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CO_2 Packaging Combined with Irradiation Decreases Nitrosamine Formation in Pork Sausage

- Research Note -

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Abstract

This study evaluated the effectiveness of different packaging methods (aerobic, vacuum and CO_2), combined with irradiation, in reducing volatile N-nitrosamine formation in pork sausage during storage. Production of nitrosodimethylamine (NDMA) and nitrosopyrrolidine (NPYR) in pork sausage was decreased by irradiation during storage at 4°C for 4 weeks. The nitrosamine concentrations were the lowest in sausage with CO_2 (100%) packaging. These results indicate that irradiation combined with CO_2 packaging is the most effective treatment among tested for reducing the formation of volatile N-nitrosamines in cooked pork sausage during storage.

Key words: sausage, nitrosamine reduction, packaging, irradiation

INTRODUCTION

Nitrite imparts desirable flavor, color, and texture characteristics to meat products and provides protection against oxidative rancidity and pathogenic microorganisms, especially *Clostridium botulinum* (1). However, the higher the concentrations of added nitrite, the higher the residual nitrite content of the foods when consumed. There is a high correlation between residual nitrite content prior to frying and concentration of the carcinogen, N-nitrosopyrrolidine (NPYR) after frying (2).

Irradiation is known to be one of the best methods to control pathogenic microorganisms in food (3). Fiddler et al. (1) suggested that irradiation sterilization with Co-60 reduces residual nitrite in bacon prior to frying, thereby reducing volatile nitrosamines after frying. Ahn et al. (4) added support to Fiddler's suggestion in a model study and additionally confirmed that the breakdown products of N-nitrosamines did not reform in the *in vitro* human stomach model system. Ahn et al. (5) proposed the possibility of using irradiation in a real food system and determined that gamma irradiation significantly reduced nitrosodimethylamine (NDMA) and nitrosopyrrolidine (NPYR) concentrations in a model system using sausage without added ascorbic acid, a reducing agent.

Recently, Jo et al. (6) found that, in addition to reducing nitrosamines, irradiation at 5 kGy also significantly reduces residual nitrite concentrations in cooked pork sausage. The authors also reported that flushing packaging with 100% CO_2 reduced nitrite content in sausage much more than vacuum or aerobic packaging.

The objective of this study was to determine the effectiveness of different packaging methods on the reduction of volatile N-nitrosamine formation in pork sausage with different irradiation doses and storage periods.

MATERIALS AND METHODS

Sample preparation

Vacuum-packaged, refrigerated lean pork and frozen pork backfat were obtained within 48 h of slaughtering from a local meat packer and sequentially ground through 9 and 3 mm plates with a meat grinder (Model 160, Fatosa, Barcelona, Spain). An emulsion-type pork sausage was prepared by the method of Jo et al. (6). The processed samples were then vacuum-packed (75 cmHg pulled) in oxygen-impermeable nylon bags (2 mL $O_2/m^2/24$ h at 0°C; 20 cm × 30 cm; Sunkyung Co. Ltd, Seoul, Korea) with a vacuum packaging machine (Leepack, Hanguk Electronic, Kyungi, Korea), and aerobically-packaged by flushing air (ultra pure O_2 (25%) and N_2 (75%), 99.999%) into the bag without sealing. The rest of the samples were CO_2-flushed (ultra pure CO_2, 99.999%) for 9 sec into an oxygen-impermeable nylon bag and sealed. All samples were refrigerated at 4°C until irradiation.

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Irradiation and storage

Irradiation was performed the next morning after sample treatment in a Co-60 gamma irradiator (point source, AECL, IR-79, MDS Nordion International Co., Ltd, Ottawa, Canada) with a source strength of 100 kCi. The dose rate was 83 Gy/min at $12 \pm 0.5^{o}C$ and the applied absorbed doses were 0, 2.5, 5 and 10 kGy. Dosimetry was performed using 5 mm-diameter alanine dosimeters (Bruker Instruments, Rheinstettem, Germany), and the free radical signal was measured using a Bruker EMS 104 EPR Analyzer. The actual dose was within ±2% of the target dose. Temperature effects of irradiation were controlled for by placing a non-irradiated control sample outside the irradiation chamber. The control and irradiated pork sausages were transferred to a 4°C refrigerator and analyses were started after 3 h.

N-nitrosamine determination

N-nitrosamines in the sausage were extracted by the methods of Raoul et al. (7), with some modifications, using an Extrelut^{R} NT3 pre-packed glass column (Merck, Darmstadt, Germany) with added Extrelut^{R} packing materials (Merck). Authentic standards of nitrosodimethylamine (NDMA) and nitrosopyrrolidine (NPYR) were purchased from Sigma Co. (St. Louis, MO, USA). The concentrations of volatile N-nitrosamines were quantitatively determined by gas chromatography (GC, Model 5890II, Hewlett-Packard Co., Wilmington, DE, USA) coupled to a thermal energy analyzer (TEA, Thermo Electron Model 502B, Waltham, MA, USA). 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, USA), which was introduced into the ceramic pyrolysis tube at the end of the 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, increased to 100°C at 5°C/min. The injection volume was 2 μL. N-nitrosodipropylamine (1 ppm) was used as an internal standard for extraction efficiency. The mean recovery values for the internal standards obtained from all samples were 91.2±7.05%.

Statistical analysis

Each experiment was performed in duplicate and analysis of variance (ANOVA) was performed using SAS software (8). Duncan's multiple range test was used to compare differences among mean values. Mean values and pooled standard errors of the mean (SEM) were reported with significance level defined at $p < 0.05$.

RESULTS AND DISCUSSION

Nitrosodimethylamine (NDMA) concentrations in non-irradiated cooked pork sausage with either aerobic, vacuum or CO_2 packaging were not different at week 0 (Table 1), and were similar to the reduction of residual nitrite concentrations seen in previous studies (6,9). However, after 4 weeks of storage, the NDMA content in sausage with aerobic packaging without irradiation increased more than 3 fold above week 0 (Table 1). Irradiation at 10 kGy significantly reduced the NDMA content in vacuum packed sausage ($p < 0.05$). NDMA in irradiated sausage with CO_2 packaging was undetectable regardless of irradiation doses, although the detectable concentration was absorbed at the initial stage of storage. These results indicate that the CO_2 packaging lowers the residual nitrite content (9) which, in turn, reduces the NDMA content significantly.

On the other hand, it has been shown that the oxidation-reduction potential of turkey breast meat is initially decreased by irradiation when aerobically and vacuum-packed, but vacuum packed turkey breast had a much lower oxidation-reduction potential in the initial stage and during storage (10). It is hypothesized that the reduction state, combined with direct physical effects produced by irradiation in vacuum or CO_2 packaging, may change NO_2^- to NO, which is not a nitrosating agent (11), and would stay in gaseous state or change to other compounds by further reaction. Francis (11) reported that neither nitrite nor nitrous acid (HONO) are nitrosating agents, but are intermediates in the formation of nitrosating agents such as dinitrogen trioxide (N_2O_3), dinitrogen tetraoxide (N_2O_4) and the nitrous acidium ion (H_2O+NO). Francis (11) noted that nitrosation can also be the result of direct or indirect transfer of a nitric oxide radical (NO ·) which can be inhibited by redox compounds such as ascorbate and vitamin E. Further investigation to support the theory is being conducted in our laboratory by studying the pro-

Table 1. Nitrosodimethylamine (NDMA, ppb) concentrations in irradiated cooked pork sausage during storage at 4°C[1),2)]

Storage (wk)	Packaging	Irradiation dose (kGy)				SEM[4)]
		0	2.5	5.0	10.0	
0	Aerobic	5.05	5.22	3.62	2.96	1.16
	Vacuum	4.56	4.61	3.62	2.60	0.92
	CO_2	5.12	2.10	3.50	ND[3)]	1.76
	SEM[5)]	1.61	1.24	0.92	1.50	
4	Aerobic	16.39^{x}	12.93^{x}	11.03^{x}	7.97	7.46
	Vacuum	12.57^{ax}	15.58^{ax}	5.2^{abxy}	ND^{b}	2.88
	CO_2	ND^{y}	ND^{y}	ND^{y}	ND	-
	SEM[5)]	2.13	1.97	2.30	5.50	

1)Different letters (a,b) within the same row indicate significant differences ($p < 0.05$).
2)Different letters (x-z) within a column with the same storage method indicate significant differences ($p < 0.05$).
3)Not detected; detection level at 1.0 ppb.
4)Pooled standard errors of the mean (n = 12).
5)(n = 9).

Table 2. Nitrosopyrrolidine (NPYR, ppb) concentrations in cooked pork sausage during storage at 4°C[1),2)]

Storage (wk)	Packaging	Irradiation dose (kGy)				SEM[4)]
		0	2.5	5.0	10.0	
0	Aerobic	3.03	1.28	ND[3)]	ND	0.78
	Vacuum	1.61	1.02	0.39	0.88	0.53
	CO_2	2.79[a]	ND[b]	ND[b]	ND[b]	0.55
	SEM[5)]	1.07	0.11	0.29	0.59	
4	Aerobic	24.88[ax]	4.13[b]	3.33[bxy]	ND[b]	3.49
	Vacuum	12.72[axy]	ND[b]	11.99[ax]	3.07[b]	2.22
	CO_2	ND[y]	ND	ND[y]	ND	-
	SEM[5)]	2.86	2.39	2.40	1.77	

1)Different letters (a, b) within the same row indicate significant differences ($p < 0.05$).
2)Different letters (x-z) within a column with the same storage method indicate significant differences ($p < 0.05$).
3)Not detected; detection level at 1.0 ppb.
4)Pooled standard errors of the mean (n = 12).
5)(n = 9).

duction of radicals using Electron Spin Resonance (ESR) and changes in NO gas composition in packaged sausage.

Concentrations of nitrosopyrrolidine (NPYR) in cooked pork sausage with CO_2 packaging was also effectively reduced by irradiation (Table 2). The NPYR content increased significantly during 4 weeks of storage ($p < 0.05$) in aerobically or vacuum packaged sausage. However, irradiation significantly reduced NPYR concentrations, especially in the sausage packed with CO_2, in which NPYR was undetectable (Table 2). Recently, the allowable levels of nitrite for use in foods were lowered and a much tighter control of manufacturing processes was instituted. Furthermore, ascorbate and erythorbate are being used at maximum levels to inhibit formation of nitrosamines, and a nitrosamine monitoring program for bacon has been established (12). Thus, the nitrosamines in cured meat products might be negligible. However, this study provides meaningful evidence that irradiation can be used to reduce or eliminate the formation of toxic nitrosamines, if present.

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