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Effects of malondialdehyde-induced protein modification on water functionality and physicochemical state of fish myofibrillar protein gel

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ABSTRACT

Effects of malondialdehyde (MDA)-induced modification on water distribution in fish myofibrillar proteins (MP) gels were investigated using nuclear magnetic resonance (NMR) and magnetic resonance imaging (MRI). The oxidative modifications of MP gels were evaluated by surface hydrophobicity, gel strength, water holding capacity (WHC), scanning electron microscopy (SEM) and SDS-PAGE. Influence of heating procedure on water distribution and functional properties of modified MP gels was also investigated. Results from NMR and MRI indicated that the water functionality was strongly affected by the modification level, which corresponded to the changes of water holding capacity of MP upon MDA modification. Compared with unmodified MP gels, the T_2 relaxation times of modified sample increased significantly, indicating an alteration of water-protein interaction upon MDA-induced modification. The fraction of P_{23} declined from 7.66% to 0.15% as the MDA addition increased from 0 to 50 mM. Moreover, the relaxation components T_{2b} disappeared with the addition of MDA mainly due to enhanced protein flexibility and surface hydrophobicity. Besides, the P_{23} (free water) of heated MP samples increased by 5.41 times compared with that of unheated MP samples.

1. Introduction

The water properties of meat products can reflect the ability of holding water and influence the product quality, yield and cost (Rosenvold & Andersen, 2003). In meat, the majority of the water is located in the spaces between the myofibrillar filaments. The gelation of myofibrillar proteins is largely responsible for the textural properties and water holding capacity of the meat products (Bertram et al., 2007; Zheng et al., 2015). The gelation ability of myofibrillar proteins has been well documented in terms of factors influencing gelation properties including differences of MP type and source, pH, ionic strength, processing parameters, temperature, pressure and the interactions of MP with fat and additives (Carini, Curti, Spotti, & Vittadini, 2010; Li et al., 2014a; Ni et al., 2014; Stangierski & Baranowska, 2015). In addition, previous studies have shown that protein oxidation could alter the secondary and tertiary structures of MP, resulting in unfolding of the protein structure and increasing of the protein-protein interactions, and eventually change the MP gelling and water holding properties (Zhou, Zhao, Zhao, Sun, & Cui, 2014; Wu, Zhang, & Hua, 2009).

Real meat system may contain organic peroxides like lipoperoxides resulting from polyunsaturated fatty acid oxidation. It is highly unlikely that the oxidation and peroxidation of lipids and proteins take place independently (Wu et al., 2009). Studies have shown that the lipid oxidation would take place faster and hence, it is more likely that lipid-derived radicals and hydroperoxides promote protein oxidation (Esterbauer, Schaur, & Zollner, 1991). Malondialdehyde (MDA) is the most abundant individual aldehyde resulting from the oxidation of ω-3 and ω-6 fatty acids (Vandemoortele & De Meulenaer, 2015). It is well document that MDA modifies the protein-protein interaction, and further leads to shifts in the functional properties of myofibrillar proteins in processed muscle foods (Zhou, Sun, & Zhao, 2015). On the other hand, MDA can react with amino groups of proteins and thus produce strong intermolecular cross-links of the Schiff base type and Michael addition type (Vandemoortele & De Meulenaer, 2015). However, the water-protein interaction influenced by MDA-induced modification of MP gels has only been studied on a very limited basis.

Nuclear magnetic resonance (NMR) relaxometry could provide direct information about characterization of water mobility and distribution, and has been used for determination of WHC in meat (Bertram, Kristensen, & Andersen, 2004). Studies have shown that the ^{1}H NMR signal is related to chemical and diffusive exchange between water protons and protons from other components (Han, Wang, Xu, & Zhou, 2014). During meat storage and processing, changes occurring on T_2

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relaxation in fish meat can provide further insight into proteins' behavior (Bertram, Purslow, & Andersen, 2002; Bertram et al., 2004). Therefore, the aim of the present study was to investigate the effect of MDA-induced modification on water distribution and mobility in MP gels before and after heat treatment using NMR and MRI. The protein structural changes after modification were assessed and discussed in relation to the water distribution of MDA-modified MP gels.

2. Materials and methods

2.1. Materials and chemicals

Fresh silver carp was purchased at a local market in Wuxi city, China. 1, 1, 3, 3 – tetramethoxypropane was purchased from Sigma-Aldrich (St Louis, MO, USA). All other chemicals were of analytical reagent grade.

2.2. Extraction of MP

MP was extracted from the dorsal muscle of silver carp by homogenizing the fish mince with 4 volumes of cold distilled water (<4 °C) according the method of Park, Xiong, Alderton, and Ooizumi (2006). After addition of 0.1 M NaCl, the pH of the MP suspension was adjusted to 6.25 before centrifugation. The pellet was finally suspended in 20 mM sodium phosphate buffer (pH 6.0), and its protein content was determined according to the Biuret method using BSA as standard.

2.3. Preparation of MDA solution

MDA solution was freshly prepared by hydrolyzing 1, 1, 3, 3 - tetramethoxypropane according to the method described by Wu et al. (2009) with minor modifications. Firstly, 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 the MDA solution. Finally, the MDA solution was diluted to 100 mL using 50 mM PB (pH 6.0). The concentration of MDA was determined by spectrophotometric measurements of the dilution 10^{-5} at 267 nm and calculated using the $\varepsilon = 31{,}500$.

2.4. MDA modification of myofibrillar protein and gelation

MP suspension (40 mg/mL, in 50 mM sodium phosphate buffer, pH 6.0) was mixed with different concentrations of MDA (0, 1, 3, 5, 10, 25, 50 mM L^{-1}, final concentration). The mixtures were immediately transferred into tightly sealed glass vials and incubated at 25 °C in the dark for 24 h. After incubation, a series of MDA treated MP gel (MDA-MP) samples was obtained. Furthermore, all samples obtained after incubation were heated from 25 to 80 °C at 1 °C/min increments in a water bath. The heated MP gels (HMDA-MP) 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.

2.5. Protein surface hydrophobicity

Protein surface hydrophobicity of MP was determined using bromophenol blue (BPB) according to the description of Chelh, Gatellier, and Sante-Lhoutellier (2006). To 1 mL of myofibril suspension, 200 μL of 1 mg/mL BPB was added and mixed well. A control, without myofibrils, was prepared substituting 1 mL of 20 mM phosphate buffer. Samples and control were mixed at room temperature, for 10 min and then centrifuged for 15 min at 2000*g*. The absorbance of the supernatants was measured at 595 nm against a phosphate buffer blank. The amount of BPB bound, given by the following formula, where A is absorbance at 595 nm,

$$\text{BPB bound } (\mu g) = 20\ \mu g \times (A_{control} - A_{sample})/A_{control}$$

2.6. Gel strength

The gel strength of the MP gels was measured using a cylinder measuring probe (P/0.5) attached to a TA.TX2 texture analyzer (TA-XT plus, Stable Micro Systems, Ltd., Surrey, United Kingdom) at a constant probe speed of 1.0 mm/min at room temperature (25 ± 1 °C). The gel strength is defined as the initial force required disrupting the gels. All samples were tested in triplicate.

2.7. Water holding capacity (WHC)

The WHC values of the MP gels were determined according to the method of Xia, Kong, Xiong, and Ren (2010) with slight modifications. The MP gels (3 g) were centrifuged at 8000*g* for 30 min at 4 °C and the WHC (%) was expresssed as the final weight of the centrifugation as a percentage of the weight before centrifugation.

2.8. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis

SDS-PAGE was performed on the MP gels according to the method described by Flores et al. (2006). The diluted MP sol was mixed with certain volumes of sample buffer (with and without 5% β-mercaptoethanol (β-ME)) to obtain a theoretical concentration of 1 mg/mL protein. Samples were vortexed and incubated at room temperature overnight. The ones with β-ME were boiled for 3 min before centrifugation (10,000*g*, 10 min). Then, 15 μL of protein sample was loaded onto the polyacrylamide gel made of 12% running gel and 5% stacking gel. Electrophoresis was run using an SE 250 Mighty Small II vertical slab gel electrophoresis unit (Hoefer Scientific Instruments, San Francisco, CA, USA).

2.9. Microstructure

Scanning electron microscopy (SEM) was performed to examine the structural characteristics of oxidized MP gels (Quanta-200, FEI Ltd., Netherlands) according to the method described by Haga and Ohashi (1984) with slight modifications. Gel cubes ($4 \times 4 \times 3$ mm^3) were fixed in 0.1 M sodium phosphate buffer (pH 7.0) containing 2.5% glutaraldehyde for 24 h at 4 °C. Fixed samples were dipped in 0.1 M phosphate buffer (pH7.0) for 10 min and then postfixed in the buffer containing 1% osmium tetraoxide for 5 h at 4 °C. Samples were washed 3 times with 0.1 M phosphate buffer and then dehydrated in graded solutions. Then the samples were vacuum-freeze-dried and applied to the SEM.

2.10. Water distribution in MP gels with low-field ^1H NMR relaxation time (T_2)

Low field ^1H NMR measurements were performed according to Li et al. (2014a), with minor modification. Gels formed in the glass bottles (20 mm × 40 mm) were placed in 30 mm NMR cylindrical glass tubes and then inserted into the NMR probe of a low field NMR analyzer (PQ001, Niumag Electric Corporation, Shanghai, China) with a resonance frequency for protons of 100 KHz and a magnetic field strength of 0.5 ± 0.08 T. Transverse (T_2) relaxation was measured using the Carr–Purcell–Meiboom–Gill pulse sequence (CPMG) with 4 scans, 3000 echoes, 13.8 s between scans, and 200 μs between pulses of 90° and 180°. The data were analyzed by applying multi-exponential fitting of T_2 relaxation data with the MultiExp Inv Analysis 4.09 (Niumag Electric Corporation, Shanghai, China).