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Effect of Grape Pomace Powder Addition on TBARS and Color of Cooked Pork Sausages during Storage

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Abstract

To determine the effects of grape skin and seed pomace (GSP) additions on the lipid oxidation susceptibility and the color change of cooked pork sausages, the chemical characteristics of GSP itself and the addition for two different levels of GSP (0.5 and 1.0% GSP, respectively) to sausages were examined. Both the redness and blueness of the GSP were significantly reduced as the pH level was increased from 5 to 7, but a reverse result was determined in the color tint and yellowness ($p<0.05$). The GSP polyphenol and flavonoid contents were influenced by the percentages of methanol solvents, and more flavonoids were established when 100% of methanol was applied as a solvent to the GSP. But, similar results were not observed in the polyphenol of GSP. In cooked pork sausages, significant decreases in the lightness and redness were found in both the 0.5% and 1.0% of GSP sausages during the storage period ($p<0.05$). However, an incompatible effect was observed in terms of yellowness, which increased as compared to the control sausage after 6 days of storage. The 0.5% addition of GSP decreased the levels of TBARS ($p<0.05$), but the ability of GSP to minimize lipid oxidation was not dose dependent. Therefore, the results indicated that the GSP is an efficient suppressor of lipid oxidation and has latent effects as a natural antioxidant when 0.5% of GSP is added to the cooked pork sausages.

Key words: grape skin and seed pomace, pork sausage, polyphenol, flavonoid, lipid oxidation

Introduction

Increasing shelf life is one of the major strategies towards improving the economic value of pork and pork products. Efforts are being made to extend the shelf life of pork and its products by reducing the number of free radicals formed as a result of the interaction between unsaturated fatty acids and initiators (Angelo, 1996). Synthetic phenolic antioxidants, including butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), are commonly used in the pork industry, and they minimize free radical generation in pork products. In sausages, to maintain sausage quality, 0.003-0.01% BHA and BHT are added to fresh (based on fat content) or dry (based on total weight) sausages, respectively, which is allowed in the US under USDA regulations (Ahn *et al*., 2002; Sebra-

nek *et al*., 2005). By adding synthetic antioxidants to pork sausages, free radicals, as very reactive and unstable components, can be scavenged or chelated, thereby delaying deterioration in the flavor, color, and texture during storage (Nam and Ahn, 2003; Shin, 2006). For this reason, synthetic antioxidants are commonly used.

However, due to concerns over the safety of synthetic antioxidants by health-conscious consumers, many meat processors are now searching for alternative natural antioxidants (Shin *et al*.,2011), and plant extracts are generally added to pork products since many naturally derived compounds possess antioxidant and antimicrobial characteristics (Ahn *et al*., 2007; Sáyago-Ayerdi *et al*., 2009a). Grape extract contains a wide range of polyphenols and has been added to meat products in many studies. The effectiveness of grape polyphenols to retard lipid oxidation in minced fish and cooked pork and chicken has been reported (Carpenter *et al*., 2007; Lau and King, 2003; Sánchez-Alonso and Borderías, 2008; Sáyago-Ayerdi *et al*., 2009a). However, its effectiveness is limited due to the fact that the grape extract used is composed of polyphenols, which are hydrophilic. A new approach is neces-

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sary. Grape skin and seed pomace (GSP), which are generally discarded after juicing, seems to contain several flavonoids, including (+)-catechins, (−)-epicatechin, and procyanidins (Sáyago-Ayerdi et al., 2009b). It is believed that most polyphenols which remain in GSP are hydrophobic, and thus may be very effective components to stabilize free radicals in pork and pork products (Stamatis et al., 2001).

Therefore, the aim of this study was to evaluate the potential effects of GSP as a natural antioxidant and colorant in cooked pork sausages, as confirmed by TBARS and CIE L*, a*, and b*.

Materials and Methods

GSP preparation

All GSP was acquired in a local wine farm, freeze-dried for 2 d and then ground (Mini blender, Ya Hong Electronic Co., China). Ten grams of freeze-dried GSP was mixed with 100 or 75% methanol and then incubated for 24 h at 4°C (VS-8480SR, Vision Scientific Co., Korea). All incubated samples were then filtered and used for polyphenol, flavonoid, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) determination, but only 20% methanol was used as a solvent to obtain GSP extract for color determination according to pH.

GSP color determination

All GSP incubated with 20% methanol for 24 h was filtered and collected, and individual GSP extracts were then read at 420, 520, and 620 nm using a spectrophotometer (Shimadzu 1600-UV spectrophotometer, Shimadzu Co., Japan) to calculate the color tint and yellow, red, and blue pigment percentages (Almela et al., 1995).

Color intensity (CI) = $A_{420} + A_{520} + A_{620}$
Color tint = (A_{420} / A_{520})
Percentage of yellow = $[100 \times (A_{420} / CI)]$
Percentage of red = $[100 \times (A_{520} / CI)]$
Percentage of blue = $[100 \times (A_{620} / CI)]$

Polyphenol, flavonoid, anthocyanin, and DPPH determination

The total polyphenol content of GSP was measured as described by Juan and Chou (2010). Briefly, 0.1 mL of GSP extract and 1 mL of Folin-Ciocalteu reagent were mixed together and vortexed for 3 min. 300 uL of 1 N sodium carbonate anhydrous (Na_2CO_3) was added, vortexed, and left at room temperature for 90 min. 2 mL of

double distilled water (DDW) was then added. The final mixture was read at 725 nm, and the results were expressed as mg/mL using gallic acid as a standard.

For flavonoid determination, a total of 0.5 mL of GSP extract and 1.5 mL of methanol mixture were vortexed, and 10% aluminum chloride ($AlCl_3$) was added. The solution was then vortexed for a further 5 min, and 0.1 mL of 0.1 M potassium acetate (CH_3COOK) and 2.8 mL of DDW were then added and mixed. The final mixture was then read at 510 nm and expressed as mg/g. Quercetin was employed as a standard (Juan and Chou, 2010).

For anthocyanin determination, a modified version of the protocol described by Montes et al. (2005) was conducted. Briefly, 1 g of GSP was added to 100 mL of an ethanol and hydrochloric acid mixture (85:15), and the solution was then shaken at 4°C for 24 h. Individual extracts were then filtered and read at 535 nm, and the reading value was calculated as follows:

Anthocyanin μg/g = [(absorbance at 535 nm × solvent volume) / sample weight] × (1 / 65.1)

A modification of the assay by Park et al. (2013) was performed, and 0.1 mL of GSP extract and 0.9 mL methanolic DPPH (0.1 mM) were mixed, vortexed, and left at an ambient temperature for 15 min. The optical density of each GSP extract at 525 nm was read and expressed as mM due to the use of ascorbic acid as a standard.

Cooked pork sausage preparation

A total 14 kg of commercial pork loins was purchased and immediately trimmed to remove visible fat. All pork loins were then cut to an appropriate size and coarsely ground (KitchenAid Professional 600 & KitchenAid Food Grinder Stand Mixer Attachment, KitchenAid, USA). The coarse ground pork was divided into four different groups (2.20 kg/group), including a control (CON), positive control (0.01% sodium nitrite and 0.05% ascorbic acid, POS), 0.5% GSP (05G), and 1.0% GSP (10G) (sausage × treatment × storage day = 6 × 4 × 4). Two different concentration of GSP was pre-determined based on the results completed by Shin (2006). Pork fat and non-meat ingredients, including ice, salt, phosphate, and sugar were added and mixed (Table 1). Rough mixtures were then finely ground and blended with sodium nitrite/ascorbic acid or freeze dried GSP powder in a paddle stand mixer (KitchenAid Professional 600 & KitchenAid Food Grinder Stand Mixer Attachment, KitchenAid, USA) for 2 min to achieve a uniform dissemination of each ingredient throughout the

Table 1. Composition of pork sausage blends[1]

Ingredients	Pork Sausage (%)			
	CON	POS	05G	10G
Meat Ingredients				
Lean pork meat	73.0	73.0	73.0	73.0
Pork fat	12.0	12.0	12.0	12.0
Nonmeat ingredients				
Ice	12.76	12.70	12.26	11.76
Phosphate	0.24	0.24	0.24	0.24
Salt	1.5	1.5	1.5	1.5
Sugar	0.5	0.5	0.5	0.5
Sodium nitrite	-	0.01	-	-
Antioxidants				
Ascorbic acid	-	0.05	-	-
Grape skin and seed pomace (GSP)	-	-	0.5	1.0
Total	100	100	100	100

[1]Treatment: CON=no sodium nitrite and vitamin C, POS=0.01% sodium nitrite and 0.05% vitamin C, 05G=no sodium nitrite and 0.5% grape residual product, 10G=no sodium nitrite and 1.0% grape residual product.

ground pork loin matrix. The ground pork loin matrix for the control had equivalent mixing steps, but sodium nitrite, ascorbic acid, and freeze dried GSP powder were not incorporated during mixing. 10 cm long sausages and weighing 90 g were processed and cooked at 180°C in a convection oven (Convection FC34-1, Equipex Ltd., USA). Individual sausages were then taken out after reaching an internal temperature of 74°C and cooled at 4°C for 30 min. After cooling, two sausages were packaged together on a foam tray with a linear low-density polyethylene film (LLD-PE, Clean Sense Wrap, Cleansense, Korea) and stored in a 4°C refrigerator (Icepia, CLK Corporation, Korea) for 10 d. A total of 6 sausages per treatment were used to measure pH, objective color evaluation, thiobarbituric acid reactive substances (TBARS), and shear force on days 1, 3, 6, and 10 of storage.

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

For pH of the cooked pork sausages, a 10 g sausage and 90 mL DDW were mixed and homogenized. Duplicate readings per sample were obtained using a pH meter (Orion 420A+, Thermo Electron Co., USA). The average value of readings was reported, and the pH meter was calibrated with standard buffers at pH 4.0 and 10.0 on a daily basis.

To determine the objective color space values of the pork loin sausages, each sample sausage was cut into 2 cm sections and bloomed for 10 min. Individual CIE L* (lightness), a* (redness), and b* (yellowness) color space values were then determined using a colorimeter (Minolta Chroma Meter CR-300, Minolta Co., Ltd., Ramsey, NJ), which was calibrated daily with a white tile (Y=94.3, x=0.3130 and y=0.3199). Two different readings were taken per sample, and the average of CIE L*, a*, and b* was reported.

TBARS and shear force determination

The amount of malondealdehyde (MDA) was established using a procedure described by Buege and Aust (1978). Briefly, 5 g of sausage, 15 mL of DDW, and 0.1 mL of BHA/BHT were homogenized and then placed in a dark room for 15 min. Only 1 mL of homogenate per sample was mixed together with 2 mL of a TCA and TBA mixture, and boiling was conducted for 15 min. All samples were then cooled and centrifuged. The supernatant was read, calculated, and expressed as mg malonaldehyde/kg of cooked pork sausage.

$$TBARS = Abs\ 530\ nm \times 7.8\ (conversion\ factor)\ mg\ malonaldehyde/kg\ pork\ sausage$$

To evaluate the shear force of the cooked pork sausages, pork sausages were formed into $1.5 \times 1.5 \times 1.5\ cm^3$ shapes and tempered at room temperature for 30 min. Individually shaped samples were then cut once using an Instron 3343 (US/MX50, A&D Co., USA) fitted with a Warner Bratzler shearing device, which generates a cross-head speed of 100 mm/min. At least fifteen different shear force values per treatment were collected, and the average value determined for each treatment was reported as kg/cm^2.

Statistical analysis

Statistical analysis was performed using SAS software (Version 6.12, Cary, NC, USA, 1998), and a significant difference was observed by Analysis of Variance (ANOVA) using GLM procedure, followed by Duncan's Multiple Range Test with a predetermined significance level of $p < 0.05$.

Results and Discussion

Color of GSP by pH

To evaluate the GSP color at pH 5 to 7, freeze dried GSP powder was dissolved in 20% methanol, and the color tint, yellowness, redness, and blueness of GSP were determined, as described in Table 2. The GSP color tint was gradually increased, showing lighter color tint of GSP at pH 7 ($p < 0.05$). The results are similar to those of Bakker

Table 2. Effect of pH values from pH 5 to pH 7 on color of grape residual product powder dissolved in 20% methanol

	Grape Residual Product					SEM	*p*-value
	pH						
	5	5.5	6	6.5	7		
Color (%)							
Color Tint	1.26[c]	1.27[bc]	1.29[bc]	1.31[b]	1.35[a]	0.01	0.01
Yellowness	41.28[c]	41.56[bc]	41.93[bc]	42.22[b]	42.90[a]	0.23	0.01
Redness	32.83[a]	32.63[ab]	32.45[ab]	32.20[bc]	31.81[c]	0.14	0.01
Blueness	25.89[a]	25.81[ab]	25.62[b]	25.57[b]	25.29[c]	0.30	0.01

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

and Timberlake (1997), which showed that malvidin 3-glucoside, an anthocyanin prepared from *Vitis vinifera* grapes, had a progressive lighting ability at pH 1.5 to 5.0 (Heredia *et al*., 1998; Shin, 2006). The malvidin 3-glucoside transforms to vitisin A through interaction with pyruvic acid (Romero and Bakker, 1999), and malvidin 3-glucoside concentration in grape may be reduced. Hence, the 3-monoglucosides with malvidin, which give a dark color in grape, may then influence the lightness of pork sausages when stored (Van Buren *et al*., 1974). A similar tendency was determined for the yellowness of GSP, and xanthylium-like derivatives formed by the oxidation of non-aldehyde polymers in grape may be factors influencing GSP's own yellow color (Santos-Buelga *et al*., 1995). In contrast to the color tint and yellowness of GSP, the redness and blueness were negatively affected by the pH variation and were reduced when the pH was increased from 5 to 7 ($p<0.05$).

GSP polyphenol, flavonoid, anthocyanin, and DPPH determination

The sugar and acidity of GSP were 5.15 and 0.13, respectively, and it was observed that GSP can influence the traditional characteristics of pork sausages when it is added as an ingredient (Table 3). The polyphenol level of GSP was high in 75% methanol extraction compared to 100% methanol extraction, but an opposite result was determined for flavonoids. A remarkably high flavonoid concentration of 0.47 mg/g was observed from 100% GSP extracts. Yin *et al*. (2008) postulated that the antioxidant activity of flavonoids is accomplished due to the hydroxyl groups on β-ring of flavonoids, indicating that the flavonoids are one of the major compounds scavenging reactive oxygen species (ROS) (Molina *et al*., 2003; Pietta, 2000). In spite of this, the level of DPPH was not in agreement with the assertions of Pietta (2000) and Yin *et al*. (2008), and DPPH was not significantly altered in accordance with the amount of flavonoid, whether derived from 100 or 75% methanol solvents.

Table 3. Determination of polyphenol, flavonoid and anthocyanin in grape residual product powder sampled by 100 or 75% methanol

	Grape Residual Product			SEM	*p*-value
	Methanol (%)				
	100	75			
Sugar content	-	-	5.15	1.33	-
Acidity	-	-	0.13	0.01	-
Polyphenol (mg/g)	2.15[b]	2.25[a]	-	0.02	0.01
Flavonoid (mg/g)	0.47[a]	0.34[b]	-	0.02	0.01
Anthocyanin (ug/g)	-	-	0.28	0.01	-
DPPH (mM)	0.11	0.11	-	0.01	0.45

[a,b]Mean values within a row followed by the same letter are not significantly different ($p>0.05$).

GSP effects on pH and CIE L*, a*, and b* of cooked pork sausage

The pH of cooked pork sausages was not altered for up to 6 d, but all pH values were elevated to the range of 6.12 to 6.19 on day 10 of storage based on the pHs of day 3 of storage (Table 4). Individual pH values of pork sausages were higher in the order of CON > POS > 05G > 10G on day 3 of storage, but the order was CON > 05G > POS = 10G on day 6 of storage. Their pH values were significantly different from one another on both occasions ($p<0.05$). All color space values of cooked pork sausages were affected due to the addition of sodium nitrite and GSP. Compared to the control sausages on day 10 of storage, the lightness of 05G and 10G sausages were reduced. It seems that the lightness of GSP sausage was influenced by both the amount of 3-monoglucosides contained in GSP (Van Buren *et al*., 1974) and the scavenging abilities of polyphenols, which breakdown heme pigments during storage (Ruberto *et al*., 2007; Sánchez-Alonso *et al*., 2008; Sánchez-Alonso and Javier Borderías, 2008). Therefore, it was concluded that CIE L* depends on the dose of GSP addition, and significantly lower CIE L* values were determined in 10G sausages than those determined in 05G sausages. A similar tendency was observed in CIE a* of 05G and 10G sausages. GSP sausages showed

Table 4. Effect of grape residual product powder addition on pH and CIE L*, a*, and b* of cooked pork sausages stored at 4°C for 10 days

Storage day	Pork Sausage				SEM	*p*-value
	CON	POS	05G	10G		
pH						
1	6.27	6.26	6.26	6.26	0.01	0.98
3	6.11[a]	6.02[b]	5.94[bc]	5.90[c]	0.02	0.01
6	6.05	6.01	6.02	6.00	0.01	0.21
10	6.19[a]	6.13[c]	6.15[b]	6.12[c]	0.01	0.01
Lightness (CIE L*)						
1	75.27[a]	74.21[b]	73.41[bc]	72.99[c]	0.23	0.01
3	76.10[a]	75.13[a]	74.15[b]	72.28[c]	0.33	0.01
6	76.44[a]	75.03[b]	74.01[c]	72.66[d]	0.32	0.01
10	75.28[a]	74.68[a]	73.43[b]	72.21[c]	0.28	0.01
Redness (CIE a*)						
1	0.92[d]	6.64[a]	1.60[b]	1.22[c]	0.49	0.01
3	0.90[c]	6.39[a]	1.47[b]	1.17[bc]	0.48	0.01
6	1.37[b]	6.22[a]	1.28[b]	1.23[b]	0.45	0.01
10	1.37[b]	6.12[a]	1.28[b]	1.22[b]	0.44	0.01
Yellowness (CIE b*)						
1	13.20[a]	9.41[c]	12.55[b]	12.50[b]	0.32	0.01
3	12.81[a]	9.12[b]	12.79[a]	12.34[a]	0.33	0.01
6	13.02[a]	8.79[b]	12.55[a]	12.51[a]	0.36	0.01
10	13.38[a]	8.79[b]	12.52[b]	12.60[b]	0.39	0.01

[l]Treatment: CON=no sodium nitrite and vitamin C, POS=0.01% sodium nitrite and 0.05% vitamin C, 05G=no sodium nitrite and 0.5% grape residual product, 10G=no sodium nitrite and 1.0% grape residual product.
[a-c]Mean values within a row followed by the same letter are not significantly different (*p*>0.05).

Table 5. Effect of grape residual product powder addition on TBARS and shear force of cooked pork sausages stored at 4°C for 10 days

Storage day	Pork Sausage				SEM	*p*-value
	CON	POS	05G	10G		
TBARS (mg MDA/kg)						
1	1.12[a]	0.54[d]	0.71[c]	0.89[b]	0.05	0.01
3	1.49[a]	0.63[d]	0.78[c]	0.93[b]	0.07	0.01
6	1.50[a]	0.69[d]	0.91[c]	1.12[b]	0.06	0.01
10	1.81[a]	0.76[d]	0.96[c]	1.30[b]	0.09	0.01
Shear force (g/cm^2)						
1	0.61	0.79	0.79	0.71	0.03	0.06
3	0.59	0.74	0.77	0.73	0.03	0.06
6	0.58[b]	0.73[a]	0.68[a]	0.78[a]	0.02	0.01
10	0.63[b]	0.78[a]	0.81[a]	0.81[a]	0.02	0.01

[l]Treatment: CON=no sodium nitrite and vitamin C, POS=0.01% sodium nitrite and 0.05% vitamin C, 05G=no sodium nitrite and 0.5% grape residual product, 10G=no sodium nitrite and 1.0% grape residual product.
[a-d]Mean values within a row followed by the same letter are not significantly different (*p*>0.05).

reduced CIE a* values compared to POS sausages, which is thought to be due to the anthocyanin present in grape skin (Boulton, 2001; Kobayashi *et al*., 2004) being removed with the grape juice (Rababah *et al*., 2008), resulting in the majority of bioactive compounds remaining in GSP being fat soluble. One fat-soluble bioactive compound of GSP is carotenoid, which generates a yellow color in grape skin (Mendes-Pinto *et al*., 2005; Mortensen, 2006). In line with this, a significant decrease in CIE a* values and a contrasting increase in CIE b* values of 05G and 10G cooked pork sausages tended to be observed after storage up to 6 day compared to those of POS sausages.

GSP effects on TBARS and shear force of cooked pork sausage

The scavenging properties of GSP were proven, as evidenced by TBARS in Table 5. The addition of GSP to cooked pork sausages seems to prolong the quality over

time when 0.5% GSP was added to raw pork sausages which were then cooked. Such results were continuously maintained for up to 10 d of storage, but TBARS of 0.5% GSP sausages was not lower than sausages containing 0.05% ascorbic acid and 0.01% sodium nitrite. TBARS of 10G sausages was higher than that of 05G sausages. Similar TBARS was noticed by Sáyago-Ayerdi *et al.* (2009a) in raw chicken hamburgers, but not in cooked ones. According to their study, 1.0% grape antioxidant dietary fiber (GADF) addition was more effective to prolong the shelf life of both raw and cooked chicken hamburgers than 2.0% GADF for up to 13 d of storage. Either salt or GSP ingredients, as described in Table 1, releases iron of the heme protein of pork (Sáyago-Ayerdi *et al.*, 2009b) or contains ferric and ferrous ions (Danilewicz, 2003), thereby accelerating lipid oxidation. The catalysis of pork sausage lipid oxidation also seems to be hastened due to the interactions of pro-oxidant flavonoids (Aguirrezábal *et al.*, 2000; Dangles *et al.*, 2000; Joubert *et al.*, 2005; Procházková *et al.*, 2011), but this interaction must be minor because of limited storage. Therefore, the addition of 1.0% GSP to pork sausages can generate more lipid oxidation than might be expected. However, structural breakdown due to lipid oxidation did not affect shear force values. No significantly different shear forces were determined for up to 3 d of storage ($p>0.05$), but after that, a difference was established among control and treatment groups only ($p<0.05$).

Conclusions

The GSP color was influenced and seemed to have effects on polyphenol and flavonoid contents due to the variation of pH and methanol solvent, respectively. The addition of 0.5% GSP in pork sausages retards lipid oxidation, but 1% GSP did not effectively scavenge ROS formation due to the confirmation of TBARS. Therefore, due to concerns regarding the safety and toxicity of BHA and BHT in pork meat-based foods, 0.5% GSP could prompt antioxidant activities in the cooked pork sausages.

Acknowledgements

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