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Transglutaminase and high pressure effects on heat-induced gelation of Alaska pollock (*Theragra chalcogramma*) surimi

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ABSTRACT

A prior high pressure processing (HPP) treatment has previously been shown to enhance subsequent (at atmospheric pressure) low temperature 'setting' (glutamyl-lysine, non-disulfide protein crosslinking) induced by transglutaminase (TGase; endogenous or added microbial enzyme). This enhanced setting induces greater gel strength and deformability of subsequently cooked surimi gels. We sought to determine if 25 °C incubation carried out simultaneous with, rather than following, HPP might similarly induce a setting effect on subsequently cooked gels. Pollock surimi pastes (± added microbial TGase) were subjected to HPP 200, 300, or 400 MPa; at 5 °C (little or no setting effect expected during HPP at this temperature) for 30 min or at 25 °C (optimal setting temperature for pollock pastes) for 30, 60, or 120 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 (25 °C for 30, 60, or 120 min), followed by cooking (90 °C 20 min) ($^{pressure}P_{°C/time} > S_{time} > C$). With no microbial TGase added to the raw paste (e.g., endogenous TGase only), $^{300MPa}P_{5°C/30min} > S_{25°C/120min} > C$ induced highest gel fracture stress and strain. The same treatment but with HPP at 200 or 400 MPa gave only slightly lower fracture stress (gel strength). Increasing the temperature of the HPP treatment to 25 °C ($^{300MPa}P_{25°C/30min} > S_{25°C/120min} > C$), even with longer HPP time ($^{300MPa}P_{25°C/60\ or\ 120min} > C$), gave weaker gels, similar to those obtained by setting and cooking without a prior HPP treatment ($S_{25°C/60min} > C$). Thus, attempting to induce TGase crosslinking by setting at 25 °C during HPP treatment actually seemed detrimental to gel strength development. However, when HPP was carried out at 25 °C and microbial TGase was added, gel strength and deformability (fracture stress, strain) were enhanced above that of all other treatments tested. All treatments containing microbial TGase evidenced enhanced protein polymerization. Scanning electron microscopy revealed a more dense and fibrous structure in such gels, and reduction of free thiol (SH) groups was noted as a result of microbial TGase addition.

1. Introduction

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. During heating (cooking), the meat proteins unfold (denature) due to the violent movement of molecules at elevated temperature (Pothakamury et al., 1995) and form an ordered, three-dimensional network stabilized by covalent, hydrophobic, and hydrogen bonds (Lee and Lanier, 1995; Whiting, 1988). Protein denaturation, leading to gelation, can alternatively be induced at cold temperatures by high pressure processing (HPP) because of the imparted decrease in volume of the protein in solution. HPP induces the exposure of buried SH groups, which subsequently are involved in the formation of disulfide bond stabilized protein aggregates (Van der Plancken et al., 2005), and also induces the formation of additional hydrogen bonds and hydrophobic interactions between proteins.

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 or added transglutaminase (TGase) to form covalent glutamyl-lysine crosslinks between proteins (Lanier, 2000). A high pressure processing (HPP) treatment just prior to the setting step has been shown to promote this crosslinking and gel strengthening even more. HPP presumably induces non-thermal denaturation of the meat proteins, giving better subsequent access

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of TGase to the glutamine and lysine residues it targets, thereby promoting additional protein crosslinking (Gilleland et al., 1997; Uresti et al., 2006; Hwang et al., 2007; Hsieh et al., 2009). Both endogenous (in fish meat) or added (microbial-derived) TGase is remarkably stable under high-pressure treatment (up to 300 MPa) (Gilleland et al., 1997; Ashie and Lanier, 1999; Menéndez et al., 2006).

The combined use of HPP + low temperature setting + higher temperature cooking (carried out in this sequence) can thus optimize the gelation process for fish proteins (Colmenero, 2002). The objective of the present work was to determine whether the setting step (crosslinking by TGase, endogenous or added) can be as effective, or possibly even more effective, in enhancing gel strength of cooked gels when it is conducted simultaneously with 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 Alaska pollock (*Theragra chalcogramma*) containing 4% sucrose, 4% sorbitol and 0.3% sodium tripolyphosphate, was purchased from Trident Seafoods (Seattle, Wash., 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 74.4% (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 (Esturk et al., 2004). 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. The same procedure was used to prepare samples containing 1 g/kg of Activa® powder containing microbial TGase (MTGase; Activa brand, Ajinomoto USA, Ft. Lee NJ), which was added to the surimi mince during the mixing with salt. 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 19 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 Fig. 1) was adjusted to either 25 °C (to presumably enhance the rate of protein crosslinking or setting) or 5 °C (presumably a low rate of setting in Alaska pollock paste) (Kamath et al., 1992; Esturk et al., 2004). Any subsequent setting treatment (designated as S in Fig. 1; carried out at atmospheric pressure) was at 25 °C, for the designated time, in a water bath. Cooking (designated as C in 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 25 °C for 60 min, then cooked at 90 °C for 20 min and subsequently cooled in ice 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 $^{\text{pressure}}P_{^\circ C/\text{time}} > C$; $^{\text{pressure}}P_{^\circ C/\text{time}} > S_{^\circ C/\text{time}} > C$; $^{\text{pressure+MTGase}}P_{^\circ C/\text{time}} > C$; or $^{\text{pressure+MTGase}}P_{^\circ C/\text{time}} > S_{^\circ C/\text{time}} > C$.

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

Samples for this analysis were prepared by the same 19 treatment combinations as before (Fig. 1), but with omission of the final cooking step, as the intent was to monitor only effects of HPP and/or setting. 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 19 treatment combinations as before (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.

Solubilized samples were prepared following the recommendations developed for NuPAGE Bis–Tris high-performance electrophoresis (Invitrogen, Carlsbad, Calif., U.S.A.) using 4X LDS sample buffer and 0.5% dithiothreitol (DTT) as a reducing agent (Engelhorn and Updyke 1996). Protein was applied at 6 μg per lane. Electro-