ANTIOXIDANT ACTION OF *GANGHWAYAKSSUK* (*ARTEMISIA PRINCEPS* PAMP.) IN COMBINATION WITH ASCORBIC ACID TO INCREASE THE OXIDATIVE STABILITY IN RAW CHICKEN NUGGETS

Hwang K. E.[1], Choe J. H. [1], Kim H. W.[1], Song D. H.[1], Choi Y. S[2] and Kim C. J.[1]

[1] Department of Food Science and Biotechnology of Animal Resources, Konkuk University, Seoul, South Korea;
[2]Food and Biological Resources Examination Division, Korean Intellectual Property Office, Daejeon, South Korea

Abstract – Raw chicken nuggets containing various levels of *Ganghwayakssuk* ethanolic extract (GE) in combination with ascorbic acid (Aa) were evaluated for oxidative stability during refrigerated storage (4°C) by measuring pH, peroxide value (POV), conjugated dienes (CD), thiobarbituric acid reactive substances (TBARS). The pH values of raw samples were significantly affected by the addition of GE (p<0.05). The antioxidant combination of Aa + GE demonstrated a definite beneficial reduction in lipid oxidation in raw samples. Aa + GE 0.1 was most effective in inhibiting lipid oxidation (POV, CD and TBARS) of raw chicken nuggets. The results suggest that the Aa + GE combination has strong antioxidant activity when added to raw chicken nuggets.

Key Words – antioxidant effects, *Ganghwayakssuk* (*Artemisia princeps* Pamp.), ascorbic acid.

I. INTRODUCTION

Increasing consumer demand for functional and safe food has focused attention on lipid oxidation of food as an important quality characteristic that influences color, odor and nutritional value, and so consumer acceptability of the final products [1]. In particular, chicken meat tends to oxidize because of relatively high levels of unsaturated fatty acids and natural antioxidants, such as tocopherol, are low [2]. Consequently, the food industry has continuously sought to develop new formulations designed to extend shelf life and improve food safety. Utilization of synthetic antioxidants has been limited because of their health risks, toxicity and carcinogenic effects [3]. For these reasons, the replacement of synthetic antioxidants with natural antioxidants has become a priority. Among the natural antioxidants, *Ganghwayakssuk* (*Artemisia princeps* Pamp) commonly known as mugwort, is one of the medicinal plants, mainly cultivated in Ganghwa Island, Korea. *Ganghwayakssuk*, which is distributed on Ganghwa Island, has more polyphenol, eupatilin and flavonoids than plants in other regions [4]. The plant contains bioactive compounds such as phenolics, alkaloids, vitamins A, B_1, B_2, and C as well as various minerals [5]. Ascorbic acid (vitamin C) has been also used for increasing the shelf-life of meat and meat products. However, ascorbic acid either promotes or inhibits lipid oxidation reactions in meat products, depending on its concentration [6]. Also, ascorbic acid acts as a synergist when used in combination with other antioxidants by promoting their antioxidant effects.

The purpose of this study was to evaluate the synergistic effect of the natural antioxidants available in *Ganghwayakssuk* in combination with ascorbic acid on the inhibition of lipid oxidation.

II. MATERIALS AND METHODS

2.1. Preparation of Ganghwayakssuk ethanolic extracts (GE)

Ten grams of ground leaves were extracted with 200 mL of 50% ethanol overnight in a shaker at room temperature. The extracts were filtered through filter paper and evaporated with a rotary evaporator (EYELA N-1000, Rikakikai, Tokyo, Japan) at < 50°C.

2.2. Raw chicken nugget preparation and processing

Fresh chicken breast meat (Muscularis pectoralis) and chicken skin were purchased from a local market. The chicken materials were initially ground through an 8-mm plate. The first step for the chicken nugget was to prepare the binder meat. Chicken breast meat (17%) was

homogenized and ground in a silent cutter (Nr-963009, Scharfen, Witten, Germany). A mixture of 1.5% NaCl, 0.3% sodium tripolyphosphate, and 3% ice were mixed with the chicken breast meat and then added to chicken skin. After emulsification of the binder meat, each portion of ground chicken breast meat was mixed with binder meat and *Ganghwayakssuk* ethanolic extracts was added (w/w) according to the following formulations: Control (no antioxidant added), Aa (0.05% ascorbic acid), GE 0.2 (0.2% *Ganghwa-yakssuk* ethanolic extract), Aa + GE 0.01 (0.05% ascorbic acid + 0.01% *Ganghwayakssuk* ethanolic extract), Aa + GE 0.05 (0.05% ascorbic acid + 0.05% *Ganghwayakssuk* ethanolic extract) and Aa + GE 0.1 (0.05% ascorbic acid + 0.1% *Gang-hwayakssuk* ethanolic extract). Raw chicken nuggets were packaged refrigerated at 4°C and used for further analysis at 0, 3, 7, 12 and 15 days.

2.3. 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, Staufen, Germany) was determined with a pH meter (Model 340, Mettler-Toledo GmbH, Schwerzenbach, Switzer-land).

2.4. Lipid extraction for peroxide value (POV) and conjugated dienes (CD)

Lipid extraction was conducted as previously described (Folch et al., 1957) using a chloroform: methanol solvent system (2:1).

2.5. Peroxide values (POV)

Lipid extracted from the sample was determined by AOAC methods (2007). The lipid sample (0.5 g) was treated with 25 mL of solvent mixture (acetic acid:chloroform mixture; 3:2). The mixture was shaken thoroughly and 1 mL of saturated potassium iodide solution was added. The mixture was kept in the dark for 10 min, 30 mL of distilled water was added, and the mixture was mixed. One milliliter of starch solution (1%, w/v) was added as an indicator. POV was determined by titrating the iodine liberated from potassium iodide with standardized 0.01 N sodium thiosulfate solutions.

2.6. Conjugated dienes (CD)

CD were determined using a modified method adapted from Juntachote et al. (2006). Extracted sample lipids (0.015 g) were massed into a 25 mL volumetric flask, brought to volume with iso-octane and mixed. The CD concentration was calculated using a 25,000 M^{-1} cm^{-1} molar extinction coefficient. The results are expressed as μmol per mg meat lipid sample.

2.7. Thiobarbituric acid-reactive substances (TBARS)

Lipid oxidation was assessed using the TBARS method of Tarladgis, Watts, Younthan, & Dugan (1960) with minor modifications. Absorbances were measured using a UV/VIS spectrophotometer (Optizen 2120 UV plus, Mecasys Co. Ltd., Seoul, South Korea) at 538 nm against a blank prepared with 5 mL distilled water and 5 mL TBA reagent. TBARS values were calculated from a standard curve (8-50 nmol) of malondialdehyde (MDA), which was freshly prepared by acidification of 1,1,3,3-tetraethoxy propane (TEP). Reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA). TBARS levels were calculated as mg MDA/kg samples.

2.8. Statistical analysis

An analysis of variance was performed on all the variables measured using the general linear model (GLM) procedure of the SAS statistical package (SAS, Cary, NC, USA) (2008). Duncan's multiple range tests ($p<0.05$) was used to determine differences between treatment means.

III. RESULTS AND DISCUSSION

Fig.1 shows the pH values of raw chicken nuggets formulated with various levels of GE in combination with Aa and then subjected to refrigerated storage for 15 days. The pH values of raw chicken nuggets ranged from 6.15-6.32. The pH values of control and all treated samples slight decreased as storage progressed. At the end of storage, the pH values of the raw chicken nugget were higher in GE 0.2 than in the other treatments and control.

Since the primary lipid oxidation products are hydroperoxides, it seemed reasonable to determine the concentration of peroxide in the meat and meat products to deduce the level of oxidation. The POV of raw chicken nuggets containing various GE levels in combination with Aa stored for 0, 3,

7, 12 and 15 days are given in Table 1. The raw samples presented a maximum POV after 7 days storage at 4°C, which was followed by a decline. On the other hand, the POV of the samples with GE 0.2, Aa + GE 0.05 and Aa + GE 0.1 increased during storage.

CD was evaluated based on the hydroperoxides formed in the extracted lipids of the raw chicken nugget. Presently, the concentration of CD on raw chicken nuggets increased significantly (p<0.05) for control and all treatments with the exception of the samples GE 0.2, Aa + GE 0.05 and Aa + GE 0.1 during the first 12 days of storage and then decreased until the end of storage (Table 2).

The malondialdehyde (MDA) contents in raw chicken nuggets containing various GE levels in combination with Aa stored for 0, 3, 7, 12, and 15 days is shown in Table 3. TBARS values represent the content of secondary lipid oxidation products, mainly aldehydes and carbonyls of hydrocarbons, that contribute to off-flavors and flavors in meat. As the storage period progressed, the TBARS values are increased significantly (p<0.05) in control and all the treated raw samples. However, when antioxidants mixture (GE and Aa) were added, the treated samples resisted lipid oxidation and displayed much lower TBARS values than the control on all days (p<0.05).

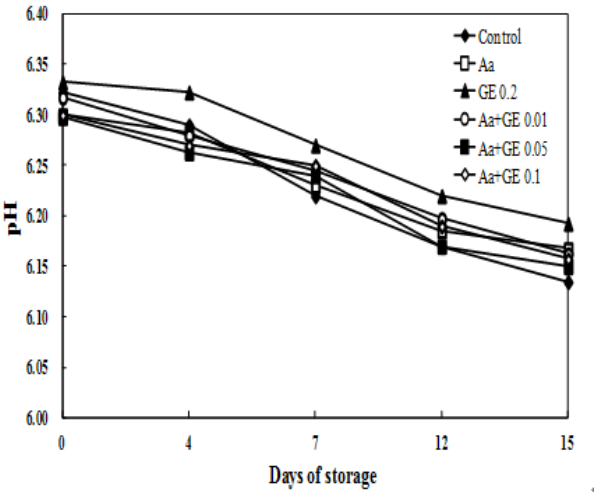

Fig.1. pH values of raw chicken nuggets treated with various amounts of added *Ganghwayakssuk* ethanolic extract (GE) in combination with ascorbic acid (Aa) during refrigerated storage at 4°C.
(◆) Control, no antioxidant, (□) Aa, raw chicken nugget containing ascorbic acid 0.05%, (▲) GE 0.2, raw chicken nuggets containing *Ganghwayakssuk* ethanolic extract 0.2%, (○) Aa + GE 0.01, raw chicken nuggets containing ascorbic acid 0.05% and *Ganghwayakssuk* ethanolic extract 0.01%, (■) Aa + GE 0.05, raw chicken nuggets containing ascorbic acid 0.05% and *Ganghwayakssuk* ethanolic extract 0.05%, (◇) Aa + GE 0.1, raw chicken nuggets containing ascorbic acid 0.05% and *Ganghwayakssuk* ethanolic extract 0.1%.

Table 1. POV (meq of active O_2/ kg meat) in raw chicken nuggets formulated with various amounts of added *Ganghwayakssuk* ethanolic extract (GE) in combination with ascorbic acid (Aa) during refrigerated storage at 4°C.

Treatment[1]	Days of storage				
	0	3	7	12	15
Control	17.20± 0.54^{Ae}	23.20± 0.50^{Ac}	27.02± 0.47^{Aa}	25.33± 0.48^{Ab}	21.15± 0.57^{Dd}
Aa	17.05± 0.50^{Ae}	21.15± 0.60^{Bc}	24.55± 0.46^{Ba}	23.72± 0.56^{Bb}	20.41± 0.49^{Ed}
GE 0.2	13.25± 0.57^{Cd}	19.83± 0.49^{Dc}	20.28± 0.50^{Ec}	21.16± 0.50^{Cb}	24.38± 0.47^{Ba}
Aa+GE 0.01	16.16± 0.68^{Bd}	20.45± 0.55^{Cc}	23.75± 0.56^{Ca}	21.20± 0.42^{Cb}	20.13± 0.47^{Ec}
Aa+GE 0.05	16.08± 0.64^{Bd}	19.92± 0.51^{Dc}	21.38± 0.51^{Db}	20.42± 0.55^{Dc}	25.27± 0.56^{Aa}
Aa+GE 0.1	12.60± 0.28^{De}	19.28± 0.49^{Ed}	20.08± 0.61^{Ec}	21.28± 0.50^{Cb}	23.75± 0.53^{Ca}

All values are mean ± SD of the three replicates.
$^{A–E}$Means sharing different letters in the same column are significantly different (p<0.05).
$^{a–d}$Means sharing different letters in the same row are significantly different (p<0.05).
[1]Treatments are the same as in Fig.1.

Table 2. CD (μmol/mg meat) in raw chicken nuggets formulated with various amounts of added *Ganghwayakssuk* ethanolic extract (GE) in combination with ascorbic acid (Aa) during refrigerated storage at 4°C.

Treatment[1]	Days of storage				
	0	3	7	12	15
Control	0.537± 0.029^{Ac}	0.641± 0.023^{Ab}	0.770± 0.021^{Aa}	0.650± 0.021^{b}	0.520± 0.026^{Dc}
Aa	0.468± 0.018^{Bd}	0.592± 0.036^{Bc}	0.721± 0.017^{Ba}	0.663± 0.030^{b}	0.611± 0.025^{Cc}
GE 0.2	0.425± 0.015^{Ce}	0.512± 0.021^{Cd}	0.624± 0.030^{Cc}	0.669± 0.014^{d}	0.747± 0.030^{ABa}
Aa+GE 0.01	0.452± 0.032^{BCe}	0.568± 0.027^{Bd}	0.699± 0.025^{Ba}	0.659± 0.032^{b}	0.600± 0.023^{Cc}
Aa+GE 0.05	0.469± 0.030^{Be}	0.533± 0.014^{Cd}	0.598± 0.028^{Dc}	0.673± 0.032^{b}	0.766± 0.028^{Aa}
Aa+GE 0.1	0.442± 0.032^{BCe}	0.516± 0.019^{Cd}	0.563± 0.025^{Ec}	0.647± 0.027^{b}	0.738± 0.021^{Ba}

All values are mean ± SD of the three replicates.
$^{A–E}$Means sharing different letters in the same column are significantly different (p<0.05).

[a–d]Means sharing different letters in the same row are significantly different (p<0.05).
[1]Treatments are the same as in Fig.1.

Table 3. TBARS values (mg malondialdehyde/kg meat) in raw chicken nuggets formulated with various amounts of added *Ganghwayakssuk* ethanolic extract (GE) in combination with ascorbic acid (Aa) during refrigerated storage at 4°C.

Treatment[1]	Days of storage				
	0	3	7	12	15
Control	0.29± 0.02^{Ae}	0.38± 0.02^{Ad}	0.44± 0.03^{Ac}	0.54± 0.03^{Ab}	0.59± 0.02^{Aa}
Aa	0.24± 0.02^{Be}	0.34± 0.02^{Bd}	0.41± 0.02^{ABc}	0.50± 0.03^{Bb}	0.56± 0.02^{Ba}
GE 0.2	0.20± 0.01^{Dd}	0.22± 0.02^{Ed}	0.30± 0.03^{DEc}	0.34± 0.02^{CDb}	0.47± 0.02^{Ca}
Aa+GE 0.01	0.22± 0.02^{Ce}	0.30± 0.02^{Cd}	0.36± 0.03^{BCc}	0.48± 0.02^{Bb}	0.57± 0.02^{Ba}
Aa+GE 0.05	0.18± 0.02^{DEe}	0.27± 0.03^{Dd}	0.33± 0.03^{CDc}	0.34± 0.02^{Cb}	0.48± 0.02^{Ca}
Aa+GE 0.1	0.17± 0.01^{Ec}	0.20± 0.02^{Ec}	0.27± 0.03Eb	0.32± 0.02^{Da}	0.34± 0.02^{Da}

All values are mean ± SD of the three replicates.
[A–E]Means sharing different letters in the same column are significantly different (p<0.05).
[a–d]Means sharing different letters in the same row are significantly different (p<0.05).
[1]Treatments are the same as in Table 1.

IV. CONCLUSION

An antioxidant mixture of GE and Aa was highly effective in inhibiting lipid oxidation (POV, CD and TBARS formation) in raw chicken nuggets during refrigerated storage at 4°C. Due to concerns regarding the safety and toxicity of synthetic antioxidants, the combination of *Ganghwayakssuk* ethanol extract and ascorbic acid may prove useful as a safe and natural health promoting antioxidant for the food industry. The best results were gained utilizing raw chicken nuggets with Aa + GE 0.1. These data strongly suggest that GE in combination with Aa act on synergetic effects and may provide reasonable antioxidant source.

ACKNOWLEDGEMENTS

This research was supported (608001-05-2-SB310) by Rural Development Administration (Republic of Korea). The authors also were partially supported by the Brain Korean 21 (BK 21) Project from Ministry of Education and Human Resources Development (Republic of Korea).

REFERENCES

1. Sánchez-Escalante, A., Djenane, D., Torrescano, G., Beltrán, J. A., & Roncales, P. (2003). Antioxidant action of borage, rosemary, oregano, and ascorbic acid in beef patties packaged in modified atmosphere. Journal of Food Science, 68(1), 339-344.
2. Gatellier, P., Gomez, S., Gigaud, V., Berri, C., Bihan-Duval, EL., & Santé-Lhoutellier, V. (2007). Use of a fluorescence front face technique for measurement of lipid oxidation during refrigerated storage of chicken meat. Meat Science, 76(3), 543-547.
3. Buxiang, S., Fukuhara, M., (1997). Effects of co-administration of butylated hydroxytoluene, butylated hydroxyanisole and flavonoids on the activation of mutagens and drug-metabolizing enzymes in mice. Toxicology, 122(1-2), 61-72.
4. Ruy, S. N., Han, S. S., Yang, J. J., Jeong, H. G., Kang, S. S., (2005). Variation of eupatilin and jaceosidin content of mugwort. Korean Journal of Crop Science. 50(5), 204-207.
5. Lee, S. J., Chung, H. Y., Lee, I. K., & Yoo, I. D. (1999). Isolation and identification of flavonoids from ethanol extracts of *Artemisia vularis* and their antioxidant activity. Korean Journal of Food Science and Technology, 31(3), 815-822.
6. Djenane, D., Sanchez-Escalante, A., Beltran, J. A., & Roncales, P. (2001). Extension of retail display life of fresh beef packaged in modified atmosphere by varying lighting conditions. Journal of Food Science, 66(1), 181-186.