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Extreme pH treatments enhance the structure-reinforcement role of soy protein isolate and its emulsions in pork myofibrillar protein gels in the presence of microbial transglutaminase ☆

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ABSTRACT

Alkali (pH_{12}) and acid ($pH_{1.5}$) pH-treated soy protein isolates (SPI) were incorporated (0.25–0.75% protein) into sols of myofibrillar protein (MP, 3%, in 0.6 M NaCl at pH 6.25) with or without 0.1% microbial transglutaminase (TG) to investigate the potential as meat processing ingredients. Static and dynamic rheological measurements showed significant enhancements of MP gelling ability by the inclusion of $pH_{1.5}$-treated as well as preheated SPI (90 °C, 3 min). A 7-h incubation with TG accentuated the gel-strengthening effect by these SPI samples. The B subunit in 11S of SPI was the main component manifesting structure reinforcement in the mixed gels. The MP gelling properties were also greatly improved ($P<0.05$) by the addition of 10% canola oil emulsions stabilized by pH-treated SPI. The principal force in the MP gels incorporated with pH-treated SPI was hydrophobic patches; in the presence of TG, cross-linking of previously dissociated A and B subunits of 11S was also intimately involved.

1. Introduction

The quality of a processed meat product is determined by the performance of the specific functional ingredients. Protein isolates are widely used as binders to improve meat products' yield and texture, as surfactants to enhance the emulsion stability upon heating, and as a meat replacement to reduce the formulation cost. Soy protein isolate (SPI) is a low-cost protein additive widely utilized as an emulsifier, moisture retainer, and filler/binder to improve the functional properties of comminuted and emulsified muscle foods (Josquin, Linssen, & Houben, 2012; Pietrasik & Li-Chan, 2002).

The usefulness of SPI as a functional additive in processed muscle foods is dictated, to a large extent, by its interaction and compatibility with muscle proteins, notably myosin. Haga and Ohashi (1984) observed interactions between SPI and myosin in heat processing and suggested disulfide bonds being a significant force involved. Peng, Dayton, Quass, and Allen (1982) claimed that the interaction did not occur between native soy protein and myosin, but between dissociated soy 11S and myosin heavy chains. In comparison, the presence of β-conglycinin suppressed the aggregation of myosin heavy chain between 50 °C and 100 °C (Peng & Nielsen, 1986). The latter finding was supported by the study that showed an antagonistic effect of 7S globulins in myofibrillar protein gels where the presence of β-conglycinin seemed to hinder the self-aggregation of myosin heavy chain (Feng & Xiong, 2002, 2003). Accordingly, different attempts have been made to modify the native structure of soy protein to enhance its performance and suitability in muscle foods.

The failure to improve the textural characteristics of processed meats by native SPI can be explained by the lack of interaction with muscle proteins under normal meat processing conditions (heated up to 75 °C) where neither 7S nor 11S globulins show structural changes, namely, the exposure of reactive groups. Soy β-conglycinin (7S) and glycinin (11S) in low-salt aqueous solutions do not unfold until the heating temperature reaches ~77 °C and ~92 °C, respectively (Jiang, Xiong, & Chen, 2010). Preheat treatment is the simplest method to improve the functional performance of SPI in comminuted meats whose quality depends upon protein gelation (Hung & Smith, 1993). Heating brings about a number of desirable physicochemical changes in proteins, such as denaturation (unfolding), dissociation of subunits, association of reactive components, and aggregation that facilitates gel formation. Conversely, heating could have unwanted consequences. For example, the emulsifying property of soy protein, an important functionality in comminuted meats, tends to decrease if the protein is preheated (Aoki, Taneyama, & Inami, 1980). Moreover, thermal treatments can generate dark color and reduce the dispersibility of soy protein in aqueous solutions (Rakosky, 1970).

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An alkaline or acidic pH treatment, also referred to as the 'pH-shifting process', involves the exposure of a substance to extreme pH followed by neutralization. pH treatments are an alternative, novel approach to the modification of protein structures without generating much undesirable effect. This process has been reported to improve the functionality of SPI through the induction of subunit dissociation and unfolding of native structure (Jiang, Chen, & Xiong, 2009; Wagner & Gueguen, 1999). The separated subunits could reaggregate to form heterogeneous soluble polymers that exhibit excellent emulsifying activity and film-forming capacity (Jiang, Xiong, Newman, & Rentfrow, 2012; Jiang et al., 2010).

Emulsified comminuted meats, such as frankfurters and bologna, are a major type of composite products widely consumed worldwide. Because of the health concern, recent interest has focused on partial replacements of animal fat with vegetable oils (Carmona, Ruiz-Capillas, Jimenez-Colmenero, Pintado, & Herrero, 2011; Youssef & Barbut, 2010, 2011). One unique benefit of incorporating oil-in-water preemulsion in comminuted meats is that the size of oil droplets can be controlled, therefore, the rheology and textural attributes of cooked meat products in which preemulsions are imbedded can be significantly enhanced (Wu, Xiong, & Chen, 2011).

Because pH-shifting is capable of improving the emulsifying capacity of SPI, a preemulsion prepared with treated SPI when incorporated into chopped meat would give rise to a more stable product. Comminuted meats with preemulsions have a reduced chance of phase separation (bulk oil physically separated from the structure of the meat gel) and have a propensity to remain stable throughout the range of environmental conditions encountered during processing, storage and consumption (Josquin et al., 2012). The addition of transglutaminase (TG) could further accentuate the functional properties of this type of composite products because cross-linking reactions are conducive to cohesive gels and stable emulsions (Ramírez-Suárez & Xiong, 2003; Trespalacious & Pla, 2007). The structural unfolding of proteins induced by the pH treatments would allow greater reactivity of TG with its target substrate amino acid residues (glutamine, lysine) thereby producing a more interactive, viscoelastic gel or meat network.

The purpose of the present study was to enhance the gelling capacity of myofibrillar proteins by means of incorporation of pH-treated SPI and the preemulsions. Microbial TG was also included to create a composite gel system with further enhanced rheological properties and stability.

2. Materials and methods

2.1. Materials

Fresh pork loins (*Longissimus lumborum*) were excised from eight randomly selected pork carcasses (24 h postmortem) processed at the University of Kentucky Meat Laboratory, a USDA-inspected facility. Slices (chops) of approximately 1.27 cm thick were cut perpendicular to the fiber direction from the loins, individually vacuum sealed, and stored in a −30 °C freezer before use.

SPI was prepared from soybeans (Taiwan 292) using alkaline pH (8.0) extraction followed by isoelectric precipitation (pH 4.5) and then neutralization (pH 7.0) according to Jiang et al. (2009). The protein content of lyophilized SPI, determined by the Biuret procedure calibrated to the Kjeldahl method as standard, was found to be 92% (w/w).

2.2. Preparation of myofibrillar protein (MP)

MP was isolated from thawed pork muscle through washing (3 times) with 0.1 M NaCl, 50 mM sodium phosphate buffer (pH 7.0) as described elsewhere (Xiong, 1993). The pellet was washed 2 more times with 0.1 M NaCl solution. All isolation steps were conducted in a 4 °C walk-in room. Protein concentration of the final pellet (MP, ~7% protein) was determined by the Biuret method using bovine serum albumin as standard (Gornall, Bardawill, & David, 1949).

2.3. Preparation of pH-treated SPI

Suspensions of SPI (3% protein, pH 7.0) were adjusted to pH 1.5 with 2 M HCl, or pH 12 with 2 M NaOH. After holding at the specific pH for 1 h at room temperature to unfold, the solutions were neutralized with 2 M HCl to pH 7 and held at this pH for 1 h to allow partial refolding. To remove salts introduced from the pH adjustments, protein from the treatments at both pH was precipitated at pH 4.5, washed 3 times, and then re-solubilized at pH 7.0. Thereafter, protein solutions were lyophilized and stored at 4 °C until use. Native SPI (without the pH treatment) was used as control, and preheated native SPI (10% protein in 90 °C, 3 min) was used as a comparison.

2.4. Preparation of mixed protein sols

For the protein gel system, mixtures of MP (final protein concentration 3% after combination) with native, preheated, $pH_{1.5}$-treated, and pH_{12}-treated SPI (final concentrations 0.25, 0.5, and 0.75% after combination) were prepared in 0.6 M NaCl, 50 mM phosphate (pH 6.25) to yield MP:SPI ratios of 12:1, 6:1, and 4:1. Two different types of gels were prepared, i.e., regular gels and emulsion gels. The regular gels consisted of 3% MP and different concentrations of SPI as mentioned above in 0.6 M NaCl, 50 mM phosphate (pH 6.25). For emulsion gels, the preemulsion made from SPI and canola oil was added to the MP sol in 0.6 M NaCl, 50 mM phosphate (pH 6.25) by gently stirring with a glass rod to prepare a mixture of 3% MP, 0.75% SPI, and 10% lipid. The preemulsion was made by homogenization of the mixture of canola oil and SPI (final 26.7% oil and 2% protein — adjusted to pH 7.0) for 2 min at room temperature with a PT 10/35 Polytron homogenizer (Brinkman Instruments Inc., Westbury, NY, USA) at speed setting of 6. The emulsion was used immediately after preparation. The two types of MP/SPI protein sols were stored at 4 °C for 15 h to reach a maximum protein solubility (Ramírez-Suárez, Xiong, & Wang, 2001), then incubated with or without 0.1% TG preparation (99% maltodextrin and 1% TG, Ajinomoto, Teaneck, NJ, USA) for 0, 1, and 7 h at 4 °C.

2.5. Gelation and testing of gel properties

Prior to gelation, protein sols were centrifuged (2000 *g*, 5 min) to remove bubbles. Aliquots of 5 g of the samples were placed into glass vials (16.5 mm i.d.). Gels were formed by heating from 5 to 73 °C at a rate of 1 °C/min in a linear-heating water bath. After reaching the target temperature (73 °C), the gels were removed from the water bath and chilled in an ice slurry. Before the gel strength measurement, gels in the vials were equilibrated at room temperature for 1 h, then tested by back-extrusion with a stainless steel flat-surface rod (12.5 mm dia.) at a crosshead speed of 20 mm/min on a Model 4301 Instron Universal Testing Instrument (Instron Corp., Canton, MA, USA). The penetration force, defined as the force required to rupture the gel, was expressed as gel strength. Triplicate samples from each replicate trial were tested.

2.6. Rheology

Protein sols were subjected to oscillatory shear analysis with a Bohlin VOR rheometer (Bohlin Instruments, Inc., Cranbury, NJ, USA) to examine the dynamic formation of a protein network during gelation. Protein sols were heated from 20 to 80 °C at a 1 °C/min heating rate. The actual temperature of the samples was measured with a thermocouple. During heating samples, loaded between two parallel plates (1 mm gap), were continually sheared in an oscillatory mode at a fixed frequency of 0.1 Hz with a maximum strain of 0.02. Changes