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The effectiveness of clove extracts in the inhibition of hydroxyl radical oxidation-induced structural and rheological changes in porcine myofibrillar protein

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ABSTRACT

Oxidation is a major cause of protein quality deterioration during the storage and processing of food. This study investigated the effects of clove extract (CE) on structural and rheological changes in porcine longissimus myofibrillar proteins (MP) and the effects of oxidizing radicals produced by a Fenton reaction system (FRS). Increased oxidation time was accompanied by increased carbonyl content, reduced Ca-ATPase activity, decreased enthalpy of denaturation, decreased thermal transition temperatures ($P < 0.05$), and increased protein susceptibility to thermal aggregation. The addition of CE significantly inhibited carbonyl formation ($P < 0.05$), enhanced solubility and thermal stability, and improved the gel formation ability (storage modulus, loss modulus) of MP. The protective effect of CE on protein denaturation was demonstrated by its efficacy in maintaining Ca-ATPase activity and decreasing the degree of protein aggregation. Overall, the hydroxyl radical-induced loss of the structural and functional properties of MP was significantly reduced by the presence of CE.

1. Introduction

Protein oxidation has been studied in human biology and the medical domain, but only to a limited degree with regards to food quality. Protein oxidation may decrease food quality by reducing juiciness and tenderness, expediting flavour deterioration and discoloration and possibly forming toxic compounds (Xiong, 2000; Utrera, Morcuende, & Estévez, 2014). The formation of protein oxidation products is influenced by the nature of the proteins present in the product and food matrix. The changes caused by protein oxidation consist of the formation of carbonyls, intra- and intermolecular cross-linking through the formation of disulphide bonds and dityrosine, decreased protein solubility, and fragmentation of the peptide backbone (Xiong, 2000; Utrera, Parra, & Estévez, 2014). Oxidation might also play a key role in enhancing the inactivation of proteolytic enzymes and thereby influences meat tenderness (Estévez, 2011).

The practical and efficient way to reduce and prevent oxidation and quality deterioration in meat and meat products is to use antioxidants into the products (Kong et al., 2010). Recently, there has been an increasing interest in the use of natural antioxidants, especially those that are extracted from plant sources due to their ability to act as metal ion chelators, free radical terminators, singlet oxygen quenchers, and reducing agents (Mathew & Abraham, 2006). Spices and herbs (phenolic-rich) are known to display effective antioxidative activity in different food systems towards lipid and protein oxidation (Madsen et al., 1997; Karre et al., 2013; Ahmad Shah et al., 2014). Clove (*Syzygium aromaticum*), belonging to the Myrtaceae family, has been widely used in the flavouring industries, fragrance and cosmetics (Baskaran et al., 2010). Clove extract (CE) is isolated from the buds of clove and is not harmful when consumed in food products. Radha Krishnan et al. (2014) reported that the addition of CE could effectively inhibit microbial growth, decrease lipid oxidation, maintain or improve sensory attributes and extend the shelf-life of raw chicken meat during refrigerated storage. Shan et al. (2009) suggested that the natural extracts from cinnamon stick, oregano, clove, pomegranate peel and grape seed applied to raw pork could help to reduce the total bacterial count and retard lipid oxidation during storage at ambient temperature for up to 9 days, and CE exhibited the most effective antibacterial property and the highest antioxidant activity. However it is unknown whether this extract may have a positive influence against protein oxidation.

Reactive oxygen species (ROS), especially free radicals involving metmyoglobin (Frederiksen et al., 2008), and transition metal ions (Rowe et al., 2004) are the major initiators of oxidation. Our previous research showed that the addition of polyphenolic-rich black currant extracts inhibited carbonyl formation during the chilled storage of raw

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pork patties (Jia et al., 2012). Kong et al. (2010) assessed the antioxidant activity of thirteen common spice extracts in a cooked pork patties and liposome system and showed that six of the spice extracts (clove, liquorice, nutmeg, rosemary, cassia bark, and ground cardamom), where were identified as having a higher total phenolic content, were strong inhibitors of TBARS formation. CE exhibited the greatest reducing power and a strong DPPH scavenging activity. Cooked pork patties including these spice extracts had markedly decreased oxidation and an off-flavour as well as a more stable red colour (Kong et al., 2010). Therefore, the application of spice extracts may endue meat processors a good chance to develop unique products with improved health benefits and enhanced food quality.

Myofibrillar protein (MP) is the major protein fraction responsible for many of the physicochemical and functional properties of meat products (Lanier, 1986). The structure of proteins greatly affects the functional properties of muscle proteins. The antioxidant potential of spices has been verified by these and other related researches. However, few studies have aimed to contact the antioxidant compounds with spice extracts and their antioxidant mechanisms to the suppression of structural and functional deterioration in MP. The objective of the present work was to observe the stabilizing effect of CE on relieved oxidative deterioration in MP under oxidative conditions generally experienced in meat processing. We used a Fenton reaction system (FRS) to simulate these conditions and subsequently determined the structural and functional changes, including carbonyls, Ca-ATPase activity, solubility, thermal stability, rheological analysis and protein cross-linking in MP prepared with or without CE.

2. Materials and methods

2.1. Materials

Pork longissimus muscle was purchased from a local Meat Corporation (Harbin, China) within 12 h of slaughter. The samples were placed in iced coolers and transported to the laboratory. Clove (*Eugenia caryophyllata*) was purchased from a local pharmacy (Harbin, China). The dried clove buds were ground to a fine powder in a Kenwood Multi-Mill (Kenwood Ltd., Havant, UK) and passed through a 24-mesh sieve. All reagents and chemicals were analytical grade. Sodium dodecyl sulphate (SDS), 2,4-dinitrophenylhydrazine (DNPH), and piperazine-N,N′-bis (2-hydroxypropanesulphonic acid) (PIPES) were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Preparation of clove extract

The CE was prepared according to Zhang et al. (2009). An aliquot of pulverized and dried clove (100 g) was mixed into 800 mL of 95% (*v*/*v*) ethanol in enclosed flasks with constant shaking (100 rpm) for 12 h. After filtration with Whatman No. 2 filter paper, the residue was re-extracted with an additional 400 mL of 95% ethanol for an additional 12 h and then filtered. The combined filtrates were concentrated on a rotary evaporator (RE-52AA, Yarong Biochemical Analysis Co., Ltd., Shanghai, China) at 50 °C with a vacuum pump, and the extracts were freeze dried.

2.3. GC–MS analysis of clove extract

GC–MS analysis was carried on an Agilent-7890A gas chromatographer with an HP 5975 mass spectrometer detector according to Radha Krishnan et al. (2014). HP-5Ms capillary column (30.0 m × 0.25 mm × 0.25 μm) was used. Helium was the carrier gas with a flow rate of 1.0 mL/min, and injection volume was 1 μL. Initially temperature was kept at 60 °C for 5 min, and then raised to 250 °C in steps of 10 °C/min. Compounds in the CEs were identified by matching their retention times and mass spectra with those of pure compounds whenever possible. NIST (National Institute of Standards and Technologies) USA, Mass Spectra Library was also used as a reference.

2.4. Preparation of myofibrillar protein

Myofibrillar protein (MP) was extracted from longissimus muscle by the method of Xia et al. (2009) within 36 h after slaughter, and the pH of muscle was about 5.6–5.8. The protein concentration in MP was measured by the Biuret method. The final pellet (MP) was stored in a tightly capped bottle, kept on ice, and utilized within 24 h.

2.5. Oxidation of samples

The above MP pellet was diluted to 20 mg/mL with pH 6.25 of 15 mM PIPES buffer containing 0.6 M NaCl. The CE was incorporated into the MP suspension at concentrations of 0, 0.1, 0.5, and 1 mg/mL (CE dry weight/solution volume). All of the protein samples were oxidized at 4 °C for 1, 3, and 5 h in FRS that consisted of 10 μM $FeCl_3$, 10 mM H_2O_2, and 0.1 mM ascorbic acid. Oxidation was ended by addition of EDTA/Trolox C/propyl gallate (1 mM each). The nonoxidized MP solution containing EDTA/Trolox C/propyl gallate was used as the control.

2.6. Carbonyl content determination

Carbonyl contents were measured according to the method of Xia et al. (2009) by reaction with DNPH. Absorbance for the DNPH-treated sample and an HCl control was measured at 365 nm. The carbonyl conformation was expressed as μmol of DNPH fixed/g of protein.

2.7. Ca-ATPase activity determination

Ca-ATPase activities of MP were determined according to Wells et al. (1979) with slight modifications. MP samples were diluted to 3.0 mg/mL protein. The protein suspension (0.2 mL) was mixed with 2.0 mL of the reaction solution. After reaction at 25 °C for 10 min, 1.0 mL of 100 g/L trichloroacetic acid was added to stop the reaction. The mixture was subsequently centrifuged at 2500 ×*g* for 5 min, and 1 mL of the supernatant was reacted with 3.0 mL of 6.6 g/L ammonium molybdate in 0.75 M sulphuric acid, followed by the addition of 0.5 mL of freshly prepared 100 g/L $FeSO_4$ in 0.15 M sulphuric acid. The mixture was then allowed to react for colour development for 2 min. The absorbance of the liberated inorganic phosphate was read at 700 nm to determine the Ca-ATPase activity. The results were expressed as mmol phosphate/g protein. A series of NaH_2PO_4 solutions (0.0–1.0 mM) were used to prepare the standard curve for phosphate calculation.

2.8. Differential scanning calorimetry

All MP samples (20 mg/mL) were diluted in 5 volumes of 15 mM PIPES (pH 6.25), then were centrifuged at 4000 ×*g* for 15 min at 4 °C. The MP pellets were redissolved in 15 mM PIPES (pH 6.25) containing 0.6 M NaCl to get final protein suspensions at a concentration of 40 mg/mL. The thermal stability of MP was measured according to Chen et al. (2013) using a differential scanning calorimeter (DSC) (Perkin Elmer Co. Ltd., Waltham, CA, USA).

2.9. Protein solubility

Protein solubility was determined according to Kong et al. (2013). The MP samples were diluted to protein concentration of 2 mg/mL with 25 mM phosphate buffer (pH 6.25) containing 0.6 M NaCl, followed by centrifugation at 5000 ×*g* for 15 min at 4 °C to measure protein solubility.