

# Characteristics of low-nitrite pork emulsified-sausages with paprika oleoresin solution during refrigerated storage

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Received: Aug 5, 2020  
Revised: Nov 27, 2020  
Accepted: Dec 7, 2020

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## Competing interests

No potential conflict of interest relevant to this article was reported.

## Funding sources

This work was supported by technology development program (# PJ013809022018) for Rural Development Administration, Korea.

## Acknowledgements

This work was supported by technology development program (# PJ013809022018) for Rural Development Administration, Korea.

## Abstract

The objective of this study was to evaluate quality characteristics of low-nitrite emulsified-sausages (ESs, < 75 ppm) containing paprika oleoresin solution (POS) for replacing sodium nitrite ( $\text{NaNO}_2$ ). Pork ESs were prepared with four treatments (reference (REF), 150 ppm  $\text{NaNO}_2$ ; TRT1, 0 ppm  $\text{NaNO}_2$  + 0.1% POS; TRT2, 37.5 ppm  $\text{NaNO}_2$  + 0.1% POS; and TRT3, 75 ppm  $\text{NaNO}_2$  + 0.1% POS). The physicochemical and texture properties, microbial counts, residual nitrite and thiobarbituric acid reactant substances (TBARS) were measured during refrigerated storage of 35 days. Although TRT2 and TRT3 had lower levels of  $\text{NaNO}_2$ , they had higher redness and yellowness than REF ( $p < 0.05$ ). Microbial counts of total bacterial counts and *Enterobacteriaceae* of TRT2 and TRT3 were similar to those of REF ( $p > 0.05$ ). Expressible moisture percentages (EM, %) of TRT2 and TRT3 were lower than those of REF ( $p < 0.05$ ). TBARS values of TRT2 and TRT3 were not different from those of REF ( $p > 0.05$ ). Among treatments, TRT1 had the highest TBARS values ( $p < 0.05$ ). In conclusion, 0.1% POS in combination with 37.5 ppm  $\text{NaNO}_2$  would have quality characteristics similar to those of REF. Therefore, approximately 3/4 of the initial nitrite level could be replaced with 0.1% POS, and eventually developed healthier pork products.

**Keywords:** Low-nitrite emulsified sausage, Paprika oleoresin solution, Quality characteristics

## INTRODUCTION

Sodium nitrite ( $\text{NaNO}_2$ ) is an essential curing ingredient for the manufacture of meat products, such as ham and sausages. When nitrite is added to the manufacture of meat products, it reacts with myoglobin in muscles, resulting in the formation of a pink color pigment, named nitrosohemochrome [1,2]. Because the color of meat products is one of important purchasing factors for consumers [3]. Nitrite is highly related to the commercial value of meat products. In addition, it also improves sensory characteristics by providing unique flavor to meat products [4,5], and extend the shelf-life of meat products by inhibiting the growth of microorganisms and toxin production of *Clostridium botulinum*, which produced the toxin that causes food poisoning [6,7].

However, if excess  $\text{NaNO}_2$  was taken by eating foods containing nitroso compounds, N-nitrosamine, a carcinogen, might be produced by combining  $\text{NaNO}_2$  and amino acids or secondary amines in

**Availability of data and material**

Upon reasonable request, the datasets of this study can be available from the corresponding author.

**Authors' contributions**

Conceptualization: Kim GH, Chin KB.  
Data curation: Kim GH.  
Formal analysis: Kim GH, Chin KB.  
Investigation: Kim GH.  
Writing - original draft: Kim GH.  
Writing - review & editing: Chin KB.

**Ethics approval and consent to participate**

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

human's digestive system [8,9]. Furthermore, processed meats are classified as carcinogens by the World Health Organization (WHO) [10]. In general, consumers had limited knowledge about nitrite being added to meat products [11]. To satisfy consumers who want to eat healthier meat products and increase their preference for meat products, it is necessary to replace nitrite or reduce the level of nitrite by replacing the color development function and storage extension function of nitrite. For this purpose, studies have been conducted using red beet [12], anka rice [13], *Opuntia ficus-indica* pigment [14], and purple sweet potato powder [15].

Paprika (*Capsicum annuum* var. *angulosum*) is an annual plant of Solanaceae *Capsicum* and *Annum*. It has considerable amounts of natural antioxidants, such as vitamin C and E, tocopherol, and carotenoids [16]. In addition, it has been reported that capsanthin, a red pigment carotenoid in red paprika fruit, could enhance the redness of meat products [17]. Considering these findings, paprika might be suitable as a natural substance that can substitute and reduce the addition amount of nitrite because it can increase the redness of meat products and improve their shelf-life.

Paprika oleoresin is a lipophilic matrix that is produced by processing paprika fruit. It is mainly composed of glyceride, liposoluble polyphenolic antioxidants and carotenoid pigments and used as a colorant for meat-based meals in the food industries [18,19]. Paprika oleoresin has excellent coloring capacity because it is composed of high carotenoid concentration [18,20,21]. Therefore, it is enough to represent the coloring effect of paprika [22]. Considering the antioxidant effect and color stability of paprika oleoresin, the addition of that to the meat products might reduce or replace the content of  $\text{NaNO}_2$ . However, there were not many studies regarding these topics. Therefore, the objective of this study was to evaluate quality characteristics, and storage properties of low-nitrite pork emulsified-sausage (ES) added with paprika oleoresin solution (POS) for the development of low-nitrite pork products.

## MATERIALS AND METHODS

### Materials

Raw meat and fat used in the experiment were from 1st grade castrated crossbreed (Landrace × Yorkshire). Back fat was purchased from a retail meat market (Hyundai Retail Meat Market, Gwangju, Korea). After removing connective tissues and external fat, raw hams were minced using meat chopper (M-12S, Korea Fuji Kogyo, Hwaseong, Korea), vacuum packaged, and stored frozen until utilized. Fat was cut off from the surface of the raw material, ground using a meat grinder, and stored at  $-50^\circ\text{C}$ . Paprika oleoresin was obtained from Kalsec (Kalamazoo, MI, USA). Sunflower seed oil used to make POS was purchased from a local supermarket. Paprika oleoresin and sunflower seed oil were diluted to approximately 20 times (pH 3.43, CIE color value was  $L^* = 52.1$ ,  $a^* = 55.4$ ,  $b^* = 54.1$ , main ingredient is lipid), respectively.

### Preparation of emulsified-sausages

Detailed formulation for ESs is listed in Table 1. After raw meat was mixed with curing ingredients with ice water using a hood mixer (Mixer, HMC-401, Hanil Electric, Seoul, Korea), fat and POS were added and emulsified. The stuffed meat batter was heated in a constant temperature water bath (WB-22, Daihan Scientific, Seoul, Korea) at  $75^\circ\text{C}$  for 30 min, cooled, and stored at  $10^\circ\text{C}$  until analyzed. Quality characteristics of ESs were measured on the 0, 3, 7, 14, 21, 28, and 35 days after preparation.

### pH and color measurements

The pH values of sausages were determined using a Minolta Color Reader (CR-10, Minolta, To-

**Table 1.** Formulation of pork emulsified-sausages with various content of sodium nitrite and paprika oleoresin solution

Ingredients (%)	Treatment			
	REF	TRT1	TRT2	TRT3
Raw pork meat	60.0	60.0	60.0	60.0
Fat	20.0	20.0	20.0	20.0
Water	18.0	18.0	18.0	18.0
Non meat ingredients				
Salt	1.50	1.50	1.50	1.50
Sodium tripolyphosphate	0.40	0.40	0.40	0.40
Sodium erythorbate	0.05	0.05	0.05	0.05
Sodium nitrite	0.015	0.00	0.00375	0.0075
Paprika oleoresin solution				
Oleoresin paprika	0.00	0.005	0.005	0.005
Sunflower seed oil	0.00	0.095	0.095	0.095
Total	100.0	100.0	100.1	100.1

REF, reference (150 ppm sodium nitrite); TRT1, treatment 1 (0 ppm sodium nitrite, 0.1% paprika oleoresin solution), TRT2, treatment 2 (37.5 ppm sodium nitrite, 0.1% paprika oleoresin solution); TRT3, treatment 3 (75 ppm sodium nitrite, 0.1% paprika oleoresin solution).

kyo, Japan). Measurements for pH were performed 5 times per sample, and the means of the values were calculated.

The color values were measured by CIE L\*, CIE a\*, and CIE b\* using a Minolta Color Reader (CR-10, Minolta, Osaka, Japan). After color values were measured six times, results were averaged for these six measurements (CIE color value of the standard white plate was L\* = 94.8, a\* = 1.0, b\* = 0.1).

### Proximate composition

Proximate composition was determined according to the AOAC [23]. Moisture content (%) was determined with the dry oven method. Fat content (%) was determined with the Soxhlet extraction method and the protein content (%) was determined with the Kjeldahl method.

### Cooking loss (CL)

CL (%) was measured based on the weight of sausage before and after heating. It was calculated by substituting the following formula.

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

### Texture profile analysis

Samples were prepared by setting a shape of 1.25 cm in diameter and 1.3 cm in height. Hardness (gf), springiness (mm), gumminess, chewiness, and cohesiveness of each sample were measured ten times with an Instron Universal Machine (Model 3344, Instron, Canton, MA, USA).

### Microbiological analysis

Microbial counts were performed by mixing 10 g of homogenized sausage samples with 90 mL of sterile distilled water and diluting them to in appropriate proportion. Total plate count (TPC) and violet red bile (VRB) agar were used for the determination of a total number of bacteria

and *Enterobacteriaceae*, respectively. After applying diluted samples onto agar plates, plates were incubated for 48 hrs at 37°C. Results are expressed as log CFU/g.

### Expressible moisture (EM)

EM (%) was prepared by cutting the sausage into a rectangular parallelepiped shape of about 1.5 g. The sample was wrapped in three equal pieces of Whatman #3 filter paper, placed into a conical tube, and centrifuged at 1,660×g (VS-5500, Vision Science, Gyeongsan, Korea) for 15 min. The amount of water liberated from the sample in the filter paper was then measured and substituted into the following formula.

$$\text{Expressible moisture (\%)} = \frac{\text{Expressible water weight of filter paper (g)}}{\text{Sample weight (g)}} \times 100$$

### Residual nitrite

Residual nitrite level (ppm) was determined by modifying the method of AOAC [23]. Briefly, approximately 5 g of ground sausage sample was mixed with 300 mL of distilled water and heated for 1 hr in a constant temperature water bath (WB-22, Daihan Scientific) at 100°C. After filtration, the sample was diluted with distilled water to make a mixture of 500 mL. Approximately 2.5 mL of sulfanilamide solution was added to 25 mL of this mixture, vortexed, and allowed to react at room temperature for about 5 min. Then, 2.5 mL of N-(1-Naphthyl) ethylene dihydrochloride solution was added to 25 mL of this mixture, vortexed, and allowed to react at room temperature for about 5 min. The absorbance was measured using a spectrophotometer (Model UV-1601, Shimadzu, Kyoto, Japan) at a wavelength of 540 nm. The measured absorbance was assigned to a standard curve obtained by measuring the absorbance of nitrite solution to derive the residual amount of nitrite.

### Thiobarbituric acid substances (TBARS)

TBARS was measured using the method of Sinnhuber and Yu [24]. Briefly, 2 g of ground sausage sample, 3 mL of thiobarbituric acid, and 17 mL of trichloroacetic acid (100 mg/mL) were mixed by vortexing, homogenized, and heated in a water bath (WB-22, Daihan Scientific) at 100°C for 30 min. After heating, samples were cooled at room temperature, then 5 mL of the supernatant of the sample and 5 mL of chloroform were vortexed together for 1 min, mixed, and centrifuged at 3,000 rpm (Model VS-5500, Vision Science) for about 5 min. Then, 3 mL of the supernatant of the sample was mixed with 3 mL of petroleum ether for 1 min by vortexing. The mixture was then centrifuged at 3,000 rpm for 10 min. The absorbance of the reaction product was measured using a spectrophotometer (Model UV-1601, Shimadzu) at a wavelength of 532 nm. The TBARS value was calculated by substituting the measured absorbance into the following equation.

$$\text{TBARS value (mg of MDA/kg of sample)} = \frac{\text{O.D. value} \times 9.48}{\text{Sample weight (kg)}}$$

### Statistical analysis

The whole experiments were performed in triplicate. The mean and standard deviation of the mean were calculated using IBM SPSS Statistics 23 (SPSS, Chicago, IL, USA). This experiment was carried out with two-way ANOVA (treatments × storage time). If the interaction between two factors were observed ( $p < 0.05$ ), then the data were separated by treatments within the storage time or storage time within the treatments. If no interaction between two factors were observed ( $p > 0.05$ ), then the data were pooled by treatments or storage time. Post ANOVA was performed by Duncan's

multiple range test at significance level of 0.05.

## RESULTS AND DISCUSSION

### pH and color measurement

Since results of pH and color had no interaction between the treatment and storage time, data were pooled by treatment in a storage time and storage time in a treatment. Results of pH among all treatments and storage time were not different (Table 2). As shown in Table 2, L\* values of TRT1 were the highest among all treatments ( $p < 0.05$ ), while those of other treatments were similar ( $p > 0.05$ ). Measured a\* values were higher in the order of TRT3 > TRT2 > REF > TRT1 ( $p < 0.05$ ). Although lower levels of NaNO<sub>2</sub> were added into TRT2 and TRT3 compared to the level added to REF (150 ppm NaNO<sub>2</sub>), a\* values of TRT2 and TRT3 were higher than those of REF due to the additional effect of POS, whereas b\* values were highest in TRT1 and lowest in REF. The addition of POS increased the yellowness which indicated that POS might increase both a\* and b\* values. In a previous study, when approximately 1% paprika oleoresin was added to the oil for marinating chicken breast, a\* and b\* values of treatments containing POS were increased by 0.87 and 3.82, respectively [26]. This is because carotenoid, a red and yellow pigment, present in paprika oleoresin. L\*, a\*, and b\* values of all treatments did not show significant change during refrigerated storage ( $p > 0.05$ ).

### Proximate composition

There were no significant differences in water, fat, or protein contents (%) among all treatments ( $p > 0.05$ ), the addition of POS did not significantly affect proximate compositions of ESs (data are not shown). This result might be due to the lower level (0.1%) of POS added into the sausage. According to Yusop et al. [19], even though the contents of paprika oleoresin was increased up to 3 times, the marinated chicken breast prepared with paprika oleoresin didn't change in moisture and fat contents (%).

**Table 2.** The pH and color values of emulsified-sausages with various content of sodium nitrite and paprika oleoresin solution

	pH	CIE L*	CIE a*	CIE b*
Treatment				
REF	6.06 ± 0.07 <sup>a</sup>	73.6 ± 0.73 <sup>b</sup>	10.7 ± 0.53 <sup>c</sup>	6.19 ± 0.74 <sup>d</sup>
TRT1	6.05 ± 0.09 <sup>a</sup>	74.9 ± 1.04 <sup>a</sup>	6.60 ± 0.46 <sup>d</sup>	9.62 ± 0.56 <sup>a</sup>
TRT2	6.08 ± 0.08 <sup>a</sup>	73.9 ± 1.37 <sup>b</sup>	11.7 ± 0.57 <sup>b</sup>	7.51 ± 0.20 <sup>c</sup>
TRT3	6.08 ± 0.08 <sup>a</sup>	73.2 ± 0.89 <sup>b</sup>	12.3 ± 0.58 <sup>a</sup>	7.86 ± 0.32 <sup>b</sup>
Storage time (d)				
0	6.08 ± 0.08 <sup>a</sup>	73.8 ± 1.04 <sup>a</sup>	10.5 ± 2.18 <sup>a</sup>	7.74 ± 1.56 <sup>a</sup>
3	6.11 ± 0.09 <sup>a</sup>	73.9 ± 1.36 <sup>a</sup>	10.2 ± 2.42 <sup>a</sup>	7.80 ± 1.48 <sup>a</sup>
7	6.10 ± 0.08 <sup>a</sup>	73.9 ± 1.36 <sup>a</sup>	10.3 ± 2.57 <sup>a</sup>	7.79 ± 1.55 <sup>a</sup>
14	6.05 ± 0.12 <sup>a</sup>	74.1 ± 1.06 <sup>a</sup>	10.4 ± 2.39 <sup>a</sup>	7.87 ± 1.40 <sup>a</sup>
21	6.03 ± 0.06 <sup>a</sup>	73.8 ± 1.28 <sup>a</sup>	10.3 ± 2.34 <sup>a</sup>	7.72 ± 1.25 <sup>a</sup>
28	6.06 ± 0.06 <sup>a</sup>	73.9 ± 1.12 <sup>a</sup>	10.3 ± 2.32 <sup>a</sup>	7.95 ± 1.10 <sup>a</sup>
35	6.05 ± 0.06 <sup>a</sup>	74.1 ± 1.41 <sup>a</sup>	10.3 ± 2.44 <sup>a</sup>	7.69 ± 1.21 <sup>a</sup>

<sup>a-d</sup>Mean having same superscripts in a same column are not different ( $p > 0.05$ ).

REF, reference (150 ppm sodium nitrite); TRT1, treatment 1 (0 ppm sodium nitrite, 0.1% paprika oleoresin solution), TRT2, treatment 2 (37.5 ppm sodium nitrite, 0.1% paprika oleoresin solution); TRT3, treatment 3 (75 ppm sodium nitrite, 0.1% paprika oleoresin solution).

### Cooking loss

As shown in Table 3, CL (%) were not significantly different among treatments ( $p > 0.05$ ). It indicated that different additional levels of NaNO<sub>2</sub> and POS had no effect on CL of ESs. Kim and Chin [27] reported that CL of pork sausage with paprika powder were similar to those of control without paprika powder.

### Texture profile analysis

The addition of POS did not affect the textural properties such as hardness, springiness, gumminess, chewiness, or cohesiveness ( $p > 0.05$ ) (Table 3). In a previous study, Bázan-Lugo et al. [17] evaluated quality characteristics of sausages added with or without 1.5% of paprika powder, and reported that the similar hardness and springiness were measured, regardless of the addition of paprika powder. Revilla and Quintana [28] also reported that the addition of paprika did not affect the textural properties of chorizo type of sausage ( $p > 0.05$ ). Based on these results, the paprika powder might not affect the textural properties of the meat products.

### Microbiological analysis

Table 4 shows microbiological counts of pork emulsion type sausages added with POS. Total microbial counts (TPC) of TRT1 were higher than those of other treatments ( $p < 0.05$ ). This probably due to the absence of NaNO<sub>2</sub>, which might have antimicrobial activity against many microbial species including *Pseudomonas aeruginosa* [29]. TRT2 and TRT3 showed similar total microbial counts to REF, indicating that addition of POS might have the antimicrobial activity. De Oliveira et al. [30] inoculated *Clostridium perfringens* into mortadella-type sausages added with 0, 100, and 200 ppm of NaNO<sub>2</sub>, respectively, and they found that the higher the NaNO<sub>2</sub> content, the lower the number of microorganisms. The addition of 200 ppm of NaNO<sub>2</sub> into the sausage was lower than those with 100 ppm after 30 days of storage ( $p < 0.05$ ). This means that higher ingoing level of nitrite could retard microbial growth more quickly. Microbial counts of *Enterobacteriaceae* higher than 10<sup>2</sup> CFU/g were detected in TRT1. However, they were not detected in REF, TRT2, or TRT3. Carotenoids present in paprika could inhibit the growth of microorganisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger*, *Aspergillus flavus*, *Penicillium chrysogenum*, *Rhizopus oryzae*, and *Saccharomyces* [29]. Paprika extracts including paprika oleoresin can destroy microorganisms in the biofilm [31]. Therefore, the addition of POS seems to reduce total bacterial counts and the microbial counts of *Enterobacteriaceae* counts. In this study, total bacterial counts

**Table 3.** The cooking loss and texture profile analysis of pork emulsified-sausages with content of sodium nitrite and paprika oleoresin solution

	Treatment			
	REF	TRT1	TRT2	TRT3
Cooking loss (%)	2.05 ± 1.53 <sup>a</sup>	1.73 ± 0.37 <sup>a</sup>	1.35 ± 0.15 <sup>a</sup>	1.32 ± 0.69 <sup>a</sup>
Hardness (gf)	3,688 ± 381 <sup>a</sup>	3,226 ± 418 <sup>a</sup>	3,529 ± 339 <sup>a</sup>	3,650 ± 380 <sup>a</sup>
Springiness (mm)	4.32 ± 0.40 <sup>a</sup>	4.42 ± 0.19 <sup>a</sup>	4.42 ± 0.16 <sup>a</sup>	4.48 ± 0.34 <sup>a</sup>
Gumminess	36.2 ± 1.43 <sup>a</sup>	36.8 ± 2.03 <sup>a</sup>	36.6 ± 2.50 <sup>a</sup>	35.9 ± 3.79 <sup>a</sup>
Chewiness	154 ± 2.24 <sup>a</sup>	157 ± 4.70 <sup>a</sup>	154 ± 1.84 <sup>a</sup>	154 ± 3.31 <sup>a</sup>
Cohesiveness	0.01 ± 0.00 <sup>a</sup>			

REF, reference (150 ppm sodium nitrite); TRT1, treatment 1 (0 ppm sodium nitrite, 0.1% paprika oleoresin solution), TRT2, treatment 2 (37.5 ppm sodium nitrite, 0.1% paprika oleoresin solution); TRT3, treatment 3 (75 ppm sodium nitrite, 0.1% paprika oleoresin solution).

<sup>a</sup>Mean having same superscripts in a same row are not different ( $p > 0.05$ ).

**Table 4.** The microbiological counts (Log CFU/g) and expressible moisture (%) of pork emulsified-sausages with content of sodium nitrite and paprika oleoresin solution

	TPC	VRB	EM
Treatment			
REF	2.04 ± 2.03 <sup>b</sup>	< 2.00 <sup>b</sup>	18.8 ± 0.76 <sup>b</sup>
TRT1	2.72 ± 2.05 <sup>a</sup>	2.05 ± 1.97 <sup>a</sup>	19.0 ± 1.07 <sup>a</sup>
TRT2	2.19 ± 2.03 <sup>b</sup>	< 2.00 <sup>b</sup>	18.5 ± 0.89 <sup>c</sup>
TRT3	2.03 ± 2.04 <sup>b</sup>	< 2.00 <sup>b</sup>	18.5 ± 0.84 <sup>c</sup>
Storage time (d)			
0	< 2.00 <sup>d</sup>	< 2.00 <sup>d</sup>	17.7 ± 0.18 <sup>f</sup>
3	< 2.00 <sup>d</sup>	< 2.00 <sup>d</sup>	18.0 ± 0.39 <sup>e</sup>
7	< 2.00 <sup>d</sup>	< 2.00 <sup>d</sup>	18.0 ± 0.33 <sup>e</sup>
14	2.30 ± 1.40 <sup>c</sup>	< 2.00 <sup>d</sup>	18.5 ± 0.37 <sup>d</sup>
21	3.90 ± 0.24 <sup>b</sup>	3.31 ± 0.32 <sup>c</sup>	19.2 ± 0.42 <sup>c</sup>
28	4.31 ± 0.27 <sup>ab</sup>	3.78 ± 0.37 <sup>b</sup>	15.5 ± 0.38 <sup>b</sup>
35	4.61 ± 0.32 <sup>a</sup>	4.17 ± 0.48 <sup>a</sup>	20.1 ± 0.39 <sup>a</sup>

<sup>a-d</sup>Mean having same superscripts in a same column are not different ( $p > 0.05$ ).

REF, reference (150 ppm sodium nitrite); TRT1, treatment 1 (0 ppm sodium nitrite, 0.1% paprika oleoresin solution); TRT2, treatment 2 (37.5 ppm sodium nitrite, 0.1% paprika oleoresin solution); TRT3, treatment 3 (75 ppm sodium nitrite, 0.1% paprika oleoresin solution).

TPC, total plate counts; VRB, violet red agar; EM, expressible moisture.

were detected at more than  $10^2$  CFU/g from 14 days, and the microbial counts of *Enterobacteriaceae* counts were detected at more than  $10^2$  CFU/g from 21 days.

### Expressible moisture

As shown in Table 4, EM (%) of TRT1 were the highest among all treatments ( $p < 0.05$ ). These results might be partially due to the lowest water holding capacity of sausage without NaNO<sub>2</sub>. Thus, NaNO<sub>2</sub> might be combined with other curing agents to improve water holding capacity in meat products [32]. On the other hand, EMs of TRT1 and TRT2 were lower than those of REF ( $p < 0.05$ ).

### Residual nitrite (RN)

Table 5 shows results of RN (ppm) of pork ESs with POS during refrigerated storage. Since RN was an interaction between treatment and storage day ( $p < 0.05$ ), data were separated out by treatment in a storage time and storage time in a treatment. Amounts of RN decreased gradually with increased storage time ( $p < 0.05$ ). RN levels of TRT2 on 0 and 3 days were similar ( $p > 0.05$ ). All subsequent values were different from each other on each measurement day ( $p < 0.05$ ). Since TRT1 did not contain NaNO<sub>2</sub>, no RN was present during the storage period, and its RN levels were lower than those in other treatments ( $p < 0.05$ ). Roberts [33] determined the microbiological role of nitrates and nitrites using bacon, and reported that higher RN tends to be higher antimicrobial activity. According to this result, REF is expected to have long-term antimicrobial activity. Even though the residual amount was lower at 35 days, TRT2 and TRT3 didn't differ from REF in terms of microbial counts. These results might be due to the influence of POS. As shown in Table 5, RN levels of ESs were proportional to added levels of NaNO<sub>2</sub>. Thus, RN levels of ESs at 35 days of storage were significantly influenced by the amount of NaNO<sub>2</sub> added. If the RN level is low (< 20 ppm), the possibility of producing N-nitrosamine also tends to be lower. Ahn et al. [34] reported that higher volatile N-nitrosamine levels (199 ppb) detected in pork sausage with higher RN level

**Table 5.** The residual nitrite (ppm) of pork emulsified-sausages with content of sodium nitrite and paprika oleoresin solution

Treatments	Storage time (d)						
	0	3	7	14	21	28	35
REF	21.2 ± 1.03 <sup>Aa</sup>	17.8 ± 0.17 <sup>Ab</sup>	14.9 ± 0.26 <sup>Ac</sup>	9.64 ± 0.22 <sup>Ad</sup>	8.68 ± 0.07 <sup>Ae</sup>	6.78 ± 0.07 <sup>Af</sup>	4.88 ± 0.09 <sup>Ag</sup>
TRT1	ND	ND	ND	ND	N D	ND	ND
TRT2	6.90 ± 0.23 <sup>Ca</sup>	5.45 ± 1.26 <sup>Cab</sup>	4.16 ± 0.34 <sup>Cb</sup>	3.01 ± 0.65 <sup>Cc</sup>	1.69 ± 0.34 <sup>Cd</sup>	0.83 ± 0.22 <sup>Ce</sup>	0.55 ± 0.03 <sup>Cf</sup>
TRT3	9.45 ± 0.11 <sup>Ba</sup>	8.95 ± 0.06 <sup>Bb</sup>	7.18 ± 0.09 <sup>Bc</sup>	5.58 ± 1.45 <sup>Bd</sup>	4.17 ± 0.14 <sup>Be</sup>	2.87 ± 0.28 <sup>Bf</sup>	1.91 ± 1.14 <sup>Bg</sup>

REF, reference (150 ppm sodium nitrite); TRT1, treatment 1 (0 ppm sodium nitrite, 0.1% paprika oleoresin solution), TRT2, treatment 2 (37.5 ppm sodium nitrite, 0.1% paprika oleoresin solution); TRT3, treatment 3 (75 ppm sodium nitrite, 0.1% paprika oleoresin solution).

<sup>A-C</sup>Mean having same superscripts in a same column are not different ( $p > 0.05$ ).

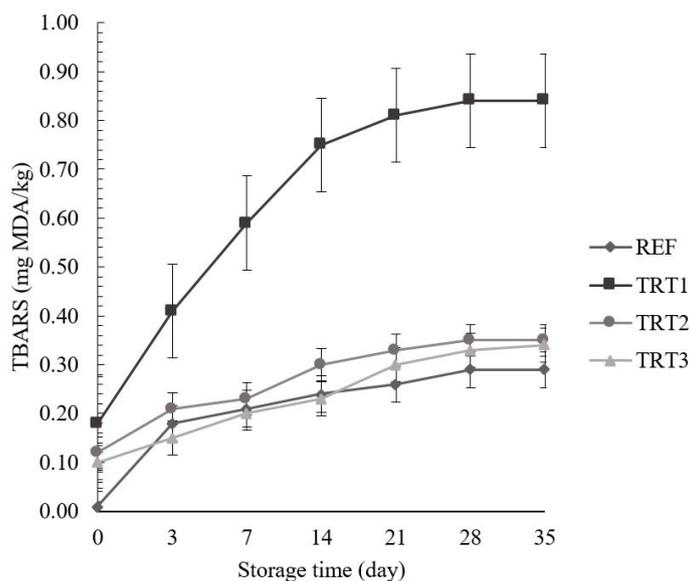
<sup>a-d</sup>Mean having same superscripts in a same row are not different ( $p > 0.05$ ).

ND, not detected.

(104 ppm), whereas those with lower volatile N-nitrosamine levels (81.8 ppm) had lower volatile N-nitrosamine levels (28.8 ppb).

### Thiobarbituric acid reactant substances (TBARS)

Fig. 1 shows the results of measured TBARS of pork ESs during storage days. There were no significant differences in TBARS values among REF, TRT2, and TRT3 during storage periods from day 0 to 35 days ( $p > 0.05$ ). TBARS values of TRT1 increased rapidly from 0 to 3 days, while those of other treatments, including REF increased slightly during the storage time. REF and TRT2 showed no changes in TBARS value from 21 days to the end of storage ( $p > 0.05$ ). However, TRT2 and TRT3 did not change in TBARS values from 14 days to the end of the measurement ( $p > 0.05$ ). It is known that nitrite has ability to effectively delay the oxidation of cured meat [7]. In this study, TBARS values of TRT1 were the highest among all treatments. however, those of TRT2 and TRT3 added with nitrite at 37.5 ppm and 75 ppm, respectively, and treatments with POS were



**Fig. 1.** The changes of thiobarbituric acid reactive substances of pork with content of sodium nitrite and paprika oleoresin solution. REF, reference (150 ppm sodium nitrite); TRT1, treatment 1 (0 ppm sodium nitrite, 0.1% paprika oleoresin solution), TRT2, treatment 2 (37.5 ppm sodium nitrite, 0.1% paprika oleoresin solution); TRT3, treatment 3 (75 ppm sodium nitrite, 0.1% paprika oleoresin solution).

not different from those of REF ( $p > 0.05$ ). Kim et al. [35] reported that 0.32% of oleoresin paprika added to pork patties could effectively inhibit lipid oxidation to replace  $\text{NaNO}_2$ . This suggests that TRT2 and TRT3 might have similar levels of lipid oxidation, although added levels of nitrite were lower than the level added to REF. These results also suggest that POS might have antioxidant activity in meat products.

## CONCLUSION

The addition of 0.1% POS increased the redness and yellowness of the pork ESs. Microbial counts and lipid oxidation of ESs added with 37.5 or 75 ppm  $\text{NaNO}_2$  and 0.1% POS were similar to those of ESs added with 150 ppm  $\text{NaNO}_2$ . As a result, ESs added with 0.1% POS and 37.5 ppm  $\text{NaNO}_2$  are similar quality characteristics to REF. Therefore, approximately 3/4 of ingoing level of  $\text{NaNO}_2$  could be replaced with 0.1% POS in the manufacture of regular-fat sausage.

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