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Application of Gamma Irradiation for the Reduction of Residual Nitrite and Nitrosamine in Meat Products

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Abstract

Nitrite, a curing agent of meat products, is precursors of carcinogenic N-nitrosamines during processing of meat products or under human stomach conditions as well as having its own toxicity. Some researches have been conducted to evaluate the effects of ionizing radiation on the reduction of residual nitrite and N-nitrosamines in an aqueous model system and cured meat products with different packaging methods during storage. These results showed that the gamma irradiation was effective in reducing the residual nitrite and N-nitrosamines in an aqueous model system as well as meat products. Especially, irradiation combined with vacuum or modified atmosphere packaging was more effective in nitrite and N-nitrosamines reduction than aerobic packaging during storage. The objective of this review is to introduce the irradiation technology for the application of reducing the residual nitrite and N-nitrosamine contents in meat products.

Key words : nitrite, N-nitrosamine, gamma irradiation

Introduction

Nitrite is one of the very important additives in the cured meat production process in terms of desirable color, texture, lipid oxidation, and especially for preventing a toxin formation by *Clostridium botulinum* (Cassens, 1997). Recent evidence has suggested that the nitrite is a bactericidal for gastrointestinal, oral and skin pathogenic bacteria when ingested and mixed with gastric acid (Douglas, 2002). But significant concerns exist because nitrite may react with amines and amino acids to produce N-nitrosamines, which are known to be carcinogenic, mutagenic and teratogenic (Mirvish et al., 1972; Schweinsberg and Burkle, 1985; Shahidi et al., 1994). The formation of N-nitrosamines in cured meat products is dependent on the cooking method, residual and/or added nitrite concentration, ascorbate or α-tocopherol concentration, nitrosamine precursors, slice thickness, preprocessing procedures and conditions, moisture content, lean to adipose tissue ratio, presence of nitrosation catalysts and inhibitors, and possibly the smoking process (Miller et al., 1989). Among the numerous chemical carcinogens that have been detected in foods and drinks, N-nitrosamines are distinguished by being very potent. Volatile N-nitrosamines induce tumors in a variety of organs, including the liver, lung, kidney, bladder, pancreas, esophagus and tongue depending on the species, but not in the skin, brain, colon or bone. For example, N-nitrosodimethylamine at the levels of 20 ppm can induce liver cancer in a human (Lijinsky, 1999). The formation of N-nitrosamines in foods occurs due to an addition of nitrite, smoking, drying with combustion gas, salting, pickling, fungal contamination or food contact materials (Tricker et al., 1989). Therefore, the nitrite in meat products is a primary problem in the formation of carcinogenic volatile N-nitrosamines under high-temperature condition.

Ionizing radiation is known to be the best method to destroy pathogenic and spoilage microorganisms without compromising the nutritional properties and sensory quality of the food (WHO, 1999) and its use is gradually increasing worldwide. The U.S. Food and Drug Administration has approved the use of ionizing radiation in fresh and frozen beef at levels of 4.5 and < 7.0 kGy, respectively (Olson, 1998). The Joint FAO/IAEA/WHO study group (1999) concluded that food irradiated to any dose appropriate to achieve the intended technological objective is both safe to consume and nutritionally adequate. In addition, the

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application of irradiation for reducing the toxic or undesirable compounds such as volatile N-nitrosamines (Ahn et al., 2002b), food allergy (Lee et al., 2001), and production of low-salted fermented foods (Byun et al., 2000) has been reported recently besides the sanitary purpose of the technology. In particular, gamma-irradiation induced the radiolysis of nitrite and volatile N-nitrosamines (Ahn et al., 2002a). This review introduces the irradiation technology for the application of reducing the residual nitrite and nitrosamine contents in meat products.

Radiolytic Characteristics of Nitrite by Gamma Irradiation in Model System

Ahn et al. (2003b) reported that sodium nitrite in dissolved water was reduced about 50% by 10 kGy irradiation, and the complete degradation was shown over 40 kGy (Table 1). The correlation coefficient was 0.9995; therefore, the degradation occurred in a dose dependent manner. Chemically, NO_2 produces NO radical by UV irradiation; however, the NO radical is difficult to detect. It might be that the amount and life of the NO radical vary according to method of irradiation, including UV, α-, β-, λ- or X-ray, due to the differences of their energies and wavelengths. Reduction of nitrite to NO can occur under acidic, highly reduced conditions, or energy input. NO is a free radical, formed endogenously in many biological roles, such as neurotransmission, blood clotting, blood pressure control and in the immune system's ability to kill tumor cells as a biological messenger (Cassens, 1995), while a neurotoxic effect, as a precursor of peroxynitrite anion formation, was also reported (Tabrizi-Fard et al., 1999).

Generally, the nitrosation reaction is predominant in acidic conditions (pH 2~4) by activated nitrite (Francis, 2000). Otherwise, 40 kGy-irradiated nitrite solution formed low concentrations of nitrosodimethylamine (NDMA), and about 49, 44, 44, or 39% of NDMA formed at pH 2, 3, 4 and 6, respectively, compared with the non-irradiated control (Ahn et al., 2003b). Only the residual nitrite after irradiation was nitrosated to NDMA. This indicated that irradiated nitrite can't form the carcinogenic NDMA in the model system because the NO radical might be rapidly disappeared in the aqueous state. Fiddler et al. (1981) suggested that irradiation sterilization at 30 kGy reduced residual nitrite in bacon prior to frying, thereby reducing volatile nitrosamines after frying, and destroying preformed volatile nitrosamines, if present, in the bacon prior to irradiation. Actually, gamma irradiation is effective for reducing residual nitrite level in meat products, and the low concentration of N-nitrosamine by irradiation is maintained after storage (Ahn et al., 2002a). In conclusion, gamma irradiation can be used for reducing nitrite and furthermore, radiolytically destroyed nitrite could not form carcinogenic N-nitrosamine, even in a model human stomach condition.

Effect of Gamma Irradiation on the Residual Nitrite in Meat Products with Different Packaging Methods

Irradiation treatment reduced the nitric contents of meat products and, especially, nitric contents were decreased under vacuum or CO_2 packaging by gamma ray as a does-dependant manner. Ahn et al. (2004) reported that gamma irradiation reduced the residual nitrite contents in sausage both aerobic and vacuum packaging (Table 2). After 4 weeks storage at 4℃, decrease of residual nitrite contents were shown in all samples, and the radiation effect was still found. Residual nitrite contents of sausage irradiated at 5 kGy or above were lower than that of non-irradiated control at aerobic and vacuum packaging conditions. The vacuum packaging was more effective for reducing nitrite in sausage irradiated at 0 to 5 kGy after storage. Similarly, Ahn et al. (2002a) found that residual nitrite levels of sausages with vacuum packaging were lower than that of aerobic packaging during storage.

Jo et al. (2003) reported that the residual nitrite was detected in the sausage with 156 ppm of $NaNO_2$ ranging from 60 to 70 ppm in freshly made and irradiated samples packaged aerobically or vacuum. However, the sausage with CO_2 packa-

Table 1. Degradation of sodium nitrite in deionized water by gamma irradiation (Unit : mg/kg)

	Irradiation dose (kGy)								SEM[1)]	r^2 [2)]	F-value	Pr>F
	0	5	10	15	20	25	30	40				
Sample[3)]	100[a4)]	63.6[b]	50.8[c]	35.9[d]	20.7[e]	12.5[f]	4.57[g]	0.28[h]	0.684	1.00	2481	0.0001

[1)] Standard error of the means (n=24).
[2)] Correlation coefficient.
[3)] The concentration of sodium nitrite was 100 mg/kg in deionized distilled water.
[4)] Different letters (a-h) within a same row differ significantly (p<0.05).

Table 2. Residual nitrite levels (ppm) of irradiated emulsion-type cooked pork sausage for 4 weeks at 4℃

Storage	Packaging	Irradiation does (kGy)				SEM[1)]
		0	5	10	20	
0 week	Air	81.6[a]	74.8[ab]	63.0[bc]	56.5[c]	3.07
	Vacuum	72.6[a]	68.1[b]	64.9[c]	54.1[d]	0.76
	SEM[2)]	1.78	2.08	2.95	2.54	
4 weeks	Air	57.3[ax]	37.5[bx]	27.6[c]	27.1[c]	1.12
	Vacuum	40.7[ay]	29.0[by]	25.4[bc]	19.9[c]	1.63
	SEM	0.40	0.95	2.30	1.21	

[a-c:] Different letters within a same row differ significantly ($p<0.05$).

[x, y:] Different letters within a same column differ significantly ($p<0.05$).

[1)] SEM: Standard error of the means (n=8).

[2)] SEM: Standard error of the means (n=4).

ging showed a lower residual nitrite content at 0 week ($p<0.05$). After 4 weeks, there was no difference in the residual nitrite content found in sausage using the same packaging method but the sausage with CO_2 packaging showed still lower the residual nitrite content than those with aerobic or vacuum packaging. Nitrite reduction by irradiation is probably due to its reaction with the hydroxyl radical produced by the radiolysis of water. CO_2 or a mixture of CO_2/N_2 packaging was more effective for reducing the nitrite level compared to N_2 packaging. Even though all packagings were done in an anaerobic environment, the oxidation-reduction potential of each packaging might be different (Simie, 1983). When the environment is in the reduced state, the nitrite can be easily converted to nitric oxide, resulting in lower residual nitrite levels with anaerobic packaging. Consequently, changing the redox potential to a reduced state in CO_2 packaging may increase the possibility of transforming the nitrite ion to nitric oxide, resulting in residual nitrite reduction in the sausage.

Effect of Gamma Irradiation on the Residual Nitrite Combined with Ascorbic Acid

Ascorbic acid in cured meats plays a role as a reducing agent for a nitrite scavenger, as an enhancer of NO-Mb development, and as an inhibitor of carcinogenic N-nitrosamine formation. Thus, residual ascorbic acid allowed the nitrite to be transformed to nitric oxide, resulting in a gradual reduction of residual nitrite in sausage during storage. Cassens (1997) reported that residual nitrite level in commercial frankfurters had declined up to 10 ppm, about 80% reduction since the mid-1970s, and also found that a high level of residual ascorbic acid was present in cured meats. The study also found that the mean value of ascorbic acid levels of commercial frankfurters was 209 ppm, or nearly 40% of the maximum allowable addition of 550 ppm. Fiddler et al. (1981) found that the reduction of nitrite by sodium ascorbic acid appeared to be enhanced by the irradiation process. Even though ionizing radiation can only cause partial conversion of ascorbic acid to dehydroascorbic acid, which has an activity equal to that of ascorbic acid, the loss of ascorbic acid was much lower than that of other cooking methods (Stewart, 1980). These results indicated that the combined effects of irradiation and maximum addition of ascorbic acid will be effective for reducing residual nitrite level in meat products.

Breakdown of N-Nitrosamines by Gamma Irradiation in Model Solution System

The major N-nitrosamines found in food system are NDMA and nitrosopyrrolidine (NPYR) (Lijinsky, 1999). Many volatile N-nitrosamines are stable to heat, but they can be cleaved photolytically by UV irradiation because of their chemical properties (Shuker and Tannenbaum 1983; Deng et al. 1998). Ahn et al (2002b) found that the solvent system had different effects on volatile N-nitrosamines after gamma irradiation (Table 3). NDMA and NPYR dissolved in distilled water were easily broken down by gamma irradiation compared with dichloromethane and ethanol, and all of the volatile N-nitrosamines were undetectable after 5 kGy or above. NDMA and NPYR showed 65 to 84% breakdown at 2.5 kGy and NPYR was the most sensitive to gamma irradiation. Water is important for breakdown of those compounds, because water is required for hydrophotolysis of nitrosamines by UV irradiation (Shuker and Tannenbaum, 1983). In foods, water plays an important role as an universal solvent for most chemical components (Blanshard

Table 3. Residual nitrosodimethylamine and nitrosopyrrolidine levels dissolved in different solvents after gamma irradiation

(Unit : ppb)

Dose (kGy)	DW[1)]		DCM		EtOH	
	NDMA[2)]	NPYR	NDMA	NPYR	NDMA	NPYR
0	1000	1000	1000	1000	1000	1000
2.5	352±5[3)]	157±1	448±4	-	706±5	683±7
5	-[4)]	-	15±1	-	385±2	296±2
7.5	-	-	1±1	-	341±3	81±1
10	-	-	-	-	196±1	56±2
15	-	-	-	-	46±2	-
20	-	-	-	-	-	-
25	-	-	-	-	-	-

[1)] The N-nitrosamine were dissolved in distilled water (DW), dichloromethane (DCM), or ethanol (EtOH), respectively.

[2)] NDMA: nitrosomethylamine, NPYR: nitrosopyrrolidine.

[3)] Mean ± standard deviation.

[4)] Not detected.

Table 4. N-nitrosodimethylamine (NDMA) and N-nitrosopyrrolidine (NPYR) levels (ppb) of irradiated emulsion-type cooked pork sausage during storage for 4 weeks at 4℃

	Storage (week)	Packaging	Irradation dose (kGy)				SEM[1)]
			0	5	10	20	
NDMA	0	Air	2.97	1.21	ND	ND	0.880
		Vacuum	1.58[ab]	2.06[a]	ND[b]	ND[b]	0.423
		SEM[2)]	0.336	1.043	0.838		
	4	Air	1.25	1.16	ND	ND	0.626
		Vacuum	3.65	2.64	0.61	ND	0.911
		SEM	1.469	0.315	0.431		
NPYR	0	Air	2.66[a]	ND[b]	ND[b]	ND[b]	0.065
		Vacuum	1.77	0.64	0.45	ND	0.438
		SEM	0.281	0.315	0.329		
	4	Air	2.67	0.90	2.29	ND	1.297
		Vacuum	1.81[a]	0.67[ab]	ND[b]	ND[b]	0.431
		SEM	0.720	0.791	1.616		

[a, b]: Different letters within a same row differ significantly ($p<0.05$).

ND: Not detected.

[1)] SEM: Standard error of the means (n=8).

[2)] SEM: Standard error of the means (n=4).

and Lillford, 1987); therefore volatile nitrosamines may exist in an aqueous solution in foods. The results indicated that volatile N-nitrosamines in water were easily destroyed by gamma irradiation at doses of 5 kGy or above. Therefore, this effect demonstrated that gamma irradiation on foods may potentially reduce concentrations of carcinogenic volatile N-nitrosamines in foods. It is also expected that this application of gamma irradiation technology will be applied to other industries having nitrosamine problems, such as elastic rubber netting used in ham processing or baby bottle nipples (Fiddler et al, 1998)

Effect of Gamma Irradiation on the Residual N-Nitrosamines in Meat Products

It was reported that the N-nitrosamine level is 10 ppb or none in the US commercial meat products (Cassens, 1995). Meanwhile, Park et al. (1998) reported that NDMA level in Korean commercial pork sausages were ranged from 0.5~36.8% ppb. If the increasing trend of meat products in Korea is considered, it is important to secure chemical and microbiological safety associated with meat products. Jo et al. (2003) reported that the NDMA was not detected in the sausage without $NaNO_2$ at 0 week; however, the NDMA was detected at 4 weeks with aerobic packaging in the non-irradiated sample. It was revealed that sodium chloride and phosphate enhance the nitrosamine formation in meat products (Rywotycki, 2002). Ahn et al. (2004) reported that NDMA contents in sausage with aerobic and vacuum packaging were decreased by irradiation and were not detected by irradiation at 10 kGy or higher dose after irradiation immediately. And also irradiation reduced NPYR contents in sausage, and the NPYR was not detected by irradiation at 5 kGy or higher. After 4 weeks of storage at 4℃, irradiation decreased NPYR contents in sausage with vacuum packaging, while the packaging effect was not found during storage (Table 4). Similarly, Hu and Song (1988) reported that dried shrimp bran contained 54.6 ppb prior to irradiation, but only 29.3, 8.8, 11.4, and 1.9 ppb in 5, 10, 15, and 20 kGy of irradiation. The addition of sodium ascorbate is essential in reducing nitrosamines formation (Fiddler et al., 1981). These results indicated that irradiation reduced the volatile N-nitrosamines, and accordingly irradiation can be utilized to improve the functionality of foods including extension of shelf life.

In conclusion, irradiation is possible means to reduce residual nitrite and carcinogenic N-nitrosamines in meat products. Even though, ascorbic acid was effective in reducing nitrite and N-nitrosamine levels in meat products, gamma irradiation or a combination of various packaging conditions might be helpful to increase its reduction capacity. Thereby, irradiation technology, apart from improving microbial safety, can be a very useful way to reduce toxic chemical compounds such as nitrite and N-nitrosamine, which are present in food or are produced during processing.

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