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Physicochemical and structural properties of composite gels prepared with myofibrillar protein and lard diacylglycerols

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ABSTRACT

The objective of this study was to investigate the physicochemical and structural properties of composite gels prepared with porcine myofibrillar protein (MP) and lard, glycerolized lard (GL) or purified glycerolized lard (PGL). The gels prepared with MP and GL or PGL had significantly higher penetration force and water-holding capacity (WHC) than the gel with lard ($P < 0.05$) and formed a more compact and orderly microstructure. Compared with the distributions of T_2 relaxation times of the pure MP gel, T_{21} and T_{22} of the gels that were prepared with GL or PGL moved in the direction of slower relaxation time, which suggests that the water mobility in the gel system was restricted. The presence of lard, GL and PGL did not affect the participating proteins in composite gels. The presence of GL and PGL altered the secondary and tertiary structures of MP in composite gels, which changed the gel properties. In general, the composite gels that were prepared with MP and GL or PGL showed improved gel quality.

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1. Introduction

Myofibrillar protein (MP) has notably important biological functions, and its gel-forming ability plays a key role in processed meat products and significantly affects the texture and sensory characteristics of the final products (Sun & Holley, 2011). Ziegler and Acton (1984) noted the MP gel formation is responsible for the formation of a three-dimensional gel matrix because of the association of protein during heat processing. The formed protein gels are notably important for their contribution to meat binding, fat immobilization and water entrapment in meat products (Wu, Xiong, Chen, Tang, & Zhou, 2009). In addition, Mendoza, García, Casas, and Selgas (2001) have reported that animal fats play an important role in providing good mouthfeel and juiciness in meat products and are stabilized by a proteinaceous membrane (Gordon & Barbut, 1992). However, overconsumption of fat causes health problems such as obesity, hypertension and cardiovascular heart diseases (Nejat, Polotsky, & Pal, 2010). To improve human health, low-fat meat foods have been developed in the meat industry without compromising on the texture and mouthfeel.

Triacylglycerols (TAGs) are the main component of pork fat. Previous studies have found that TAGs can be converted into diacylglycerols (DAGs) through the enzymatic glycerolysis of fat with glycerol (Cheong, Zhang, Xu, & Xu, 2009; Miklos, Xu, & Lametsch, 2011). Miklos et al. (2011) reported that DAGs could improve the food texture and water retention. In addition, DAGs have been reported to significantly suppress abdominal and visceral fat accumulation and reduce body weight (Maki et al., 2002; Meng, Zou, Shi, Duan, & Mao, 2004). Meanwhile, the safety of DAGs has been confirmed through several animal and human studies (Morita & Soni, 2009). Therefore, DAGs may totally or partially replace animal fat in the processing of meat products. Some reports show that lard-based diacylglycerols can be applied as a fat replacer in meat emulsions and fermented sausages (Miklos et al., 2011, 2014; Mora-Gallego et al., 2013). Our previous study investigated the emulsifying properties and oxidative stability of emulsions of MP and different lipids (lard, GL and PGL). The results revealed that lard diacylglycerols enhanced the emulsifying abilities and had no adverse effects on the oxidation stability of the emulsions that were prepared with MP (Diao, Guan, Zhao, Chen, & Kong, 2016).

The interaction between fat and the MP gel matrix plays a determinant role in the stability of cooked meat products. Xiong and Kinsella (1991) revealed that fat globules of various sizes and concentrations could reinforce the milk protein-based gel matrix. Wu et al. (2009) reported that lipid types and concentrations could alter the rheological and microstructural properties of MP-lipid composite gels. However, to our knowledge, no literature has been reported about the interaction between myofibrillar proteins and DAGs in composite-gel formation during the heating process. Therefore, in this study, we attempt to describe the effect of lard DAGs on the gel strength, water-holding capacity, gel-forming proteins and microstructures of composite gels that

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were prepared with MP and lard DAGs. The secondary and tertiary structures of MP, mobility of water molecules and molecular forces in composite gels were elucidated.

2. Materials and methods

2.1. Materials

Fresh pork back fat and pork loin muscle were obtained from the Beidahuang Meat Corporation (Harbin, Heilongjiang, China). Sodium dodecyl sulfate (SDS), piperazine-1 and 4 bisethanesulfonic acid (PIPES) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and reagents in this work were obtained from commercial sources and were of analytical grade.

2.2. Preparation of lard diacylglycerols (DAGs)

Lard DAGs were prepared according to Diao et al. (2016). Lard was extracted by heating the backfat at 120 °C. The reaction mixture, which included glycerol, melted lard and Lipozyme RMIM, was used in the glycerolysis reaction under the following conditions: 1:1 M ratio of lard to glycerol, 14:100 (W/W) of enzyme-to-lard substrate ratio and 500 rpm magnetic stirring speed. First, the reaction mixture was incubated at 65 °C for 2 h and transferred to 45 °C for 8 h. After the reaction, the enzyme was filtered to yield the glycerolized lard (GL), whose DAG content was 61.8%. The GL was purified using the two-step wiped film molecular distillation (SPE10, manufactured in Haiyuan biochemical equipment Co. Ltd., Wuxi, China). The purified glycerolized lard (PGL), which had a higher DAG content (82.0%), was obtained in the second purification step.

2.3. Preparation of myofibrillar protein (MP)

MP was extracted from the pork loin muscle according to the described procedure (Xia, Kong, Liu, & Liu, 2009). The final protein concentration was measured using the Biuret method with bovine serum albumin (Sigma Chemical Co., St. Louis, MO) as a standard. The MP was maintained at 2–4 °C and used within 48 h.

2.4. Preparation of MP and fat composite gel

Predetermined amounts of melted fats (lard, GL and PGL at 45 °C) were separately mixed with a myofibril solution (1% protein in 0.6 M NaCl, 50 mM PIPES, pH 6.0). These mixtures were placed in a 35 °C water bath for 5 min to guarantee that the fats maintained liquid and were subsequently homogenized at 10,000 rpm for 1 min with an IKA T18 Ultra-Turrax (IKA-Werke GmbH & Co., Staufen, Germany). The pre-emulsified fats were immediately used after preparation.

A predetermined amount of MP was dissolved in 50 mM PIPES (pH 6.0), which contained 0.6 M NaCl, to form a protein solution. Then, each specific amount of pre-emulsified fat was added into the protein solution by gently stirring with a glass rod to produce composites with fat contents of 4%, 8% and 12% (w/w). Simultaneously, the total amount of MP was maintained constant (4%). Then, aliquots of 15 g of MP and lard, GL and PGL composites were separately poured into 25 mm (inner diameter) × 40 mm (length) glass vials and covered with aluminum foil. These composites were stored at 2–4 °C for one night to reach maximum protein solubility (Ramírez-Suárez, Xiong, & Wang, 2001), subsequently equilibrated at room temperature (25 ± 1 °C) for 30 min and heated in a water bath at 72 °C for 10 min. After heating, the formed gels were cooled and stored in crushed ice for 2 h prior to further analysis. Some prepared gel samples were allowed to equilibrate at ambient temperature (approximately 23 °C) for 30 min to measure these gel properties: gel strength, water-holding capacity, molecular forces, gel-forming proteins, low-field NMR analysis and gel microstructure. Other aliquots of gel samples were lyophilized to investigate the secondary and tertiary structural changes of MP in composite gels using Fourier transform infrared spectra and intrinsic fluorescence measurement.

2.5. Gel strength

The MP and fat composite gels were penetrated with a flat-surface cylindrical probe (P/0.5, 12 mm in diameter), which was attached to a Model TA-XT2 texture analyser (Stable Micro Systems Ltd., England, U.K.) at a test speed of 1 mm/s over a 10 mm displacement. The required penetration force (N) to rupture the gels was expressed as the gel strength (Xiong & Brekke, 1991).

2.6. Gel water-holding capacity

The water-holding capacity (WHC) of the gels was determined using a centrifugal method. Briefly, the gel samples (5 g) were placed into a centrifuge tube and centrifuged at 10,000 × *g* for 15 min at 4 °C. The surface of the gels was soaked using filter paper. WHC (%) was expressed as the ratio between the gel weights after centrifugation and before centrifugation, which was multiplied by 100. The supernatant was collected and used to analyse the gel-forming proteins in section *2.8*.

2.7. Low-field nuclear magnetic resonance analysis

The nuclear magnetic resonance relaxation of the gel samples was measured using an LF-NMR analyser minispec mq 20 (Bruker Optik GmbH, Germany) to determine the mobility and proportion of different fractions of water molecules in the gel system without destroying the gel structure. Approximately 2 g of composite gel was placed in an NMR glass tube (1.8 cm in diameter and 18 cm in height). The analyser was operated at a magnetic field strength of 0.47 T and a proton resonance frequency of 20 MHz. The transverse relaxation time (T_2) was measured using the Carr-Purcell-Meiboom-Gill pulse sequence (CPMG). For each sample, 16 scans were obtained at a 2 s interval with 3000 echoes in total. The relaxation data were treated using the CONTIN software that was provided with the equipment which resulted in the corresponding distributions of relaxation times from the decay curve. The mean apparent relaxation time (T_{2i}) and amplitude (A_{2i}) for each detected population in CONTIN were recorded.

2.8. Identification of gel-forming proteins

The solutions that gathered from the WHC measurement were used to perform the sodium-dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to monitor the composition of uncoagulated proteins in the complex gels according to the method of Laemmli (1970). For SDS-PAGE, a resolving gel of 12% acrylamide and a stacking gel of 5% acrylamide were used. The amount of loaded samples per lane was 12 μL. The following proteins were used as the molecular weight standards: myosin (200.0 kDa), β-galactosidase (116.0 kDa), phosphorylase b (97.2 kDa), serum albumin (66.4 kDa), ovalbumin (44.3 kDa), carbonic anhydrase (29.0 kDa) and trypsin inhibitor (20.1 kDa).

2.9. Molecular forces in composite gels

The major molecular forces that were involved in the composite gels were evaluated according to the method of Jiang and Xiong (2013). Different dissolving solutions were used: 8 M urea + 50 mM sodium phosphate (pH 7.0) to analyse the hydrogen bond; 0.5% (w/v) SDS + 50 mM sodium phosphate (pH 7.0) to analyse the total non-covalent forces; 0.25% (v/v) β-mercaptoethanol + 50 mM sodium phosphate (pH 7.0) to analyse the disulfide bands. Samples (1 g) of composite gels were homogenized in 9 mL of various solvents at a speed of 13,500 rpm for 20 s. The homogenates were heated at 80 °C for 1 h to dissolve the gel-forming protein, chilled to room temperature and subsequently

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