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ARTICLE

Effect of Addition of *Allium hookeri* on the Quality of Fermented Sausage with Meat from Sulfur Fed Pigs during Ripening

Eun-Yeong Song, Chang-Won Pyun, Go-Eun Hong, Ki-Won Lim[1], and Chi-Ho Lee*

Department of Food Science and Biotechnology of Animal Resource, Graduate School of Konkuk University, Seoul 143-701, Korea

[1]Department of Physical Education, Konkuk University, Seoul 143-701, Korea

Abstract

The effect of the addition of *Allium hookeri* on the quality of fermented sausage made with meat from sulfur fed pigs was examined, throughout a 60 d ripening period. There were two treatments in animal management: normal feed fed pigs, and sulfur fed pigs given 0.3% sulfur mixed normal feed. Fermented sausage manufactured with meat from normal feed fed pigs, and with meat from sulfur fed pigs, and 1% *A. hookeri*-containing fermented sausage processed with meat from sulfur fed pigs, were determined at 1 d, 15 d, 30 d, and 60 d. The meat qualities in fermented sausage were measured by DPPH radical scavenging activity (DPPH), $ABTS^+$ radical scavenging activity ($ABTS^+$), total phenolic acids, and total flavonoid contents. Fermented sausage made from pigs that had been fed with 0.3% sulfur was protected from oxidation by reduced free radical, as shown by the significant increase in DPPH and $ABTS^+$ values, compared with fermented sausage made from normal feed fed pigs ($p<0.05$). *A. hookeri*-added fermented sausage with sulfur fed pork was shown to increase the values in DPPH, $ABTS^+$, total phenolic acid, and total flavonoid contents, by comparison with both the control sausage, and sausage with sulfur fed pork, at 60 d. These results suggest that *A. hookeri* in meat from sulfur fed pigs could be a source of natural addition, to increase quality in the food industry.

Keywords: meat quality, *Allium hookeri*, fermented sausage, sulfur supplement, bioactive compound

Introduction

Fermented sausage is traditional in Europe, as it has a special texture and flavor made through bacterial growth, acid formation, lipolysis, proteolysis, and oxidation (Johansson *et al*., 1994). Fermentation occurs when lactic acid bacteria synthesize lactic acid from glucose in the sausage and also in pH decreases occur due to lactic acid which protects against growth of pathogenic bacteria, modifies the meat protein due to water flowing out, and decreases water activity (Kim *et al*., 1996). The initial enzymes and microorganisms in meat decompose the meat protein and fat resulting in a unique flavor (Hammes and Knauf *et al*., 1994). When *Staphylococcus carnosus* and *Staphylococcus xylosus* are added to fermented sausage with lactic acid, the taste and smell become stronger (Johansson *et al*., 1994).

Recent developments in the field of long-term storage of fermented sausage have led to a renewed interest in protecting against oxidation. Oxidative modifications in meat products can induce loss of essential amino acids and decrease digestibility (Morzel *et al*., 2006; Santé-Lhoutelier *et al*., 2008). Lipid oxidation induces changes in quality such as odor and taste (Nieto *et al*., 2013) and protein oxidation in meat products affects gelation, emulsification, viscosity, solubility, and rehydration (Xiong, 2000). Synthetic antioxidants such as butylated hydrozyanisole, butylated hydroxytoluene, and propyl gallate are generally used in the food industry but questions have been raised about the safety of prolonged use of synthetic antioxidants with known potential adverse health effects.

Sulfur is an important component as part of the essential amino acids cysteine and methionine, which forms a protein block in connective tissues and muscle. Sulfur protects cells from damage due to radical activity such as oxidation because thiols formed in the body are related to reduced oxidation (Han *et al*., 1996). Sulfur compounds such as sulfoxide, disulfides, trisulfides, and thiophenes are involved in decreasing lipid content (Sainani *et al*.,

*Corresponding author: Lee Chi-Ho, Department of Food Science and Biotechnology of Animal Resource, Graduate School of Konkuk University, Seoul 143-701, Korea. Tel: +82-2-450-3681, Fax: +82-2-453-1948, E-mail: leech@konkuk.ac.kr

1979) and inhibiting microorganism activities (Subrahmanyan *et al*., 1958) but sulfur should not be eaten directly due to side effects; therefore, sulfur should be taken indirectly in food with a high sulfur concentration (Choi and Kim, 2002). Confirmation of pig and chicken quality fed sulfur has been increasing in the meat industry (Lee *et al*., 2009; Lee *et al*., 2013).

Allium species such as garlic (*Allium sativum* L.) and onion (*Allium cepa*) have been used to increase flavor and taste because they are a rich source of sulfur (Cavallito and Bailey *et al*., 1994). *Allium* spp. possesses antioxidant activity due to their rich phenolic and flavanoid contents, which protect against bacterial infection (Kim *et al*., 2012; Yin and Cheng, 1998). They also have antifungal activity due to chitinases that decompose glycosidic bonds of chitin in cell walls of fungi (Vergawen *et al*., 1998; Yin and Tsao, 1999). *Allium* spp. also have high inulin type fructant content, which affects serum lipid, blood glucose, and the gastro-intestinal environment of humans (Causey *et al*., 2000). According to the medical dictionary of Myanmar, *A. hookeri* can be used to treat cancer or inflammation because of methyl sulfonyl methane (Bae and Bae, 2012). *A. hookeri* is known of widely used medicinal food as possessing a lot of contents such as protein, glucose, fiber, ascorbic acid, phytosterol, total phenol than onion (Ayam, 2011). Experiments in regard to bioactive substance of *A. hookeri* have performed and recently reported anti inflammatory effects of *A. hookeri* root (Kim *et al*., 2012).

Experiments to identify effective ways to improve meat quality of fermented sausage with natural agents have become a central issue in the meat industry. The effect of *A. hookeri* on quality of fermented sausage made of meat from sulfur fed pigs has not been investigated during ripening. The objective of this study was to confirm the effects of addition in a fermented sausage made from meat from sulfur fed pigs with added *A. hookeri.*

Materials and Methods

Animal management

Three way crossbred pigs (Landrace, Yorkshire, and Duroc) were used in this study. They were allotted into four pens (15 pigs per pen) and pigs were allocated into two dietary treatments from Farm (Paju-si, Gyeonggi-do, Korea). Pigs were fed a commercial diet, of which the chemical composition is given in Table 1, were used to make the control group of fermented sausage. A 0.3% sulfur mixed commercial diet was fed to pigs used in the test group. The two diets contained the same composition at the same rates until 90 d, but differed at 90 d as control pigs were fed a normal diet and the meat from sulfur fed pigs were fed 0.3% sulfur until 180 d. The pigs were slaughtered at an average final weight of 120 kg and no significant difference was observed between the two groups. Part of pork loin was used to be sausage.

Table 1. Chemical composition of the supplemented feed

Chemical composition	Contents (%)	
	Normal feed	Sulfur mixed feed
Crude protein	16	16
Crude fat	4.48	4.48
Crude ash	4.03	4.03
Crude fiber	3.99	3.99
Ca	0.40	0.40
P	0.80	0.80
Total lysine	0.86	0.86
Sulfur	-	0.3
DE (Mcal/kg)	0.345	0.345

DE, digestible energy

Sample preparation and design

The *Pentosaceus pentosaceus* ATCC 33314 culture was purchased from KCTC (Korean Collection for Type Culture) and the freeze dried *Staphylococcus carnosus* was obtained from Chr. Hanscn's. The starter culture was incubated in MRS broth (Oxoid, Surrey, England) with 5% (w/v) glucose until *P. pentosaceus* ATCC 33314 reached a concentration of 10^6 microorganisms/g before being added to the grinding sausage. *P. pentosaceus* ATCC 33314 was injected at 6-7 Log CFU/g and the start culture powder of *S. carnosus* was used at a concentration > 10^7 microorganisms/g.

Purchased from local market, the *A. hookeri* was cut and frozen in deep freezer to dry. The dried powder of *A. hookeri* was stored at -80°C until used. The sausages were divided into three different batches. Back fat and lean pork of pigs fed with normal feed were used as the control (Con), sausage made from 0.3% meat from sulfur fed pigs was the test 1 (T1) group, and adding 1% dried *A. hookeri* to T1 was the test 2 (T2) group.

Each group followed the ingredients shown in Table 2. Three different batches of fermented sausage were produced on same day. As approximately 3 cm×3 cm of raw meats were cut between -4°C and -8°C after slaughter, samples for making sausage were frozen in a deep freezer immediately and thawed 1 d before preparation. The meat and fat were chopped. The ground back fat and lean pork were mixed with prepared starters and other ingredients.

Table 2. Ingredients used for the fermented sausage (%)

	Con	T1	T2
Lean Pork	75	75	75
Pork Back Fat	25	25	25
	100	100	100
Salt	2	2	2
Glucose	1	1	1
Sugar	0.50	0.50	0.50
Pepper	0.50	0.50	0.50
Nitrite	0.0004 (4 ppm)	0.0004 (4 ppm)	0.0004 (4 ppm)
Allium Hookeri			1 (10000 ppm)

Con, control group; T1, Fermented sausage from 0.3% sulfur fed pigs; T2, Fermented sausage from 0.3% sulfur fed pigs with added *Allium hookeri*

The sausages were stuffed in 20 cm long and 3 cm diameter collagen casings and dried at 20-25°C for seven days at a relative humidity of > 80%, were not smoked ,and then the temperature range was decreased to 15-20°C for 60 d at a relative humidity of > 50 % in the chamber (HT-A30GG3, Century, Korea). The period of fermentation was set at 60 d. Analyses were conducted at d 0, 15, 30, and 60 during fermentation.

Analysis of fermented sausage

Proximate analysis

The proximate analysis of the fermented sausage followed an AOAC (Association of Official Agricultural Chemists) method (2002). Moisture content was determined by drying in a 100°C oven. Crude protein was determined by the Kjeldahl method. Crude fat was extracted by the Soxhlet method, and crude ash was determined at 450°C by burning.

pH, water activity, and microorganisms

A 2 g sample of sausage was diluted 10 times with distilled water to measure pH with a pH meter (pH 900, Precisa Co, London, UK) after using a Bagmizer homogenizer for 90 sec (Bagmixer 400, Interscience Co., Saint-Nom-la-Bretèche, France). To assess microorganisms, a 2 g sample diluted 10 times with 0.85% sterile saline in a bag filter was homogenized with a bag mixer. Lactic acid bacteria were evaluated in MRS broth (Oxoid, Surrey, England) with 5% (w/v) glucose. Water activity was assessed with a water activity measuring device (Aqua Lab CX-2, Decagon Device Inc., Pullman, WA, USA) at 25°C (room temperature). *E. coli* test was performed in Petrifilm *E. coli* Count Plates (3M, Seoul, Korea) for 48 h at 35°C, *Staphylococcus aureus* was determined in Baird parker agar (Oxoid, Surrey, England) for 48 h at 35°C, and to confirm the presence of *Salmonella enteritidis*, a solution of sample was cultured for 24 h at 35°C in MacConkey's agar (Oxoid, Surrey, England). The counts of all colonies were in Log values when the count was in the range of 30-300 per dish.

Antioxidant analysis

A 1 g fermented sausage sample was homogenized and extracted in 10 mL of 70% ethanol (v/v), and the supernatant was used to assess antioxidant properties by DPPH and ABTS radical scavenging activities as well as phenol contents. The flavonoid analysis used a 70% methanol (v/v) solution instead of a 70% ethanol (v/v) solution.

DPPH radical scavenging activity

The free radical scavenging activity assays followed the modified method described by Blois (1958). A 900 μL aliquot of sample and 300 μL of a 100 ppm DPPH solution were reacted for 30 min. The absorbance was checked at 517 nm, and free radical scavenging activity was calculated using the following formula.

$$\text{DPPH radical scavenging activity (\%)} = (1 - \text{ABS sample}/\text{ABS control}) \times 100$$

$ABTS^+$ radical scavenging activity

The modified method described by Erel (2004) was applied to evaluate $ABTS^+$ radical scavenging activity. Before analyzing the samples, 7 mM ABTS (2,2'-azinbis-(3-ethyl-benzothiazoline-6-sulfonic acid) was reacted with 2.45 mM potassium persulfate to form $ABTS^+$ after 14 h in the dark. $ABTS^+$ had an absorbance of 0.70±0.02 at 734 nm as a diluted $ABTS^+$ solution in ethanol. A 20 μL aliquot of sample was added to 3 mL of diluted $ABTS^+$ solution. The blank used ethanol instead of sample. The $ABTS^+$ radical scavenging activity of samples was mea-

sured by absorbance with a UV spectrometer at 734 nm and calculated with the equation:

$ABTS^+$ radical scavenging activity (%) =
[(ABS control – ABS sample)/ABS control] × 100

Total phenol content

The modified method described by Singleton *et al.* (1999) was used to analyze total phenol content. Briefly, 125 μL of sample was added to 125 μL Folin-Ciocalteu phenol reagent in 0.5 mL distilled water and then mixed. A 7% Na_2CO_3 (w/v) solution was diluted with 1 mL of distilled water, added, and incubated for 1 min. The tubes remained at room temperature in the dark for 90 min. The samples were then analyzed with a UV spectrometer (Optizen 2120UV, Mecasys, Seoul, Korea) at 750 nm. Phenolic content is presented as mg GAE (gallic acid equivalent) per g using gallic acid in the standard curve.

Total flavonoid content

Flavonoid content was measured by the method described by Sakanaka *et al*. (2005). A 1.25 mL aliquot of distilled water and 75 μL of 5% (w/v) sodium nitrite were added to 150 μL of 10% (v/v) aluminum chloride for 5 min and then mixed with 250 μL of extract solution. The blank used distilled water. A 0.5 mL aliquot of 1 M sodium hydroxide and 0.6 mL distilled water were added for 6 min. After mixing the solution, absorbance was measured with a UV spectrometer at 510 nm. The units of flavonoid content were mg quercetin per g using quercetin as the standard curve.

Color value

Color values were measured as Hunter values of lightness (L^*), redness (a^*), and yellowness (b^*) on the fermented sausage cut surface using a colorimeter (Chromameter, CR-210, Minolta Co, Tokyo, Japan) following fermentation. The total color difference was described by the color value E. The L^*, a^*, b^* Hunter values of the standard plate were L^*=96.46, a^*=0.25, and b^*=2.02.

$$E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$

ΔL : difference in lightness
Δa : difference in redness
Δb : difference in yellowness

Sensory test

The sensory test results were scored from 1 to 5 with 1 representing the worst. Six categories were used such as taste, flavor, color, hardness, juiciness, and overall acceptance on the last fermentation day. Twelve panelists participated in the sensory test.

Statistical analysis

The statistical analysis was performed using SAS (Statistical Analysis System, 1999). All data are presented as mean±standard deviation from three replicates but sensory value data were n=12. Differences between means were statistically determined by Duncan's multiple range test. Differences were considered significant at $p<0.05$

Results and Discussion

Proximate analysis

The proximate analysis consisting of moisture, crude fat, crude protein, and crude ash is shown in Table 3. Moisture content in the control group was significantly lower than that in all other test groups on d 1 ($p<0.05$). Moisture content of T2 decreased significanty during 60 d of ripening and increased significantly compared to that of the other groups during the entire fermentation period ($p<0.05$). This result was regarded as a sulfur effect of *A. hookeri* and was similar with previous experiments; pigs loin supplemented with methyl sulfonyl methane during cold storage had the lowest amount of leaked meat juice (Lee *et al*., 2009). Crude protein, and crude fat contents increased significantly on d 60 compared with on d 1 and were not shown significant different among the groups on d 1, 30, and 60 ($p<0.05$). Crude ash was no significant different among the groups on d 1 but as the value of T1 and T2 was significantly increased during entire period, Crude ash in the T1 and T2 groups was significant increase compared with control on 60 d ($p<0.05$).

pH, water activity, and microorganism

The results of pH, lactic acid bacterial counts, water activity, and microorganisms on fermented sausage are shown in Table 4. The significant change of pH in the T2 was shown during 60 d of ripening ($p<0.05$). The initial and final values of pH in the T2 group were shown, the final pH being lower than the initial pH, a decreasing pH value was observed in the T2 group on d 15 and 30, as lactic acid increased due to growth of lactic acid bacteria ($p<0.05$) and the value of pH in the T2 group significantly increased to 60 d ($p<0.05$). The decreasing pH was confirmed by the lactic acid bacteria count at the same time. When the pH in T2 decreased, the maximum num-

Table 3. Effect of addition of *Allium hookeri* on the proximate analysis in fermented sausage with meat from sulfur fed pigs during 60 d of ripening

Items	Ripening time (d)	Con	T1	T2
Moisture (%)	1	46.90 ± 3.93^{Ab}	55.77 ± 3.07^{Aa}	54.96 ± 0.72^{Aa}
	15	16.26 ± 0.06^{Bb}	18.42 ± 2.50^{Bb}	21.91 ± 0.94^{Ba}
	30	12.83 ± 0.54^{BCba}	12.67 ± 0.68^{Cba}	14.43 ± 1.91^{Ca}
	60	9.78 ± 0.47^{Cb}	10.51 ± 0.51^{Cba}	11.05 ± 0.28^{Da}
Crude Protein (%)	1	17.31 ± 0.68^{Cns}	18.34 ± 1.80^{C}	17.92 ± 0.19^{D}
	15	27.83 ± 1.63^{Bb}	28.51 ± 0.71^{Bab}	31.01 ± 0.77^{Ca}
	30	34.14 ± 1.08^{Ans}	34.37 ± 1.43^{A}	34.63 ± 0.44^{B}
	60	36.44 ± 2.95^{Ans}	38.47 ± 3.17^{A}	37.33 ± 0.01^{A}
Crude Fat (%)	1	23.41 ± 4.65^{Bns}	21.55 ± 1.86^{C}	20.20 ± 2.38^{C}
	15	40.59 ± 1.67^{Aa}	38.11 ± 0.47^{Bb}	36.30 ± 1.21^{Bbc}
	30	41.24 ± 3.46^{Ans}	39.51 ± 0.99^{AB}	39.62 ± 1.41^{AB}
	60	42.12 ± 0.48^{Ans}	41.97 ± 1.33^{A}	40.35 ± 0.80^{A}
Crude Ash (%)	1	2.80 ± 0.02^{Ca}	2.62 ± 0.19^{Dab}	2.66 ± 0.02^{Dab}
	15	4.83 ± 0.43^{Ba}	4.49 ± 0.48^{Cb}	4.79 ± 0.29^{Ca}
	30	5.22 ± 0.12^{Ans}	5.30 ± 0.39^{B}	5.38 ± 0.22^{B}
	60	5.12 ± 0.23^{Ac}	6.26 ± 0.32^{Aa}	6.04 ± 0.05^{Aab}

All values are means±standard deviations (n=3).
^{NS,ns}: not significant
$^{a-d}$: Small letters within the same row are significantly different among groups ($p<0.05$)
$^{A-D}$: Capitals within the same column are significantly different during the period ($p<0.05$)
Con: Control group
T1: Fermented sausage from 0.3% sulfur fed pigs
T2: Fermented sausage from 0.3% sulfur fed pigs with added *Allium hookeri*

Table 4. Effect of addition of *Allium hookeri* on the pH, water activity, and microbial counts in fermented sausage with meat from sulfur fed pigs during 60 d of ripening

Items	Ripening time (d)	Con	T1	T2
pH	1	5.92 ± 0.05^{NSab}	5.88 ± 0.01^{ABb}	5.97 ± 0.07^{Ca}
	15	5.92 ± 0.02^{b}	5.87 ± 0.02^{Ab}	5.68 ± 0.08^{Ba}
	30	5.88 ± 0.09^{b}	5.94 ± 0.04^{Bb}	5.40 ± 0.02^{Aa}
	60	5.96 ± 0.03^{c}	5.94 ± 0.02^{ABb}	5.62 ± 0.01^{Ba}
Lactic acid bacteria (Log CFU/g)	1	6.39 ± 0.09^{Bns}	6.44 ± 0.03^{AB}	6.58 ± 0.11^{C}
	15	6.67 ± 0.05^{Ab}	6.30 ± 0.74^{ABb}	8.12 ± 0.07^{ABa}
	30	6.35 ± 0.10^{Bb}	6.64 ± 0.04^{Aab}	8.32 ± 0.73^{Aa}
	60	6.51 ± 0.04^{AB}	6.15 ± 0.03^{B}	7.30 ± 0.15^{BC}
Water Activity	1	0.993 ± 0.003^{Ans}	0.993 ± 0.006^{A}	0.994 ± 0.002^{A}
	15	0.822 ± 0.003^{Bns}	0.835 ± 0.004^{B}	0.835 ± 0.020^{B}
	30	0.664 ± 0.002^{Cb}	0.641 ± 0.003^{Cc}	0.734 ± 0.002^{Ca}
	60	0.575 ± 0.002^{Db}	0.538 ± 0.005^{Dc}	0.605 ± 0.004^{Da}
E. coli (Log CFU/g)	1	-	-	-
	15	-	-	-
	30	-	-	-
	60	-	-	-
Staphylococcus aureus (Log CFU/g)	1	-	-	-
	15	-	-	-
	30	-	-	-
	60	-	-	-
Salmonella spp. (Log CFU/g)	1	-	-	-
	15	-	-	-
	30	-	-	-
	60	-	-	-

All values are means±standard deviations (n=3).
^{NS,ns}: Not significant
$^{a-d}$: Small letters within the same row are significantly different among groups ($p<0.05$)
$^{A-D}$: Capitals within the same column are significantly different during the period ($p<0.05$) Con: Control group
T1: Fermented sausage from 0.3% sulfur fed pigs
T2: Fermented sausage from 0.3% sulfur fed pigs with added *Allium hookeri*

ber of lactic acid bacteria at the lowest pH was observed in T2. It seems like that *A. hookeri* in fermented sausage induces a fast reduction of pH.

The lactic acid bacteria counts in T2 increased significantly to 8.12±0.07 Log CFU/g at 15 d, 8.32±0.73 Log CFU/g at 30 d and significantly showed a slight decrease at 60 d (p<0.05). These results suggest that *A. hookeri* contributed to the decreased pH by increasing the number of lactic acid bacteria as allium plants are an excellent source of lactic acid bacteria such as inulin oligosaccharides (Causey *et al*., 2000).

Water activity decreased significantly during 60 d of ripening (p<0.05). Water activity was not significantly different among the groups on d 1 or 15 (p>0.05) but a significant difference appeared between T2 and the other groups on d 30 and 60 (p<0.05). Huff-Lonergan *et al.* (2010) explained that water activity is affected by various factors and possibly by oxidation. The increase in T2 water activity was regarded as an antioxidant effect because oxidation during storage of meat products changes water activity and affects tenderness and juiciness (Baron *et al*., 2007; Bertram *et al*., 2007; Eymard *et al*., 2009; Fuentes *et al*., 2010; Kim *et al*., 2010; Lund *et al*., 2007; Rowe *et al*., 2004; Ventanas *et al*., 2007).

Pathogenic microorganisms were not detected in any of the groups during the fermentation period. Allium plants have antifungal activity (Jeon *et al*., 2001; Tsen *et al.,* 2000; Vergawen *et al*., 1998; Yin and Tsao, 1999).

Antioxidant analysis

The antioxidant analysis of fermented sausage from meat from sulfur fed pigswith added *A. hookeri* is shown in Table 5.

DPPH decreased significantly in the initial and the final values during the 60 d of ripening (p<0.05). T2 had significantly higher values than control on d 1 (p<0.05). In particular, the T1 and T2 groups had significantly increased DPPH values than those of the control on d 60 (p< 0.05).

$ABTS^+$ radical scavenging activity was not significantly different on d 1 (p>0.05). The T1 and T2 groups increased significantly compared with that in the control on d 60 (p<0.05).

T1 group had protection against oxidation due to scavenging free radicals because sulfur in meat from sulfur fed pigsforms thiol groups to reduce oxidation (Han *et al*., 1996). T2 showed antioxidation effects in fermented sausage during storage and the DPPH and ABTS results were different because DPPH · is a free radical, whereas ABTS·+ is a cation radical, so the coupling between DPPH

Table 5. Effect of addition of *Allium hookeri* on the DPPH radical scavenging activity, $ABTS^+$ radical scavenging activity, total phenol content, and total flavonoid contents in fermented sausage with meat from sulfur fed pigs during 60 d of ripening

Items	Ripening time (d)	Con	T1	T2
DPPH radical scavenging activity (%)	1	$82.14±0.45^{Ab}$	$82.69±0.23^{Aab}$	$83.04±0.00^{Aa}$
	15	$80.39±0.568^{Bns}$	$81.35±0.00^{AB}$	$80.99±0.31^{AB}$
	30	$79.44±0.65^{Bab}$	$80.14±1.35^{ABa}$	$78.13±0.48^{Cb}$
	60	$77.27±0.55^{Cb}$	$80.54±1.17^{Ba}$	$80.47±1.14^{BCa}$
$ABTS^+$ radical scavenging activity (%)	1	$26.98±0.36^{Cns}$	$28.70±2.22^{B}$	$30.23±1.25^{C}$
	15	$27.65±0.52^{Cc}$	$30.75±0.22^{ABab}$	$31.71±0.36^{BCa}$
	30	$32.86±0.30^{Ans}$	$31.90±0.95^{AB}$	$33.57±0.66^{AB}$
	60	$31.28±0.22^{Bc}$	$32.19±0.36^{Ab}$	$35.39±0.29^{Aa}$
Total Phenol Content (mg/g GAE)	1	$0.20±0.03^{Db}$	$0.27±0.01^{Ca}$	$0.26±0.03^{Da}$
	15	$0.52±0.01^{Cb}$	$0.50±0.05^{Bb}$	$0.66±0.08^{Ca}$
	30	$0.70±0.02^{Bb}$	$0.71±0.05^{Ab}$	$0.89±0.01^{Ba}$
	60	$0.84±0.05^{Ab}$	$0.84±0.09^{Ab}$	$1.02±0.06^{Aa}$
Total Flavonoid Content (μg/g Quercine)	1	$8.60±1.80^{Cb}$	$9.77±2.71^{Cb}$	$17.37±1.75^{Ba}$
	15	$40.20±1.80^{Ab}$	$47.23±1.75^{Ab}$	$59.50±1.80^{Aa}$
	30	$39.93±1.75^{Ba}$	$40.77±2.70^{Bb}$	$82.33±1.75^{Aa}$
	60	$36.67±175^{ABb}$	$40.20±1.80^{Bb}$	$95.20±6.64^{Aa}$

All values are means±standard deviations (n=3).

^{NS,ns}: Not significant

$^{a-d}$: Small letters within the same row are significantly different among groups (p<0.05)

$^{A-D}$: Capitals within the same column are significantly different during the period (p<0.05)

Con: Control group

T1: Fermented sausage from 0.3% sulfur fed pigs

T2: Fermented sausage from 0.3% sulfur fed pigs with added *Allium hookeri*

and ABTS are distinct (Lee *et al.*, 2005). The ABTS assay assesses not only lipophilic antioxidants but also hydrophilic compounds (Kim and Lee, 2009).

Total phenol contents (TPC) and total flavonoid contents (TFC) on d 60 increased significantly in all groups compared with the value on d 1 ($p<0.05$), suggesting that macromolecules decomposed to make new compounds during fermentation. The TPC of T1 and T2 increased significantly on d 1 ($p<0.05$). TPC of T2 was significantly higher than that of all three groups during d 60 of ripening ($p<0.05$). TFC of T2 increased significantly on d 1 but only T2 increased significantly on d 60 ($p<0.05$).

The results suggest that adding *A. hookeri* resulted in abundant antioxidant agents in the fermented sausage with meat from sulfur fed pigs. The TCP and TFC of T2 scavenging activity results were high, as free radicals were scavenged by polyphenols such as superoxide, peroxyl, and hydroxyl radicals (Hogan *et al.*, 2009) but the reduction of free radicals in T1 was considered an effect of sulfur. Polyphenol contents are highly correlated with total antioxidant capacity (R^2=0.9773) (Prasad *et al*., 2009). Phenol compounds have various structures and molecular weights, which are associated with original flavor and an antioxidative effect, and are also related with protein for physiological activities (Shahidi and Pegg, 1993). Phenolic and flavonoid compounds such as phenol, phenolic acid, and phenylpropanoid inhibit lipid oxidation and protein oxidation (Ganhão *et al*., 2010; Jongberg *et al.,* 2011; Liu *et al.*, 2009; Loliger, 1983). Consequently, it is likely that *A. hookeri* provided a protective effect against oxidation of meat products through phenol compounds during storage.

Color values

The color value results of fermented sausage are shown in Table 6. *L** (lightness) decreased significantly in all groups because water was lost from the stored sausage ($p<0.05$), which the color of sausage was getting dark while removing including water in sausage. The *L** values of the T1 group on d 30 and 60 were not significantly different because the moisture content in the T1 group was not significantly different between d 30 and 60 ($p>0.05$). The T2 group showed lower *L** values resulting from formation of a dark color by quinines of polyphenols oxidized in plant materials (Liu *et al.,* 2009). Zhang *et al*. (2013) reported that a decrease of *L** values is induced by pigments in the plant or oxidation of phenolic compounds in meat products. These data indicate that *A. hookeri* had polyphenol compounds that affected storage of fermented sausage.

The *a** (redness) value of all groups increased significantly over the entire period ($p<0.05$). Generally, an inc-

Table 6. Effect of addition of *Allium hookeri* on the color values in fermented sausage with meat from sulfur fed pigs during 60 d of ripening

Surface color	Ripening time (d)	Con	T1	T2
*L** (Lightness)	1	60.07 ± 1.65^{Ans}	60.96 ± 1.06^{A}	60.48 ± 0.24^{A}
	15	57.87 ± 0.58^{ABns}	59.35 ± 1.64^{AB}	52.59 ± 1.75^{B}
	30	54.72 ± 2.10^{Bns}	54.48 ± 0.97^{C}	51.84 ± 0.62^{B}
	60	47.29 ± 0.79^{Cb}	56.11 ± 2.58^{BCa}	46.69 ± 1.32^{Cb}
*a** (Redness)	1	2.51 ± 0.02^{Ca}	2.98 ± 0.22^{Ba}	-5.55 ± 0.25^{Cb}
	15	3.47 ± 0.74^{Cb}	6.43 ± 0.65^{Aa}	-0.90 ± 1.10^{Bc}
	30	6.83 ± 0.89^{Ba}	5.54 ± 0.86^{Aab}	0.30 ± 0.20^{Bb}
	60	9.75 ± 0.60^{Aa}	6.70 ± 0.09^{Ab}	4.23 ± 0.49^{Ac}
*b** (Yellowness)	1	11.45 ± 0.91^{NSb}	10.47 ± 2.37^{ABb}	17.32 ± 0.16^{Aa}
	15	10.26 ± 0.75^{b}	8.10 ± 0.77^{Bb}	13.72 ± 0.27^{Ba}
	30	9.71 ± 0.59^{b}	6.89 ± 2.11^{Bb}	16.38 ± 0.40^{Aa}
	60	11.00 ± 0.29^{b}	12.46 ± 0.52^{Ab}	14.59 ± 1.13^{Ba}
E	1	35.92 ± 1.93^{Cns}	36.64 ± 1.20^{C}	39.53 ± 0.31^{C}
	15	39.60 ± 0.51^{BCns}	38.11 ± 1.77^{BC}	45.42 ± 1.72^{B}
	30	42.95 ± 2.24^{Bns}	42.64 ± 0.62^{A}	46.90 ± 0.56^{B}
	60	50.87 ± 0.84^{Aa}	42.16 ± 2.35^{ABb}	51.49 ± 1.51^{Aa}

All values are means±standard deviations (n=3).

^{NS,ns}: Not significant

$^{a-d}$: Small letters within the same row are significantly different among groups ($p<0.05$)

$^{A-D}$: Capitals within the same column are significantly different during the period ($p<0.05$)

Con: Control group

T1: Fermented sausage from 0.3% sulfur fed pigs

T2: Fermented sausage from 0.3% sulfur fed pigs with added *Allium hookeri*

Table 7. Effect of addition of *Allium hookeri* on the sensory attributes in fermented sausage with meat from sulfur fed pigs on d 60 of ripening

Items	Con	T1	T2
Taste	3.00±0.74^{b}	4.00±0.43^{a}	2.75±1.22^{b}
Flavor	3.17±0.94^{ab}	3.67±0.65^{a}	2.67±0.98^{b}
Color	3.42±0.79^{a}	3.92±0.52^{a}	2.67±1.15^{b}
Hardness	3.58±0.67^{ns}	3.50±0.52	3.67±0.98
Juiciness	3.00±0.85^{ns}	3.25±0.87	3.25±1.14
Overall acceptance	3.08±0.67^{b}	4.08±0.52^{a}	3.00±1.04^{b}

All values are means±standard deviations (n=12).
^{ns}: Not significant
$^{a-d}$: Small letters within the same row are significantly different among groups (p<0.05)
Con: Control group
T1: Fermented sausage from 0.3% sulfur fed pigs
T2: Fermented sausage from 0.3% sulfur fed pigs with added *Allium hookeri*

reasing a^* value in meat products is attributed to a reaction of myoglobin and nitrites that form nitrosomyoglobin in the fermented sausage (Fista *et al.,* 2004). The T2 group on d 0 and 15 had a negative a^* value because the original color of *A. hookeri* prevented an increase in the a^* value. The a^* value of T2 decreased significantly on d 60 compared with the others (p<0.05). Fernández-López *et al*., 2007 explained the a^* value is related to lipid oxidation, as the antioxidant effect contributed to reduce the a^* value of T2.

Changes of T2 in b^* (yellowness) were significantly increased compared with control or T1 for entire days (p< 0.05). The E value was not significantly different among the groups on d 0, 15 and 30, but increased significantly in all groups throughout fermentation compared with those observed initially (p<0.05) because of the Maillard reaction (increased browning) (Jee *et al*., 1999).

Sensory test

The results of the sensory test are shown in Table 7. The T1 group (0.3% sulfur fed pigs) had significantly better taste and flavor and also had high overall acceptance compared to the others (p<0.05). Sensory testing in previous reports indicated that lean pork from meat from sulfur fed pigs was better in color, drip loss, marbling score, aroma, and acceptability compared with lean pork from commercial feed fed pigs (Lee *et al*., 2009). The T2 sensory scores showed very similar results of overall acceptance value with the control group. These results were expected, as the fermented flavor of *A. hookeri* was unfamiliar.

Conclusions

This study was designed to investigate the effect of *A. hookeri* in fermented sausage made with meat from sulfur fed pigs. Adding *A. hookeri* to fermented sausage processed from sulfur fed pigs increased radical scavenging activities. *A. hookeri* had increasing meat quality on the fermented sausage, as it increased DPPH free radical scavenging and $ABTS^{+}$ reducing activities, total phenol content, and total flavanoid content compared to those of the control and also provided a good growth environment for lactic acid bacteria compared to that in the other groups. Adding *A. hookeri* resulted in predominant antioxidant capacity in the fermented sausage during fermentation period so it is anticipated that *A. hookeri* could help to prolong the storage period of meat products as a naturally occurring antioxidant agent. Therefore, these results suggest that *A. hookeri* in meat from sulfur fed pigs may play very important roles to increase meat quality during fermentation.

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