# TOXICOLOGICAL SAFETY OF EMULSION-TYPE SAUSAGE CURED BY PLASMA-TREATED WATER

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**Abstract – The objective of this study was to determine the toxicological safety of cured meat products manufactured by plasma-treated water (PTW) as nitrite source. The emulsion type sausage cured with PTW revealed that there was non-toxicity by *Salmonella* mutagenicity assay (Ames test) and acute toxicity with Balb/c mice for 24 h of diet. These toxicological experiments suggest the possibility to use PTW as a nitrite replacer.**

**Key Words – Sausage, Plasma, Toxicological safety**

## I. INTRODUCTION

Consumers prefer the cured meat products with natural curing over conventional curing because of the concerns about synthetic curing agents including sodium nitrite [1]. Therefore, the market of naturally cured meat product has been grown rapidly [2]. However, the use of vegetable concentrate, especially celery concentrate, in curing process has defects including added high cost and time for incubation step compared with the use of sodium nitrite.

Cold plasma, ionized gas, is an emerging non-thermal sterilization technology. The researchers in previous study showed that that plasma interacted with liquid and resulted in the acidification of liquid and generation of reactive oxygen and nitrogen species including nitrate ($NO_3^-$) and nitrite ($NO_2^-$) [3].

Therefore, the objective of this study was to identify the toxicological safety of plasma-treated water (PTW) as a nitrite source in meat products.

## II. MATERIALS AND METHODS

*Plasma treatment of water*

To produce air discharge irradiated solution (ADIS), 500 mL distilled water containing 1% sodium pyrophosphate (w/v) was irradiated by a surface dielectric barrier discharge (S-DBD) in atmospheric air for 4 hr. As illustrated in Fig. 1(a), a plasma device for providing air discharge consists of a pair of powered electrode and ground electrode, and an alumina plate with 0.6 mm thickness is installed between two electrodes. Fig. 1(b) presents real images of ground electrode involving patterns with 3 × 3 mm size. A bipolar square waveform with 15 kHz was applied to powered electrode. Total discharge area is ca. 20 $cm^2$, while the average power is 3.14 W and the peak power is 200 W.

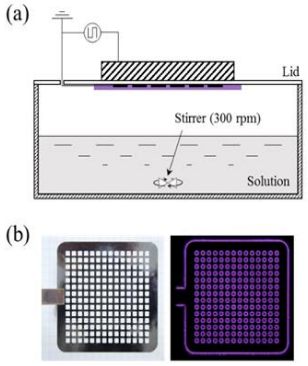



Fig. 1. Schematic drawing of plasma apparatus (a), real images of ground electrode (b).

*Manufacture of emulsion-type sausage*

Emulsion-type sausages were prepared using pork hind leg meat and back fat obtained from a commercial butcher's (Seoul, Korea). Meat was ground using a meat grinder with 6 mm plate. Ground meat was mixed with back fat, iced water, and additives in bowl cutter depending on the each formula of three treatments (control, emulsion sausage cured with no nitrite source; SCP, emulsion sausage cured with PTW; SCS, emulsion sausage cured with sodium nitrite). The concentration of nitrite ion in both treatments was maintained at 70 mg/kg. After storage, meat batter was stuffed collagen casing (2.5 cm of diameter). Sausages were cooked in water-bath at 80°C for 30 min until internal temperature of the sausage

reached 75°C. Cooked sausages were vacuum-packaged in a low-density polyethylene/nylon bags. Packaged sausage was pasteurized in 85°C hot water for 2 min then cooled in 10°C water.

*Mutagenicity*

The mutagenicity was performed with 70% ethanol extracts of each sample. The *Salmonella* mutagenicity assay (Ames test) was performed according to the methods of Ames et al. [4] and Maron and Ames [5].

*Acute toxicity*

To test acute toxicity of emulsified-type sausage, this study procured 8-wk old female Balb/c mouse (Oriental, Japan). Group I mice were administrated the normal diet (solid feed for mouse) and group II mice were administrated normal diet with SCS and group III mice were administrated normal diet with SCP and group IV mice were given lipopolysaccharide (LPS). All diets were supplied unrestrictedly. Five mice are randomly choose in one group and raised for 24 h (temperature 25℃, humidity 55%, illuminance 300~500 lux) and then scarified to detect the serum tumor necrosis factor (TNF)-α and peyer's patch counts. Levels of mouse TNF-α in the mouse blood serum were measured by ELISA Development kit (Mouse TNF-α DuoSet, R&D Systems, Minneapolis, MN). Peyer's patch were directly counted in small intestine with the unaided eye.

*Statistical analyses*

Data was analyzed using SAS software (Release 8.01, SAS Institute, Inc., Cary, NC, USA). Statistical analysis was performed by One-way Analysis of Variance (ANOVA). When significant differences were detected, the differences among the mean values were determined by the Duncan's multiple comparison test at a confidence level of $p < 0.05$.

## III. RESULTS AND DISCUSSION

*Mutagenicity assay*

There was no mutagenicity that all samples with a concentration up to 3 mg per plate (Table 1). The number of revertant colonies of the positive control showed more than about 30 and three times higher than of the sample when using the TA98 and TA100 strains, respectively. There was no difference in the number of revertant colonies between the sodium nitrite added and the plasma-treated water added emulsion-type sausages.

Table 1. Revertant colonies in the *Salmonella* Typhimurium reversion assay of the emulsion-type sausage using plasma-treated water

| Sample* | Dose (μg/plate) | Number of revertant colonies (His+) per plate | | | |
|---|---|---|---|---|---|
| | | TA98 (-S9) | TA98 (+S9) | TA100 (-S9) | TA100 (+S9) |
| Control | 188 | 15±5 [a] | 30±1 | 102±47 | 150±28 |
| | 375 | 19±1 | 27±5 | 123±10 | 178±15 |
| | 750 | 25±1 | 27±2 | 118±1 | 178±6 |
| | 1,500 | 29±5 | 44±3 | 136±4 | 137±10 |
| | 3,000 | 38±4 | 56±1 | 182±32 | 182±23 |
| SCS | 188 | 10±1 | 24±8 | 157±7 | 176±15 |
| | 375 | 18±3 | 28±4 | 131±3 | 140±29 |
| | 750 | 20±2 | 25±4 | 111±21 | 138±3 |
| | 1,500 | 22±1 | 28±3 | 134±24 | 177±9 |
| | 3,000 | 27±8 | 37±12 | 261±3 | 266±51 |
| SCP | 188 | 10±1 | 31±8 | 144±53 | 214±16 |
| | 375 | 12±2 | 33±5 | 163±13 | 185±18 |
| | 750 | 24±0 | 33±6 | 139±25 | 206±6 |
| | 1,500 | 17±8 | 26±7 | 164±33 | 221±13 |
| | 3,000 | 24±1 | 46±11 | 259±47 | 329±38 |
| Negative control | 70% Ethanol | 26±5 | 37±4 | 207±19 | 2204 |
| Positive control | 4-NQO | 637±56 | | | |
| | 2-AA | | 1102±73 | | |
| | SA | | | 886±47 | |
| | 2-AA | | | | 1965±71 |

Abbreriviations: 4-NQO, 4-Nitroquinoline-1-oxide; SA, Sodium azaide; 2-AA, 2-Aminoanthracene.

*Control, emulsion sausage cured with no nitrite source; SCP, emulsion sausage cured with PTW; SCS, emulsion sausage cured with sodium nitrite

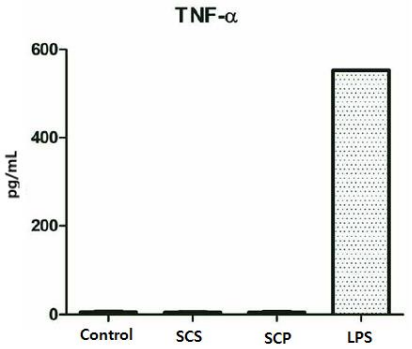
[a]Values are the mean ± S.D. ($P<0.05$).

*Acute toxicity*

TNF has been implicated in a diverse range of inflammatory, infectious and malignant conditions as an important parameter of inflammatory diseases. TNF is not usually detectable in healthy individuals, but elevated serum and tissue levels are found in inflammatory and infectious conditions [6,7] and serum levels correlate with the severity of infections [8,9].

TNF-α value less than 10 ng/mL was measured in both control and sausage groups, which shows that inflammatory response in mouse was not observed when compared to LPS, positive control group, and there were no differences in the length of intestine with control, SCS, and SCP (Fig. 2).

As a result of measuring the number of Peyer's patches and length of intestine, this study found that the foregoing was similar to the number of Peyer's patches of all the mouse (Table 2).




*Control, sausage cured without nitrite source; SCP, emulsion sausage cured with plasma-treated water; SCS, sausage cured with sodium nitrite

Fig 2. TNF-α value (pg/mL) in Balb/c mice supplemented the emulsion-type sausage orally.

Table 2. Length of intestine (cm) and Peyer's patches in Balb/c mice supplemented the emulsion-type sausage orally.

| Sample* | Length of intestine (cm) | Peyer's patches |
|---|---|---|
| Control | 49.8 | 8.8 |
| SCS | 49.5 | 8.4 |
| SCP | 49.9 | 8.8 |

*Control, sausage cured without nitrite source; SCP, emulsion sausage cured with plasma-treated water; SCS, sausage cured with sodium nitrite

## IV. CONCLUSION

In conclusion, emulsion type sausages cured with PTW are nontoxic as assessed thorough the mutagenicity and acute toxicity and can be applied as a potential nitrite replacer.

## ACKNOWLEDGEMENTS

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