of inhibitor solution was added to 300 μL of bovine pancreas trypsin (20 μg/mL) and 150 μL of distilled water and preincubated at 37 °C for 10 min. 750 μL of 0.4 mg/mL BAPNA in 50 mM Tris–HCl buffer (pH 8.2) containing 20 mM $CaCl_2$ and pre warmed to 37 °C was then added and the reaction mixture was incubated at 37 °C for 10 min. The reaction was terminated by adding 150 μL of 30% (v/v) acetic acid. Absorbance was read at 410 nm and the inhibitory activity was expressed as the percent decrease in $OD_{410}$ compared to the control.

### 2.5. Papain inhibition assay

Papain inhibition was determined according to the method of Abe, Domoto, Arai, Abe, and Iwabuchi (1994) with some modification. To 2 mL of 0.25 M sodium phosphate buffer (pH 6.0) containing 2.5 mM EDTA and 25 mM β-mercaptoethanol (βME) was added 0.1 mL of papain solution (100 μg/mL) in 25 mM sodium phosphate buffer (pH 7.0) and 2 mL of inhibitor solution. After preincubation at 37 °C for 5 min, 0.2 mL of 2 mM BANA was added to initiate the reaction. After 10 min of incubation, 1 mL of cold 2% HCl in ethanol was added to stop the reaction. 1 mL of 0.06% ρ-dimethylamino-cinnamaldehyde was then added to develop color. Absorbance was read at 540 nm and the inhibitory activity was expressed as the percent decrease in $OD_{540}$ compared to the control.

### 2.6. Pacific whiting protease inhibition assay

Pacific whiting protease inhibition was determined according to the method of Benjakul and Visessanguan (2000) using fish juice (the supernatant recovered after centrifuging Pacific whiting mince at 5000*g* for 30 min) that was heated to 60 °C for 3 min and centrifuged at 7800*g* for 15 min according to the method of An, Morrissey, Fan, and Hartley (1995) as an enzyme source. Enzyme activity was determined using casein as a substrate according to the method of An, Seymour, Wu, and Morrissey (1994). The substrate mixture consisted of 2 mg casein in 0.625 mL of 0.2 M McIlvaine's buffer (0.2 M sodium phosphate and 0.1 M citric acid, pH 5.5) containing 0.1 mM βME adjusted to 1.25 mL with distilled deionized water. 100 μL of inhibitor solution was added to 100 μL of enzyme and preincubated at 55 °C for 5 min. The enzyme-inhibitor mixture was then added to the substrate mixture (prewarmed to 55 °C) and incubated for 10 min. The reaction was stopped by the addition of 200 μL of cold 50% trichloro acetic acid (TCA). The mixture was centrifuged at 8000*g* for 5 min (Sorvall Biofuge fresco, Kendro Laboratory Products, Newtown, CT, USA) and the TCA-soluble peptides in the supernatant were measured by the method of Lowry, Rosebrough, Farr,