Food Hydrocolloids 40 (2014) 153–162

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Gelation of salted myofibrillar protein under malondialdehyde-induced oxidative stress

Feibai Zhou, Mouming Zhao, Guowan Su, Chun Cui, Weizheng Sun*

College of Light Industry and Food Sciences, South China University of Technology, Guangzhou 510640, China

ARTICLE INFO

Article history:
Received 10 October 2013
Accepted 3 March 2014

Keywords:
Myofibrillar protein
Malondialdehyde
Protein oxidation
Gelation
FTIR

ABSTRACT

This study presented the formation of cold-set myofibrillar protein gel under oxidative stress originated from malondialdehyde (MDA) in the presence of 0.6 M NaCl. Heating procedure was also applied for further evaluation of gel properties. Protein carbonyl content was used to evaluate protein oxidation. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed to explain the formation of the gel. Gel properties were compared in range of MDA/NaCl concentration with use of gel strength, water-holding capacity (WHC), scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR) measurements. Results confirmed the occurrence of protein oxidation in the system applied. Myosin was greatly involved in gel formation through non-disulfide covalent bond. Meanwhile, significant improvements in gel strength, WHC, the network structure of highly porous with well-defined shapes were observed with increasing addition of MDA/NaCl. Furthermore, with the addition of MDA no more than 10 mM, the gel formed in the presence of 0.6 M NaCl was stable to heat treatment, while the higher MDA could cause gel collapse, which was believed to be due to the excessive covalent bond existed. The gelation procedure in this study involved simultaneous protein oxidation and internal cross-linking. The gel was formed on the premise of the swelling of myofibrillar protein under certain ionic strength and the intermolecular cross-links formed via MDA.

1. Introduction

Formation of protein gels in processed muscle foods is very important, which influences the texture and sensory characteristics of the final products (Acton, Ziegler, Burge Jr., & Froning, 1982). Myofibrillar proteins, contributing to 55–65% of total muscle protein, or 10% of skeletal muscle weight, are excellent gelling agents that are largely responsible for the textural and structural characteristics of meat products (Acton et al., 1982).

With the increasing understanding that food proteins are sources and targets for reactive oxygen species (ROS) and other peroxide, protein oxidation is currently one of the most important research topics within the Food Science field. In recent years, the effect of natural oxidation of myofibrillar proteins on their texture and gelation properties during meat processing has attracted more attention. Liu, Xiong, and Butterfield (2000) reported that oxidation promoted interactions of myofibrils with soy protein isolate (SPI), thus resulting in >30% increase in the elasticity of myofibril/SPI composite gels. Also, it has been proposed that mild oxidation altered the mode of myosin aggregation in favor of an elastic gel network formation (Xiong, Blanchard, Ooizumi, & Ma, 2010). Thus, moderate modifications caused by protein oxidation might result in unfolding of protein structure and increase accessibility to effectively initiate protein–protein interactions, which enhance the gel network.

Numerous studies had shown that lipid oxidation led to modification of proteins in biological systems (Louise & Harding, 1993). Furthermore, how lipid-derived radicals and hydroperoxides promote protein oxidation in food systems has attracted special attention for decades (Mercier, Gatellier, Viau, Remignon, & Renerre, 1998; Xiong, Park, & Ooizumi, 2008; Zhou, Zhao, Zhao, Sun, & Cui, 2014). Malondialdehyde (MDA), which is naturally generated under meat processing conditions, is the most abundant individual aldehyde resulting from lipid peroxidation (Adams, De Kimpe, & van Boekel, 2008). Recent studies have shown that MDA could bind to proteins (Xiong et al., 2008), which modifies the protein–protein interaction, and further lead to shifts in the functional properties of myofibrillar proteins in processed muscle foods (Xiong, Decker, Faustman, & Lopez-Bote, 2000, pp. 85–111). On one hand, as reactive oxidation products of lipid peroxidation, MDA

* Corresponding author. Tel./fax: +86 20 22236089.
E-mail address: fewzhsun@scut.edu.cn (W. Sun).

http://dx.doi.org/10.1016/j.foodhyd.2014.03.001
0268-005X/

could oxidatively modify side chains and polypeptide backbone of protein, resulting in its conformational changes (Zhao, Chen, Zhu, & Xiong, 2012). On the other hand, as aldehyde, MDA can react with amino groups of proteins and thus produce strong intermolecular cross-links of the Schiff base type (Chio & Tappel, 1969). Therefore, it is reasonable to hypothesize that these oxidative changes in proteins might bidirectionally affect the gelling properties of the whole myofibrillar protein, leading to the formation of gel without heating.

The structure of myofibrillar protein varies with the ionic strength (Offer & Trinick, 1983). Also, among all the factors affecting gelation properties of myofibrillar proteins, ionic strength has long been considered highly important (Xiong, Lou, Wang, Moody, & Harmon, 2000). Furthermore, there is evidence that salt promotes oxidation of myofibrillar protein as well as muscle tissue (Liu, Xiong, & Chen, 2011). The oxidation at high salt concentrations could also significantly improve MTGase-mediated myofibrillar protein gelation (Li, Xiong, & Chen, 2013). However, it is not clear how MDA-induced oxidation, coupled with salt treatments, alter the gelation of myofibrillar protein.

The objective of this study was to test the hypothesis that MDA affect the gelation of myofibrillar protein under different ionic strength conditions. Heating procedure was further applied for better explaining the mechanism involved. The consequential physicochemical changes of myofibrillar protein and the gel properties were also investigated to elucidate the structure-modifying effect of MDA.

2. Materials and methods

2.1. Materials and chemicals

Longissimus muscle from three pork carcasses (48 h postmortem) was purchased from a local commercial abattoir (Zhongshan, China) and the pigs were slaughtered about 6 months of age following standard industrial procedures. Fat was trimmed away and muscle was cut into cubes, minced, vacuum packaged (ca. 100 g) and frozen at −80 °C until use (used within 1 week). MDA solution was prepared by acid treatment of 1,1,3,3-tetramethoxypropane obtained from Sigma–Aldrich Chemical Co. (St Louis, MO, USA). All other chemicals were analytical reagent grade or better.

2.2. Preparation of myofibrillar protein

Raw muscle was thawed at 4 °C and then used for the myofibrillar protein preparation according to the method of Park, Xiong, Alderton, and Ooizumi (2006). The pH of myofibrillar protein suspension at 0.1 M NaCl in the last wash was adjusted to 6.25 before centrifugation. The pallet was finally suspended in 50 mM sodium phosphate buffer (PB) (pH 6.0) and the protein concentration was determined by the Biuret method using BSA as standard.

2.3. Preparation of MDA solution

MDA stock solution was freshly prepared by hydrolyzing 1,1,3,3-tetramethoxypropane according to the method described by Wu, Zhang, and Hua (2009) with minor modifications. Briefly, 8.4 mL (50 mM) 1,1,3,3-tetramethoxypropane was mixed with 10.0 mL 5.0 M HCl and 31.6 mL distilled water and incubated at 40 °C in the dark for 30 min. After acidic hydrolysis, the resulting mixtures were adjusted with NaOH (6 M) solution to pH 6.0 to obtain MDA stock solution. The stock solution was then diluted to 100 mL with 50 mM PB (pH 6.0). The concentration of MDA was determined by spectrophotometric measurements of the dilution 10^{-5} at 267 nm ($\varepsilon = 31{,}500$).

2.4. MDA-modified myofibrillar protein with salt at different levels

Myofibrillar proteins were divided into three groups that were subjected to different treatments (A, B, and C) as detailed in Fig. 1. In treatment A and B, myofibrillar proteins suspension (40 mg/mL, in 50 mM PB, pH 6.0) in the presence or absence of 0.6 M NaCl, were mixed with different concentrations of MDA (0, 0.5, 2.5, 5, 10, 25, 50 mM). The resulting mixtures were immediately transferred into tightly sealed glass vials (2.5 cm inner dia 5 cm height) and incubated at 25 °C in the dark for 24 h. After incubation, a series of treated samples with different states were obtained. To further investigate the effect of different NaCl concentrations on gelation, as shown in treatment C, myofibrillar protein suspension (40 mg/mL) in 50 mM PB (pH 6.0) containing 10 mM MDA was mixed with NaCl at different levels (0, 0.2, 0.4 and 0.6 M). The incubation condition used was the same as that for treatment A and B.

Furthermore, all samples obtained after treatments (A, B, and C) were heated from 25 to 80 °C at 1 °C/min increments in a water bath. After heating, the samples were chilled in an ice slurry and then stored at 4 °C overnight before any measurements.

Samples before and after heating were expressed as MDA-modified myofibrillar protein (MDA-MP) and heated MDA-modified myofibrillar protein (HMDA-MP), respectively. In addition, HMDA-MP in treatment A (Fig. 1) without MDA was selected as the control.

2.5. Determination of carbonyl content

Carbonyl groups were determinated by reactivity with 2, 4 dinitrophenylhydrazine (DNPH) to form protein hydrazones (Oliver, Ahn, Moerman, Goldstein, & Stadtman, 1987). Samples were homogenized and diluted in 7 volumes (w/v) of distilled water. Briefly, the DNPH-reacted (1 h) samples were recovered by centrifugation after 10% TCA precipitation, and then washed with ethanol: ethyl acetate (1:1, v:v) solution three times. The final pellets were dissolved in 6 M guanidine hydrochloride. The absorbance was read at 370 nm for carbonyl content against a blank of HCl-treated protein sample. The amounts of carbonyl were expressed as nmol of DNPH equivalents/mg of protein using an absorption coefficient of 21 mM^{-1} cm^{-1} for protein hydrazones.

2.6. SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS–PAGE was performed on MDA-MP and HMDA-MP according to the method described by Flores et al. (2006). Briefly, 2 mg of lyophilized samples were dissolved in 500 μL of sample buffer with and without 5% β-mercaptoethanol (β-ME) and the

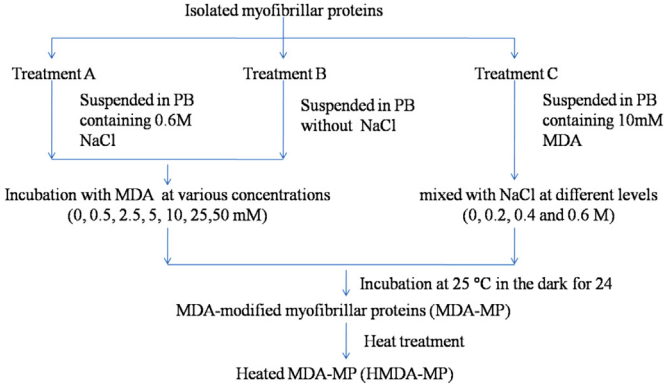

Fig. 1. Flowchart of three treatments applied. All suspensions were made with 50 mM sodium phosphate buffer (PB) at pH 6.0.