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RESEARCH NOTE

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Influence of Curing and Heating on Formation of N-Nitrosamines from Biogenic Amines in Food Model System using Korean Traditional Fermented Fish Product

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Abstract The *myeolchi-jeot* samples were divided into different groups with or without the supplementation with biogenic amines. Subsequently, the samples were placed in an oven at 80°C for 1 hr to allow the chemical reaction to proceed, and then were analyzed for N-nitrosamine contents using GC-TEA. N-nitrosamine was not detected in any of the *myeolchi-jeot* samples which had been treated with/without sodium nitrite. On the other hand, the yield of N-nitrosopyrrolidine from 1,000 mg/kg of putrescine and spermidine in the *myeolchi-jeot* samples (treated with 5 mg/kg of sodium nitrite) was 0.002 and 0.014%, respectively. N-nitrosamine was not produced from any other biogenic amines like, histamine, tyramine, cadaverine and spermine. In addition, curing and heating were the factors which influenced the formation of N-nitrosamine during the nitrosation of biogenic polyamines. For the formation of N-nitrosamine in the food systems, treatment with sodium nitrite and heating at appropriate temperature along with the satisfied supplementation of biogenic polyamines are required.

Keywords: biogenic amines, N-nitrosamine, *myeolchi-jeot*, Korean salted and fermented anchovy, GC-TEA

Introduction

Biogenic amines are reported to be toxic to humans, and the frequently observed food borne intoxications are known to be caused by histamine (a type of biogenic amine) (1). Typical symptoms of histamine intoxication are nausea, respiratory distress, hot flushes, sweating, heart palpitation, headache, burning sensations in the mouth and hypotension (2). Histamine also possesses a powerful biological activity. It serves as a primary mediator of the immediate symptoms noted in allergic responses (3, 4). Tyramine causes a rise in blood pressure (5) and has been identified as the major mutagenic precursor (6). Biogenic amines which act as precursors of nitroso compounds possess an additional health risk. They have also been recognized as precursors for carcinogenic nitroso compounds (7). Secondary amines are well known to form carcinogenic N-nitrosamines upon reaction with nitrosating compounds. However, primary biogenic amines can convert to secondary amines, by heating as well as during storage at room temperature, and can further react with nitrite (8). Putrescine (8, 9), cadaverine (8), spermidine (7, 9), spermine and agmatine (7) are reported to be potentially carcinogenic, as they can be converted to respective nitrosamines.

In Korea, *myeolchi-jeot*, a Korean traditional salted and fermented anchovy product, is taken not only as a side dish, but also as an ingredient in *kimchi*. For preparing *myeolchi-jeot*, salt (generally, sun-dried salt) should be added at the level of over 20% to raw anchovy, and then should be fermented for few months to develop the taste (10). Hence it is known to contain relatively high concentration of amino acids which can be the source for biogenic amine formation. Biogenic amine formation during the ripening of *myeolchi-jeot* (11) has already been reported. Therefore, *myeolchi-jeot* that contains a nitrite derived from high concentration of salt can be a potential matrix for N-nitrosamine formation. However, no information is available on the occurrence of N-nitrosamine related to biogenic amines. This study was carried out to evaluate safety aspects related to biogenic amines along with N-nitrosamines. Studies were performed to estimate the role of biogenic amine as precursors, for the formation of N-nitrosamines in *myeolchi-jeot* in which chemical reaction was accelerated by adding sodium nitrite at high temperature.

Materials and Methods

Sample preparation To prepare *myeolchi-jeot*, a Korean salted and fermented anchovy, salt was added at the level of 15% to raw anchovy that contained approximately 5% salt, and the salted anchovy was fermented for 1 month. This served as a *myeolchi-jeot* sample. Ten grams of *myeolchi-jeot* sample was weighed into a flask, and 1,000 mg/kg of different biogenic polyamine hydrochloride salts and 5 mg/kg of sodium nitrite were added directly to the sample. The sample was then placed in an 80°C oven for 1 hr to induce chemical reaction, and kept at -25°C until extraction was followed. The *myeolchi-jeot* sample and the sample prepared by adding sodium nitrite served as control. The N-nitrosodipropylamine (1 mg/kg) was used as an internal standard, and 100 mg/kg of various N-nitrosamines served as external standards.

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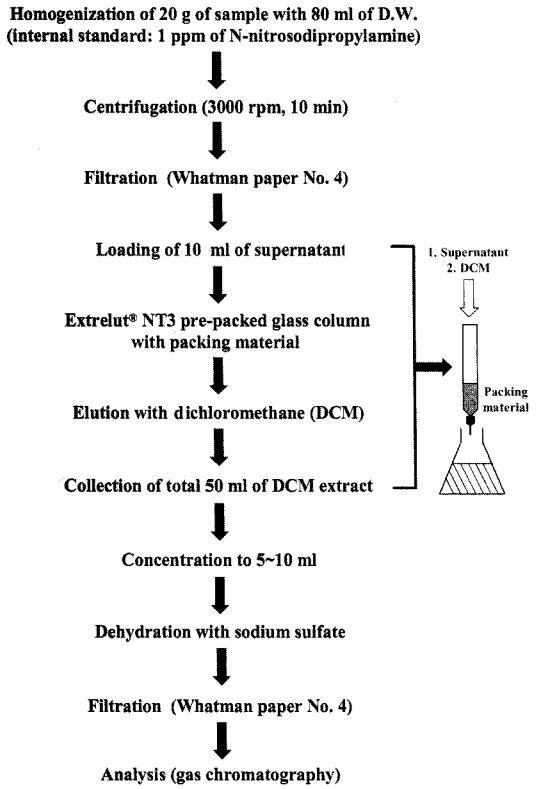

Fig. 1. Flow diagram illustrating the extraction of N-nitrosamine in food model system.

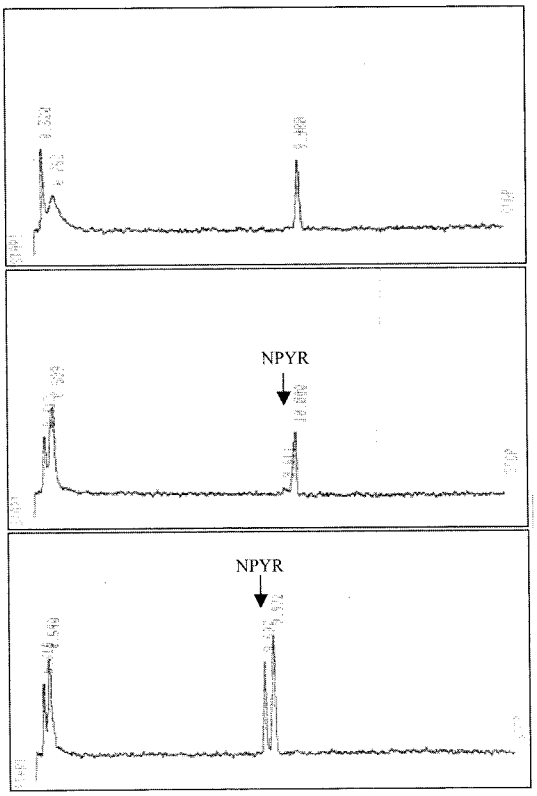

Fig. 2. Chromatogram of the extract of *myeolchi-jeot*, Korean salted and fermented anchovy, heated at 80°C for 1 hr. NPYR: N-nitrosopyrrolidine. Upper: Sample treated with 5 mg/kg of $NaNO_2$ (control), Center: Sample treated with 1,000 mg/kg of putrescine and 5 mg/kg of $NaNO_2$, Lower: Sample treated with 1,000 mg/kg of spermidine and 5 mg/kg of $NaNO_2$.

Extraction of N-nitrosamine The *myeolchi-jeot* samples were homogenized with distilled water, and filtered. Then, the filtrate was extracted by the method of Raoul *et al.* (12) with some modifications using Extrelut® NT3 pre-packed glass column (Merck KGaA, Darmstadt, Germany) added with Extrelut® packing materials (Merck).

Determination of volatile N-nitrosamine The concentration of volatile N-nitrosamines was determined quantitatively by gas chromatography (GC, Model 5890II, Hewlett-Packard Co., Wilmington, DE, U.S.A.) coupled to a thermal energy analyzer (TEA, Thermo Electron Model 502B, Waltham, MA, U.S.A.). Analyses were carried out with a non-polar SPB-5 fused silica capillary column (0.53 mm i.d. × 30 m, Supelco Co. Bellefonte, PA, U.S.A.), which was introduced into the ceramic pyrolysis tube by the end of TEA. Helium was used as the carrier gas at a flow rate of 3.5 ml/min. The injection port was set at 220 °C and the temperature of the column port was ramped (50 °C for 5 min, and then increased to 100°C at 5°C/min). The sample injection volume was 2 μl.

Results and Discussion

The gas chromatograms of the dichloromethane extracts of the reaction mixtures from putrescine and spermidine treated with sodium nitrite and reacted for 1 hr at 80°C are shown in Fig. 2. N-nitrosamine was not detected in any of the *myeolchi-jeot* samples which had been treated with/without sodium nitrite. The yield of N-nitrosopyrrolidine in *myeolchi-jeot* sample treated with 5 mg/kg of sodium nitrite and 1,000 mg/kg of putrescine/spermidine was 0.002 and 0.014%, respectively (Table 1). N-nitrosamine was not produced from any other biogenic amines like, histamine, tyramine, cadaverine and spermine.

Various authors have suggested that curing and heating are the factors, which influence the formation of N-nitrosamines during the nitrosation of biogenic polyamines. Bill *et al.* (9) reported that N-nitrosopyrrolidine was formed from spermidine and putrescine, on reaction with 0.005 M sodium nitrite at a pan temperature of 170°C for 10 min

Table 1. Production of N-nitrosamine in *myeolchi-jeot*, Korean salted and fermented anchovy, heated at 80°C for 1 hr

Samples[1)]	NPYR[2)] (ppb)	NPYR (% yield)
Control	-	-
A	-	-
B	-	-
C	-	-
D	25.96	0.002%
E	143.82	0.014%

[1)]A: Control treated with 5 mg/kg of $NaNO_2$, B: Sample treated with 1,000 mg/kg of histamine, tyramine and spermine, and 5 mg/kg of $NaNO_2$, C: Sample treated with 1,000 mg/kg of cadaverine and 5 mg/kg $NaNO_2$, D: Sample treated with 1,000 mg/kg of putrescine and 5 mg/kg $NaNO_2$, E: Sample treated with 1,000 mg/kg of spermidine 5 mg/kg $NaNO_2$.
[2)]NPYR: N-nitrosopyrrolidine.

with a theoretical yield of 1.0 and 0.04%, respectively. N-nitrosopyrrolidine (yield = 2.76%) was also produced from putrescine, when treated in 0.1 M acetate buffer with 0.05 M sodium nitrite at 100°C for 1 hr (8). Cadaverine yielded 0.02% of N-nitrosopiperidine under the similar conditions. The nitrosation of spermidine and spermidine·3HCl in 0.2 M acetate buffer with 0.09 M sodium nitrite at pH 3.5 at 80°C for 1 hr, yielded 0.6 and 0.62% of N-nitrosopyrrolidine, respectively (13). In our study, we observed that sodium nitrite and heat along with high levels of biogenic polyamines were required to catalyze the formation of N-nitrosamines in the food system used. From this point of view, it is evident that to avoid the formation and/or accumulation of N-nitrosamines, salted and fermented fish products should neither be cured nor heated. Even though salted and fermented fish products could be manipulated without either artificial curing or heating, but still there are ways to revert back the formation of N-nitrosamines from biogenic amines in salted and fermented fish products. Sodium nitrite can be introduced to raw anchovy during rinsing and/or salting of anchovy with sea water on board and at a fishing port as sea water contains sodium nitrite. Furthermore, N-nitrosopyrrolidine can also be formed during storage at an ambient temperature, according to the report by Warthesen *et al.* (8). Hence, salted and fermented fish products such as *myeolchi-jeot* are still have a possibility of N-nitrosamine formation, because they contain excessive amounts of biogenic amines (11) and have enough chances to encounter sodium nitrite. Also, the possibility of N-nitrosamine formation can be reinforced by storage for a long period of time.

Myeolchi-jeot has also been used as an ingredient in preparing *kimchi* for a long time in Korea. Like biogenic amines (14), *myeolchi-jeot* may also transfer the formed N-nitrosamines to *kimchi*. It is therefore important to carefully monitor the formation of N-nitrosamine in both *myeolchi-jeot* and *kimchi* to ensure their safety for human consumption.

In summary, the yields of N-nitrosopyrrolidine produced from putrescine and spermidine in *myeolchi-jeot* system were 0.002 and 0.014%, respectively. To form N-nitrosamine, a satisfied quantity of biogenic polyamines and sodium nitrite, and heating at appropriate temperature were required.

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