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Protective effect of porcine plasma protein hydrolysates on the gelation of porcine myofibrillar protein exposed to a hydroxyl radical-generating system

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ABSTRACT

This study investigated the effect of different concentrations of porcine plasma protein hydrolysates (PPPH) on the gelation of porcine myofibrillar protein (MP) exposed to a hydroxyl radical-generating system (HRGS). Compared to non-oxidized MP, the oxidative modification led to a decreased penetration force and water holding capacity (WHC) ($P < 0.05$). Meanwhile, addition of PPPH (1.5 mg/mL) reduced the loss of MP gel strength and WHC induced by HRGS ($P < 0.05$). Low-field nuclear magnetic resonance results suggested that the addition of PPPH facilitated the hydration of the protein. The results concerning molecular forces revealed that hydrophobic interactions, hydrogen bonds, and disulphide bonds are the primary forces in gel formation. Non-reducing electrophoresis indicated that the addition of PPPH reduced protein loss and aggregation. The addition of PPPH promoted the formation of a more smooth and homogeneous gel network. Our results demonstrated that PPPH effectively retarded oxidation-induced MP gel deterioration and could be used as an antioxidant in meat products.

1. Introduction

The properties of comminuted meat products, such as a desirable texture and water-binding capacity, are based on the heat-induced gelling and rheological behaviour of the proteins. Salt-soluble myofibrillar protein (MP) plays a remarkable role in imparting these properties to meat products [1]. Meat proteins are susceptible to oxidation, especially during meat processing and storage, and protein oxidation negatively affects the MP gelling. Protein oxidation is initiated by reactive oxygen species (ROS), especially free radicals, and is characterized by conformational changes, reduced solubility, aggregation, and a general loss of functional properties [2]. Tunhun et al. [3] reported that the oxidation of fish protein during washing decreased its ability to dimerize and form a gel. Huang et al. [4] reported that protein oxidation reduces the gelling capacity of porcine MP, which contributes most to the loss of texture in dumpling meat fillings. More importantly, Wang et al. [5] reported that, at high malondialdehyde oxidative concentrations (25–50 mM), the gelling quality of MP in silver carp was weakened, probably because of the formation of excessive covalent bonds. However, Lu et al. [6] proved that mild protein oxidation caused by frozen storage or treatment with 0.1 mM H_2O_2 promotes bighead carp MP gel formation.

Of various antioxidative strategies, the most effective approach to inhibiting protein oxidation is the application of antioxidants [7]. Protein hydrolysates prepared with different kinds of enzymes are increasingly used as natural antioxidants in the food industry and provide many health benefits, which have been mainly attributed to the bioactive peptides in them. Porcine plasma protein hydrolysates (PPPH), hydrolysed with alcalase for 5 h, show strong radical-scavenging, Cu^{2+}-chelation and reducing power abilities [8]. In rats, PPPH has strong antioxidant activity in protecting the liver against CCl_4-induced oxidative damage [9]. Li et al. [10] reported that a combination of zein hydrolysates and sage extract exhibits a significant synergism against oxidation in liposomes. Li et al. [11] studied the effect of combined whey protein isolate hydrolysates and cryoprotectants on inhibiting the loss of quality in common carp (*Cyprinus carpio*) surimi during freezing. Their results showed that treatments with combined protein hydrolysates and cryoprotectants decrease the extent of protein oxidation and limit protein structural changes.

Although there have been studies on the inhibition of MP oxidation with protein hydrolysates, few studies have studied the influence of protein hydrolysates on the gelling of oxidized MP. The aim of this study was to study the influence of different

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concentrations of PPPH on the gelling properties, including gel penetration force, water holding capacity (WHC), and microstructure and surface morphology of porcine MP under controlled oxidizing conditions created with a hydroxyl radical-generating system (HRGS). Moreover, a possible protective mechanism was proposed by analysing molecular forces in the gel, water distribution and data from sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE).

2. Materials and methods

2.1. Materials

Porcine plasma protein, purchased from Beidahuang Meat Corporation (Harbin, Heilongjiang, China), contained 85% protein as determined by the Kjeldahl method of standard procedures (AOAC, 2000). Pork longissimus muscle (1 d post mortem) was obtained from a local commercial store (Harbin, Heilongjiang, China). The samples were kept on ice and transported to the laboratory and used on the same day. Piperazine-*N*,*N* bis, and Piperazine-1,4-bis(2-ethanesulfonic acid) (PIPES) was purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Preparation of porcine plasma protein hydrolysates

PPPH were prepared on the basis of Liu et al. [8]. The PPPH samples were desalted, lyophilized, sealed in polyethylene bags and then stored at 4 °C.

2.3. Preparation of myofibrillar protein

MP was prepared as previously described in Ref. [12]. All steps were carried out in a 4 °C cooling room. The protein concentration of prepared MP (68 mg/mL) was measured by the Biuret method using bovine serum albumin as a standard [13]. The MP solution was stored in a capped bottle, kept on ice, and utilized within 48 h.

2.4. Oxidation of samples

MP was suspended in a 15 mM PIPES buffer containing 0.6 M NaCl (pH 6.25) to a concentration of 30 mg/mL. PPPH was incorporated into the MP suspension at concentrations of 0, 0.3, 0.75, and 1.5 mg/mL (that is, 0%, 1%, 2.5%, and 5% of MP protein, respectively). Oxidation was conducted for all of the samples at 4 °C for 5 h in a system that generated hydroxyl radicals and consisted of 10 μM $FeCl_3$, 0.1 mM ascorbic acid and 10 mM H_2O_2. A mixture of 1 mM butylated hydroxyanisole, 1 mM Trolox C, and 1 mM EDTA was added to terminate the oxidation reaction. To get rid of the oxidizing reagents, protein suspensions were washed once with 5 times the volume of the aqueous protein solution, and then centrifuged at 10,000*g* for 5 min. The protein content of oxidized MP (OxiMP) with different concentrations of PPPH was assessed using the Biuret method. The non-oxidized MP solution was used as a control. All of the samples were stored at 4 °C for at least 12 h to reach the maximum protein solubility.

2.5. Gel strength analysis

The MP and OxiMP/PPPH samples were diluted to 40 mg/mL with 50 mM PIPES buffer containing 0.6 M NaCl (pH 6.25). MP solution (20 mL) was placed in a 25 × 40 mm (length × diameter) glass vial. Gels were formed in a 72 °C water bath for 10 min and cooled immediately in ice slurry. Before the measurement of gel strength, the gels in the vials were equilibrated at 25 °C for 1 h. The gel strength of the samples was measured with a TA-XT2 texture analyser (Stable Micro Systems Ltd., Godalming, UK). The gels were axially penetrated to a depth of 12 mm at a speed of 50 mm/min with a P/0.5 flat-surface cylindrical probe (12 mm in diameter) using the gel capsule penetration mode. The penetration force, defined as the force required to rupture the gel, was expressed as gel strength.

2.6. Water holding capacity

The gel water holding capacity (WHC) was measured in accordance with the method of Salvador et al. [14] with some modifications. Briefly, 5 g aliquots of the gels prepared in Section 2.5 were centrifuged at 10,000*g* for 10 min at 4 °C. The supernatant was abandoned and the total weight of the sediment and the tube was recorded. WHC (%) was calculated by dividing the gel weight after centrifugation by the gel weight before centrifugation then multiplying by 100.

2.7. Low field nuclear magnetic resonance

The gel samples (10 g) were prepared as described in Section 2.5 in NMR glass tubes (1.8 cm in diameter) and heated for 10 min in a 72 °C water bath. After heating, the gels were immediately cooled to room temperature (25 °C). The transverse relaxation time (T_2) measurements were performed using a low field nuclear magnetic resonance (LF-NMR) analyser minispec mq 20 (Bruker Optik GmbH, Germany) with a magnetic field strength of 0.47 T and a resonance frequency for protons of 20 MHz. T_2 was measured using the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence [15,16], which scanned 16 times automatically for each sample. The repetition time between two scans was 2 s. The corresponding relaxation times (T_{2b}, T_{21}, and T_{22}) were reflected by the CONTIN algorithm (Bruck Crop.) using a multi-exponential decay curve [17].

2.8. Differential scanning calorimetry

The thermal properties of the MP and OxiMP/PPPH suspensions (40 mg/mL) were measured using a Model Q20 differential scanning calorimetry (DSC) machine (TA Instruments, Inc., New Castle, DE, USA). A thermal scan was conducted from 30 to 90 °C at a constant heating rate of 10 °C/min. Peak transition temperature (T_{max}) and total transition enthalpy changes (ΔH) were recorded from the endothermic peaks observed in the thermograms. Each sample was analysed in duplicate.

2.9. Electrophoresis

The MP and OxiMP/PPPH samples were subjected to sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Non-reducing electrophoresis without β-mercaptoethanol (βME) was conducted to evaluate the changes of protein subunits on the basis of Flores et al. [18]. All of the MP samples were diluted to a final concentration of 2 mg/mL with 15 mM PIPES buffer containing 0.6 M NaCl (pH 6.25). Diluted samples (500 μL) were mixed with 500 μL of pH 6.8 sample dissolution buffer containing 0.125 M Tris–HCl, 4% SDS, and 20% glycerol. Next, the non-reducing agent, 0.5 mM *N*-ethylmaleimide (NEM) (100 μL), was also added to the samples. The mixture was heated in boiling water for 3 min to completely dissolve the protein, chilled at room temperature and packed into small centrifuge tubes. Then, 12 μL of each sample was loaded into each well of a 3% acrylamide stacking gel and a 12% acrylamide resolving gel. The intensity of the bands was analysed and evaluated using peak area as detected with Image J2 software.

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