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Research Article

Exploring Natural Alternatives to Nitrites in Pork Meat Patty: A Study on the Effects of Gromwell Root Extract and Lettuce Powder as Substitutes

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The aim of this study was to assess the feasibility of substituting nitrite in pork meat patties with gromwell root extract and lettuce powder. We made pork patties with gromwell root extract and lettuce powder (control, no addition; positive control, addition of 50 ppm sodium nitrite + 0.1% ascorbic acid; T1, 0.3% gromwell root extract; T2, 0.1% lettuce powder; T3, 0.3% gromwell root extract + 0.1% lettuce powder) and investigated whether gromwell root extract and lettuce powder could serve as nitrite substitutes. Addition of gromwell root extract and lettuce powder improved WHC and CL and storage stability. Furthermore, addition of gromwell root extract reduced lightness and yellowness and increased redness. However, it had a negative effect on the sensory evaluation of flavor, bitterness, and off odor. In conclusion, gromwell root extract and lettuce powder showed positive potential as substitutes for nitrite in pork meat patties.

1. Introduction

Global meat consumption in Asia increased by 2.7 times between 1956 and 2006 [1]. From 1992 to 2016, global meat consumption increased by more than 500%, and steady growth is predicted [2]. Meat has a rich nutritional matrix that creates an environment favorable for the growth of meat deterioration bacteria and common foodborne diseases [3].

Processed pork meat products are vulnerable to oxidative reactions due to added heme iron, salt, and the relative plentiful of endogenous phospholipids [4]. Therefore, nitrite, with antioxidant and antimicrobial effects, is used in many meat products. Nitrite is an indispensable additive in meat product manufacturing as it plays a role in expressing and fixing meat color, the antioxidant effect of lipids, the flavor formation of unique cured meat, and antimicrobial action against *Clostridium botulinum*, the most important food-poisoning bacterium [5]. However, nitrites can react with amines at low pH in the body to form N-nitroso compounds, and many substances, including nitrosamine, are considered carcinogenic [6]. That is why many studies have measured residual nitrite in pork meat products [7, 8], and health concerns of using nitrite have increased consumer demand for organic and natural meat products; as a result, the meat industry is actively developing alternatives to nitrites [9].

Previous studies have reported using cherry tomato paste [10], drone pupa meal [11], red beet powder [12], polygoni multiflori radix [13], lemon seed essential oil and pitaya peel extract [8], and sage essential oil [14] as nitrite substitutes.

Gromwell (*Lithospermum erythrorhizon* Sieb. et Zucc.) is a perennial herbaceous plant with red pigment in the root integument and has been used as an herb for medication in eastern medicine [15, 16]. Gromwell contains a large amount of shikonin, a naphthoquinone-based compound, and its derivatives. This substance has a purple color and is known to be associated with the pharmacological effects of

gromwell, such as antioxidant, antibacterial, anti-inflammatory, and immunomodulatory actions [15]. Also, gromwell has nitrite scavenging ability [17].

Lettuce (*Lactuca sativa* L.) is a 1- to 2-year-old herb belonging to *Asteraceae* and is cultivated nationwide in Korea. It contains vitamins A, B, C, and K, carotenoids, fiber, and polyphenolic compounds, so it exerts physiological activities, such as antibacterial and antioxidant effects [18]. In addition, lettuce contains nitrite and nitrate, and the contents of nitrite and nitrate were identified through several previous studies [19–21].

In this study, we added gromwell root extract, lettuce powder, and nitrite to pork patties and compared them to investigate their potential as nitrite replacements. There has been neither research to date on using gromwell as a substitute for nitrite, and no previous studies have investigated the combined use of gromwell and lettuce.

Our goal is to expand the range of potential substances that can replace nitrite in pork meat patties. This would benefit everyone, including those in the meat processing industry who are interested in health and public health issues.

2. Materials and Methods

2.1. Gromwell Root Extract. The gromwell root extract manufacturing method is shown in Figure 1. Gromwell root (herb and woodcutter, Dangjin-si, Korea) and 95% ethanol (Ethanol Supplies World Co., Ltd., Jeonju-si, Korea) were put into a bottle at a ratio of 100 g : 1000 ml and extracted at room temperature for 196 hours. The extract was filtered using Whatman no. 2 ∅ 150 mm (Korea Ace Scientific Co., Ltd., Seoul, Korea). The filtrate was concentrated in a vacuum rotary evaporator (N-1300E-W, EYELA, Tokyo, Japan) at 40°C and lyophilized (FDU-2100, EYELA, Tokyo, Japan).

2.2. Manufacturing of Patties. The material mixture of the patties is shown in Table 1. All patties had the same amount of ground pork meat, ice, salt, and pepper. 70 g of ground pork meat (Manpyeong Livestock Products, Cheongju, Korea) and 0.8 g of 99.0% sodium chloride (Samchun Chemicals, Pyeongtaek, Korea) were added first, followed by 0.16 g of pepper (Ottogi Co., Ltd., Anyang, Korea). Additives are added according to each treatment. Types of additives are gromwell root extract, lettuce powder (Baeksefood, Seoul, Korea), sodium nitrite (Junsei Chem. Co., Tokyo, Japan), and ascorbic acid (Sigma Aldrich, Darmstadt, Germany). The amount of additives added to each treatment is shown in Table 1.

The meat was kneaded for a total of 3 minutes, and ice was added 5 times for 30 seconds each to add a total of 80 g. Ice was added to prevent denaturing by heat from the hands and kneading action. After that, it was molded into 80 g patties. Finally, 8 patties of 80 g per treatment were made. The 5 treatment groups are as follows: control (no addition), positive control (addition of 0.005% sodium nitrite and 0.1% ascorbic acid), T1 (addition of 0.3% gromwell root extract), T2 (addition of 0.1% lettuce powder), and T3 (addition of 0.3% gromwell root extract and 0.1% lettuce powder). All patties were refrigerated.

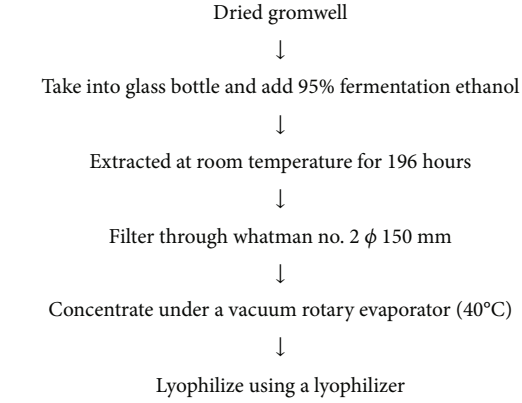

Figure 1: Gromwell root extraction procedure.

2.3. Proximate Analysis. Moisture, ash, fat, and protein components of the patties were determined prior to storage according to methods approved by AOAC [18, 22]. 1 g of the sample was dried overnight at 105°C in a forced air oven (SH-DO-100 FG, Samheung, Seoul, Korea) to a constant weight to determine the amount of moisture. To determine the ash content, a 1 g sample was placed in a porcelain dish and heated in a muffle furnace (MF2-12 GF, Jeio tech, Daejeon, Korea) at 540°C for 10 hours. To estimate crude fat, a 0.5 g sample was mixed with 25 ml of Folch solution, which is a combination of 99.5% chloroform (Samchun Chemicals, Pyeongtaek, Korea) and 99.5% methanol (Samchun Chemicals, Pyeongtaek, Korea) in a 2 : 1 ratio. The mixture was then refrigerated at 4°C for 24 hours, after which it was filtered using Whatman no. 2 paper and cleaned with 5 ml of the Folch solution. Next, 10 ml of deionized water was added to the filtrate, and the sample was centrifuged at 3000 rpm at room temperature for 20 minutes (Union 55R, Hanil Science Co., Ltd., Daejeon, Korea). The upper layer consisting of water and methanol was removed using a pipette, and the remaining mixture was left to evaporate overnight in a hood. Finally, the weight of the residue was measured. To measure proteins, the Kjeldahl method was used. A 0.5 g sample was mixed with 25 mL of 98% sulfuric acid (Merck, Darmstadt, Germany), and the mixture was heated in a flask. The flask was then connected to a distillation apparatus to adsorb the ammonia component of the sample using boric acid (Sigma Aldrich, Darmstadt, Germany) in the flask. Finally, titration with 0.05 M sulfuric acid was performed.

2.4. pH. Using a Stomacher (400 Lab Blender, London, England), homogenize 5 g of the material for 30 seconds with 50 ml of deionized water, and then measure it using a pH meter (Orion Star A211, Thermo Scientific, USA). Repeat 3 times and calculate the mean value.

2.5. Water-Holding Capacity (WHC). Water-holding capacity was calculated according to the method of Laakkonen et al.'s measurement [23]. Weigh a 2 ml test tube with a small hole, and accurately weigh 0.5 ± 0.005 g of sample into the test tube. The sample was subjected to a temperature of 80°C in a water bath (SW-90 MW, Sangwoo Scientific,

Table 1: Formula of patty.

Ingredient		Meat (g)	Ice (g)	Sodium chloride (g)	Pepper (g)	Gromwell root extract (g)	Lettuce powder (g)	Sodium nitrite (g)	Ascorbic acid (g)	Total (g)
Treatments	Control	70	10	0.8	0.16	—	—	—	—	80.96
	Positive control	70	10	0.8	0.16	—	—	0.004	0.081	81.045
	T1	70	10	0.8	0.16	0.244	—	—	—	81.204
	T2	70	10	0.8	0.16	—	0.081	—	—	81.041
	T3	70	10	0.8	0.16	0.244	0.081	—	—	81.285

Bucheon-si, Korea) for 20 minutes and then allowed to cool down to room temperature for 10 minutes. Following a 10 min, 2,000 rpm, 4°C centrifugation, the weight was recorded, and the water retention was calculated using the formula below.

$$\text{WHC} = \left(\frac{100}{\text{moisture}}\right) \times (\text{moisture} - \text{free moisture}).$$

$$\begin{aligned}\text{Free moisture} = {} & (\text{sample weight (g) before centrifugation} \\ & - \text{sample weight (g) after centrifugation}) \\ & \times 100/(\text{fat coefficient} \times \text{sample weight (g)}).\end{aligned}$$

$$\text{Fat coefficient} = 1 - \text{fat (\%)}/100. \quad (1)$$

2.6. Cooking Loss (CL). CL was measured by referring to the method used by Park et al. [24]. Samples were cut into a steak shape of about 80 g and cooked in a water bath at 70°C for 40 min, and CL show the difference in weight before and after heating in percentage (%).

$$\text{CL (\%)} = \frac{[\text{Before heating weight (g)} - \text{after heating weight (g)}]}{\text{Before heating weight (g)}} \times 100. \quad (2)$$

2.7. Color Measurements. Patty color was standardized using a spectrocolorimeter (JX-777, Color Techno System Co., Ltd., Tokyo, Japan), and whiteboard was measured (L^*, 94.04; a^*, 0.13; b^*, −0.51). The present method involves using a white fluorescent lamp (D65) as the light source to measure the L^* value for brightness, the a^* value for red, and the b^* value for yellow, according to the Hunter Laboratory color scheme. Repeat 3 times and calculate the mean value.

2.8. Texture Profile Analysis. Texture was analyzed using a rheometer (Model Compac-100, Sun Scientific Co., LTD. Tokyo, Japan). Samples were equilibrated to room temperature prior to texture mapping analysis. Two compression cycle measurements were used to generate force versus time graphs. The crosshead moves at 200 millimeters per minute, and the load cell weighs 10 kg. The Bourne-described curves [25] were used to calculate the characteristics of cohesiveness, chewiness, and hardness. To obtain the cohesiveness value, divide the area under the second compression curve by the area under the first compression curve. To calculate chewiness, multiply gumminess by springiness. And gumminess is hardness multiplied by cohesiveness. The maximum force of the first compression is defined as hardness.

2.9. Sensory Evaluation. Sensory evaluation was measured by referring to the method used by Kim et al. [26]. The sample was baked in a frying pan heated using a portable butane stove (DYC-3000, Daeyang, Busan, Korea) at the same intensity for 3 minutes and 30 seconds on each side and then cooled down at room temperature for 1 hour. Sensory evaluation was performed on samples shaped to 1 × 1 × 1 (height × width × length) cm. Eight sensory inspectors who had received sensory test training evaluated 7 items, including color, flavor, bitterness, juiciness, off-odor, texture, and total preference. A 5-point scale was used. Redness was rated on a 5-point scale, with 5 points for red and 1 point for gray. The other tests were scored on a 5-point scale where a perfect score was 5 points, with 5 points indicating the best and 1 point indicating the worst.

2.10. 2-Thiobarbituric Acid Reactive Substance (TBARS). TBARS was measured using Witte et al. method [27]. For the sample 5 g, homogenize 25 ml of deionized water and cold 10% perchloric acid which diluted 70% perchloric acid (Samchun Chemicals, Pyeongtaek, Korea) 15 ml in 20 seconds of homogenizing at 10,000 rpm to use homogenizer (AM-7, Nissei, Izumichom, Tokyo). The homogenate was filtered using Whatman no.2 filter paper, and 5 ml of 2-thiobarbituric acid (Sigma Aldrich, Darmstadt, Germany) solution with a concentration of 0.02 M and 5 ml of the filtrate were thoroughly mixed and placed in a cool, dark place for 16 hours. Blank used deionized water and 5 ml of 0.02 M 2-thiobarbituric acid solution. Thereafter, the absorbance at 529 nm was measured using a spectrophotometer (mobi, MicroDigital Co., Ltd., Seongnam, Korea). The TBA content is represented in mg of MDA per kg of sample (mg MDA/kg). The standard curve used at this time is $x - 0.0011$ ($r = 0.999$), $y = 0.1975$, and $x =$ TBA value, and $y =$ absorbance is calculated.

2.11. Peroxide Value (POV). POV was measured by referring to the method of Folch [28] and Park [29]. After finely chopping the sample, take 1.0 g of the sample into an Erlenmeyer flask with a stopper. After completely dissolving the sample by adding 10 ml of chloroform, 15 ml of CH_3COOH was added and mixed. A saturated KI solution was prepared by dissolving 99.5% potassium iodide (Samchun Chemicals, Pyeongtaek, Korea) and deionized water in a ratio of 7 : 3.

After adding 1 ml of saturated KI solution, stoppering, and homogenizing for about 1 minute, it was left in the dark for 10 minutes at room temperature. After homogenizing again with 30 ml of deionized water, 1 ml of a 1% starch solution (BIOZOA Biological Supply, Seoul, Korea) indicator was added, and titration was performed with $Na_2S_2O_3$ (Samchun Chemicals, Pyeongtaek, Korea) solution with a concentration of 0.01 M until it became colorless. A blank test was conducted in parallel with this experiment.

2.12. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Capacity. DPPH radical scavenging capacity was measured using Brand-Williams et al.'s [30] method. To obtain samples, blanks, and references, the mixture of 45 ml of methanol and 5 g of patties was homogenized and then subjected to filtration using Whatman no. 2 filter paper. After that, each sample was prepared: sample, homogenate 2 ml + DPPH solution (0.2 mM DPPH solution, BIOZOA Biological Supply, Seoul, Korea) 1 ml + methanol 2 ml; blank, 99% methanol 5 ml; reference, DPPH 1 ml + methanol 4 ml. Cover with aluminum foil to prevent exposure to light, and let it sit at room temperature in a dark environment for a duration of 20 to 30 minutes and then take measurements at a wavelength of 517 nm. After averaging the obtained values, they were substituted into the formula to obtain the value of the capacity of radical scavenging.

$$\text{Radical scavenging capacity} = \left\{1 - \frac{(\text{Average of samples})}{\text{Absorbance of reference}}\right\} \times 100. \tag{3}$$

2.13. Total Microbial Count (TMC). TMC was measured by referring to the method used by Park et al. [24]. TMC uses a serial dilution method. Combine 5 g of the sample with 45 ml of a peptone (Bacto^{tm} Peptone solution, Becton, Dickinson and Company, New Jersey, USA) solution with a concentration of 0.1% then use a stomacher bag to homogenize the mixture for 30 seconds. Afterwards, plate count agar (Difco^{tm} Plate Count Agar, Becton, Dickinson and Company, New Jersey, USA) medium was added with serially diluted samples, and the mixture was incubated for 48 hours at 37°C. Following incubation, count the colonies. Log cfu/g is the unit used to indicate the overall plate count.

2.14. Volatile Basic Nitrogen (VBN). The VBN was determined using Pearson's method [31]. A 5 g sample was mixed with 45 ml of deionized water. After homogenization at 10,000 rpm for approximately 20 seconds, filter the homogenate through Whatman no.2 filter paper. Introduce 1 ml of boric acid solution with a concentration of 0.01 M and 3 drops of Conway solution, which is a mixture of 0.066% bromocresol green (Samchun Chemicals, Pyeongtaek, Korea) and 0.066% methyl red (Samchun Chemicals, Pyeongtaek, Korea), into the inside of the Conway unit. Then, add 3 ml of filtrate outside the Conway unit. Following this, 1 ml of K2CO3 (Samchun Chemicals, Pyeongtaek, Korea) with a concentration of 50% is added to the outside of the Conway unit, and it is then incubated for 120 minutes at 37°C. After the incubation period, the boric acid solution inside the Conway unit is titrated using sulfuric acid with a concentration of 0.01 M. The resulting value for VBN is expressed in milligrams per 100 grams of the sample (mg%).

$$28.014 = 0.01\ \text{M H2SO4 1 ml},$$
$$\text{VBN} = \frac{(\text{ab}) \times 100 \times 28.014 \times F}{\text{Sample amount}}. \tag{4}$$

The variables used are a, which represents the volume of sulfuric acid added in milliliters (ml); b, which denotes the quantity of sulfuric acid added to the blank sample in ml; and f, which represents the amount of N necessary to react with 1 ml of 0.01 M H2SO4.

2.15. Statistical Analysis. Statistical analysis was performed using GLM (general linear model) of the SAS 9.4 program (statistics analytical system). For comparison between treatment averages, a significance test ($p < 0.05$) was conducted through Duncan's multiple tests.

3. Results and Discussion

3.1. Proximate Analysis. Table 2 shows the proximate analysis of patties with added gromwell root extract and lettuce powder. In the control group, ash and fat contents were lower than other treatments ($p < 0.05$). The T3 group was higher fat content than the control, positive control, and T1 groups ($p < 0.05$).

The composition of typical lettuce, excluding moisture, is about 28% of crude protein, 6% of crude fat, 11% of crude fiber, 43% of nitrogen-free extract, and 11% of crude ash [32]. Since the lettuce powder used in this experiment was a dried product, the moisture content was excluded. The ash content of gromwell was 9.61% [33]. The control group was thought to have a low ash content because it was not treated with gromwell root extract, lettuce powder, nitrite, or ascorbic acid.

The reason for the lower fat content of the control group is thought to be due to the moisture content. Moisture loss occurs due to the physical force applied when manufacturing the patties. Lettuce powder was added to groups T2 and T3, and the WHC of meat increased as the strength of the protein network increased [34]. However, the addition of dietary fiber may have interfered with the gel network formed by protein-water or protein-protein, resulting in a reduction in the strength of the gel in the product [35]. The positive control group had a lower pH than the control group due to the addition of ascorbic acid (Table 3). Moisture loss increased as the lattice-shaped space between myofibrils became smaller when the pH approached the isoelectric point (pH 5 for pork meat) [5, 36]. Moisture in meat is divided into bound water, combined water, and free water based on the binding pattern with protein [5]. Free water accounts for about 75% of the water in meat and can easily be exuded to the surface of the meat in response to external shock due to its free thermodynamic movement [5]. The excessive exudation of free water can cause protein loss because free water contains water-soluble proteins [5].

Table 2: Proximate analysis for patties with gromwell root extract and lettuce powder.

Treatments[1)]	Fat (%)	Moisture (%)	Ash (%)	Protein (%)
Control	4.87 ± 0.44^{c}	74.11 ± 3.15	0.53 ± 0.30^{c}	20.47 ± 2.46
Positive control	6.80 ± 0.92^{b}	74.05 ± 0.86	1.09 ± 0.08^{b}	18.05 ± 0.87
T1	7.02 ± 0.39^{b}	74.05 ± 0.83	1.32 ± 0.07^{ab}	17.58 ± 1.19
T2	8.10 ± 0.73^{ab}	72.75 ± 1.68	1.20 ± 0.24^{ab}	17.93 ± 1.47
T3	9.00 ± 1.13^{a}	71.16 ± 3.14	1.51 ± 0.27^{a}	18.32 ± 3.61

[a-c]There is a significant difference between values within the same column that have different superscripts, based on their means ± standard deviations ($p < 0.05$). [1)]Control, no addition; positive control, addition of 50 ppm sodium nitrite + 0.1% ascorbic acid; T1, 0.3% gromwell root extract; T2, 0.1% lettuce powder; T3, 0.3% gromwell root extract + 0.1% lettuce powder.

Table 3: pH, WHC, and CL for patties with gromwell root extract and lettuce powder.

Treatments[1)]	pH	WHC (%)	CL (%)
Control	5.66 ± 0.02^{d}	48.50 ± 3.02^{b}	27.90 ± 0.60^{a}
Positive control	5.60 ± 0.01^{e}	50.63 ± 1.31^{ab}	28.00 ± 1.36^{a}
T1	5.73 ± 0.01^{a}	53.98 ± 3.45^{a}	22.91 ± 2.25^{b}
T2	5.71 ± 0.00^{b}	54.09 ± 1.48^{a}	28.01 ± 1.45^{a}
T3	5.68 ± 0.01^{c}	51.90 ± 2.35^{a}	23.64 ± 1.98^{b}

[a-e]There is a significant difference between values within the same column that have different superscripts, based on their means ± standard deviations ($p < 0.05$). [1)]Control, no addition; positive control, addition of 50 ppm sodium nitrite + 0.1% ascorbic acid; T1, 0.3% gromwell root extract; T2, 0.1% lettuce powder; T3, 0.3% gromwell root extract + 0.1% lettuce powder.

Most of the juiciness of meat is due to free water [5]. The sensory evaluation results of juiciness in the T1 group were not significant, but it was evaluated as lower than the other treatments (Table 4). This finding could have been due to the escape of more free water in this treatment group compared to the other groups. Therefore, the control group tended to have higher moisture and protein contents than the other treatments. Accordingly, the proportion of fat was relatively low. Lettuce excluding moisture contains about 6% of crude fat [32]. Therefore, it is considered that the T3 group, with lettuce powder added, showed a higher fat content than the control, positive control, and T1 groups.

3.2. pH, WHC, and CL. Table 3 shows the pH, WHC, and CL of patties with added gromwell root extract and lettuce powder. The positive control group showed a lower pH value than the control group ($p < 0.05$). The T1, T2, and T3 groups which added gromwell root extract, lettuce powder, or both had higher pH value than the control and positive control groups ($p < 0.05$).

The positive control group had a lower pH than the control group because ascorbic acid was added. Natural color mainly causes color fading and discoloration due to the interaction between the ingredients in the food itself. In the case of gromwell root extract pigments, it is orange in acidic conditions but changes from red to purple as the pH increases [35]. The gromwell root extract used in the experiment had high redness and low yellowness values in the patty color measurements (Table 5) and showed a deep red color to the naked eye. The pH of cut lettuce according to the storage days was 6.0 on day 1 and 6.5 on day 15 [37]. Therefore, it is considered that the T1, T2, and T3 groups showed higher pH values than the control and positive control groups.

The T1, T2, and T3 groups had higher WHC value than the control group ($p < 0.05$). And WHC measurements of patties generally showed a tendency that the higher the pH, the higher the WHC. This result was due to the WHC decreased as the lattice-shaped space between myofibrils became smaller as the pH approached the isoelectric point (pH 5 for pork meat) [5, 36].

The cooking loss of patties in the T1 and T3 groups with added gromwell root extract had lower than the control, positive control, and T2 groups ($p < 0.05$), indicating a negative correlation with WHC. Cooking loss increases as the pH decreases and the surface-to-weight ratio of meat increases [38], and moisture loss occurs as the binding force between protein and water molecules weakens. When the pH is high, the ability to retain water increases, so CL also decreases. However, when the pH is low, protein approaches the isoelectric point, making it easier to release water, so CL also increases [5, 38, 39]. This is the reason why the T1 and T2 groups had lower CL value than the control and positive control groups. In general, dietary fiber absorbs and stores moisture. However, during the heating process, some of the soluble dietary fiber can be hydrolyzed and lost, while the molecular structure of some of the insoluble dietary fiber may also be broken, leading to a decrease in the overall amount of dietary fiber [40]. As an example of this, the CL value of sausages with 3% spinach powder was significantly lower than that of sausages without anything added [41]. The crude fiber content of lettuce excluding moisture is 11% [32]. It is thought that T2 with lettuce powder added showed a higher CL value than T1 for this reason, and T3 also showed a higher tendency than T1 in CL value.

In conclusion, when gromwell root extract was added, pH and WHC increased, and CL decreased. Furthermore, while adding lettuce powder increased pH and WHC, it did not have a significant effect on CL.

3.3. Color Measurements. Table 5 shows the color of patties with added gromwell root extract and lettuce powder. The T1 and T3 groups, which added gromwell root extract, show low lightness and yellowness values and a higher redness

Table 4: Sensory evaluation for patties with gromwell root extract and lettuce powder.

Treatments[1)]	Color	Flavor	Bitter	Juiciness	Off odor	Texture	Total preference
Control	2.37 ± 0.91^{bc}	3.37 ± 0.74^{a}	4.25 ± 1.16^{a}	3.25 ± 0.88	4.12 ± 0.99^{a}	3.37 ± 0.74	4.00 ± 0.92^{a}
Positive control	3.12 ± 0.64^{b}	3.87 ± 0.99^{a}	3.87 ± 1.45^{a}	3.12 ± 0.83	4.00 ± 0.92^{a}	3.37 ± 0.74	3.75 ± 0.88^{a}
T1	4.25 ± 1.03^{a}	1.50 ± 1.06^{b}	2.00 ± 1.06^{b}	2.62 ± 0.91	1.87 ± 1.35^{b}	2.62 ± 0.51	2.12 ± 1.45^{b}
T2	2.12 ± 0.64^{c}	3.25 ± 0.46^{a}	3.62 ± 1.30^{a}	2.75 ± 0.70	3.5 ± 0.75^{a}	3.25 ± 0.70	3.19 ± 0.37^{a}
T3	4.37 ± 0.51^{a}	1.62 ± 0.74^{b}	2.12 ± 0.99^{b}	3.12 ± 0.83	2.25 ± 1.16^{b}	3.00 ± 0.75	2.12 ± 0.79^{b}

Color: 1: gray; 5: red. Otherwise: 1: very poor; 5: very good. [a-c]There is a significant difference between values within the same column that have different superscripts, based on their means ± standard deviations ($p < 0.05$). [1)]Control, no addition; positive control, addition of 50 ppm sodium nitrite + 0.1% ascorbic acid; T1, 0.3% gromwell root extract; T2, 0.1% lettuce powder; T3, 0.3% gromwell root extract + 0.1% lettuce powder.

Table 5: CIE color (L^*, a^*, and b^*) for patties with gromwell root extract and lettuce powder.

Treatments[1)]	$L^{*2)}$	$a^{*3)}$	$b^{*4)}$
Control	55.33 ± 3.41^{a}	5.41 ± 1.37^{c}	12.29 ± 1.38^{b}
Positive control	49.64 ± 1.11^{b}	8.37 ± 1.07^{b}	14.43 ± 0.30^{a}
T1	45.19 ± 0.87^{c}	15.68 ± 1.59^{a}	9.49 ± 1.35^{c}
T2	52.4 ± 2.00^{ab}	8.10 ± 0.30^{b}	14.75 ± 1.20^{a}
T3	31.25 ± 3.41^{d}	14.70 ± 0.93^{a}	4.06 ± 0.67^{d}

[a-d]There is a significant difference between values within the same column that have different superscripts, based on their means ± standard deviations ($p < 0.05$). [1)]Control, no addition; positive control, addition of 50 ppm sodium nitrite + 0.1% ascorbic acid; T1, 0.3% gromwell root extract; T2, 0.1% lettuce powder; T3, 0.3% gromwell root extract + 0.1% lettuce powder. [2)]L^*: lightness; [3)]a^*: redness; [4)]b^*: yellowness.

value than the control, positive control, and T2 groups ($p < 0.05$). The T2 group, which added lettuce powder, showed high redness and yellowness value than the control group ($p < 0.05$). But there was no difference between T2 and positive control groups.

Gromwell root is known to contain shikonin and its derivatives, which are red-purple pigments [15, 42]. Therefore, the high red color of the T1 and T3 groups' patties was due to shikonin, which also resulted in low lightness and yellowness values in T1 and T3 groups. The T2 group supplemented with lettuce powder showed significantly greater redness than the control group, but there was no significant difference compared to the positive control group, according to the results. Redness of meat can be increased by inhibiting metmyoglobin formation using ascorbic acid, which acts as a reducing agent [43]. Lettuce is known to contain ascorbic acid [18], so it is possible that the result was due to the ascorbic acid present in the lettuce powder.

Thus, when gromwell root extract is added to the pork patty, the redness is significantly increased, while the brightness and yellowness are reduced. On the other hand, adding lettuce powder to the pork patties results in an increase in both redness and yellowness.

3.4. Texture Profile Analysis. Table 6 shows the texture profile analysis of patties with added gromwell root extract and lettuce powder. The T3 group had lower values in hardness and chewiness than other treatments.

The addition of dietary fiber may have caused interfered with the gel network formed by protein-water or protein-protein, resulting in a reduction in the strength of the gel in the product [35]. Moreover, hardness was negatively correlated with fiber content [44]. The lettuce powder added to the T3 group contains dietary fiber, and the fiber of the lettuce powder destabilized the patty which may have contributed to the lower hardness value. The degree of chewiness is a secondary characteristic that is influenced by the level of hardness [45, 46]. Therefore, if the hardness is low, chewiness is also low. Since the hardness of the T3 group was low, it is considered that the chewiness was also low.

The higher the moisture content, the higher the springiness [47]. Texture profile analyses were measured after cooking the patties. Since the CL value of the T3 group was lower than that of the T2 and positive control groups, it can be inferred that it has a high water content, and thus, a high springiness value was obtained (Tables 3 and 6, $p < 0.05$).

3.5. Sensory Evaluation. Table 4 shows the sensory evaluation of patties with added gromwell root extract and lettuce powder. The T1 and T3 groups which added gromwell root extract had higher meat color values than control, positive control, and T2 groups ($p < 0.05$), because gromwell root contains a large amount of red-colored shikonin and its derivatives [15]. In terms of flavor, bitterness, and off-odor, the T1 and T3 groups with added gromwell root extract received lower scores than control, positive control, and T2 groups ($p < 0.05$).Gromwell's unique flavor in the patty was perceived negatively. However, adding gromwell to gangjeong did not adversely affect the taste or overall acceptability [48], suggesting that the preference for gromwell-added patties can be increased depending on the cooking method. While the T2 group showed significantly lower color scores than the other treatment groups, there were no significant differences between the T2 group and the control group in terms of the other characteristics, particularly overall preference. Therefore, it can be said that the addition of 0.1% lettuce powder did not decrease the preference for the patties.

Thus, the addition of gromwell root extract to the pork patty had a positive effect on color but had a negative effect on flavor, bitterness, and off odor. The addition of lettuce powder did not affect sensory evaluation.

3.6. TBARS, POV, and DPPH Radical Scavenging Capacity. TBARS, POV, and DPPH radical scavenging capacity were measured to confirm the antioxidant capacity of gromwell

Table 6: Texture profile analysis for patties with gromwell root extract and lettuce powder.

Treatments[1)]	Hardness (kg)	Springiness (%)	Cohesiveness (%)	Chewiness (kg)
Control	2.27 ± 0.04^{a}	66.30 ± 5.68^{ab}	64.71 ± 6.55	1.47 ± 0.03^{a}
Positive control	2.15 ± 0.05^{ab}	63.21 ± 4.58^{b}	61.31 ± 4.29	1.31 ± 0.03^{ab}
T1	2.26 ± 0.08^{a}	67.05 ± 2.49^{ab}	66.55 ± 3.67	1.48 ± 0.05^{a}
T2	2.09 ± 0.03^{ab}	63.48 ± 4.20^{b}	64.47 ± 4.80	1.34 ± 0.02^{ab}
T3	1.45 ± 0.04^{b}	71.08 ± 4.20^{a}	66.17 ± 6.45	0.94 ± 0.02^{b}

[a-b]There is a significant difference between values within the same column that have different superscripts, based on their means ± standard deviations ($p < 0.05$). [1)]Control, no addition; positive control, addition of 50 ppm sodium nitrite + 0.1% ascorbic acid; T1, 0.3% gromwell root extract; T2, 0.1% lettuce powder; T3, 0.3% gromwell root extract + 0.1% lettuce powder.

root extract and lettuce powder in pork patties. Figure 2 shows the TBARS, POV, and DPPH radical scavenging capacity of patties with added gromwell root extract and lettuce powder.

TBARS measurements on 1 day of storage showed that the T2 group had lower values than control group ($p < 0.05$). On 3 days of storage, the T3 group had lower values than the control and positive control groups ($p < 0.05$). On 7 days of storage, T1 had lower values than the control and positive groups ($p < 0.05$). Also, the T1, T2, and T3 groups were lower than control groups ($p < 0.05$).

POV measurements did not show any differences between the treatment groups on days 1 and 3 of storage. On day 7 of storage, the T1 and T3 groups had lower values than the T2 group ($p < 0.05$), but no significant differences were found between the T2, control, and positive control groups. However, on day 7, the T1 and T3 groups exhibited lower POV values compared to the other treatment groups ($p < 0.05$).

On DPPH radical scavenging capacity measurement, during all storage days, it was higher in the order of positive control, T1, T2, T3, and control groups ($p < 0.05$).

Since phenolic compounds have a phenolic hydroxyl group capable of accepting free radicals, they can bind to proteins and other macromolecules, providing antioxidant properties [49]. Gromwell root contained phenolic compounds and exhibits high antioxidant activity [50]. Also, gromwell contained 1.7 times more polyphenols than ginseng and 2.6 times more than sealwort [51]. Furthermore, shikonin from gromwell has been shown to scavenge reactive oxygen species [52]. Acetylshikonin, β, β-dimethylacrylshikonine, and shikonin in gromwell acted as antioxidants in lard [53].

Lettuce contained 22-48 mg/kg of nitrite at ambient temperature (25°C) and 21-40 mg/kg when refrigerated (10°C), depending on storage time (0-48 hours) [54]. Lettuce contains nitrates and nitrites [19], and ascorbic acid present in lettuce acts as a reducing agent that promotes nitrite reduction [55]. In addition, lettuce contains fat-soluble antioxidants such as lutein and tocopherol, as well as water-soluble antioxidants such as phenolic acid, anthocyanidin, caffeic, caftaric, and chicoric acid [56–58]. Thus, these factors may explain the observed antioxidant effect in patties that have been supplemented with gromwell root extract and lettuce powder.

Based on the results of TBARS and DPPH radical scavenging measurements, it was shown that gromwell root extract and lettuce powder have antioxidant abilities in pork patties. In addition, the antioxidant effect was better in the treatment group added separately than in the combination of gromwell root extract and lettuce powder.

3.7. TMC and VBN. To investigate the antimicrobial properties of gromwell root extract and lettuce powder on pork patties, TMC and VBN were confirmed. Figure 3 shows the TMC and VBN of patties with added gromwell root extract and lettuce powder.

On TMC measurements, in all storage days, the positive control group showed lower values than all other treatments ($p < 0.05$). The T1 and T2 groups showed lower values than the control group on all storage days ($p < 0.05$). The T3 group showed a higher value than the T1 and T2 groups on day 1 ($p < 0.05$). Also, the T3 group showed a lower value than the control group on the 3rd day ($p < 0.05$).

On VBN measurements, on days 1 and 7, the T1, T2, and T3 groups were lower than the control group ($p < 0.05$). Also on day 7, there was no difference between the T1 and T2 groups and the positive control group, and the T3 group was greater than the positive control group ($p < 0.05$).

Gromwell contains a large amount of shikonin, a naphthoquinone-based compound with antimicrobial properties [15]. Shikonin has bactericidal properties [59]. Shikonin showed antibacterial effect against food-poisoning bacteria, such as *E. coli*, *B. cereus*, *S. aureus*, and *V. parahaemolyticus*, as well as antimicrobial effects against both gram-negative and positive bacteria [60]. Lettuce contains nitrate and nitrite [19], as well as ascorbic acid, which is a reducing agent that can promote nitrite reduction to NO [18]. Nitrite and NO can interfere with heme transport by impairing cyt c maturation E, resulting in impaired cytochromes c biosynthesis, resulting in antibacterial action [61]. Lettuce's phenolic acid is effective in either killing or preventing the growth of microorganisms through multiple mechanisms, such as modifying the permeability of the bacteria plasma membrane, directly impacting microbial metabolism, and depriving them of the necessary substrates for growth [62]. The T1 and T2 groups added with gromwell root extract and lettuce powder through these materials showed lower values than the control group in TMC measurement ($p < 0.05$).

The VBN values of the treatments showed a similar trend to the TMC values. Microbes break down meat

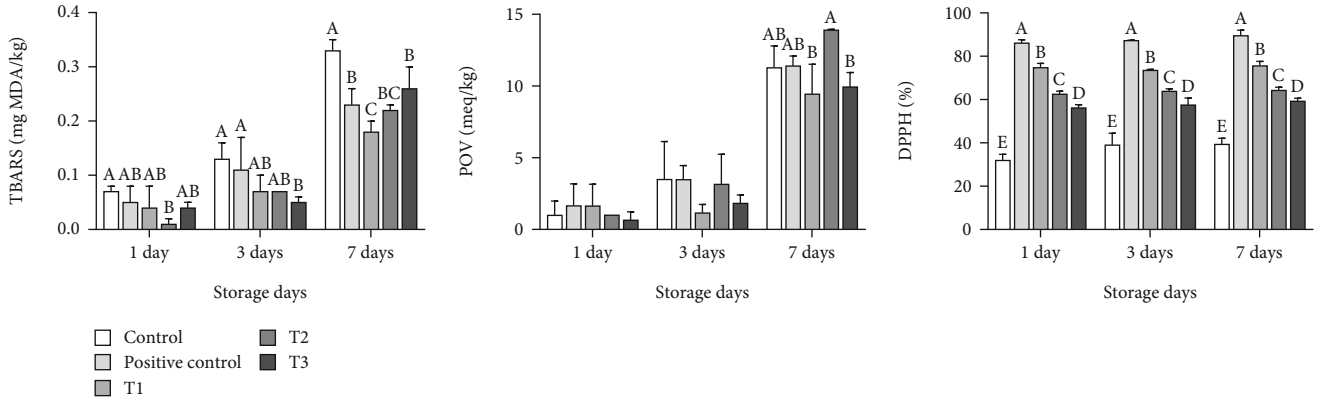

Figure 2: TBARS, POV, DPPH for patties with gromwell root extract and lettuce powder. There is a significant difference between patties with different additives that have different letters (A–E), based on their means ± standard deviations ($p < 0.05$). Control, no addition; positive control, addition of 50 ppm sodium nitrite + 0.1% ascorbic acid; T1, 0.3% gromwell root extract; T2, 0.1% lettuce powder; T3, 0.3 % gromwell root extract + 0.1% lettuce powder.

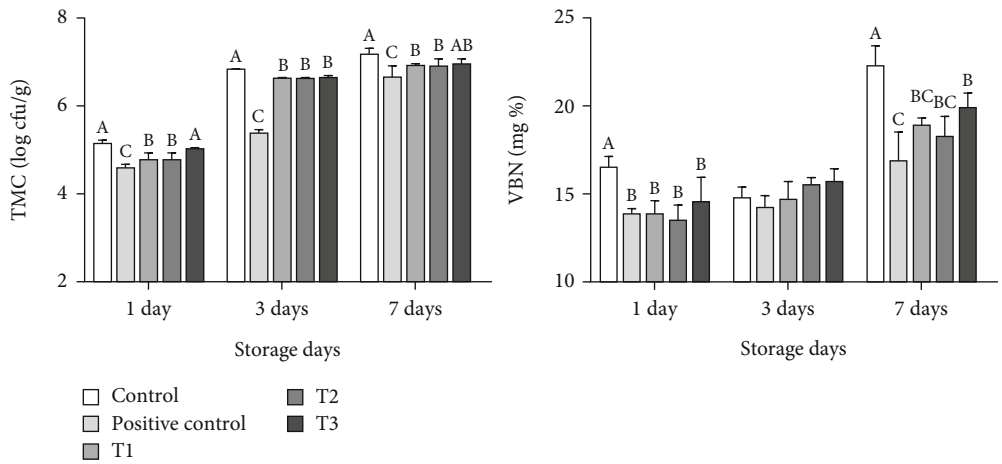

Figure 3: TMC and VBN for patties with gromwell root extract and lettuce powder. There is a significant difference between patties with different additives that have different letters (A–C), based on their means ± standard deviations ($p < 0.05$). Control, no addition; positive control, addition of 50 ppm sodium nitrite + 0.1% ascorbic acid; T1, 0.3% gromwell root extract; T2, 0.1% lettuce powder; T3, 0.3% gromwell root extract + 0.1% lettuce powder.

proteins into amines, ammonia, and other alkaline nitrogenous substances that can be measured by the VBN measurement [63]. Additionally, as spoilage bacteria and endogenous enzyme activities increased, the VBN value also increased [8]. Therefore, VBN values in meat are highly correlated with microbial counts. For fresh meat, the Korean Food Code specifies a VBN value of less than 20 mg/% [64].

TMC and VBN measurements confirmed that gromwell root extract and lettuce powder have antimicrobial effects on pork patties. The antimicrobial effect was found to be better when gromwell root extract and lettuce powder were used separately rather than used together.

4. Conclusion

The addition of gromwell root extract to pork patties (T1) had positive effects on WHC, CL, and meat color. Furthermore, in the storage experiments measuring TBARS, DPPH radical scavenging capacity, TMC, and VBN, it was shown to improve storage stability with antioxidant and antimicrobial effects. However, it had a negative effect on flavor, bitterness, and off-odor of sensory evaluation. This disadvantage could potentially be solved by adding spices or seasoning or changing the cooking method. When lettuce powder was added to pork patties (T2), WHC and storage stability were improved, and there was no difference in sensory evaluation compared to the control group. The addition of gromwell root extract and lettuce powder to pork patties (T3) had a positive effect on WHC, CL, and meat color. However, in storage experiments, it tended to be less effective than patties with separately added gromwell root extract and lettuce powder (T1 and T2). And it had a bad effect on flavor, bitterness, and off-odor of sensory evaluation.

In summary, gromwell root extract and lettuce powder have the potential to replace nitrites in pork meat patties. Gromwell is a good option when antioxidant, antimicrobial, WHC, CL, and red meat color effects are required, while lettuce is a good option when antioxidant and antimicrobial

effects are required without affecting its organoleptic properties. Adding gromwell root extract and lettuce powder together tends to have lower antioxidant and antimicrobial effects than adding them separately. Therefore, when using gromwell and lettuce as nitrite substitutes, it is recommended to use either 0.3% gromwell root extract or 0.1% lettuce powder alone, rather than combining both.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of Interest.

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