



## Antioxidant Activities of *Achyranthes japonica* Nakai Extract and Its Application to the Pork Sausages

J. H. Park<sup>1</sup>, S. N. Kang<sup>1</sup>, D. Shin<sup>2</sup>, I. C. Hur<sup>3</sup>, I. S. Kim<sup>3</sup> and S. K. Jin<sup>3,\*</sup>

<sup>1</sup> Swine Science and Technology Center, Gyeongnam National University of Science and Technology, Jinju 660-758, Korea

**ABSTRACT:** Influence of *Achyranthes japonica* Nakai Extract (AJNE) on properties of pork sausages were studied in the present investigation. AJNE was added to sausages alone or in combination with ascorbic acid to obtain a comparative analysis on properties of control and ascorbic acid added-sausages. Results showed that addition of 0.05% AJNE led to a decrease in color L\* and whiteness (W), and an increase in color b\* of pork sausage samples ( $p < 0.05$ ). Although color a\* of pork sausages containing AJNE was not significantly different, ascorbic acid added-sausages were highest amongst other treatments ( $p < 0.05$ ). Sausages containing AJNE had lower non-heme iron values and peroxide value (POV) than control sausages ( $p < 0.05$ ); however, high nitrosomyoglobin content was observed in AJNE added-sausages ( $p < 0.05$ ). Ascorbic acid led to a decrease in residual nitrite concentration of sausages ( $p < 0.05$ ), but no difference was found in AJNE added-sausages. Free radical scavenging analysis showed that AJNE did not affect 1,1-diphenyl -2-picrylhydrazyl (DPPH) activity of sausages, whereas ascorbic acid added-sausages showed relatively higher activity among the samples ( $p < 0.05$ ). Addition of AJNE had no influence on texture properties of sausages. In sensory evaluation, AJNE treatment had significant effects on color ( $p < 0.05$ ), but no significant effects on aroma, flavor, springiness, juiciness, and overall acceptability. In conclusion, the addition of AJNE, as a natural supplement may offer natural antioxidants for pork sausages, and appears to be particularly effective in inducing changes in non-heme iron concentration, POV value and nitrosomyoglobin content. (**Key Words:** *Achyranthes japonica* Nakai Extract, Peroxide Value, Pork Sausage, Nitrosomyoglobin, Sensory Evaluation)

### INTRODUCTION

Natural food additives derived from herbs and spices have been recognized and used for centuries in food preservation. In recent years, the increasing interest of consumers for natural foods or foods free from artificial chemicals has raised the demand for meat products. Therefore, functional properties of numerous plant extracts have been investigated, and many herbs, spices, and their extracts have been proven to possess high antimicrobial and antioxidant activity with respect to their functional properties in meat products (Wu et al., 2006; Kong et al., 2007; Wojdylo et al., 2007; Rounds et al., 2012). The most

important of these bioactive plants substances are flavonoids, alkaloids, tannins, saponins, glycosides, phenolic compounds and essential oils (Cowan, 1999; Torres et al., 2002; Medina et al., 2003).

The use of plant extracts is an effective way to minimize lipid oxidation in meat products, thus maintaining nutritional quality (Tanabe et al., 2002). Some studies have demonstrated that shelf-life and meat quality can be improved by using natural antioxidants during storage of meat products (Chouliara et al., 2007; Martin-Diana et al., 2008). Therefore, plant extracts are important ingredients of the quality of meat products because it affects the color, flavor, texture, and nutritional value of these foods.

*Achyranthes japonica* Nakai (AJN) is a perennial herb that has a wide distribution in East Asia including Korea, China, and Japan and mainly used as a medicinal plant (Jung et al., 2007). AJN has various physiological effects including the control of blood circulation, the removal of extravasated blood, and the inteneration of joint actions in humans and experimental animals (Marcone et al., 2003). The root of AJN contains various active components,

\* Corresponding Author: Sang Keun Jin. Tel: +82-55-751-3584, Fax: +82-55-751-3689, E-mail: skjin@gntech.ac.kr

<sup>2</sup> Department of Animal Science, Chonbuk National University, Jeonju 561-756, Korea.

<sup>3</sup> Department of Animal Resources Technology, Gyeongnam National University of Science and Technology, Jinju 660-758, Korea.

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including phytoecdysteroid, saponin, polysaccharide, 20-hydroxyecdysone, and inokosterone and has also been demonstrated to exhibit the highest inhibitory effect against *Clostridium difficile* amongst various herb extracts (Jung et al., 2007). Although plant extracts induced changes in physicochemical properties with apparent effect on meat quality have widely been reported, the effect of AJNE on the quality of sausages has not been studied yet.

Thus, this study was performed to investigate the potential possibility of antioxidant by measuring meat color, non-heme iron, POV, residual nitrite, nitrosomyoglobin, DPPH, texture properties and sensory evaluation.

## MATERIALS AND METHODS

### Preparation of *Achyranthes japonica* Nakai extract

Air dried AJN roots were purchased from local herbal market. After grinding for 1 min, AJN powder was boiled in distilled water (1:10) for 3 h. The mixture was then filtered, and evaporated in a vacuum at a temperature of 70°C using a rotary evaporator (RW-0525G, Heidolph, Germany). The extract was stored at 4°C until used.

### Total phenol content

Total phenol content in sample extracts was determined spectrophotometrically according to the Folin-Ciocalteu method (Singleton and Rossi, 1965). Because catechin is one of the polyphenol compounds, total phenol content of extract from *Achyranthes japonica* Nakai was expressed as microgram catechin equivalents/milligram extract ( $\mu\text{g CE/mg}$ ). Typically, 150 ml of sample at a concentration of 1 mg/ml, 2.4 L of deionized water, and 150 ml of 0.25 N Folin-Ciocalteu reagents were combined in a plastic vial, and then mixed well using a vortex mixer. The mixture was allowed to react for 3 min, and then 300 ml of 1 N  $\text{Na}_2\text{CO}_3$  solution was added, and mixed well. The solution was incubated at ambient room temperature (23°C) in a dark place for 2 h. The absorbance was measured at 725 nm using a spectrophotometer (Hewlett Packard 8452A, Diode Array, Santa Clara, CA, USA). Additional dilution was done if the absorbance value measured was over the linear range of the standard curve ( $Y = 0.0016X + 0.0424$ ,  $R^2 = 0.9999$ ).

### Total flavonoid content

Total flavonoid content was determined using the method of Chun et al. (2003) with minor modifications. Exactly, 0.25 ml of sample (1 mg/ml) was added to a tube containing 1 ml of double-distilled water. Subsequently, 0.075 ml of 5%  $\text{NaNO}_2$ , 0.075 ml of 10%  $\text{AlCl}_3$  and 0.5 ml of 1 M NaOH were added at 0, 5 and 6 min, sequentially. Finally, the volume of the reacting solution was made up to 2.5 ml with double-distilled water. The absorbance of the

solution was recorded at a wavelength of 410 nm, and was detected using the Ultrospec 2100 pro spectrophotometers. Quercetin, a ubiquitous flavonoid present in many plant extracts, was used as a standard to quantify the total flavonoid content of water extract. Results were expressed as microgram quercetin equivalents/milligram extract ( $\mu\text{g QE/mg}$ ).

### Experimental design and sausage processing

Pork loins were obtained from a local slaughter house 24 h post mortem. The meat was ground after removal of visible fat and connective tissue. Experimental sausages were manufactured under the conditions described by Jin et al. (2002). Four treatments of boiled pork sausages were prepared (Table 1). The control (CON) was prepared with the addition of sodium nitrite without AJNE or ascorbic acid. The other three treatments were prepared with 0.05% ascorbic acid (T1), 0.05% AJNE (T2), and 0.025% ascorbic acid plus 0.025% AJNE (T3). All the samples were stored in refrigerator at 10°C for 7 d.

### Evaluation of sausage meat color

Color properties (CIE  $L^*$ ,  $a^*$  and  $b^*$ ) of cooked pork sausages were evaluated by Minolta colorimeter (CR-300, Tokyo, Japan) calibrated against a white plate ( $L^* = 93.5$ ,  $a^* = 0.3132$ , and  $b^* = 0.3198$ ) at 7 d storage period. The W (whiteness) was calculated using the following formula:  $L^* - 3b^*$  (Park, 2005). Three replicate measures were performed on each sample.

### Determination in non-heme iron concentration

Non-heme iron concentration in sausages was determined by following the modified method of Carter (1971). Four gram of ground meat and 12 ml of deionized

**Table 1.** The sausage formulation as affected by ascorbic acid and AJNE

Ingredients (%)	Treatments			
	CON	T1	T2	T3
Lean pork loin	72.40	72.40	72.40	72.40
Pork back fat	11.20	11.20	11.20	11.20
Ice	13.80	13.75	13.75	13.75
Salt	1.39	1.39	1.39	1.39
Sodium nitrite	0.01	0.01	0.01	0.01
Phosphate	0.24	0.24	0.24	0.24
Sugar	0.50	0.50	0.50	0.50
MSG <sup>1</sup>	0.06	0.06	0.06	0.06
Seasoning <sup>2</sup>	0.40	0.40	0.40	0.40
L-ascorbic acid	-	0.05	-	0.025
AJNE	-	-	0.05	0.025
Total	100	100	100	100

<sup>1</sup> Monosodium L-Glutamate (Shinwon Chemical Co. Ltd., Seoul, Republic of Korea).

<sup>2</sup> Bologna (Taewon Food Co., Kyungkido, Republic of Korea).

distilled water were added to a disposable test tube. The sample mixture was vortexed, and 1 ml of 11.3% TCA was added. After centrifugation at 4,000 rpm for 15 min, 2 ml of supernatant was mixed with 0.8 ml of 10% ammonium acetate and 0.2 ml of ferroin color reagent. Iron concentrations of the supernatant were determined by spectrophotometer and non-heme iron concentration was calculated by subtracting the value of heme iron from total iron.

#### Determination of peroxide value

The peroxide value (POV) was determined as described in the AOAC (2000). Typically, 5 g of sample was weighed in a 50 ml glass tube, and to this 30 ml of acetic acid-chloroform mixture (3:2 v/v) was added. After incubation at 60°C for 5 min in a water bath, the mixture was filtered using No. 1 Whatman filter paper. Subsequently, 0.5 ml of potassium iodide solution was added to the filtrate, which was further analyzed using an automatic titrator equipped with pH meter and stirrer. The titration was allowed to run against standard solution of 0.1 N sodium thiosulfate. POV was expressed as milliequivalent (meq) of active oxygen per kg of sausage.

#### Determination of nitrite concentration

Residual nitrite concentration was determined by a spectrophotometric method (AOAC, 2000). Typically, 5 g sample of minced sausage was transferred to a 500 ml volumetric flask containing 40 ml of hot water (80°C), and the volume was made up to 300 ml with hot water. After thorough mixing, the flask was heated in a steam bath for 2 h before adding more water to bring the volume to 500 ml. The mixture was then filtered (0.45 µm filter), and an aliquot (45 ml) was transferred to a 50 ml volumetric flask together with 2.5 ml of sulphanilamide (SUL), and set aside for 5 min; then 2.5 ml of N-ethylenediamine dihydrochloride (NED) was added. After waiting for 1.5 min for color development, the absorbance was determined at 540 nm against a blank comprising of 45 ml of water, 2.5 ml of SUL and 2.5 ml of NED.

#### Determination of nitrosomyoglobin concentration

Nitrosomyoglobin concentration in the sausages was determined according to the method of Bozkurt and Erkmen (2004). Typically, 10 g of minced sample was added to solvent mixture of 40 ml of acetone and 3 ml of water, mixed and allowed to stand for 5 min. After filtration and centrifugation for 5 min at 1,790×g, the absorbance value was read at 540 nm with respect to a blank solution. The result was expressed as mg/kg nitroso haem pigments.

#### DPPH radical scavenging activity

The DPPH scavenging activity of sausages was

measured using a spectrophotometer (Akowuah et al., 2005). A diluted extract of concentration of 0.15 ml was added to 0.9 ml of the 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. Percentage of DPPH scavenging activity (% SA) was calculated from the equation  $(1 - (\text{absorbance of extract} / \text{absorbance of control})) \times 100$ .

#### Texture property

Mechanical properties of sausage samples (1.5×1.5×1.5 cm) were measured using the EZ Test-500N texture analyzer (TA-XTZ-5, Shimadzu Co., Japan) equipped with a cylindrical plunger (diameter 5 mm, depression speed 60 mm/min) and a 500 N load cell. Texture parameters measured included hardness, cohesiveness, springiness, chewiness and adhesiveness.

#### Sensory evaluation

Sensory evaluation was performed by 15 panels on the 7th day of storage. Sausage samples, cut into 2 cm cubes, were presented to each panelist. The color, aroma, flavor, springiness, juiciness, and overall acceptability were evaluated using a 9-point scale (1 = extreme dislike, 9 = extreme like).

#### Statistical analysis

Measurements of all parameters were performed by 8 replications. All data were analyzed by the general linear model (GLM) of SAS (version 9.1), and the post-hoc test was performed using Duncan's multiple range test with  $p < 0.05$  as the minimum acceptable probability for differences between means.

## RESULTS

#### Total phenolic and flavonoid content

Total phenolic and flavonoid contents in AJNE were measured to be  $25.38 \pm 0.02$  CE µg/mg and  $26.27 \pm 3.95$  QE µg/mg, respectively (Table 2).

#### Sausage color

A significant decrease in the color CIE L\* values of sausage containing ascorbic acid, AJNE and ascorbic acid plus AJNE was observed when compared with control sausage after being stored for 7 d ( $p < 0.05$ ) (Table 3).

**Table 2.** Total phenolic and flavonoid content in water extracts of *Achyranthes japonica* Nakai

	Total phenolic content (CE µg/mg extract)	Flavonoid content (QE µg/mg extract)
<i>A. japonica</i> Nakai	$25.38 \pm 0.02$	$26.27 \pm 3.95$

n = 3, All values are mean ± standard deviation.

**Table 3.** Color of sausages containing *Achyranthes japonica* Nakai

	CIE L*	a*	b*	W
CON <sup>1</sup>	81.38±0.46 <sup>A</sup>	6.53±0.64 <sup>B</sup>	7.79±0.41 <sup>B</sup>	58.01±1.22 <sup>A</sup>
T1	80.64±0.37 <sup>B</sup>	7.71±0.33 <sup>A</sup>	7.83±0.19 <sup>B</sup>	57.14±0.66 <sup>B</sup>
T2	80.71±0.43 <sup>B</sup>	6.52±0.66 <sup>B</sup>	7.40±0.53 <sup>A</sup>	55.51±1.70 <sup>C</sup>
T3	80.44±0.34 <sup>B</sup>	7.37±0.39 <sup>A</sup>	7.91±0.30 <sup>B</sup>	56.71±0.95 <sup>B</sup>

<sup>1</sup>CON: no ascorbic acid and AJNE; T1: 0.05% ascorbic acid; T2: 0.05% AJNE; T3: 0.05% ascorbic acid plus 0.05% AJNE.

<sup>A-C</sup> Means±SD are significantly different within the same column (p<0.05).

Whereas, an increase in CIE a\* values in ascorbic acid and ascorbic acid plus AJNE added-sausages was observed. However, addition of 0.05% AJNE into pork sausage had no effect on a\* value; whereas a significant increase in b\* value was observed. The whiteness (W value) was also significantly lower among treatments due to the addition of AJNE in pork sausages (p<0.05).

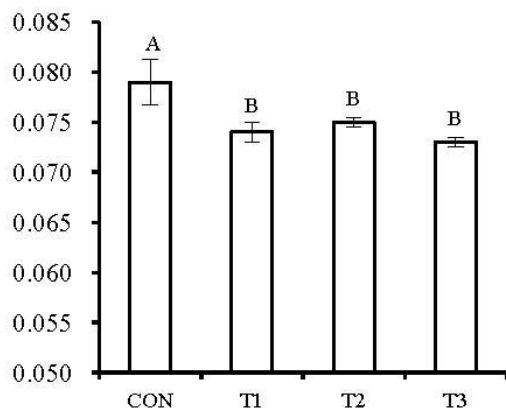
#### Non-heme iron value

Non-heme iron concentrations in cooked sausages are shown in Figure 1. Non-heme iron concentrations in sausages were found to range from 0.073 to 0.079 mg/100 g. The highest value of non-heme iron was observed in control group (0.079 mg/100 g) when compared with ascorbic acid group (0.074 mg/100 g), AJNE group (0.075 mg/100 g) and ascorbic acid plus AJNE group (0.073 mg/100 g) (p<0.05).

#### POV value

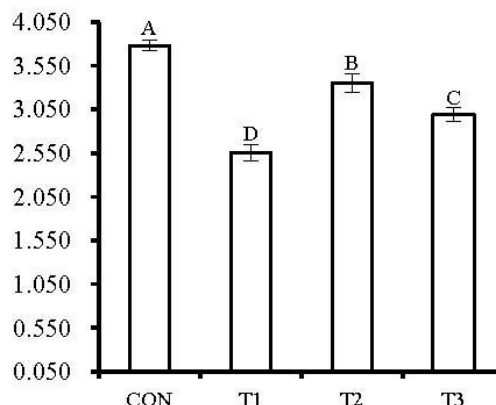
The differences in POV amongst different treatments are shown in Figure 2. After 7 d of storage, POV value of sausage with AJNE was 3.35 meq/kg and showed lower value than control sausage (3.78 meq/kg) (p<0.05).

#### Non-heme iron (mg/100 g)



**Figure 1.** Non-heme iron content in sausages containing *Achyranthes japonica* Nakai. <sup>1</sup>CON: no ascorbic acid and AJNE; T1: 0.05% ascorbic acid; T2: 0.05% AJNE; T3: 0.05% ascorbic acid plus 0.05% AJNE. <sup>A-B</sup> Means±SD with the different letters are significantly different (p<0.05).

#### POV (meq/kg)



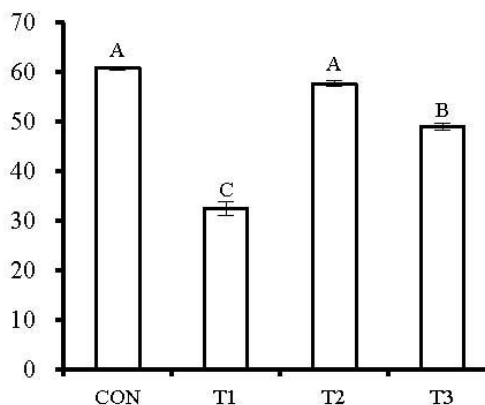
**Figure 2.** POV of sausages containing *Achyranthes japonica* Nakai. <sup>1</sup>CON: no ascorbic acid and AJNE; T1: 0.05% ascorbic acid; T2: 0.05% AJNE; T3: 0.05% ascorbic acid plus 0.05% AJNE. <sup>A-D</sup> Means±SD with the different letters are significantly different (p<0.05).

Significant differences were also observed in ascorbic acid group (2.55 meq/kg) and ascorbic acid plus AJNE group (2.99 meq/kg) (p<0.05). The evaluated POV value of the four different treatments was in the order; control>T2>T3>T1.

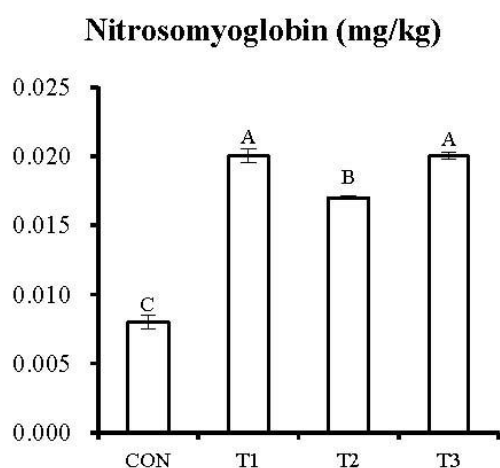
#### Residual nitrite value

Residual nitrite of approximately 60.7 ppm was found in the sausage of the control group (Figure 3). The addition of ascorbic acid and ascorbic acid plus AJNE into sausages led to a significant decrease (32.4 and 49.9 ppm, respectively) in residual nitrite level (p<0.05). Addition of 0.05% AJNE (57.59 ppm) had no effect on the residual

#### Residual nitrite (ppm)



**Figure 3.** Residual nitrite concentration in sausages containing *Achyranthes japonica* Nakai. <sup>1</sup>CON: no ascorbic acid and AJNE; T1: 0.05% ascorbic acid; T2: 0.05% AJNE; T3: 0.05% ascorbic acid plus 0.05% AJNE. <sup>A-C</sup> Means±SD with the different letters are significantly different (p<0.05).



**Figure 4.** Nitrosomyoglobin content in sausages containing *Achyranthes japonica* Nakai. <sup>1</sup> CON: no ascorbic acid and AJNE; T1: 0.05% ascorbic acid; T2: 0.05% AJNE; T3: 0.05% ascorbic acid plus 0.05% AJNE. <sup>A-C</sup> Means±SD with the different letters are significantly different (p<0.05).

nitrite level of pork sausages.

#### Nitrosomyoglobin value

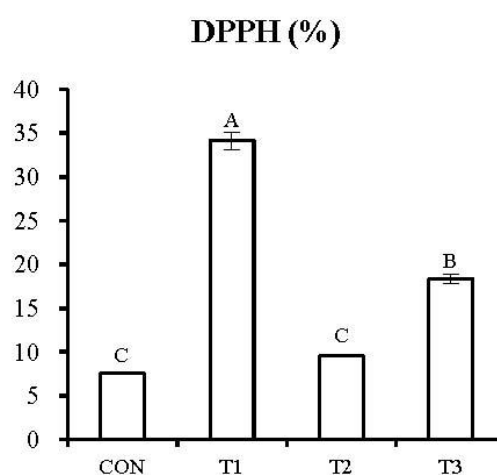
The nitrosomyoglobin content in all the treatments (0.02, 0.017 and 0.02 mg/kg in ascorbic acid group, AJNE group and ascorbic acid plus AJNE group, respectively) was increased when compared with the control group (0.008 mg/kg), and the highest myoglobin content was observed for the sausage containing ascorbic acid and AJNE plus ascorbic acid (p<0.05) (Figure 4).

#### DPPH value

The DPPH free radical scavenging activity of ascorbic acid group and ascorbic acid plus AJNE group was 34.13% and 18.37%, respectively, and these values were higher than control group, which showed 7.53% of activity (p<0.05) (Figure 5). However, no change in the DPPH values was observed for the sausage containing AJNE only.

#### Texture properties

The effect of addition of AJNE on texture properties were measured, and the results are provided in Table 4. All profiles (hardness, cohesiveness, springiness, chewiness and adhesiveness) measured were not influenced by the



**Figure 5.** The DPPH free radical scavenging activity of sausages containing *Achyranthes japonica* Nakai. <sup>1</sup> CON: no ascorbic acid and AJNE; T1: 0.05% ascorbic acid; T2: 0.05% AJNE; T3: 0.05% ascorbic acid plus 0.05% AJNE. <sup>A-C</sup> Means±SD with the different letters are significantly different (p<0.05).

addition of AJNE, ascorbic acid and AJNE plus ascorbic acid.

#### Sensory evaluation

The influence of AJNE, ascorbic acid and AJNE plus ascorbic acid on sensory evaluation scores of sausages is shown in Table 5. The treatments with ascorbic acid and AJNE plus ascorbic acid had a higher score for internal color than the control and AJNE added-sausages (p<0.05). Aroma, flavor, springiness, juiciness and overall acceptability were not significantly different amongst treatments.

## DISCUSSION

The higher intensity of redness of sausages containing nitrite is due to the fact that nitrite is reduced to nitric oxide (NO) by ascorbic acid, and reacts with myoglobin to form the red nitrosomyoglobin (Adams, 1997). As expected, the sausages produced with ascorbic acid had the highest value for redness, but the presence of AJNE prevented any increase in redness. The whiteness was also significantly low among treatments due to the addition of AJNE in pork sausages. The dark color of AJNE might be responsible for

**Table 4.** Texture properties of sausages containing *Achyranthes japonica* Nakai

	Hardness (kg)	Cohesiveness (%)	Springiness (mm)	Chewiness (kg,mm)	Adhesiveness
CON <sup>1</sup>	4.30±1.16	4.45±1.01	6.25±1.59	22.20±5.09	1.55±0.69
T1	4.60±1.24	4.00±1.03	6.60±1.68	26.20±6.27	1.45±0.50
T2	4.25±1.40	4.35±1.36	6.10±1.29	23.70±4.99	1.45±0.50
T3	4.40±1.37	4.35±1.08	5.85±1.51	24.60±5.52	1.45±0.50

<sup>1</sup> CON: no ascorbic acid and AJNE; T1: 0.05% ascorbic acid; T2: 0.05% AJNE; T3: 0.05% ascorbic acid plus 0.05% AJNE.

**Table 5.** Sensory evaluation of sausages containing *Achyranthes japonica* Nakai

	Color	Aroma	Flavor	Springiness	Juiciness	Overall acceptability
CON <sup>†</sup>	6.65±0.47 <sup>B</sup>	6.80±0.54	7.10±0.39	7.00±0.33	7.05±0.44	6.85±0.47
T1	7.60±0.46 <sup>A</sup>	7.10±0.46	7.20±0.59	6.95±0.37	7.15±0.47	7.30±0.48
T2	6.65±0.47 <sup>B</sup>	6.85±0.71	7.10±0.39	7.00±0.24	7.05±0.37	7.05±0.55
T3	7.35±0.41 <sup>A</sup>	6.90±0.46	6.95±0.60	7.05±0.16	7.25±0.26	7.05±0.60

<sup>†</sup>CON: no ascorbic acid and AJNE; T1: 0.05% ascorbic acid; T2: 0.05% AJNE; T3: 0.05% ascorbic acid plus 0.05% AJNE.

<sup>A,B</sup> Means±SD are significantly different within the same column (p<0.05).

the negative decrease in L\* and a\* values, respectively. Interestingly, the results of our study performed with AJNE were similar to the previous reports of Du and Ahn (2002), which reported the decrease in sausage redness between plant extract treatments (e.g. rosemary, sesamol, gallic acid) and control. Moreover, the present findings suggest that AJNE had no direct effect on the improvement of color L\*, a\* and W in comparison with control sausages.

Dietary iron presents as two major forms, heme and non-heme iron (Kongkachuichai et al., 2002). Although plant materials contain only non-heme iron, meat products contain both heme and non-heme iron. Based on the report that ferrous iron in general had a significant effect on the rate at which oxymyoglobin was converted into metmyoglobin (oxidative state of myoglobin) in the absence of lipid (Allen and Cornforth, 2006), high iron ions could induce lipid oxidation. Decreased non-heme iron in this study due to the addition of AJNE may be related to the reduction of myoglobin oxidation in sausage.

An increase in the POV value in sausages was observed with 0.05% AJNE treatment, showing the same pattern as the non-heme iron values. Currently, there is a lack of useful data regarding the presence of AJNE in sausages, but lipid oxidation reduction could be attributed to the presence of polyphenols, rich in anthocyanins (Yoshida et al., 1999; Han et al., 2005) as well as other saponins, inokosterone, ecdysterone and eleanolic acid bisdesmoside (Hahn and Lee, 1991; Ida et al., 1994) in plant extracts. The finding of the present report is in agreement with the findings of the several other studies, which have reported, the successful inhibition of lipid oxidation in many processed meat products using natural antioxidants of plant origin (Formanek et al., 2003; O'Grady et al., 2008; Nunez de Gonzalez et al., 2008; Devatkal et al., 2010). Another explanation for decrease in POV values in AJNE added-sausages is also possible. The AJN produced in Korea also contains 977 ppm of sulphite (Kim et al., 2000a) which is a food additive used widely in the food industry as a preservative. It might be expected that sulphite in AJN might influence the POV value. These results suggest that 0.05% AJNE would be sufficient to inhibit the generation of POV, although did not have the same effect as 0.05% ascorbic acid in cooked pork sausages.

The presence of residual nitrate in the meat products to which only nitrite has been added is attributed to the oxidation of nitrite to nitrate (Honikel, 2008). Kim et al. (2000b), Ha et al. (2001) and Cho et al. (2006) reported that a steady decrease in the nitrite content was observed in meat products produced with plant extract. This could be due to the high reactivity of nitrite, which allowed its reactions with active polyphenols, flavonoids and ascorbic acid present in the plant extract. In the present study, 0.05% ascorbic acid resulted in significantly lowering residual nitrite. However, the results of AJNE addition were not similar to the reported data, and AJNE had no effect on the residual nitrite concentration in sausages.

It is well known that myoglobin is converted into nitrosomyoglobin during the curing of sausages (Varnam and Sutherland, 1995). Ascorbic acid is generally used to increase the rate of conversion, and thus, the formation of nitrosomyoglobin is also increased. The current study identified that the addition of AJNE also influenced the nitrosomyoglobin formation, although AJNE was less effective than ascorbic acid in the rate of conversion.

Previous studies have reported that phenolic compounds in spices and herbs significantly contributed to their antioxidant properties (Shan et al., 2005; Wong et al., 2006; Wu et al., 2006). Similar to these findings, Naveena et al. (2008) reported free radical scavenging activity in juice, extract, seed and powder of certain plants. Jayawardana et al. (2011) have also reported a significant relation between phenolic content and antioxidant effect of plant extracts in pork sausages. Kwon et al. (2008) reported that ANJ root showed relatively high antioxidant activities. However, in this study, DPPH values of AJNE added-sausages were slightly higher but no differences in statistically were observed between the AJNE treatments and control unlike the effect of ascorbic acid.

Rowe et al. (2004) reported that retarded lipid oxidation of muscle fibers was related to textural properties and sensory evaluations of sausage. However, in the present study, the decreased POV values in the sausages with the added AJNE were not closely associated with texture properties. The results also suggested that the addition of ascorbic acid and AJNE plus ascorbic acid in pork sausages resulted in high scores for color value, but addition of

0.05% AJNE had no effect on the color, aroma, flavor, springiness, juiciness, and overall acceptability of the sausage. All the sausages were found to be of moderately good overall acceptability. The sausage containing AJNE and control were rated as 6.85 and 7.05 based on overall acceptability respectively, thus indicating that they were moderately good.

## CONCLUSIONS

Ascorbic acid was demonstrated to have an effect on the meat color, non-heme iron, POV, residual nitrite, nitrosomyoglobin and DPPH values. The addition of ascorbic acid plus AJNE showed intermediate values in these measurements. Use of AJNE at 0.05% concentration effectively suppressed the POV increment, and appeared to have an effect on non-heme iron concentration and nitrosomyoglobin content in cooked pork sausages. Addition of 0.05% AJNE in cooked pork sausages resulted in low scores for color values in sensory evaluation, but did not affect other parameters including overall acceptability. Thus, the inclusion of AJNE, as a natural ingredient may offer a natural alternative for cooked pork sausages with nitrite. In future, further research regarding additional alterations in the concentration of AJNE in sausages is needed to improve the quality of product and consumer acceptance.

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