



ARTICLE

## Effects of Six Different Starter Cultures on Mutagenicity and Biogenic Amine Concentrations in Fermented Sausages Treated with Vitamins C and E

Hyeong Sang Kim<sup>1</sup>, Seung Yun Lee<sup>2</sup>, Hea Jin Kang<sup>2</sup>, Seon-Tea Joo<sup>3</sup>, and Sun Jin Hur<sup>2,\*</sup>

<sup>1</sup>Department of Animal Life and Environment Science, Hankyong National University, Anseong 17579, Korea

<sup>2</sup>Department of Animal Science and Technology, Chung-Ang University, Anseong 17546, Korea

<sup>3</sup>Institute of Agriculture & Life Science, Gyeongsang National University, Jinju 52828, Korea



OPEN ACCESS

Received July 9, 2019

Revised August 19, 2019

Accepted September 16, 2019

\*Corresponding author : Sun Jin Hur  
Department of Animal Science and Technology, Chung-Ang University, Anseong 17546, Korea  
Tel: +82-31-670-4673  
Fax: +82-31-670-3108  
E-mail: [hursj@cau.ac.kr](mailto:hursj@cau.ac.kr)

\*ORCID  
Hyeong Sang Kim  
<https://orcid.org/0000-0001-7054-2989>  
Seung Yun Lee  
<https://orcid.org/0000-0002-8861-6517>  
Hea Jin Kang  
<https://orcid.org/0000-0001-6765-3434>  
Seon-Tea Joo  
<https://orcid.org/0000-0002-5483-2828>  
Sun Jin Hur  
<https://orcid.org/0000-0001-9386-5852>

**Abstract** This study was performed to determine changes in mutagenicity and biogenic amine concentrations in sausages fermented with six different starter cultures treated with vitamins C and E. Six different types of fermented sausages with different combination of starter cultures were manufactured. T1, *Pediococcus acidilactici*; T2, *P. pentosaceus* and *Staphylococcus carnosus*; T3, *S. carnosus*, *S. xylosum*, *Debaryomyces hansenii*, *Lactobacillus curvatus*, and *P. pentosaceus*; T4, *S. carnosus* and *L. sakei*; T5, *S. xylosum* and *L. plantarum*; and T6, *Penicillium nalgiovensis*. After treatment with vitamins C and E in fermented sausages, changes in mutagenicity and biogenic amine concentrations were measured. The sausages fermented with *Staphylococcus xylosum* and *Lactobacillus plantarum* starter cultures showed the most effective antimutagenic activity ( $p < 0.05$ ). The mutagenicity was further decreased in the sausages treated with vitamins C and E ( $p < 0.05$ ), regardless of the starter cultures. The use of *Pediococcus acidilactici*, *S. xylosum*, *L. plantarum*, and *Penicillium nalgiovensis* as starter cultures was effective in decreasing biogenic amine concentrations ( $p < 0.05$ ). In addition, vitamin E was more effective in decreasing the biogenic amine concentrations than vitamin C. In conclusion, we recommend the use of *S. xylosum* and *L. plantarum* as starter cultures, in addition to the use of vitamins C and E, to reduce the potential risk of meat mutagens in fermented sausages.

**Keywords** starter culture, fermented sausage, mutagenicity, vitamin C, vitamin E, biogenic amine

## Introduction

As consumers' interest in functional food and safety has increased, products that promote health and wellness have developed (Tyopponen et al., 2003). Dairy products fermented by probiotic bacteria are well-known nutritional foods (Saxelin, 2000).

Therefore, use of probiotics to fermented meat products could increase health benefits (De Vuyst et al., 2008; Lucke, 2000).

Biogenic amines are of worry in fermented meat products because of their toxicological effects on the blood pressure, intestinal system, and nervous system. Usual symptoms of biogenic amine poisoning, especially tyramine include headache, migraine, and increased blood pressure (Ladero et al., 2010). In addition, some studies were performed to evaluate the effects of the cytotoxicity of individual compounds on the *in vitro* mutagenicity and toxicity of biogenic amines (Badolo et al., 1998). Thus, excessive intake of these biogenic amines may cause health problems (Ten Brink et al., 1990). In addition to their toxicity, some biogenic amines can react with nitrite in meat products to form potentially harmful nitrosamines (Santos, 1996). These problems can be more serious for consumers with reduced monoamine and diamine oxidase activities; these enzymes are responsible for biogenic amine detoxification (Bardocz, 1995).

Vitamins C and E are representative natural antioxidants. These antioxidants can decrease the risk of various diseases, including cancer (Decker et al., 2000). Mitsumoto et al. (1991) reported that ascorbic acid scavenges oxygen and inhibits radical formation at double bonds.  $\alpha$ -Tocopherol (vitamin E) is also an crucial antioxidant that can retard color changes and lipid oxidation in animal fats and meat products (Dziezak, 1986; Morrissey et al., 1994).

Anti-mutagenicity of L-ascorbic acid and  $\alpha$ -tocopherol in sausage samples has been reported by Pourazrang et al. (2002). They reported that addition of L-ascorbic acid (>500 ppm) and  $\alpha$ -tocopherol (>250 ppm) to pork sausages decreased mutagenicity to 61% and 54%, respectively. However, to the best of our knowledge, there are no studies on the effects of different starter cultures on mutagenicity in fermented sausages treated with vitamins C and E. Therefore, the objective of this study was to evaluate the effects of six different starter cultures on mutagenicity and biogenic amine contents in fermented sausages treated with vitamins C and E.

## Materials and Methods

### Manufacture of fermented sausages

Six different types of fermented sausages were prepared (Kim and Hur, 2017). Fresh pork back-fat and ham were purchased from whole-sale meat market (Jeonju, Korea). The sausage was manufactured with pork ham (84.145%), pork back fat (9.4%), salt (1.0%), sodium erythorbate (0.045%), sodium nitrite (0.01%), pepper mix (2.3%), red pepper sauce (3%), and sugar (0.1%). Trimming, chopping, and additive mixing (CE93, RUHLE GMBH, Grafenhausen, Germany) were performed. After that, starter cultures were inoculated. According to our previous study (Kim and Hur, 2017), the following starter cultures were selected: T1, *Pediococcus acidilactici*; T2, *Staphylococcus carnosus* and *P. pentosaceus*; T3, *P. pentosaceus*, *Lactobacillus curvatus*, *Debaryomyces hansenii*, *S. xylosus*, and *S. carnosus*; T4, *L. sakei* and *S. carnosus*; T5, *L. plantarum* and *S. xylosus*; and T6, *Penicillium nalgiovensis*. These starter cultures were purchased from Almi Ges. M.g.H & Co GK (Offering, Austria). Each starter culture was prepared as instructor's guideline and inoculated at  $10^4$  CFU/g into the sausage batter (the bacteria were suspended in 50 mL of deionized water before inoculation). Subsequently, the sausage batter was stuffed into a pork small intestine casing (Naturin Viscofan Co, Tajonar-Navarra, Spain) and placed in a fermentation chamber (FT 50, Frigomeccania, Parma, Italia). The first fermentation step involved 8 days of fermentation at 4°C. The second fermentation step involved 28 days of fermentation at 10°C. The relative humidity (RH) values of the first and second fermentation steps were 70%–80% and 60%–70%, respectively. After fermentation, the fermented sausages were immersed in the same volume of deionized water, 0.1% vitamin C solution, 0.1% vitamin E solution, and 0.05% vitamin C and E solution (v/v) and then shaken at 4×g and 20°C for 4 h. The fermented sausage samples were stored at 4°C

overnight before use.

### Mutagenicity test

Before measuring the mutagenicity of the fermented sausages, extraction step was performed (Weisburger et al., 2002). Briefly, 10 mL of methanol was added to 1 g of each fermented sausage sample and homogenized (HG-15D, Labtech, Ltd., Jeonju, Korea) for 1 min at 11,200×g and then centrifuged (Combi 514R, Hanil Scientific Inc., Gimpo, Korea) for 5 min at 10,000×g. Then, a rotary evaporator was used to remove the methanol. Dimethyl sulfoxide (5 mL) was added to residue. Mutagenicity was measured using the Ames test with *Salmonella* Typhimurium TA98, which is a frameshift mutation strain (Maron and Ames, 1983). *S. Typhimurium* TA98 strain was purchased from Moltax (Molecular Toxicology Inc., Boone, USA). *S. Typhimurium* culture (0.1 mL,  $1\text{--}2\times 10^8$  cells), each sample (0.1 mL), and maintained overnight, and Aroclor 1254-induced Sprague-Dawley rat liver S9 mix (0.5 mL) were added to 2 mL of top agar containing biotin and histidine (0.05 mM each), and then the mixture was vortexed and poured onto a minimal glucose agar plate (25 mL). After the solidification, the agar plate was incubated for 36–48 h (37°C). The mutagenicity was expressed as the mean number of revertants per agar plate ( $n=3$ ). 4-Nitro-*o*-phenylenediamine (2.5 µg/plate) was used as positive control.

### Determination of biogenic amines

A total of 8 biogenic amines—tryptamine, phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine, and spermine—were determined using high-performance liquid chromatography (HPLC; HP Agilent 1100; Hewlett Packard Co., Palo Alto, CA, USA) with an Eclipse XDB-C<sub>8</sub> column (150 mm×4.6 mm; 5 µm) using a method of Eerola et al. (1993). Internal standard (1,7-Diaminoheptane, 1 mg mL<sup>-1</sup>, 125 µL) was added to sausage samples (1 g) and homogenized with 5 mL of 0.4 M perchloric acid with homogenizer (HG-15D, Labtech, Ltd., Jeonju, Korea) at 11,200×g. Then the mixture was centrifuged for 5 min at 1,008×g and filtered through Whatman No. 2 filter paper. Extraction procedure was repeated twice, and combined supernatants was adjusted to 20 mL with 0.4 M perchloric acid. Extracted sample (1 mL) was alkalized by adding 200 µL of 2 N NaOH, and buffered by adding 300 µL of saturated sodium bicarbonate. Dansyl chloride solution (2 mL, 10 mg mL<sup>-1</sup>) was added and incubated at 40°C for 45 min. An 100 µL of ammonia solution (10%) was added to remove residual dansyl chloride. After 30 min, 5 mL of acetonitrile was added to the mixture and centrifuged 5 min at 700×g, and the supernatant was filtered through 0.25 µm PTFE membrane filter, before HPLC injection. The mobile phase consisted of acetonitrile (solvent A) and water (solvent B). The gradient began at 50% and ended at 90% of A in 20 min. The volume of the injected sample and the flow rate were 20 µL and 1.0 mL/min, respectively. The detection wavelength was set at 254 nm. The retention times of tryptamine, phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine, and spermine were 8.07, 9.42, 9.96, 10.63, 10.90, 14.19, 15.08, and 18.80 min, respectively.

### Statistical analysis

The statistical analysis was performed for changes in the mutagenicity and biogenic amine concentrations in the fermented sausages using two-way analysis of variance (ANOVA) with IBM SPSS 22.0 software (Chicago, USA) as factors for six combinations of starter cultures (T1, T2, T3, T4, T5, and T6) and vitamin treatments (vitamin C, vitamin E, and vitamins C and E). All experiments were performed in triplicate. The Student–Newman–Keuls multiple range test was used to compare the mean values. Significant differences ( $p<0.05$ ) between the mean values of the mutagenicity and biogenic amine

concentrations were calculated.

## Results

The mutagenicity of the 6 different types of fermented sausages is shown in Fig. 1A–C. The mean values of mutagenicity for the 6 fermented sausages in the control group were 220, 273, 247, 383, 173, and 200. Among the treatments, T4, sausages fermented with *S. carnosus* and *L. sakei* starter cultures, showed the highest mutagenicity, and T5, sausages fermented with *S. xylosum* and *L. plantarum* starter cultures, showed the lowest mutagenicity (Fig. 1B). However, their mutagenicity values were decreased by the addition of vitamin C and/or E ( $p < 0.05$ ), regardless of the starter cultures. In particular, vitamin E reduced mutagenicity more effectively than vitamin C (Fig. 1C;  $p < 0.05$ ).

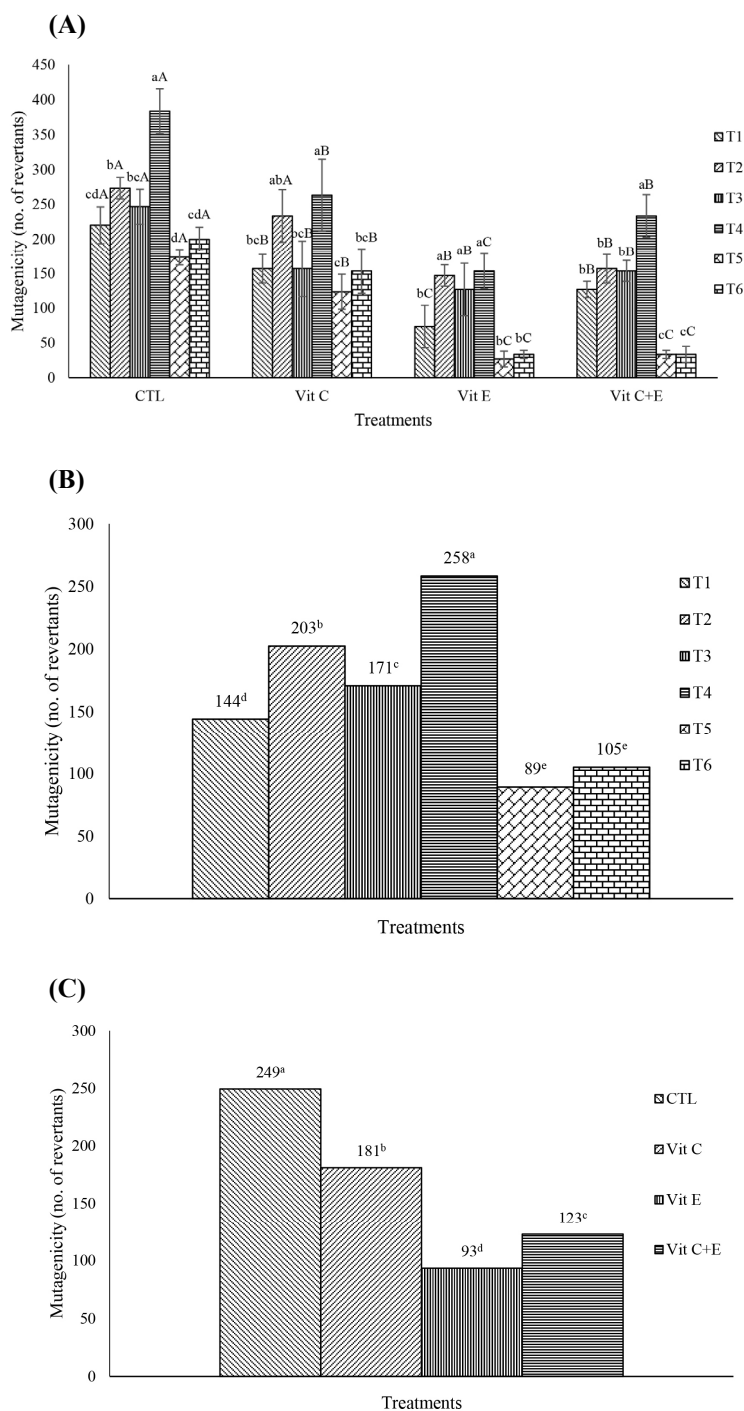
The levels of biogenic amines are shown in Fig. 2A–C. In this study, tyramine was the only biogenic amine found in the fermented sausages. The mean levels of tyramine in the 6 different fermented sausages were 14.1, 84.9, 79.9, 99.5, 13.0, and 13.2 mg kg<sup>-1</sup>. T4, sausages fermented with *L. sakei* and *S. carnosus* starter cultures, showed the highest biogenic amine concentration, and T1, T5, and T6, sausages fermented with *P. acidilactici*, *S. xylosum* and *L. plantarum*, and *P. nalgiovensis* starter cultures, respectively, showed the lowest biogenic amine concentration (Fig. 1B;  $p < 0.05$ ). The concentrations of biogenic amines in the six different fermented sausages were decreased by the addition of vitamin C and/or E ( $p < 0.05$ ). Similar to the mutagenicity results, addition of vitamin E effectively reduced the concentration of biogenic amines (Fig. 2C;  $p < 0.05$ ).

The correlations with factors that affect mutagenicity are listed in Table 1. Among the factors, starter cultures showed the highest correlation ( $r = 0.81$ ) with mutagenicity. In contrast, biogenic amine ( $r = 0.54$ ), vitamin C ( $r = 0.22$ ), and vitamin E ( $r = 0.60$ ) showed relatively low correlations. In addition, biogenic amines showed high correlations with starter cultures ( $r = 0.85$ ) and mutagenicity ( $r = 0.54$ ), whereas the effects of vitamin C ( $r = 0.15$ ) and vitamin E ( $r = 0.34$ ) on the concentration of biogenic amines were relatively low.

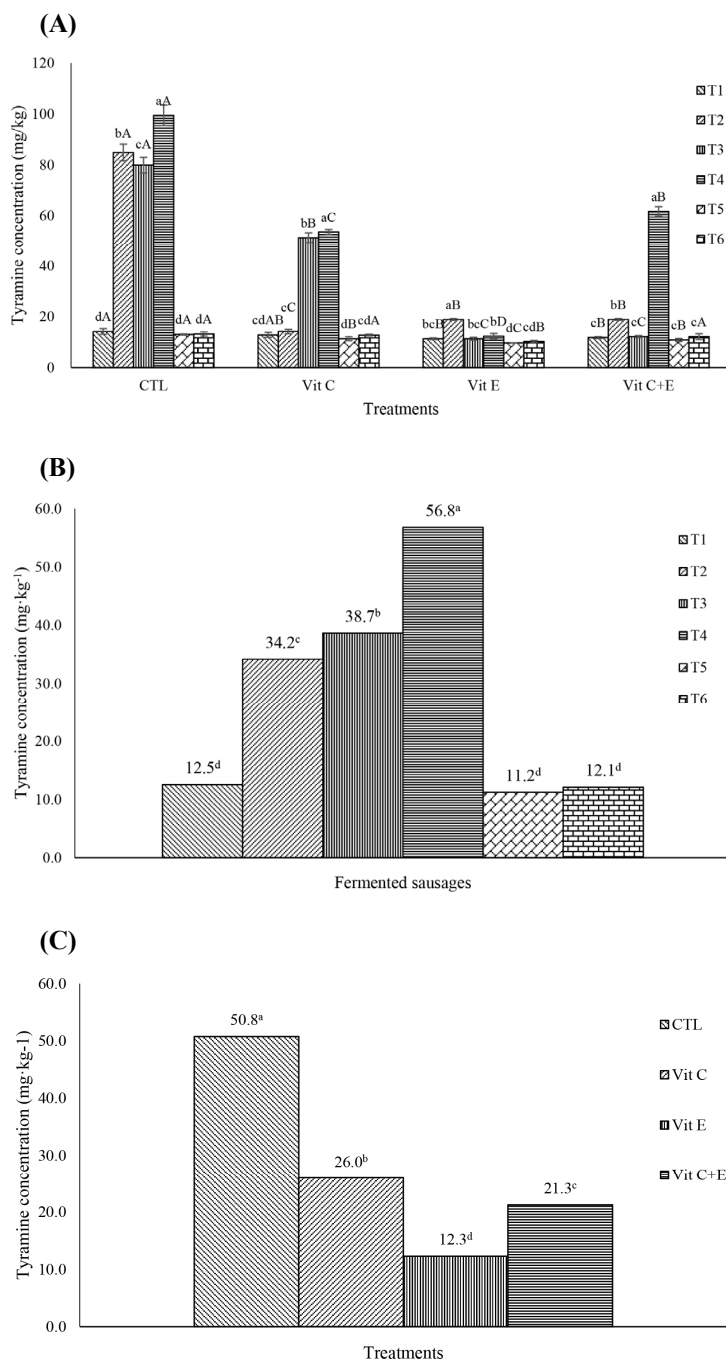
Among the treatments, T4, which had the highest mutagenicity, showed the highest biogenic amine concentration, however the other treatments did not showed similar patterns after the vitamin C and/or E treatments. Therefore, the correlation between mutagenicity and biogenic amine content was not high ( $r = 0.54$ ).

## Discussion

The use of appropriate starter cultures for the production of fermented sausages is necessary to control mutagenicity and biogenic amine concentrations. Some starter cultures has antimutagenic effect (Lankaputhra and Shar, 1998) by desmutagen factor (Hosono et al., 1987). Gram-positive and -negative bacteria have antimutagenicity by binding mutagens to their peptidoglycan layer and outer layer, respectively (Zhang and Ohta, 1991). In the case of biogenic amines, it is naturally produced compounds in the process of decarboxylation of the amino acid components in fermented foods (Ten Brink et al., 1990). Factors that control the formation of biogenic amines in fermented sausage include the use of amine-oxidizing or non-amine-forming starter cultures (Nieto-Arribas et al., 2009), control of pH (Hu et al., 2007) and temperature (Shalaby, 1996), however inhibiting decarboxylase activity is the most crucial factor (Wendakoon and Sakaguchi, 1995). Therefore, the use of amine-negative starter cultures can reduce the accumulation of biogenic amines (Bover-Cid et al., 2000). In this study, the six different combination of starter cultures may be affected the antimutagenicity and formation of biogenic amine by various factors, such as rates of Gram-positive and -negative bacteria, amine-oxidizing starter culture, pH, temperature, and



**Fig. 1. Mutagenicity (no. of revertants) of fermented sausages as affected by six combinations of starter cultures and vitamins treatments.** (A) Mutagenicity (no. of revertants) of various fermented sausages. <sup>a-d</sup> Means with different superscripts within the same treatment are different ( $p < 0.05$ ). <sup>A-C</sup> Means with different superscripts within the same fermented sausages are different ( $p < 0.05$ ). (B) Mutagenicity (no. of revertants) of six different fermented sausages. <sup>a-e</sup> Means with different superscripts within the different fermented sausages are different ( $p < 0.05$ ). (C) Mutagenicity (no. of revertants) of fermented sausages treated with vitamins C and E. Treatments are described in Fig. 1A. <sup>a-d</sup> Means with different superscripts within different treatment are different ( $p < 0.05$ ). Treatments: T1, *Pediococcus acidilactici*; T2, *Pediococcus pentosaceus* and *Staphylococcus carnosus*; T3, *Staphylococcus carnosus*, *Staphylococcus xylosus*, *Debaryomyces hansenii*, *Lactobacillus curvatus*, and *Pediococcus pentosaceus*; T4, *Staphylococcus carnosus* and *Lactobacillus sakei*; T5, *Staphylococcus xylosus* and *Lactobacillus plantarum*; T6, *Penicillium nalgiovensis*; CTL, fermented sausages (FS) with 6 different starter cultures; Vit C, FS immersed in the same volume of 0.1% Vit C solution; Vit E, FS immersed in the same volume of 0.1% Vit E solution; Vit C+E, FS immersed in the same volume of 0.05% Vit C and 0.05% Vit E solutions.



**Fig. 2. Tyramine concentration of fermented sausages as affected by six combinations of starter cultures and vitamins treatments.** (A) Concentrations of tyramine (mg kg<sup>-1</sup>) in various fermented sausages. <sup>a-d</sup> Means with different superscripts within the same treatment are different (p<0.05). <sup>A-D</sup> Means with different superscripts within the same fermented sausages are different (p<0.05). (B) Concentrations of tyramine (mg kg<sup>-1</sup>) in six different fermented sausages. <sup>a-d</sup> Means with different superscripts within the different fermented sausages are different (p<0.05). (C) Concentrations of tyramine (mg kg<sup>-1</sup>) in fermented sausages treated with Vitamins C and E. <sup>a-d</sup> Means with different superscripts within different treatment are different (p<0.05). Treatments: T1, *Pediococcus acidilactici*; T2, *Pediococcus pentosaceus* and *Staphylococcus carnosus*; T3, *Staphylococcus carnosus*, *Staphylococcus xylosum*, *Debaryomyces hansenii*, *Lactobacillus curvatus*, and *Pediococcus pentosaceus*; T4, *Staphylococcus carnosus* and *Lactobacillus sakei*; T5, *Staphylococcus xylosum* and *Lactobacillus plantarum*; T6, *Penicillium nalgiovensis*; CTL, fermented sausages (FS) with 6 different starter cultures; Vit C, FS immersed in the same volume of 0.1% Vit C solution; Vit E, FS immersed in the same volume of 0.1% Vit E solution; Vit C+E, FS immersed in the same volume of 0.05% Vit C and 0.05% Vit E solutions.

**Table 1. Correlation between various parameters**

	Mutagenicity	BA	Starter cultures	Vitamin C	Vitamin E
Mutagenicity	1				
BA	0.54	1			
Starter cultures	0.81	0.85	1		
Vitamin C	0.22	0.15	-	1	
Vitamin E	0.60	0.34	-	-	1

inhibition of decarboxylase activity.

In previous studies, several vitamins, including vitamins C and E, have been shown to possess antimutagenic activities (Ramel et al., 1986). Vitamins C and E are known to be radical scavengers and strong antioxidants (Ramel et al., 1986). Inhibition of the mutation caused by oxygen radicals is possible with vitamins C and E. Vitamin C reduced the mutagenicity of activated aflatoxin B<sub>1</sub> in *S. Typhimurium* TA100 more than 50% (Bhattacharya et al., 1987) by prevention of its metabolic activation (Firozi et al., 1986). Raina and Gurtoo (1985) reported that vitamin C reduced mutagenicity. Vitamin C showed antimutagenicity of sodium nitrite and spermidine mixture in the Ames test (Kokatnur et al., 1978). Experiments performed using bacterial cells showed vitamin E activity opposition to aflatoxin B<sub>1</sub> mutagenicity in the TA100 and TA98 strains (Raina and Gurtoo, 1985), and antimutagenic activity was discovered with high levels of vitamin E. Shamberger et al. (1979) reported that vitamin E inhibited mutagenicity by the direct-acting mutagen malondialdehyde, which is produced during lipid oxidation, in a number of *S. Typhimurium* strains. Possibly, vitamin E prevents the conversion of promutagens into mutagens by inactivation of the free oxy radicals (Colin et al., 1991). Thus, antioxidant activity, including radical scavenging activity of vitamins C and E, may be the major mechanism for decreasing mutagenicity in fermented sausages. In particular, the use of vitamin E, a lipophilic antioxidant, was more effective in lowering mutagenicity than that of vitamin C, a hydrophilic antioxidant.

Formation of biogenic amine by the microorganisms is dependent on the availability of the amino acid substrate, level of decarboxylase activity, and specific bacterial strain(s) present (De Las Rivas et al., 2008; Suzzi and Gardini, 2003). Tyramine is the most abundant biogenic amine in fermented sausages (Coisson et al., 2004; Komprda et al., 2004). Santos (1996) reported that the toxic level of tyramine is 100–800 mg kg<sup>-1</sup>. Considering the already known harmful effects of biogenic amines (Ladero et al., 2010), it is crucial for the meat industry to produce safe fermented meat products with an appropriate level of biogenic amine. In this study, tyramine was the predominant amine among the biogenic amines and showed correlation with the mutagenicity of the fermented sausages ( $r=0.54$ ). Badolo et al. (1998) have reported the mutagenicity and *in vitro* toxicity of biogenic amines, and the effects were analyzed based on the cytotoxicity of individual compounds. Bozkurt and Erkmén (2004) reported that sausages containing food preservatives, such as ascorbic acid (500 ppm) and  $\alpha$ -tocopherol (200 ppm) significantly reduced biogenic amine formation. Especially, diaminoxidase activity of vitamins can degrade biogenic amine concentrations (Maintz et al., 2006). Therefore, high concentrations of biogenic amines in fermented meat and meat products may increase mutagenicity, and using antioxidants such as vitamins C and E may reduce the concentrations of biogenic amines and mutagenicity.

Certain starter cultures used in sausage fermentation can also retard the development of biogenic amines (Latorre-Moratalla et al., 2007). The microorganisms possess the decarboxylation ability may be used as starter cultures in fermented sausages (Spicka et al., 2002). *S. xyloso* and *L. curvatus* delay cadaverine and putrescine formation during the fermentation



and storage of dry fermented sausages (Bover-Cid et al., 2001). *S. carnosus*, *S. xylosum*, and *L. sakei* have also been used during the manufacture of dry sausages and were found to inhibit the accumulation of biogenic amines (Bover-Cid et al., 2001). Combined starter cultures induce a synergistic effect for the reducing biogenic amines (Hu et al., 2007). The use of combined starter cultures results in a pH reduction (Hu et al., 2007), which may be another factor that contributes to reducing the accumulation of biogenic amines. Due to increased decarboxylase activity of bacteria against acidic pH, biogenic amine concentration can be increased in the low pH condition (Bover-Cid et al., 2001). In this study, pH values of fermented sausages with six different starter cultures were 5.08, 4.92, 5.24, 4.96, 5.07, and 5.66 for T1, T2, T3, T4, T5, and T6, respectively. However, the highest biogenic amine concentration was observed in T4, which showed significantly lower pH than other treatments ( $p < 0.05$ ). This means that although pH may affect the formation of biogenic amines, other factors must also be considered. Lactobacilli are also capable to reduce biogenic amines. Dapkevicius et al. (2000) reported the ability of lactic acid bacteria to degrade biogenic amines. Therefore, the use of bacteria which has oxidizing enzymes or amine-oxidizing activity can reduce biogenic amine concentrations when it is difficult to control biogenic amine concentration and remove already formed biogenic amines in fermented meat products. For that reason, treatment with vitamins C and E could be useful for reducing biogenic amine levels in various sausages fermented with starter cultures.

Komprda et al. (2004) reported that the sum of all biogenic amines in fermented sausages produced with *P. pentosaceus* and *S. carnosus* was higher than that in fermented sausages produced with other starter cultures ( $p < 0.05$ ). In this study, various starter cultures were used to manufacture fermented sausages, and they showed a wide range of biogenic-amine reduction effects. As a result, the use of *S. carnosus* and *L. sakei* as starter cultures increased the mutagenicity and biogenic amine concentrations in the fermented sausages, whereas the combination of *S. xylosum* and *L. plantarum* starter cultures was the most effective in decreasing the mutagenicity and biogenic amines concentrations in the fermented sausages.

## Conclusion

In this study, the fermented sausages containing *S. xylosum* and *L. plantarum* showed the lowest mutagenicity value. In addition, the fermented sausages treated with vitamin E alone decreased mutagenicity to a greater extent than the fermented sausages treated with vitamin C and a combination of vitamins C and E. The levels of biogenic amines decreased after the addition of vitamins C and E ( $p < 0.05$ ). In particular, vitamin E was more effective in decreasing the concentration of biogenic amines than vitamin C. The correlation between starter culture and concentrations of tyramine ( $r = 0.85$ ) was higher than that of the other parameters ( $p < 0.05$ ). In conclusion, we recommend the use of *S. xylosum* and *L. plantarum* as starter cultures for fermented sausages with vitamins, especially vitamin E, to reduce potential meat mutagens.

## Conflicts of Interest

The authors declare no potential conflict of interest.

## Acknowledgements

This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through High Value-added Food Technology Development Program funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (118039-03-2-WT031).



## Author Contribution

Investigation: Kim HS. Writing original draft: Kim HS, Hur SJ. Writing review & editing: Kim HS, Lee SY, Kang HJ, Joo ST, Hur SJ.

## Ethics Approval

This article does not required IRB/IACUC approval because there are no human and animal participants.

## References

- Badolo L, Gelbcke M, Dubois J, Hanocq M. 1998. Synthesis and cytotoxicity studies of new analogues of polyamines. *Die Pharmazie* 53:15-19.
- Bardocz S. 1995. Polyamines in food and their consequences for food quality and human health. *Trends Food Sci Technol* 6:341-346.
- Bhattacharya RK, Francis AR, Shetty TK. 1987. Modifying role of dietary factors on the mutagenicity of aflatoxin B<sub>1</sub>: *In vitro* effect of vitamins. *Mutat Res Genet Toxicol* 188:121-128.
- Bover-Cid S, Izquierdo-Pulido M, Vidal-Carou MC. 2000. Mixed starter cultures to control biogenic amine production in dry fermented sausages. *J Food Protect* 63:1556-1562.
- Bover-Cid S, Izquierdo-Pulido M, Vidal-Carou MC. 2001. Effect of the interaction between a low tyramine-producing *Lactobacillus* and proteolytic staphylococci on biogenic amine production during ripening and storage of dry sausages. *Int J Food Microbiol* 65:113-123.
- Bozkurt H, Erkmén O. 2004. Effects of temperature, humidity and additives on the formation of biogenic amines in sucuk during ripening and storage periods. *Food Sci Technol Int* 10:21-28.
- Coisson JD, Cerutti C, Travaglia F, Arlorio M. 2004. Production of biogenic amines in “Salamini italiani alla cacciatora PDO”. *Meat Sci* 67:343-349.
- Colin C, Narbonne JF, Migaud ML, Grolier P, Cassand P, Pellissier MA. 1991. Lipid peroxidation and benzo[*a*]pyrene activation to mutagenic metabolites: *In vivo* influence of vitamins A, E and C and glutathione in both dietary vitamin A sufficiency and deficiency. *Mutat Res* 246:159-168.
- Dapkevicius MLE, Nout MR, Rombouts FM, Houben JH, Wymenga W. 2000. Biogenic amine formation and degradation by potential fish silage starter microorganisms. *Int J Food Microbiol* 57:107-114.
- De Las Rivas B, Gonzalez R, Landete JM, Munoz R. 2008. Characterization of a second ornithine decarboxylase isolated from *Morganella morganii*. *J Food Protect* 71:657-661.
- De Vuyst L, Falony G, Leroy F. 2008. Probiotics in fermented sausages. *Meat Sci* 80:75-78.
- Decker EA, Livisay SA, Zhou S. 2000. Mechanisms of endogenous skeletal muscle antioxidants: Chemical and physical aspects. In *Antioxidants in muscle foods*. Decker EA, Faustman C, Lopez-Bote CJ (ed). Wiley-Interscience, New York, NY, USA. pp 25-60.
- Dziedzic JD. 1986. Preservatives: antioxidants. *Food Technol* 40:94-102.
- Eerola S, Hinkkanen R, Lindfors E, Hirvi T. 1993. Liquid chromatographic determination of biogenic amines in dry sausages. *J AOAC Int* 76:575-577.

- Firozi PF, Aboobaker VS, Bhattacharya RK. 1986. Modulation by certain factors of metabolic activation of aflatoxin B<sub>1</sub> as detected *in vitro* in a simple fluorimetric assay. *Chem Biol Interact* 58:173-184.
- Hosono A, Yoshimura A, Otani H. 1987. Antimutagenic activity of cellular component of *Streptococcus faecalis* IFO 12965. *Neth Milk Dairy J* 41:239-245.
- Hu Y, Xia W, Liu X. 2007. Changes in biogenic amines in fermented silver carp sausages inoculated with mixed starter cultures. *Food Chem* 104:188-195.
- Kim HS, Hur SJ. 2017. Effect of six different starter cultures on the concentration of residual nitrite in fermented sausages during *in vitro* human digestion. *Food Chem* 239:556-560.
- Kokatnur MG, Murray ML, Correa P. 1978. Mutagenic properties of nitrosated spermidine. *Exp Biol Med* 158:85-88.
- Komprda T, Smela D, Pechova P, Kalhotka L, Stencil J, Klejdus B. 2004. Effect of starter culture, spice mix and storage time and temperature on biogenic amine content of dry fermented sausages. *Meat Sci* 67:607-616.
- Ladero V, Calles-Enriquez M, Fernandez M, Alvarez MA. 2010. Toxicological effects of dietary biogenic amines. *Curr Nutr Food Sci* 6:145-156.
- Lankaputhra WEV, Shah NP. 1998. Antimutagenic properties of probiotic bacteria and of organic acids. *Mutat Res* 397:169-182.
- Latorre-Moratalla ML, Bover-Cid S, Aymerich T, Marcos B, Vidal-Carou MC, Garriga M. 2007. Aminogenesis control in fermented sausages manufactured with pressurized meat batter and starter culture. *Meat Sci* 75:460-469.
- Lucke FK. 2000. Utilization of microbes to process and preserve meat. *Meat Sci* 56:105-115.
- Maintz L, Benfadal S, Allam JP, Hagemann T, Fimmers R, Novak N. 2006. Evidence for a reduced histamine degradation capacity in a subgroup of patients with atopic eczema. *J Allergy Clin Immun* 117:1106-1112.
- Maron DM, Ames BN. 1983. Revised methods for the Salmonella mutagenicity test. *Mutat Res* 113:173-215.
- Mitsumoto M, Cassens RG, Schaefer DM, Arnold RN, Scheller KK. 1991. Improvement of color and lipid stability in beef *longissimus* with dietary vitamin E and vitamin C dip treatment. *J Food Sci* 56:1489-1492.
- Morrissey PA, Buckley DJ, Sheehy PJA, Monahan FJ. 1994. Vitamin E and meat quality. *Proc Nutr Soc* 53:289-295.
- Nieto-Arribas P, Poveda JM, Sesena S, Palop L, Cabezas L. 2009. Technological characterization of *Lactobacillus* isolates from traditional Manchego cheese for potential use as adjunct starter cultures. *Food Control* 20:1092-1098.
- Pourazrang H, Moazzami AA, Bazzaz BSF. 2002. Inhibition of mutagenic N-nitroso compound formation in sausage samples by using L-ascorbic acid and  $\alpha$ -tocopherol. *Meat Sci* 62:479-483.
- Raina V, Gurtoo HL. 1985. Effects of vitamins A, C, and E on aflatoxin B<sub>1</sub>-induced mutagenesis in *Salmonella typhimurium* TA-98 and TA-100. *Teratog Carcinog Mutagen* 5:29-40.
- Ramel C, Alekperov UK, Ames BN, Kada T, Wattenberg LW. 1986. Inhibitors of mutagenesis and their relevance to carcinogenesis: Report by ICPEMC expert group on antimutagens and desmutagens. *Mutat Res Genet Toxicol* 168:47-65.
- Santos MS. 1996. Biogenic amines: Their importance in foods. *Int J Food Microbiol* 29:213-231.
- Saxelin M. 2000. Clinical documentation, product development and marketing of *Lactobacillus GG*. *Scand J Food Nutr* 44:102.
- Shalaby AR. 1996. Significance of biogenic amines to food safety and human health. *Food Res Int* 29:675-690.
- Shamberger RJ, Corlett CL, Beaman KD, Kasten BL. 1979. Antioxidants reduce the mutagenic effect of malonaldehyde and  $\beta$ -propiolactone: Part IX, antioxidants and cancer. *Mutat Res Genet Toxicol* 66:349-355.
- Spicka J, Kalac P, Bover-Cid S, Krizek M. 2002. Application of lactic acid bacteria starter cultures for decreasing the

- biogenic amine levels in sauerkraut. *Eur Food Res Technol* 215:509-514.
- Suzzi, G, Gardini F. 2003. Biogenic amines in dry fermented sausages: A review. *Int J Food Microbiol* 88:41-54.
- Ten Brink B, Damink C, Joosten HMLJ, Huis in 't Veld JH. 1990. Occurrence and formation of biologically active amines in foods. *Int J Food Microbiol* 11:73-84.
- Tyopponen S, Petaja, E, Mattila-Sandholm T. 2003. Bioprotectives and probiotics for dry sausages. *Int J Food Microbiol* 83:233-244.
- Weisburger JH, Veliath E, Larios E, Pittman B, Zang E, Hara Y. 2002. Tea polyphenols inhibit the formation of mutagens during the cooking of meat. *Mutat Res Genet Toxicol Environ Mutagen* 516:19-22.
- Wendakoon CN, Sakaguchi M. 1995. Inhibition of amino acid decarboxylase activity of *Enterobacter aerogenes* by active components in spices. *J Food Protect* 58:280-283.
- Zhang XB, Ohta Y. 1991. Binding of mutagens by fractions of the cell wall skeleton of lactic acid bacteria on mutagens. *J Dairy Sci* 74:1477-1481.