



Effects of Grapefruit Seed Extract on Oxidative Stability and Quality Properties of Cured Chicken Breast

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Abstract

This study investigated the antioxidative and functional effects of a curing agent containing grapefruit seed extract (GSE) on the quality and storage characteristics of chicken breast. The total polyphenol and total flavonoid contents of GSE were 45.06 mg/g and 36.06 mg/g, respectively. The IC₅₀ value of 2,2-diphenyl-1-picrylhydrazyl hydroxyl scavenging of GSE was 333.33 µg/mL. The chicken breast comprised six groups: no-treatment (N), 0.2% ascorbic acid + 70 ppm sodium nitrite (C), 0.05% GSE (G0.05), 0.1% GSE (G0.1), 0.3% GSE (G0.3), and 0.5% GSE (G0.5). The pH and cooking loss of cured chicken breast decreased with increasing GSE levels, and the water holding capacity increased with increasing GSE levels. The hardness and chewiness of GSE-treated chicken breast were higher than those of N and C. Hunter's L and a color values increased significantly after GSE addition. Moreover, 0.1% GSE (G0.1) increased the flavor and total acceptability scores. The 2-thiobarbituric acid and volatile basic nitrogen values of the 0.5% GSE group decreased significantly compared with those of C group. Total microbial counts of GSE-treated chicken breast were higher than those of C, but that lower than those of N. Adding GSE to chicken breast delayed lipid peroxidation and had antimicrobial effects during cold storage. GSE improved shelf life and palatability; therefore, it could be used as a natural antioxidant and functional curing agent ingredient in meat products.

Keywords grapefruit seed extract, cured chicken breast, antioxidative effect, quality properties, stability characteristics

Introduction

In Korea, meat consumption per person has increased rapidly from 11.3 kg in 1980 to 51.4 kg in 2014. Chicken consumption was 15.4 kg in 2014, which was the second highest after pork; subsequently, the growth in chicken consumption has exceeded that of beef and pork (Korea Meat Trade Association, 2015). While the major type of chicken consumption previously was whole chicken, sales of prime cuts, such as breast meat and wing, have increased recently (Jung *et al.*, 2013). Chicken breast contains 23.3% of protein, which is higher than of beef or pork, and contains about 0.4% of fat and 102 kcal per 100 g (Rural Development Administration, 2011); thus chicken breast is a low-fat and high-protein food compared to other parts of the chicken. Those requiring weight control or a balanced

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diet for muscular strengthening are highly interested in chicken breast, which is also sold as convenience food, such as canned meat or smoked products.

The growth of microorganisms in stored processed meat products leads to deterioration in quality; therefore, food additives are used to improve shelf life and palatability. Widely used food additives for chicken meat and products include sodium chloride, phosphates, ozone, nisin, and sorbic acid (Ko *et al.*, 2005; Lim and Yang, 2014; Muhlisin *et al.*, 2016; Tan and Ockerman, 2006; Thakur *et al.*, 1994). Sorbic acid and its salts have been used widely as preservatives for food manufacturing and processing, livestock feed, drugs, cosmetics, and tobacco because of their antibacterial activities against microorganisms (Thakur *et al.*, 1994). Nisin produced by *Lactococcus lactis* strains also has been reported in extending the shelf life of chicken products (Tan and Ockerman, 2006). Nitrate and nitrite increase the shelf life of products by preventing acidification (Duncan and Foster, 1968); by inactivating *Clostridium botulinum*, the causative microorganism for food poisoning, by inhibiting its toxin production (Johnston *et al.*, 1969); and by preventing lipid peroxidation (Eakes *et al.*, 1975). Despite the regulation of food additives through the Food Sanitation Act, there has been a growing recognition that food additives have negative effects on the human body through interaction with food components (Barnes, 1975; Fiddler *et al.*, 1972). Thus, the use of food additives has been restricted, and various studies have been conducted to substitute synthetic food additives with substances isolated from natural sources that do not harm the human body.

Grapefruit (*Citrus paradisi* Macf.) is a citrus fruit that contains high levels of vitamins, minerals, and dietary fiber. The seeds and peel of grapefruits are rich sources of antioxidative components including flavonoids, vitamin C, carotenoid, citric acid, and limonoid (Vanamala *et al.*, 2006). Grapefruit seed extract (GSE) refers to the material extracted from grapefruit seeds using water, glycerin, and ethyl alcohol. GSE contains vitamin C, tocopherol, and naringin. These components of GSE have antibacterial and antioxidative effects in various foods, and prevent lipid peroxidation and inhibit off-flavors, which improves the freshness and shelf life of foods. In addition, GSE promotes the stabilization of fat-soluble vitamins and colorant materials, without affecting the taste, smell, or colors of foods or livestock feed (Bae, 2002). In addition, GSE, as a natural food preservative, is barely toxic and is non-corrosive. GSE is a natural organic mixture without color

and odor, and is safer than sodium benzoate or potassium sorbate, as shown by its LD₅₀ value of 2,900 mg/kg (Park and Kim, 2006). The addition of natural substances containing various functionalities as sub-ingredients in processed meats is expected to prevent the rancidity of lipid components, a cause of quality deterioration, and to eliminate meat product-specific odors.

Therefore, the objective of this study was to investigate the effects of chicken breast prepared with a curing agent containing various levels of GSE during storage at 4°C on oxidative stability and quality properties.

Materials and Methods

Materials

Grapefruit seed extract (DF-100, QUINABRA-Quimica Natural Brasileira Ltd., Brazil) (Harich, 1985) was purchased from FA Bank Co. (Korea). According to the manufactured, this extract comprised 49.49% GSE, 50% glycerin, and 0.51% naringin (solvent).

Assay of total polyphenol and flavonoid contents of grapefruit seed extract

Total polyphenol content was measured by the Folin-Denis method (1912). A mix of 1 mL of GSE and 2 mL of Folin reagent in a test tube was incubated at room temperature for 3 min, to which 2 mL of 10% Na₂CO₃ was added and mixed, followed by incubation at 30°C for 40 min. Thereafter, the optical density was measured at 760 nm using a UV-spectrophotometer (Shimadzu UV-1601 PC, Japan). A standard curve was made using tannic acid in a series of final concentrations of 0, 6.25, 12.5, 25, 50, and 100 µg/mL and this calibration-curve was used to calculate total polyphenol contents of the samples. Total flavonoid contents were measured by a modified Davis method (Chae *et al.*, 2002). Two mL of diethylene glycol was added to 1 mL of each GSE sample, followed by addition of 20 µL of 1 N NaOH and incubation in a water bath at a 37°C for 1 h; the OD was then measured at 420 nm using a UV-spectrophotometer. A standard curve was made using rutin at final concentrations of 0, 6.25, 12.5, 25, 50, and 100 µg/mL, and the total flavonoid contents were calculated using this calibration curve.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH radical scavenging activity of GSE was measured by the method of Blois (1958). One mL of 0.2

mM DPPH was mixed with 1 mL GSE at each concentration (0.125, 0.25 and 0.5 mg/mL) in a test tube, and incubated at 37°C for 30 min, followed by measurement of the OD at 517 nm using a UV-spectrophotometer (Shimadzu UV-1601PC). As a positive control, the same method was applied to vitamin C, a natural antioxidant, and butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), as synthetic antioxidants, to compare activities. The DPPH radical scavenging activity of GSE was calculated by the equation $(1 - \text{OD of group with sample addition} / \text{OD of group with no sample}) \times 100$.

Sample preparation

A total of 60 broilers (Ross broiler) aged 6 wk (approximately 1.6-1.8 kg live weight) were purchased from a poultry farm affiliated with Cheongam Food Co., (Korea). The birds were stunned and killed by conventional neck cut. Individual carcasses were trimmed for the breast meat (*pectoralis major*) by removing feather, bones, skin and connective tissues. After post-mortem 24 h, the breast meat blended with the curing agent. As shown in Table 1, the curing agent was made by blending water, salt, and sugar, with which sorbic acid, nitrite, or GSE was mixed. The experimental groups were divided into a no-treatment control (N, normal), positive control with 0.2% sorbic acid + 70 ppm sodium nitrite (C, positive control), a 0.05% GSE group (S0.05), a 0.1% GSE group (G0.1), a 0.3% GSE (G0.3), and a 0.5% GSE group (G0.5). Treatment groups with corresponding curing agent within polythene bags were kept at 4°C for 24 h, and then used as the test samples.

Proximate composition

Proximate composition analysis of chicken breast treated with GSE was performed according to the method of the Association of Official Analytical Chemists (AOAC)

(2005). The moisture content was determined according to the 105°C atmospheric heat drying method. The crude protein content was estimated by the micro-Kjeldahl method. The crude fat content was determined according to the soxhlet extraction method, and the crude ash content was determined by AOA method 923.03 determined. Each test was replicated three times for each experimental group.

pH measurement

pH was measured by the Khalil (2000) method, in which a 10-g sample was mixed with 100 mL of distilled water and homogenized with a Stomacher® (400 Lab blender, England) for 30 s, followed by measurement using a pH-meter (WTW pH 720, Germany).

Measurement of water holding capacity (WHC)

The WHC was measured following the method of Laakkonen *et al.* (1970). A 2-mL tube with a tiny hole was weighed, and 0.5±0.05 g sample was added into the tube, followed by measurement of the combined weight of the sample and the tube before heating in an 80°C water bath (HB-205SW, Hanbaek Scientific Co., Korea) for 20 min, which was then cooled to room temperature for 10 min. The sample was centrifuged at 6,710 ×g at 4°C for 10 min, and then the % of the remaining sample weight to that of the sample before heating was calculated.

Measurement of cooking loss

To measure the cooking loss, chicken breast samples (approximately 50 g, 1 cm thick) stuffed into each centrifuge tube and was heated at 75°C using a constant-temperature water bath (HB-205SW, Hanbaek Scientific Co., Korea) for 30 min until the core temperature of the samples reached at 72°C and then the cooked samples were cooled for 30 min. After measuring its weight, the weights before and after heat treatment were compared, and the

Table 1. Formulation of chicken breast meat blending (%)

Ingredients	Treatments ¹⁾					
	N	C	G0.05	G0.1	G0.3	G0.5
Salt	3.33	3.33	3.33	3.33	3.33	3.33
Sugar	0.87	0.87	0.87	0.87	0.87	0.87
Sorbic acid		0.2				
Sodium nitrite		0.007				
Grapefruit seed extract			0.05	0.1	0.3	0.5
Water	95.80	95.593	95.75	95.70	95.50	95.30
Total	100	100	100	100	100	100

¹⁾N: No treatment (normal), C: 0.2% sorbic acid + 70 ppm sodium nitrite (positive control), G0.05: 0.05% grapefruit seed extract, G0.1: 0.1% grapefruit seed extract, G0.3: 0.3% grapefruit seed extract, G0.5: 0.5% grapefruit seed extract.

weight reduction caused by cooking (%) was calculated as the cooking loss.

Texture profile analysis

To measure the texture characteristics of chicken breast, heat-cooked chicken breast was cooled and cut into 1 cm (wide) × 1 cm (long) cubes. Mastication, shear force, and cutting tests were performed using a Rheometer (Compac-100, Sun Scientific Co., Japan), for which the Rheology Data System (RDS Ver 2.01) program was used. Three samples from each treatment group were subjected to three repeated measurements, from which mean values were calculated. The measurement was performed under the following conditions: table speed, 110 mm/min; graph interval, 20 m/s; and load cell, 10 kg (max).

Color measurement

Meat color was measured using a Spectrocolorimeter (Model JX-777, Color Techno. System Co., Japan) that was calibrated using a white board (L, 94.04; a, 0.13; b, -0.51). A cool white fluorescent lamp (D65) was used as the light source. Colors were expressed as the L value for lightness, the a value for redness, and the b value for yellowness, following the color system of Hunter Laboratory. Mean values were calculated after five repeated measurements.

Sensory evaluation

Sensory evaluation was conducted using a trained 10 undergraduate and graduate students studying for food-related degree who were already knowledge about sensory test. Saltiness, tenderness, juiciness, flavor, and overall acceptability were evaluated using a 5-point scale method. Saltiness, flavor, and overall acceptability (1 = extremely undesirable, 5 = extremely desirable), tenderness (1 = extremely tough, 5 = extremely tender), and juiciness (1 = extremely dry, 5 = extremely juicy) of the samplings were evaluated. Samples were cooked by heating to 72°C (temperature inside the chicken breast meat) using pan-frying, and then each sample was cut into 2 cm (wide), 2 cm (long), and 1.5 cm (thick) in samples, which were supplied to the participants on a white plate for evaluation. Drinking water was provided to the participants before evaluating the next sample (Keeton, 1983).

Determination of 2-thiobarbituric acid (TBA) value

The TBA value was obtained using a modified extraction method after Witte *et al.* (1970), in which a 10-g sample was homogenized together with 15 mL of 10% cold per-

chloric acid and 25 mL of deionized water at 10,000 rpm for 10 sec in a homogenizer (AM-Series). The homogenate was filtered through a qualitative filter paper No. 2 (Advantec). Five milliliters of the filtrate and 5 mL of 0.02 M TBA solution were mixed completely, followed by incubation under cool and dark conditions for 16 h. The OD was then measured at 529 nm using a spectrophotometer (DU-650, Beckman, USA). Deionized water was used as a blank. The TBA value was expressed in mg malonaldehyde (MA)/kg. A standard curve was constructed that conformed to the equation: $y = 0.1975x - 0.0011$ ($r = 0.999$), where $y = OD$ and $x = TBA$, which was used to calculate the TBA values of the samples.

Volatile basic nitrogen (VBN) value

The VBN content was measured by the micro-diffusion method using a Conway unit (Short, 1954). A 10-g sample was homogenized with 90 mL distilled water at 10,000 rpm in a homogenizer (AM-Series) for about 30 s, and the homogenate was filtered using a Qualitative filter paper No. 2 (Advantec). The filtrate (1 mL) was added to the outer chamber of the Conway unit, and then 1 mL of 0.01 N boric acid solution and three drops of indicator (0.066% methyl red + 0.066% bromocresol green) were added to the inner chamber. After applying glycerin to the contact part of the lid, the unit was closed with the lid, and 1 mL of 50% K_2CO_3 was injected into the outer chamber, followed by immediate sealing. Thereafter, the unit was stirred horizontally and then incubated at 37°C for 120 min. After incubation, boric the acid solution in the inner chamber was titrated with 0.02 N H_2SO_4 . The VBN value was expressed as mg per 100 g sample (mg%).

Microbiological analysis

Total microbial counts were obtained using a serial dilution method. Ten grams of sample was homogenized with 90 mL of 0.1% peptone solution in a Stomacher® (400 Lab blender, Seward) for 30 s. Serially diluted samples were inoculated on plate count agar media and cultured at 37°C for 48 h (Short, 1954), followed by counting using a colony counter. The total microbial count was expressed as log colony forming units (CFU)/g.

Statistical analysis

In the statistical analysis, variance analysis of the data was performed using the GLM (general linear model) procedure of in the SAS program (2002), and a significance test on mean differences of treatment groups was conduc-

Table 2. Contents of total polyphenol and total flavonoid in grapefruit seed extract

	Total polyphenol (mg/g)	Total flavonoid (mg/g)
Grapefruit seed extract ¹⁾	45.06±6.42 ¹⁾	36.06±3.46

¹⁾All values are expressed as mean±SE of triplicate determinations.

Table 3. DPPH radical scavenging activity of grapefruit seed extract

Samples	DPPH radical scavenging activity (%)	IC ₅₀ (µg/mL)
0.125 ¹⁾	9.85±0.40 ⁴⁾⁵⁾	
0.25	37.50±1.13 ^b	333.33±14.12 ^a
0.5	70.83±2.47 ^a	
BHT ²⁾		98.52±2.44 ^b
BHA ³⁾		92.01±4.88 ^b
Vitamin C		58.24±3.28 ^c

¹⁾Grapefruit seed extract: 0.125 ppm (0.125 mg/mL), 0.25 ppm (0.25 mg/mL), and 0.5 ppm (0.5 mg/mL).

²⁾BHT: Butylated hydroxytoluene.

³⁾BHA: Butylated hydroxyanisole.

⁴⁾All values are expressed as mean±SE of triplicate determinations.

⁵⁾Different superscript letters indicate significant differences at $p < 0.05$ by Duncan's multiple range test.

ted at the 5% level using Duncan's multiple range test.

Results and Discussion

Total polyphenol and total flavonoid contents, and DPPH radical scavenging activity of grapefruit seed extract

The total polyphenol and total flavonoid contents of the GSE were 45.06 mg/g and 36.06 mg/g, respectively (Table 2). Oh *et al.* (2003) reported that the total polyphenol and total flavonoid contents in citrus fruits were 20.9-53.1 mg/g and 12-48 mg/g, respectively, which were similar to

those of the GSE used in this study. DPPH radical scavenging activity depending on the GSE concentration and the IC₅₀ value (the sample concentration that removes 50% of DPPH radicals) of GSE are shown in Table 3. The DPPH radical scavenging activities of GSE were 9.85% at 0.125 mg/L, 37.50% at 0.25 mg/L, and 77.83% at 0.5 mg/L, indicating that the DPPH radical scavenging activity increased significantly as the concentration of the extract increased. The IC₅₀ value of GSE was 333.33 µg/mL, whereas those of control groups were 98.52 µg/mL for BHT, 92.01 µg/mL for BHA, and 58.24 µg/mL for vitamin C, showing that the GSE groups had a lower DPPH radical scavenging activity than the control groups. The DPPH radical scavenging activity is attributable to antioxidative materials, such as phenolic compounds and flavonoids, in plant extracts; therefore, the DPPH radical scavenging activity has been used as an indicator of antioxidative activity (Aoshima *et al.*, 2004). Similar to this study, Ahn *et al.*, (2007) reported that citrus fruits had high DPPH radical scavenging activities because of their high total polyphenol content. Thus, GSE, as a natural antioxidant extracted from plant materials, should be useful as a healthy functional food material.

Proximate composition of cured chicken breast prepared with grapefruit seed extract

The proximate analysis of the cured chicken breast meat prepared by addition of a series of GSE concentration is presented in Table 4. The moisture contents of the treatment groups (C, G0.05, G0.1, G0.3, and G0.5) were lower than that of the no-treatment control (N). According to Offer and Trinick (1983), the addition of curing agents such as salt and phosphate increased the osmotic pressure, which leached moisture out of the tissues, resulting in a reduction in moisture content. However, in this study,

Table 4. Proximate composition, pH, WHC and cooking loss of cured chicken breast prepared with different concentrations of grapefruit seed extract

Items	Treatments ¹⁾					
	N	C	G0.05	G0.1	G0.3	G0.5
Moisture (%)	79.52±0.16 ²⁾³⁾	74.26±0.23 ^b	76.51±0.11 ^b	76.01±0.18 ^b	75.25±0.20 ^b	75.06±0.36 ^b
Crude protein (%)	18.60±0.45 ^c	23.22±0.05 ^a	20.79±0.06 ^b	21.09±0.11 ^b	22.34±0.08 ^b	21.90±0.31 ^b
Crude lipid (%)	1.92±0.50 ^a	1.36±0.09 ^c	1.49±0.14 ^b	1.59±0.06 ^b	1.58±0.10 ^b	1.86±0.04 ^b
Crude ash (%)	1.09±0.57 ^{ab}	1.36±0.03 ^a	1.21±0.08 ^{ab}	1.31±0.05 ^{ab}	0.83±0.04 ^b	1.18±0.28 ^{ab}
pH	6.01±0.01 ^c	6.28±0.02 ^a	6.29±0.01 ^a	6.26±0.01 ^a	6.12±0.01 ^b	5.90±0.01 ^c
WHC (%)	64.79±5.14 ^{ab}	67.73±8.03 ^a	65.89±5.69 ^{ab}	53.78±5.00 ^b	62.32±7.06 ^{ab}	55.32±8.07 ^{ab}
Cooking loss (%)	14.61±0.22	14.00±0.91	16.58±2.65	17.70±3.51	18.65±3.99	17.55±0.68

¹⁾Treatments: See the legend of Table 1.

²⁾All values are expressed as mean±SE of triplicate determinations.

³⁾Values with different superscripts in the same row are significantly different ($p < 0.05$) between groups by Duncan's multiple range test.

there were no significant differences between the moisture contents of the treatment groups. Contents of crude protein, crude fat, and crude ash were 18.60-23.22%, 1.36-1.92%, and 0.83-1.36%, respectively, which tended to increase as the GSE concentration increased, although there was no difference among the experimental groups.

pH, water holding capacity (WHC), and cooking loss of cured chicken breast prepared with grapefruit seed extract

pH, WHC, and cooking loss of the cured chicken breast prepared by the addition of a series of GSE concentrations are presented in Table 4. The pH values of the no-treatment control (N), positive control (C), and the groups with GSE (G0.05, G0.1, G0.3, and G0.5) were 5.90, 6.28, 6.24, 6.26, 6.19, and 6.06, respectively, showing that the no-treatment control (N) had the lowest pH. Addition of the curing agent raised the pH significantly, as shown by the higher pH in the positive control (C) compared with that in the no-treatment control (N). As the GSE concentration increased, the pH of the chicken breast tended to decrease; the groups treated with 0.05% GSE (G0.05) and 0.1% GSE (G0.1) showed no significant difference in pH from the positive control (C). This probably reflected the acidity of the GSE (pH 2.53). Meat pH affects meat quality, because the pH affects WHC, meat color, texture, freshness, and shelf-life (Honikel *et al.*, 1986). Son *et al.* (2009), in contrast to our results, reported that a quality evaluation of sliced low fat sausage treated with GSE and sodium lactate found no significant difference in pH among the treatment groups. In this study, the pH levels of the GSE groups were similar to that of the positive control treated with sorbic acid and sodium nitrite (C); thus, GSE is expected to be applicable as a functional curing agent.

The WHC values of no-treatment control (N), positive control (C), and the groups treated with GSE (G0.05, G0.1, G0.3, and G0.5) were 59.78, 66.73, 61.32, 62.32, 64.79, and 65.89%, respectively, showing that the positive control (C) had the highest WHC (Table 4). The WHC tended to increase as the GSE concentration increased; in particular, the group treated with 0.5% GSE (G0.5) had the highest WHC. The WHC of meats and processed meat products is increased by high pH, addition of more salty, processing before rigor mortis, and addition of phosphate (Sadler and Swan, 1997). The WHC is reduced by protein denaturation during cooking of meat products, which compromises their palatability in terms of juiciness, cooking loss, and physical properties. Similar to the results of this

study, Lim *et al.*, (2014) reported that the control group of pork sirloin without a curing agent showed the lowest WHC, and that pork sirloin treated with salt, phosphate, or sodium bicarbonate as curing agents had a higher WHC. In our study, the group treated with 0.5% GSE (G0.5) showed no significant difference in WHC from the positive control treated with sorbic acid and sodium nitrite (C); therefore, GSE, as a functional curing agent ingredient, would improve the WHC in the development of meat products.

Results of the cooking loss assays for the cured chicken breast treated with a series of GSE concentrations are shown in Table 4. The cooking loss rates for the no-treatment control (N), the positive control (C) and the groups treated with GSE (G0.05, G0.1, G0.3, and G0.5) were 18.65, 15.01, 17.70, 17.55, 16.58, and 15.61%, respectively, showing that all groups treated with GSE had lower cooking losses compared with the no-treatment control (N). With the increasing levels of GSE, cooking loss tended to become lower, and the group treated with 0.5% GSE (G0.5) showed the lowest cooking loss. Cooking loss is also a method to measure the WHC of meat, and moisture loss during cooking can affect sensory characteristics, including tenderness and juiciness; therefore, it is recommended to be careful not to lose moisture and fat to raise the quality of meat products (Winger and Fennema, 1976). In this study, the no-treatment control (N) had the lowest pH and the highest cooking loss, whereas the positive control (C) had the highest pH and the lowest cooking loss; thus, we speculated that cooking loss was also affected by pH. In addition, when sorbic acid and sodium nitrite were substituted by GSE, the cooking loss from chicken breast decreased, which suggested that GSE should be applied as a functional curing agent ingredient.

Texture properties of cured chicken breast prepared with grapefruit seed extract

The results of the hardness, springiness, cohesiveness, and chewiness texture properties analysis of the cured chicken breast treated with a series of GSE concentrations are presented in Table 5. For hardness, the groups treated with GSE (G0.05, G0.1, G0.3, and G0.5) tended to be harder than the no-treatment control (N) and the positive control (C), and the group treated with 0.5% GSE (G0.5) was significantly harder than the other treatment groups. For springiness, the group treated with 0.3% GSE (G0.3) was significantly more springy than the other treatment groups. For cohesiveness, there was no significant difference bet-

Table 5. Textural properties of cured chicken breast prepared with different concentrations of grapefruit seed extract

Textural properties	Treatments ¹⁾					
	N	C	G0.05	G0.1	G0.3	G0.5
Hardness (kg)	1.92±0.34 ^{2)bc3)}	1.71±0.12 ^c	2.08±0.24 ^b	2.18±0.13 ^b	2.18±0.22 ^b	2.48±0.13 ^a
Springiness (%)	48.09±4.81 ^b	44.38±3.64 ^c	46.93±5.00 ^b	49.19±1.44 ^b	52.73±2.93 ^a	47.50±8.52 ^b
Cohesiveness (%)	44.02±3.60 ^a	39.26±4.05 ^c	42.94±6.03 ^{ab}	44.50±4.93 ^a	43.46±7.05 ^{ab}	45.81±4.59 ^a
Chewiness (g)	206.72±21.05 ^d	247.84±22.23 ^c	282.17±24.33 ^a	299.00±24.98 ^a	262.87±27.29 ^{ab}	274.74±11.21 ^{ab}

¹⁾Treatments: See the legend of Table 1.

²⁾All values are expressed as mean±SE of triplicate determinations.

³⁾Means in the same row not sharing a common letter are significantly different ($p<0.05$) by Duncan's multiple range test.

Table 6. Changes in Hunter's value of cured chicken breast prepared with different concentrations of grapefruit seed extract

Hunter color	Treatments ¹⁾					
	N	C	G0.05	G0.1	G0.3	G0.5
L	52.17±4.48 ^{2)bc3)}	53.99±1.60 ^c	52.46±4.19 ^c	57.63±5.69 ^{ab}	58.78±2.11 ^a	57.21±3.29 ^{ab}
a	6.49±0.21 ^c	8.16±0.24 ^a	6.66±2.70 ^c	7.23±0.93 ^b	7.99±1.57 ^b	8.07±0.78 ^a
b	1.02±0.32 ^a	3.17±0.64 ^a	-3.44±2.45 ^b	-1.92±1.85 ^b	-0.82±2.14 ^b	-1.10±0.77 ^b

¹⁾Treatments: See the legend of Table 1.

²⁾All values are expressed as mean±SE of triplicate determinations.

³⁾Values with different superscripts in the same row are significantly different ($p<0.05$) between groups by Duncan's multiple range test.

ween the groups treated with GSE (G0.05, G0.1, G0.3, and G0.5) and the no-treatment control (N); however, GSE addition tended to result in higher cohesiveness. For chewiness, the GSE-treated groups were significantly more chewy than the no-treatment control (N), and the group treated with 0.1% GSE (G0.1) showed the highest chewiness. Texture is a food property that affects consumers' opinions on palatability and preference significantly. Textural characteristics of meat products depend on various factors, including the fat or moisture content, the condition of the raw meat, the type of additives, and the level of protein denaturation by heating. Kim and Ahn (2014) reported that a significant difference in chewiness between the group treated with nitrite and the group treated with grapefruit extract accounted for the difference in palatability due to easiness of chewing, because the group with grapefruit extract had a relatively lower pH, making proteins closer to their isoelectric points, and subsequently resulting in a low WHC. In this study, the groups with GSE showed lower pH than the positive control, and, accordingly, they also had a lower WHC. Hardness and chewiness were higher in the group treated with higher GSE concentrations than in those in the no-treatment control and the positive control.

Changes in color of cured chicken breast prepared with grapefruit seed extract

The color measurement results of the cured chicken breast prepared with different GSE concentrations are

presented in Table 6. The L value for lightness tended to increase as the GSE concentration increased in comparison with the no-treatment control (N) and the positive control (C); the group treated with 0.3% GSE (G0.3) had the highest L value. The positive control (C) had the highest a value (redness). The cured chicken breast groups treated with GSE (G0.05, G0.1, G0.3, and G0.5) had higher a values than the no-treatment control (N), while there was no significant difference between the group treated with 0.5% GSE (G0.5) and the positive control (C). The positive control (C) had the highest b value (yellowness). While the groups treated with GSE (G0.05, G0.1, G0.3, and G0.5) had lower b values than the no-treatment control (N); the b value increased as the GSE concentration became higher. Kang *et al.* (2013) reported that it would be more beneficial for production of smoked duck meat if materials such as L-ascorbic acid, which increase the red color of meat indirectly, are used rather than chemical additives, such as nitrite or duck seasoning, which affect the red color directly, because those meats would have a higher chance of being chosen by consumers. In this study, GSE addition increased lightness and redness of the cured chicken breast, which suggested that GSE addition to meat products would have a positive effect on meat color.

Sensory evaluation of cured chicken breast prepared with grapefruit seed extract

The cured chicken breast prepared with different concentration of GSE was cooked by heating, and then sub-

jected to sensory evaluation, including saltiness, tenderness, juiciness, flavor, and overall acceptability (Table 7). The no-treatment control (N) had the lowest values for saltiness and tenderness. The values for saltiness and tenderness were lower in the groups treated with GSE (G0.05, G0.1, G0.3, and G0.5) than in the positive control (C), although there was no significant difference. The no-treatment control (N) had the lowest value for juiciness, and GSE addition significantly increased juiciness; however, there was no significant difference among the groups treated with GSE (G0.05, G0.1, G0.3, and G0.5). The flavor values were higher in the positive control (C) and the groups treated with GSE (G0.05, G0.1, G0.3, and G0.5) compared with that for the no-treatment control (N), among which the group treated with 0.1% GSE (G0.1) showed the highest flavor value. There were no significant differences among the groups treated with the various GSE concentrations. The group treated with 0.1% GSE (G0.1) also showed the highest overall acceptability. The groups treated with GSE (G0.05, G0.1, G0.3, and G0.5) showed higher overall acceptability than the no-treatment control (N), while there was no significant difference depending on the GSE concentration. In summary, GSE addition increased the saltiness, tenderness, juiciness, flavor, and overall acc-

eptability significantly compared with the no-treatment control, and substitution of sorbic acid and sodium nitrite with GSE resulted in no significant differences in saltiness, tenderness, flavor, and overall acceptability. Thus, these results suggested that GSE could be used as an alternative to chemical food additives.

Changes in the TBA value of cured chicken breast prepared with grapefruit seed extract

Changes in lipid peroxidation during storage of the cured chicken breast prepared with a series of GSE concentrations are presented in Table 8. The TBA value measured on the day of production was 0.14-0.23 mg MA/kg, which increased significantly with storage time, reaching 0.29-0.56 mg MA/kg on the 10th day of storage. The TBA value was significantly lower in the groups treated with GSE (G0.05, G0.1, G0.3, and G0.5) on the day of production than in the no-treatment control (N), while there was no significant difference among the GSE groups. The TBA values of all experimental groups tended to increase significant as the storage time increased. On the 10th day of storage, the no-treatment control (N) showed the highest value, while the groups treated with 0.3% GSE (G0.3) and 0.5% GSE (G0.5) showed lower values than the positive

Table 7. Sensory evaluation of cured chicken breast prepared with different concentrations of grapefruit seed extract

Sensory characteristics ²⁾	Treatments ¹⁾					
	N	C	G0.05	G0.1	G0.3	G0.5
Saltiness	2.33±0.57 ^{3) b4)}	3.20±0.50 ^a	3.00±0.41 ^a	3.10±0.54 ^a	3.10±0.74 ^a	3.00±0.70 ^a
Tenderness	2.16±0.28 ^b	3.33±0.28 ^a	3.30±0.44 ^a	3.10±0.22 ^a	3.50±0.50 ^a	3.40±0.41 ^a
Juiciness	2.00±0.00 ^c	3.66±0.28 ^a	3.10±0.22 ^b	3.10±0.41 ^b	3.20±0.27 ^b	3.10±0.22 ^b
Flavor	2.83±0.28 ^b	3.10±0.50 ^{ab}	3.30±0.27 ^a	3.50±0.41 ^a	3.15±0.27 ^a	3.10±0.67 ^{ab}
Overall acceptability	2.46±0.28 ^c	3.25±0.50 ^a	3.40±0.27 ^a	3.50±0.44 ^a	3.35±0.22 ^{ab}	3.20±0.67 ^{ab}

¹⁾Treatments: See the legend of Table 1.

²⁾Traits: saltiness, flavor, and overall acceptability (1 = extremely undesirable, 5 = extremely desirable), tenderness (1 = extremely tough, 5 = extremely tender), and juiciness (1 = extremely dry, 5 = extremely juicy).

³⁾All values are expressed as mean±SE of triplicate determinations.

⁴⁾Means in the same row not sharing a row letter are significantly different (*p*<0.05) by Duncan's multiple range test.

Table 8. Changes of TBA values for cured chicken breast prepared with different concentrations of grapefruit seed extract during 10 d of storage at 5°C

Storage time (d)	Treatments ¹⁾					
	N	C	G0.05	G0.1	G0.3	G0.5
0	0.23±0.01 ^{2) a3)D4)}	0.16±0.01 ^{cD}	0.18±0.01 ^{bD}	0.15±0.01 ^{cD}	0.15±0.02 ^{cD}	0.14±0.01 ^{cD}
3	0.30±0.01 ^{aC}	0.25±0.01 ^{cC}	0.27±0.00 ^{abC}	0.25±0.01 ^{bC}	0.23±0.01 ^{cC}	0.22±0.01 ^{cC}
7	0.43±0.01 ^{aB}	0.29±0.01 ^{bB}	0.31±0.04 ^{bB}	0.30±0.01 ^{bB}	0.28±0.01 ^{bB}	0.26±0.01 ^{cB}
10	0.56±0.09 ^{aA}	0.31±0.01 ^{cA}	0.35±0.01 ^{bA}	0.32±0.01 ^{bA}	0.30±0.01 ^{cA}	0.29±0.01 ^{cA}

¹⁾Treatments: See the legend of Table 1.

²⁾All values are expressed as mean±SE of triplicate determinations.

^{3)a-d}Means with different superscripts within a row differ significantly (*p*<0.05).

^{4)A-D}Means with different superscripts within a column differ significantly (*p*<0.05).

control (C). Kim and Ahn (2014) investigated the effects of grapefruit extract and lactic acid bacteria from kimchi on lipid peroxidation during the storage of fermented sausage, in which the group treated with the grapefruit extract showed superior outcomes compared with the group treated with nitrite and ascorbic acid until the 7th day of storage. It was suggested that these results were attributable to the excellent antioxidative effect of the high levels of flavonoids contained in grapefruits (Kanner, 1994). Kim *et al.* (1994) added DF-100 (GSE, a natural plant additive), potassium sorbate (a synthetic preservative), and sodium erythorbate (a synthetic antioxidant) to ham and sausage, followed by the measurement of lipid peroxidation, and found no differences. In the present study, the TBA value tended to be lower with increasing GSE concentration, and the group treated with 0.5% GSE (G0.5) had a lower TBA value than the positive control treated with sorbic acid and sodium nitrite (C). Thus, it was expected that the addition of approximately 0.5% GSE to meat products would inhibit lipid peroxidation and would have a positive effect on the development of meat products.

Changes in VBN contents of cured chicken breast prepared with grapefruit seed extract

Changes in the VBN content of the cured chicken breast prepared with a series of GSE concentrations during storage are presented in Table 9. The VBN values measured on the day of production were 10.39-11.66 mg%, and the VBN content increased significantly with storage time, reaching 15.54-18.93 mg% on the 10th day of storage. The groups treated with GSE (G0.05, G0.1, G0.3, and G0.5) tended to have lower VBN contents on the day of production than the no-treatment control (N) and had no significant difference compared with the positive control (C). On the 10th day of storage, the VBN value became lower as the GSE concentration increased, such that the group

treated with 0.5% GSE (G0.5) had the lowest VBN value. Park and Kim (2010) measured the VBN contents of chicken meat treated with mulberry leaves and dandelion extract, and found lower VBN contents in the treated groups. It was speculated that these results were attributable to the high polyphenol and flavonoid contents in mulberry leaves and dandelion extract, which delayed protein degradation. The extended shelf life of cured chicken breast meats treated with GSE seemed to be attributable to the antibacterial activity of GSE (Lim *et al.*, 2009) and the antioxidative activity of phenolic compounds, such as polyphenols and flavonoids. These results suggested that GSE delayed protein degradation to some extent.

Changes in total aerobic bacteria

Changes in the total aerobic bacterial count in the cured chicken breast prepared with a series of GSE concentrations during storage are presented in Table 10. There were differences in total aerobic bacterial count depending on GSE addition and storage time. Comparisons among the GSE-treated groups showed that the groups treated with 0.3% GSE (G0.3) 0.5% GSE (G0.5) had the lowest aerobic bacterial counts, while the no-treatment control (N) had the highest. The lowest total aerobic bacterial count was 3.06-3.65 Log CFU/g, which was found on the day of production, whereas the highest value was 6.93-7.79 Log CFU/g after 10 d of storage; thus, the total aerobic bacterial count increased significantly during storage. Kang *et al.* (2009) analyzed the total aerobic bacterial count of mechanically deboned chicken meats treated with different curing agents, in which the total aerobic bacterial count increased in all treatment groups when stored at 4°C for 7 d; however, groups treated with curing agents showed significant reductions in the count compared with the control group. In particular, the nitrite treatment group showed the largest reduction, which was similar to the result of this

Table 9. Changes of VBN content for cured chicken breast prepared with different concentrations of grapefruit seed extract during 10 d of storage at 5°C

	Storage time (d)	Treatments ¹⁾					
		N	C	G0.05	G0.1	G0.3	G0.5
VBN (mg%)	0	11.66±0.69 ^{2)a3)D4)}	10.39±0.19 ^C	11.06±0.27 ^{abC}	10.93±0.00 ^{bC}	10.79±0.41 ^{bC}	10.75±0.15 ^{bC}
	3	14.70±0.15 ^{aC}	11.75±0.31 ^{cC}	13.26±0.15 ^{bb}	12.62±0.27 ^{bb}	11.80±0.72 ^{cB}	12.07±0.27 ^{cB}
	7	15.79±0.41 ^{aB}	12.14±0.57 ^{dB}	14.41±0.15 ^{bb}	12.85±0.57 ^{cB}	12.57±0.57 ^{cdB}	12.50±0.38 ^{dB}
	10	18.93±0.96 ^{aA}	15.70±0.41 ^{bcA}	16.16±0.15 ^{bA}	16.00±0.57 ^{bA}	15.84±0.54 ^{bA}	15.44±0.83 ^{cA}

¹⁾Treatments: See the legend of Table 1.

²⁾All values are expressed as mean±SE of triplicate determinations.

^{3)a-d}Means with different superscripts within a row differ significantly ($p<0.05$).

^{4)A-D}Means with different superscripts within a column differ significantly ($p<0.05$).

Table 10. Changes of total aerobic counts for cured chicken breast prepared with different concentrations of grapefruit seed extract during 10 d of storage at 5°C

	Storage time (d)	Treatments ¹⁾					
		N	C	G0.05	G0.1	G0.3	G0.5
Total microbial count (Log CFU/g)	0	3.65±0.15 ^{2)a3)D4)}	3.21±0.10 ^{BD}	3.24±0.01 ^{BD}	3.11±0.04 ^{BCD}	3.07±0.05 ^{CD}	3.06±0.02 ^{CD}
	3	5.15±0.06 ^{aC}	4.45±0.13 ^{bcC}	4.90±0.00 ^{bcC}	4.80±0.00 ^{bcC}	4.47±0.14 ^{cC}	4.40±0.05 ^{cC}
	7	6.99±0.04 ^{aB}	6.36±0.05 ^{bbB}	6.97±0.10 ^{abB}	6.81±0.14 ^{abB}	6.50±0.19 ^{bbB}	6.37±0.12 ^{bbB}
	10	7.79±0.03 ^{aA}	7.03±0.01 ^{cA}	7.37±0.14 ^{baA}	7.35±0.13 ^{baA}	7.15±0.09 ^{bcA}	7.05±0.11 ^{bcA}

¹⁾Treatments: See the legend of Table 1.

²⁾All values are expressed as mean±SE of triplicate determinations.

^{3)a-d)}Means with different superscripts within a row differ significantly ($p<0.05$).

^{4)A-D)}Means with different superscripts within a column differ significantly ($p<0.05$).

study, in which the positive control treated with sorbic acid and nitrite (C) showed a low count. Our results showed that GSE had an inhibitory effect against bacterial proliferation when used to treat chicken breast. Choi *et al.* (2014) analyzed the total aerobic bacterial count in marinated pork treated with GSE during storage, which demonstrated the value of GSE as a natural preservative: the total bacterial count decreased significantly with increasing GSE concentration. Thus, GSE added to chicken breast has an antibacterial activity that inhibits bacterial proliferation, indicating that GSE is useful as a natural preservative to prevent the decomposition and deterioration caused by microorganisms, which should extend the storage time of meat products.

Conclusions

This study found that the total polyphenol and total flavonoid contents of GSE were 45.06 mg/g and 36.06 mg/g, respectively, and the IC₅₀ value of DPPH radical scavenging activity was 333.33 µg/mL, indicating the excellent antioxidative effect of GSE. GSE addition to chicken breast resulted in a higher WHC and lower cooking loss, and had positive effects on the texture properties, leading to high scores in the sensory evaluation. During storage, lipid peroxidation, microbial proliferation, and VBN production were inhibited in the cured chicken breast prepared with GSE, resulting in a shelf life similar to that of the positive control treated with sorbic acid and sodium nitrite, which demonstrate the potential of GSE as a functional curing agent ingredient for processed meat products. The results of the present study suggest that GSE, as a natural antioxidant and functional curing agent ingredient, should not only help to improve the shelf life and palatability, but also positively affect consumer choice, if applied to meat products as an alternative to chemical food

additives.

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