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Effect of phosvitin on lipid and protein oxidation in ground beef treated with high hydrostatic pressure

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ABSTRACT

In this study, we aimed to examine the effect of phosvitin on lipid and protein oxidation of raw and cooked ground beef treated with high hydrostatic pressure (HHP). Ground beef patty with 0, 500, or 1000 mg phosvitin/kg meat was treated with HHP at 0.1, 300, or 600 MPa. Half of the patties were used in a raw meat analysis, and the other half were used in a cooked meat analysis. Phosvitin and HHP treatment at 300 MPa synergistically reduced microbial growth, and HHP treatment at 600 MPa reduced microbial counts to undetectable levels (<1 log CFU/g) throughout the length of the study in all samples. Phosvitin delayed lipid and protein oxidation in HHP-treated cooked and raw ground beef, respectively. However, phosvitin had no effect on the color changes of raw ground beef attributable to HHP. The results indicated that phosvitin could enhance the stability of lipids and proteins but not color changes of raw ground beef caused by HHP.

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1. Introduction

Nowadays, consumers are demanding not only convenient but also high-quality foods that are safe, nutritious, and attractive. Processed meat products such as ground meat and ready-to-eat meat are sufficient to meet the consumer's demand for convenience. However, certain processing steps such as cutting, grinding, and packaging can increase the risk of microbial contamination in meat (Bover-Cid, Belletti, Garriga, & Aymerich, 2011); this decreases the shelf life of such meat products.

High hydrostatic pressure (HHP) processing is a technology that can improve the microbiological safety and shelf life of meat and meat products (Bover-Cid et al., 2011; Jung, Jung, Lee, Kang, Lee, Kim, & Jo, 2012; Jung, Kang, Kim, Nam, Ahn, & Jo, 2012; Kruk, Yun, Rutley, Lee, Kim, & Jo, 2011). HHP minimizes destruction of nutrients because it is a non-thermal technology (Kimura et al., 1994). Thus, HHP could satisfy the consumer's demand with respect to safety and has an advantage in pasteurizing pre-packed foods. However, acceleration of lipid and protein oxidation is a serious problem when meat and meat products are subjected to HHP (Cheah & Ledward, 1997; Fuentes, Ventanas, Morcuende, Estevez, & Ventanas, 2010; Orlien, Hansen, & Skibsted, 2000). Lipid and protein oxidation deteriorate meat quality parameters such as flavor, color, texture, and nutritional value during storage (Mielnik, Olsen, Vogt, Adeline, & Skrede, 2006; Xiong, 2000). Cheah and Ledward (1997) reported that lipid oxidation in HHP-treated meat was catalyzed by the free iron released from either heme or non-heme compounds via HHP. In addition, oxidizing lipids and metal ions are the major causes of protein oxidation in meat (Estevez, Kylli, Puolanne, Kivikari, & Heinonen, 2008). Therefore, control of lipid and protein oxidation in HHP-treated meat and meat products is required to produce high-quality products. Besides, the use of natural sources to improve food quality is more attractive for consumers than the synthetic methods, which could be rejected by consumers (Naveena, Sen, Vaithiyanathan, Babji, & Kondaiah, 2008).

Phosvitin is a natural phosphoglycoprotein in yolk granules (Tarborsky, 1963). It comprises about 7% of the yolk protein (Mecham & Olcott, 1949). Phosvitin has a characteristic amino acid composition, of which greater than 50% is serine and most of the serine residues are phosphorylated (Clark, 1985). This specific structure of phosvitin makes it a potent metal chelator. Two moles of organic phosphorus from phosvitin can bind an iron molecule, and a phosvitin molecule can bind a maximum of 65–70 iron molecules under optimal conditions (Tarborsky, 1963; Webb, Multani, Saltman, Beach, & Gray, 1973). The ability of phosvitin to act as a metal chelator enables it to be used as an antioxidant. Ishikawa, Yano, Arihara, and Itoh (2004) reported that phosvitin inhibited the generation of iron-catalyzed hydroxyl radicals from the Fenton reaction. The inhibition of iron-catalyzed lipid oxidation in meat by adding phosvitin was confirmed (Lee, Han, & Decker, 2002; Lu & RC, B., 1986). Therefore, iron-catalyzed lipid and protein oxidation in HHP-treated meat can be inhibited by phosvitin. However, no study has been performed on the use of phosvitin as an antioxidant for HHP-treated meat.

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Recently, Ko, Nam, Jo, Lee, and Ahn (2011) reported a method for the separation of phosvitin from egg yolk. This method was simple and cost effective, and the purity of the separated phosvitin was competitive, with its high purity as a commercial standard. In fact, commercially available phosvitin is too expensive to be used as a functional ingredient in the food industry. Therefore, the objective of this study was to examine the effect of phosvitin that was separated from egg yolk on lipid and protein oxidation, color, and microbial growth in HHP-treated raw and cooked ground beef.

2. Materials and methods

2.1. Sample preparation

Phosvitin was prepared from chicken eggs, according to the method described by Ko et al. (2011). The lyophilized phosvitin was dissolved in distilled water before use. The frozen beef (inside rounds) imported from the U.S. was obtained from a local market (Daejeon, Korea) and ground by a grinder to achieve homogeneous sample. The ground beef was divided into 3 treatment groups: (1) control (GBP 0), ground beef without phosvitin; (2) GBP 500, ground beef with 500 mg of phosvitin/kg meat; and (3) GBP 1000, ground beef with 1000 mg of phosvitin/kg meat. After adding phosvitin, the ground beef (2 kg) was mixed by hand for 1 min and subsequently mixed using a food mixer (M-12S, Hankook Fujee Industries Co., Hwaseong, Korea) for 2 min. Aliquots of ground beef (50 g) were individually vacuum-packaged in a low-density polyethylene/nylon vacuum bags (10 cm ×10 cm; oxygen permeability of 22.5 mL/m^2/24 h atm at 60% RH/25 °C; water vapor permeability of 4.7 g/m^2/24 h at 100% RH/25 °C) using a vacuum-packaging machine (FJ-600XL; Hankook Fujee Industries Co., Hwaseong, Korea) at −650 mm Hg. The vacuum-packaged ground beef was equally divided into 2 groups: the first half was used for the raw meat analysis, and the second half was used for the cooked meat analysis. The packaged meats were cooked in a 75 °C water bath for 30 min, cooled at room temperature for 2 h, and then re-packaged in vacuum bags after removing exudate.

2.2. HHP process and storage

The samples in the vacuum bags were placed in a pressure vessel (Quintus food processor 6; ABB Autoclave Systems, Inc., Columbus, OH, USA) that was submerged in hydrostatic fluid medium (Water glycol type; Houghton International Inc., Allentown, PA, USA) and pressurized at 300 MPa and 600 MPa for 5 min, with the initial temperature of the pressure vessel set at 15 ± 3 °C. The rate of pressurization was 5–7 MPa/s, and the pressure in the chamber was released within 10 s. The control samples were maintained under atmospheric pressure (0.1 MPa) in a styrofoam box to keep the temperature around 10 ± 2 °C while the other samples were treated. Following the HHP process the bags were cut in one side of the packaging material and opened by hand to allow air exposure to accelerate oxidation. The beef samples for each treatment were packaged individually for each storage day with 2 observation numbers, and one-layer stored in the dark condition at 4 °C for 10 days.

2.3. Total aerobic bacterial counts

The samples (10 g) were blended with sterile saline (90 mL) for 2 min by using a stomacher (BagMixer® 400; Interscience Ind., St. Nom, France). A series of decimal dilutions was prepared using sterile saline. Each diluent (0.1 mL) was spread in triplicate on tryptic soy agar plates (Difco Laboratories, Detroit, MI, USA). The plates were incubated at 37 °C for 48 h, and the microbial counts were expressed as log CFU/g.

2.4. Lipid oxidation

Lipid oxidation in samples was measured using 2-thiobarbituric acid reactive substance (TBARS) values, according to the modified method described by Stalikas and Konidari (2001). The sample (3 g) was added to 9 mL of 1 N NaOH with 100 μL of 7.2% butylated hydroxyl toluene and was hydrolyzed in a shaking water bath at 60 °C for 1 h. The hydrolysate was filtered (filter paper, Whatman no. 1) after stirring with 6 mL of 40% trichloroacetic acid (TCA) by vortexing for 30 s. The filtrate (1 mL) was mixed with 20 mM 2-thiobarbituric acid (1 mL), and the mixture was kept in a boiling water bath (90 °C) for 40 min, cooled, and then centrifuged at 2090 ×g for 15 min. The absorbance of the supernatant was measured at 532 nm with a spectrophotometer (DU 530; Beckman Instruments Inc., Fullerton, CA, USA). The amount of malondialdehyde was calculated using a standard curve prepared from tetraethoxypropane, and the TBARS value was reported as mg malondialdehyde/kg meat.

2.5. Protein oxidation

Protein oxidation in the samples was measured by carbonyl content, and protein carbonyls were determined by derivatizing them with 2,4-dinitrophenylhydrazine (DNPH), as described by Fagan, Sleczka, & Sohar (1999). The sample (2.0 g) was homogenized in 15 mL of pyrophosphate buffer (pH 7.4; 2.0 mM $Na_4P_2O_7$, 10 mM Tris–maleate, 100 mM KCl, 2.0 mM $MgCl_2$, and 2.0 mM EGTA) by using a homogenizer (T25b; Ika Werke Gmbh & Co. KG, Janke & Kunkel, Staufen, Germany). The homogenate of each sample was divided into 2 test tubes, of which one was used to determine carbonyl content and the other was used as a protein blank. Chromophores from the sample were removed by washing the sample (2 mL) with 4 mL of HCl–acetone (3:100) (V/V) twice, followed by washing with 2 mL of 10% TCA twice. The samples were derivatized for 30 min with 4 mL of 10 mM DNPH in 2.0 N HCl, and the protein blanks were prepared by adding 4 mL of 2.0 N HCl, instead of DNPH solution. Excess DNPH was removed by washing with 4 mL of 20% TCA once, followed by 5 washes with 4 mL of 10 mM HCl in ethanol–ethyl acetate (1:1) (V/V). The pellets were solubilized in 4 mL of 6.0 M guanidine hydrochloride and 20 mM potassium dihydrogen phosphate (pH 2.3) at 4 °C for 24 h. The absorbance of the protein blank was measured at 280 nm by using a spectrophotometer (DU 530; Beckman), and the amount of protein was calculated using a standard curve that was prepared using bovine serum albumin. The carbonyl content was calculated using an absorption coefficient of 22,000 $M^{-1} \cdot cm^{-1}$ at 370 nm for the formed hydrazones. The carbonyl content was reported as nmol carbonyl per mg protein.

2.6. Instrumental color measurements

The lightness (L^*), redness (a^*), and yellowness (b^*) of the samples were measured using a spectrophotometer (CR-300; Minolta Inc., Tokyo, Japan). Measurements were taken perpendicularly to the sample in a quartz cell (3 cm × 1.5 cm) at 2 different locations per sample.

2.7. Statistical analysis

All experiments in this study were performed 3 individual trials with 2 observation numbers. Analysis of variance was performed using the raw data, and the mean values and standard error of the means (SEM) were calculated using the Statistical Analysis System (SAS, 2002). Differences among the means were determined using Duncan's multiple range test with $P < 0.05$.

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