FACTORS AFFECTING THE FORMATION OF CARCINOGENIC N-NITROSOPIPERIDINE (NPIP) IN CURED MEAT MODEL SYSTEM

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Abstract - N-Nitrosopiperidine (NPIP) are a group of carcinogens which have been detected in various cured meat products. NPIP formation can occur by a direct nitrosation of the piperidine which is a cyclic secondary amine found in spices. The objective of this research was to study the factors such as pH (3, 5, 7, 11) and temperature (30, 50, 95 °C) on the formation of carcinogenic of NPIP in cured meat model system. NPIP levels were analyzed by gas chromatography with flame ionization detector (GC-FID). The levels of NPIP were 187.0, 24.9, 20.9, and 19.8 ppm at pH 3, 5, 7 and 11, respectively. The NPIP formation at 30, 50 and 95 °C were 26.8, 85.8, 233.6 ppm, respectively. Therefore, decrease pH and increase temperature tended to increase NPIP levels in cured meat model system. In our study, cured meat model system at pH 3 and temperature 95 °C showed the highest levels of NPIP.

Keyword - N-Nitrosopiperidine, Nitrosamine, Meat product, Cured meat model system

I. INTRODUCTION

N-nitrosamines are a group of human carcinogen based on sufficient evidence of carcinogenicity in experimental animals [7] and classified as probable human carcinogens. The formation of N-Nitrosamines in meat is a complex process and depends on the reaction between nitrite and secondary amines [12]. Nitrite is an essential for developing typical cured meat color, flavor and texture and for protect against microorganisms that can cause food poisoning, such as *Clostridium botulinum* [2].

N-Nitrosamines are commonly found in cured meat products include N-nitroso-dimethylamine (NDMA), N-nitrosopyrrolidine (NPYR), N-nitrosopiperidine (NPIP) and N-nitrosodiethylamine (NDEA), occurred generally at low levels (<5 ppb) but at high levels up to 20 ppb has been reported in some cured meat products [8]. The formation of N-nitrosamines in meat products depends on the method of cooking, cooking temperature and time [6]. Using high temperature during cooking e.g. frying, baking has been reported to result in an increase in N-nitrosamines in cured meat products [10,13].

NPIP formation can occur by a direct nitrosation of the piperidine which is a cyclic secondary amine found in black pepper, white pepper, paprika and nutmeg usually used as ingredients in cured meat products [3]. Yurchenko and Molder [14] reported the NPIP levels in some cured meat products e.g. smoked sausage (0.89 ppm), ham (1.79 ppm), and bacon (1.23 ppm). However, there is still little information on the factors affecting the formation of NPIP. Therefore, the purpose of our study is to investigate the factors such as pH and temperature on the formation of carcinogenic of NPIP in cured meat model system. Four pH levels (3, 5, 7 and 11) and two temperatures (50 and 95 °C) were studied as the wide range to understand the formation of NPIP.

II. MATERIALS AND METHODS

2.1 Effect of pH on NPIP formation in model system

The cured meat model was prepared as follows: 0.04 M $NaNO_2$, 0.05 M Sodium citrate and 0.1 M Piperidine (1 mL each) were mixed together. The mixture was then adjusted pH to 3, 5, 7, 11 with 1 M HCl. After that, the mixture was incubated at 70 °C for 3.5 hr. Then the reaction was stop by adjusting pH to 11 with 1 M NaOH.

2.2 Effect of temperature on NPIP formation in model system

The cured meat model was prepared as follows: 0.04 M $NaNO_2$, 0.05 M Sodium citrate and 0.1 M Piperidine (1 mL each) were mixed together. The mixture was then adjusted to pH 3 with 1 M HCl. After that, the mixture was incubated at 30, 70, 95 °C for 3.5 hr. Then the reaction was stop by adjusting pH to 11 with 1 M NaOH.

2.3 Extraction of NPIP

The extraction of NPIP was performed by followed the method by Tanaka *et al* (1988). [12] with some modifications. The model system from 2.1 and 2.2 (3 mL) was mixed with 1 g NaCl, and 100 µl of N-Nitrosoethylamine (NDEA) was added as an internal standard. After that, 4 mL dichloromethane was added and stirred for 30 min at room temperature. Then the solution dried with sodium sulfate, and the sodium sulfate was removed moisture by filtration. The sample was then analyzed by GC-FID.

2.4 Determination of NPIP by GC-FID

GC-FID analyses were performed on an Agilent 6890 Plus. Chromatographic separation was achieved with an Agilent DB-1 column (60 m x 0.320 mm x 0.25 µm). The concentrated sample (1µl) was injected into a GC-FID. Helium was used as the carrier gas at a flow rate of 3.5 ml/min. The injection port was set at 250 °C and the temperature of the column port were ramped; 40 °C for 4 min, increased to 120 °C at 5 °C/min and then increased to 250 °C at 15 °C/min. The retention time was 21.73 for NPIP under this chromatographic condition [1].

2.5 Statistical analysis

The experiment was independently performed at least in duplicate. Duplicate measurements taken on the same experimental unit were averaged for statistical analysis. The difference of mean was determined in Duncan's test using the general linear model in the statistical analysis system program and means were considered significantly different at $P<0.05$. The statistical analysis was performed by using SPSS version 16.0 with 95% confidence level.

III. RESULTS AND DISCUSSION

The chromatogram of NPIP standard and NDEA (internal standard) are shown in Figure 1. The effect of pH on NPIP formation is shown in Figure 2. It is obvious that pH is significantly affected the formation of NPIP in cured meat model system. NPIP levels at pH 3, 5, 7 and 11 were 187.0, 24.9, 20.9, and 19.8 ppm, respectively. The results showed that decreasing of pH caused a significant increase in NPIP contents. When pH decreased, the nitrite (NO_2) was converted to nitrous acids (HNO_2), which is a precursor of the nitrosation [9]. Douglass [5] reported that the nitrosation occurred faster at pH 3.0 – 3.4. On the other hand, at pH 7.5 – 11.0 the nitrosation occurred at slower rate.

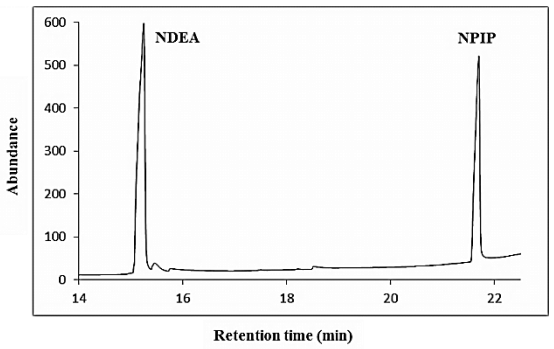

Figure 1. GC Chromatogram of NPIP standard at 500 ppm (1 µL injection) using FID detection. NDEA (500 ppm) was used as internal standard.

The effect of temperature on NPIP formation is shown in Figure 3. Three temperature levels, such as 30, 50 and 95 °C, were evaluated to investigate NPIP formation. NPIP levels at temperature of 30, 50 and 95 °C were 26.8, 85.8, 233.6 ppm, respectively. Increasing the level of NPIP at higher temperature could be due to the dissociation of N_2O_3 to NO radical which acts as direct nitrosating agents [13].

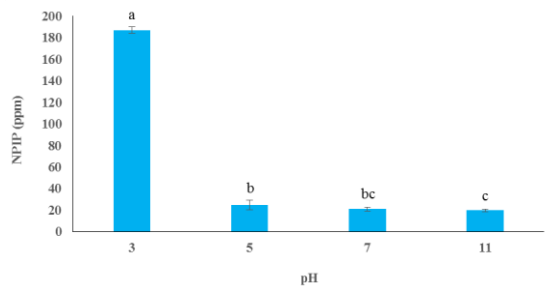

Figure 2. N-Nitrosopiperidine (NPIP) levels in cured meat model system at pH 3, 5, 7 and 11 (temperature 70 °C)

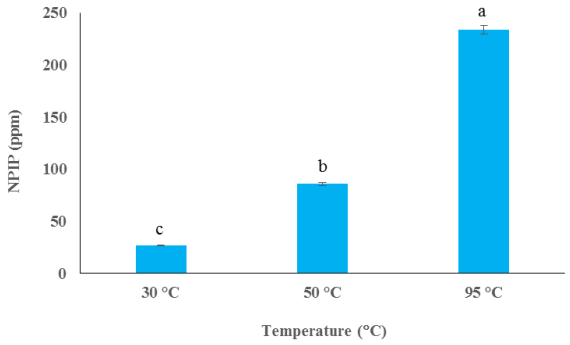

Figure 3. N-Nitrosopiperidine (NPIP) levels in cured meat model system at temperature of 30, 50 and 95 °C (pH 3)

IV. CONCLUSION

In the present study, the formation of N-Nitrosopiperidine (NPIP) was dependent on pH and temperature. Decrease pH and increase temperature tended to bring up the levels of NPIP in cured meat model system. In our study, cured meat model system at pH 3 and temperature 95 °C showed the highest levels of NPIP.

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