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Effects and mechanism of modified starches on the gel properties of myofibrillar protein from grass carp

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ABSTRACT

The effects and mechanism of cassava starch (CS), hydroxypropylated cassava starch (HCS) and cross-linked hydroxypropylated cassava starch (CHCS), on gel properties of myofibrillar protein (MP) from grass carp were investigated using texture analyzer, dynamic rheometer, scanning electron microscopy (SEM) and circular dichroism (CD). Water holding capacity (WHC) of MP gels increased from 70% to ca. 90% with an increase in additive amount of modified starches (MS) and HCS improved WHC most effectively. The MS (HCS and CS) at proper additive amount (1.5 wt%) improved the textural properties of MP gels significantly ($P < 0.05$). In rheological test, all MS-MP solutions showed viscoelastic nature of MP, but adding MS resulted in much higher G', suggesting the effect of MS (especially 2.5 wt% CHCS) was considerable and equal to increasing protein concentration. The SEM photographs showed the MP gel with 2.5 wt% CHCS or 0.5 wt% HCS formed compact and homogeneous network, while the gel with CS agglomerated and the agglomerate phenomenon occurred more obviously with increasing additive amount. The addition of MS, especially CHCS, promoted the heat–induced conformational transition from the α-helix companied with β-turn to β-sheet, leading to the MP molecules stretching out.

1. Introduction

Myofibrillar proteins are a kind of structural proteins which have very important biological functions. They can regulate muscle contraction and affect meat tenderness, and their gel properties can greatly influence the structural characteristics of meat products, caking properties, appearance and yield of products [1,2]. Compared with saltwater fish proteins, freshwater fish proteins have poor gelling capability and tend to modori, which limit the processing characteristics of freshwater fish to a large extent [3]. In order to improve the quality of fish protein gels, exogenous additives, such as calcium chloride, transglutaminase, polyphenols, pectins, carrageenan, non-muscle proteins and starches, are necessary during processing [4–10].

Starches are the most common additives in fish products processing. It can not only decrease the additive amount of fish meat in products and reduce the cost, but also improve the gel strength and water holding capacity (WHC) of protein gels as well as the appearance and taste of products [11–13]. At present, it is generally believed that starch granules absorb water and swell during heating, filling into the meat protein network structure. The additive amount of starch is of great significance for improving gel properties [14].

Having inherited the characteristics of native starch, modified starch (MS) is a series of starch derivatives through physical methods, chemical methods or enzymatic processing which attempts to change the molecular size and physico–chemical properties for specific application [15,16]. Hydroxypropylated starch is a kind of nonionic modified starch produced from etherification reaction between starch and propylene epoxide under strong alkaline conditions [17]. Cross-linked hydroxypropylated starch is a kind of double modified product through hydroxypropyl etherification and crosslinking [18]. Compared with native starch, hydroxypropylated starch and cross-linked hydroxypropylated starch have lower gelatinization temperatures, higher water swelling capability and paste clarity values, better anti-retrogradation characteristics and dispensability in cold water as well as product reconstituting properties [19,20]. Thus, hydroxypropylated starch and cross-linked hydroxypropylated starch can be used to improve the quality of fish protein products. But there are hardly any reports about the influences of hydroxypropylated starch and cross-linked hydroxypropylated starch on the gel properties and molecular structures of myofibrillar protein from fishes. Therefore, the aim of this study was to compare the effects of three kinds of starch (cassava

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starch CS, hydroxypropylated cassava starch HCS and cross-linked hydroxypropylated cassava starch CHCS) on gel properties of myofibrillar protein (MP) from grass carp and discuss the underlying molecular mechanism. The WHC of MS-MP complex gels was determined through high-speed centrifugation. The dynamic and static features of texture were measured by dynamic rheometer with temperature ramp pattern and texture analyzer, respectively. The morphology and microstructure of complex gels were observed by scanning electron microscopy (SEM). The changes of secondary structure during heating were monitored by using circular dichroism spectrometer. This work will contribute useful information to the effects of MS with different chain structures on MP gel formation, and provide a reference for development of surimi product with high gel strength.

2. Materials and methods

2.1. Materials

The cassava starch (CS), hydroxypropylated cassava starch (HCS) and cross-linked hydroxypropylated starch (CHCS) were provided from Hangzhou Starpro Starch Co. Ltd (Hangzhou, Zhejiang, China). The degree of hydroxypropyl substitution (DHS), defined as moles of hydroxypropyl groups per mole of glucose residue, could be calculated from the hydroxypropyl content by spectrophotometric method [19]. The degree of cross-linking (DC), expressed as moles of phosphorus per mole of glucose residue, could be calculated from phosphorus content by spectrophotometric method [18]. In the present work, DHS of HCS and CHCS were determined to be 0.12 and 0.16, respectively. DC of CHCS was determined to be 0.001. All chemicals used were of analytical grade.

2.2. Extraction of myofibrillar protein

Grass carp was purchased from a fish market in Wuhan, Hubei, China. Each of fresh fishes weighing about 2.5 kg was washed with its scale, head and organs removed. The white meat on the back was collected and stirred into surimi. The surimi was rinsed with 4 times the volume of low phosphate buffer (0.05 mol/L potassium chloride, 3.38 mmol/L sodium dihydrogen phosphate, 15.5 mmol/L disodium hydrogen phosphate, pH 7.5) and then centrifuged at 4000 r/min 4 °C. The obtained precipitate was rinsed and centrifuged twice using the above method. After that, the precipitates were extracted for 22 h at 4 °C with 3 times the volume of high phosphate buffer (0.45 mol/L potassium chloride, 3.38 mmol/L sodium dihydrogen phosphate, 15.5 mmol/L disodium hydrogen phosphate, pH 7.5), and then centrifuged for 10 min at 12,000 r/min using a refrigerated centrifuger. The supernatant liquid was precipitated again with 10 times the volume of cold water for 30 min. After the obtained supernatant was removed, the suspension at the bottom was centrifuged for 10 min at 10,000 r/min 4 °C. The precipitate was washed again with 10 times the volume of cold water and centrifuged for 15 min at 10,000 r/min 4 °C. Finally, the resulted precipitate, namely MP was collected and dissolved in 0.6 mol/L potassium chloride and stored in a refrigerator at 4 °C.

2.3. Preparation of modified starch-myofibril protein (MS-MP) complex gel

The MP concentration was measured by Lowry method using bovine serum albumin (BSA) as a reference, and its concentration was adjusted to 40 mg/mL with 0.6 mol/L potassium chloride. CS, HCS and CHCS were added into the MP solutions with 0.5, 1.5 and 2.5 wt% (starch/protein), respectively. The solutions were stirred for 2 h and then centrifuged at 4 °C, 1000 r/min for 5 min to remove the bubbles. After that, the obtained solutions were poured into a casing with a diameter of 25 mm, and heated by two-step, i.e. at 40 °C for 60 min and at 90 °C for 30 min. Finally, the obtained cylindrical complex gels were cooled to ambient temperature for further analysis of water holding capacity, texture and scanning electron microscopy.

2.4. Determination of water holding capacity

The water holding capacity (WHC) was determined by a centrifugal method. An amount of gel was put into a weighed centrifuge tube (W_0). The tube together with gel was weighed (W_1) and centrifuged for 5 min at 10,000 r/min 4 °C. The supernatant was discarded and the tube with pellet was inverted for 10 min to allow supernatant to drain. After 10 min, the remaining water on the surface of gel was soaked up using filter papers, and the tube with pellet was weighed (W_2) [21]. WHC (%) was expressed as gram of water retained per 100 g of myofibril protein gel by the following equation:

$$\text{WHC} \quad (\%) = \frac{(W_2 - W_0)}{(W_1 - W_0)} \times 100$$

2.5. Determination of textural properties

Textural profile analysis (TPA) of complex gel was performed on a Texture Analyser (TA-XT. Plus, Stable Micro Systems, UK) equipped with probe P/36R. The MS-MP complex gel was cut into a cylinder with a diameter of 20 mm and height of 20 mm, then compressed at compression degree of 50% with the pre-test speed and test speed at 1 mm/s and the post-test speed at 10 mm/s. Trigger type was set at auto with 5.0 g trigger force. The data acquisition rate was 50 pps. All samples were performed in octuplicate and repeated at least once [22].

2.6. Determination of dynamic rheological properties

Dynamic rheological properties of MS-MP mixture solutions were monitored during heating by a rheometer (AR 2000ex, TA Instruments, New Castle, DE, US) using a parallel-plate geometry (diameter 40 mm). The MS-MP mixture solutions were prepared, similar to the MS-MP complex gels, except for heating. The parallel-plate was equilibrated to 20 °C beforehand, and the samples were placed between the 2 plates with a gap of 1 mm. In order to avoid sample dehydration, the exposed edge was covered with a thin layer of liquid paraffin. Then the samples were subjected to the following temperature ramp procedures: heating from 20 °C to 90 °C at 1.5 °C/min, and then cooling to 20 °C at 5 °C/min. This temperature ramp tests were done at a fixed frequency of 1 Hz with a fixed stress of 1 Pa to ensure the integrity of gel networks in the linear viscoelastic region. Storage modulus (G') and loss modulus (G'') were obtained using the rheometer data-analysis software [23].

2.7. Scanning electron microscopy

The complex gels were cut into slices with a thickness of 1 mm, fixed with 2.5% glutaraldehyde containing 0.1 mol/L sodium phosphate (pH 7.3) at 4 °C for one night, and then washed three times using the above phosphate buffer. Then, the complex gels were dehydrated using 30%, 50%, 70%, 80%, 90% and 100% ethanol successively. The dehydrated samples were immersed in isoamyl acetate in order to remove ethanol by substitution, and dried by the critical point drying method. Afterwards, they were sputtered with platinum using an ion sputter coater (IB-5, EIKO Ltd., Tokyo, Japan). The samples were observed and photographed in a scanning electron