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Effect of trypsin treatments on the structure and binding capacity of volatile compounds of myosin

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ABSTRACT

In order to investigate the mechanism between flavor binding and proteins degradation during meat processing, the influence of different trypsin contents on the structure of myosin and the adsorption capacity on aldehydes and ketones was determined. The 1% treatment produced subfragment 2 (S2), light meromyosin (LMM) and decreased 18 and 16 kDa light chains; 5% and 10% treatments produced 100 and 65 kDa new bands and more S2, LMM and cleaned light chains. With the rising trypsin contents, β-sheet, β-turn, random coil, hydrophobicity and total sulfydryl content increased; solubility, α-helix and free percentages of aldehydes and ketones decreased. The increase of absorbing capacity could be attributed to the increased hydrophobicity and total sulphydryl and the unfolding of secondary structures by exposing reactive amino and thiol groups and hydrophobic sites; the decreased solubility was related to the increased hydrophobicity. The trypsin-dose dependent proteolysis of myosin increased the retention of volatile compounds.

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1. Introduction

Flavor is an essential aspect of the quality of foods and one of the principle factors involved in a consumer's purchase decision. It is necessary to have a thorough understanding of flavors and changes in flavors (Heng et al., 2004). Flavor depends on the headspace concentration of volatile flavor compounds, flavor perception et al. The concentration of the free volatile substances in the gas phase depends on several factors such as their physicochemical properties, content and the interactions with the other food constituents (Perezjuan, Flores, & Toldra, 2007). It is well known that the generation and deterioration of meat flavor through interact with others like carbohydrates, lipids, and proteins (Heng et al., 2004; Tan & Siebert, 2007). Food proteins have little flavor of their own, but the interaction of proteins with volatile compounds significantly changes their headspace (HS) concentration. Both reversible binding and irreversible binding can occur between proteins and flavor compounds (Pérez-Juan, Flores, & Toldrá, 2006).

Proteins have the potential to interact with flavor compounds in a variety of ways due to the considerable variation in the amino acid side chain structures; these include ionic bonding, hydrogen bonding, hydrophobic bonding, schiff bases and fragmentation of the peptide backbone. Different proteins have different proportions of amino acids of particular side chain types, leading to distinctly different interactions (Tan & Siebert, 2007). The significant role of the protein structure in binding and release of aroma was investigated in many studies. Wang and Arntfield (2014) demonstrated that salt extracted meat proteins exhibited higher flavor binding abilities than alkaline extracted ones. The conformational changes of protein in thermal treatments caused the increase of interactions between protein and flavor compounds. Shimizu, Saito, and Yamauchi (1985) suggested that the structural changes of β-lactoglobulin due to pH adjustment influenced its adsorption properties. Zhou, Zhao, Cui, and Sun (2015) suggested that oxidation could alter the secondary and tertiary structure of myofibrillar proteins, leading to changes in physicochemical state of proteins and thus their functional properties. However, the exact association between the flavor binding and protein degradation during processing is far from being understood.

The degradation of the muscle proteins as a result of the action of endogenous peptidases has been studied intensively during processing in dry-cured meat products and during conditioning of fresh meat (Toldrá & Flores, 1998). It influenced texture and produced free amino acids and other low-molecular weight compounds. These free amino acids took part not only in taste directly, but also indirectly in flavor development because they

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are precursors of many odorants (Jurado, García, Timón, & Carrapiso, 2007). At the same time, a more pronounced action of the enzymes can be observed during the full process of dry-cured ham; most of the muscle enzymes are very stable and active (Toldrá & Flores, 1998). Myosin is the most important part of myofibrillar proteins that constitutes approximately 55–60% of the myofibrillar fractions in muscle tissues of mammals, birds and fish. It is present in a complex form with actin and other proteins and easy to be disintegrated by various proteolysis systems (Visessanguan, Ogawa, Nakai, & An, 2000). Mora, Sentandreu, and Toldrá (2011) indicated that cleaved fragments of myosin light chain isoforms have been already identified in dry-cured ham; they considered that the degradation of myosin could be very important to flavor development during processing in Spanish dry-cured ham. In addition, the proteolysis of myosin has been demonstrated to be related to the gel properties in many works (Visessanguan & An, 2000).

To the best of our knowledge, most studies on myofibrillar degradation have focused on tenderness, gel properties and flavor development, but little information about the role of myosin degradation on its binding characters of flavor is available. Trypsin, a model protease, which can cleave myosin heavy chain (MHC) to produce subfragment 2 (S2), light meromyosin (LMM) and other well known fragments, was used to study the effect of hydrolysis on the functional properties of myosin usually (Collins, Cote, & Korn, 1982; Levitsky, 2004; Togashi, Kakinuma, Nakaya, Ooi, & Watabe, 2002). Therefore, the main objective of the present study was to evaluate the influence of different concentrations of trypsin on the adsorption capacity of myosin on aldehydes and ketones and determine the effect of trypsin treatments on the secondary structure, surface hydrophobicity, solubility and the total sulfydryl content of myosin.

2. Materials and methods

2.1. Materials

The 3-methyl butanal, pentanal, heptanal, nonanal, 2-pentanone, 2-heptanone, 2-octanone, 2-nonanone were obtained from Sigma-Aldrich (Sigma, USA). The purity of the standard flavor compounds was between 98 and 99.7%. Trypsin (250 U/mg, Porcine Pancreas) and Phenylmethylsulfonyl fluoride (PMSF) were purchased from Solarbio Company (Shanghai, China). The purity of PMSF was above 99.0%. Landrace castrated boars (4–5 month with body weight of 96 ± 5 kg) were slaughtered in a local abattoir. Within 2 h postmortem, *Biceps femoris* muscles free of fat and connective tissue were taken and minced, vacuum-packaged and frozen stored (−20 °C) until use.

2.2. Preparation of myosin

Myosin was prepared according to the method of Hwang et al. (1990) with slight modification. One hundred grams samples were taken, thawed, homogenized with 5 volumes of 6 mM potassium phosphate buffer (pH 7.0) at 10,000 rpm for 3 × 10 s while cooled on ice with a DY89-I high speed homogenizer (Scientz co., Ningbo, China) and centrifuged at 11,500*g* for 10 min at 4 °C with a refrigerated centrifuge (Hunan Xiangyi Laboratory Instrument Development Co., Changsha, China). After repeating the homogenizing and centrifuging procedure, 2 volumes of 7.5 mM ATP solution (pH 6.4) containing 0.675 M KCl, 7.5 mM $MgCl_2$ and 0.15 mM DTT were added to the precipitate; the mixture was held at 0 °C for 10 min. After centrifugation at 11,500*g* for 10 min, the supernatant was precipitated by adding 9 volumes of ice-cold distilled water and centrifuged again under the same conditions. The precipitate was dissolved in 0.2 vol of 0.12 M Tris-maleate (pH 7.5) containing 3 M KCl and 0.6 mM DTT; 0.1 vol of 110 mM ATP (pH 7.5) containing 55 mM $MgCl_2$ and 5.5 mM EGTA was added to the mixture. The fraction obtained at 45–55% saturation with ammonium sulfate was dissolved in 20 mM Tris-maleate (pH 7.5) containing 0.5 M KCl and 0.1 mM DTT and dialyzed at the same buffer. After centrifugation at 11,500*g* for 60 min, the supernatant was freeze-dried as a purified myosin powder.

2.3. The hydrolysis of myosin with trypsin

The concentration of myosin was adjusted to 20 mg/mL with 0.6 M KCl and 20 mM potassium phosphate buffer (pH 7.0). Trypsin stock solution (0.5 mg/mL) was prepared fresh at 2–8 h before each experiment; appropriate volumes were added to myosin solution to give the final concentrations of 0%, 1%, 5% and 10% (w/w) of trypsin per gram basis of myosin as required. Proteolysis was terminated after incubated for 20 min at 37 °C; the reaction was ended by adding PMSF (Jacobson & Rosenbusch, 1976). The final concentration of 0% trypsin was defined as control; the treatments with the final concentrations of 1%, 5% and 10% trypsin were defined as 1%, 5% and 10% treatments, respectively. The hydrolyzed samples were dialyzed in 6 mM potassium phosphate buffer containing 0.6 M KCl (pH 7.0) overnight and freeze-dried.

2.4. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis

The concentration of freeze-dried samples was adjusted to 5 mg/mL with 0.6 M KCl and 20 mM potassium phosphate buffer (pH 7.0). The SDS-PAGE was done and analyzed according to our previous work (Cao et al., 2013). Each lane was loaded with 50 μg of protein for samples.

2.5. Raman spectroscopy

The myosin after freeze-drying was used for Raman analysis (Renishaw inVia Reflex Raman spectrometer, Renishaw Company, France) according to Alix, Pedanou, and Berjot (1988) with slight modification. An Argon Ion Laser emitted at a wavelength of 532 nm was used as the excitation source. The scattered radiation was collected at 180° to the source; typical spectra were recorded at 1 cm^{-1} resolution with 500–2100 cm^{-1} scans with 12 mW of laser power. The Phe ν-ring band located near 1003 cm^{-1} was used as internal standard for normalization of the spectra. The contents of secondary structures (α-helix, random coil, β-sheet, β-turn and other structures) were calculated according to Susi and Byler (1988) using PeakFit 4.12 software (SeaSolve Software Inc., USA).

2.6. The determination of the solubility of hydrolyzed myosin samples

The solubility of hydrolyzed myosin samples was determined according to the method of Petruccelli and Anon (1994) with slight modification. Two mg of Samples after freeze-drying was added in 1 mL of 6 mM potassium phosphate buffer containing 0.6 M KCl (pH 7.0) with a WH966 occasional vortex agitation (Kanghua Biochemical instrument corp., Shanghai, China) for 1 h at room temperature. The solutions were centrifuged at 10,000*g* for 30 min at 4 °C. The content of protein in the supernatant was determined using BCA Assay Kit (Beyotime Biotechnology Corp., China). The assays were done for triplicate repeat. The solubility was calculated using the following equation.

$$\text{The solubility of myosin } (\%) = [\text{The concentration of protein solution in supernatant } (\text{mg/mL})/2(\text{mg/mL})] \times 100.$$

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