INTRODUCTION

Chicken meat provides relatively low fat content and high nutritional value. Moreover, chicken lipids have relatively high levels of unsaturated fatty acids, which are considered a health benefit by consumers. However, higher unsaturated fatty acids content may affect the oxidative stability of chicken meat because the unsaturated fatty acids tend to oxidize easily. Lipid oxidation limits the shelf life of meat and meat products by reducing quality and accelerating deterioration. This may produce changes in meat quality parameters such as color, flavor, odor, texture, and even nutritional value (Bonoli et al., 2007; Nieto et al., 2011).

In an increasingly competitive marketplace, the meat industry is continually searching for natural ingredients to prevent rancidity and maintain the nutritional and quality properties of meat products rather than synthetic food additives, such as butylated hydroxy anisole, butylated hydroxy toluene, propyl gallate, and tertiary butylhydroquinone. The continuous use of such synthetic chemicals may cause health hazards such as teratogenic and carcinogenic effects in laboratory animals and primates. Because of concerns about the toxicological safety of artificial additives, the use of spices and herbs has increased considerably in the last few decades (Hathway, 1966; Branen, 1975).

Ganghwayakssuk (Artemisia princeps Pamp.) commonly known as mugwort, is a medicinal plant, mainly cultivated on Ganghwa Island, Korea (Hwang et al., 2011). Mugwort is widely used as a food or food additive in Korea. It can be found in markets in various forms such as cakes, emulsified sausages, sauces, and noodles (Park and Kim, 2006; Kim and Kim, 2010; Kim, 2011; Oh and Park, 2012). Ganghwayakssuk contains bioactive compounds such as phenolics, alkaloids, and vitamins A, B1, B2, and C as well as various minerals (Hwang et al., 2011).

Ascorbic acid (vitamin C; Aa) is very effective for increasing the shelf life and stabilizing the color of meat and meat products. It has also been used as a meat surface treatment alone or in combination with other antioxidants to stabilize meat color during display. However, Aa either promotes or inhibits lipid oxidation reactions in meat products, depending on its concentration. Ascorbic acid acts as a synergist when used...
in combination with other antioxidants by promoting their antioxidant effects (Djenane et al., 2002).

In the present study, lipid oxidation was investigated in raw chicken patties prepared with 2 different antioxidants, a ganghwayakssuk extract (GE), Aa, and their combinations during 12 d at 4°C.

MATERIALS AND METHODS

Preparation of the GE

In a preliminary study, total phenolic content, 2,2-diphenyl-1-picrylhydrazyl radical scavenging, reducing power, and ferrous iron chelating ability of GE were influenced by ethanol concentration, and the GE extracted with 50% ethanol resulted in the greatest antioxidant effects (Hwang et al., 2011). Therefore, this concentration was used in this study on raw chicken patties. Commercial samples of dried ganghwayakssuk were purchased from a local market on Ganghwa Island. After the leaves were separated from the dried ganghwayakssuk, they were ground using a blender (KA-2610, Jworld Tech, Seoul, South Korea) for 1 min. Ten grams of ground leaves was 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, Maidstone, UK) and evaporated with a rotary evaporator (EYELA N-1000, Rikakikai, Tokyo, Japan) at <50°C. The products represented the GE.

Preparation of the Antioxidant Combination

An antioxidant combination of Aa (Sewoo Inc., Seoul, South Korea) and GE (pH, 6.06 ± 0.02; L* value, 29.11 ± 0.07; a* value, 1.46 ± 0.21; b* value, −0.80 ± 0.05) was prepared according to the following formulations: control (no antioxidant added), Aa (0.05% Aa), GE 0.05 (0.05% GE), GE 0.1 (0.1% GE), GE 0.2 (0.2% GE), Aa + GE 0.05 (0.05% Aa + 0.05% GE), Aa + GE 0.1 (0.05% Aa + 0.1% GE), and Aa + GE 0.2 (0.05% Aa + 0.2% GE).

Preparation of Raw Chicken Patties

Fresh chicken breasts (pectoralis major) with chicken skin were obtained locally. Chicken carcasses contain roughly 15% skin and 2 to 4% fat. Chicken skin without adipose tissue contains approximately 20 to 30% fat (Sheu and Chen, 2002). In this study, chicken skin by-products were used as fat in the raw chicken patties. The chicken materials were initially ground through an 8-mm plate. The ground tissue was then placed in polyethylene bags, vacuum packaged using a vacuum packaging system (FJ-500XL, Fujee Tech, Seoul, South Korea), and stored at 0°C until required. Suitable amounts of the meat and chicken skin were tempered at 4°C for 24 h before preparing the chicken patties. A process flow diagram for the chicken patty is shown in Figure 1. A portion of the raw chicken patty was packaged as mentioned earlier, stored under refrigeration (4°C), and used for further analysis (0, 3, 7, and 12 d).

Lipid Oxidation

Lipid extraction was conducted according to the method of Folch et al. (1957) using a chloroform:methanol solvent system (2:1). Lipid extracts were evaporated and concentrated with a rotary evaporator (EYELA N-1000, Rikakikai), and the extracted lipids were used for the conjugated diene (CD) and peroxide value (POV) analyses.

The CD values 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 isooctane, and mixed. The absorbance was read at 234 nm with isooctane used as the blank. The CD concentration was calculated using a 25,000 M−1·cm−1 molar extinction coefficient. Results are expressed as micromoles per milligram of meat lipid sample.

Lipids extracted from the sample were determined by the method of AOAC International (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%, wt/vol) was added as an indicator. The POV was determined by titrating the iodine liberated from potassium iodide with standardized 0.01 N sodium thiosulfate solutions. The POV was calculated as follows:

POV (mEq/kg) = (S − B) × F × N × 1,000/W,
where S is the titration amount of the sample, B is the titration amount of the blank, F is the titer of 0.01 N sodium thiosulfate, N is normality of sodium thiosulfate, and W is sample weight (g). The results are expressed as milliequivalents of peroxide O₂ per kg of meat.

Lipid oxidation was assessed in triplicate using the TBA reactive substance (TBARS) method of Tarladgis et al. (1960) with minor modifications. A 10-g sample was blended with 50 mL of 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 of 4 N HCl and several drops of a silicone o/w antifoam agent (KMK-73, Shin-Etsu Silicone Co. Ltd., Seoul, South Korea). The mixture was distilled, and 50 mL of the distillate was collected. Five milliliters of 0.02 M 2-thiobarbituric acid in 90% acetic acid (TBA reagent) was added to a vial containing 5 mL of the distillate and mixed well. The vials were capped and heated in a boiling water bath for 30 min to develop the chromogen and then cooled to room temperature. Absorbance was 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 of distilled water and 5 mL of TBA reagent. The TBARS values were calculated from a standard curve (8–50 nmol) of malondialdehyde (MDA), which was freshly prepared by acidifying 1,1,3,3-tetraethoxy propane. Reagents were obtained from Sigma-Aldrich (St. Louis, MO). The TBARS levels were calculated as milligrams of MDA per kilogram of sample.

**Color Measurement**

Color changes in the patties during storage were monitored with a colorimeter (Chroma meter CR-210, Minolta, Tokyo, 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 chromatic difference between a sample and a white standard reflectance plate, color difference (∆E*), was calculated using the following equation: 

\[
\Delta E^* = [(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2]^{1/2}
\]

where \( L^* \) is the lightness value, \( a^* \) is the redness value, \( b^* \) is the yellowness value, and \( L^*, a^*, b^* \) are the reflectance values of the sample and the standard, respectively. 

Additionally, hue angle (H°) was calculated with the following formula: 

\[
H^\circ = \tan^{-1}\left(\frac{b^*}{a^*}\right)
\]

Hue angle was estimated as described by Hunt et al. (1991). Color readings were measured on 5 randomly chosen spots on the chicken patties and were used as an estimate of meat discoloration.

**Microbial Counts**

To determine the bacterial count in each sample during storage, 25-g samples were aseptically transferred to a sterile stomacher bag containing 225 mL of 0.1% peptone water followed by pummeling the samples in a stomacher (Masticater-Paddle-Blender, IUL Instrument, Barcelona, Spain) for 2 min. The homogenates were serially diluted with 0.1% peptone water. The diluents (1 mL) were then placed in Petri dishes, and 20 mL of plate count agar (Difco, Sparks, MD) was poured over the diluents. After the medium solidified, the plates were incubated at 37°C for 48 h, and colonies that developed on the plates were manually counted.

**Statistical Analysis**

Eight samples were analyzed from each type of raw chicken patties and antioxidant combination tested. Each parameter [color, color difference, H°, CD, POV, TBARS, and total viable count (TVC)] was determined 3 times in each sample. In tables and figure, mean values are shown. Data analysis was carried out with the GLM procedure in the SAS statistical package (SAS Institute Inc., 2010). A 2-way ANOVA test was carried out to determine significant differences among raw chicken patties depending on the type of antioxidant and type of storage. Significant interactions were found, so independent one-way ANOVA tests were carried out on each variable.

**RESULTS AND DISCUSSION**

The results from the CD analysis in raw chicken patties are illustrated in Figure 2. The CD results showed that the control, Aa, and GE 0.05 had significantly increased CD (P < 0.05) during 7 d of refrigeration, which decreased until the end of storage. This was in agreement with previous studies, in which the concentration of CD increased significantly in cooked chicken meat treated with and added as a natural antioxidant combination (oregano, sage, and honey) during 48 h, followed by a decrease thereafter (Sampaio et al., 2012). After 12 d of refrigeration, raw chicken patties treated with Aa + GE 0.2 were the most resistant to oxidation, as evidenced by the lowest value, followed by GE 0.1, GE 0.2, Aa + GE 0.05, and Aa + GE 0.1. The increase in CD formation was observed until the end of storage (12 d). The antioxidant mixture (Aa + GE) retarded lipid oxidation during storage. This effect was expected due to the synergistic effect of Aa with GE. Several researchers have reported the strong antioxidant effect of Aa and other natural antioxidant mixtures (Sánchez-Escalante et al., 2001; Serdaroglu and Yildiz-Turp, 2004). According to Sampaio et al. (2012), the lipid oxidation mechanism suggested by CD formation precedes the TBARS formation during refrigeration of meat samples. However, our results indicate that adding Aa and GE to raw chicken patties increased the resistance to lipid oxidation compared with that in the control, as shown by the lower CD content during storage.

The POV is a useful method to determine the initial process of lipid oxidation, and a product is considered...
rancid when POV of 20 to 40 mEq/kg is reached (Hassan and Fan, 2005). Figure 3 shows the POV results as affected by the various levels of GE in combination with Aa. The trend was similar to the CD measurement in which the strength of antioxidative protection increased with increasing GE concentration in raw samples. The raw samples with control, Aa, and GE 0.01 presented a maximum POV after 7 d of storage at 4°C, which was followed by a decline. Treatments containing GE 0.2, Aa + GE 0.05, Aa + GE 0.1, and Aa + GE 0.2 maintained POV at <20 mEq/kg for 3 d and <40 mEq/kg on d 12. The increase was probably due to the faster rate of formation of new hydroperoxides than degradation of hydroperoxides into secondary oxidation products. Decomposition of hydroperoxides into secondary products increases at a higher rate as lipid oxidation progresses, as compared with the formation of new hydroperoxides, resulting in decreased POV (Teets andWere, 2008). These data agree with those of Hassan and Fan (2005) who reported that the POV of mechanically deboned chicken meat treated with a polyphenol extract from cocoa leaves increases and thereafter decreases with storage time. Similar increases followed by decreases in POV over time were found by Soyer et al. (2010) in chicken meat during frozen storage. In the present study, the antioxidant combination (Aa + GE) appeared to effectively control peroxide levels of raw chicken patties during the entire storage period (P < 0.05). Samples containing Aa + GE 0.2 were more effective in lowering the increase in POV in raw chicken patties than the other treatments (P < 0.05).

In contrast to POV and CD, TBARS measures MDA a secondary product formed by oxidation of unsaturated fatty acid after reacting with TBA to form a pink complex (TBA chromogen) with maximum absorbance at 538 nm (Hassan and Fan, 2005). The result of TBARS analyses is shown in Figure 4. As the storage period progressed, lipid oxidation in the control and all tested samples increased significantly (P < 0.05). However, TBARS values were significantly lower (P < 0.05) at any day of storage in samples containing GE (either alone or with Aa), so TBARS formation was almost totally inhibited. This effect may have depended on the concentration of GE because the highest concentration of antioxidant (GE 0.2 and Aa + GE 0.2) retarded the oxidation process efficiently by maintaining TBARS values during 12 d of storage. These results agree with those of Sáyago-Ayerdi et al. (2009) who found that lipid oxidation was most efficient in 2% grape antioxidant dietary fiber added raw hamburger compared with that in 1% grape antioxidant dietary fiber raw chicken hamburger. Kim (2011) reported that a mugwort-treated emulsified pork sausage significantly inhibits lipid oxidation during 28 d of storage. The antioxidant capacity of the GE extracts could be attributable to the presence of natural antioxidants. Mugwort contains many ingredients with antioxidant and antimicrobial effects such as polyphenols (Kim, 2011). A relationship between phenolic content and antioxidant activity has been described (Velioğlu et al., 1998). Thus, the antioxidant effect of GE may be related to its phenolic constituents. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutral-
izing free radicals, quenching singlet oxygen, or decomposing peroxides. This further inhibits degradation to more active oxidizing forms, such as MDA (Baydar et al., 2004).

According to our results, Aa showed a synergistic antioxidant effect when used in combination with GE. Production of TBARS was slightly inhibited in samples treated with Aa compared with the control. However, the combination of GE and Aa led to effective inhibition of TBARS formation ($P < 0.05$). The TBARS value of the GE 0.2 treatment was significantly similar to the samples with Aa + GE 0.05 ($P < 0.05$). These results suggest that when combined with Aa 0.05%, GE 0.05% showed synergistic effects on lipid oxidation. Serdaroğlu and Yildiz-Turp (2004) showed that Aa functions synergistically during antioxidation when combined with other antioxidants by boosting their antioxidant effects. Those authors noted that Aa (500 ppm) was completely ineffective for preventing lipid oxidation in chicken patties. However, a mixture of Aa (500 ppm) and tocopherol (200 ppm) was the most resistant to lipid oxidation, as evidenced by the lowest TBARS values compared with that in the other treatments. Several authors have suggested that an antioxidant combination would more effectively inhibit lipid oxidation than the use of any single antioxidant, and synergism might result from the action of mixed free radical scavengers or the combined action of free radical scavengers and metal chelators (Calvert and Decker, 1992; Lee et al., 2006).

Colors expressed as $a^*$ values (redness), color difference ($\Delta E$), and $H^*$ (90° = yellow, 180° = green, and 0° = blue) were measured to evaluate the changes in color. Figure 4 shows the changes in TBARS reactive substance values (mg of malondialdehyde/kg of meat) on raw chicken patties formulated with antioxidant combination during refrigerated storage at 4°C. Control: no antioxidant, ($\bullet$) Aa: raw chicken patties containing ascorbic acid (Aa) 0.05%, (▲) GE 0.05: raw chicken patties containing ganghwa-akssuk extracts (GE) 0.05%, (○) GE 0.1: raw chicken patties containing GE 0.1%, (◇) GE 0.2: raw chicken patties containing GE 0.2%, (●) Aa + GE 0.05: raw chicken patties containing Aa 0.05% and GE 0.05%, (▲) Aa + GE 0.1: raw chicken patties containing Aa 0.05% and GE 0.1%, and (●) Aa + GE 0.2: raw chicken patties containing Aa 0.05% and GE 0.2%.

Figure 5. Changes in $a^*$ (A), hue angles (B), and color difference (C) values on raw chicken patties formulated with antioxidant combination during refrigerated storage at 4°C. Control: no antioxidant, ($\bullet$) Aa: raw chicken patties containing ascorbic acid (Aa) 0.05%, (▲) GE 0.05: raw chicken patties containing ganghwa-akssuk extracts (GE) 0.05%, (○) GE 0.1: raw chicken patties containing GE 0.1%, (◇) GE 0.2: raw chicken patties containing GE 0.2%, (●) Aa + GE 0.05: raw chicken patties containing Aa 0.05% and GE 0.05%, (▲) Aa + GE 0.1: raw chicken patties containing Aa 0.05% and GE 0.1%, and (●) Aa + GE 0.2: raw chicken patties containing Aa 0.05% and GE 0.2%.
higher concentrations. This could be attributed to the values in the samples with GE increased slightly at increasing levels of GE (Figure 2C). The $H^\circ$ and $\Delta E$ in chicken samples increased with increasing rice bran fiber level (Choi et al., 2011). Ascorbic acid is very effective for maintaining redness of irradiated ground beef. Additionally, Aa leads to declining myoglobin oxidation and improves the color stability in chicken meat (Ahn and Nam, 2004). The $H^\circ$ values and $\Delta E$ of raw samples showed some significant differences in relation to the amount of GE added (Figure 5B, and C). Higher $H^\circ$ values result in a more brown color (Hunt et al., 1999). The initial $H^\circ$ indices of the control, and all treated samples ranged from 47.79 to 62.12 with counts increasing from 53.52 to 66.00 after 12 d (Figure 5B). Among all samples, formulations with GE had higher $H^\circ$ values compared with that of the control. Similar results were observed in pork batter formulated with rice bran fiber, in which the $H^\circ$ value was higher than that in the control with increasing rice bran fiber level (Choi et al., 2011). As storage time increased, $H^\circ$ values (Figure 5B) of all samples increased slightly (shifted from red to yellow), whereas lightness, redness, and yellowness values decreased during storage ($P < 0.05$). This result agreed with the observation that $H^\circ$ values increased over time, resulting from a decrease in $a^*$ relative to $b^*$, which has been used to follow meat discoloration (Luciano et al., 2009). The $\Delta E$ in chicken samples increased with increasing levels of GE (Figure 2C). The $H^\circ$ and $\Delta E$ values in the samples with GE increased slightly at higher concentrations. This could be attributed to the brownish color of the GE. According to Mitsumoto et al. (2005), discoloration of chicken meat patties occurs after adding natural antioxidants such as tea catechins.

The TVC of control and all treated samples were 3.5 to 3.8 log cfu/g with increasing counts to 5.3 to 6.7 log cfu/g after 12 d (Table 1). Samples treated with GE 0.2 and Aa + GE 0.2 exhibited significantly lower counts during storage ($P < 0.05$). Kim (2011) obtained similar results; microbial populations decreased significantly after adding a 1% mugwort extract compared with a control emulsified sausage ($P < 0.05$). Karabegović et al. (2011) noted that extracts of various mugworts possess antimicrobial activity and that the differences in antimicrobial activity of various mugworts can result in differences in their qualitative and quantitative component, which may be attributed to different growth environments and extraction conditions. Among all samples, treatment with Aa + GE 0.2 delayed TVC growth during the entire storage period ($P < 0.05$). These results agree with the findings of Djenane et al. (2002), who noted a small but significant ($P < 0.05$) inhibitory effect of an antioxidant combination (500 ppm of rosemary extract + 500 ppm of Aa) on psychrotroph growth when applied to the surface of beef steaks. However, in contrast to our results, several researchers have reported that adding Aa dose not inhibit bacterial spoilage of meat and meat products (Sánchez-Escalante et al., 2001; Djenane et al., 2002). This trend of controlling TVC may be due to the synergistic effect of the functional ingredients available in ganghwayakssuk in combination with Aa.

In conclusion, results of the present study demonstrate the positive effects of GE, added individually or in combination with Aa, on retarding lipid oxidation and inhibiting microbial growth of raw chicken patties during refrigeration storage for 12 d. This combination could have commercial use to improve shelf life of raw chicken patties. The Aa + GE 0.2 (0.05% Aa + 0.2% GE) was most effective for delaying lipid oxidati-
tion (CD, POV, and TBARS formation). Because of concerns regarding the safety and toxicity of synthetic antioxidants, the combination of GE and Aa may prove useful as a safe and natural health-promoting antioxidant for the food industry.

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