CHANGES IN PHYSICOCHEMICAL AND MICROBIOLOGICAL PROPERTIES OF DRY-CURED SAUSAGES FROM SULFUR-FED PORK DURING FERMENTATION

Min-Gu Ju, Ji-Han Kim, Go-Eun Hong, Su-Jung Yeon, Na-Yeon Lee, Won-Young Cho, Hyun-Joo Jang, Chi-Ho Lee

Department of Food Science and Biotechnology of Animal Resources, Konkuk University, Korea Jmg0608@hanmail.net

Abstract - This study is performed to evaluate the quality of dry-cured sausages that processed with sulfur-fed pigs. The dry-cured sausages were designed as two groups; first group was manufactured with processed sulfur-fed pigs (SP), other group was manufactured with nonprocessed sulfur fed pigs (NP). The pH of SP was significantly lower than that of NP, aw of SP was significantly higher than that of NP at 14, 21 day (p < 0.05). Lipid oxidation was found be more vulnerable in the NP than SP (p < 0.05). The lightness and yellowness of NP were significantly higher than that of SP, whereas redness of NP was lower than SP (p <0.05). Total plate count of SP was lower than that of NP (p < 0.05). SP significantly increased mono unsaturated fatty acid (p < 0.05), and have a tendency to decrease saturated fatty acid of the dry-cured sausages. Glutamic acid contents of SP were significantly higher than that of NP in free amino acid analysis (p < 0.05). Sulfur-fed pork improved the quality and extend shelf life of meat products by depressing the harmful bacteria and lipid oxidation.

Keywords : sulfur-fed pigs, lipid oxidation, antimicrobial effect, MUFA, free amino acid

I. INTRODUCTION

Sulfur is a major inorganic element and essential for the entire biological field because of its incorporation into amino acids, proteins, enzymes, vitamins, and other biomolecules and unlike humans and monogastric animals, plants can use inorganic sulfur and synthesize sulfur-containing amino acids such as methionine and cysteine (Lioudmila, Robert, and Tapan, 2003). MSM (methylsufonylmethane) is the representative non-toxic sulfur compound. It is extracted from plants involved in the formation of sulfur-containing amino acids, such as methionine, cysteine and taurine (Total Health, 1998). These sulfur compounds are quickly evacuated from the body and the toxicity test with rats, for 90days even to pay a 1.5~2.0g/kg did not have any problems (Magnuson et al., 2007; Otsuki et al., 2002).

A lot of scientists have experiment on increasing meat quality (Jose *et al.*, 2005; Lee *et al.*, 2009; Kim *et al.*, 2015)

One of the most important things of improving meat quality is reducing lipid oxidation (Buckley et al. 1995). Meat products that processed with dietary sulfur fed animals are well known as great antioxidant effect (Lee et al., 2009; Kim et al., 2015). Song et al. (2013) reported that pigs which had eaten feeds containing high concentration of sulfur had a lot of antioxidants in their body. Also they are known as decrease of saturated fatty acid and increase of poly unsaturated fatty acid due to the

supplementation with dietary sulfur (Hiroaki, 2006; Danielle et al., 2014). Several researchers reported that the ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA) is important factor of indicator for cardiovascular disease prevention (Guo et al., 2010; Colussi et al., 2014; Kim et al., 2015). Dry cured sausages are one of the oldest and famous meat products in southern Europe because of a dry climate such as Spain, Italy, France etc (Pérez-Alvarez et al, 1999; Pastorelli et al., 2015). However, these meat products have high fat contents (20~40% lard of dry- cured sausage formulation). Because of this reason, they are susceptible to lipid oxidation prevention than other meat products, so they are not recommended for health aspect (Utrilla et al., 2014). Moreover, saturated fatty acid and cholesterol which increase the rate of cardiovascular disease are distributed high in dry-cured sausages (AHA, 2000; USDA, 2000; Mugerza et al., 2004). These problems of dry-cured sausages might be solved for using dietary sulfur-fed pork as a raw meat, because dietary sulfur-fed pork can decrease saturated fatty acids and increase polyunsaturated fatty acids of meat products (Kim et al., 2015). Therefore, processed sulfur-fed pork might affect fat quality of dry-cured sausages. Also, meat products that processed from dietary sulfur-fed pork have high moisture contents and water-holding capacity than general meat products (Park et al., 2003; Lee et al., 2009; Kim et al., 2015). This paper presents the results for comparing the sensory properties and antioxidant effects of drycured sausages processed sulfur-fed pigs (SP) and normal pigs (NP). For finding out the quality of drycured sausages made from dietary sulfur-fed pork, we experienced proximate composition, water activity, pH, TBARS (Thiobarbituric acid reactive substances), microbial analysis, fatty acid composition, TPA (textual profile analysis) and sensory test of SP and NP.

II. METERIALS AND METHODS

Animal experiment

The sample used in the present study was obtained from a population of pigs fed the same composition of basal diet, according to Kim et al. (2015). Total 60 three-way crossbred pigs (Duroc \times Landrace \times Yorkshire) from Yang-Ju Livestock Federation in Republic of Korea were raised for 174 days. The animal experiment was managed by the animal care committee of Konkuk University of Seoul, Republic of Korea. Non-processed sulfur-fed pigs (NP) were fed a commercial-compound diet; 0.3% processed sulfur-fed pigs (SP) were fed a mixed diet until 121.01 ± 1.26 kg BW. The elemental sulfur concentration was 97.9% in processed sulfur according to the Korea Feed Ingredients Association. The feeding system and watering processes were by automatic circulation.

Preparation of dry-cured sausages

Raw pork meat (sirloin) and pork back fat were purchased at butcher 24hr postmortem. The drycured sausages were produced according to a recipe described by Sanchez-Zapata et al (2013). First, pork meat was trimmed to remove visible fats and connective tissue. And then, pork meat and pork back fat were ground through 5mm plate respectively. After grinding, basic ingredients were added into meat and fat. The formulation of dry-cured sausage is presented in Table 1. Also, starter culture, **Staphylococcus** carnosus and Lactobacillus plantarum were added when basic ingredients were added. Two cultures are mixed 1:1 (CFU). The mixtures were mixed for 5 minutes by hands. Finally, mixtures were filled with collagen casing and hung in a room for 30 days with humidifier and air conditional which can adjust the temperature. The

ripening conditions are that first day is temp and RH of 20 ± 2 °C and $90 \pm 5\%$, and that following days are temp and RH of 12 ± 2 °C and $75 \pm 5\%$. The final products were used for experiments.

Table 1. Formulation of dry-cured sausages.

	formulation
Pork sirloin (%)	60
Pork Back Fat (%)	40
Total	100
Water (%)	10
Salt (%)	2
Black pepper (%)	0.05
Sodium ascorbate (%)	0.05
Sodium nitrite (%)	0.015
Glucose (%)	1

Proximate composition of dry-cured sausages.

Moisture, crude protein, crude fat and ash contents of dry-cured sausages were experienced by AOAC (1995).

Water activity (a_w) *and pH measurement*

Water activity was measured at 25°C using a water activity device (Aqua Lab CX-2, Decagon Device Inc, Germany).

pH was measured on a suspension homogenized 2g sample and 18mL distilled water for 1 minute with pH meter device (pH 900, Precisa Co, Dietikon, Switzerland).

Color measurement

Color was measured using Handy colorimeter 9 (NR-300, Nippon Denshoku, Tokyo, Japan). The machine was calibrated with a white square plate (CIE L* = +94.48, a* = -0.67, b* = +3.31). Values of CIE L*(lightness), CIE a*(redness), and CIE b*(yellowness) was determined at 30 days.

Thiobarbituric acid reactive substance

Lipid oxidation was determined according to TBA (2-thiobarbituric acid) method of Whitte et al (1977). First, 2g of sample was homogenized at 12,000 rpm for 1 minute with 10mL of 10% TCA (trichloroacetic acid) and 10mL of distilled water. After homogenizing, the solution was filtered through a filter paper (Whatman No. 1, Whatman Inc., USA). 5mL of filtered solution was mixed with 5mL of TBA (2-thiobarbituric acid 2.88g/L) and then, the mixed solution was placed at 90°C water bath for 10 minutes. After 30 minutes for cooling, the solution was measured absorbance with spectrophotometer (UV/Vis Spectrophotometer, Mecasys Co., Korea) at 532nm. Thiobarbituric acid reactive substance (TBARS) values were determined from a standard curve of malondialdehyde.

Microbial analysis

2g of sample was homogenized with 18mL distilled water for 90 seconds then the supernatant was diluted for inoculation onto Petrifilm and MRS agar. Total aerobe counts were inoculated onto petrifilm which can count total aerobic bacteria (3M Petrifilm, USA) for 24 hr at 35°C. Lactic acid bacteria were inoculated on MRS agar (OXOID, England) for 24 hr at 35°C.

Fatty acid composition

The fatty acid analysis was experimented by AOAC (1995). Lipid extraction was conducted following the method of Folch *et al.* (1957), with slight modifications. Briefly, 25 mg of samples were mixed with potassium hydroxide (KOH) in methanol and heated. After cooling, 1 mL of isooctane solution and saturated sodium chloride (NaCl) were mixed with the solution. Chromatographic conditions were as follows: initial oven temperature, 100°C (held for 4 min); ramping at 3°C/min to 240°C (held for 15 min).

The injector and detector were maintained at 225°C and 285°C, respectively. The flow rate of helium was 0.75 mL/min, and 1 μ L of solution was injected in split mode (200:1). Nonadecanoic acid methyl ester, as an internal standard, at 0.3 mg/mL, was added to the samples prior to fat extraction and methylation. The isooctane layer was dehydrated with anhydrous sodium sulfate and analyzed by gas chromatography (GC) (5,890, Agilent Technologies, USA). An SP-2560 column (100 m × 0.25 mm × 0.2 um) was used with a flame ionization detector

Free amino acid composition

A standard method from the National Agricultural Products Quality Management Service was used in analysis. 50ml sample was hydrolyzed and hydrolyzed sample was condensed for removal of hydrochloric acid by rotary evaporator. And then, 50ml distilled water was added to the condensed sample and analyzed using an automatic amino acid analyzer (S2100, S4300, S5200, SYKAM, Germany).

Statistical analysis

ANOVA (one way analysis of variance) and Independent *t* test were used for analyzing results of data. p < 0.05 were considered significant, and trend was noted at p < 1.0 by SPSS 19.0.

III. RESULTS AND DISCUSSION

Proximate composition

Proximate composition of finished dry-cured sausages is shown in Table 2. Many scientists reported that meat products, made with sulfur-fed pork or cattle, had higher moisture contents and lower fat contents than meat products made with normal pork or cattle (Kim *et al.*, 2015; Daniell,

Steven & Stephanie, 2014; Lee *et al.*, 2009). However, these two contents of SP and NP was not significantly different (p > 0.05) in this report. Protein and ash contents of SP were higher than those of NP (p < 0.05).

Table 2. Proximate composition of dry-cured
sausages

Composition	NP	SP	SEM	p-value
Moisture	25.54	23.16	0.21	0.17
Protein	22.59	23.53	2.34	0.04
Fat	36.75	38.66	1.28	0.46
Ash	4.23	5.20	0.20	0.01
TBA	1.94	0.96	0.05	0.01
Weight loss	0.34	0.35	0.01	0.10

SEM = standard error of the means. NP, control group; SP, processed sulfur group.

pH and water activity (aw)

Fig. 1 shows aw and pH during the dry-curing process. Both samples of aw decreased in dry-curing process, as expected in typical dry-cured sausages (Baldini *et al.*, 1981). Aw of samples had no significant difference (p > 0.05) at the 0 and 7 day. However, aw of SP was significantly higher than aw of NP at the 14 and 21 day (p < 0.05). These results of 14 and 21 day may be explained by higher water holding capacity of processed sulfur fed pigs (Park *et al.*, 2003; Lee *et al.*, 2009). At the end of the process (30 day), there was no significant difference between NP and SP.

Graph of pH value showed decrease until 21 days in all samples. However, between 21 and 30 day, samples of pH value were slightly increased or remained stable. Globally pH downs from 5.7 to 4.66. This pH evolution is similar to the other dry-cured sausages (Gimeno *et al.*, 1999; Fernández-López et al., 2007; Sánchez-Zapata et al., 2013). These results are because of microbial activity of lactic acid bacteria (Fernández-López et al., 2007). The lactic acid bacteria metabolizes sugar in meat and presents lactic acid. The pH value of two samples had no significant difference at 0, 1 and 7 day, while pH value of SP was significantly lower than that of NP at 14 and 21 day (p < 0.05). Generally, high pH value of meat and meat product is due to hydrolysis of urea and accumulation of ammonia, sulphides, indole and amines (Hernández-Jover et al., 1996). On the other hands, low pH value in meat product can inhibit pathogenic microorganism and decrease biogenic amines (Suzzi et al., 2003). Therefore, the lower pH group of SP than group of NP might have correlation of antimicrobial activity.





SEM = standard error of the means. NP, control group; SP, processed sulfur group.

Lipid oxidation

TBA value of final product was shown in Table 2. TBA value of NP was significantly higher than that of SP (p < 0.05). Some scientists reported that sulfur containing compounds such as cysteine, glutathione and lipoic acid could protect against oxidative stress in biological systems (Eiserich *et al.*, 1994; Komarnisky et al., 2003). These compounds can be antioxidants through the scavenging and reduction of various oxidants. Some researchers found that glutathione, which is an important antioxidant by preventing damage caused by free radicals, peroxides and lipid peroxides (Pompella *et al.*, 2003), was formed from transsulfuration pathway during feeding sulfur containing feeds. (Kim et al., 2015; Song et al., 2013; Gethin, 2012; Salinas and Wong, 1999). Similar results showed an antioxidant effect of sulfur-fed porks applied in meat products. (Cho *et al.*, 2015; Kim *et al.*, 2015; Ismail *et al.*, 2009)

Changes in color

Table 3. shows the color of the final products. The lightness (L^{*}) of NP was significantly higher than that of SP (p < 0.05), while the redness (a^{*}) of NP was significantly lower than that of SP at the end of the processing (p < 0.05). The yellowness of the NP was significantly higher than that of SP (p < 0.05). Some researchers found that the yellowness was increased when the lipid oxidation was increased during processing (Ruiz *et al.*, 2002; Kim *et al.*, 2015). Therefore, the lower yellowness of SP could be related to the lower lipid oxidation in dry-cured sausages.

Table 3. Physicochemical properties of dry-curedsausages.

Parameter	NP	SP	SEM	<i>p</i> -value
TPC	6.49	6.05	0.07	0.02
LAB	8.14	8.06	0.04	0.31
L*	54.40	47.56	0.33	0.004
a*	7.92	12.64	0.50	0.004
b*	21.34	15.37	0.72	0.008

SEM = standard error of the means.

NP, control group; SP, processed sulfur group.

Microbial properties

Microbial properties of finished dry-cured sausages are shown in Table 3. Total plate counts (TPC) of SP were significantly lower than those of NP (p < 0.05). These results might be caused by the lower pH value of SP than NP. Similar researches that lower TPC by using sulfur-fed pork were reported by Kim *et al.*, (2014). In general, TPC can be used for parameter of deterioration of meat products (Ciuciu *et al.*, 2011). Hence, these results can say dry-cured sausages processed with sulfur-fed pork can prevent spoilage of microorganisms. Lactic acid bacteria (LAB) counts of SP and NP had no significant difference in finished products (p > 0.05).

Fatty acid composition

The fatty acid composition of dry-cured sausages is shown in Table 4. The SFA (saturated fatty acids) content of SP was lower than that of NP, while the MUFA (monounsaturated fatty acids), especially oleic acid of SP was significantly higher than that of NP (p < 0.05). In the sensual, according to Lunt and Smith (1991), meat products that have more oleic acid were evaluated better taste and flavor in sensory test. Also, Cameron and Enser (1991) found out that better taste in meat when MUFA was higher and PUFA was lower in meat. However, PUFA/SFA of significant difference between SP and NP was not shown.

Table 4. Fatty acid composition of dry-curedsausages.

	NP	SP	SEM	<i>p</i> -value
C14:0	1.62	1.08	0.28	0.42
C16:0	23.56	23.99	0.19	0.27
C18:0	12.33	11.28	0.58	0.27
C20:0	0.19	0.12	0.04	0.36
C16:1n7	2.42	2.47	0.17	0.12
C18:1n9	43.51	46.34	0.69	0.03
C20:1n9	1.04	1.01	0.01	0.06
C18:2n6	12.13	12.03	0.16	0.69
C20:2n6	0.53	0.18	0.09	0.18
C18:3n3	0.67	0.66	0.01	0.40
C20:3n3	0.08	0.10	0.09	0.90
\sum SFA	37.78	36.46	0.44	0.16
\sum MUFA	46.96	49.82	0.70	0.03
$\sum PUFA$	13.59	12.96	0.28	0.23
$\sum w6$	12.83	12.21	0.26	0.18
$\sum w3$	0.75	0.76	0.08	0.98
w6/w3	17.30	16.58	1.69	0.78
PUFA/SFA	0.36	0.35	0.01	0.80
MUFA/SFA	1.24	1.37	0.02	0.04

SEM = standard error of the means.

NP, control group; SP, processed sulfur group.

Free amino acid composition

Free amino acid composition of dry-cured sausages

was shown in Table 6. Free amino acid composition is parameter of meat quality (Kim et al., 2015). During the ripening process of dry-cured sausages, proteins are degraded into large peptides, and then large peptides are degraded into oligopeptides, at last, they are degraded into free amino acids (Domínguez et al., 2016). Glutaminc acid, Asparagines, Histidine, Glycine, Arginine, Alanine, Taurine, Tyrosine, Valine, Tryptophan, Isoleucine and Leucine contents of SP were significantly higher than those of NP (p < 0.05). Among these amino acids, glutamic acid has synergy effect with salt in increasing Umami taste (Nishimura and Kato, 1988). Aspartic acid, Serine, Glutamine, Threonine, GABA, Methionine, Phenylalanine and Lysine contents have no significant difference between SP and NP.

Table 5. Free amino acid composition of dry-cured sausages

	NP	SP	SEM	<i>p</i> -value
Aspartic acid	9.94	9.40	0.12	0.49
Glutamic acid	811.69	886.42	8.41	0.02
Asparagines	152.77	175.49	4.01	0.02
Serine	34.93	42.06	1.73	0.12
Glutamine	201.47	229.11	3.15	0.01
Histidine	112.01	122.79	1.62	0.03
Glycine	229.39	285.74	2.31	0.01
Threonine	268.52	249.61	9.01	0.39
Arginine	99.38	116.50	4.16	0.05
Alanine	894.52	1018.24	8.89	0.01
Taurine	1859.19	1940.78	6.89	0.01
GABA	28.41	30.58	2.12	0.60
Tyrosine	47.19	72.80	1.60	0.01
Valine	304.24	351.79	3.31	0.02
Methionine	152.46	181.15	10.07	0.13
Tryptophane	26.32	37.90	0.69	0.01
Phenylalainine	247.44	249.84	6.54	0.81
Isoleucine	290.84	312.04	2.10	0.03
Leucine	555.02	627.23	10.38	0.01
Lysine	285.59	310.71	7.66	0.16
Proline	246.59	223.77	3.52	0.02

SEM = standard error of the means.

NP, control group; SP, processed sulfur group.

IV. CONCLUSION

Dry-cured sausages made from sulfur-fed pork had better depression effects of antimicrobial and antioxidant than made from normal pork. Also, the SP group had more monounsaturated fatty acid, especially oleic acid than NP group. The free amino acid of SP group was more than of NP group. In conclusion, the supplementation with processed sulfur to pigs improves nutritional quality of meat products and extends shelf-life of dry-cured sausages.

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