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## Effects of Lemon Extract Powder and Vinegar Powder on the Quality Properties of Naturally Cured Sausages with White Kimchi Powder

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**Abstract** This study investigated the effects of lemon extract powder and vinegar powder on the physicochemical and microbiological characteristics of pork sausages naturally cured using white kimchi powder during storage for 30 days. Six batches were included: control (0.01% sodium nitrite and 0.05% sodium ascorbate); treatment 1 (0.3% white kimchi powder and 0.5% lemon extract powder); treatment 2 (0.3% white kimchi powder and 1.0% lemon extract powder); treatment 3 (0.3% white kimchi powder and 0.5% vinegar powder); treatment 4 (0.3% white kimchi powder and 1.0% vinegar powder); and treatment 5 (0.3% white kimchi powder, 0.5% lemon extract powder, and 0.5% vinegar powder). Treatment 2 had significantly lower pH values and higher cooking loss than the other batches ( $p < 0.05$ ). Treatments 1, 2, and 5 had similar ( $p > 0.05$ ) CIE  $a^*$  as the control, while treatments 3 and 4 showed significantly lower values ( $p < 0.05$ ). The residual nitrite content in naturally cured products was lower than the control ( $p < 0.05$ ), while treatments 1 and 2 showed significantly higher nitrosyl hemochrome content and curing efficiency ( $p < 0.05$ ). TBARS values were similar for all treatments and the control ( $p > 0.05$ ). Treatments 1 and 2 showed significantly reduced aerobic plate counts (APC;  $p < 0.05$ ) than the control and other treatments. However, across all batches, TBARS values and APC significantly increased during storage ( $p < 0.05$ ). Our results suggest that lemon extract powder, rather than vinegar powder, may offer a promising alternative for supplementing the functions of nitrite in naturally cured sausages.

**Keywords** lemon extract powder, vinegar powder, sodium nitrite, white kimchi powder, naturally cured sausages

### Introduction

Nitrite is one of the most commonly used curing agents; it has multiple beneficial effects on the cured meat, including improved color, antimicrobial properties, unique flavor, and inhibition of lipid oxidation (Cassens, 1995; Pegg and Shahidi, 2000; Sindelar and Milkowski, 2011). These effects can be achieved in cured meat by adding 50–100 ppm of nitrite (Sebranek, 2009). However, consumers have a negative perception of nitrite in meat products due to health concerns related to the potential for

the formation of carcinogenic nitrosamine (Bedale et al., 2016; Jeong, 2016). Thus, consumers and the meat processing industry continue to favor clean-label meat products with natural ingredients (Asioli et al., 2017; Bedale et al., 2016; Yong et al., 2021).

As an alternative for synthetic nitrite, the use of plant-based materials such as vegetable powders with high nitrate content and a nitrate-reducing starter culture can result in equivalent quality characteristics to products containing sodium nitrite (Sebranek and Bacus, 2007). However, the residual nitrite content in naturally cured meat products is generally lower than that in traditionally cured products (Gabaza et al., 2013; Jeong et al., 2020a; Jeong et al., 2020b; Sebranek and Bacus, 2007; Yong et al., 2021), which could result in poor color stability, lipid oxidation, and microbiological safety during storage (Jackson et al., 2011; Jeong et al., 2020a; Lee et al., 2018; Siekmann et al., 2021). Therefore, it is important to consider alternative means to compensate for low residual nitrite levels in naturally cured meat products. Natural ingredients from fruits and vegetables have been proposed as suitable alternatives for synthetic food additives preferred by health-conscious consumers (Aziz and Karboune, 2018; Price et al., 2013; Sebranek et al., 2012; Shah et al., 2014).

Kimchi is widely recognized, not only for its high nutritional value, but also for its numerous health benefits such as the promotion of lactic acid bacteria during fermentation. Kimchi has also been reported to contain beneficial dietary fibers, minerals, amino acids, vitamins, and phytochemicals (Kim and Shin, 1997; Patra et al., 2016). Furthermore, kimchi is a rich natural source of nitrite/nitrate because of the high nitrate content in its raw materials (Kang et al., 2016). Recently, Choi et al. (2020) attempted to replace synthetic nitrite and ascorbate with white kimchi powder and acerola juice powder in cooked pork products. The addition of these natural ingredients effectively improved the cured meat color, compared to traditionally cured products, although there was limited inhibition of lipid oxidation. Thus, further research is needed to find a solution that can supplement the antioxidant effect by replacing sodium nitrite and ascorbate in meat products.

Lemon and vinegar are rich natural sources of organic acids and other bioactive compounds and may be promising alternatives for synthetic nitrite and ascorbate. Lemon juices and extracts have very low pH due to the high levels of acids such as ascorbic acid, citric acid, and malic acid (Baseler, 2009; Burdurlu et al., 2006). The high levels of acetic acid in vinegar can lower the pH of meat products. Lemon juice and vinegar may thus act as reductants, subsequently accelerating the curing reactions during processing (Lavieri et al., 2014; Sebranek, 1979). In addition, these natural supplements have antioxidative effects against lipid oxidation and can delay microbial growth (Fernández-López et al., 2005; Sebranek et al., 2012; Xi et al., 2011). Although some studies have investigated the inhibitory effect on microorganisms in traditionally or naturally cured meat products using lemon juice and vinegar (King et al., 2015; Li et al., 2012), the effects of lemon extract powder and vinegar powder on the quality characteristics of meat products cured with white kimchi powder have not been reported. We hypothesized that lemon extract powder or vinegar powder would increase the nitrite reactions and enhance the antioxidant as well as antimicrobial activity in naturally cured sausages with white kimchi powder, without synthetic additives. Furthermore, the addition of these ingredients may improve the quality and extend shelf-life by replacing or supplementing the function of sodium nitrite and ascorbate in naturally cured meat products with less nitrate.

Therefore, the aims of this study were to investigate the effect of treating naturally cured meat with lemon juice powder or vinegar powder on: (1) the color and pigment properties related to meat curing and (2) the inhibition of lipid oxidation and antimicrobial activities. We also evaluated the possibility of replacing these natural powders with sodium nitrite and sodium ascorbate, by comparing them with traditionally cured sausages.

## Materials and Methods

### Preparation of white kimchi powder and other materials

Chinese cabbages and radishes grown in the five regions of South Korea (Chungcheong-do, Gangwon-do, Gyeonggi-do, Gyeongsang-do, and Jeolla-do) were purchased and randomly selected for white kimchi production. Garlic, ginger, fermented shrimp, solar salt, and refined salt were purchased from local retail markets (Busan, Korea). Fermented white kimchi was prepared according to the standardized recipe for Korean food (Institute of Traditional Korean Food, 2008). After 2 wk of fermentation at 0°C, white kimchi was powdered as described in previous studies (Choi et al., 2020) using a freeze vacuum dryer (PVTFD10R, Ilshinbiobase, Yangju, Korea) at -40°C for 2 d and filtered through a 30-mesh sieve (Test sieve BS0600, Chunggye Sieve, Gunpo, Korea). The white kimchi powder prepared had a pH of 6.24 and 19,634 ppm sodium nitrate. It was vacuum-packaged and stored in a freezer at -18°C until use.

A starter culture (S-B-61, Christian Hansen, Milwaukee, WI, USA) comprising *Staphylococcus carnosus*, sodium nitrite (S2252, Sigma-Aldrich, St. Louis, MO, USA), sodium ascorbate (#35268, Acros Organics, Geel, Belgium), lemon extract powder (#13101000018, Jung Ang Tafla, Yesan, Korea), and vinegar powder (Verdad® Powder N6, Corbion NV, Amsterdam, The Netherlands) were purchased from commercial suppliers. Other ingredients and spices were obtained from an ingredient supplier (Taewon Food Industry, Ansan, Korea).

### Processing of ground pork sausages

Fresh pork ham (*M. biceps femoris*, *M. semitendinosus*, and *M. semimembranosus*) muscles and backfat were purchased from a local meat processor (Pukyung Pig Farmers Livestock, Gimhae, Korea) at 24–48 h postmortem. After trimming and cutting into squares of approximately 4–5 cm, the raw materials were stored at -18°C and processed within 1 month. A total of 35 kg of meat per replicate was prepared for manufacturing the ground pork sausages, and sausage processing was replicated thrice. Before processing, frozen pork ham and backfat were completely thawed at 2°C–3°C and ground using a chopper (TC-22 Elegant plus, Tre Spade, Torino, Italy) equipped with an 8-mm plate and sequentially with a 3-mm plate. The ground materials were randomly assigned to one of six treatment groups (Table 1): control (0.01% sodium nitrite and 0.05% sodium ascorbate); treatment 1 (0.3% white kimchi powder and 0.5% lemon extract powder); treatment 2 (0.3% white kimchi powder and 1% lemon extract powder); treatment 3 (0.3% white kimchi powder and 0.5% vinegar powder); treatment 4 (0.3% white kimchi powder and 1% vinegar powder); and treatment 5 (0.3% white kimchi powder, 0.5% lemon extract powder, and 0.5% vinegar powder). The nitrate content from the white kimchi powder was calculated to be 60 ppm for naturally cured sausages (treatments 1 to 5), which contained 0.03% starter culture to promote the conversion of the natural nitrate in the white kimchi powder to nitrite. Intentionally, any phosphates were not incorporated because this study was to investigate the quality of meat products with a clean-label concept by replacing sodium nitrite and sodium ascorbate with natural ingredients. The ground pork meat and fat were mixed with 1.5% NaCl using a mixer (5KSM7990, Whirlpool, St. Joseph, MI, USA) for 3 min and the other ingredients along with ice/water were added into the mixer and mixed again for an additional 7 min (Table 1). The meat mixtures from each treatment were manually stuffed into 24-mm cellulose casings (NOJAX® Cellulose Casings, Viskase Companies, Lombard, IL, USA) using a stuffer (TRE SPADE® MOD.5/V Deluxe, FACEM S.p.A, Torino, Italy). The control samples were then placed in a refrigerator (C110AHB, LG Electronics, Changwon, Korea) at 2°C–3°C for 1 h, while the naturally cured batches were incubated at 40°C for 2 h in an incubator (C-IB4, Changshin Science, Pocheon, Korea). All samples were then cooked to an internal temperature of 75°C in a water bath

**Table 1.** The formulation for naturally cured pork sausages with white kimchi powder as a natural nitrate

Materials and ingredients (%)	Treatments <sup>1)</sup>					
	Control	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Pork ham	70.00	70.00	70.00	70.00	70.00	70.00
Pork backfat	15.00	15.00	15.00	15.00	15.00	15.00
Ice/water	15.00	15.00	15.00	15.00	15.00	15.00
Sub total	100.00	100.00	100.00	100.00	100.00	100.00
Salt	1.50	1.50	1.50	1.50	1.50	1.50
Sugar	1.00	1.00	1.00	1.00	1.00	1.00
Mustard powder	0.15	0.15	0.15	0.15	0.15	0.15
Pepper	0.25	0.25	0.25	0.25	0.25	0.25
Onion powder	0.10	0.10	0.10	0.10	0.10	0.10
Garlic powder	0.10	0.10	0.10	0.10	0.10	0.10
Sodium nitrite	0.01	-	-	-	-	-
Sodium ascorbate	0.05	-	-	-	-	-
White kimchi powder	-	0.30	0.30	0.30	0.30	0.30
Starter culture	-	0.03	0.03	0.03	0.03	0.03
Lemon extract powder	-	0.50	1.00	-	-	0.50
Vinegar powder	-	-	-	0.50	1.00	0.50
Total	103.16	103.93	104.43	103.93	104.43	104.43

<sup>1)</sup> Treatments: Control, 0.01% sodium nitrite+0.05% sodium ascorbate; treatment 1, 0.3% white kimchi powder+0.5% lemon extract powder; treatment 2, 0.3% white kimchi powder+1.0% lemon extract powder; treatment 3, 0.3% white kimchi powder+0.5% vinegar powder; treatment 4, 0.3% white kimchi powder+1.0% vinegar powder; and treatment 5, 0.3% white kimchi powder+0.5% lemon extract powder+0.5% vinegar powder.

(MaXturdy 45, Daihan Scientific, Wonju, Korea) set to 90°C. The products were then immediately cooled for 20 min on slurry ice. Samples from each treatment were individually vacuum-packaged into polyethylene/nylon pouches using a vacuum packaging machine (M6-TM, Leepack, Incheon, Korea) and stored at 2°C–3°C for 30 d in the dark until analysis. Measurements for all dependent variables, except cooking loss, were made in duplicate on 0, 15, and 30 d.

### Determination of pH values and cooking loss

Five grams of the samples were blended with 45 mL of distilled water and homogenized for 1 min at 8,000 rpm using a homogenizer (DI 25 basic, IKA®-Werke GmbH & Co. KG, Staufen, Germany). The pH of the homogenates was measured using a pH meter (Accumt® AB150, Thermo Fisher Scientific, Singapore, Singapore).

The sausages were weighed before cooking and again after cooking and cooling. After peeling the casing from the cooked sausages, the drips of each sausage and casing were removed using a paper towel, and then the weight was measured to determine the cooking loss. The cooking loss was calculated using the following equation:

$$\text{Cooking loss (\%)} = \frac{\text{Sausage weight before cooking (g)} - \text{Sausage weight after cooking (g)}}{\text{Sausage weight before cooking (g)}} \times 100$$

### CIE color measurements

The Commission Internationale de l'Eclairage (CIE) lightness ( $L^*$ ), redness ( $a^*$ ), and yellowness ( $b^*$ ) of the cut surfaces of the samples were measured using a chromameter (CR-400, Konica Minolta Sensing, Osaka, Japan; 8 mm aperture) with illuminant C and 2° observer angle. Each cooked sample was cut parallel to the longitudinal axis and divided into two slices. Color measurements were performed on the fresh-cut surface at two random locations per sliced sample. Four readings per treatment were performed for each replicate. Before measurement, the chroma meter was calibrated using a white calibration tile ( $L^*$ : +94.90,  $a^*$ : -0.41,  $b^*$ : +3.88).

### Determination of nitrate and residual nitrite content

The nitrate ion ( $\text{NO}_3^-$ ) content in the prepared white kimchi powder was analyzed using the zinc reduction method (Merino, 2009). Results were converted in terms of concentration of sodium nitrate (ppm). The residual nitrite content in the cooked pork sausages was analyzed using AOAC method 973.31 (AOAC, 2016). The calibration curve was prepared using sodium nitrite as a standard (S2252, Sigma-Aldrich, St. Louis, MO, USA) and the result of residual nitrite content was reported in ppm.

### Determination of nitrosyl hemochrome, total pigment, and curing efficiency

Nitrosyl hemochrome and total pigment content were measured after extraction using 80% acetone and acidified acetone solution, respectively, according to the method described by Hornsey (1956). The absorbance of the filtrate was measured at 540 nm ( $A_{540}$ ) and 640 nm ( $A_{640}$ ) using a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan), and the following equations were used to calculate the nitrosyl hemochrome content and total pigment content. Finally, the curing efficiency was calculated as a percentage of the ratio of nitrosyl hemochrome to total meat pigment content.

$$\text{Nitrosyl hemochrome (ppm)} = A_{540} \times 290$$

$$\text{Total pigment (ppm)} = A_{640} \times 680$$

$$\text{Curing efficiency (\%)} = \frac{\text{Nitrosyl hemochrome (ppm)}}{\text{Total pigment (ppm)}} \times 100$$

### Determination of thiobarbituric acid reactive substances (TBARS)

Lipid oxidation was analyzed according to the distillation method described by Tarladgis et al. (1960). Malondialdehyde (MDA) released by lipid oxidation first reacted with 0.02 M 2-thiobarbituric acid (TBA) solution and the absorbance was measured at 538 nm using a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). The obtained results were multiplied by a factor of 7.8 to calculate the TBARS value in milligrams of MDA per kilogram of cooked samples.

### Microbiological analysis

The total aerobic plate counts (APC), *E. coli*, and coliforms for cooked samples were determined by the procedure recommended by the Food Code of the Ministry of Food and Drug Safety (MFDS, 2021). The samples (25 g) were diluted

with 225 mL of 0.85% sterile saline solution in a sterile stomacher bag to achieve ten-fold dilution and homogenized for 2 min. One milliliter of the homogenized extract was serially diluted in 9 mL of sterile saline solution. One milliliter of the sample aliquot or diluent was plated onto an aerobic count plate (Petrifilm™ Aerobic Count Plate, 3M, St. Paul, MN, USA) and an *E. coli*/coliform count plate (Petrifilm™ *E. coli*/Coliform Count Plate, 3M). The plates were incubated at 35±2°C for 48 h. After incubation, the colonies on the plates were counted. The final counts were calculated by multiplying the observed counts by the dilution factor and reported as Log colony-forming units (CFU)/g following log transformation.

### Statistical analysis

The experiments were replicated thrice with independent replicates on different days. The experimental design was a split-plot design, with the six treatments (control and five naturally cured treatments) representing the whole plot factor and the three storage periods (0, 15, and 30 days) representing the split-plot factor. The fixed effects for the treatments and the storage period and their interactions were performed using the PROC GLIMMIX procedure of the SAS program (SAS, 2012). For significant differences in the model, least square mean was separated ( $p < 0.05$ ) by pairwise comparisons using the LINES options in the PROC GLIMMIX procedure of SAS.

## Results and Discussion

### The pH and cooking loss

The treatment (T) were found to significantly affect the pH of the product ( $p < 0.0001$ ; Table 2). In contrast, the storage period (S) did not affect ( $p > 0.05$ ) the pH of the product. No two-way interactions (T×S) were observed between these two effects on pH ( $p > 0.05$ ). Pork sausages cured with natural ingredients (treatments 1 to 5) showed lower ( $p < 0.05$ ) pH than the traditionally cured control (Table 3). As the concentration of lemon extract powder or vinegar powder increased, the pH of naturally cured sausages significantly decreased ( $p < 0.05$ ). Treatment 2 (1.0% lemon extract powder) resulted in the lowest ( $p < 0.05$ ) pH of the product. In our preliminary study, the measured pH of raw pork meat, white kimchi powder, lemon extract powder, and vinegar powder were 5.80, 6.24, 3.23, and 5.91, respectively. Therefore, it is possible that the pH of the natural ingredients directly affected that of the final products; furthermore, the lemon extract powder, which had the lowest pH of the

**Table 2.** Significance of main and interaction effects of lemon extract powder and vinegar powder on the quality properties of naturally cured pork sausages with white kimchi powder

Main and interaction effects <sup>1)</sup>	Dependent variables													
	pH	Cooking loss	CIE L*	CIE a*	CIE b*	Nitrite	Nitrosyl hemochrome	Total pigment	Curing efficiency	TBARS	APC	<i>E. coli</i> counts	Coliform counts	
Treatment (T) <sup>2)</sup>	**	**	**	*	**	**	**	*	*	NS	**	NS	NS	
Storage day (S) <sup>3)</sup>	NS	–	**	NS	*	**	**	NS	*	**	**	NS	NS	
T×S	NS	–	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	

<sup>1)</sup> Main and interaction effects: \*  $p < 0.05$ , \*\*  $p < 0.0001$ .

<sup>2)</sup> Treatment: Control, 0.01% sodium nitrite+0.05% sodium ascorbate; treatment 1, 0.3% white kimchi powder+0.5% lemon extract powder; treatment 2, 0.3% white kimchi powder+1.0% lemon extract powder; treatment 3, 0.3% white kimchi powder+0.5% vinegar powder; treatment 4, 0.3% white kimchi powder+1.0% vinegar powder; treatment 5, 0.3% white kimchi powder+0.5% lemon extract powder+0.5% vinegar powder.

<sup>3)</sup> Storage day: All samples were stored at 2°C–3°C for 30 days in the dark until analysis. Measurements for all dependent variables except for cooking loss were made on 0, 15, and 30 days.

CIE L\*, lightness; CIE a\*, redness; CIE b\*, yellowness; Nitrite, residual nitrite content; TBARS, 2-thiobarbituric acid reactive substances; APC, aerobic plate counts; NS, not significant.

**Table 3.** Effects of lemon extract powder and vinegar powder on pH values, cooking loss, and CIE color of naturally cured pork sausages with white kimchi powder

Main effects	Dependent variables				
	pH	Cooking loss (%)	CIE L*	CIE a*	CIE b*
Treatment <sup>1)</sup>					
Control	6.12 <sup>A</sup>	5.57 <sup>D</sup>	66.33 <sup>A</sup>	8.90 <sup>A</sup>	8.78 <sup>E</sup>
Treatment 1	5.92 <sup>D</sup>	9.78 <sup>B</sup>	65.66 <sup>B</sup>	8.81 <sup>A</sup>	10.73 <sup>A</sup>
Treatment 2	5.84 <sup>E</sup>	11.43 <sup>A</sup>	65.48 <sup>B</sup>	8.94 <sup>A</sup>	10.78 <sup>A</sup>
Treatment 3	5.99 <sup>B</sup>	6.15 <sup>D</sup>	65.22 <sup>C</sup>	8.51 <sup>B</sup>	9.99 <sup>C</sup>
Treatment 4	5.96 <sup>C</sup>	5.78 <sup>D</sup>	64.99 <sup>C</sup>	8.57 <sup>B</sup>	9.80 <sup>D</sup>
Treatment 5	5.92 <sup>D</sup>	7.68 <sup>C</sup>	65.23 <sup>C</sup>	8.84 <sup>A</sup>	10.49 <sup>B</sup>
SEM	0.01	0.27	0.46	0.26	0.05
Storage day <sup>2)</sup>					
Day 0	5.96 <sup>A</sup>	-	64.88 <sup>C</sup>	8.83 <sup>A</sup>	10.04 <sup>B</sup>
Day 15	5.96 <sup>A</sup>	-	65.69 <sup>B</sup>	8.72 <sup>A</sup>	10.07 <sup>B</sup>
Day 30	5.95 <sup>A</sup>	-	65.88 <sup>A</sup>	8.73 <sup>A</sup>	10.18 <sup>A</sup>
SEM	0.01		0.45	0.26	0.04

<sup>1)</sup> Treatment: Control, 0.01% sodium nitrite+0.05% sodium ascorbate; treatment 1, 0.3% white kimchi powder+0.5% lemon extract powder; treatment 2, 0.3% white kimchi powder+1.0% lemon extract powder; treatment 3, 0.3% white kimchi powder+0.5% vinegar powder; treatment 4, 0.3% white kimchi powder+1.0% vinegar powder; and treatment 5, 0.3% white kimchi powder+0.5% lemon extract powder+0.5% vinegar powder.

<sup>2)</sup> Storage day: All samples were stored at 2°C–3°C for 30 days in the dark until analysis. Measurements were made on 0, 15, and 30 days.

<sup>A-E</sup> Means within a column with different superscript letters are significantly different ( $p < 0.05$ ).

ingredients tested, also had the most drastic effect on the pH of the product. These results are similar to those of Xi et al. (2012), who found combinations of different levels of cranberry powder (pH 2.2) with cherry powder or lime powder decreased the pH of naturally cured frankfurters. However, the observed decrease in pH in this study may have a significant impact on accelerating the curing reaction by simulating reducing conditions (Sebranek, 1979; Sebranek, 2009).

The treatment significantly affected the cooking loss as well ( $p < 0.0001$ ) (Table 2). Treatments 1 and 2 (lemon extract powder at 0.5% and 1.0%, respectively) and treatment 5 (lemon extract powder at 0.5% and vinegar powder at 0.5%) resulted in higher cooking loss than the control ( $p < 0.05$ ), whereas treatments 3 and 4 (vinegar powder at 0.5% and 1.0%, respectively) resulted in a level of cooking loss similar to that seen in the control ( $p > 0.05$ ) (Table 3). Treatment 2 caused the most cooking loss ( $p < 0.05$ ), suggesting that lemon extract powder may affect cooking loss by lowering the pH of naturally cured sausages. Sebranek and Bacus (2007) reported that lemon juice solids or powder, which are major sources of citric acid, could lower the pH of natural or organic products, as phosphates cannot be used to improve the water-binding properties of such products. Therefore, improving water retention is a crucial requirement when dealing with natural ingredients. Consistent with our results, Choi et al. (2020) attempted to use white kimchi powder and acerola juice powder to replace sodium nitrite and sodium ascorbate in indirectly cured meat products. They found that the products showed significantly decreased cooking yield when treated with acerola juice powder with a pH of 3.3.

### Instrumental color

Both treatment (T) and storage period (S) significantly affected the CIE L\* ( $p < 0.0001$ ), but no two-way interactions were

observed between the two main effects ( $T \times S$ ,  $p > 0.05$ ) (Table 2). The naturally cured sausages (treatments 1 to 5) showed lower ( $p < 0.05$ ) CIE  $L^*$  than the control (Table 3). When vinegar powder was added at 0.5% and 1.0% (treatments 3 and 4) and when lemon extract powder and vinegar powder were added in combination (both at 0.5%) (treatment 5), the CIE  $L^*$  of the product was significantly lower ( $p < 0.05$ ) than when only lemon extract powder was used (treatments 1 and 2). Studies on the use of natural ingredients in meat products have revealed that product color achieved using celery juice concentrate (Horsch et al., 2014), cranberry powder (Xi et al., 2012), and dog rose extract (Vossen et al., 2012) is darker than that achieved with traditional methods. Sullivan et al. (2012a) reported that treatment with a blend of vinegar, lemon, and cherry powder resulted in lower  $L^*$  in nitrite/nitrate-cured hams, which is consistent with our results. The CIE  $L^*$  also increased ( $p < 0.05$ ) with storage period (Table 3). This increase in CIE  $L^*$  may be related to the light scattering effect caused by an increase in water loss due to poor water retention during storage (O'Keeffe and Hood, 1981; Wu et al., 2020). These results were consistent with those of Fernández-López et al. (2005), who treated beef meatballs with lemon extracts and orange extracts.

The CIE  $a^*$  represent the redness of the product and is an important parameter as it is related to the nitrosyl hemochrome content in the cured meat products (AMSA, 2012; Barbut, 2010). In this study, the CIE  $a^*$  of cured pork sausages were affected only by the treatment ( $T$ ,  $p < 0.05$ ; Table 2), and no effects of the storage period ( $S$ ) or two-way interactions ( $T \times S$ ) were significant ( $p > 0.05$ ). The CIE  $a^*$  ranged from 8.51 to 8.94, with no differences in the CIE  $a^*$  between the control and treatments 1, 2, and 5 ( $p > 0.05$ ) (Table 3). Similar results were reported by Xi et al. (2012), who reported that the use of 1% cranberry powder did not affect the redness of naturally cured frankfurters, compared to sodium nitrite-added products. Lavieri et al. (2014) found similar results for alternatively cured frankfurters using cranberry powder, dried vinegar, and a blend of lemon juice and vinegar concentrate. However, treatments 3 and 4 (0.5% and 1% vinegar powder, respectively) resulted in lower redness (lower CIE  $a^*$ ,  $p < 0.05$ ) than the control and other treatments. Vinegar powder at 0.5% and 1.0% only reduced CIE  $a^*$  by 0.39 units and 0.33 units (4.4% and 3.7% reduction), respectively, compared to the control, although this was statistically significant. Nonetheless, the low redness in products treated with vinegar powder may be a reflection of the low nitrosyl hemochrome found in this study. Therefore, combining white kimchi powder with lemon extract powder was more effective for improving cured color properties in naturally cured sausages than combining white kimchi powder with vinegar powder. Furthermore, in all sausages tested in this study, the CIE  $a^*$  showed no changes over storage ( $p > 0.05$ ), suggesting that the redness remained stable during storage, regardless of nitrite/nitrate source and supplemental natural ingredients.

CIE  $b^*$  were affected by both treatment ( $T$ ,  $p < 0.0001$ ) and storage period ( $S$ ,  $p < 0.05$ ; Table 2), although two-way interactions ( $T \times S$ ,  $p > 0.05$ ) were not significant. All the treatment groups (treatment groups 1 to 5) showed higher CIE  $b^*$  than the control ( $p < 0.05$ ), indicating more yellowness (Table 3). This high yellowness of naturally cured sausages could be attributed to the plant pigments present in the plant-derived powders, as has been observed in several researches previously (Horsch et al., 2014; Jeong et al., 2020b; Jin et al., 2016; Nowak et al., 2016; Xi et al., 2012). Regardless of the concentration, when lemon extract powder was used on cured sausages (treatments 1 and 2), higher CIE  $b^*$  were observed than all other batches ( $p < 0.05$ ). This was likely due to the influence of carotenoid pigments, which are known to be present in citrus fruits (Viuda-Martos et al., 2010; Yokoyama and Vandercook, 1967). Consistent with our results, Fernández-López et al. (2005) reported increased yellowness in beef meatballs treated with citrus extracts, which they attributed to the presence of carotene in the citrus extracts. We found no change ( $p > 0.05$ ) in CIE  $b^*$  during storage for the first 15 d, although they showed significant increase ( $p < 0.05$ ) by 30 d (Table 3).



### Residual nitrite, nitrosyl hemochrome, total pigment, and curing efficiency

The residual nitrite content in the cured pork sausages was affected by treatment (T,  $p < 0.0001$ ) and storage period (S,  $p < 0.0001$ ) (Table 2). A two-way interaction (T×S) effect was also significant ( $p < 0.05$ ) in this case (Table 4). The control sausages had the highest ( $p < 0.05$ ) residual nitrite content (18.23 ppm), and about 82% of the initial nitrite content was depleted during processing. This is a higher reduction in nitrite content than reported in a previous study on frankfurters (~75%) (Xi et al., 2012). The naturally cured products (treatments 1 to 5) had significantly lower ( $p < 0.05$ ) residual nitrite content than the control, which is consistent with several previous studies (Jeong et al., 2020a; Jeong et al., 2020b; Viuda-Martos et al., 2010; Xi et al., 2012). Among the naturally cured sausages, there were no differences ( $p > 0.05$ ) in residual nitrite content between the treatments 1 and 2, which treated with different concentrations of lemon extract powder. However, in the products treated with vinegar powder, the residual nitrite content tended to decrease as vinegar powder concentration increased, with treatment 4 showing a lower ( $p < 0.05$ ) residual nitrite content than the control and treatments 1 and 2. Jackson et al. (2011) reported that naturally cured frankfurters treated with a cultured sugar and vinegar blend had lower residual nitrite than those treated with a vinegar, lemon, and cherry powder blend. They speculated that the blend of cultured sugar and vinegar affected the activity of the nitrate-to-nitrite reduction (by *Staphylococcus carnosus*), thereby resulting in less nitrate-nitrite conversion. Likewise, the low residual nitrite content in vinegar-treated products might be attributed to the impaired activity of the nitrate-reducing starter culture. This may be further supported by our nitrosyl hemochrome content analysis. On 0 d, treatments 3 to 5 (vinegar powder alone or combined with lemon extract powder) showed a lower ( $p < 0.05$ ) residual nitrite content than treatments 1 and 2 (lemon extract powder alone). However, on 15 d and 30 d, there were no significant differences ( $p > 0.05$ ) in residual nitrite content among any of the naturally cured treatments. In addition, the residual nitrite content decreased ( $p < 0.05$ ) with storage period (Table 4), which was consistent with results from Hernández-Hernández et al. (2009) and Lavieri et al. (2014). Treatments 1, 2, and 5 showed significantly more reduction ( $p < 0.05$ ) in residual nitrite content on 30 d than on 0 d. However, treatments 3 and 4 (vinegar powder alone) showed no significant changes ( $p > 0.05$ ) in residual nitrite content during storage. These results indicate that the minimum nitrite content was

**Table 4.** Interaction effects of treatment (treatments 1 to 5) and storage day (0, 15, and 30 days) on nitrite content (ppm) of naturally cured pork sausages with white kimchi powder

Treatment <sup>1)</sup>	Storage day <sup>2)</sup>			
	Day 0	Day 15	Day 30	Overall mean
Control	25.11 <sup>Ax</sup>	18.00 <sup>Ay</sup>	11.61 <sup>Az</sup>	18.23 <sup>A</sup>
Treatment 1	8.23 <sup>Bx</sup>	5.54 <sup>Bxy</sup>	3.31 <sup>By</sup>	5.69 <sup>B</sup>
Treatment 2	9.13 <sup>Bx</sup>	5.00 <sup>By</sup>	3.00 <sup>By</sup>	5.70 <sup>B</sup>
Treatment 3	5.59 <sup>BCx</sup>	3.86 <sup>Bx</sup>	3.32 <sup>Bx</sup>	4.25 <sup>BC</sup>
Treatment 4	4.37 <sup>Cx</sup>	3.00 <sup>Bx</sup>	3.11 <sup>Bx</sup>	3.49 <sup>C</sup>
Treatment 5	7.77 <sup>BCx</sup>	4.42 <sup>Bxy</sup>	3.18 <sup>By</sup>	5.12 <sup>BC</sup>
Overall mean	10.03 <sup>x</sup>	6.64 <sup>y</sup>	4.59 <sup>z</sup>	

<sup>1)</sup> Treatment: Control, 0.01% sodium nitrite+0.05% sodium ascorbate; treatment 1, 0.3% white kimchi powder+0.5% lemon extract powder; treatment 2, 0.3% white kimchi powder+1.0% lemon extract powder; treatment 3, 0.3% white kimchi powder+0.5% vinegar powder; treatment 4, 0.3% white kimchi powder+1.0% vinegar powder; and treatment 5, 0.3% white kimchi powder+0.5% lemon extract powder+0.5% vinegar powder.

<sup>2)</sup> Storage day: All samples were stored at 2°C–3°C for 30 days in the dark until analysis. Measurements were made on 0, 15, and 30 days.

<sup>A–C</sup> Means within a column with different superscript letters are significantly different (SEM: 0.78;  $p < 0.05$ ).

<sup>x–z</sup> Means within a row with different superscript letters are significantly different (SEM: 0.59;  $p < 0.05$ ).

maintained during storage, which is consistent with the results of Baseler (2009) for Canadian-style bacon. Meanwhile, lemon and vinegar are known to be rich in organic acids, including citric acid and acetic acid, and various bioactive compounds with low pH (Simpson and Sofos, 2009), which can convert nitrite to nitric oxide by acting as acidulants (Pegg and Shahidi, 2000; Sebranek and Bacus, 2007). This may explain our observation of accelerated reduction of residual nitrite content in this study.

Nitrosyl hemochrome is a stable pink pigment formed in cured meat products after cooking and is important for the development of the typical cured meat color (Cassens, 1997; Claus and Jeong, 2018; Suman and Joseph, 2013). The nitrosyl hemochrome content in the cured pork sausages was affected by both treatment (T,  $p < 0.0001$ ) and storage period (S,  $p < 0.0001$ ), but no significant effects of interaction (T×S) were observed ( $p > 0.05$ ; Table 2). Among the naturally cured meat products, treatments 1 and 2 (lemon extract powder at 0.5% and 1.0%, respectively) had higher ( $p < 0.05$ ) nitrosyl hemochrome content than the control and the other treatments; increased lemon extract powder concentration (0.5% vs. 1%) resulted in an increased nitrosyl hemochrome content as well ( $p < 0.05$ ) (Table 5). These results suggest that the lemon extract powder promotes curing reactions by decreasing the pH, resulting in the rapid development of cured color. In fact, even a pH reduction by just 0.2 units has been shown to double the rate of cured color formation (Sebranek, 1979). This trend was expected from the results of our pH analysis as well, which is consistent with the results of Choi et al. (2020), who reported increased nitrosyl hemochrome content in pork products treated with white kimchi powder by adding celery juice powder with a low pH. However, we also observed low pH in treatments 3 and 4 (vinegar powder at 0.5% and 1.0%, respectively),

**Table 5. Effects of lemon extract powder and vinegar powder on nitrosyl hemochrome, total pigment, curing efficiency, TBARS, and aerobic plate counts of naturally cured pork sausages with white kimchi powder**

Main effects	Dependent variables				
	Nitrosyl hemochrome (ppm)	Total pigment (ppm)	Curing efficiency (%)	TBARS (mg MDA/kg)	Aerobic plate counts (Log CFU/g)
Treatment (T) <sup>1)</sup>					
Control	40.20 <sup>C</sup>	52.02 <sup>C</sup>	77.22 <sup>AB</sup>	0.093 <sup>A</sup>	2.06 <sup>A</sup>
Treatment 1	42.15 <sup>B</sup>	54.06 <sup>AB</sup>	77.93 <sup>AB</sup>	0.109 <sup>A</sup>	1.64 <sup>D</sup>
Treatment 2	43.73 <sup>A</sup>	55.19 <sup>A</sup>	79.21 <sup>A</sup>	0.098 <sup>A</sup>	1.61 <sup>D</sup>
Treatment 3	39.32 <sup>D</sup>	52.76 <sup>BC</sup>	74.53 <sup>DC</sup>	0.087 <sup>A</sup>	1.86 <sup>B</sup>
Treatment 4	37.83 <sup>E</sup>	51.45 <sup>C</sup>	73.47 <sup>D</sup>	0.093 <sup>A</sup>	1.84 <sup>B</sup>
Treatment 5	40.19 <sup>C</sup>	52.59 <sup>BC</sup>	76.37 <sup>BC</sup>	0.093 <sup>A</sup>	1.73 <sup>C</sup>
SEM	2.52	2.10	1.99	0.008	0.03
Storage day (S) <sup>2)</sup>					
Day 0	41.49 <sup>A</sup>	53.15 <sup>A</sup>	77.98 <sup>A</sup>	0.071 <sup>C</sup>	1.70 <sup>C</sup>
Day 15	39.82 <sup>B</sup>	52.56 <sup>A</sup>	75.69 <sup>B</sup>	0.097 <sup>B</sup>	1.79 <sup>B</sup>
Day 30	40.39 <sup>B</sup>	53.32 <sup>A</sup>	75.69 <sup>B</sup>	0.119 <sup>A</sup>	1.88 <sup>A</sup>
SEM	2.51	2.06	1.88	0.007	0.03

<sup>1)</sup> Treatment: Control, 0.01% sodium nitrite+0.05% sodium ascorbate; treatment 1, 0.3% white kimchi powder+0.5% lemon extract powder; treatment 2, 0.3% white kimchi powder+1.0% lemon extract powder; treatment 3, 0.3% white kimchi powder+0.5% vinegar powder; treatment 4, 0.3% white kimchi powder+1.0% vinegar powder; and treatment 5, 0.3% white kimchi powder+0.5% lemon extract powder+0.5% vinegar powder.

<sup>2)</sup> Storage day: All samples were stored at 2°C–3°C for 30 days in the dark until analysis. Measurements were made on 0, 15, and 30 days.

<sup>A–E</sup> Means within a column with different superscript letters are significantly different ( $p < 0.05$ ).

TBARS, 2-thiobarbituric acid reactive substances; MDA, malondialdehydes.

but their results of nitrosyl hemochrome analysis were different from that of the lemon extract powder-treated batches. In treatments 3 and 4, the nitrosyl hemochrome content decreased ( $p < 0.05$ ) when the vinegar powder concentration increased from 0.5% to 1.0%, and was lower ( $p < 0.05$ ) than that in the control and other treatments. As the vinegar powder contained in the formulation of these products might inhibit the reducing activity of the starter culture, it is possible that the nitrate-to-nitrite conversion may have been insufficient. Eventually, insufficient nitrite levels may result in fewer nitrite reactions and subsequently low cured pigment content in the final products (Terns et al., 2011). This could be possibly explained by the low CIE  $a^*$  and residual nitrite content that we observed. However, the nitrosyl hemochrome content was not significantly different between treatment 5 (lemon extract powder and vinegar powder) and the synthetic nitrite-treated control. This was similar to the results reported by Jackson et al. (2011). These results suggest that when lemon extract powder is used alone or in combination with vinegar powder for naturally cured sausages, nitrosyl hemochrome formation may increase, resulting in rapid development of cured meat color comparable to traditionally cured products. In this study, nitrosyl hemochrome content decreased ( $p < 0.05$ ) at 15 d and 30 d of storage, compared to 0 d (Table 5). Similarly, Terns et al. (2011) reported that nitrosyl hemochrome content of indirectly cured sausages treated with celery juice powder and cherry powder as well as sodium nitrite-treated controls decreased during storage.

The total pigment content of the pork sausages was affected by the treatment (T,  $p < 0.05$ ) (Table 2), but not by the storage period (S,  $p > 0.05$ ). No significant two-way interaction effects (T×S,  $p > 0.05$ ) were observed. The total pigment content was higher ( $p < 0.05$ ) in treatments 1 and 2 than in the control (Table 5), but there were no significant differences ( $p > 0.05$ ) in total pigment content between the control and the other treatments. These results indicate that the treatment of naturally cured pork sausages with lemon extract powder or vinegar powder could facilitate the development of cured meat color, which in turn affects the total pigment content in the finished product. Jeong et al. (2020b) and Sullivan et al. (2012b) have reported a positive correlation between nitrosyl hemochrome content and total pigment content in naturally cured products.

Treatment (T) and storage period (S) both significantly affected ( $p < 0.05$ ) the curing efficiency of the pork sausages (Table 2), but no significant two-way interaction (T×S) effect ( $p > 0.05$ ) was observed. There were no significant differences ( $p > 0.05$ ) in curing efficiency between the control and treatments 1, 2, and 5. The control and treatments 1 and 2 showed higher ( $p < 0.05$ ) curing efficiency than treatments 3 and 4 (Table 5). The curing efficiency observed in treatments 1 (77.93%) and 2 (79.21%) were higher than the previously reported value for spinach powder treatment (73.00%), but lower than that for radish powder treatment (83.05%) (Jeong et al., 2020a). However, naturally cured sausages tested in this study had higher curing efficiency (73.47%–79.21%) than those measured in commercially available frankfurters (31.3%–46.2%) (Sullivan et al., 2012b). These high curing efficiencies despite the absence of nitrite and ascorbate suggest that nitrate derived from white kimchi powder could be an effective replacement for nitrite in an indirect meat curing system. Further, treatment of naturally cured sausages with lemon extract powder alone or in combination with vinegar powder may provide cured meat characteristics comparable to traditionally processed products. The curing efficiency decreased significantly between 0 d and 15 d of storage ( $p < 0.05$ ) (Table 5). This was likely due to the low nitrosyl hemochrome content; curing efficiency is defined as the percentage of nitroso pigment content to the total pigment content (AMSA, 2012). Consistent with our results, Terns et al. (2011) reported that the curing efficiency of indirectly cured and conventionally cured sausages decreased as the storage period increased.

### **Thiobarbituric acid reactive substances (TBARS) and microbiological analysis**

Treatment (T) had no significant effect ( $p > 0.05$ ) on TBARS values (Table 2), although they were significantly affected by the storage period (S,  $p < 0.0001$ ). No two-way interaction effects were observed (T×S,  $p > 0.05$ ). None of the treatments

(treatments 1 to 5) had TBARS values that were significantly different from that of the sodium nitrite-treated control ( $p > 0.05$ ) (Table 5), irrespective of the concentration or combination of natural ingredients added. In contrast, a previous study reported that products cured with white kimchi powder or celery powder showed higher TBARS values than those treated with sodium nitrite (Choi et al., 2020). Regarding the high TBARS values of products treated with vegetable powders, Jeong et al. (2020a) speculated that the low nitrite content could cause this pattern. Another possibility, proposed by Choi et al. (2020), is that the low pH induced by acerola juice powder and white kimchi increased TBARS values. Because TBARS values are known to have a negative correlation with pH (Yasosky et al., 1984), and high TBARS values were expected in all naturally cured products exhibiting low pH. However, the TBARS values showed no significant differences between the treatments and the control. This could be due to the inhibition of lipid oxidation caused by the combination of lemon extract, vinegar powder, and white kimchi powder. Nitrite exerts a strong and effective antioxidant activity in naturally cured and traditionally cured meat products (Kim et al., 2017; Pegg and Shahidi, 2000; Xi et al., 2012). Organic acids found in plant-based ingredients have also been known to have antioxidative as well as antimicrobial effects (Ben Braïek and Smaoui, 2021; Theron and Lues, 2007). However, some natural ingredients have also shown prooxidant effects in meat products (Hernández-Hernández et al., 2009; Lin et al., 2011; Vossen et al., 2012). Fortunately, the lemon extract powder and vinegar powder used in this study caused no prooxidant effects. Our findings therefore indicate that treating naturally cured sausages with lemon extract powder, alone or in combination with vinegar powder, and white kimchi powder could supplement the antioxidative effect of nitrite, without requiring treatment with sodium nitrite and ascorbate. As expected, TBARS values increased ( $p < 0.05$ ) with the storage period (Table 5). Previous studies have shown that storage of meat products treated with natural extracts causes increased lipid oxidation (Fernández-López et al., 2005; Sebranek et al., 2005; Wenjiao et al., 2014), which is consistent with our results. Nevertheless, the TBARS values (0.119 mg MDA/kg) on 30 d of storage were much lower than the threshold levels for detecting rancidity in cooked meat (0.5 to 1.0 mg MDA/kg) (Tarladgis et al., 1960), indicating that all the cured sausages retained their quality properties during refrigerated storage.

APC were affected by treatment ( $p < 0.0001$ ) and storage period ( $p < 0.0001$ ; Table 2). Regardless of the concentration and combination of lemon extract powder and vinegar powder, all naturally cured sausages had a lower ( $p < 0.05$ ) APC than the traditionally cured control (Table 5). Among the naturally cured products, treatments 1 and 2 (0.5% and 1.0% lemon extract powder, respectively) showed the most effective ( $p < 0.05$ ) inhibition of aerobic bacteria (APC of 1.64 and 1.61 Log CFU/g, respectively), followed by treatment 5 (1.73 Log CFU/g). Treatments 3 and 4 showed the highest ( $p < 0.05$ ) APC (1.86 and 1.84 Log CFU/g). Lemon extract and vinegar have been shown to have antimicrobial activity by inhibiting the growth of microorganisms (Jackson et al., 2011; King et al., 2015; Sullivan et al., 2012a). King et al. (2015) suggested that organic acids, mainly citric acid and acetic acid, found in lemon extract and vinegar may inhibit microbial growth. Citric acid has also been shown to have more effective antimicrobial activity than lactic or acetic acid (Simpson and Sofos, 2009). Thus, the reduced APC in treatments 1 and 2 may be due to the citric acid in lemon extract. Likewise, vinegar powder also showed antimicrobial activity in pork sausages cured with white kimchi powder, although its effect was significantly less drastic than that of lemon extract ( $p < 0.05$ ). Indeed, the combination of lemon extract powder and vinegar powder (treatment 5) resulted in better reduction of APC than vinegar powder alone (treatments 3 and 4). The APC of the products was 1.70 Log CFU/g on 0 d, which increased significantly to 1.88 Log CFU/g on 30 d ( $p < 0.05$ ) (Table 5). Although there was some aerobic bacterial growth in vacuum-packed products during storage under refrigeration, the growth was very delayed. Our results may reflect the synergistic effect of lemon extract powder and vinegar powder on antimicrobial activity, along with nitrite in cured sausages. However, in this study, *E. coli* and coliform bacteria were not detected in the control or in the naturally cured sausages,

regardless of the storage period (data not shown). This implies that the cooking step was sufficiently completed in the sausage manufacturing process, resulting in hygienic packaging and storage. Our results suggest that the lemon extract powder and vinegar powder effectively inhibited microbial growth, and could be promising natural alternatives to compensate for concerns related to microbial safety caused by low amounts of residual nitrite in naturally cured sausages.

## Conclusion

This study investigated the effects of lemon extract powder or vinegar powder on the curing reaction, antioxidant activity, and antimicrobial activity of sausages cured naturally using white kimchi powder during refrigerated storage. The application of lemon extract was effective at promoting nitrite reactions in naturally cured sausages, thus increasing cured pigment content, total pigment content, and curing efficiency, compared to traditionally cured controls. In contrast, the use of vinegar powder did not positively affect the cured meat characteristics of the products. However, despite the low nitrite content in naturally cured sausages, the addition of both powders resulted in antioxidant stability and antibacterial effects. In particular, regardless of the concentration, the lemon extract powder treatments resulted in stronger antibacterial effects. Similar to previous studies on traditionally and naturally cured meat products, residual nitrite and nitrosyl hemochrome content decreased during storage, while TBARS values and APC increased. Therefore, lemon extract powder, rather than vinegar powder, would be a promising supplemental ingredient to improve the cured color properties, lipid oxidation, and antimicrobial activity of naturally cured pork sausages. Further studies are necessary to improve the water retention of naturally cured meat products supplemented with other natural ingredients.

## Conflicts of Interest

The authors declare no potential conflicts of interest.

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## Author Contributions

Conceptualization: Jeong JY. Data curation: Bae SM. Formal Analysis: Bae SM, Gwak SH, Yoon J. Methodology: Bae SM, Gwak SH, Yoon J. Software: Bae SM. Validation: Bae SM, Jeong JY. Investigation: Bae SM, Gwak SH, Yoon J, Jeong JY. Writing – original draft: Bae SM, Jeong JY. Writing – review & editing: Bae SM, Gwak SH, Yoon J, Jeong JY.

## Ethics Approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

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