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Short Communication of Novel Application of Food Irradiation

- Review -

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Abstract

Irradiation of food is not only used for sanitation purposes but can be used for processing techniques to reduce or eliminate toxic or undesirable compounds on food. Irradiation was effective to reduce the allergenicity of food by modification of the structure of proteins causing allergy reactions. Volatile N-nitrosmaine was reduced or eliminated by irradiation in the model system study and the breakdown products by irradiation did not recombine under human stomach conditions (pH 2,3, and 4,37°C). The possibility of residual chlorophyll b reduction by irradiation was also found, and the model study indicated that irradiation be used to destroy chlorophyll b, resulting in protection from photooxidation in oil without acceleration of lipid oxidation during irradiation. In this paper, several on-going research projects for the application of food irradiation as a new processing technique are introduced, including reduction of food allergens, breakdown of volatile N-nitrosamine and residual chlorophyll b.

Key words: food irradiation, allergenicity, nirtosamine, chlorophyll b

INTRODUCTION

Food irradiation is known to be the best method for controlling pathogenic microorganisms and also one of the best alternatives to chemical fumigants or preservatives usually used for sanitation treatment in international trade (1). Irradiation technology has been officially adopted by international organizations (WHO/IAEA/FAO) and experts (1) for its wholesomeness and economic benefits. After the US Food and Drug Administration (FDA) approved food irradiation on raw meat to control food-borne pathogens and extend product shelf-life (2), the use of this technology in related industry is expanding worldwide.

Besides the sanitary purposes, efforts for application of irradiation in reducing the toxic or undesirable materials such as food allergens (3), carcinogenic volatile N-nitrosamines (4), color enhancement of meat products with reduced nitrite (5) or salt content reduction of traditional Korean fermented foods (6) were introduced. Wierbicki and Brynjolfsson (7) reported previously that irradiation sterilization with ^{60}Co and ^{137}Cs reduced nitrite and preformed volatile nitrosamine level in cured meat products. This research also implicated possibilities for reducing nitrite and nitrosamines in a wide range of food systems. Since then, no research related to effects of gamma irradiation on volatile N-nitrosamines has been reported. Pesticide residue and polychlorinated biphenyl (PCB) reduction by irradiation were studied, and Bachman and Gieszczynska (8) reported that 30 kGy of irradiation decreased 25% of organochlorine pesticides, but when the same irradiation dose was applied to the luncheon meat, which contained added pesticides, the reduction was not found.

There is a great potential for reducing toxic or undesirable compounds by irradiation technology. Studies in this field have not been well documented. In this paper, some research results from our laboratory, which are related to the reduction of undesirable compounds in food processing, are introduced.

REDUCTION OF FOOD ALLERGIES

The phenotypic expression and natural history of food allergies vary widely according to the patient's age, disease presentation and type of food. Prevention of food allergies might be achieved by altering the dietary factors responsible for the sensitization and phenotypic expression of the disease (9). The hydrolysis of allergens by proteolytic enzymes and development of recombinant foods with modified DNA have been studied to eliminate protein allergens from allergenic foods (10). These approaches can be used only in extremely limited foodstuffs.

Meanwhile, the structural modification of food proteins by radiation was observed (11) and these results have indicated that ionizing radiation could change antigenicity by the destruction or modification of antibody (Ab) binding epitopes in food antigens/allergens (12).

Binding abilities (allergenicities) of most irradiated proteins were changed with different slopes of the inhibition curves in Ci-ELISA formatted with patients IgE. The patients IgE did not recognize the irradiated allergens very well, depending on the dose. When quantifying allergens in irradiated solutions, the amount of intact allergens in an irradiated solution was reduced by gamma irradiation depending upon the dose (Table 1). These results indicate that epitopes on allergens were structurally altered by gamma irradiation and IgE did not recognize well antigen-determinant sites on

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allergens.

At the evaluation on the effects of gamma irradiation to the whole shrimp muscle, binding ability of shrimp hypersensitive patients IgE (SHP-IgE) to allergens in sarcoplasmic and myofibrillar protein fractions decreased, depending upon the dose (Fig. 1). Interestingly, IgE-specific proteins existed in both fractions. The conformational modification of allergens was caused by gamma irradiation at the shrimp muscle. Above 7 kGy, the binding ability of patients IgE was below 50% in both fractions. It is clinically important to compare the differences of the reactivities of SHP-IgE to isolated and then irradiated STM (Table 1) with those of IgE to irradiated shrimp.

The results indicate that there is a possibility in reducing antigenicities of the most common food allergens exposed to gamma irradiation.

VOLATILE *N*-NITROSAMINE BREAKDOWN BY IRRADIATION

N-nitroso compounds are composed of nitrosamines and nitrosamides, and most nitrosamines are volatile and carcinogenic (13). Volatile N-nitrosamines presents in many foodstuffs (14,15), rubber products (16) and tobacco (17). Many volatile N-nitrosamines are stable to heat, but they can be cleaved photolytically by UV irradiation because of their chemical properties. Much research has been performed to inhibit nitrosamine formation with dietary compounds such as ascorbic acid (18), green tea (19) and phenol compounds (20).

Table 1. Residual concentration (μg/mL) of gamma-irradiated food allergens detected by Ci-ELISA based with IgE in patients' sera

Irradiation dose (kGy)	Shrimp tropomyosin	Milk BLG[1)]	Egg OVA[2)]
0	10	10	10
3	5.34	3.52	1.95
5	4.21	1.12	0.36
10	3.35	0.36	0.03

[1)]Milk betalactoglobulin.
[2)]Egg ovalbumin.

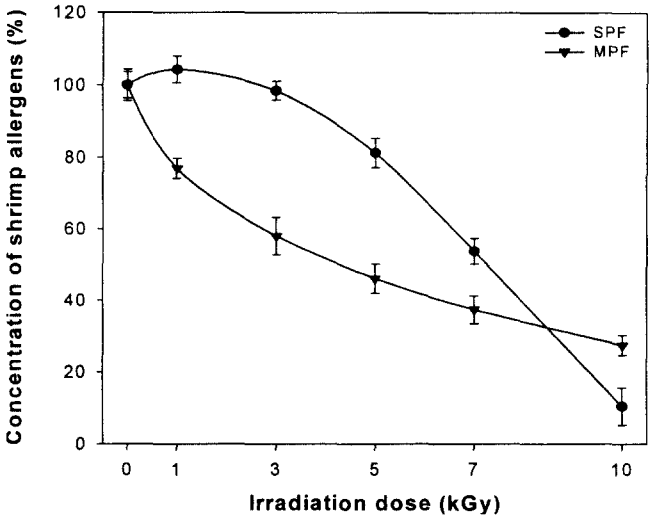

Fig. 1. Concentration of allergens in gamma-irradiated shrimp extracts detected by shrimp-hypersensitive patients' IgE. The concentration was measured by Ci-ELISA formatted with shrimp tropomyosin as standard allergen. SPF and MPF indicate sarcoplasmic protein fraction and myofibrillar protein fraction, respectively.

The residual amounts of nitrosodimethylamine (NDMA) and nitrosopyrrolidine (NPYR) dissolved in different solvents and following gamma irradiation are shown in Table 2. The results show that the solvent had different effects on volatile N-nitrosamines after gamma irradiation. NDMA and NPYR dissolved in distilled water were easily broken down by gamma irradiation and all of the VNAs were undetectable after irradiation of 5 kGy or above (Table 2). NDMA and NPYR showed 65~84% breakdown at 2.5 kGy and NPYR was more sensitive to gamma irradiation. Water is important for breakdown of those compounds, because water is required for hydrophotolysis of nitrosamines by UV irradiation.

The NDMA in dichloromethane was broken down to the level of 448 ppb (g/L) at 2.5 kGy and NPYR was completely broken at the same dose. The NDMA required a dose of 10 kGy or above to achieve 99% breakdown by gamma irradiation.

NDMA and NPYR dissolved in ethanol were comparatively stable to gamma irradiation. At the dose of 20 kGy, NDMA and NPYR showed 100% breakdown, respectively. Therefore, ethanol was the most resistant in nitrosamine breakdown among solvents used in this study for breakdown of volatile nitrosamines, and the NPYR was still more sensitive nitrosamine to gamma ray in this solvent. Although these solvents improved the stability of VNAs, gamma irradiation could be applied to destroy VNAs.

The breakdown products of NDMA and NPYR dissolved in distilled water by 5 kGy irradiation were reacted under human stomach conditions, which was set at pH 2, 3, and 4 at 37°C (Table 3). Neither NDMA nor NPYR were found in these samples.

The breakdown products from NDMA by irradiation were not recombined with pH 2, 3 and 4 but, in the presence of

Table 2. Residual nitrosodimethylamine (NDMA) and nitrosopyrrolidine (NPYR) levels (ppb) dissolved in different solvents when exposed to gamma irradiation[1)]

Irradiation dose (kGy)	Distilled water		Dichloromethane		Ethanol	
	NDMA[2)]	NPYR	NDMA	NPYR	NDMA	NPYR
0	1000	1000	1000	1000	1000	1000
2.5	352±5[1)]	157±1	448±4	-	706±5	683±7
5.0	-[2)]	-	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)]Mean standard deviation.
[2)]Not detected.

Table 3. Nitrosodimethylamine (NDMA) and nitrosopyrrolidine (NPYR) formation (ppb) from breakdown products of irradiated NDMA and NPYR at 5 kGy in different pHs[1]

	pH		
	2	3	4
NDMA			
None	-[2]	-	-
$NaNO_2$	$459 \pm 32^{3)a}$	474 ± 27^{a}	66 ± 8^{b}
NPYR			
None	-	-	-
$NaNO_2$	678 ± 41^{a}	779 ± 37^{a}	94 ± 11^{b}

[1]The breakdown products of NDMA and NPYR were reacted with 50 mM sodium nitrite (1 mL) or none at different pHs, and different letters (a,b) within a row are significantly different ($p < 0.05$).
[2]Not detected.
[3]Mean of triplicates standard deviation.

sodium nitrite, NDMA was formed at the same pHs. The recombined NDMA was 459 and 474 ppb at pH 2 and 3, respectively. The weaker nitrosation occurred at pH 4 which means that the pH is one of the important factor for nitrosation. The irradiated NPYR showed similar results (Table 3).

The breakdown products from NDMA and NPYR by gamma rays did not recombine *in vitro*, but recombined in the presence of nitrite, indicating that gamma irradiation has a potential to be applied in real food systems without reformation in the human stomach condition. Further identification of irradiation-induced breakdown compounds is important and should be performed.

CHLOROPHYLL b BREAKDOWN

The presence of green pigments of the chlorophyll in soybean oil is of interest not only because of their impact on finished product color but also because of their potential role in oxidative stability (21). Except for virgin olive oil where a greenish color is tolerated, an excessive amount of chlorophyll (>20 g/g) is considered undesirable but it is difficult to remove by conventional bleaching processes (22). Chlorophyll pigments not only impart an undesirable color to vegetable oils but impair the hydrogenation process (23) and to promote oxidation in the presence of light although they may be antioxidants in dark conditions (23). Oil processors routinely obtain apparent chlorophyll values by the AOCS spectrophotometric method (24).

Chlorophyll b content of nonirradiated and uncovered samples with aluminum foil was 2.91 ppm immediately after irradiation and decreased during 6 hr photooxidation to about a half amount (Table 4). The sample irradiated at 20 kGy or that irradiated at 20 kGy with N_2-bubbling destroyed all the chlorophyll contained in the sample. The foiled sample, which was treated to avoid photooxidation during storage, showed no difference during 6 hrs of photooxidation in the nonirradiated control, and maintained the amount that was added in the solution. But the irradiated sample detected no chlorophyll content by HPLC analysis regardless of N2-bubbling treatment.

Table 4. Residual chlorophyll content (ppm) of 20 kGy-irradiated and photooxidized linoleic acid solution (1% in methanol) containing chlorophyll b (3 ppm) by HPLC

	Irradiation dose (kGy)	Photooxidation time (hr)[1]					
		0	1	2	4	6	SEM[2]
Uncovered	0	$2.91^{a3)}$	2.99^{a}	2.34^{a}	1.81^{b}	1.51^{b}	0.144
	20	-[4]	-	-	-	-	-
	20 / N_2[5]	-	-	-	-	-	-
Covered[6]	0	2.88	3.05	3.02	2.85	2.73	0.108
	20	-	-	-	-	-	-
	20 / N_2	-	-	-	-	-	-

[1]Light intensity was 3,300 lux at 25°C.
[2]Pooled standard errors of the mean (n=12).
[3]Different letters (a,b) within a row differ significantly ($p<0.05$).
[4]Not detected.
[5]Sample was bubbled with ultra pure N_2 during 20 kGy-irradiation.
[6]Sample bottle was covered with aluminum foil to avoid photooxidation.

The absorbance spectra of the sample irradiated with or without N_2-bubbling differ significantly from the nonirradiated control (data not shown). The spectrum of the nonirradiated sample had two significant peaks at 658 nm and 436 nm but no peak was detected in irradiated samples.

The peroxide value (POV) of the unfoiled sample without irradiation increased slowly during storage because of photooxidation (Table 5). The POVs of 20 kGy-irradiated samples were dramatically increased by irradiation and stayed in all photooxidation time but with no difference during storage. It indicates that irradiation increased lipid oxidation but chlorophyll breakdown in the sample irradiated at 20 kGy did not develop the photooxidation when exposed to light. Irradiated samples that were treated by N_2-bubbling did not increase the POV at all, suggesting that N_2-bubbling can completely stop or inhibit the lipid oxidation during the irra-

Table 5. Peroxide value of photooxidized linoleic acid solution (1% in methanol) containing chlorophyll b (3 ppm) by gamma irradiation

	Irradiation	Photooxidation time (hr)[1]					
		0	1	2	4	6	SEM[3]
Uncovered	0 kGy	0^{cy}	24.0^{cy}	27.5^{cy}	49.7^{by}	79.0^{ay}	4.86
	20 kGy	1243.7^{x}	1251.6^{x}	1286.3^{x}	1253.4^{x}	1268.8^{x}	11.44
	20 / N_2[5]	0^{y}	0^{y}	0^{z}	0^{z}	0^{z}	-
	SEM[4]	7.81	12.90	3.82	3.38	3.03	
Covered[2]	0 kGy	0^{y}	0^{y}	0^{y}	0^{y}	0^{y}	-
	20 kGy	1251.7^{x}	1275.4^{x}	1262.1^{x}	1251.8^{x}	1266.5^{x}	17.40
	20 / N_2	0^{y}	0^{y}	0^{y}	0^{y}	0^{y}	-
	SEM[4]	10.41	6.19	3.88	11.10	14.82	

[a,b]Different letters within a row differ significantly ($p<0.05$).
[x-z]Different letters within a column with the same foiling/unfoiling differ significantly ($p<0.05$).
[1]Light intensity was 3,300 lux at 25°C.
[2]Sample bottle was covered by aluminum foil to avoid photooxidation.
[3]Pooled standard errors of the mean. n = 20,[4]n = 12.
[5]Sample was bubbled with ultra pure N_2 during 20 kGy- irradiation.

diation process and photooxidation during light storage.

The POV of the foiled sample did not develop the oxidation at all in the nonirradiated control (Table 5), indicating that the photooxidation is one of the main reasons of the increase of POV in nonirradiated unfoiled sample. The POV value of the 20-kGy irradiated sample with N_2-bubbling stayed 0 during whole storage regardless of covering effect by aluminium foil which means that irradiation destroy all chlorophyll, resulting in complete protection of photooxidation. The sample with 20 kGy irradiation without N_2-bubbling had significantly high POV from the beginning.

CONCLUSION

Results indicate that food irradiation technology has a great potential to reduce or eliminate toxic or undesirable compounds in food. Further research to identify the breakdown products induced by irradiation and its safety evaluation should be performed. Applications to real food systems are also needed.

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