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Immunological Assay to Detect Irradiated Beef

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Abstract

Competitive indirect enzyme linked immunosorbent assay (Ci-ELISA) was used to obtain the preliminary data for the detection of irradiated beef. Ci-ELISA was individually formatted with polyclonal antibodies produced from 2 kinds of bovine proteins, myosin and bovine serum albumin (BSA). Beef round, loin and tender loin were vacuum-packaged and subdivided into 3 groups of 1) irradiation; 2) irradiation and chilled at 4°C for 7 day; 3) irradiation and frozen at 20°C for 2 months to observe the changes under different storage and/or distribution conditions. Irradiation was performed at 3, 5 and 7 kGy. Protein solutions prepared from the sample were tested by formatted Ci-ELISA. Detected concentrations of myosin and BSA decreased with the increased irradiation dose in all samples with different reduction rates. Myosin was more susceptible to freezing than BSA. Samples irradiated at 5 kGy or above could be differentiated from non-irradiated ones by Ci-ELISA. These results indicate that immunological assay can be used as a detection method for irradiated beef.

Key words: Ci-ELISA, myosin, BSA, irradiated beef, detection method

INTRODUCTION

Muscle foods, meat and meat products, are composed of many proteins and are used as one of the major supplements of proteins in foodstuff. Meats are contaminated with various types of pathogens. Food irradiation is a physical method of processing that is comparable to heat treatment used to avoid microbial growth (1). US Food and Drug Administration and US Department of Agriculture permitted gamma irradiation of fresh (4.5 kilogray (kGy)≥) and frozen beef (7.0 kGy≥) in Dec. 2 1997 (2) and in Feb. 22 2000 (3), respectively. The import and distribution of irradiated beef in domestic markets is expected. Therefore, it is necessary that the pertinent regulations should be established. In addition, detection methods should be developed to identify irradiated beef from non-irradiated beef (4,5).

The effects of ionizing radiation have been studied on meat and meat proteins (6,7). A protein might be structurally changed by ionizing radiation (8-10). There are several factors leading to denaturation of protein by irradiation. The factors are the local conformation of amino acids in the peptide chain, its accessibility to the water radiolysis products, and the amino acid sequence itself where some particular amino acid residues may be more susceptible to radiolysis.

Immunological analysis has been used to monitor the structural modification of epitopes on a protein antigen (Ag) under physico-chemical conditions (11,12). Lee (13) reported that gamma-irradiated bovine myosin was differently recognized by antibody (Ab) produced from non-irradiated myosin, when determined by enzyme linked immunosorbent assay (ELISA) with 3 Abs produced individually from myosin and its subfragments. Also, heavy meromyosin subfragment-1 (MS-1) was more susceptive to radiation than the whole myosin molecule and myosin rod (subfragment-2 and light meromyosin).

This work investigated the effects of gamma irradiation on the conformational changes of beef proteins, myosin and bovine serum albumin (BSA), by competitive indirect-ELISA (Ci-ELISA) to verify whether it is suitable or not for the detection of irradiated meat.

MATERIALS AND METHODS

Preparation of proteins, meat and antibodies

Myosin was prepared from post mortem bovine M. *Semitendinosus* by modification of the method described previously (13). Concentration of myosin was adjusted to 2 mg/mL with 0.6 M NaCl, 10 mM phosphate buffer (PB), pH 7.0 (high salt buffer, HSB). Isolated BSA was purchased from Sigma Chemical Co. (St Louis, MO, USA). Chilled beef round, loin and tender loin were purchased from domestic market and vacuum-packaged before irradiation.

Polyclonal anti-MS-1 IgG and anti-BSA IgG were individually prepared from rabbits immunized by the method described previously (14).

Gamma irradiation and storage

Gamma irradiation was carried out in a cobalt-60 irradiator equipped with 100 kCi activity at 10±0.5°C and operated at a dose rate of 7.0 kGy h-1. The applied dose levels were 0, 3, 5, and 7 kGy. The absorbed dose was monitored with

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both free-radical and ceric/cerous dosimeters (15). After irradiation, all samples were divided into 3 groups: 1) immediately after irradiation; 2) chilled storage at 4°C for 7 days; and 3) frozen storage at -20°C for 2 months to monitor the changes of myosin and BSA under the conditions of the storage and distribution.

Formation of Ci-ELISA and standard curves for quantification

Ci-ELISA was formatted by the method described previously (13). The concentration of Ag for coating to well (Maxisorp, Nunc, Kamstrup, Denmark) was 10 μg/mL, and 1% (w/v) gelatin in 0.01 M phosphate buffered saline (PBS, pH 7.4) was used as a blocking solution for reducing nonspecific binding of Ab. Final dilutions of IgG and standard myosin were 1:5,000 diluent (0.2 μg/mL) and 500 to 0.1 μg/mL diluents, respectively. As a secondary IgG, 1:20,000 diluted horseradish peroxidase (HRP) conjugated goat anti-rabbit IgG (Sigma Chemical Co.) was used. Substrate solution was 0.04% o-phenylenediamine (OPD, Sigma Chemical Co.) in 0.1 M phosphate-citrate buffer with 0.04% hydrogen peroxide (v/v, 35% H_2O_2, Merck-Schuchardt, Munchen, Germany), pH 5.0 for running the color reaction for 20 min before stopping reaction by the addition of 2.0 M H_2SO_4. All steps were performed at 37°C, except for coating step performed overnight at 4°C. After incubation, the wells were washed three times with 0.01 M PBS with 0.1% (v/v) of tween 20. Absorbance was measured at 492 nm by an ELISA reader (CERES UV-900C, BIO-TEK instruments Inc., MI, USA). Formation of Ci-ELISA for anti BSA-IgG was also performed as above.

Preparation of muscle protein solutions

Two gram of sample was removed and homogenized in 20 mL of cold HSB, pH 7.0 by a homogenizer with a cold jacket. The homogenate was centrifuged at 9,000×*g* for 20 min at 4°C and supernatant was filtered through Whatman No. 3 filter paper (Whatman International Ltd., England) to remove insoluble particles. Filtrates were used as protein solution for quantifying myosin and BSA and also used to quantify protein content in each solution by a method described previously (16). Non-irradiated beef parts were used as a control. All protein solutions were serially diluted with HSB and concentrations of myosin and BSA in diluted sample solutions were determined by Ci-ELISA. All samples were triplicate and experiments were repeated 5 times.

Statistical analysis

Data from protein solubility and Ci-ELISA were analyzed by the general linear procedures, least square means, and Duncan's multiple range test as programmed by SAS® software (17).

RESULTS AND DISCUSSION

Formation of Ci-ELISA

When the diluted concentrations of primary IgG and secondary IgG were 1:5,000 and 1:20,000, respectively, and standard curves of anti-MS-1 IgG and anti-BSA IgG were obtained with the detection range from 250 to 3.9 μg/mL and from 125 to 1.0 μg/mL to myosin and BSA, respectively (Fig. 1 and 2). The limit of detection of both Ags was 0.1 μg/mL. This result indicated that Abs used to detect a meat protein were highly sensitive to a specific antigen and might be effectively used as an analytical method, compared with other research (18-20).

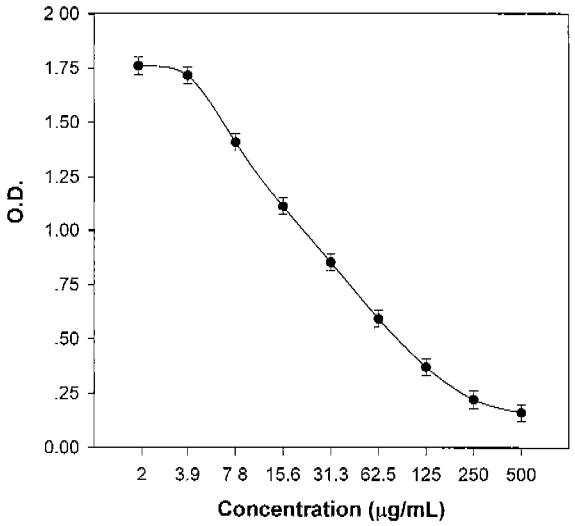

Fig. 1. Standard curve for quantification of myosin molecules in protein solutions prepared from gamma-irradiated beef samples. The curve was made by Ci-ELISA formatted with anti-MS-1 IgG produced from rabbits to myosin subfragment-1 (myosin head part) as primary antibody. Detection range is from 250 to 3.9 μg/mL and the concentration of detection limit is 0.1 μg/mL.

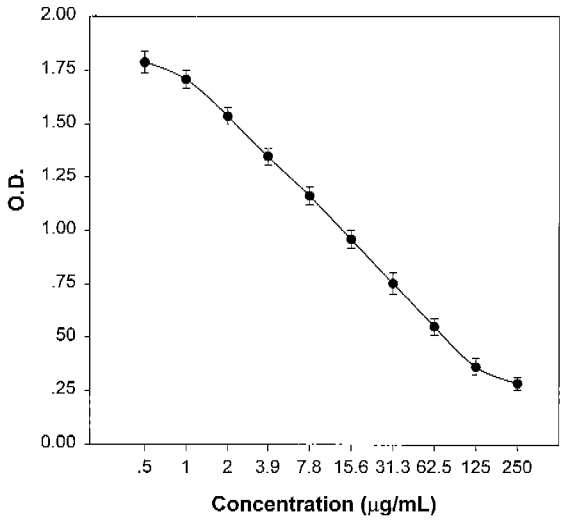

Fig. 2. Standard curve for quantification of bovine serum albumin (BSA) in protein solutions prepared from gamma-irradiated beef samples. The curve was made by Ci-ELISA formatted with anti-BSA IgG produced from rabbits to BSA as primary antibody. Detection range is from 125 to 1.0 μg/mL and the concentration of detection limit is 0.1 μg/mL.

Effect of gamma irradiation on protein solubility

Though protein solubility of beef samples slightly increased by gamma irradiation, statistical differences were not observed in most samples except for 7 kGy-irradiated samples (Table 1). The decrease of the solubility was observed in irradiation and frozen treatments. Differences in the solubility among beef parts might be due to the differences of the composition of each sample. To measure correct intact Ag concentration in sample solutions by Ci-ELISA, protein concentration of the solution must be determined, because large differences among the concentration of samples can interfere with data from ELISA (13). Irradiation and chilling treatments increased the protein solubility more than other treatments. This result was supported by a report that beef tenderness was improved and the protein solubility increased by gamma irradiation (21). This result was statistically evaluated, and these solutions were used as sample solution of Ci-ELISA without any adjustments of protein concentration, except for the one-fourth dilution to meet in detection ranges of standard curves.

Changes of Ag by irradiation treatment

Intact Ags decreased in all irradiated samples depending upon the dose (Table 2). In 7 kGy-irradiated beef round, a myosin molecule was detected at 42.2 μg/mL with the reduction rate of 5.59 compared with 78.3 μg/mL of non-irradiated one. The reduction rate was calculated by the slope of the regression curve of the detected concentration of Ag, and indicates radiation sensitivity of Ag in samples. The precision of this assay can be evaluated by the application of the reduction rate (11). The binding ability of anti-MS-1 IgG to myosin molecules in 7 kGy irradiated loin and tender loin also decreased to 56.9 and 61.9%, respectively. The concentration of BSA ranged from 48.9 to 51.3 μg/mL in non-irradiated samples, but that of 7 kGy was in the range of 24.3 to 31.7 μg/mL. Binding ability of anti-BSA IgG decreased to 49.7 to 61.8% in 7 kGy-irradiated samples.

The reduction rate of the binding ability by gamma irradiation and the concentration of Ags detected differed from sample to sample. The reason may be deduced from the differences of proximate compositions among samples including protein contents (13).

Changes of Ag by irradiation and chilled storage treatment

Large amounts of myosin was detected in non-irradiated samples, compared to other treatments (Table 3). This result could be explained by the fact that myosin molecules may be more easily dissolved by the cleavage and destruction of muscle structure by proteolytic enzymes in meat during storage (/or ageing) (22). MS-1 was denatured by both irradiation and chilling treatment more than other treatments. The reduction rates of the detected concentration were 6.28, 5.10 and 4.57 in round, loin and tender loin, respectively. During storage, myosin molecules were attacked by the various enzymes and fragmented (23). Though samples were stored at a chilling temperature of 4°C, several enzymes, such as calcium acti-

Table 1. Protein solubility[1] of gamma-irradiated beef parts in the condition of different storages

Treatments	Samples	Irradiation dose (kGy)			
		0	3	5	7
Irradiation	Round	$3.4^{AJ)}$	3.5^{AB}	3.6^{B}	3.6^{B}
	Loin	3.4^{A}	3.4	3.4	3.5^{B}
	Tender loin	3.0^{A}	3.0	3.0	3.1^{B}
Irradiation and chilling	Round	3.6	3.6	3.6	3.5
	Loin	3.4	3.3	3.5	3.5
	Tender loin	3.0	3.2	3.1	3.1
Irradiation and frozen	Round	2.5^{A}	2.6^{AB}	2.6^{AB}	2.7^{B}
	Loin	2.1^{A}	2.1	2.2^{B}	2.2
	Tender loin	1.9	1.9	2.0	2.0

[1)]Protein concentrations (mg/mL) in sample solutions were determined by protein assay kit.

[2)]Means (n=5) in the same row with different superscript differ significantly ($p \leq 0.05$).

Table 2. Concentrations[1)] of myosin and bovine serum albumin in protein solutions[2)] prepared from gamma-irradiated beef parts

Proteins	Samples	Irradiation dose (kGy)				Reduction rate of intact Ag[3)]
		0	3	5	7	
Myosin	Round	$78.3^{A4)}$	60.2^{B}	50.9^{BC}	42.2^{C}	5.59
	Loin	66.2^{A}	48.6^{B}	45.1^{B}	37.7^{C}	3.96
	Tender loin	59.2^{A}	48.5^{B}	43.5^{B}	36.7^{C}	3.17
Bovine serum albumin	Round	51.3^{A}	45.6^{A}	37.8^{B}	31.7^{B}	2.85
	Loin	49.2^{A}	40.5^{B}	32.8^{B}	25.4^{C}	3.41
	Tender loin	48.9^{A}	38.7^{B}	32.5^{BC}	24.3^{C}	3.47

[1)]Concentrations (μg/mL) of myosin in sample solutions were determined by Ci-ELISA.

[2)]Protein solution was diluted 4 times with high salt buffer (pH 7.4) to be correctly detected in the detection range of standard curve.

[3)]Reduction rate was calculated by the slope of regression curve of Ag concentration detected by Ci-ELISA.

[4)]Means (n=5) in the same row with different superscript differ significantly ($p \leq 0.05$).

Table 3. Concentrations[1)] of myosin and bovine serum albumin in protein solutions[2)] prepared from beef parts gamma-irradiated and chilled at 4°C for 10 days

Proteins	Samples	Irradiation dose (kGy)				Reduction rate of Intact Ag[3)]
		0	3	5	7	
Myosin	Round	$96.5^{A4)}$	72.3^{B}	64.5^{B}	51.5^{C}	6.28
	Loin	81.6^{A}	70.8^{B}	52.6^{C}	48.3^{C}	5.11
	Tender loin	79.1^{A}	64.5^{B}	57.3^{B}	46.5^{C}	4.57
Bovine serum albumin	Round	52.6^{A}	46.9^{A}	32.2^{B}	25.6^{B}	4.07
	Loin	50.5^{A}	42.7^{B}	31.9^{B}	23.4^{C}	3.94
	Tender loin	51.8^{A}	41.6^{B}	30.7^{C}	22.5^{C}	4.26

[1)]Concentrations (μg/mL) of each protein in sample solutions were determined by Ci-ELISA.

[2)]Protein solution was diluted 4 times with high salt buffer (pH 7.4) to be correctly detected in the detection range of standard curve.

[3)]Reduction rate was calculated by the slope of regression curve of Ag concentration detected by Ci-ELISA.

[4)]Means (n=5) in the same row with different superscript differ significantly ($p \leq 0.05$).

vated factors and cathepsin, could hydrolyze the myofibrillar proteins. The greater decrease of the reduction rates in this treatment might be induced from the combination of irradiation and aging at chilled condition.

The concentration of BSA decreased by gamma irradiation, depending upon the increase of the irradiation dose (Table 3). BSA was affected by chilled storage, as the case of myosin, even though not more severe than in myosin. BSA of 7 kGy-irradiated samples was less than the half concentration of non-irradiated one.

Changes by irradiation and frozen treatment

Beef loin and tender loin were more susceptible to irradiation and frozen treatment than beef round, in the case of myosin. However, significant differences were not observed in BSA (Table 4). MS-1 was about 54.2, 48.9 and 47.6 μg/mL in non-irradiated and frozen beef round, loin and tender loin, respectively, and this result was a decrease of about 30% to the non-irradiation treatment. Lee (13) reported that the myosin molecule denatures during frozen storage, and the rate of denaturation accelerates by the extended frozen storage period. The combination treatment of irradiation and freezing might affect denaturation of MS-1 more than an irradiation treatment alone. The detected concentration of myosin (Table 2, 3) and protein contents (Table 1) in all samples decreased in this treatment more than other treatments. This reduction may be induced by insolublization of myofibrillar proteins caused by frozen denaturation (24). BSA was not affected by freezing (Table 2). This result is supported by some reports that sarcoplasmic proteins are not well affected by freezing compared with myofibrillar proteins (25).

In conclusion, immunoassay can be applied to the detection of irradiated beef at 5 kGy or above. Unfortunately, 3 kGy-irradiated beef was not detected with a great reproducibility by Ci-ELISA with Abs, even though statistical differences were recognized in several samples. An Ab with high specificity and affinity, like a monoclonal Ab, is required for differentiating irradiated beef from non-irradiated beef. To produce the Ab, at first, a target protein (Ag) should be considerably selected from irradiated food and be sufficiently studied on the surface epitopes of B cells (13).

Table 4. Concentrations[1)] of myosin and bovine serum albumin in protein solutions[2)] prepared from beef parts gamma-irradiated and frozen at -20°C for 2 months

Proteins	Samples	Irradiation dose (kGy)				Reduction rate of intact Ag[3)]
		0	3	5	7	
Myosin	Round	$54.2^{A4)}$	38.2^{B}	27.5^{C}	15.4^{D}	5.51
	Loin	48.9^{A}	31.5^{B}	26.4^{BC}	17.5^{C}	4.38
	Tender loin	47.6^{A}	32.2^{B}	21.9^{C}	14.2^{C}	4.82
Bovine serum albumin	Round	55.4^{A}	48.2^{A}	36.8^{B}	32.5^{B}	3.45
	Loin	51.8^{A}	46.5^{A}	37.2^{B}	24.6^{C}	3.84
	Tender loin	50.7^{A}	43.7^{AB}	33.7^{B}	27.8^{B}	3.38

[1)]Concentrations (μg/mL) of each protein in sample solutions were determined by Ci-ELISA.
[2)]Protein solution was diluted 4 times with high salt buffer (pH 7.4) to be correctly detected in the detection range of standard curve.
[3)]Reduction rate was calculated by the slope of regression curve of Ag concentration detected by Ci-ELISA.
[4)]Means (n=5) in the same row with different superscript differ significantly ($p \leq 0.05$).

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