Korean J. Food Sci. Ani. Resour.
Vol. 31, No. 5, pp. 693~700(2011)
DOI http://dx.do.org/10.5851/kosfa.2011.31.5.693

ARTICLE

Evaluation of Antioxidant Effects of Vitamins C and E Alone and in Combination with Sorghum Bran in a Cooked and Stored Chicken Sausage

Daekeun Shin[1,2], Han-Sul Yang[3], Byoung-Rok Min[4], Carlos Narciso-Gaytán[5], Marcos X. Sánchez-Plata[1,6], and Ciro A. Ruiz-Feria[1]*

[1]*Department of Poultry Science, 2472 TAMU, Texas A&M University, College Station, TX 77843-2472, USA*
[2]*Swine Science and Technology Center, Gyeongnam National University of Science and Technology, Jinju 660-758, Korea*
[3]*Division of Animal Science and Technology, Gyeongsang National University, Jinju 660-701, Korea*
[4]*Department of Agriculture, Food & Resource Science, University of Maryland Eastern Shore, Princess Anne, MD 21853, USA*
[5]*Colegio de Postgraduados Campus Córdoba, Km. 348 Carr. Fed. Córdoba-Veracruz. Congregación Manuel León. Amatlán de los Reyes, Ver. Apartado Postal 143 Córdoba, Ver. C.P. 94946, Mexico*
[6]*Inter-American Institute for Cooperation on Agriculture, Miami, FL 33126, USA*

Abstract

To evaluate the antioxidant effects of vitamin C, vitamin E and sorghum bran, alone or in combination on chicken sausages, 9 kg of chicken thigh meat was prepared. All thigh meat was divided into seven different batches as follows; no antioxidant (CON); vitamin C (VTC), vitamin E (VTE) or sorghum bran (SOR) at 0.02%; or three different combination ratios of vitamin C, vitamin E and sorghum bran at 0.02% (VT2, 2:1:1; VT4, 4:1:1; VT6, 6:1:1). All cooked sausages were stored at 4°C, and six sausages per treatment were used for chemical analysis on five different storage days. As the addition of vitamin E was increased, sausages stored for 10 d had decreased redness; thereby, VTE showed the lowest CIE a* ($p<0.05$). Sausages mixed with vitamins and sorghum bran combinations had lower peroxide and free fatty acid values ($p<0.05$) when compared to sausages without antioxidants. The TBARS were the lowest in sausages containing vitamin C, vitamin E and sorghum bran at 6:1:1 ratio, and they significantly differed to CON, VTC and SOR treatments ($p<0.05$). Therefore, our results suggest that meat mixed with vitamins and sorghum bran had more antioxidant activity than the meat mixed with only antioxidant vitamins or without antioxidants.

Key words: antioxidant, vitamin C, vitamin E, sorghum bran, chicken sausage

Introduction

One of the major concerns related to poultry processing, from an economical point of view, is the product stability during storage. For this reason, synthetic antioxidants including butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ethoxyquin and propyl gallate are commonly added to poultry products in order to extend storage in a commercial market (Kahl, 1984). The addition of synthetic antioxidants to poultry products helps in maintaining a lower market price as the storage period increases. However, due to a higher demand for natural and healthier foods, and to consumer concerns about some negative health effects of synthetic antioxidants (Okonogi *et al*., 2007), some poultry processors are now looking for alternatives to reduce or remove synthetic antioxidants from their products. However, it seems that natural antioxidants used alone do not increase shelf life as expected, leading to deterioration of poultry products at a faster rate. Therefore, a new approach to maintain the poultry product quality and to satisfy the expectations of healthy conscious consumers is necessary.

Vitamins C (ascorbic acid, VTC) and E (α-tocopherol, VTE) are naturally occurring antioxidants which can be added to poultry products and are very safe to humans. When fruit and vegetable derivatives are added to poultry products, only limited VTC or VTE is available to work as an electron donor and reduce the release of reactive oxygen species (ROS) during storage (Blokhina *et al*.,

*Corresponding author: Ciro A. Ruiz-Feria, Department of Poultry Science, 2472 TAMU, Texas A&M University, College Station, TX 77843-2472, USA. Tel: 1-979-845-2994, Fax: 1-979-845-1921, E-mail: ciro.ruiz@poultry.tamu.edu

2003). The antioxidant effects of VTE on poultry products are limited due to its lipophilic characteristics (Packer *et al*., 1979). Therefore, a strategy to increase the antioxidant effects of VTE is necessary. It has been reported that both VTC and VTE have synergistic effects when they are blended and added to poultry products (Doba *et al*., 1985). Ascorbate has been shown to prevent the pro-oxidant activity of α-tocopherol by reducing the α-tocopheroxyl radical to α-tocopherol, and then acting as a co-antioxidant (Blokhina *et al*., 2003; Packer *et al*., 1979; Peyrat-Maillard *et al*., 2001). Through this mechanism, VTC supplementation has been reported to increase or restore VTE (Padayatty *et al*., 2003; Wayner *et al*., 1986). However, VTC can also be a pro-oxidant which accelerates the release of ROS in poultry products when electrons are donated (Podmore *et al*., 1998).

Therefore, a powerful electron donor to regenerate oxidized VTC and oxidized VTE is required. Sorghum bran (SOR) may be a possible natural electron donor. The antioxidant effects of SOR were verified when 0.5% SOR was added, mixed and pre-cooked in ground beef patties and stored up to 10 d (Shin, 2006). The SOR contains both hydrophobic and hydrophilic polyphenols (Kamath *et al*., 2004) and may act as a strong electron donor to restore electrons for both water and lipid soluble vitamins. Therefore, we hypothesize that the combination of VTC, VTE and SOR will have synergistic antioxidant effects on poultry meat. Therefore, this study was conducted to evaluate the antioxidant effects of VTC C and VTE in combination with SOR at three different ratios in broiler chicken sausages.

Materials and Methods

Sample preparation

Nine kg of commercial chicken thigh meat were purchased from a retail store, trimmed, coarse ground (KitchenAid Professional 600 & KitchenAid Food Grinder Stand Mixer Attachment, KitchenAid, USA), weighed, and divided into seven different groups (1 kg/group).

Preparation of cooked chicken sausage

Pork fat and non-meat ingredients including salt, sodium tripolyphosphate (STP), starch and ice were added to each meat group as described in Table 1. The mixture was fine ground and divided into seven formulations that were treated with either no antioxidants (control), or 0.02% vitamin C (Sigma-Aldrich, USA), 0.02% vitamin E (Sigma-Aldrich, USA), 0.02% sorghum bran, or three combination ratios of vitamin C:vitamin E and sorghum bran at 2:1:1, 4:1:1 and 6:1:1, respectively (all combinations providing 0.02% of antioxidants). Each treatment was mixed in a paddle stand mixer (KitchenAid Professional 600 & KitchenAid Food Grinder Stand Mixer Attachment, KitchenAid, USA) for 2 min to achieve uniform distribution of antioxidant in the ground chicken thigh meat matrix. The control group also had a mix step, but no antioxidant was included during mixing. Sausages of 12

Table 1. Composition of chicken sausage blends[1]

Ingredients	%	Weight (g)						
		CON	VTC	VTE	SOR	VT2	VT4	VT6
Meat ingredients								
Lean chicken meat	76.48	1000	1000	1000	1000	1000	1000	1000
Pork fat	10.0	120	120	120	120	120	120	120
Nonmeat ingredients								
Salt	2.0	24	24	24	24	24	24	24
STP[2]	0.5	6	6	6	6	6	6	6
Potato starch	1.0	12	12	12	12	12	12	12
Ice	10.0	120	120	120	120	120	120	120
Antioxidants								
α-Tocopherol		-	0.24	-	-	0.06	0.04	0.03
Ascorbic acid	0.02	-	-	0.24	-	0.12	0.16	0.18
Sorghum bran		-	-	-	0.24	0.06	0.04	0.03
Total	100	1282	1282.2	1282.2	1282.2	1282.2	1282.2	1282.2

[1]Treatment: CON (control), no antioxidant; VTC, vitamin C (0.02%); VTE, vitamin E (0.02%); SOR, sorghum bran (0.02%); VT2, 2:1:1 (vitamin C: vitamin E: sorghum bran; 0.02%); VT4, 4:1:1 (vitamin C: vitamin E: sorghum bran; 0.02%); VT6, 6:1:1 (vitamin C: vitamin E: sorghum bran, 0.02%)

[2]STP, sodium tripolyphosphate

cm long and 90 g were formed (KitchenAid Sausage Stuffer Stand Mixer Attachment, KitchenAid, USA) and cooked at 180°C in a convection oven (Convection FC34-1, Equipex Ltd., Providence, RI, USA). Each sausage was removed when an internal temperature of 74°C was reached and cooled at 4°C in a refrigerator for 1 h. The sausage was packed on a foam tray (CRYOVAC®, Sealed Air Co., USA) with a provinylchloride (PVC) film (Stretchable Meat Film 55003815, Prime Source, USA) and stored at 4°C for chemical analysis on d 0, 1, 3, 6 and 10 of storage.

pH and CIE L*, CIE a* and CIE b* measurements

For pH value of the cooked chicken thigh meat sausage, duplicate readings per sample were performed using a pH meter (Corning M240, Corning, USA), and an average value was reported. Prior to use, the pH meter was calibrated with standard buffers at pH 4.0 and 7.0.

CIE L* (lightness), CIE a* (redness) and CIE b* (yellowness) color space values were determined using a colorimeter (Minolta Chroma Meter CR-300, Minolta Co., Ltd., USA). The colorimeter was calibrated daily using a white tile, which is Y=94.3, x=0.3130 and y=0.3199. Three different readings were obtained, and the average of CIE L*, CIE a* and CIE b* color space values was reported.

Non-heme iron content determination

The amount of non-heme iron content was determined using a protocol conducted by Cater (1971) and modified by Ahn *et al*. (1993). Briefly, 4 g of sample from each treatment was homogenized (Tissumizer, Tekmar Co., USA). One and a half mL of homogenized sample was transferred and mixed with 0.5 mL of 2% ascorbic acid. The mixture was left at room temperature for 5 min. At the end of 5 min, 1 mL of trichloroacetic acid (TCA) was added, mixed and centrifuged. Two mL of supernatant was removed, to which 0.8 mL of ammonium acetate and 0.2 mL of ferroin color reagent were added and mixed. The contents of the mixture were centrifuged again. Supernatant was read, calculated and reported as μg non-heme iron/g of cooked chicken thigh sausage.

Peroxide value determination

Peroxide value was determined by a procedure described by the AOAC (1990) method 956.33. A five g sample was added to a 30 mL of acetic acid and chloroform mixture and incubated at 60°C for 5 min in a water bath. After incubation, sample was filtered (Whatman No. 1, Whatman International Ltd., UK), and 0.5 mL of saturated potassium iodide was added, and shaken for 5 min. Then 30 mL of double distilled water (DDW) was added to the sample and titrated with a 0.01 N sodium thiosulfate ($Na_2S_2O_3$). Titration was stopped when yellow color of sample was cleared. Subsequently, 0.5 mL of 1% starch was obtained and re-titrated with 0.001 N $Na_2S_2O_3$ until purple color was disappeared. Peroxide value of sample was calculated as below:

$$\text{PV (Milliequivalents peroxide/kg sample)} = (S \times N \times 1000)/\text{Sample weight (g)}$$

Where S=mL of $Na_2S_2O_3$ and N=normality of $Na_2S_2O_3$

Free fatty acid determination

Free fatty acid was determined using a method provided by the AOAC (1990) method 940.28. Briefly, 5 g of sample was added to 50 mL of warmed ethanol. The mixture was shaken gently for 5 min to dissolve the fat. Five drops of phenolphthalein was added to the sample and titrated with a 0.01 N sodium hydroxide (NaOH). The end point of titration was determined at the permanent appearance of a faint pink color. Free fatty acid content was calculated as follows:

$$\%\ \text{FFA} = (V \times N \times F \times 100)/\text{Sample weight} \times 1000$$

Where V=mL of NaOH, N=normality of NaOH, and F= equivalent weight of the FFA expressed in oleic acid equivalents (1 acid group, M.W. = 282).

Thiobarbituric acid reactive substances (TBARS) determination

The TBARS value was measured to determine the degree of lipid oxidation using a method described by Buege and Aust (1978). A sample of 5 g was mixed with 15 mL of DDW and 0.1 mL of BHA/BHT. The mixture was homogenized, vortexed and left in the dark place at room temperature for 15 min. Then 1 mL of homogenate was mixed with 2 mL of TCA and thiobarbituric acid (TBA). The mixture was boiled, cooled and centrifuged at 2,000 rpm for 10 min. Sample was read, calculated and expressed as mg malonaldehyde/kg of cooked chicken thigh meat.

$$\text{TBARS value} = \text{Abs 530 nm} \times 7.8\ \text{(conversion factor)}\ \text{mg malonaldehyde/kg sample}$$

Statistical analysis

All parameters were analyzed in duplicates of six sausage samples per treatment. At each storage time, data was analyzed as a one-way ANOVA. When the treatment effect was significant, means were separated using the Duncan's Multiple Range Test (SAS, Version 6.12, USA) with a predetermined significance level of $p<0.05$. Correlation analysis by the Pearson correlation coefficient was also conducted to determine the relationship between pH, lightness, non-heme iron content, peroxide value, free fatty acid value and TBARS value after 10 d of storage.

Results

pH and CIE L*, CIE a* and CIE b* values

The pH and the lightness of the cooked chicken sausages were not different among treatments regardless of the storage time (Table 2). The pH values ranged from 6.68 to 6.78, and the lightness values ranged from 67.50 to 72.62 for all treatments. Although redness (CIE a* values) was similar among all treatments at d 0 and 1, sausages treated with VTC were redder when compared to CON sausages and sausages treated with VTE and SOR on d 3 of storage ($p<0.05$). The CIE a* values for sausages treated with VTC and vitamins and sorghum bran combination (VT6) were similar but higher than sausages treated with VTE and SOR after 10 d storage (Table 2). The yellowness (CIE b* values) was higher in the CON sausages compared to the other sausages on 0 d of stor-

Table 2. pH and CIE L^*, CIE b^* and CIE a^* of cooked chicken sausages containing three different antioxidants or their combination during storage 10 days at 4°C

Treatment[1]	Storage day				
	0	1	3	6	10
pH					
CON	6.76±0.02	6.76±0.05	6.72±0.07	6.72±0.05	6.69±0.03
VTC	6.73±0.06	6.74±0.03	6.73±0.05	6.72±0.05	6.68±0.02
VTE	6.77±0.05	6.79±0.04	6.77±0.07	6.72±0.06	6.72±0.02
SOR	6.78±0.05	6.77±0.05	6.75±0.04	6.74±0.07	6.70±0.03
VC2	6.77±0.05	6.77±0.03	6.74±0.05	6.72±0.07	6.69±0.03
VC4	6.75±0.07	6.78±0.03	6.73±0.05	6.71±0.08	6.69±0.04
Lightness (L)*					
CON	70.97±1.43	72.62±0.97	71.34±2.30	70.22±2.07	70.21±0.86
VTC	69.74±2.45	70.43±1.37	69.51±2.55	69.93±1.29	69.73±1.35
VTE	69.36±2.60	70.90±2.01	70.46±1.54	69.27±1.55	70.03±2.55
SOR	68.57±1.98	71.18±1.75	69.68±0.82	68.34±1.52	68.95±2.39
VC2	70.99±0.64	71.69±0.70	70.00±1.30	70.95±0.50	69.61±1.47
VC4	69.46±1.92	71.10±1.82	71.14±0.77	70.72±1.92	68.60±2.54
VC6	69.51±2.13	70.75±1.34	69.11±2.55	69.38±1.75	67.50±2.06
Redness (a)*					
CON	7.35±1.52	6.86±1.10	6.63±0.51^{b}	6.53±1.18^{c}	7.51±1.10^{abc}
VTC	7.28±1.34	7.43±0.78	8.06±0.99^{a}	8.12±1.14^{a}	8.16±0.61^{a}
VTE	6.51±0.50	6.55±0.96	6.56±1.20^{b}	7.44±0.89^{abc}	6.45±0.51^{c}
SOR	7.27±0.69	6.50±0.78	6.73±1.07^{b}	6.64±1.03^{bc}	6.95±1.05^{bc}
VC2	7.29±0.67	6.66±0.84	7.62±0.84^{ab}	7.99±0.40^{a}	7.26±0.63^{abc}
VC4	7.79±0.96	7.88±1.09	7.57±0.83^{ab}	7.52±1.01^{abc}	7.86±1.06^{ab}
VC6	7.28±1.15	7.37±0.78	7.73±1.10^{ab}	7.81±0.79^{ab}	8.12±1.01^{a}
Yellowness (b)*					
CON	10.72±1.05^{a}	12.14±1.05	12.10±0.92	11.40±1.15	10.93±1.21
VTC	8.78±0.54^{c}	10.17±1.03	10.68±0.35	10.14±0.83	10.34±1.52
VTE	8.85±0.86^{c}	10.92±1.28	11.32±1.61	11.43±1.60	10.94±0.78
SOR	9.18±1.00^{bc}	11.29±1.81	11.48±1.86	10.73±1.22	10.66±1.48
VC2	8.80±0.70^{c}	10.49±1.39	10.25±1.10	10.51±0.88	10.07±0.48
VC4	9.19±0.79^{bc}	11.10±1.29	11.61±0.92	10.94±0.87	10.55±0.95
VC6	10.13±0.79^{ab}	11.43±0.78	11.133±0.63	10.408±1.20	10.813±0.80

Data represent mean±SD.

[1]Treatment: CON (control), no antioxidant; VTC, vitamin C (0.02%); VTE, vitamin E (0.02%); SOR, sorghum bran (0.02%); VT2, 2:1:1 (vitamin C: vitamin E: sorghum bran; 0.02%); VT4, 4:1:1 (vitamin C: vitamin E: sorghum bran, 0.02%); VT6, 6:1:1 (vitamin C: vitamin E: sorghum bran, 0.02%)

[a-c]Mean values within a column followed by the same letter are not significantly different ($p>0.05$).

age. However, significantly different CIE b* values were not determined after 0 d of storage (Table 2).

Non-heme iron, peroxide and free fatty acid values

The non-heme iron content (NHI) of the sausages was not affected by treatments at 0, 1, 6 and 10 d, but at 3 d, sausages treated with the vitamins and sorghum bran combination (VT6) had lower levels of NHI when compared to all the other treatments, except VTC (Table 3). In contrast, the peroxide value (POV) of cooked chicken sausages was affected by treatments at 0, 3, 6 and 10 d ($p<0.05$) (Table 3). At 0 d the lowest POV was found in the VTE sausages, followed by the VTC and VT2 sausages, whereas the highest POVs were found in the CON, SOR and VT6 sausages. At 3, 6 and 10 d, all POVs were lower for all antioxidant treatments when compared to the CON sausages. The FFA content was not different among treatments at 0 or 1 d of storage. However, at 3, 6, or 10 d of storage, addition of antioxidants, alone or in combination, reduced the amount of FFA, compared with the CON sausages.

Thiobarbituric acid reactive substances (TBARS) values

The TBARS values were similar among treatments at 0 and 3 d of storage. However, at 1 and 6 d, the addition of antioxidants, alone or in any combination, reduced the TBARS values as compared with the CON. After 10 d of storage the sausages with a combination of antioxidants (VT4 and VT6) had lower levels of TBARS than sausages in the CON, VTC or SOR groups ($p<0.05$) (Table 4).

Pearson correlation coefficients of parameters

The pHs were positively correlated with lightness, but negatively correlated with TBARS and POV (Table 5).

Table 3. Non-heme iron (NHI, μg/g), peroxide (POV, meq O_2/kg) and free fatty acid (FFA, % oleic acid) of cooked chicken sausages containing three different antioxidants or their combination during storage 10 d at 4°C

Treatments[1]	Storage d				
	0	1	3	6	10
Non-heme iron (NHI, μg/g)					
CON	0.107±0.004	0.109±0.004	0.107±0.001[a]	0.107±0.007	0.112±0.004
VTC	0.102±0.003	0.108±0.005	0.106±0.003[bc]	0.109±0.007	0.109±0.002
VTE	0.105±0.003	0.108±0.002	0.107±0.002[ab]	0.106±0.008	0.112±0.003
SOR	0.104±0.004	0.108±0.004	0.107±0.001[ab]	0.107±0.006	0.110±0.002
VT2	0.103±0.005	0.107±0.004	0.106±0.002[ab]	0.104±0.006	0.109±0.005
VT4	0.105±0.006	0.107±0.003	0.107±0.001[ab]	0.107±0.008	0.108±0.003
VT6	0.106±0.004	0.107±0.003	0.105±0.002[c]	0.103±0.003	0.111±0.006
Peroxide value (POV, meq O_2/kg)					
CON	0.18±0.01[a]	0.21±0.03	0.25±0.05[a]	0.43±0.08[a]	0.52±0.06[a]
VTC	0.14±0.01[c]	0.18±0.06	0.19±0.02[b]	0.34±0.07[b]	0.36±0.05[bc]
VTE	0.12±0.01[d]	0.16±0.04	0.19±0.04[b]	0.26±0.06[c]	0.28±0.03[d]
SOR	0.16±0.02[ab]	0.24±0.10	0.21±0.03[b]	0.33±0.08[bc]	0.39±0.04[b]
VT2	0.14±0.01[c]	0.18±0.06	0.19±0.02[b]	0.29±0.04[bc]	0.31±0.04[cd]
VT4	0.15±0.02[bc]	0.19±0.06	0.19±0.04[b]	0.26±0.04[c]	0.31±0.05[cd]
VT6	0.17±0.02[ab]	0.22±0.08	0.19±0.03[b]	0.25±0.04[c]	0.31±0.04[cd]
Free fatty acid (FFA, % oleic acid)					
CON	0.148±0.047	0.132±0.032	0.131±0.028[a]	0.159±0.020[a]	0.137±0.006[a]
VTC	0.106±0.006	0.108±0.012	0.098±0.014[c]	0.114±0.005[cd]	0.114±0.010[bc]
VTE	0.114±0.014	0.110±0.010	0.105±0.007[bc]	0.110±0.009[cd]	0.107±0.010[c]
SOR	0.114±0.005	0.119±0.008	0.106±0.004[bc]	0.108±0.016[d]	0.119±0.006[b]
VT2	0.122±0.017	0.119±0.007	0.108±0.009[bc]	0.116±0.003[cd]	0.114±0.003[bc]
VT4	0.111±0.005	0.116±0.008	0.109±0.015[bc]	0.127±0.006[bc]	0.114±0.004[bc]
VT6	0.132±0.030	0.132±0.027	0.120±0.012[ab]	0.141±0.025[b]	0.121±0.008[b]

Data represent mean±SD.

[1]Treatment: CON (control), no antioxidant; VTC, vitamin C (0.02%); VTE, vitamin E (0.02%); SOR, sorghum bran (0.02%); VT2, 2:1:1 (vitamin C: vitamin E: sorghum bran; 0.02%); VT4, 4:1:1 (vitamin C: vitamin E: sorghum bran, 0.02%); VT6, 6:1:1 (vitamin C: vitamin E: sorghum bran, 0.02%)

[a-d]Mean values within a column followed by the same letter are not significantly different ($p>0.05$)

Table 4. Thiobarbituric acid reactive substances (TBARS, mg MDA/kg) of cooked chicken sausages containing three different antioxidants or their combination during storage 10 d at 4°C

Treatments[1]	Storage d				
	0	1	3	6	10
CON	2.12±1.31	2.72±0.87[a]	4.52±1.52	7.03±3.61[a]	6.38±2.25[a]
VTC	1.53±1.17	1.92±0.49[b]	4.40±1.57	2.46±1.83[b]	4.74±2.60[ab]
VTE	1.02±0.38	1.37±0.42[b]	2.82±1.05	2.87±1.24[b]	3.54±1.82[bc]
SOR	1.43±0.37	1.89±0.39[b]	4.31±1.66	3.91±1.27[b]	6.21±0.93[a]
VT2	1.17±0.63	1.80±0.67[b]	4.10±1.85	3.80±1.86[b]	3.45±1.97[bc]
VT4	1.28±0.63	1.27±0.35[b]	2.63±1.79	2.92±1.30[b]	2.86±2.28[c]
VT6	1.33±0.79	1.27±0.26[b]	2.26±1.21	2.20±1.23[b]	2.00±0.93[c]

Data represent mean±SD.
[1]Treatment: CON (control), no antioxidant; VTC, vitamin C (0.02%); VTE, vitamin E (0.02%); SOR, sorghum bran (0.02%); VT2, 2:1:1 (vitamin C: vitamin E: sorghum bran; 0.02%); VT4, 4:1:1 (vitamin C: vitamin E: sorghum bran, 0.02%); VT6, 6:1:1 (vitamin C: vitamin E: sorghum bran, 0.02%)
[a-c]Mean values within a column followed by the same letter are not significantly different (p>0.05).

Table 5. Pearson correlation coefficients between parameters[1] of cooked chicken sausages with different additives during storage 10 d at 4°C

	pH	Lightness	TBARS	NHI	POV
Lightness	**0.210 (0.0023)**[2]				
TBARS	**-0.150 (0.0299)**	**0.190 (0.0058)**			
NHI	0.001 (0.9854)	-0.044 (0.5252)	**0.236 (0.0006)**		
POV	**-0.251 (0.0003)**	-0.088 (0.2050)	**0.596 (0.0001)**	**0.441 (0.0001)**	
FFA	-0.013 (0.8472)	0.077 (0. 2716)	0.048 (0.4909)	0.107 (0.125)	**0.242 (0.0004)**

TBARS, thiobarbituric acid reactive substances; NHI, non-heme iron content; POV, peroxide value; FFA, free fatty acids. The values for the correlations are shown in the upper row; significant correlations in parenthesis and indicated in bold; P-values in parentheses are presented for the difference from zero.

The TBARS were positively correlated with changes in NHI and POV but were not significantly correlated with the FFA content of the sausages. The FFA had a positive correlation with the POV, which indicates an indirect influence on TBARS and NHI.

Discussion

Vitamin C is a common food additive intended to trap peroxyl radicals, which are the leading source of deterioration of broiler chicken meat products including sausages and patties. As reported by Fredriksen *et al*. (2009), sausages in Norway normally contain 11 to 40 mg of vitamin C/100 g due to an addition of vitamin C as antioxidant to preserve sausage color. However, the antioxidant effects of vitamin C in chicken meats are not quite as efficient as the antioxidant effects of vitamin E in chicken meat and its products (Grau *et al*., 2001; Mielnik *et al*., 2003; Packer *et al*., 1979). It is well documented that vitamin C acts as an electron donor and restores tocopheroxyl radicals to α-tocopherols (Padayatty *et al*., 2003; Wayner *et al*., 1986). Sorghum bran is another electron donor, containing polyphenols and may be capable of regenerating tocopheroxyl or ascorbyl radicals to α-tocopherols and ascorbates, respectively (Kamath *et al*., 2004). Thus, our study was conducted to evaluate and determine an efficient combination of antioxidant vitamins and sorghum bran on the antioxidant and preserving qualities of broiler chicken sausages stored at 4°C for 10 d.

Overall, the addition of vitamin C, alone or in combination with the other antioxidants, improved the redness (CIE a*) of broiler chicken sausages when compared to sausages from the VTE or SOR treatments (p<0.05) (Table 2), whereas the CIE a* values of the VTE or SOR sausages were not significantly different to that of sausages from the CON group (p>0.05). Du *et al*. (2002) indicated that feeding two different sorghum cultivars to broiler chickens enhanced the stability of CIE a* value of raw thigh meat patties when patties were stored at 4°C up to 7 d of storage, whereas Du and Ahn (2002) reported that the addition of 0.02% vitamin E to turkey thigh meat sausages did not show a significant impact on the CIE a* value. Results in this study indicate that vitamin C alone is quite effective in preserving CIE a* values in poultry sausages, and the combination of VTC with VTE and SOR does not improve its antioxidant efficacy. In addition, vitamin E or sorghum bran does not have effects on preserving the CIE a* value of poultry sausages.

However, our results indicate that the addition of antioxidants, alone or in combination, are effective in reducing the amount of FFA and POV in the poultry sausages compared with the CON, and this occurs only after 3 d of storage (Table 3). Our results also suggest that after longer storage times (6 and 10 d) vitamin E alone was better than vitamin C or sorghum bran in maintaining low levels of FFA and POV. Furthermore, the combination of different antioxidants did not have complimentary or synergistic effects on reducing the FFA and POV of poultry sausages under storage. Thus the addition of vitamin E to sausages may have delayed or blocked the hydrolysis of phospholipids to form FFAs, and as a result the formation of peroxides of FFA was delayed when compared to sausages from CON, VTC and SOR treatments (Aksu, 2007).

The TBARS, an important parameter of oxidative stress, was not affected by treatment (0 and 3 d) or was reduced by the addition of vitamins or sorghum bran, alone or in combination, compared with sausages without additives, at 1 and 6 d of storage (Table 4). However, after 10 d of storage, it was clear that the combination of vitamins C and E and sorghum bran were better than the CON or the individual additives in reducing the oxidative stress of sausages under storage. Although sausages in the VT4 and VT6 (higher proportions of vitamin C) had lower levels of TBARS, they were similar to sausages treated with VE. Jia *et al*. (1998) and Zhou *et al*. (2000) proposed that polyphenols from green tea reduce the depletion of α-tocopherol due to a strong electron donor capacity of polyphenols to tocopheroxyl radicals. Dai *et al*. (2008) reported an antioxidant synergism of green tea polyphenols, vitamin C and vitamin E, showing that the synergism was due to the regeneration of vitamin E by the green tea polyphenol and the regeneration of the latter by vitamin C. In our experiment, the polyphenols including tannin and/or anthocyanin (0.5-3.8 and/or 4-9.8 mg/g, respectively) (Awika *et al*., 2004; Awika and Rooney, 2004) from sorghum bran may have play a role in restoring oxidized vitamin E at the initial stages of storage, and at later stages of storage vitamin C may restore oxidized polyphenols, thus maintaining lower TBARS values than other treatments including CON, VTC and SOR treatments. Nevertheless, our TBARS results show that the ratio of vitamins and sorghum bran has an important effect on the antioxidant capacity, and further research is required to find the best combination to reduce the oxidative stress of stored poultry sausages.

The results in this study suggest that the addition of vitamin C and E and sorghum bran in chicken meat reduce the oxidative stress and peroxidation of sausages stored for long periods under normal refrigeration temperature, which may extend the shelf life of poultry products. Furthermore, the use of naturally occurring ingredients could be favorably viewed by the consumers. Further research is warranted to determine the optimal ratio of vitamins and sorghum bran to reduce oxidative stress.

Acknowledgment

Sorghum bran was provided by Dr. Rooney at Texas A&M University, and we thank his donation for improving our study.

References

1. Ahn, D. U., Wolfe, F. H., and Sim, J. S. (1993) Three methods for determining nonheme iron in turkey meat. *J. Food Sci.* **58**, 288-291.
2. Aksu, M. İ. (2007) The effect of α-tocopherol, storage time and storage temperature on peroxide value, free fatty acids and pH of Kavurma, a cooked meat product. *J. Muscle Foods* **18**, 370-379.
3. AOAC (1990) Official Methods of Analysis. 15th ed, Association of Official Analytical Chemists, Washington, DC.
4. Awika, J. M. and Rooney, L. W. (2004) Sorghum phytochemicals and their potential impact on human health. *Phytochemistry* **65**, 1199-1221.
5. Awika, J. M., Rooney, L. W., and Waniska, R. D. (2004) Anthocyanins from black sorghum and their antioxidant properties. *Food Chem*. **90**, 293-301.
6. Blokhina, O., Virolainen, E., and Fagerstedt, K. V. (2003) Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Ann. Bot.* **91**, 179-194.
7. Buege, J. and Aust, S. D. (1978) Microsomal lipid peroxidation. *Method Enzymol.* **52**, 302-310.
8. Cater, P. (1971) Spectrophotometric determination of serum iron at the submicrogram level with a new reagent (ferrizine). *Anal. Biochem.* **40**, 450-458.
9. Dai, F., Chen, W. F., and Zhou, B. (2008) Antioxidant synergism of green tea polyphenols with alpha-tocopherol and L-ascorbic acid in SDS micelles. *Biochimie* **90**, 1499-1505.
10. Doba, T., Burton, G. W., and Ingold, K. U. (1985) Antioxidant and co-antioxidant activity of vitamin C. The effect of vitamin C, either alone or in the presence of vitamin E or a water-soluble vitamin E analogue, upon the peroxidation of aqueous multilamellar phospholipid liposomes. *Biochim. Biophys. Acta* **836**, 298-303.
11. Du, M., Cherian, G., Stitt, P. A., and Ahn, D. U. (2002) Effect

of dietary sorghum cultivars on the storage stability of broiler breast and thigh meat. *Poult. Sci.* **81**, 1385-1391.
12. Du, M., and Ahn, D. U. (2002) Effect of antioxidants on the quality of irradiated sausages prepared with turkey thigh meat. *Poultry Sci*. **81**, 1251-1256.
13. Fredriksen, J., Løken, E. B., Borgejordet, Å., Gjerdevik, K., and Nordbotten, A. (2009) Unexpected sources of vitamin C. *Food Chem.* **113**, 832-834.
14. Grau, A., Guardiola, F., Grimpa, S., Barroeta, A. C., and Codony, R. (2001) Oxidative stability of dark chicken meat through frozen storage: influence of dietary fat and alpha-tocopherol and ascorbic acid supplementation. *Poultry Sci.* **80**, 1630-1642.
15. Jia, Z-S., Zhou, B., Yang, L., Wu, L-M., and Liu, Z-L. (1998) Antioxidant synergism of tea polyphenols and α-tocopherol against free radical induced peroxidation of linoleic acid in solution. *J. Chem. Soc. Perk. T.* **2**, 911-915.
16. Kahl, R. (1984) Synthetic antioxidants: biochemical actions and interference with radiation, toxic compounds, chemical mutagens and chemical carcinogens. *Toxicol.* **33**, 185-228.
17. Kamath, V. G., Chandrashekar, A., and Rajini, P. S. (2004) Antiradical properties of sorghum (*Sorghum bicolor* L. Moench) flour extracts. *J. Cereal Sci*. **40**, 283-288.
18. Mielnik, M. B., Aaby, K., and Skrede, G. (2003) Commercial antioxidants control lipid oxidation in mechanically deboned turkey meat. *Meat Sci.* **65**, 1147-1155.
19. Okonogi, S., Duangrat, C., Anuchpreeda, S., Tachakittirungrod, S., and Chowwanapoonpohn, S. (2007) Comparison of antioxidant capacities and cytotoxicities of certain fruit peels. *Food Chem.* **103**, 839-846.
20. Packer, J. E., Slater, T. F., and Willson, R. L. (1979) Direct observation of a free radical interaction between vitamin E and vitamin C. *Nature* **278**, 737-738.
21. Padayatty, S. J., Katz, A., Wang, Y., Eck, P., Kwon, O., Lee, J-H., Chen, S., Corpe, C., Dutta, A., Dutta, S. K., and Levine, M. (2003) Vitamin C as an antioxidant: evaluation of its role in disease prevention. *J. Am. Coll. Nutr.* **22**, 18-35.
22. Peyrat-Maillard, M. N., Bonnely, S., Rondini, L., and Berset, C. (2001) Effect of vitamin E and vitamin C on the antioxidant activity of malt rootlets extracts. *Lebensm-Wiss. Technol*. **34**, 176-182.
23. Podmore, I. D., Griffiths, H. R., Herbert, K. E., Mistry, N., Mistry, P., and Lunec, J. (1998) Vitamin C exhibits pro-oxidant properties. *Nature* **392**, 559.
24. SAS (1998) SAS/STAT Software for PC. Release 6.12, SAS Institute Inc., Cary, NC. USA.
25. Shin, D. (2006) Antioxidant, color and sensory properties of sorghum bran in pre-cooked ground beef patties varying in fat and iron content. MS thesis. Texas A&M University at College Station, USA.
26. Wayner, D. D. M., Burton, G. W., and Ingold, K. U. (1986) The antioxidant efficiency of vitamin C is concentration dependent. *Biochim. Biophys. Acta* **884**, 119-123.
27. Zhou, B., Jia, Z-S., Chen, Z-H., Yang, L., Wu, L-M., and Liu, Z-L. (2000) Synergistic antioxidant effect of green tea polyphenols with α-tocopherol on free radical initiated peroxidation of linoleic acid in micelles. *J. Chem. Soc. Perk. Trans* **2**, 785-791.

(Received 2011.7.8/Revised 1st 2011.9.7, 2nd 2011.9.27/ Accepted 2011.9.27)