INVESTIGATION OF NITROSAMINE FORMATION AT COOKING TEMPERATURE AND NITRITE CONCENTRATION BY IN VITRO GASTRIC MODEL

Jeong-Uk Eom[1], Kang-Jin Jeong[1] and Han-Sul Yang[1,2*]

[1]Division of Applied Life Science (BK21 Plus)

[2]Institute of Agriculture and Life Science, Gyeongsang National University, Jinju-aero, Jinju, Gyeongnam 52828, Republic of Korea

[*]Corresponding author email: hsyang@gnu.ac.kr

I. INTRODUCTION

Nitrosamines are considered carcinogenic substances and are found in various meat products. Also, it can be formed under various conditions such as water, cooking conditions, temperature, nitrate or nitrite, etc. [1]. Most nitrosamines in food are detected in small amounts and are not considered to impact the human body in healthy adults significantly. Some studies have suggested the potential risk of increasing nitrosamines in the stomach after consuming food. The stomach promotes nitrosation reactions due to its acidic environment, which facilitates the precursors and catalysts of nitrosation reactions. Meat products are rich in proteins that can release a large amount of secondary amines (proline, hydroxyproline, etc.) during the digestive process. Therefore, various nitrosamines may increase in the stomach after consuming meat products. Furthermore, by investigating the effect of different cooking temperatures and nitrate concentrations in meat products, it may be possible to propose guidelines to avoid the excessive formation of nitrosamines. However, information on the nitrosamines that increase in the stomach is limited. Also, no studies have been conducted under various conditions. This study investigated the effects of different cooking temperatures and nitrite levels on nitrosamine formation in cooked emulsified sausages using an *in vitro* gastric model.

Table 1. Retention time (t_R), qualitative ion pairs, and case numbers of eight NAs

No.	Compounds	Abbreviations	CAS NO.	t_R	Qualitative ions (m/z)
1	N-nitrosodimethylamine	NDMA	62-75-9	5.91	74/44
2	N-niotroso-n-methylethylamine	NMEA	10595-95-6	6.52	88/42
3	N-nitrosodiethylamine	NDEA	55-18-5	6.95	102/57
4	N-nitrosodi-n-propylamine	NDPA	621-64-7	9.82	130/43
5	N-nitrosodi-n-butylamine	NDBA	924-16-3	13.84	84/42
6	N-nitrosopiperidine	NPIP	100-75-4	14.24	114/84
7	N-nitrosopyrrolidine	NPYR	930-55-2	15.01	100/43
8	N-nitrosomorpholine	NMOR	59-89-2	16.16	116/56

II. MATERIALS AND METHODS

In this experiment, the emulsified sausages were made by grinding meat (75%) and fat (10%) into 6 mm particles and adding water (10%), salt (1.2%), sodium nitrite (0.005%, 0.01%, 0.02%, and 0.04%), phosphate (0.2%), and sugar (0.5%). The cooking conditions were applied at 80°C (pasteurisation), 120°C (sterilisation), and 200°C (as an imitation of baking and roasting) for 30 min in the oven. The two treatments consisted of non-reacted in vitro gastric models and reacted in vitro gastric models, and the amount of sample used for the analysis was 3 g.The *in vitro* gastric model was modified from the method of [2]. The extraction of nitrosamines from the emulsified sausages was performed using the method of [3]. The analysis of nitrosamines was performed using GC/MS (Agilent Technologies, Santa Clara, USA) and an HP-INNOWAX column (30 m × 0.25 mm, 0.25 μm of film thickness). Eight

representative carcinogenic nitrosamines were analyzed. Our results for *N*-nitrosodimethylamine (NDMA) and *N*-nitrosopyrrolidine (NPYR) showed changes. Qualitative data were presented in Table 1, and quantitative data were obtained by creating a standard curve using a nitrosamine standard. Data were analysed using SAS (9.4 ver., SAS Institute Inc., NC, USA) and presented as mean ± SE.

III. RESULTS AND DISCUSSION

Table 2 shows the NDMA and NPYR detection results according to the cooking temperature and nitrite addition amount. As the cooking temperature increased, NDMA in control increased, which was not detected in the gastric model at 200°C. The control and gastric model did not observe changes in NPYR with increasing cooking temperature. The changes in the amount of nitrite in control showed that more NDMA was detected as the nitrite increased. The gastric model detected the most NDMA at 120 mg/kg of nitrite. The change in the nitrite content of control resulted in the detection of NPYR only at nitrite 480 mg/kg.
The gastric model showed the highest amount of NPYR and was detected the most at nitrite 120, 480 mg/kg. In summary, at 80 and 120°C, with 120 mg/kg of nitrite, a large amount of NDMA was produced in the gastric model. The gastric model showed an explosive increase in NPYR compared to the control.

Table 2. Levels of NDMA and NPYR detected at different temperatures and nitrite concentrations in vitro gastric juice model

	Nitrite concentration (mg/kg)	Treatment	80℃	120℃	200℃
NDMA (mg/L)	0	Control	N.d	N.d	76.0±5.4
		Gastric model	N.d	N.d	N.d
	120	Control	N.d	19.1±2.4	103.4±13.6
		Gastric model	80.2±7.9	78.1±7.6	N.d
	480	Control	8.5±0.8	5.6±0.6	103.7±7.6
		Gastric model	42.3±4	44.5±4.5	N.d
NPYR (mg/L)	0	Control	N.d	N.d	N.d
		Gastric model	2.92±0.2	270.6±12.6	49.2±7.9
	120	Control	N.d	N.d	N.d
		Gastric model	1844.4±281.1	1166.7±135.5	62.0±4.4
	480	Control	23±2.46	0.7±0.08	5.3±0.49
		Gastric model	907.3±8.1	1070.1±87.3	418.7±32.0

"N.d" indicates the content of the target analysis is below the LOD. Data are presented as mean ± SE

IV. CONCLUSION

The study confirmed increased NDMA and NPYR in the *in vitro* gastric model. Therefore, the study's results raise concerns about the potential risks of consuming food due to increased NDMA and NPYR in the gastric.

REFERENCES

1. Scanlan, R. A. & Issenberg, P. (2009). N-nitrosamines in foods. Critical Reviews in Food Technology 29: 357-402.
2. Dong, H., Li, H., Liang, M., Luo, D., Liu, G., Zeng, X., Bai, W., Yang, J. & Xian, Y. (2020). Rapid determination of nine N-nitrosamines in dry-cured mackerel (Scomberomorus niphonius) using salting out homogeneous phase extraction with acetonitrile followed by GC-MS/MS. LWT - Food Science and Technology 130: 109716.
3. Wang, C., Zhao, F., Bai, Y., Li, C., Xu, X., Kristiansen, K. & Zhou, G. (2022). Effect of gastrointestinal alterations mimicking elderly conditions on in vitro digestion of meat and soy proteins. Food Chemistry 383: 132465.