THE EFFECTIVENESS OF CLOVE EXTRACT IN THE INHIBITION OF HYDROXYL RADICAL OXIDATION-INDUCED STRUCTURAL CHANGES IN PORCINE MYOFIBRILLAR PROTEINS

Qian Liu[1], Hongsheng Chen[1, 2], Jingjing Diao[2] and Baohua Kong[1*]

[1] College of Food Science, Northeast Agricultural University, Harbin, Heilongjiang 150030, China

[2] College of Food Science, Heilongjiang Bayi Agricultural University, Daqing, Heilongjiang 163319, China

Abstract – This study aimed to investigate the effects of clove extract (CE) on protein oxidation induced structural changes in porcine myofibrillar proteins (MP) which produced by a hydroxyl radical-generating system. The addition of CE significantly inhibited carbonyl formation (P<0.05), enhanced solubility 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 changes of MP was significantly reduced by the presence of CE.

Keywords – Porcine longissimus muscle; clove extract; protein structure; hydroxyl radical-generating system

I. INTRODUCTION

In meat, protein oxidation may decrease food quality by reducing juiciness and tenderness, expediting discoloration and flavour deterioration and possibly forming toxic compounds [1]. Multifarious protein oxidation products are formed according to the food matrix and the nature of the proteins present in the product, and different oxidation initiators may result in different oxidation products. The protein changes caused by oxidation consist of the formation of carbonyls and hydroperoxides, inter- and intramolecular cross-linking through the formation of dityrosine and disulphide bonds, fragmentation of the peptide backbone, and decreased protein solubility. The most practical and efficient way to prevent oxidation and quality deterioration in muscle-based foods is to incorporate antioxidants into the meat products [2]. Spices and herbs containing polyphenols are known to display antioxidative activity towards lipid and protein oxidation in different food systems [3]. Our previous research showed that the addition of phenolic-rich plant extracts prevented protein carbonyl accumulation during the storage of fresh meat [4]. Myofibrillar protein (MP) is the major protein fraction responsible for many of the physicochemical and functional properties of muscle foods. The structure of proteins greatly affects the functional properties of muscle proteins. The objective of the present work was to investigate the effects of clove extract (CE) on protein oxidation induced structural changes in MP which produced by a hydroxyl radical-generating system.

II. MATERIALS AND METHODS

Materials. Pork longissimus muscle was obtained within 12 h of slaughter from Beidahuang Meat Corporation (Harbin, Heilongjiang, China). The samples were kept on ice and transported to the laboratory and used on the same day. Clove (*Eugenia caryophyllata*) was purchased from a local traditional Chinese pharmacy (Harbin, China). Sodium dodecyl sulphate (SDS), piperazine-N, N bis, and piperazine-N, N'-bis (2-hydroxypropanesulfonic acid) (PIPES) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All chemicals and reagents were analytical grade.

Preparation of clove extract. The clove extract was prepared according to the method of Zhang,

Kong, Xiong, and Sun [5].

Preparation of myofibrillar protein. MP was prepared from porcine longissimus muscle according to the procedure of Xia, Kong, Liu, and Liu [6].

Oxidation of samples. The MP pellet was quantitatively diluted to a final protein concentration of 20 mg/mL in 15 mM piperazine-N,N-bis(2-ethanesulfonic acid) (PIPES) buffer containing 0.6 M NaCl (pH 6.25). The CE was incorporated into the MP suspension at concentrations of 0.1, 0.5, and 1 mg/mL. The control sample did not contain any antioxidant. All of the protein samples were oxidized at 4 °C for 1, 3, and 5 h in HRGS that consisted of 10 μM $FeCl_3$, 0.1 mM ascorbic acid and 10 mM H_2O_2. Oxidation was terminated by adding propyl gallate/Trolox C/EDTA (1 mM each). The nonoxidized MP solution containing propyl gallate/Trolox C/EDTA was used as the control.

Carbonyl content. Carbonyl groups were measured by reaction with 2, 4-dinitrophenylhydrazine (DNPH) to form protein hydrazones according to Xia, Kong, Liu, and Liu [6].

Ca- ATPase activity determination. Ca-ATPase activities of MP were determined according to Wells, Werber, and Yount [7] with slight modifications.

Protein solubility. The MP solution (2 mg/mL protein in 25 mM phosphate buffer containing 0.6 M NaCl, pH 6.25) was centrifuged at 5000 × *g* at 4 °C for 15 min to determine protein solubility.

Electrophoresis. The MP samples were also subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) as described by Laemmli [8] to assess the extent of covalent protein cross-linking.

Statistical analysis. All of the experiments were conducted in triplicate for each sample. Three independent experimental trials (replications) were conducted. The data were analysed using the General Linear Models procedure of the Statistix 8.1 software package (Analytical Software, St. Paul, MN, USA) for microcomputers. An analysis of variance was performed to determine the significance of the main effects. The significant differences (P < 0.05) between the means were identified using Tukey's procedures.

III. RESULTS AND DISCUSSION

Protein carbonyls. As shown in Fig. 1, incubation of proteins with the oxidative system significantly increased (P<0.05) the protein carbonyl content; the carbonyl was more pronounced at longer reaction times for all samples.

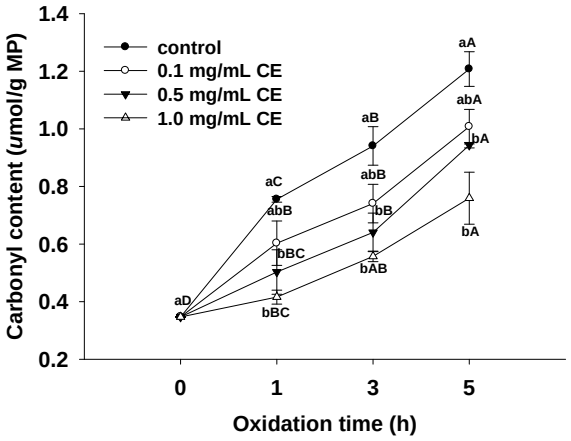

Fig. 1. Influence of clove extraction (CE) on carbonyl formation in MP solution incubated in HRGS at 4°C for 1, 3 or 5 h

Incorporation of CE into the MP suspension (20 mg/mL) prior to oxidation significantly inhibited carbonyl formation (P<0.05); the antioxidant effect was more intense when higher

levels of CE were added. Carbonyl formation is one of the major chemical consequences when muscle proteins are exposed to an oxidizing environment. In the present study, CE addition significantly inhibited (P<0.05) the formation of protein carbonyls.

Ca-ATPase Activity. The Ca-ATPase activity of the porcine longissimus MP samples showed a significant decrease with increased oxidation time (P<0.05) (Fig. 2). The Ca-ATPase activity of nonoxidized MP was 0.95 mmol/g, and this decreased (P<0.05) by 40.35%, 58.63%, and 81.22% when oxidized in HRGS for 1, 3, and 5 h, respectively. Incorporation of CE into the MP suspension could significantly reduce the loss of Ca-ATPase (P<0.05).

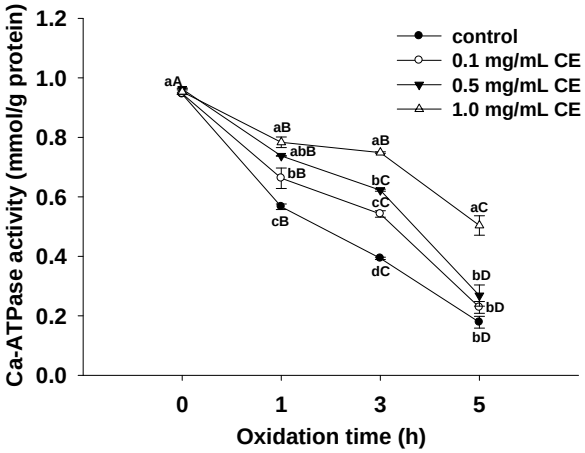

Fig. 2. Influence of clove extraction (CE) on Ca-ATPase activity in MP solution incubated in HRGS at 4°C for 1, 3 or 5 h

Ca-ATPase activity is closely related to the reactivity of SH groups in the myosin globular heads of muscle myofibrils and has been used as an indicator of myosin integrity [9]. The decrease in Ca-ATPase activity can be attributed to the conformational changes of the myosin globular head as well as the aggregation of this protein. MP rearrangements due to protein-protein interactions induced by oxidation contributed to the loss in ATPase activity. Our results revealed that the incorporation of CE could inhibit the decrease in Ca-ATPase activity, indicating that CE had protective effects on muscle proteins during oxidation.

Protein solubility. The protein solubility of the MP samples generally decreased with increasing oxidation time (P<0.05) (Fig. 3). Specifically, the solubility of the control (nonoxidized) MP (74.06%) dropped to 66.07%, 54.67%, and 45.57% (P<0.05) after exposure to HRGS for 1, 3, and 5 h, respectively. Incorporation of CE into the MP solution significantly retarded the loss of protein solubility. At 5 h, the protein solubility was 47.42%, 50.92%, and 57.55% in the 0.1 mg/mL, 0.5 mg/mL, and 1.0 mg/mL CE groups (P<0.05), respectively.

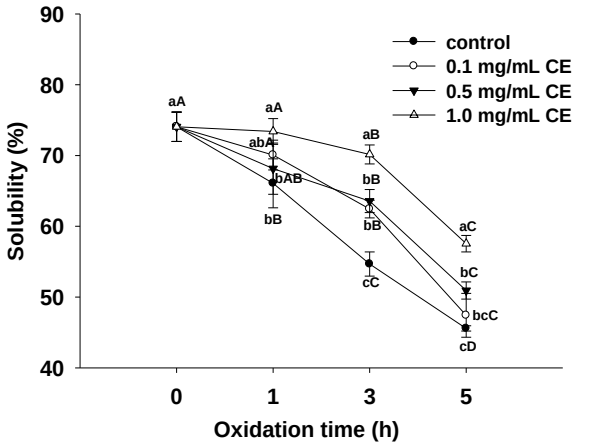

Fig. 3. Influence of clove extraction (CE) on the solubility of MP solution incubated in HRGS at 4°C for 1, 3 or 5 h.

Proteolytic changes. SDS-PAGE of MP subjected to varying degrees of oxidation with or without CE demonstrated an obvious loss in intact myosin heavy chains, actin, and tropomyosin bands with increasing oxidation time (Fig. 4). For nonoxidized samples, there were barely detectable polymers at the top of the stacking gel in the nonreducing (-βME) gel. Upon oxidation, high MW polymers, which barely entered the stacking gel, became

accentuated. These polymers were largely derived from myosin, as the amount of MHC (especially at 3 and 5 h of oxidation) drastically decreased. Concomitantly, actin also showed decreases after oxidation, which may also contribute to the increased band intensity at the top of the stacking gel. In the reducing (+βME) gel, the lost myosin and actin bands due to oxidation were mostly recovered in the control group and were almost completely recovered in the CE group, indicating that these polymers were largely formed by disulphide bonds.

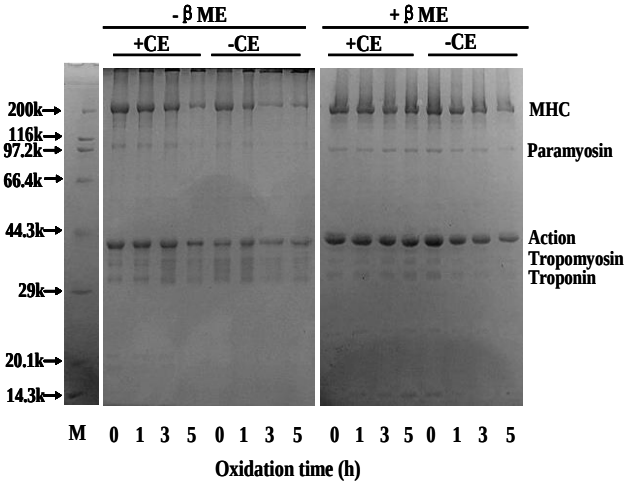

Fig. 4. Influence of clove extraction (CE) on the SDS-PAGE pattern of MP solution incubated in HRGS at 4°C for 1, 3 or 5 h. Samples were prepared in the presence (+βME) or absence (−βME) of 10% β ME.

ACKNOWLEDGEMENTS

This study was supported by the National Natural Science Foundation of China (grant no. 31471599) and the Research Item of Department of Education in Heilongjiang (grant no. 12541574).

REFERENCES

1. Utrera, M., Morcuende, D., & Estévez, M. (2014). Temperature of frozen storage affects the nature and consequences of protein oxidation in beef patties. Meat Science 96: 1250-1257.
2. Kong, B. H., Zhang, H. Y., & Xiong, Y. L. (2010). Antioxidant activity of spice extracts in a liposome system and in cooked pork patties and the possible mode of action. Meat Science 85: 772-778.
3. Madsen, H. L., Bertelsen, G., & Skibsted, L. H. (1997). Antioxidant activity of spices and spice extracts. American Chemical Society 1: 176-187.
4. Jia, N., Kong, B. H., Liu, Q., Diao, X. P., & Xia, X. F. (2012). Antioxidant activity of black currant *(Ribes nigrum L.)* extract and its inhibitory effect on lipid and protein oxidation of pork patties during chilled storage. Meat Science 91: 533-539.
5. Zhang, H. Y., Kong, B. H., Xiong, Y. L., & Sun, X. (2009). Antimicrobial activities of spice extracts against pathogenic and spoilage bacteria in modified atmosphere packaged fresh pork and vacuum packaged ham slices stored at 4 °C. Meat Science 81: 686-692.
6. Xia, X. F., Kong, B. H., Liu, Q., & Liu, J. (2009). Physicochemical change and protein oxidation in porcine longissimus muscle as influenced by different freeze-thaw cycles. Meat science 83: 239-245.
7. Wells, J. A., Werber, M. M., & Yount, R. G. (1979). Inactivation of myosin subfragment one by cobalt (II) /cobalt (III) phenanthroline complexes 2. Cobalt chelation of two critical SH groups. Biochemistry 18: 4800-4805.
8. Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685.
9. Roura, S. I., Monteccia, C., Goldemberg, A. L., Trucco, R. E., & Crupkin, M. (1990). Biochemical and physicochemical properties of actomyosin from pre- and post-spawned Hake stored ice. Journal of Food Science 55: 688-692.