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## Effects of antioxidant combinations on shelf stability of irradiated chicken sausage during storage



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### HIGHLIGHTS

- We evaluate the combined effects of gamma irradiation and antioxidant combination.
- Gamma irradiation is effective in reducing the nitrite levels.
- Combination of antioxidants might be helpful in enhancing the oxidative stability.

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### ABSTRACT

This study was conducted in order to investigate the combined effects of gamma irradiation (0, 2.5, and 5 kGy) and antioxidant combination, mugwort extract (ME) and ascorbic acid (Aa), on the pH, total color difference ( $\Delta E$ ), hue angle ( $H^\circ$ ), 2-thiobarbituric acid-reactive substances (TBARS) values, residual nitrite contents, and sensory evaluation in chicken sausage during storage. The pH values and sensory properties, except for color, of chicken sausage were not significantly affected by adding ME or treating irradiation during storage. However,  $\Delta E$  and  $H^\circ$  values of samples containing ME (either alone or with Aa) were higher than that of control, whereas irradiation had no significant effect during storage. A combination of ME+Aa (0.2% ME+0.05% Aa) was effective at delaying lipid oxidation in irradiated chicken sausage. In addition, nitrite contents were reduced by gamma ray as a dose dependent manner and, particularly in ME+Aa was most effective in decreasing the residual nitrite. Our results suggested that gamma irradiation combined with an antioxidant mixture is a useful technology for reducing the residual nitrite and retarding the lipid oxidation in chicken sausage.

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### 1. Introduction

Food irradiation techniques are the most effective methods for destroying pathogenic and spoilage microorganisms without compromising to the nutritional properties of foods; further, utilization of such techniques is increasing worldwide. However, irradiation accelerates free radical reactions, thereby leading to the possibility of lipid oxidation, odor generation and color changes, which may engender negative consumer responses (Ahn et al., 1997).

Lipid oxidation in irradiated meat and meat products can be delayed using several techniques and various packaging methods, such as vacuum packaging and MAP (Modified Atmosphere

Packaging) or low temperature, such as chilled storage condition. In the food processing industry, the application of useful antioxidants has become an effective means to inhibit the lipid oxidation of meat and meat products. Moreover, natural antioxidants sources have been tested for their antioxidant activities, resulting to be more effective for inhibiting lipid oxidation compared to artificial additives (Seol et al., 2010).

Mugwort (*Artemisia princeps* Pamp.) is a perennial plant that is widely distributed in Japan, Korea, China and Europe. In Oriental countries, including Korea, mugwort is widely used as a food or food additive. This plant contains bioactive compounds, such as phenolics, vitamins A, B<sub>1</sub>, B<sub>2</sub> and C as well as various minerals (Hwang et al., 2011).

Ascorbic acid is a reducing agent, which increases shelf-life and stabilizes the color of meat and meat products. Also, the combination of ascorbic acid with other antioxidants is effective in inhibiting

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the oxidative reaction as well as providing a decrease of residual nitrite in meat products (Fiddler et al., 1981).

Therefore, the purpose of this study was to investigate the combined effects of gamma irradiation (0, 2.5, and 5 kGy) and antioxidant combination, mugwort extracts (ME) and ascorbic acid (Aa), for chicken sausage during refrigerated storage.

## 2. Materials and methods

### 2.1. Preparation of ME

Commercial samples of dried mugwort were purchased from a local market. After separating the leaves from the dried mugwort, they were ground using a blender for 1 min. Ten gram of ground leaves were extracted with 200 ml of 50% ethanol overnight (24 h) in a shaker at room temperature. The extracts were filtered through filter paper no. 1 and evaporated with a rotary evaporator at  $< 50$  °C. The products represented the ME.

### 2.2. Preparation of the antioxidant

An antioxidant combination of ascorbic acid (Aa; Sewoo Inc, Seoul, South Korea) and mugwort extracts (ME: pH,  $6.06 \pm 0.02$ ;  $L^*$ -value,  $29.11 \pm 0.07$ ;  $a^*$ -value,  $1.46 \pm 0.21$ ;  $b^*$ -value,  $-0.80 \pm 0.05$ ) was prepared according to the formulations: Control (no antioxidant added), ME (0.2% ME), and ME+Aa (0.2% ME+0.05% Aa).

### 2.3. Preparation of samples

Fresh chicken breast meat and pork back fat were initially ground through an 8-mm plate. Three batches consisted of differing in composition with respect to the addition of antioxidant (see Section 2.2). For each batch of emulsion sausage, chicken breast meat (60%) pork back fat (20%), ice (20%), sodium chloride (NaCl, 1.5%), sodium tripolyphosphate (0.3%), and sodium nitrite ( $\text{NaNO}_2$ , 150 ppm), antioxidant were emulsified using silent cutter, and then the each batter was stuffed into collagen casings using stuffer. The chicken sausage was heated at  $75 \pm 1$  °C (central temperature) for 30 min in a smoke chamber. After heated, the cooked chicken sausages were vacuum-packaged in a polyethylene/nylon bag ( $2 \text{ ml O}_2/\text{m}^2/24 \text{ h}$  at 0 °C). All samples were stored at 4 °C before irradiation.

### 2.4. Gamma irradiation

Gamma irradiation of chicken sausage was carried out using a cobalt-60 irradiator. The source strength was approximately 100 kCi with a dose rate of 5 kGy/h at room temperature. Dosimetry was performed using 5 mm diameter alanine dosimeters. The doses applied in this study were 0, 2.5 and 5 kGy, and the actual doses were within  $\pm 2\%$  of target dose. The samples were immediately stored at 4 °C for 4 weeks.

### 2.5. Analysis

The pH values of samples were measured in a homogenate prepared with 5 g of sample and distilled water (20 ml) using a pH meter.

Color changes in the chicken sausage during storage were monitored with a colorimeter using an 8-mm diameter measuring area and a 50-mm diameter illumination area. Color was expressed with  $L^*$  (100=white, 0=black),  $a^*$  (positive=redness, negative=greenness), and  $b^*$  (positive=yellowness, negative=blueness) values. The colorimetric difference between a sample and a white standard reflectance plate, total color difference ( $\Delta E$ ), was calculated using the equation:  $\Delta E = [(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2]^{1/2}$  ( $L^* = 97.83$ ,

$a^* = -0.43$ ,  $b^* = +1.98$ ). Additionally, hue angle ( $H^\circ$ ) was calculated with the following formula: ( $H^\circ$ ;  $\text{Tan}^{-1}(b^*/a^*)$ ). Color readings were measured on ten randomly chosen spots on the chicken sausage and were utilized as an estimate of meat discoloration.

Lipid oxidation was assessed in sample triplicates using the 2-thiobarbituric acid (TBA) method of Tarladgis et al. (1960) with minor modifications and was expressed as milligrams of malondialdehyde (MA) per kilogram of sausage. A 10 g sample was blended with 50 ml distilled water for 2 min and then transferred to a distillation tube. The cup used for blending was washed with an additional 47.5 ml of distilled water, which was added to the same distillation flask with 2.5 ml 4 N HCl and a few drops of antifoam agent. The mixture was distilled, and 50 ml of the distillate was collected. Five ml of 0.02 M TBA in 90% acetic acid (TBA reagent) was added to test tubes containing 5 ml of the distillate and mixed well. The tubes were capped and heated in a boiling water bath for 30 min to develop the chromogen and cooled to room temperature. Absorbance was measured at 538 nm against a blank prepared with 5 ml distilled water and 5 ml TBA reagent using a UV/VIS spectrophotometer.

Residual nitrite content was determined according to the Diazo coupling method (Korea Food and Drug Administration (KFDA), 2013) and is expressed as ppm per kilogram of sausage. Briefly, a 10 g of ground sample was placed in a 200 ml volumetric flask to which approximately 150 ml of preheated distilled water was added. The solution was combined with 10 ml 0.5 N sodium hydroxide and 10 ml 12% ammonium thiosulfate and then heated in a boiling water bath at 80 °C for 30 min. After cooling, the solution was added up to 200 ml by ammonium acetate buffer 20 ml and distilled water. The sample was incubated for 10 min at room temperature and filtered through filter paper No. 1. After filtration, 1.5 ml sulphanilamide solution and 1.5 ml *N*-(1-naphthyl) ethylenediamine dihydrochloride reagent were added to the tube containing 20 ml of filtrate and kept at room temperature for 30 min. The absorbance at 538 nm was read in a UV/VIS spectrophotometer. The residual nitrite content was calculated by a standard curve using nitrite solution.

The color, odor, and overall acceptability of chicken sausages (irradiated and non-irradiated) were evaluated using 9-point hedonic rating scale described by Lawless and Heymann (1999). Twenty five panelists were chosen from among the graduate students and faculty of the Department of Food Sciences and Biotechnology of Animal Resources, University of Konkuk using the following criteria: ages between 20 and 35, non-smokers, regular consumers of sausage products. The panelists were instructed to clean their plate by consuming a cracker and sipping water between evaluations. The scale includes the following ranking: 1=extremely unacceptable, 2=very much unacceptable, 3=moderately unacceptable, 4=slightly unacceptable, 5=between acceptable and unacceptable, 6=slightly acceptable, 7=moderately acceptable, 8=very much acceptable and 9=extremely acceptable. A mean score of 7 or above indicates an acceptable product. A mean score below 5.0 marks the end of chicken sausage shelf-life (Mexis et al., 2009).

### 2.6. Statistical analysis

An analysis of variance was performed on all the variables measured using the general linear model (GLM) procedure of the SAS (2010) statistical package. Duncan's multiple range test ( $p < 0.05$ ) was used to determine the differences between treatment means.

## 3. Results and discussion

The pH changes of irradiated chicken sausage treated with an antioxidant mixture are presented in Table 1. Both antioxidants

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