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High pressure effects on heat-induced gelation of threadfin bream (*Nemipterus* spp.) surimi

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ABSTRACT

We sought to determine, in threadfin bream surimi system, if 40 °C incubation carried out simultaneous with, or following, HPP might also induce a more effective setting on subsequently cooked gels. Threadfin bream surimi pastes were subjected to HPP 200 or 300; at 5 °C (little or no setting effect expected during HPP at this temperature) for 15 min or at 40 °C (optimal setting temperature for threadfin bream pastes) for 15 or 30 min. Pastes were then directly cooked (90 °C for 20 min) ($^{pressure}P_{°C/time}$ > C), or first allowed to undergo setting at atmospheric pressure (40 °C for 60 min), followed by cooking (90 °C 20 min) ($^{pressure}P_{°C/time}$ > S_{time} > C). With or without followed setting treatment at 40 °C, HPP treatment at 40 °C, or even lower pressure at 5 °C for shorter time ($^{200MPa}P_{5°C/15min}$ > $S_{40°C/60min}$ > C) produce weak cooking gels. Two opposite actions of cross-linked polymer (CP) and degraded protein (DP) was observed during the HPP treatment. The given HPP treatment was sure to triggered the detriment of protein in combined treatments, the followed setting treatment after HPP enhanced the degradation of protein as well. The formation of disulfide bonds during HPP and cooking treatments was retarded, which was attributed to the influence of protein degradation behavior. Scanning electron microscopy revealed, with giving a prior HPP treatment, the fiberlike structure gradually disappeared and the compact microstructure increased in final cooking gel.

1. Introduction

Texture is a critical preference factor in describing the functional characteristics for most surimi-based foods. The fracture properties (texture) of surimi-based foods are particularly impacted by the heat processing step(s) used during conversion of a raw surimi paste to a cooked gel. A so-called 'setting' treatment prior to cooking, which is pre-incubation of a salted surimi paste at temperatures between 0 and 40 °C, can result in stronger cooked gels as this pre-incubation period allows endogenous transglutaminase (TGase) to form covalent glutamyl-lysine cross-links between proteins (Lanier, 2000). Threadfin bream (*Nemipterus* spp.) is a typical tropical fish species and the second largest resource used for surimi processing after Alaska pollock (*Theragra chalcogramma*). For some intrinsic differences of surimi system, the gellation behavior of threadfin bream surimi seems more complex than that of Alaska Pollock surimi. Under atmospheric pressure, the pre-incubation of threadfin bream paste at 40 °C for a short duration showed an increase of gel strength because of TGase activity. Extended incubation time at 40 °C, however, resulted in a decrease in force and deformation due to endogenous proteinase(s) (Yongsawatdigul and Park, 2004; Yongsawatdigul et al., 2002; Benjakul et al., 2004).

The combined use of pressure and temperature is considered that can offer promising possibilities for the gelation process, can thus be used to improve the gelation process for fish proteins (Colmenero, 2002). A high pressure processing (HPP) treatment prior to the setting step has been shown to promote this crosslinking and gel strengthening. In the cross-linking reaction via a transglutaminase, a methylene group of the glutamine residue is considered to be necessary to confer substrate properties and this hydrophobic methylene group is essential for interaction with a hydrophobic region near the active site of transglutaminase (Folk, 1983). HPP is presumed to induce non-thermal denaturation of the meat proteins, giving better subsequent access of TGase to the glutamine and lysine residues it targets, thereby promoting additional protein crosslinking (Gilleland et al., 1997; Hwang et al., 2007). Except the formation of cross-link by endogenous TGase, when heating at high temperatures (>40 °C), disulfide bonding (S–S) is an important covalent bond thought to contribute to gel formation of proteins (Lanier et al., 2013). Pressure-induced

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unfolding also can enhance the exposure of buried free SH groups, which involve in the formation of disulfide bond stabilized protein aggregates (Van der Plancken et al., 2005).

The objective of the present work was to determine, in threadfin bream surimi protein system (containing crosslinking by TGase and degradation by endogenous proteinase(s)), whether the setting step can be as positive, or possibly even detrimental, in influencing gel strength of cooked gels when it is conducted simultaneously with or after the HPP step, by elevating temperature and extending the time of the HPP process.

2. Materials and methods

2.1. Frozen surimi

Frozen surimi commercially prepared from threadfin bream (*Nemipterus* spp.) containing 4% sucrose, 4% sorbitol and 0.3% sodium tripolyphosphate, was purchased from Aquamar Inc. (Rancho Cucamonga, CA, U.S.A.). Blocks (10 kg) frozen at −50 °C were impact-shattered, and the pieces randomized. Portions (1 kg) were vacuum packed in oxygen impermeable bags and stored at −20 °C until needed. Moisture content was 76.4% (AOAC, 1984). The surimi was subsequently used within 3–6 mo of frozen storage at −20 °C.

2.2. Preparation of surimi pastes

Frozen surimi was tempered at room temperature for 30 min before cubing with a knife while frozen. The surimi was then chopped for 15 s to reduce particle size followed by blending with NaCl and ice in a vertical cutter/mixer (Stephan Machinery Corp., Columbus, OH). Ice and NaCl were added to adjust final moisture (total) and salt (added) content of all formulas to 78% and 2%, respectively. The pastes were chopped at 2100 rpm under vacuum and controlled at a constant temperature near 0 °C. Pastes were then vacuum packaged and extruded from a slit made in the vacuum bag within a sausage stuffer into a stainless steel tube (22.7 cm length, 1.9 cm i.d.) for torsion testing, or into a polystyrene bag for chemical measurement and microscopy (raw paste). All stainless steel tubes were sprayed inside with a lecithin-based release agent to prevent gel adhesion. All sample tubes were placed and in a polystyrene bag, and all the stuffed polystyrene bags were evacuated and sealed for the subsequent treatments.

2.3. Combination treatments

The experimental treatment combinations are given in Table 1 and Fig. 1B. A Model 1P-2-22-60 HPP unit (Autoclave Engineers Inc., Erie, PA), consisting of a liquid pump feeding a sealable compression vessel, was employed for HPP of pastes while still contained in the metal tubes or bags (both vacuum packaged for this treatment). The temperature of the compression fluid and chamber for HPP treatment (designated as P in Table 1 and Fig. 1) was adjusted to either 40 °C (to presumably enhance the rate of protein crosslinking or setting) or 5 °C (presumably a low rate of setting in threadfin bream paste). Any subsequent setting treatment (designated as S in Table 1 and Fig. 1; carried out at atmospheric pressure) was at 40 °C, for the designated time, in a water bath. Cooking (designated as C in Table 1 and Fig. 1) was subsequently carried out in a water bath at 90 °C for 20 min, followed by cooling in ice water for 20 min. The control sample consisted of paste incubated at 40 °C for 60 min, then cooked at 90 °C for 20 min and subsequently cooled in ice water for 20 min. Gels were removed from the tubes and held refrigerated in a sealed container until tested for fracture properties.

Designation of the particular combination treatments was by the notations $^{\text{pressure}}P_{\text{°C/time}} > C$; $^{\text{pressure}}P_{\text{°C/time}} > S_{\text{°C/time}} > C$.

Table 1
Experimental treatments.

	Symbol	Treatment
1.	Control ($S_{40°C/60min} > C$)	0.1 MPa; setting 40 °C/60 min; cooking 90 °C/20 min
2.	$^{200MPa}P_{5°C/15min} > S_{40°C/60min} > C$	200 MPa/5 °C/15 min; setting 40 °C/60 min; cooking 90 °C/20 min
3.	$^{300MPa}P_{5°C/15min} > S_{40°C/60min} > C$	300 MPa/5 °C/15 min; setting 40 °C/60 min; cooking 90 °C/20 min
4.	$^{200MPa}P_{40°C/30min} > C$	200 MPa/40 °C/30 min; cooking 90 °C/20 min
5.	$^{300MPa}P_{40°C/30min} > C$	300 MPa/40 °C/30 min; cooking 90 °C/20 min
6.	$^{200MPa}P_{40°C/15min} > S_{40°C/60min} > C$	200 MPa/40 °C/15 min; setting 40 °C/60 min; cooking 90 °C/20 min
7.	$^{300MPa}P_{40°C/15min} > S_{40°C/60min} > C$	300 MPa/40 °C/15 min; setting 40 °C/60 min; cooking 90 °C/20 min

2.4. Fracture stress and strain of gels

Torsion testing was performed using a Hamann Torsion Gelometer (Gel Consultants, Raleigh, N.C., U.S.A.) to determine the fracture shear stress (strength) and fracture shear strain (cohesiveness or deformability) of gels (Park, 2005). All gels were equilibrated to room temperature before cutting into 2.54 cm length sections and grinding these to a 1 cm minimum diameter capstan shape for testing. A minimum of 7 gel samples were subjected to torsional fracture for each treatment.

2.5. Quantification of thiol groups

Except frozen surimi was used as the control, samples of each step in other 6 treatment combinations as before (Table 1 and Fig. 1) for this analysis were prepared. Changes in the free thiol groups (SH) of proteins were determined using Ellman's reagent according to Beveridge et al. (1974) with slight modification. Briefly, Tris–glycine (Tris–Gly) buffer was prepared by dissolving 10.4 g of Tris, 6.9 g of glycine, and 1.2 g of EDTA in 1 L of water and adjusted to pH 8.0. GuHCl/Tris–Gly solution contained 5 M guanidine hydrochloride, urea–GuHCl/ Tris–Gly solution contained 5 M guanidine hydrochloride and 8 M urea. Ellman's reagent contained 4 mg of 5,5′ dithiobis-2-nitrobenzoic acid in 1 mL of Tris–Gly buffer at pH 8.0 and was freshly prepared each day.

Samples (0.3 g) were solubilized in test tubes with 10 mL buffer (20 mM Tris, 8 M urea, 1% sodium dodecyl sulfate (SDS), 1 mM EDTA pH 8) by continuous shaking for 16–24 h at room temperature. The sample solutions (100 μL) were then mixed with urea–GuHCl/Tris–Gly solution (1.0 mL) and Ellman's reagent (20 μL), and the absorbance read at 412 nm. Results were calculated against a cysteine standard curve. Three replicates were run for each determination.

2.6. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE)

Gels were solubilized according to Lee et al. (1997). Samples were prepared by the same 7 treatment combinations as before (Table 1 and Fig. 1), but with omission of the final cooking step, as the intent was to monitor only effects of HPP and/or setting. Samples (0.3 g) were solubilized in test tubes with 15 mL urea buffer (20 mM Tris, 8 M urea, 2% sodium dodecyl sulfate (SDS), and 2% β-mercaptoethanol pH 8) during a heating treatment in boiling water for 2 min followed by continuous shaking for 16–24 h at room temperature.