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Thermal gelation and microstructural properties of myofibrillar protein gel with the incorporation of regenerated cellulose

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ABSTRACT

The aim of this study was to evaluate water holding capacity, texture, rheological properties and microstructure of myofibrillar protein (MP) gel with the addition of regenerated cellulose (RC) during the heat-induced process. Control samples had the lowest water holding capacity and a disordered microstructure compared to MP-RC emulsion composite gel ($P < 0.05$). Increasing the concentration of stable RC emulsions from 0% to 30% significantly improved the water holding capacity and enhanced the viscoelastic properties of MP composite gel during heat induced process. In addition, the three-dimensional network structure was more uniform and compact for samples prepared with stable RC emulsion. Consequently, significantly higher hardness was found in MP composite gel ($P < 0.05$). These findings show that stable RC emulsion could be used as additives to enhance microstructure, texture and functional quality of meat products.

1. Introduction

Many researches have been conducted to explore the gel formation of myofibrillar proteins (MPs) as a basis to produce acceptable emulsified meats products with lower cost and better nutritional value. MPs play a significant role in the processing of heat-induced gel products, which influence fat and water retention, yield, texture and cohesion of meat products with emulsion composite gel type (Hong & Xiong, 2012; Sun & Holley, 2011; Westphalen, Briggs, & Lonergan, 2006). Likewise, MPs affect the emulsification properties and the rheological behaviors of comminuted meat products. According to Wu, Xiong, Chen, Tang, and Zhou (2009), the interactions between emulsified fat globules and proteins in aqueous phase influenced the overall properties of MP gel. However, the gel function could be favorably improved by using additives that forms more bonding in meat matrix and has a more stable protein matrix (Zhang, Xiao, Himali, Lee, & Ahn, 2010). Factors influencing protein-protein interaction during the heat-induced gelation properties have been well studied for MPs from different species including beef, pork and poultry (Chen, Xu, & Wang, 2007; Gao, Kang, Zhang, Li, & Zhou, 2015; Hong & Xiong, 2012; Sun, Wu, Xu, & Li, 2012; Zhao et al., 2014). Changes in rheological characteristics depended on the combinations of MPs, fats and transglutaminase with or without non-meat proteins which significantly affected gel texture during thermal gelation (Sun & Holley, 2011; Xiong, 2007; Zhao et al., 2014). Emulsified fat and peanut oil improved gel network, hardness, and water holding capacity (WHC) of MPs during heating process. Sun et al. (2012) and Wu et al. (2009) showed that the gelation properties of MP isolate and pea protein isolate were improved by adding microbial transglutaminase during heat induced process.

In this study we highlighted the use of regenerated cellulose (RC) from microcrystalline cellulose through acid dissolution and water regeneration with strong surface activities due to the presence of hydrophobic and hydrophilic regions (Glasser, Atalla, Blackwell, & Jr, 2012; Jia et al., 2014). Our previous research has demonstrated that RC emulsion could effectively improve the quality of emulsified sausage as animal fat substitute (Hu et al., 2016). RC emulsions were stabilized by sodium caseinate with multi-functional properties due to the excellent emulsion stabilizing properties. The use of RC emulsion improved the stabilization of oil and water emulsion systems by reducing the interfacial tensions and enhancing the thickness of absorption layer around fat globules (Hu et al., 2015). Due to the special function of stable RC emulsions, it is important to understand its effects on WHC, texture

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and rheological characteristic during the thermal gelation process of MP gel matrix. To further elucidate the role of stable RC emulsions in meat systems, the current study was conducted to investigate the activity of stable RC emulsion on WHC, texture, storage modulus and microstructure of MPs composite gels during the heat induced process.

2. Materials and methods

2.1. Regenerated cellulose emulsion preparation

Our previous study carried on at National Center of Meat Quality and Safety Control, Nanjing Agricultural University measured the emulsion stability formulated with various levels of RC, sodium caseinate (Tabo company, Zhengzhou, China), and soybean oil (incorporating vitamin E, Suguo supermarket, Nanjing, China) (Hu et al., 2015). The stable RC emulsion used in this study was produced according to Hu et al. (2016). About 15.0 g/kg RC, 20.0 g/kg sodium caseinate and 300 g/kg soybean oil (SFA: 160 g/kg; MUFA: 25 g/kg; PUFA: 59 g/kg) were mixed. One part of sodium caseinate was mixed with eight parts of water for 4 h for complete dissolution. After dissolution, 300 g/kg of oil was added and homogenized at 15,000 rpm for 1.5 min in ice bath (Ultra TurraxT25 BASIS, Germany). The remaining water and RC were mixed with the preformed emulsion at 10,000 rpm for 1.5 min in ice bath. The stable RC emulsion was used within 24 h after preparation.

2.2. Extraction of MP

MP was extracted from pork *longissimus dorsi* at 4 °C using a modified procedure reported by Fu, Liu, Zhang, and Li (2015) with minor modifications. At first, 250 g of pork meat was ground twice using a Waring grinder for 7 s at 2000 rpm (Model Nr. 8010ES, Waring Commercial, New Hartford, Conn. USA). The minced samples were homogenized for 30 s with 4 vol (ml/g) of cold isolation buffer (10 mmol/L potassium phosphate, 0.1 mol equi/L NaCl, 2 mmol/L MgCl2, and 1 mmol/L EGTA at pH 7). After centrifugation at 714 × g for 15 min at 4 °C, the pellet was washed twice with 4 vol (ml/g) of same buffer. The myofibrillar pellet was washed three times with 4 vol of 0.1 mol equi/L NaCl. The myofibrillar suspension was filtered and the pH was adjusted to 6.0 with 0.1 mol equi/L HCl prior to the third centrifugation. MP suspensions (5 mg/mL) were prepared in 15 mmol/L piperazine-N, *N*′-bis (2-ethanesulfonic acid) (pH 6) buffer containing 0.6 mol equi/L NaCl.

2.3. Preparation of MP composite gel

The respective concentrations of stable RC emulsions (7.5 g/100 g, 15 g/100 g, 22.5 g/100 g and 30 g/100 g) were added into the MP solution (6% concentration) to produce MP-RC emulsion. Control samples were prepared without RC emulsion. The MP-RC emulsions standardized in 0.6 mol/L NaCl were stirred and then centrifuged at 121 × g for 5 min to ensure sample homogeneity. MP-RC emulsions prepared above were placed and heated in a water bath from 20 °C to 80 °C and stayed at 80 °C for 20 min according to the procedure described by Sun, Huang, Hu, Xiong, and Zhao (2014) with slight modifications. The obtained composite gels were stored at 4 °C overnight prior to the evaluation of WHC, texture, thermal rheology and scanning electron microscopy.

2.4. WHC

The WHC of prepared composite gel was analyzed according to Shao, Zou, Xu, Zhou, and Sun (2015) with minor modifications. About 5 g of composite gel samples were centrifuged at 10,000 × g at 4 °C for 10 min. WHC was presented as the percentage of pellet composite gel weight after centrifugation relative to original composite gel weight. All data were measured in triplicate.

2.5. Thermal rheological measurements during gelation

Dynamic rheological measurements of MP and stable RC emulsion composite gel were performed using a rheometer (Physica MCR301, Anton Paar Corporation, Austria). Viscoelastic properties of composite gel were measured according to the method described by Westphalen, Briggs, and Lonergan (2005) with slight modification. Samples were loaded between two 50 mm diameter parallel plates with a 1 mm gap. To induce gel formation, samples were stored at 20 °C for 2 min and heated from 20 °C to 80 °C at the heating rate of 1 °C/min. Prior to the heating process, the exposed composites were sealed with silicone oil to avoid any interference from extrinsic factors. The measurements during the heating process were performed at constant frequency of 0.1 Hz and a strain of 0.3% was used to monitor the storage modulus (G′). Changes in the storage modulus (G') were consistently recorded and all data were collected from triplicate treatments.

2.6. Textural properties of MP composite gel

TPA was performed using a TA-XT plus texture analyzer (Stable Micro Systems Co. Ltd. Surrey, England) with probe P/36R. The composite gel samples stored at 4 °C were equilibrated at 25 °C for 30 min. The gel samples were cut into cylinder (diameter of 15 mm and height of 20 mm) and then exposed at compression degree of 50% with pre-test and test speed set at 1 mm/s and the post-test speed set at 5 mm/s with trigger set auto at 5.0 g trigger force. The following parameters of textural properties were expressed as hardness, chewiness, springiness and cohesiveness (Gao, Zhang, & Zhou, 2015).

2.7. Microstructure evaluation

The measurements of composite gel samples were performed using SEM (Hitachi S−3000N, Hitachi High Technologies Corp. Tokyo, Japan) according to the procedure described by Hong and Chin (2013) with some modifications. Cubic gel samples ($3 \times 3 \times 3$ mm^3) taken from MP−RC emulsion composite gels were fixed in 0.1 mol/L phosphate buffer (pH 7.0) containing 2.5% glutaraldehyde and cooled at 4 °C overnight. The fixed samples were washed with phosphate buffer B (0.1 mol/L, pH 7.0), post-fixed for 5 h in phosphate buffer B with Osmium tetroxide (1%, OsO_4), and washed three times with phosphate buffer B for 10 min, followed by dehydration in ascent concentrations of ethanol (50%, 60%, 70%, 80%, 90%, 95% and three times with absolute ethanol) for 10 min. Each gel sample was frozen dried, sputter-coated with 10 nm of gold and palladium, and observed at an accelerating voltage of 15 kV.

2.8. Statistical analysis

Statistical analyses of all data were performed using Statistical Analysis System (SAS 8.2, Cary, NC, USA). Analysis of variance (ANOVA) was employed to determine significant treatment effects by the inclusion of stable RC emulsions. Significant differences at the level of $P < 0.05$ between means were identified using Duncan's multiple range test procedure.