

Effect of Dietary Processed Sulfur Supplementation on Water-holding Capacity, Color, and Lipid Profiles of Pork

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Abstract

This study was performed to investigate the effect of dietary processed sulfur supplementation on water-holding capacity, color, and lipid profiles of pork according to the level of dietary processed sulfur (0%, CON; 0.3%, S). The pigs were slaughtered at an average final weight of 120 kg, and the *longissimus dorsi* muscles were collected from the carcasses. As results, pork processed with sulfur had significantly higher moisture and ash contents compared to those of CON but lower crude fat, pH, expressible drip, lower redness and yellowness, and greater lightness. Pork processed with sulfur showed significantly lower total lipid content, triglycerides, and atherosclerosis index but significantly higher high-density lipoprotein cholesterol. Feeding processed sulfur significantly lowered myristic acid, heptadecanoic acid, and stearic acid contents, whereas monounsaturated fatty acids and oleic acids were significantly higher compared to those in the CON. Higher amounts of polyunsaturated fatty acids and n-6 fatty acids were observed in the pork processed with sulfur than that of the CON. Therefore, supplementing pigs with dietary sulfur improved nutrient and meat quality.

Keywords: processed sulfur, fatty acids, cholesterol, triglyceride, pork

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Introduction

Appearance determines how consumers perceive quality and significantly influences their purchasing behavior (Glitsch, 2000). Grunert (1997) reported that fat content and color are the most important product characteristics that consumers base their quality evaluations regarding the appearance of meat. Moreover, Grunert *et al.* (2015) investigated traditional open markets and Western style supermarkets in China and reported that consumers consistently base their choices predominantly on intrinsic cues, such as fat content and meat color when purchasing pork ribs. This consumer perception of meat, with a focus on color, packaging, and amount of visible fat, was reported by Troy (2010). Therefore, meat color and fat content still play major roles as quality indicators. Consumers expect that meat should have an attractive bright red color, low visible fat, and look appealing. Thus, improving color and enhancing meat eating quality are of utmost importance.

Jang *et al.* (2006) reported that meat from pigs whose diets were supplemented with methyl sulfonyl methane (MSM) were lighter in color than that from untreated pigs. Furthermore, pigs fed sulfur receive higher sensory evaluations scores (Dryden, 1970). However, consumers are now emphasizing overall healthiness as well as sensory-oriented qualities (Grunert, 2006). The increased consumption of meat and high-calorie foods produces health problems and diseases (Shin *et al.*, 2006), such as high blood pressure, obesity, cancer, heart disease, arteriosclerosis, and cardiovascular diseases. Therefore, consumers have become much more health and nutrition conscious (Resurreccion, 2004).

Epidemiological studies have provided some convincing evidence that increasing dietary consumption of *Allium* species (garlic, onion, and chive) reduces the risk of cancer (Milner, 2001; Zhang *et al.*, 2015) because these plants have sulfur-containing compounds (Nicastro, 2015). Sulfur compounds have antioxidant, anti-inflammatory, antimicrobial, and anticancer activities (Battin and Brumaghim, 2009). Leustek *et al.* (2000) and Stipanuk (2004) reported that sulfur biomolecules exert important functions in all living organisms, including free radical scavenging, enzyme function, DNA methylation and repair, regulation of gene expression, protein synthesis, remo-

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deling of extracellular matrix components, lipid metabolism, and detoxification in plants and animals. Sulfur is an essential mineral for plant growth and development (Corpas and Barroso, 2015) and is also used in feed for steers, ducks, and chickens. However, as sulfur is toxic when injected directly, it needs to be processed for detoxification or used in the natural form of MSM (Kim *et al.*, 2006; Lee *et al.*, 2010).

Circulating palmitic acid and stearic acid are associated with adiposity, triglycerides, and a higher risk for diabetes (Ma *et al.*, 2015). Moreover, fatty acids are related to human health, as improving the proportion of unsaturated fatty acids and reducing the proportion of saturated fatty acids is beneficial to health, such as preventing atherosclerosis and hypertension (Decker and Shantha, 1994; Engler *et al.*, 1991). Massive intake of oleic acid can reduce serum triglycerides (TG) and cholesterol, and have a beneficial effect on preventing arteriosclerosis (Grundy, 1986). Studies that have reported on using processed sulfur in raw *longissimus dorsi* (LD) meat and its effect on fatty acids and cholesterol have provided incomplete information. Thus, this study was performed to investigate the effects of processed sulfur on fatty acid composition, triglycerides, and the cholesterol content in pork.

Materials and Methods

Animals and Experimental Design

Three-way crossbred pigs (Landrace, Yorkshire, and Duroc) from a farm in Paju-si, Gyeonggi-do, Korea were assigned randomly to either of two treatments based on the level of dietary processed sulfur (0% and 0.3%). Details of the feed and basal diet composition are given in Table 1 (Song *et al.*, 2014). Processed sulfur was purchased from Jungmin Co., Ltd (Korea) and contained 97.93% elemental sulfur. Untreated pigs were used as the control group (CON), whereas the S group was supplied with 0.3% processed sulfur for 3 mon before shipment. The pigs were slaughtered at a mean final weight of 120 kg, and the LD muscle was collected from the carcasses. The muscles were stored in vacuum packaging at 80°C until use.

Proximate Composition

Moisture, ash, protein, and fat contents were determined according to the official method (AOAC, 1995).

pH

LD muscle samples (2 g) were prepared with 18 mL distilled water and measured in a Bag Mixer 400 (Interscience

Table 1. Ingredient composition of the experimental diets

Ingredients (%)	Contents (%)	
	Normal Feed	Sulfur Mixed Feed
Crude Protein	16.00	16.00
Crude Fat	4.48	4.48
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
Processed Sulfur	-	0.30
Digestible Energy (Mcal/kg)	3.45	3.45

Co, St Nom la Bretèche, France); pH was determined using a pH meter (pH 900, Precisa Co, Switzerland). All determinations were performed in triplicate.

Determination of Expressible Drip

Expressible drip was determined according to Benjakul's method (2003). A 0.3 g sample was weighed (A) and placed between two pieces of filter paper (Whatman No. 1; Maidstone, UK). A pressure of 9.9 kg/cm² was applied to the samples and maintained for 1 min. The samples were removed from the paper and weighed (B). Expressible drip was calculated using the following equation:

$$\text{Expressible drip (\%)} = (\text{weight of A} - \text{weight of B}) / \text{weight of A} \times 100$$

Color Measurements

Samples color was measured using a colorimeter (NR-300, Nippon Denshoku, Japan). The instrument was calibrated before the measurements using the standard white plate supplied with the instrument. The color values were recorded as lightness (CIE L*), redness (CIE a*), and yellowness (CIE b*) values. The samples were exposed to air for 30 min before the measurements, and then analyzed with 10 replications.

Lipid Analysis

Lipid Extraction

A method modified from Folch *et al.* (1957) was used to extract lipids. Total meat lipids were extracted with chloroform: methanol (2:1 v/v), using a homogenizer stirrer (HS-30E, DAIHAN Scientific Co., Ltd., China). The samples were shaken and centrifuged at 3,000 rpm and 4°C for 20 min using a VS-550 multi-tube carrier refrigerated centrifuge (no. 9 rotor, Vision Scientific, Korea).

The lipid phase was separated and evaporated using N₂ gas; the dried lipids were weighed and stored at 4°C until needed for the total cholesterol (TC), high-density cholesterol (HDL-C), and TG analyses.

TC, HDL-C, and TG Analyses

TC, HDL-C, and TG contents of each lipid sample were measured using a kit (Asan Pharm. Co., Ltd., Korea). The lipid extracts were mixed with 2 mL 2% Triton X-100 (in chloroform), according to the procedure described by De Hoff *et al.* (1978) and evaporated with N₂ gas. A 2 mL aliquot of distilled water was added to each tube. Next, 0.02 mL lipid was mixed with 3 mL enzyme reagent from the TC kit. The mixture was heated at 37°C for 10 min in an oven and tested with a spectrophotometer (OPTIZEN 2120UV, Mecasys Co., Ltd., Korea) at 500 nm. The TG analysis was performed using the same method as for TC, except a 550 nm wavelength was used with a TG kit. Well before the HDL-C analysis, 0.2 mL lipid was mixed with 0.2 mL separation reagent. The samples were left to stand for 10 min and then centrifuged at 3,000 rpm and 4°C for 20 min. A 0.1 mL aliquot of the top layer was mixed with enzyme reagent, and the analysis was performed using the same method as for TC.

Low-density lipoprotein cholesterol (LDL-C) and Atherosclerosis Index (AI) Analyses

LDL-C was calculated following an equation in Friedewald *et al.* (1972):

$$\text{LDL C} = \text{TC} - (\text{HDL C} + \text{TG} / 5)$$

The AI was calculated following an equation in Cho *et al.* (2001):

$$\text{AI} = (\text{TC} - \text{HDL-C}) / \text{HDL-C}$$

Fatty Acid Analysis

The fatty acid analysis was conducted using a Korean Food Standards Codex (2014) method. A sample containing 100-200 mg of lipids was placed in a Mojonnier flask and shaken with 100 mg pyrogallol, 2 mL triundecanoin (C11:0), and 2 mL ethanol, followed by adding 10 mL 8.3 M hydrochloric acid. After shaking, the mixture was refluxed for 40 min (80°C) in a shaking water bath. After cooling the sample to room temperature, 25 mL of diethyl ether and 25 mL of anhydrous petroleum ether were added, and the mixture was stirred for a further 5 min. After the samples had cooled at room temperature for 1 h, the lay-

ers were separated; the ether layer was collected in a beaker and evaporated with N₂ gas in a 40°C water bath. The residue was dissolved in 2 mL chloroform and 2 mL diethyl ether and transferred to a test tube before being evaporated again with N₂ gas in a 40°C water bath. Next, 2 mL of 7% BF₃/L MeOH and 1 mL toluene were added. The mixture was heated at 105°C for 45 min in an oven and cooled again to room temperature. Five mL distilled water, 1 mL hexane, and 1 g sodium sulfate anhydride were mixed with the solution before the top layer was collected in a sample vial containing 1 g sodium sulfate anhydride. The samples were analyzed using a gas chromatograph GC (model CP-3800; Varian Analytical Instruments, Walnut Creek, USA) equipped with a flame ionization detector and fitted with a SP-2560 fused silica capillary column (100 m × 0.25 mm ID, 0.2 μm d_p). The column temperature was programmed to start at 100°C for 4 min, and then increase at 3°C/min from 100 to 240°C for 15 min. The injector and detector temperatures were 225 and 285°C, respectively. Fatty acids were identified by comparing the relative retention times of fatty acid methyl ester (FAME) peaks from the samples with standards from Supelco (USA).

Statistical Analysis

All data were analyzed using SPSS/PC Statistics 18.0 software (SPSS Inc., USA) and one-way analysis of variance. Significant differences between the CON and S groups were analyzed using the independent *t*-test. All data are presented as mean ± standard deviation, and *p*-values < 0.05 were considered significant.

Results and Discussion

Proximate Analysis

The results of proximate analysis, including moisture, ash, crude protein, and crude fat are shown in Table 2. The LD muscles of pigs fed sulfur had lower amounts of fat (*p*<0.05), but higher moisture and ash contents than those in the CON group (*p*<0.05). Crude protein content tended to be higher between the S and CON groups (*p*>0.05). A similar result regarding decreased fat and increased protein contents in pig meat was reported by Zheng (2004). Additionally, supplementing chicks with a sulfur amino acid results a linear decrease in body fat according to Boomgaardt and Baker (1973).

Expressible Drip

Expressible drip of LD muscle from pigs treated with

Table 2. Proximate analysis consisting of moisture, ash, crude protein, crude fat contents of the *longissimus dorsi* (LD) from pigs fed processed sulfur

Contents	Treatment ¹⁾	
	CON	S
Proximate Compositions (%)		
Moisture	70.87±0.62 ^b	72.42±0.44 ^a
Ash	1.22±0.05 ^b	1.33±0.06 ^a
Crude Protein	24.63±0.15	24.86±0.19
Crude Fat	1.54±0.13 ^a	0.89±0.08 ^b

All values are mean±standard deviation of three replicates (n=5).

¹⁾CON, commercially formulated feed; S, control diet + 0.1% processed sulfur.

^{a,b}Means within a row with different letters are significantly different at $p<0.05$.

Table 3. pH, expressible drip, and color (CIE) values of *longissimus dorsi* (LD) from pigs fed processed sulfur

Contents	Treatment ¹⁾	
	CON	S
pH	5.74±0.01 ^a	5.71±0.01 ^b
Expressible Drip	41.03±1.00 ^a	37.15±0.79 ^b
CIE L*	46.56±2.25 ^b	49.85±0.82 ^a
CIE a*	9.10±0.45 ^a	6.78±0.72 ^b
CIE b*	9.71±0.51 ^a	8.09±0.42 ^b

All values are mean±standard deviation of three replicates (n=5).

¹⁾CON, commercially formulated feed; S, control diet + 0.1% processed sulfur.

^{a,b}Means within a row with different letters are significantly different at $p<0.05$.

processed sulfur was significantly lower compared to that of the CON ($p<0.05$) (Table 3), indicating that water-holding capacity of the S group was higher than that of the CON. Dietary sulfur also appears to play an important role in drip loss of MSM-fed pigs, which is lower than that of a control group (Lee *et al.*, 2009). Den Hertog-Meischke *et al.* (1997) reported that one of the main quality attributes of fresh meat is its water-holding capacity because it influences consumer acceptance and final product quality.

pH and Color

The pH value for the S group was significantly lower than that of the CON group ($p<0.05$), and it influenced the meat color values (Table 3). The CIE L* value of the S treated group was higher than that of the CON ($p<0.05$), whereas the CIE a* and CIE b* values were significantly lower than those in the CON ($p<0.05$). These results indicate that processed sulfur influenced pH, and that pH plays affects meat color. Jang *et al.* (2006) found similar results for pigs supplemented with MSM, as pH, redness, and yellowness decreased but lightness increased. The

Table 4. Total lipid content expressed as a percentage and lipid class composition (mg/dL) in *longissimus dorsi* (LD)

Contents	Treatment ¹⁾	
	CON	S
Total Lipid	125.91±4.69 ^a	106.67±3.73 ^b
TG	214.84±25.76 ^a	113.54±12.46 ^b
TC	251.86±49.30	182.24±30.27
HDL	64.94±4.52 ^b	75.40±4.10 ^a
LDL	143.95±42.33	84.13±29.18
AI	2.87±0.65 ^a	1.43±0.46 ^b

All values are mean±standard deviation of three replicates (n=5).

¹⁾CON, commercially formulated feed; S, control diet + 0.1% processed sulfur.

^{a,b}Means within a row with different letters are significantly different at $p<0.05$.

most important factor influencing meat color is the extent of the reaction between myoglobin, oxygen, and enzymes, particularly when pH changes (Lawrie, 1985).

Lipid, Cholesterol, TG and AI

The lipid content and lipid class composition of LD are shown in Table 4. The S treatment group had significantly lower total lipid and TG contents than those in the CON ($p<0.05$). No significant difference in TC content was detected between the groups ($p>0.05$), but HDL-C level was higher in the S group than that in the CON ($p<0.05$). LDL-C was not different between the groups ($p>0.05$), but LDL-C content was lower in the S group than that in the CON. A similar trend was observed by Skorve *et al.* (1990), as treatment with 3-thiadicarboxylic acid and tetradecylthioacetic acid resulted in reductions in plasma and hepatic TG. Broiler chicks fed 30 g onion/kg body weight had a significantly higher HDL-C but lower TG concentrations than those in a CON group, which was due to onion containing the sulfur compounds *S*-methylcysteine sulfoxide and *S*-allylcysteine sulfoxide (Goo-darzi *et al.*, 2013). High concentrations of cholesterol in the blood can cause abnormalities in the immune mechanism and in immune cells inside blood vessels, inducing atherosclerosis (Shin *et al.*, 2013). The AI in the S group was significantly lower than that in the CON ($p<0.05$), suggesting that the meat from pigs fed processed sulfur prevents atherosclerosis. In addition, Li *et al.* (2011) reported that sulfur dioxide (SO₂) has an antioxidant effect, as found in rats with atherosclerosis that received SO₂.

Fatty Acid Composition

The fatty acid composition of the LD meat from pigs fed processed sulfur is shown in Table 5. Myristic, hepta-

Table 5. Fatty acid composition (g/100 g) of the *longissimus dorsi* (LD) muscle from pigs fed processed sulfur

Item	Fatty acid	Treatment ¹⁾	
		CON	S
C14:0	myristic	0.23±0.04 ^a	0.12±0.02 ^b
C15:0	pentadecanoic	0.12±0.01	0.10±0.01
C16:0	palmitic	0.42±0.10	0.35±0.05
C17:0	heptadecanoic	7.38±0.67 ^a	5.38±0.61 ^b
C18:0	stearic	23.07±0.35 ^a	21.17±0.34 ^b
C20:0	arachidic	4.08±0.49	4.84±0.69
SFA ²⁾		35.29±1.58	31.96±1.40
C15:1	heptdecenoic	3.35±0.16	4.02±0.41
C16:1	palmitoleic	0.3±0.05	0.2±0.01
C18:1n9t	elaidic	4.5±0.58	3.24±0.15
C18:1n9c	oleic	26.94±1.02 ^b	32.99±1.31 ^a
C20:2	eicosadienoic	2.46±0.31	2.76±0.57
MUFA ²⁾		37.54±1.82 ^b	43.19±2.16 ^a
C18:3n3	linolenic	11.34±1.27 ^b	15.65±0.07 ^a
C20:5n3	eicosapentaenoic	1.33±0.56	1.28±0.29
C22:6n3	docosahexaenoic	1.65±0.43	1.09±0.17
n3FA ²⁾		14.32±2.23	18.01±0.40
C18:2n6c	linoleic	47.56±1.63 ^b	54.24±1.45 ^a
C18:3n6	̑-linolenic	0.28±0.03 ^a	0.13±0.03 ^b
C20:4n6	arachidonic	1.34±0.22 ^a	0.87±0.12 ^b
n6FA ²⁾		49.17±1.87 ^b	55.24±1.42 ^a
PUFA ²⁾		63.49±4.10 ^b	73.25±1.43 ^a
n6FA/n3FA		3.48±0.45	3.07±0.11

¹⁾CON, commercially formulated feed; S, control diet + 0.1% processed sulfur.

²⁾SFA, sum of saturated fatty acids

MUFA, sum of monounsaturated fatty acids; PUFA, sum of polyunsaturated fatty acids; n3FA, sum of (n-3) fatty acids; n6FA, sum of (n-6) fatty acids.

^{a,b}Means within a row with different letters are significantly different at $p<0.05$.

decanoic, and stearic acid contents were significantly lower ($p<0.05$) in the S group than those in the CON but total saturated fatty acids (SFA) remained unchanged ($p>0.05$). The quantities of monounsaturated fatty acids (MUFA) were higher in the S group than those in the CON ($p<0.05$), which was due to an increase in oleic acid (C18:1 n-9). Lee *et al.* (2009) reported that pigs fed MSM have higher C18:1 n-9 content than those in a control group. High C18:1 n-9 content in meat improves taste (Lunt and Smith, 1991), confirming that adding processed sulfur to the diet can change meat quality. No significant difference in total n-3FA was observed between the S and CON groups ($p>0.05$), but linolenic and linoleic acid contents were significantly higher in the S group than those in the CON ($p<0.05$). Moreover, consumption of the processed sulfur diet led to higher total n-6FA and polyunsaturated fatty acids (PUFA) ($p<0.05$), whereas no effect on the n-6/n-3 ratio was detected ($p>0.05$). Similar results were reported by Pogge *et al.* (2014) in which SFA concentration was not different, but PUFA concentration increased in the *longissimus thoracis* as dietary S increased.

Conclusions

In this study, meat from the LD in pigs fed processed sulfur showed excellent water-holding capacity, improved color, and different lipid profiles. The sulfur-fed pork showed lower expressible drip and higher crude fat, total lipids, and cholesterol. Additionally, the sulfur-fed pork exhibited a lower AI, as the MUFA and PUFA concentrations were higher in the meat. Consequently, feeding processed sulfur to pigs may help consumers lead a more nutrition-oriented and healthy life.

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