

Application of *Ganghwa* Mugwort in Combination with Ascorbic Acid for the Reduction of Residual Nitrite in Pork Sausage during Refrigerated Storage

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Abstract

The application of *ganghwa* mugwort (GM), ascorbic acid (AC), and their combinations for reduction of residual nitrite contents was analyzed in pork sausages during storage of 28 d. Six treatments of pork sausages contained the following: Control (no antioxidant added), AC (0.05% AC), GM 0.1 (0.1% GM), GM 0.2 (0.2% GM), AC+GM 0.1 (0.05% AC + 0.1% GM) and AC+GM 0.2 (0.05% AC + 0.2% GM). Results showed that the mixture of 0.05% AC and 0.2% GM was most effective for reducing thiobarbituric acid reactive substances (TBARS) and residual nitrite contents than the control and GM added sausages alone ($p < 0.05$). The color values of all treatments were significantly affected by adding GM (either alone or with AC). Additionally, the total color difference (ΔE) and hue angle (H°) values of treatments added with GM were higher than those of the control as the amount of GM increased ($p < 0.05$). However, there were no significant differences in the pH values between the control and all treatments during the storage period ($p > 0.05$). Our results showed possible applications of antioxidant combination, for preventing the lipid oxidation and decreasing the residual nitrite levels of meat products.

Key words: antioxidant, mugwort, ascorbic acid, residual nitrite, pork sausage

Introduction

Sodium nitrite (NaNO_2) is one of the important ingredients in meat curing process. It has various roles in meat systems such as color development, lipid oxidation, flavor formation and microbiological safety. In addition, the function of nitrite is the production of the attractive pink color of cured meats, a common indicator of the quality of the cooked meat products preferred by the consumer (Zhang *et al.*, 2007).

However, nitrite can cause the formation of carcinogenic *N*-nitrosamines due to its reaction with secondary amines and amino acids in meat proteins. The potential health risks associated with nitrite in meat products calls for attention on decreasing its amounts considerably. Additionally, as consumers are concerned about the possible harmful effects of nitrite and synthetic food additives, the

meat industry is continually searching for alternative methods to reduce the concentrations of nitrite in their meat products (Vossen *et al.*, 2012; Zhang *et al.*, 2007).

The ascorbic acid works as reducing agent, which increases shelf-life and improves the color of meat and meat products. Also, the combination of ascorbic acid not only acts synergistically with other antioxidants by regenerating and restoring their antioxidant properties but also decreases the residual nitrite in meat products (Djenane *et al.*, 2002; Fiddler *et al.*, 1981).

Ganghwa mugwort (*Artemisia princeps* Pamp.) is a perennial plant 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, B₁, B₂ and C as well as various minerals (Hwang *et al.*, 2013).

Even though several studies have until now focused on the application of antioxidant combination to inhibit lipid oxidation in various meat products, scientific literature on the residual nitrite contents of sausage added with antioxidant combination is still insufficient. Thus, the objective of

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this study was to investigate the effects of two different antioxidants, a *ganghwa* mugwort (GM), ascorbic acid (AC) and their combinations in pork sausage as measured by pH, total color difference (ΔE), hue angle (H°), thiobarbituric acid reactive substances (TBARS) value, and residual nitrite contents during 28 d at 4°C.

Materials and Methods

Preparation of the *ganghwa* mugwort (GM)

Commercial samples of dried *ganghwa* mugwort were purchased from a local market on *Ganghwa* Island. After separating the leaves from the dried *ganghwa* mugwort, they were ground using a blender (KA-2610, Jworld Tech, Korea) for 1 min. Ten g 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 (Whatman International, UK) and evaporated with a rotary evaporator (EYELA N-1000, Rikakikai, Japan) at < 50°C. The products represented the GM.

Preparation of the antioxidant

The antioxidant of ascorbic acid (AC: Sewoo Inc, Korea) and *ganghwa* mugwort extract (GM: pH, 6.12±0.02; L*-value, 29.21±0.05; a*-value, 1.76±0.33; b*-value, -0.77±0.25) was prepared according to the formulations: Control (no antioxidant added), AC (0.05% AC), GM 0.1 (0.1% GM), GM 0.2 (0.2% GM), AC+GM 0.1 (0.05% AC + 0.1% GM) and AC+GM 0.2 (0.05% AC + 0.2% GM).

Preparation of pork sausage

Fresh pork ham and pork back fat were initially ground through an 8-mm plate. Six batches consisted of differing in composition with respect to the addition of antioxidant combination (see above section preparation of the antioxidant). For each batch of emulsion sausage, pork meat (60%), pork back fat (20%), and ice (20%), sodium chloride (NaCl, 1.5%), sodium tripolyphosphate (0.3%), and sodium nitrite (NaNO₂, 150 ppm), antioxidant (see above section preparation of the antioxidant) were emulsified using silent cutter (Cutter Nr-963009, Hermann Scharfen GmbH & Co, Germany), and then the each batter was stuffed into collagen casings (#240, NIPPI Inc., Japan; approximate diameter, 25 mm) using stuffer (Stuffer IS-8, Sirman, Italy). The pork sausage was heated at 75±1°C (central temperature) for 30 min in a smoke chamber. After heated, the cooked pork sausages were packaged refrigerated at 4°C and used for further analysis after 0, 7, 14, 21, and 28 d.

pH

The pH of 5 g samples blended with 20 mL distilled water for 60 s in a homogenizer (Ultra-Turrax T25, Janke and Kunkel, Germany) was determined with a pH meter (Model 340, Mettler-Toledo GmbH, Switzerland).

Color

Color changes in the sausage during storage were monitored with a colorimeter (Chroma meter CR-210, Minolta, Japan) 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 (ΔE), 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°) was calculated with the following formula: (H° ; $\tan^{-1}(b^*/a^*)$). Hue angle was estimated as described by Hunt *et al.* (1991). Color readings were measured on five randomly chosen spots on the pork sausages and were utilized as an estimate of meat discoloration.

Thiobarbituric acid reactive substances (TBARS) values

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 (KMK-73, Shin-Etsu Silicone Co., Ltd., Korea). 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 (Optizen 2120 UV plus, Mecasys Co. Ltd., Korea).

Residual nitrite contents

Residual nitrite content was determined according to the

Diazo coupling method (KFDA, 2013) and is expressed as ppm 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 (Whatman International, UK). 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 (Optizen 2120 UV plus, Mecasys Co. Ltd., Korea). The residual nitrite content was calculated by a standard curve using nitrite solution.

Statistical analysis

Statistical analysis and comparisons among means were carried out using the statistical package SPSS 18.0 (SPSS Inc., USA). All data were analysed by ANOVA to test the effects of two fixed factors: storage periods (0, 7, 14, 21, and 28 d) and sausage type (Control, AC, GM0.1, GM0.2, AC+GM0.1, AC+GM0.2). The Duncan's multiple range was applied for comparisons of means, differences were considered significant at $p < 0.05$.

Results and Discussion

pH

Addition of *ganghwa* mugwort and/or ascorbic acid in pork sausage did not significantly affect the pH values, whereas a significant effect from the storage time was no-

ted (Table 1). These results agree with the study of Aksu and Kaya (2005), who obtained similar results when the pH values are not significantly influenced by antioxidant addition. Mansour and Khalil (2000) showed no difference in pH between controls and antioxidant-added beef patties during chilled storage. Conversely, other studies have demonstrated that ground beef patties containing 0.1% ascorbic acid decreased meat pH by about 0.2 units. Ozer and Sariçoban (2010) indicated that chicken patties treated with ascorbic acid (300 mg/kg) have a lower pH value than that of the control sample and those containing BHA and α -tocopherol. In our study, the samples containing 0.05% ascorbic acid had no effects on the pH values of pork sausage during storage. These results are in accordance with the finding of Li *et al.* (2013) who showed that the pH values of dry cured sausage were not significantly affected by adding ascorbic acid (300 mg/kg) during 28 d. The pH values of control and all treated samples slightly decreased after storage 14 d ($p < 0.05$). According to Kim (2011), the pH values of sausage treated with mugwort extract decrease during storage. A study performed by Fernández-López *et al.* (2007) proposed that the pH values can be affected by many factors; however, the production of lactic acid by lactic acid bacterial growth is the major reason responsible for the pH decrease in packaged meats.

Color

Food processing industries widely utilize various methods to assess color when determining the commercial acceptability of final products. Changes in the total color difference (ΔE), and the hue angle (H° : 90°=yellow, 180°=green, and 0°=red) values of pork sausage during storage were showed in Table 2-3, respectively. The ΔE and H° values of the sausage samples indicated significant differences in relation to the level of GM because the yel-

Table 1. The pH values during storage periods of pork sausage containing *ganghwa* mugwort (GM) either alone or with ascorbic acid (AC)

Storage time (days)	Control ¹	AC	GM 0.1	GM 0.2	AC+GM 0.1	AC+GM 0.2
0	6.15±0.08 ^b	6.15±0.11 ^b	6.19±0.08 ^b	6.22±0.04 ^b	6.21±0.08 ^b	6.20±0.10 ^b
7	6.14±0.04 ^b	6.13±0.03 ^b	6.16±0.05 ^b	6.17±0.05 ^b	6.16±0.04 ^b	6.17±0.04 ^b
14	6.08±0.03 ^b	6.06±0.02 ^b	6.09±0.07 ^b	6.10±0.04 ^b	6.07±0.10 ^b	6.09±0.04 ^b
21	6.07±0.05 ^a	6.03±0.06 ^a	6.08±0.06 ^a	6.09±0.08 ^a	6.07±0.08 ^a	6.09±0.07 ^a
28	6.03±0.08 ^a	6.04±0.03 ^a	6.05±0.04 ^a	6.06±0.07 ^a	6.04±0.06 ^a	6.05±0.07 ^a

All values are mean±SD of the three replicates.

^{a-b}Means sharing different letters in the same column are significantly different ($p < 0.05$).

¹Control, no antioxidant; AC: pork sausage containing AC 0.05%, GM 0.1: pork sausage containing GM 0.1%, GM 0.2: pork sausage containing GM 0.2%, AC+GM 0.1: pork sausage containing AC 0.05% and GM 0.1%, AC+GM 0.2: pork sausage containing AC 0.05% and GM 0.2%.

Table 2. The ΔE (total color difference) values during storage periods of pork sausage containing *ganghwa* mugwort (GM) either alone or with ascorbic acid (AC)

Storage time (days)	Control	AC	GM 0.1	GM 0.2	AC+GM 0.1	AC+GM 0.2
0	25.23±0.55 ^{Ba}	24.27±0.28 ^{Aa}	26.47±0.37 ^{Ca}	28.38±1.14 ^{Ea}	26.08±0.87 ^{Ca}	27.36±0.80 ^{Da}
7	25.22±0.53 ^{Aa}	24.58±0.43 ^{Aa}	26.50±0.75 ^{Ba}	28.31±0.72 ^{Ca}	26.89±0.65 ^{Bb}	28.22±0.73 ^{Cb}
14	25.95±0.64 ^{Bb}	25.41±0.41 ^{Ab}	27.26±0.93 ^{Cb}	29.37±0.89 ^{Eb}	26.95±0.68 ^{Cb}	28.66±0.49 ^{Db}
21	26.27±0.38 ^{Bb}	25.56±0.71 ^{Ab}	28.32±0.95 ^{Cc}	29.64±0.63 ^{Db}	27.91±0.58 ^{Cc}	29.52±0.70 ^{Dc}
28	26.85±0.88 ^{Bc}	25.66±0.79 ^{Ab}	28.80±0.88 ^{Cc}	29.95±0.66 ^{Db}	28.23±0.64 ^{Cc}	30.08±0.87 ^{Dc}

All values are mean±SD of the three replicates.

^{A-E}Means sharing different letters in the same row are significantly different ($p<0.05$).

^{a-c}Means sharing different letters in the same column are significantly different ($p<0.05$).

Treatments are the same as in Table 1.

Table 3. The H° (hue angle) values during storage periods of pork sausage containing *ganghwa* mugwort (GM) either alone or with ascorbic acid (AC)

Storage time (days)	Control	AC	GM 0.1	GM 0.2	AC+GM 0.1	AC+GM 0.2
0	45.48±1.98 ^B	41.43±2.00 ^A	61.83±1.41 ^D	70.21±1.98 ^F	58.74±1.27 ^C	66.83±0.60 ^E
7	46.00±1.78 ^B	41.64±1.08 ^A	60.97±2.15 ^D	69.56±0.82 ^F	58.47±0.83 ^C	66.98±0.71 ^E
14	45.81±1.62 ^B	40.96±1.50 ^A	62.03±1.98 ^D	69.53±2.10 ^F	59.15±1.41 ^C	67.37±0.66 ^E
21	46.00±1.09 ^B	41.86±0.63 ^A	62.41±1.04 ^D	70.12±1.08 ^F	59.06±1.00 ^C	67.28±0.42 ^E
28	45.17±1.74 ^B	40.70±1.07 ^A	61.36±2.17 ^D	70.87±0.98 ^F	59.40±1.36 ^C	67.29±0.86 ^E

All values are mean±SD of the three replicates.

^{A-F}Means sharing different letters in the same row are significantly different ($p<0.05$).

Treatments are the same as in Table 1.

lowness values increased with increasing the GM amount, whereas the lightness and redness values decreased with increasing the GM content (lightness, redness and yellowness data not shown). The initial ΔE values indices of the control and all treated samples ranged from 24.27-28.38 with counts slightly increasing from 25.66-30.08 after 28 d (Table 2). The H° values of all samples ranged from 41.43-70.21 and did not have a significant effect on the storage (Table 3). Among all treatments, the GM 0.2 (either alone or with AC) had the greatest effect on the ΔE and H° values of the sausage samples; this result could be attributed to the brownish color of the GM. These results were similar to those of previous researches which showed that replacing fat by peach dietary fiber in frankfurters increased the ΔE values (Grigelmo-Miguel *et al.*, 1999). According to Choi *et al.* (2011), pork batter treated with rice bran fiber showed higher H° values with increasing rice bran fiber concentration than that in the control. Rodríguez-Carpena *et al.* (2012) reported that higher hue angle values (more green color) were correlated with higher concentrations of chlorophylls and carotenoids, which contribute to the typical green color of the derived products. Mitsumoto *et al.* (2005) showed that tea catechins were caused discoloration after the addition in chicken patties. Additionally, Karre *et al.* (2013) rec-

ommended that some natural ingredient such as plum products, grape seed extract, pine bark extract, rosemary, and spices all have been shown to affect the color of finished meat products.

TBARS

Table 4 shows that TBARS was significantly affected ($p<0.05$) by storage time and the addition of GM (either alone or with AC). However, 0, 7, and 14 d of storage, no remarkable difference was detected between the control and all treatments. TBARS production of samples treated with GM 0.1 was not much different from that of the control. In contrast to the current results, previous researches showed that treatments containing GM 0.05% and 0.1% indicate significantly lower TBARS values than the control in chicken samples during storage for 12 d (Hwang *et al.*, 2013). This trend may be due to the antioxidant activity of nitrite which plays a role in the prevention of lipid oxidation of pork sausage and inhibits oxidative stress. Additionally, Kim (2011) reported that emulsified pork sausage treated with different types of mugwort (powder, juice, and ethanol extract) significantly inhibits lipid oxidation during 28 d of storage since mugwort contains many ingredients with antioxidant and antimicrobial effects such as polyphenols.

In our study, the combination of GM and AC was effective in inhibiting lipid oxidation in pork sausage. These results are in agreement with studies by Ismail *et al.* (2008) who reported that ground beef added mixture of ascorbic acid and α -tocopherol prevented lipid oxidation the most, as evidenced by the lowest TBARS values compared to that in the other treatments. A study performed by Nam and Ahn (2003) suggested that the use of ascorbic acid combined with α -tocopherol was more beneficial in controlling lipid oxidation than the use of any single antioxidant in ground beef during storage. The synergistic effects of ascorbic acid when used in combination with other antioxidants in meat have been reported by several researchers, who proposed that the synergism might result from the action of mixed free radical scavengers or the combined action of free radical scavengers and metal chelators (Ahn *et al.*, 2004; Calvert and Decker, 1992; Djenane *et al.*, 2002).

Residual nitrite contents

The initial contents of residual nitrites in control and all treatments ranged from 49.46-57.21 with counts markedly decreasing from 25.93-41.96 ppm after 28 d (Table 5). These results confirmed the findings of Li *et al.* (2013), who reported that residual nitrite contents can be

detected approximately 50-70% of the added nitrite in the meat product immediately after manufacturing process because when nitrite is added in the meat product, it reacts with myoglobin, sulphhydryl groups, lipids and proteins. Also, residual nitrite contents declined further during storage due to oxidization to nitric acid (HNO_3) or transformation to gaseous forms (N_2O and N_2). The samples added with antioxidant treatment reduced the residual nitrite contents of pork sausage; in particular, residual nitrite contents were decreased by the combination of GM and AC. These results suggest that GM and AC in pork sausages have antioxidant properties that decrease residual nitrite. As reported by Li *et al.* (2013), reductions in residual nitrite contents were probably due to polyphenols and flavonoids, which react with the various bio-compounds present in the plant extract. Kim (2011) also observed in emulsion pork sausage, where mugwort reduced residual nitrite contents during storage periods.

The sausages containing ascorbic acid had lower nitrite contents than those of the control and samples treated with GM alone ($p < 0.05$) at each of the storage periods investigated. These data agree with those of Li *et al.* (2013), who reported that the presence of ascorbic acid makes nitrite to more quickly reduce nitrite oxide than plant polyphenols. Ahn *et al.* (2004) reported that ascorbic acid in cured

Table 4. The TBARS values (mg MA/kg) during storage periods of pork sausage containing *ganghwa* mugwort (GM) either alone or with ascorbic acid (AC)

Storage time (days)	Control	AC	GM 0.1	GM 0.2	AC+GM 0.1	AC+GM 0.2
0	0.15±0.02 ^a	0.15±0.04 ^a	0.15±0.03 ^a	0.14±0.02 ^a	0.14±0.02 ^a	0.13±0.02 ^a
7	0.19±0.02 ^b	0.19±0.02 ^b	0.19±0.03 ^b	0.19±0.02 ^b	0.18±0.02 ^b	0.17±0.02 ^b
14	0.23±0.03 ^c	0.23±0.02 ^c	0.22±0.02 ^c	0.21±0.03 ^{bc}	0.21±0.02 ^{bc}	0.21±0.02 ^c
21	0.23±0.03 ^{Bc}	0.23±0.04 ^{Bc}	0.23±0.04 ^{Bc}	0.22±0.02 ^{ABc}	0.20±0.02 ^{Ac}	0.20±0.02 ^{Ac}
28	0.29±0.04 ^{Bd}	0.28±0.02 ^{Bd}	0.28±0.02 ^{Bd}	0.25±0.02 ^{Ad}	0.24±0.02 ^{Ad}	0.25±0.02 ^{Ad}

All values are mean±SD of the three replicates.

^{A-B}Means sharing different letters in the same row are significantly different ($p < 0.05$).

^{a-d}Means sharing different letters in the same column are significantly different ($p < 0.05$).

Treatments are the same as in Table 1.

Table 5. The residual nitrite contents (ppm) during storage periods of pork sausage containing *ganghwa* mugwort (GM) either alone or with ascorbic acid (AC)

Storage time (days)	Control	AC	GM 0.1	GM 0.2	AC+GM 0.1	AC+GM 0.2
0	57.21±0.64 ^{Fe}	52.44±0.96 ^{Ce}	56.29±0.61 ^{Ec}	55.34±0.34 ^{Dc}	50.69±0.49 ^{Bc}	49.36±0.42 ^{Ae}
7	56.24±0.64 ^{Fd}	51.01±0.74 ^{Cd}	55.26±0.58 ^{Ed}	54.31±0.49 ^{Dd}	49.65±0.47 ^{Bd}	48.33±0.39 ^{Ad}
14	50.28±0.54 ^{Fc}	42.29±0.70 ^{Cc}	49.53±0.32 ^{Ec}	47.11±0.79 ^{Dc}	40.62±0.54 ^{Bc}	37.19±0.75 ^{Ac}
21	46.45±0.43 ^{Fb}	36.54±0.72 ^{Cb}	45.60±0.74 ^{Eb}	42.52±0.51 ^{Db}	34.14±0.45 ^{Bb}	30.02±0.36 ^{Ab}
28	41.96±0.78 ^{Fa}	30.90±0.78 ^{Ca}	41.57±0.64 ^{Ea}	37.19±0.47 ^{Da}	28.06±0.78 ^{Ba}	25.93±0.18 ^{Aa}

All values are mean±SD of the three replicates.

^{A-F}Means sharing different letters in the same row are significantly different ($p < 0.05$).

^{a-e}Means sharing different letters in the same column are significantly different ($p < 0.05$).

Treatments are the same as in Table 1.

meat performs as an effective reducing agent for nitrite scavenger. Our result indicates that the antioxidant combination of GM and AC will be more effective for reducing the residual nitrite level in pork sausage. The nitrite in meat products is a primary problem in formation of carcinogenic volatile N-nitrosamines. In order to reduce the intake of nitrite in meat products, the application of antioxidant combination could be a suitable alternative method to reduce residual nitrite contents in meat products. (Li *et al.*, 2013)

Conclusion

The present study showed that the antioxidant *ganghwa* mugwort (GM), ascorbic acid (AC) and their combination did not significantly influence pH, whereas the total color difference (ΔE) and the hue angle (H°) values increased significantly with increasing the GM (either alone or with AC) level. Among the all treatments, the mixture of 0.05% ascorbic acid and 0.2% *ganghwa* mugwort was effective in reducing TBARS and residual nitrite contents values compared to control. The antioxidant combination seems to be more attractive since it inhibits lipid oxidation and decreases the residual nitrites level during storage periods. Additional studies on various natural ingredients would be of great interest for studies about meat products due to the potential harmful effects of artificial additives, especially if combined with *ganghwa* mugwort, which is capable of reducing the residual nitrite contents.

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