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Heat-induced gel properties of porcine myosin in a sodium chloride solution containing L-lysine and L-histidine

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ABSTRACT

For the development of low sodium gelled meat products, the influence of L-Lys, L-His and L-Lys + L-His on the gelation behaviour and gel properties of porcine myosin in low (1 mmol/L), physiological (0.15 mol/L) and high (0.6 mol/L) NaCl solutions were studied. The dynamic rheological results indicated that the introduction of L-Lys or/and L-His increased the storage moduli change rate (ΔG′) of myosin at 1 mmol/L, 0.15 mol/L and 0.6 mol/L NaCl concentrations. The ΔG' of myosin in the presence of L-Lys was higher than that in the L-His at 1mmol/L- 0.6 mol/L NaCl. A significant increase in gel hardness was observed in the presence of L-Lys or/and L-His ($P < 0.05$). Compared to the control, L-Lys or/and L-His remarkably reduced the cook loss and improved water-holding capacity of myosin gel at 1 mM NaCl ($P < 0.05$). Nuclear magnetic resonance (NMR) revealed a distinct increase in the immobile water content with a decrease in the free water in the presence of L-Lys or/and L-His. These findings demonstrated that the introduction of L-Lys or/and L-His have a positive effect on the thermal-induced gelation behaviour and enhanced water binding capacity, contributing to an improvement in the gel properties of myosin, particularly at low NaCl concentrations.

1. Introduction

The gel-forming ability of myosin, the major protein of myofibrillar proteins (salt-soluble proteins), is important for meat products and is influenced by several factors, including ionic strength, pH, temperature, high pressure, protein concentration and ion type (Chen et al., 2014; Liu et al., 2010; Totosaus, Montejano, Salazar, & Guerrero, 2002).

With respect to ionic strength or salt concentration, it is generally accepted that myosin is soluble at high concentrations of NaCl or KCl (>0.3 mol/L), allowing good gel formation and improving the textural properties of meat products (Lee, Kang, & Chin, 2014; Zayas, 1997). Currently, a reduction of NaCl consumption is required due to the direct relationship between excessive sodium intake and an increased incidence of hypertension (McGregor, Kilcast, & Angus, 2007). However, a reduction in NaCl weakens the repulsion between the myofilaments of myosin (Hamm, 1972, p. 222) and the interaction among proteins, resulting in a uncomplete solubilization or aggregation of proteins, which would induce the lower water holding capability (WHC) and higher cook loss (Fu et al., 2012).

In recent years, the influence of amino acids on physicochemical properties of meat protein has attracted great interests. It was found that L-Arg increased the equilibrium solubility of myosin at low NaCl concentration (0.15 mol/L NaCl) (Takai, Yoshizawa, Ejima, Arakawa, & Shiraki, 2013), and improved the WHC and texture of salt-soluble protein gels in 0.17 M NaCl (Qin, Xu, Zhou, & Wang, 2015). L-His was reported to cause 80% of chicken breast myosin soluble in a low salt concentration solution (1 mmol/L KCl) (Hayakawa, Ito, Wakamatsu, Nishimura, & Hattori, 2009) due to the elongation of light meromyosin (LMM) region of myosin in the presence of L-His (Hayakawa, Ito, Wakamatsu, Nishimura, & Hattori, 2010). It was indicated that the addition of L-His contributed to the monomers formation of myosin in a low salt concentration solution, which affected the interaction among myosin during heating, resulting in a fine gel properties (Hayakawa et al.,

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2012). Recently, Guo, Peng, Zhang, Liu, and Cui (2015) found that L-Lys or L-His caused a transformation of the porcine myosin conformation and increased the surface hydrophobicity. Moreover, L-Lys was more effective than L-His in increasing the solubility of myosin (Takai et al., 2013) and used as one of additives to suppress protein aggregation (Hamada, Arakawa, & Shiraki, 2009) in a freeze-dried product. L-Lys and L-His were both belong to basic amino acid, but only L-Lys was a diamino acid (amino acid with two amino groups and one carboxylic group), which might affect the pH to contribute to the interaction with myosin (Takai et al., 2013). Though some studies were conducted on the effect of L-His on the heat-induced gelation of chicken breast myosin at low ionic strength (Hayakawa et al., 2012; Hayakawa et al., 2015), there was little information regarding the effect of L-Lys or L-Lys + L-His on gel properties of myosin. Furthermore, L-His and/or L-Lys might contribute to the salty taste of NaCl through the interactions (Zhang et al., 2014), and being as one of the flavor enhancers for reducing the sensory defects caused by NaCl reduction in meat products (Campagnol, dos Santos, & Morgano, 2011; Campagnol, dos Santos, & Terra, 2012; Zhang, Zhang, Hui, Guo, & Peng, 2015; Zhou, Li, & Tan, 2014).

Therefore, to test the potential of L-Lys or/and L-His as an alternative to reduce NaCl for gelled meat products, the present study investigated the influence of L-Lys or L-His, or L-Lys + L-His in 1 mmol/L NaCl, 0.15 mol/L NaCl and 0.6 mol/L NaCl on the dynamic rheological properties, gel hardness, WHC, cook loss and nuclear magnetic resonance (NMR) T_2 of the porcine myosin heat-induced gel, elucidating the effect of L-His or/and L-Lys on the gelation behaviour and gel properties of myosin gel.

2. Materials and methods

2.1. Myosin preparation

The pigs were handled according to the degree of the state Council of the People's Republic of China (No.525) 'Regulations on Administration of Hog Slaughter' and 'Good manufacturing practice for pig slaughter (GB/T 19479-2004)'. The carcasses were chilled for 24 h at 4 °C, after which porcine *longissimus dorsi* samples were removed from a carcass and were immediately chilled in ice. The ice-cold muscle was minced within 30 min, and the myosin was prepared according to the method described earlier (Guo et al., 2015). The myosin purity was assessed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli (1970). The purity of the myosin samples was greater than 90% as determined by densitometry (Quantity One analysis software, Bio-Rad Co., Hercules, CA, USA).

2.2. Myosin treated with NaCl containing L-Lys or L-His

Myosin was dialysed against 1 mmol/L, 0.15 mol/L and 0.6 mol/L NaCl solutions (pH 6.5) with/without 5 mmol/L L-Lys and/or 5 mmol/L L-His overnight. Then, the protein concentration of the dialysed myosin suspension was adjusted to 15 mg/ml before further analysis. The NaCl concentrations in the final solutions of 15 mg protein/ml were 1 mmol/L NaCl, 0.15 mol/L NaCl and 0.6 mol/L NaCl in the 1 mmol/L NaCl, 0.15 mol/L NaCl and 0.6 mol/L NaCl treatments, respectively. All procedures were carried out at 4 °C.

2.3. Dynamic rheological measurements

The dynamic rheological characteristics of the myosin during the heat-induced gelation were measured using a Physica MCR 301 rheometer (Anton Paar, Graz, Austria) in oscillatory mode. A 50-mm parallel steel plate geometry with a 1-mm gap was used. The samples were heated from 20 °C to 80 °C at 2 °C min^{-1} at a constant frequency of 0.1 Hz and a strain of 2%. The storage modulus (G′), loss modulus (G″) and phase shift (δ) were continuously recorded. The G′ change rate was calculated by $\Delta G' = (G_1' - G_0')/G_0'$, where G_1' is the G′ at 80 °C and G_0' is the G′ at 20 °C.

2.4. Heat-induced gel preparation

The dialysed myosin solution was transferred into a 10-ml capped plastic centrifuge tube for heating. The samples were heated in a water bath from 20 °C to 75 °C at 1 °C min^{-1} and held for 20 min. After heating, the tubes were cooled and then kept at 0 °C −4 °C overnight before analysis.

2.5. Gel hardness analyses

The gel hardness was investigated using a texture analyser TA-XT2i (Stable Micro Systems, Godalming, UK). Once a trigger force of 5 g was sensed on the sample surface, the probe (P50) penetrated the gel to a depth of 5 mm. The maximum force was recorded and used as an indication of hardness. Other parameters were as follows: pre-test speed, 1.0 mm/s; test speed, 0.5 mm/s; post-test speed, 1.0 mm/s; time between two compressions, 5.0s; and data acquisition rate, 200 points/s.

2.6. Water-holding capacity and cook loss

The WHC was based on the protocol described by Kocher and Foegeding (1993). The gel was centrifuged at 5000 × g for 10 min, and the supernatant was decanted and weighed. The WHC is the percentage of gel weight retained after centrifugation relative to its initial weight.

The cook loss was measured as described by Chen et al. (2014). The gel was removed from the beaker, wiped with filter paper and weighed. The cook loss was expressed as a percentage based on the raw stuffed net weight.

2.7. NMR spin-spin relaxation (T_2) measurements

NMR relaxation measurements were performed according to the method of Wang, Xu, Huang, Huang, and Zhou (2014) with a slight modification. Approximately 2 g of sample was placed in a 15-mm glass tube and inserted in the NMR probe of a PQ001 Niumag Pulsed NMR analyser (Niumag Electric Corporation, Shanghai, China). T_2 was measured using the Carr-Purcell-Meiboom-Gill sequence at a resonance frequency of 22.6 MHz with a τ-value of 300 μs at 32 °C. Data from 16000 echoes were acquired as 32 scan repetitions.

2.8. Statistical analysis

The data were analysed using a statistical analysis system (SAS Institute Inc., Cary, NC, USA). A variance test (ANOVA) was performed with a significance level of $P < 0.05$. Duncan's multiple range test was used to evaluate the differences between the treatments.

3. Results and discussion

3.1. Dynamic rheological

As shown in Fig. 1, the dynamic rheological properties of porcine myosin during thermal gelation were significantly affected by the introduction of L-His and L-Lys. An increase in the storage modulus (G') and a decrease in the phase shift (δ) in all treatments were