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Effect of salmon plasma protein on Pacific whiting surimi gelation under various ohmic heating conditions

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ABSTRACT

The effect of salmon blood plasma (SPP) on the gelation of Pacific whiting surimi under different ohmic heating conditions was investigated. SPP was found to significantly increase gel strength in gels heated ohmically to and held at 60° for 30 min followed by heating ohmically to 90 °C. SPP at a level of 1 g/100 g was also found to increase gel strength in gels held at 25 °C for 2 h prior to ohmic heating. This increase was not seen in gels where EDTA was added to inhibit the activity of endogenous transglutaminase (ETG). SPP also created a more pronounced setting effect as measured by dynamic rheology and SDS-PAGE. SPP was found to effectively inhibit protease activity through TCA-soluble peptide analysis. Scanning electron microcopy revealed a loosely arranged gel network caused by protease enzymes. It was reversed by the addition of SPP as well as setting at 25 °C due to ETG.

1. Introduction

In the United States, surimi is made from two types of fish: Alaska pollock (AP) and Pacific whiting (PW). Unlike AP, PW contains a high amount of protease enzymes that degrade the quality of the surimi gel when heated slowly (Klesk, Yongsawatdigul, Park, Viratchakul, & Virulhakul, 2000). The major protease enzymes found in PW are cathepsins B, H, and L. After PW has gone through the rinsing step of surimi manufacturing, most of cathepsin B and almost all of cathepsin H proteases are removed. However, cathepsin L, a protease associated with myofibrillar proteins, is not removed during the washing process and was found to be the main protease responsible for degradation of the surimi gel (An, Weerasinghe, Seymour, & Morrissey, 1994). Cathepsin L is a heat activated cysteine protease, having an optimum temperature of around 55–60 °C (Seymour, Morrissey, Peters, & An, 1994; Visessanguan, Benjakul, & An, 2003). Incubating PW surimi around this temperature range for 30 min before heating to 90 °C will result in a complete disappearance of the myosin heavy chain as well as an inability to form a gel network (Morrissey, Wu, Lin, & An, 1993; Rawdkuen, Benjakul, Visessanguan, & Lanier, 2007a). Surimi that is heated at a slow rate (such as in a water bath) also suffers protoeolytic degradation (Yongsawatdigul, Park, Kolbe, Dagga, & Morrissey, 1995). In the past, bovine blood plasma (BPP) was added to PW surimi as a protease inhibitor. This practice was discontinued however due to public fear of Bovine Spongiform Encephalopathy (BSE). Since then, BPP has been replaced by dried egg white (DEW), which contains mainly serine protease inhibitors (Yongswatdigul, Hemung, & Choi, 2014). Since DEW is not as effective as BPP at inhibiting cysteine proteases, such as cathepsin L (Yongswatdigul et al., 2014), finding an alternative inhibitor that can be used at small concentrations would be beneficial. Blood plasma from other sources, such as pork (Benjakul, Srivilai, & Visessanguan, 2001; Visessanguan, Benjakul, & An, 2000) and chicken (Rawdkuen, Benjakul, et al., 2007a; Rawdkuen, Lanier, Visessanguan, & Benjakul, 2004, 2007b), have been found to be effective inhibitors of protease enzymes found in surimi.

In addition to protease inhibitors, blood plasma also contains other proteins that may enhance the gelation of surimi. Blood plasma proteins such as fibrinogen exhibit their own gelling ability upon heating (Davila, Pares, Cuvelier, & Relkin, 2007). Also, blood plasma has been shown to contain endogenous transglutaminase (ETG) enzymes (Lorand, 2007). ETG is a naturally occurring enzyme in PW and other species of fish. ETG is a calcium dependent enzyme that mediates covalent cross linking of myofibrillar proteins, resulting in a higher gel strength (An, Peters, & Seymour, 1996).

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Since endogenous PW and AP ETG has an optimum temperature of around 25 °C, leaving surimi paste at room temperature for 1 or 2 h before heating results in stronger gels. This ETG mediated formation of non-disulfide covalent cross links before heating is known as "setting". Addition of calcium and calcium containing compounds to surimi has been shown to increase the effect of setting (Lee & Park, 1998) and the addition of calcium chelating compounds, such as EDTA, has been shown to completely inhibit setting (Kumazawa, Numazawa, Seguro, & Motoki, 1995). Since blood plasma contains ETG, it may also contribute to the setting phenomenon when added to surimi in sufficient amounts.

The activity of these two different types of enzymes (proteases and ETG), pose a problem when evaluating the quality of PW surimi gel. Traditionally, surimi is heated in a water bath to 90 °C before conducting gel texture measurement. This slow heating allows for the activity of both ETG (enhances gel strength) and proteases (lowers gel strength). Surimi crabstick, however, is manufactured in a thin sheet under gas and/or steam heating, which quickly deactivates both types of enzymes and does not allow for any activity beyond 75 °C. Therefore, these testing methods do not accurately assess the quality of the surimi seafood being produced in a thin sheet under fast heating. Rapid heating methods, such as ohmic heating, allow for a better assessment of surimi containing protease enzymes (Yongsawatdigul et al., 1995). The objective of this study was to isolate the activities of both proteases and ETG at various heating rates under ohmic heating, and evaluate the effect of salmon plasma protein (SPP) on the gelation of PW surimi in combination with these enzymes.

2. Materials and methods

2.1. Materials

Pacific whiting surimi (FA grade) without the addition of egg white, 2 months old, was obtained from American Seafoods (Seattle, WA, USA) and kept at −30 °C until used. Protein markers and other electrophoresis chemicals were purchased from Bio-Rad Laboratories (Hercules, CA, 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 g/100 mL sodium citrate (as an anti-coagulant), and gently mixed at a ratio of 9:1 (mL:mL) blood to sodium citrate (Li, Lin, & Kim, 2008; Rawdkuen, Lanier, et al., 2007b). 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 then lyophilized in a Labconco freeze drier (Kansas City, MO, USA) and regarded as salmon plasma protein (SPP). SPP was stored at −80 °C until used. Samples were not stored longer than 3 months.

2.3. Surimi gel preparation

Paste and gels were prepared according the method of Poowakanjana, Mayer, and Park (2012) with various heating methods. Partially thawed surimi was chopped at 1800 rpm for 1 min using a silent cutter (UM 5 universal, Stephan Machinery Corp, Columbus, OH, USA). After a 2 g/100 g addition of salt, surimi was chopped for an additional 1 min at 1800 rpm. Moisture content was then adjusted to 78 g/100 g using ice. At this time SPP (0, 0.5 or 1 g/100 g) as well as EDTA (0 or 0.1 g/100 g) was added. A preliminary study conducted in our laboratory indicated that 0.1 g/100 g EDTA was sufficient to completely inhibit ETG activity in PW surimi (data not shown). Following the addition of ice and other dry ingredients, surimi was chopped again for 1 min at 1800 rpm. Chopping was then continued at 3600 rpm under vacuum (40–60 kPa) for an additional 3 min and a total chopping time of 6 min. Care was taken so that the final temperature of the surimi paste was less than 15 °C. Paste was packed in a polyethylene bag and subjected to vacuum to remove any air pockets developed during packing. The paste was then stuffed into a 15 cm × 3 cm nylon tube. 3 different heating methods were used: 1. Ohmic (rapid) heating to 90 °C at a voltage gradient of 12.62 V/cm with settings of 250 V and 10 kHz to prevent the activity of both ETG and proteases (OH); 2. Ohmic heating to 60 °C followed by ohmically holding at 60 °C for 30 min before ohmically heating to 90 °C to prevent the activity of ETG and maximize the activity of proteases (60/OH); and 3. Holding in a 25 °C water bath for 2 h (to maximize the activity of ETG) followed by ohmic heating to 90 °C to prevent the activity of proteases (25/OH). Two sausages were made per heating method. Following heating, gels were placed in a plastic bag, submerged in ice water for 15 min, and stored overnight at 4 °C.

2.4. Oscillatory dynamic measurement

Surimi paste was subjected to a temperature sweep using a CVO rheometer (Malvern Instruments Ltd., Worcestershire, UK) using a cone (4° and 40 mm diameter) and plate geometry with a gap of 150 μm. Surimi gels prepared as described above were thinly cut to a thickness of 2 mm and subjected to a frequency sweep using parallel plate geometry (20 mm diameter) and a gap of 1 mm. Surimi gel (3 cm diameter) was trimmed to 2 cm using a small knife and moisture trap containing a moistened sponge was used to minimize drying of sample. Temperature sweeps were conducted from 10 °C to 90 °C at a heating rate of 2 °C/min at a fixed frequency of 0.1 Hz. Frequency sweeps were conducted from 0.1 to 10 Hz at a fixed temperature of 25 °C. A shear stress of 50 Pa, determined by stress sweep to be in the linear viscoelastic region, was used.

2.5. Fracture gel evaluation

The day after heating, gels were removed from refrigerated storage and held at room temperature for 2 h prior to testing. Gel samples were cut into 30 mm lengths and the breaking force (g) and penetration distance (mm) were determined using a texture analyzer (TA-XT plus, Texture Technologies Corp, NY, USA). Gels were punctured with a spherical probe (5 mm diameter) at 1 mm/s.

2.6. Color analysis

L*, a*, and b* values of surimi gels were determined from 30 mm samples using a Minolta colorimeter (CR-310; Minolta Camera Co. Ltd., Osaka, Japan). The instrument was standardized using a Minolta calibration plate and a Hunter Lab standard hitching file according to the method of Park (1994). Whiteness was calculated using the equation L* − 3b*.

2.7. Determination of TCA soluble peptides

Eighteen mL of 5 g/100 mL trichloroacetic acid (TCA) was added to 2 g of sample followed by homogenization for 2 min at 15,000 rpm using a Tissue Tearor homogenizer (Biospec Products Inc., Bartlesville, OK, USA). Homogenate was then held at 4 °C for 1 h before centrifugation at 8000× *g* for 5 min using a Sorvall RC-5B