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ARTICLE

# Effect of Thyme and Rosemary on The Quality Characteristics, Shelf-life, and Residual Nitrite Content of Sausages During Cold Storage

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**Abstract**

The effects of thyme and rosemary on the quality characteristics of sausages during cold storage were investigated. Sausages were prepared with thyme and rosemary powder (1 and 2%) and stored for 6 weeks at 10°C. The pH was significantly decreased in sausages by addition of thyme and rosemary compared to that observed in the control before and after storage. At 4 weeks of storage, the residual nitrite content was decreased by thyme and rosemary compared to the control. Lightness (L*) and yellowness (b*) were increased during storage, whereas redness (a*) and whiteness (W) were decreased before and after storage by addition of thyme and rosemary. The amount of TPC and lactic acid bacteria was lower at the end of storage in sausage containing thyme and rosemary. The 2, 2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging capacity of sausages was increased by addition of thyme and rosemary compared to that in the control before and after storage. In particular, T2 (0.2% thyme addition) showed the highest DPPH radical scavenging capacity during storage. In a sensory evaluation, flavor and overall acceptability were lower in sausages containing thyme and rosemary than in the control. However, at the end of storage (6 wk), aroma, flavor and overall acceptability were not significantly different among the sausage samples.

**Keywords:** thyme, rosemary, sausage, residual nitrite, lipid oxidation

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## Introduction

Natural ingredients possessing bioavailability have the advantage of being readily accepted by consumers, as they are considered natural. Several studies (Sebranek *et al*., 2005: Yu *et al*., 2002) have reported that in addition to inhibition of lipid oxidation; rosemary extracts improve the color stability of cooked turkey rolls. Erkan *et al*. (2008) reported that rosemary extract had a high phenolic content, which, in turn, contributes to high antioxidant activity. Moreover, rosemary extracts have been shown to have some antimicrobial effects (Angioni *et al*., 2004). Viuda-Martos *et al*. (2009) suggested that components of thyme essential oils show their own antioxidant activity and that these bioactive compounds having antioxidant activities may also interfere with the free radical propagation. Sodium nitrite is responsible for the unique taste, development of red color, and inhibition of microbial growth, especially that of *Clostridium botulinum*, in cured meat products. However, it can be converted to the nitrosating agent $NO^+$, which can react with biogenic amines to form carcinogenic N-nitrosamines (Honikel, 2008). Thus, excessive consumption of sodium nitrite through diet may also have harmful effects on health. Viuda-Martos *et al*. (2009) suggested that polyphenols and flavonoids, reduce the levels of residual nitrite. Thus, reduction of residual nitrite levels could be an acceptable alternative for reducing nitrite intake through processed meats to alleviate the potential risk of the formation of carcinogenic, and mutagenic N-nitroso compounds (Karolyi, 2003). Although the physiological and pharmacological functions of natural ingredients have been extensively studied, few studies have focused on their effects on the quality characteristics of sausages during storage. Therefore, the purpose of this study was to determine the effects

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of thyme and rosemary addition on the quality, shelf-life, and residual nitrite content of sausages during storage.

## Materials and Methods

### Materials

Refined salt was obtained from Woo-Il S&F Co., (Korea). Additionally, sodium nitrite was purchased from Duksan Co., (Korea). Phosphate and sausage spice were purchased from Taewon Food Co., (Korea). In addition, sugar was obtained from Cheiljedang Co., (Korea). Monosodium L-glutamate was purchased from Shinwon Chemical Co., (Korea). All other reagents were of the highest grade commercially available.

### Preparation of thyme and rosemary powder

Air-dried thyme (*Thymus vulgaris* L.) and rosemary (*Rosmarinus officinalis* L.) were purchased from a herbal market (Kumho market, Korea). The samples were washed under running tap water before being chopped into pieces. Then, they were oven-dried at 45°C for 2 d and ground to a powder. The powder was stored at -20°C until use.

### Experimental design and sausage processing

Lean pork and backfat were purchased from a local meat-processing plant. Excess fat was trimmed from the meat, and the lean muscle was diced into pieces (approximately 8 cm × 4 cm × 2 cm) and ground through an orifice with a diameter of 7 mm by using a mincer. The ground meat was cured for 30 min with phosphate and NPS using a meat mixer and then stored for 24 h at 4°C. The cured meat was placed in a bowl cutter along with ice, sugar, monosodium L-glutamate, spice and different ingredients (Table 1). Chopping was continued until the batter temperature reached 10°C. The emulsified meat batters were stuffed into PVDC casings (50-mm diameter) and placed in a cooking chamber (programmed at 65°C for 30 min, followed by 75°C for 30 min, and then 80°C for 20 min). The samples were divided into five groups: C (commercial meat), T1 (containing 0.1% thyme), T2 (containing 0.2% thyme), T3 (containing 0.1% rosemary) and T4 (containing 0.2% rosemary). The core sausage temperature was measured using a flexible internal thermometer (Temp 300, Thermo Scientific, USA). After cooling in iced water for 20 min, the sausages were stored at 10°C until use.

### pH

The pH values of a sausage homogenate prepared with 3 g of sausage sample and 27 mL of distilled water were determined using a digital pH meter (SevenEasy pH, Mettler-Toledo AG, Switzerland) equipped with an electrode calibrated with phosphate buffer at pH 4.0 and pH 7.0 at room temperature.

**Table 1. The formulation for pork sausage**

| Ingredient (%) | Treatment | | | | |
|---|---|---|---|---|---|
| | C | T1 | T2 | T3 | T4 |
| Pork loin | 67.4 | 67.4 | 67.4 | 67.4 | 67.4 |
| Fat | 21 | 21 | 21 | 21 | 21 |
| Ice | 9 | 9 | 9 | 9 | 9 |
| NPS ($NaCl:NaNO_2$=99:1)[1)] | 1.4 | 1.4 | 1.4 | 1.4 | 1.4 |
| Phosphate[2)] | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Sugar[3)] | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Monosodium L-glutamate[4)] | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Sausage spice[5)] | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 |
| Total | 100 | 99.9 | 99.8 | 99.9 | 99.8 |
| Thyme | - | 0.1 | 0.2 | - | - |
| Rosemary | - | - | - | 0.1 | 0.2 |
| Total | 100 | 100 | 100 | 100 | 100 |

[1)]Refined salt (Woo-Il S&F Co., Ulsan, Republic of Korea) and sodium nitrite (Duksan Co., Gyeongki-do, Republic of Korea).
[2)]Phosphate (Taewon Food Co., Gyeongki, Republic of Korea).
[3)]Sugar (CJ Cheiljedang Co., Incheon, Republic of Korea).
[4)]Monosodium L-glutamate (Shinwon Chemical Co. Ltd., Seoul, Republic of Korea).
[5)]Sausage spice (Taewon Food Co., Gyeongki, Republic of Korea).

### Thiobarbituric acid-reactive substances (TBARS)

TBARS values were determined using a modification of the method of Buege and Aust (1978). Sausages (5 g) were weighed in a 50-mL test tube and homogenized with 15 mL of deionized distilled water by using a Polytron homogenizer at 1000×*g* for 10 s. The sausage homogenate (1 mL) was transferred to a disposable test tube (3 × 100 mm), and butylated hydroxyanisole (50 μL, 10%) and thiobarbituric acid/trichloroacetic acid (TBA/TCA) (2 mL) were added. The mixture was vortexed and then incubated in boiling water for 15 min to develop the color. The sample was cooled in cold water for 5 min, vortexed again, and centrifuged for 15 min at 2,000×*g*. The absorbance of the resulting supernatant solution was determined at 531 nm against a blank containing 1 mL of deionized distilled water and 2 mL of TBA/TCA solution. TBARS values were calculated from a standard curve of malondialdehyde (MDA), freshly prepared by acidification of TEP (1,1,3,3-tetraethoxypropane) in the range of 0.02 to 0.3 μg/mL ($y = 0.8729x + 0.0382$, $r = 0.9961$) and were expressed as mg of malondialdehyde per kg of sample.

**Residual nitrite**

Residual nitrite levels were determined in triplicate in the sausage according to the colorimetric method of AOAC, Method No. 973.31 (Codex general method) (AOAC, 1990). Specifically, nitrite reacts with sulphanilamide to form a diazonium salt which is added to N-(1-Naphthyl)-ethylenediamine dihydrochloride (NED) to form azo dye compound, which absorbance was measured spectrophotometrically at 540 nm. Residual nitrite was determined by flow injection analysis (Ruiz-Capillas *et al*., 2007).

**Color**

The lightness L*, redness a*, and yellowness b* (CIE) of sausages were measured using a Minolta colorimeter; illuminant-C, $D_{65}$, aperture-8 mm size, standard observer-2º standard observer (Minolta Chroma Meter CR-300, Minolta Co., Ltd., Japan), which was calibrated using a white standard plate (Y = 92.8, x = 0.3134, and y = 0.3193). Sausages were cut into slices with a length of 5 cm, and the surface color of the slices was evaluated three times for each sample. The whiteness W was calculated using the following formula; L* − 3b* (Park, 2005). Color was determined five times for each sample, and the mean values were used.

**Microorganisms**

Microorganisms were analyzed with regard to total plate count (TPC) and number of lactic acid bacteria according to standard procedures (Speck, 1992), with incubation for 72 h at 37 °C. The relevant colonies on the plates were counted, and the results were expressed as colony-forming units (CFU) per gram of meat sample. The TPC and lactic acid bacteria count were then normalized by logarithm (base 10) transformations.

**Water activity (a*w*)**

Water activity (a*w*) was measured using an AquaLab Water Activity Meter Series 3TE with an internal temperature control (Decagon Devices, Inc., USA). The a*w* at each storage temperature was recorded.

**DPPH radical scavenging activity**

The 2, 2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging activity of the sausages was measured using a spectrophotometer (Akowuah *et al.*, 2005). 250 g sample was minced and homogenized in 250 mL distilled water using a Polytron homogenizer (T25-B, IKA Labortechnik, Germany) at 8,000 rpm for 5 min. The sample was filtered using cheese clothes and diluted sample (0.15 mL) added to 0.9 mL of a methanolic DPPH solution (0.1 mM). After 10 min, the absorbance of the solution was measured at 517 nm. Pure methanol was used as the control. The DPPH scavenging activity was calculated as a percentage (% SA) from the equation (1 − [absorbance of extract/absorbance of control]) × 100.

**Sensory evaluation**

Sensory evaluation was performed by a panel of 20 semi-trained tasters. Panel development followed the prescreening, screening, training, and performance evaluation phases as described previously (Cross *et al.*, 1978). The panel evaluated each treatment within each replicate in triplicate, and the evaluation was performed using samples at room temperature. Triplicate responses were measured to monitor the inherent texture variability associated with the same sample. One slice with a thickness of 0.5 cm and a diameter of 5 cm was cut into six pie-shaped wedges and presented to each panelist. The panelists chose three of the most characteristic wedges to avoid a sample containing large pieces of tissue. The color, aroma, flavor, springiness, juiciness, and overall acceptability were evaluated according to a 9-point scale: Color (9 = very good and 1 = very bad), Aroma (9 = very intense and 1 = very weak), Flavor (9 = very good and 1 = very bad), Springiness (9 = very elastic and 1 = very inelastic), Juiciness (9 = very juiciness and 1 = very dry), and Overall acceptability (9 = very good and 1 = very bad).

**Statistical analysis**

The data are expressed as the mean ± standard error of mean (SEM). Statistical analyses were conducted on three batches of sausages. Data for each batch of sausage for pH, TBARS, residual nitrite, color, microorganisms, a*w*, DPPH radical scavenging activity and sensory evaluation were analyzed using an ANOVA with SAS software (SAS Inst. Inc., USA) by the Duncan's multiple range test were used to compare the differences among means. Significant differences ($p<0.05$) between mean values of quintuplicate samples were determined for pH, TBARS, residual nitrite, color, microorganisms, a*w*, DPPH radical scavenging activity and the sensory evaluation (n=15).

## Results and Discussion

The effects of the addition of thyme and rosemary on the pH, TBARS, and residual nitrite content in sausages during storage are presented in Table 2. The pH was sig-

**Table 2. Effect of thyme and rosemary on the pH, TBARS, and residual nitrite content in sausages during storage**

| Item | Treatment[1)] | Storage (wk) | | | |
|---|---|---|---|---|---|
| | | 0 | 2 | 4 | 6 |
| pH | C | 6.45±0.01$^{Aa}$ | 6.29±0.02$^{Ab}$ | 6.10±0.02$^{Ac}$ | 5.86±0.01$^{Ad}$ |
| | T1 | 6.26±0.02$^{Ba}$ | 6.08±0.01$^{Bb}$ | 5.89±0.02$^{Bc}$ | 5.82±0.01$^{BCd}$ |
| | T2 | 6.26±0.02$^{Ba}$ | 6.09±0.00$^{Bb}$ | 5.87±0.01$^{Bc}$ | 5.84±0.01$^{Bd}$ |
| | T3 | 6.21±0.01$^{Ca}$ | 6.08±0.01$^{Bb}$ | 5.77±0.01$^{Cd}$ | 5.83±0.01$^{Bc}$ |
| | T4 | 6.20±0.01$^{Ca}$ | 6.03±0.00$^{Cb}$ | 5.77±0.02$^{Cd}$ | 5.81±0.01$^{Cc}$ |
| TBARS (mg MA/100g) | C | 0.21±0.01$^{b}$ | 0.31±0.02$^{a}$ | 0.31±0.01$^{Aa}$ | 0.29±0.02$^{a}$ |
| | T1 | 0.22±0.04$^{b}$ | 0.28±0.01$^{a}$ | 0.28±0.01$^{Ca}$ | 0.27±0.01$^{a}$ |
| | T2 | 0.21±0.01$^{b}$ | 0.30±0.01$^{a}$ | 0.29±0.02$^{BCa}$ | 0.30±0.02$^{a}$ |
| | T3 | 0.22±0.02$^{b}$ | 0.29±0.01$^{a}$ | 0.29±0.00$^{Ca}$ | 0.27±0.01$^{a}$ |
| | T4 | 0.22±0.02$^{c}$ | 0.29±0.01$^{b}$ | 0.31±0.01$^{ABa}$ | 0.27±0.01$^{b}$ |
| Residual nitrite (ppm) | C | 12.63±0.29$^{Aa}$ | 9.92±0.39$^{b}$ | 8.42±0.07$^{Ac}$ | 6.91±0.54$^{d}$ |
| | T1 | 12.04±0.19$^{Ba}$ | 9.27±1.00$^{b}$ | 7.14±0.48$^{Bc}$ | 6.90±0.33$^{c}$ |
| | T2 | 12.60±0.33$^{Aa}$ | 9.59±0.29$^{b}$ | 7.50±0.41$^{Bc}$ | 7.21±0.12$^{c}$ |
| | T3 | 13.01±0.05$^{Aa}$ | 9.91±0.49$^{b}$ | 7.16±0.15$^{Bc}$ | 7.02±0.04$^{c}$ |
| | T4 | 12.76±0.20$^{Aa}$ | 9.25±1.00$^{b}$ | 7.55±0.27$^{Bc}$ | 6.60±0.29$^{c}$ |

[1)]Treatments are the same as those in Table 1.
[A-C]Means with different superscript uppercase letters in the same column significantly differ with $p<0.05$.
[a-d]Means with different superscript lowercase letters in the same row significantly differ with $p<0.05$.

nificantly decreased in sausages by addition of thyme and rosemary compared to that in the control before and after storage. TBARS measured as the lipid oxidation value increased with storage period in all sausages. However, the TBARS value did not show any consistent trends between sausage samples during storage. Residual nitrite contents were not significantly different at the beginning of storage, but decreased by addition of thyme and rosemary compared to that in the control, at 4 wk of storage. The effects of thyme and rosemary on the color of sausages during storage are presented in Table 3 and Fig. 2. The lightness (L*) and yellowness (b*) during storage was increased, whereas the redness (a*) and whiteness (W) were decreased, both before and after storage by addition of thyme and rosemary. The effects of thyme and

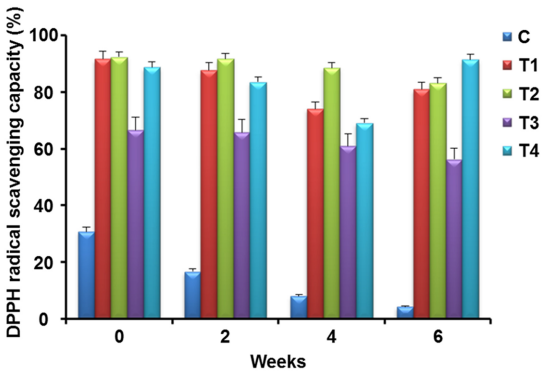


**Fig. 1. Effect of thyme and rosemary on the DPPH radical scavenging capacity in sausages during storage.** [1)]Treatments are the same as those in Table 1. Different letters are significantly different at $p<0.05$.

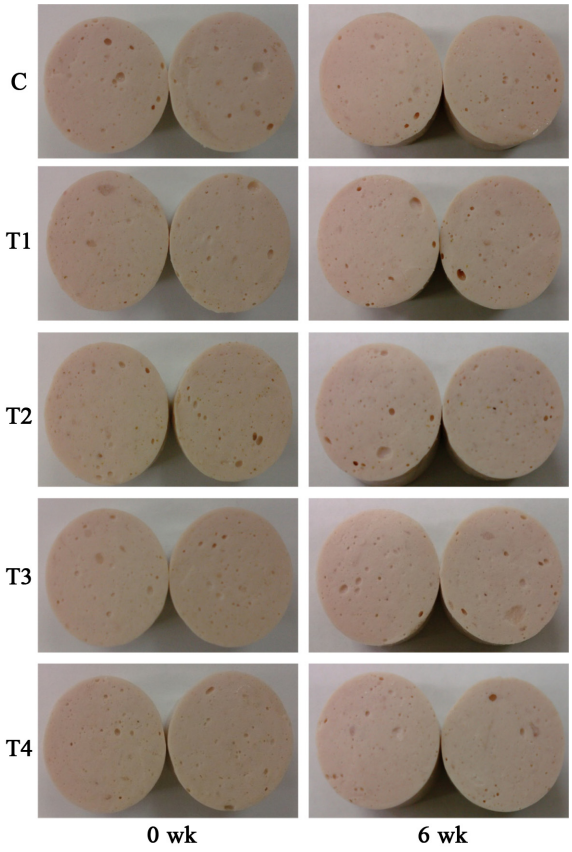


**Fig. 2. Representative images of the processed sausages.** Treatments are the same as those in Table 1.

**Table 3. Effect of thyme and rosemary on the color of sausages during storage**

| Item | Treatment[1)] | Storage (wk) | | | |
|---|---|---|---|---|---|
| | | 0 | 2 | 4 | 6 |
| L* | C | 81.34±0.39$^{AB}$ | 81.33±0.18$^{B}$ | 81.47±0.05$^{C}$ | 81.24±0.06$^{C}$ |
| | T1 | 81.57±0.19$^{A}$ | 81.46±0.38$^{B}$ | 81.87±0.24$^{B}$ | 81.31±0.28$^{C}$ |
| | T2 | 80.78±0.24$^{B}$ | 80.65±0.32$^{C}$ | 80.84±0.16$^{D}$ | 80.61±0.09$^{D}$ |
| | T3 | 81.43±0.47$^{AB}$ | 81.53±0.38$^{B}$ | 81.91±0.13$^{B}$ | 81.60±0.04$^{B}$ |
| | T4 | 82.01±0.44$^{A}$ | 82.21±0.15$^{A}$ | 82.34±0.13$^{A}$ | 82.00±0.19$^{A}$ |
| a* | C | 6.40±0.18$^{Ab}$ | 6.97±0.38$^{Aa}$ | 6.32±0.03$^{Ab}$ | 6.51±0.04$^{Ab}$ |
| | T1 | 5.49±0.09$^{Cb}$ | 5.92±0.09$^{BCa}$ | 5.80±0.19$^{Ca}$ | 5.97±0.13$^{Ba}$ |
| | T2 | 5.35±0.03$^{CDb}$ | 6.12±0.20$^{Ba}$ | 5.38±0.09$^{Db}$ | 5.45±0.16$^{Cb}$ |
| | T3 | 6.16±0.18$^{Ba}$ | 5.50±0.10$^{CDb}$ | 6.01±0.05$^{Ba}$ | 6.03±0.02$^{Ba}$ |
| | T4 | 5.13±0.09$^{D}$ | 5.01±0.56$^{D}$ | 5.27±0.07$^{D}$ | 5.12±0.02$^{D}$ |
| b* | C | 8.18±0.15$^{Ca}$ | 7.50±0.21$^{Db}$ | 8.31±0.06$^{Ca}$ | 8.25±0.07$^{Ca}$ |
| | T1 | 8.81±0.08$^{ABa}$ | 8.52±0.03$^{BCb}$ | 8.69±0.10$^{Bab}$ | 8.76±0.17$^{Ba}$ |
| | T2 | 8.81±0.07$^{ABb}$ | 8.32±0.10$^{Cc}$ | 9.13±0.12$^{Aa}$ | 9.13±0.17$^{Aa}$ |
| | T3 | 8.70±0.28$^{B}$ | 8.90±0.13$^{AB}$ | 8.61±0.08$^{B}$ | 8.68±0.09$^{B}$ |
| | T4 | 9.02±0.08$^{A}$ | 9.03±0.49$^{A}$ | 8.99±0.04$^{A}$ | 9.10±0.05$^{A}$ |
| W* | C | 56.79±0.79$^{Ab}$ | 58.83±0.58$^{Aa}$ | 56.54±0.15$^{Ab}$ | 56.49±0.23$^{Ab}$ |
| | T1 | 55.14±0.38$^{B}$ | 55.89±0.32$^{B}$ | 55.81±0.26$^{BC}$ | 55.02±0.76$^{BC}$ |
| | T2 | 54.34±0.06$^{Bb}$ | 55.68±0.53$^{Ba}$ | 53.45±0.52$^{Dc}$ | 53.22±0.55$^{Dc}$ |
| | T3 | 55.32±1.32$^{B}$ | 54.83±0.77$^{B}$ | 56.09±0.34$^{AB}$ | 55.57±0.29$^{B}$ |
| | T4 | 54.95±0.54$^{B}$ | 55.13±1.42$^{B}$ | 55.36±0.11$^{C}$ | 54.69±0.09$^{C}$ |

[1)]Treatments are the same as those in Table 1.
[A-D]Means with different superscript uppercase letters in the same column significantly differ with $p<0.05$.
[a-c]Means with different superscript lowercase letters in the same row significantly differ with $p<0.05$.
*W=L*-3b*

**Table 4. Effect of thyme and rosemary on the a*w*, TPC, and lactic acid bacteria count in sausages during storage**

| Item | Treatment[1)] | Storage (weeks) | | | |
|---|---|---|---|---|---|
| | | 0 | 2 | 4 | 6 |
| a*w* | C | 0.92±0.02 | 0.90±0.00 | 0.91±0.01 | 0.90±0.01 |
| | T1 | 0.92±0.01 | 0.90±0.00 | 0.91±0.00 | 0.90±0.00 |
| | T2 | 0.91±0.00 | 0.91±0.00 | 0.91±0.00 | 0.91±0.01 |
| | T3 | 0.91±0.01 | 0.90±0.01 | 0.92±0.01 | 0.91±0.00 |
| | T4 | 0.91±0.01 | 0.91±0.01 | 0.92±0.00 | 0.91±0.01 |
| TPC | C | 0.00±0.00 | 0.45±0.21 | 0.50±0.71 | 0.97±0.39$^{A}$ |
| | T1 | 0.00±0.00 | 0.38±0.04 | 0.24±0.34 | 0.54±0.08$^{B}$ |
| | T2 | 0.00±0.00 | 0.15±0.21 | 0.48±0.00 | 0.24±0.34$^{BC}$ |
| | T3 | 0.00±0.00 | 0.26±0.11 | 0.10±0.00 | 0.00±0.00$^{C}$ |
| | T4 | 0.00±0.00 | 0.54±0.34 | 0.63±0.46 | 0.00±0.00$^{C}$ |
| Lactic acid bacteria | C | 0.00±0.00 | 0.00±0.00 | 0.60±0.61 | 0.85±0.21$^{A}$ |
| | T1 | 0.00±0.00 | 0.15±0.21 | 0.24±0.34 | 0.83±0.11$^{Aa}$ |
| | T2 | 0.17±0.18 | 0.00±0.00 | 0.24±0.34 | 0.00±0.00$^{B}$ |
| | T3 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00$^{B}$ |
| | T4 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00$^{B}$ |

[1)]Treatments are the same as those in Table 1.
[A-C]Means with different superscript uppercase letters in the same column significantly differ with $p<0.05$.
[a-c]Means with different superscript lowercase letters in the same row significantly differ with $p<0.05$.

rosemary on the a*w*, TPC, and lactic acid bacteria count in sausages during storage are presented in Table 4. Water activity (a*w*) was not significantly different among sausage samples during storage. TPC and lactic acid bacteria count were lower at the end of storage in sausage samples containing thyme and rosemary. The effects of thyme and rosemary on the DPPH radical scavenging capacity in sausages during storage are presented in Fig. 1. The DPPH radical scavenging capacity of the sausages was increase by the addition of thyme and rosemary compared to that

**Table 5. Effect of thyme and rosemary on the sensory evaluation of sausages during storage**

| Item | Treatment[1)] | Storage (wk) | | | |
|---|---|---|---|---|---|
| | | 0 | 2 | 4 | 6 |
| Color | C | 7.42±0.38 | 7.50±0.32 | 7.25±0.52 | 7.17±0.26 |
| | T1 | 7.42±0.66 | 7.17±0.26 | 6.83±0.61 | 7.08±0.20 |
| | T2 | 7.08±0.20 | 6.83±0.41 | 6.67±0.61 | 6.92±0.20 |
| | T3 | 7.42±0.20 | 7.08±0.20 | 6.75±0.27 | 7.00±0.55 |
| | T4 | 6.83±0.52 | 6.83±0.41 | 6.67±0.41 | 6.83±0.52 |
| Aroma | C | $7.67±0.26^{A}$ | $7.33±0.26^{A}$ | $7.17±0.52^{A}$ | 7.00±0.55 |
| | T1 | $6.67±0.41^{B}$ | $6.33±0.41^{B}$ | $6.33±0.52^{B}$ | 6.67±0.41 |
| | T2 | $6.58±0.38^{B}$ | $6.17±0.41^{B}$ | $6.17±0.61^{B}$ | 6.67±0.26 |
| | T3 | $6.83±0.61^{Ba}$ | $6.75±0.61^{AB}$ | $6.33±0.41^{B}$ | 6.67±0.68 |
| | T4 | $6.83±0.68^{B}$ | $6.83±0.75^{AB}$ | $6.50±0.63^{B}$ | 6.92±0.38 |
| Flavor | C | $7.92±0.20^{Aa}$ | $7.67±0.41^{Aab}$ | $7.08±0.74^{b}$ | $7.08±0.49^{b}$ |
| | T1 | $6.75±0.69^{BC}$ | $6.42±0.49^{B}$ | 6.25±0.61 | 6.67±0.41 |
| | T2 | $6.50±0.32^{C}$ | $6.42±0.66^{B}$ | 5.83±0.93 | 6.42±0.38 |
| | T3 | $7.00±0.63^{BC}$ | $6.42±0.49^{B}$ | 6.58±0.97 | 6.58±0.38 |
| | T4 | $7.17±0.52^{B}$ | $7.00±0.45^{B}$ | 6.83±0.61 | 6.83±0.41 |
| Springiness | C | $7.83±0.41^{a}$ | $7.42±0.58^{ab}$ | $7.42±0.38^{ab}$ | $6.75±0.69^{b}$ |
| | T1 | $7.75±0.42^{a}$ | $7.58±0.58^{a}$ | $7.08±0.20^{b}$ | $7.00±0.32^{b}$ |
| | T2 | $7.67±0.61^{a}$ | $7.58±0.38^{ab}$ | $7.08±0.49^{bc}$ | $6.83±0.26^{c}$ |
| | T3 | 7.75±0.52 | 7.50±0.63 | 7.08±0.38 | 7.22±0.57 |
| | T4 | 7.58±0.49 | 7.42±0.38 | 7.17±0.52 | 7.30±0.35 |
| Juiciness | C | $7.92±0.20^{a}$ | $7.33±0.26^{b}$ | $7.33±0.52^{b}$ | $7.00±0.55^{b}$ |
| | T1 | 7.42±0.38 | 7.17±0.26 | 7.17±0.41 | 6.83±0.41 |
| | T2 | 7.25±0.76 | 7.17±0.26 | 7.17±0.68 | 6.92±0.49 |
| | T3 | 7.50±0.32 | 7.17±0.52 | 7.00±0.55 | 7.05±0.42 |
| | T4 | 7.50±0.32 | 7.08±0.49 | 7.17±0.68 | 6.83±0.26 |
| Overall acceptability | C | $8.00±0.32^{Aa}$ | $7.58±0.38^{Aab}$ | $7.17±0.41^{Abc}$ | $7.00±0.45^{c}$ |
| | T1 | $7.08±0.49^{B}$ | $6.42±0.49^{B}$ | $6.50±0.63^{AB}$ | 6.67±0.41 |
| | T2 | $6.50±0.45^{B}$ | $6.42±0.74^{B}$ | $6.17±0.61^{B}$ | 6.55±0.34 |
| | T3 | $6.92±0.74^{B}$ | $6.50±0.77^{B}$ | $6.67±0.61^{AB}$ | 6.83±0.52 |
| | T4 | $6.83±0.82^{B}$ | $7.00±0.45^{AB}$ | $6.92±0.49^{A}$ | 6.75±0.27 |

[1)]Treatments are the same as those in Table 1.

[A,B]Means with different superscript uppercase letters in the same column significantly differ with $p<0.05$.

[a,b]Means with different superscript lowercase letters in the same row significantly differ with $p<0.05$.

[2)]Color (9 = very good and 1 = very bad), Aroma (9 = very intense and 1 = very weak), Flavor (9 = very good and 1 = very bad), Springiness (9 = very elastic and 1 = very inelastic), Juiciness (9 = very juiciness and 1 = very dry), Overall acceptability (9 = very good and 1 = very bad).

observed in the control both before and after storage. In particular, T2 showed the highest DPPH radical scavenging capacity during storage. The effects of thyme and rosemary on the sensory evaluation of sausage samples during storage are presented in Table 5. In the sensory evaluation, color, springiness, and juiciness were not significantly different among the sausage samples during storage, whereas aroma, flavor, and overall acceptability were lower in sausages containing thyme and rosemary than in the control. However, at the end of storage (6 wk), the aroma, flavor, and overall acceptability were not significantly different among the sausage samples.

Phytochemicals such as polyphenol and flavonoids play a major role in antioxidant activity. Erkan *et al*. (Erkan *et al.*, 2008) reported that rosemary extract has a high phenolic content, thus leading to a high antioxidant activity. Some phenolic diterpenes such as carnosic acid, carnosol, rosmanol, rosmariquinone and rosmaridiphenol, which break free radical chains by donating hydrogen atoms, have been reported to be associated with the antioxidant activity of rosemary extracts (Aruoma *et al*., 1992). Rota *et al*. (2008) reported that the most important antioxidant compounds of thyme are the phenols thymol and carvacrol. Thus, components of thyme essential oils show their own antioxidant activity (Viuda-Martos *et al.*, 2009). However, the addition of thyme and rosemary powder to sausages did not influence the lipid oxidation value (TBARS) in this study, whereas the DPPH radical scavenging capacity of the sausage samples was dramatically increased by thyme and rosemary addition; the highest DPPH radical

scavenging capacity was observed in sausages containing 0.2% thyme. This radical scavenging capacity may result from the disruption of radical reactions by thyme and rosemary based on their flavonoid or phenolic components, which may be responsible for the apparent antioxidative activity. The different results between the previous studies and present study with regard to TBARS measured as lipid oxidation may have resulted from different amounts of flavonoids or phenolic components in thyme and rosemary. In this study, the sausage samples contained thyme and rosemary powder; therefore, the amount of flavonoids and phenolic components was lower than that in the thyme and rosemary extract and thyme essential oil used in previous studies. Therefore, we assume that thyme and rosemary extracts may be more beneficial in reducing lipid oxidation, although DPPH radical scavenging capacity was increased by thyme and rosemary powder in the sausage samples. The difference between TBARS value and DPPH radical scavenging activity in this study may be due to other factors were involved such as packaging conditions, sausage spice, salt concentration or fat contents.

In an early study, Sebranek *et al.* (1979) discussed the importance of pH for the residual nitrite level and stated that a pH decline could decrease the residual nitrite level. Honikel (Honikel, 2008) also reported that decreasing the product pH dramatically increases the rate of nitrite reduction to nitric oxide in meat curing. Deda *et al*. (Deda *et al.*, 2007) found that the residual nitrite content of frankfurters decreased with storage time in association with the decrease of pH during storage. Therefore, we consider that the reduction of residual nitrite concentration in the sausage samples may have resulted from a pH decline because the pH of the sausages was decreased by addition of thyme and rosemary in this study.

The addition of thyme and rosemary largely influenced sausage color during storage, mainly because thyme and rosemary have their own color. Thus, the lightness (L*) and yellowness (b*) increased with storage in sausage to which thyme and rosemary were added, whereas redness (a*) and whiteness (W) were decreased by addition of thyme and rosemary. This color change may have negatively affected the sensory evaluation score. Additionally, the decrease in flavor and overall acceptability scores for the sausage samples at the beginning of storage may have been the result of the distinct flavor of thyme and rosemary. Therefore, decoloration or bleaching of thyme and rosemary should be a prerequisite to prevent the alteration of color or sensory characteristics at the beginning of storage.

In this study, the total plate count and lactic acid bacteria count, which are indices of bacterial growth, were decreased by thyme and rosemary addition when determined at the end of storage. This result may reflect the antibacterial activity of thyme and rosemary. Thyme and rosemary are a rich source of polyphenolic compounds and flavonoids, such as thymol, carvacrol, terpenes or alcohol. Angioni *et al*. (Angioni *et al*., 2004) reported that rosemary extracts have some antimicrobial effects. Soluble phenols are thought to exert their antimicrobial effect by causing hyperacidification at the plasma membrane interface of the microorganism, which potentially results in disruption of the $H^+$-ATPase required for ATP synthesis (Vattem *et al.*, 2004). Moreno *et al.* (2006) also reported that the antimicrobial action of phenolic compounds was related to the inactivation of cellular enzymes, which depended on the rate of penetration of the substance into the cell or was caused by membrane permeability changes. It has been suggested that phenolic compounds destabilize the cytoplasmic membrane and also act as proton exchangers, thereby reducing the pH gradients across the cytoplasmic membrane (Gallucci *et al*., 2009; Karunanayaka *et al*., 2016). The resulting collapse of the proton motive force and depletion of the ATP pool eventually leads to cell death (Gallucci *et al*. 2009; Lee *et al*., 2016). Thus, we assumed that the antimicrobial effect of thyme and rosemary in sausages might result from phenolic toxicity to microorganisms during storage. Another possible mechanism for the inhibition of bacterial growth in the sausage samples by thyme and rosemary addition may be decreased pH. It is known that bacteria are sensitive to pH changes because the enzymes that stabilize their cell membrane and participate in ion exchange are affected by pH. Many microorganisms involved in spoilage of meat products grow better at neutral pH. Therefore, the pH decrease by thyme and rosemary addition to sausage may have been one of the main reasons for the anti-bacterial effect in this study. However, the growth of lactic acid bacteria also decreased in sausage during storage although lactic acid bacteria is acidophil bacterium. This result may be due to other factors were involved in growth of lactic acid bacteria such as water activity (a*w*), packaging condition, surface drying or additives in this study. The water activity (a*w*) is the ratio of the partial vapor pressure of water and plays an important role in microbial growth in food materials. However, the water activity did not vary among the sausage samples during storage in this study, which indicates that the water activity was not

involved in the effect of thyme and rosemary addition on microorganism in this study.

In this study, the addition of thyme and rosemary negatively influenced the sensory evaluation score during storage; in particular, the aroma, flavor, and overall acceptability were decreased by addition of thyme and rosemary. However, at the end of storage, all sensory characteristics did not differ among the sausage samples. As thyme and rosemary are herbs, their herbal flavor may have had a negative effect on sensory characteristics because the flavor of thyme and rosemary may be unfamiliar in commercial sausages. Therefore, deodorization processes such as encapsulation or steaming may be needed before addition.

## Conclusions

In this study, the lipid oxidation values in sausages were not affect by the addition of thyme and rosemary. However, the addition of thyme and rosemary increased the DPPH radical scavenging activity of the sausage samples. At the end of storage, the total plate count and lactic acid bacteria count were decreased by the addition of thyme and rosemary compared to the counts in control samples. In the sensory evaluation, the flavor and overall acceptability were lower in sausages containing thyme and rosemary than in the control. However, at the end of storage (6 wk), aroma, flavor, and overall acceptability were not significantly different among the sausage samples. Therefore, we concluded that addition of thyme and rosemary is not an effective way to improve the sensory evaluation of sausages at the beginning of storage but may beneficially affect antioxidative activity and antimicrobial activity, as thyme and rosemary are good natural sources of dietary bioactive components.

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