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Effects of Electron Beam Irradiation on Functional and Other Associated Properties of Pork Myofibrillar Salt-Soluble Proteins

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Abstract

Ground pork was irradiated with an electron beam (e-beam) at a dose of 0, 1.5, 3, 5 and 10 kGy and the changes in various functional and other associated properties of salt-soluble proteins extracted from the pork were evaluated. Irradiation did not affect turbidity and the disulfide content of pork salt-soluble protein, but the content of sulfhydryls and the hydrophobocity of salt-soluble protein increased. This indicates that protein degradation occurred when the pork was e-beam irradiated and that the sulfhydryls and hydrophobic moieties buried inside the proteins were exposed to the outside environment. However, these degraded protein molecules did not form large protein aggregates through disulfide bridges. The emulsifying capacity of the pork increased with irradiation, which could be the result from increased hydrophobicity of pork salt-soluble protein. Water holding capacity of pork was not affected by e-beam irradiation.

Key words: electron beam, pork salt-soluble proteins, functional properties

INTRODUCTION

The beneficial effects of electron beam (e-beam) irradiation on foods were well reported. These effects include pasteurization, destruction of insects, inactivation of parasites, delaying of ripening and prevention of sprouting (1). E-beam irradiation of foods has been primarily used for pasteurization (2-9). Research on the application of e-beam for pasteurization purposes has been conducted in pork (10-20), beef (7,12,15,21), turkey (10,15,18,22,23), chicken (4,24-26), cooked sausages (27), soybean paste (8) and ginseng powder (5). Industrial utilization of e-beam irradiation of foods is also increasing. It has been successfully used for frozen beef patties, poultry products, precooked processed product and papaya (28). Advantages of e-beam irradiation include its non-radioactivity and a short treatment time (a few seconds). In addition, e-beam irradiation is an environmentally-friendly method and brings about little change in temperature during the treatment (2,3). E-beam irradiation is a possible alternative pasteurization technique for some juice processing without damaging the flavors (3).

As stated, most of the research into e-beam irradiation of foods has focused on pasteurization. However, there have been few studies on the effect of e-beam irradiation on the changes of functionalities of myofibrillar proteins in meat. Both emulsifying capacity and water holding capacity are important functional properties influencing the final quality of meat. Higher emulsifying capacity increases the stability of meat emulsions and increased water holding capacity enhances many physical properties including color, texture, firmness of raw meat and juiciness and tenderness of processed meat products (29). Therefore, this study evaluated the effects of e-beam irradiation on the changes in functional and other associated properties of pork myofibrillar salt-soluble proteins.

MATERIALS AND METHODS

Preparation of ground pork

Fresh ground pork (Boston shoulder) was obtained from a local meat market. The ground pork was purchased before 48 hrs postmortem, the visible fat and connective tissue removed, made into patties (15 cm in diameter), and divided into 5 groups. Each group of ground pork patty was wrapped with polyethylene film and irradiated with different doses of e-beam and stored at 4°C. Less than 6 hours elapsed from time of purchase until the e-beam treatment. All the studies were replicated three times.

Electron beam irradiation

Ground pork was irradiated with a high voltage, Cockraft-Walton type of electron beam accelerator (Max. beam energy: 1.0 meV, Yeungnam Univ.). Irradiation doses were 1.5, 3, 5 and 10 kGy and the beam currents were

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0.15, 0.3, 0.5 and 1 mA, respectively. Conveyer speed was set at 10 Hz (2.87 cm/s). For each irradiation, it took 10 to 15 min to calibrate the instrument, but it took less than 10 seconds to irradiate the samples. There were no temperature changes after the e-beam treatment and the irradiated samples were stored at 4°C until further analyses.

Measurements of turbidities of pork salt-soluble proteins

Increased turbidity of protein solutions is the indicator of protein aggregate formation (30). Turbidity was measured by the method of Chan et al. (30). Ground pork was homogenized with 0.6 N NaCl (1:4, w/v) and left for 1 hour. An aliquot of the homogenate was transferred to a cuvette and the absorbance at 320 nm was measured with a spectrophotometer (UVICON 922, Kontron Instrument, Italy). The differences in turbidity (protein aggregate) with the changes in e-beam dose were monitored.

Measurements of sulfhydryl and disulfide contents of pork salt-soluble proteins

The content of total and reactive (surface) sulfhydryls in pork salt-soluble proteins were determined in the presence and absence of 8 M urea using Ellman's reagent (dithionitrobenzoic acid, DTNB) (31). Salt-soluble protein was extracted from pork as follows: 25 g of ground pork was homogenized (Nihon Seiki Kaisha Ltd., Japan) in 0.6 N NaCl solution (1:4, w/v) for 3 minutes and held at 4°C for 1 hour. The homogenate was centrifuged (VS 3000i, Vision Sci. Co. Ltd., Korea) at 12,000×*g* for 30 minutes and the supernatant was collected for salt-soluble protein solution. Two hundred microliter of salt-soluble protein solution was mixed with 1.5 mL of DTNB in Tris-glycine buffer (pH 8, glycine 100 mM, Tris-base 85 mM, EDTA 4 mM). The mixture was kept at room temperature for 1 hr and its absorbance was measured at 412 nm.

Disulfide content was determined using 2-nitro-5-thiosulfobenzoate (NTSB) according to the method of Thanhauser et al. (32) and Damodaran (33) with slight modification. The stock solution of NTSB was made by solubilizing 0.1 g of DTNB in 10 uL of 1 M sodium sulfite (Na_2SO_3) solution. This stock solution was diluted 100 times with tris base containing 2.5 M guanidine thiocyanate (Gu · SCN) to make NTSB assay solution. Two hundred microliter of salt-soluble protein solution was mixed with 1.5 mL of NTSB assay solution and the mixture was incubated in the dark for 25 minutes. The absorbance was measured at 412 nm as described for sulfhydryl measurement. The contents of sulfhydryl and disulfide were calculated from A_{412} values using an extinction coefficient of 13,600 $M^{-1}cm^{-1}$ and expressed as μmole/g of protein. The protein concentrations were measured by the method of Lowry et al. (34).

Measurements of surface hydrophobicities of pork salt-soluble proteins

Protein surface hydrophobicity was measured with the fluorescent probe, 1-anilo-8-naphthalene sulfonate (ANS) as described by Li-Chan et al. (35). Salt-soluble protein solutions were serially diluted with 0.01 M phosphate buffer (pH 9.0) at a concentration of 0.00125, 0.0025, 0.005, 0.01 and 0.02% and aliquots of ANS were added to the protein solutions. Fluorescence intensities of the solutions were measured with a fluorescence spectrometer (Fluology 3, Jobinyron-Spex, Horiba group, Co. Ltd., USA) with the excitation and emission wavelengths of 390 and 470 nm, respectively. Surface hydrophobicity was expressed as a slope of changes in fluorescent intensity against protein concentration gradients.

Measurements of emulsifying capacities of pork

Twenty five gram aliquots of each of the irradiated ground pork samples were mixed with 100 mL of NaCl solution (0.6 N) and homogenized for 2 minutes. In order to create a meat emulsion state, 12.5 g of homogenate was mixed with 37.5 mL of NaCl solution and the first aliquot of soybean oil (50 mL) was added and blended. Additional aliquots of oil were slowly added to the meat and blended until the emulsion was visibly broken. Emulsifying capacities were expressed as grams of total oil added per gram of meat sample.

Measurements of water holding capacities of pork

Water holding capacity of ground pork was determined using a centrifugation method (36). One and a half grams of pork were wrapped with triple layers of filter paper and inserted into test tubes (30×90 mm). Test tubes containing samples were centrifuged at 2,000 ×*g* (VS-3000i, Vision Scientific Co. Ltd., Korea) for 10 minutes. The liquid that separated from the pork was measured by subtracting the sample weight after centrifugation from the weight before centrifugation. Water holding capacity was calculated as a percentage of water content of sample remaining in the sample out of the total water content of the samples. The moisture contents of the samples were measured by the drying oven method (105°C, 15 hrs).

Statistical analyses

All analyses were performed with SAS Version 8.01, 2001 (SAS Institute, Inc., Cary, NC). Analyses of variances and Duncan's multiple range tests were used to determine the significances of differences among the

means.

RESULTS AND DISCUSSION

Effect of electron beam irradiation on the changes of turbidities of pork salt-soluble proteins

Turbidities of pork salt-soluble protein solutions, as measured by the absorbances at 320 nm after various electron beam treatments are shown in Table 1. Turbidity decreased with increased electron beam irradiation. Turbidity of ground pork protein solution with 10 kGy e-beam treatment was significantly ($p<0.05$) lower than those of other treatments and the turbidity of pork protein irradiated with 5.0 kGy was significantly ($p<0.05$) lower than those of control and 1.5 kGy e-beam irradiated pork. Gill and Conway (37) demonstrated an increase in turbidity when fish myosin was heated, which was the direct result of the formation of larger myosin aggregates. Chan et al. (30) also observed an increase in turbidity with increased temperature in cod and herring myosin. Lee et al. (38) reported that when bovine and porcine blood plasma protein solutions were irradiated with γ-ray, it first caused a breakdown of polypeptide chains into low molecular weight compounds and secondly led to a conversion of these low molecular weight compounds into higher molecular weight aggregates. These aggregates were formed with protein-protein crosslinking, hydrophobic, electrostatic interaction and disulfide formation (39). In our previous research on the effect of e-beam treatment on the changes in SDS-PAGE pattern of pork myofibrillar protein, the breakdown of protein (mainly myosin and actin) occurred (40), but further conversion of low molecular weight compounds to higher molecular weight aggregates did not proceed. The level of energy produced by and the penetration depth of the electron beam are lower than those of heat and γ-ray (3). Our above finding of the above result may have occurred because e-beam irradiation did not generate as much energy as heating or γ-irradiation, and the energy was insufficient to permit the formation of aggregates.

Table 1. Turbidities of ground pork with different doses of e-beam irradiation

Treatments	Turbidity (Abs. at 320 nm)
Control	$0.83\pm0.04^{1)a2)}$
1.5	0.82 ± 0.02^{a}
3	0.75 ± 0.75^{ab}
5	0.69 ± 0.05^{b}
10 kGy	0.57 ± 0.01^{c}

[1)]Values are mean ± standard deviations. All the values are means of 3 replicates.
[2)]Values in the same column bearing different superscripts are significantly different ($p<0.05$).

Effect of electron beam irradiation on the changes of sulfhydryl and disulfide contents of pork salt-soluble proteins

Electron beam irradiation dose dependantly increased sulfhydryl contents of pork protein and was significantly higher ($p<0.05$) in 10 kGy e-beam irradiated pork protein compared to other treatments (Table 2). Electron beam irradiation has been reported to degrade proteins in our previous research (40) and other report (41). It is presumed that when the pork is e-beam irradiated, protein degradation or denaturation occurs and the sulfhydryls that were buried inside the protein molecule are exposed to the exterior environment leading to an increase in the total sulfhydryl content. This result is consistent with the finding of Ishizaki et al. (42) that an increase in reactive (surface) sulfhydryl of sardine and pork muscle treated with UV radiation was due to protein unfolding.

The disulfide contents of pork protein with various e-beam doses are shown in Table 3. Disulfide content did not increase even though the e-beam dose increased from 1.5 to 10 kGy. This finding that increased sulfhydryls (Table 2) did not result in the later increase of disulfides in pork protein (Table 3) suggests that the protein degradation occurred, but the further formation of large molecular weight aggregates from the degraded peptides by disulfide bridges did not proceed with the e-beam treatment. This hypothesis is supported with our previous data that turbidity (aggregate formation) did not

Table 2. Sulfhydryl (-SH) contents of salt-soluble proteins in pork irradiated with different doses of e-beam

Treatments	Total-SH	Reactive-SH
Control	$7.78\pm0.12^{1)a2)}$	5.69 ± 0.16^{a}
1.5	7.97 ± 0.18^{a}	5.72 ± 0.15^{a}
3	7.84 ± 0.11^{a}	5.81 ± 0.11^{a}
5	8.01 ± 0.10^{a}	5.93 ± 0.18^{a}
10 kGy	8.29 ± 0.06^{b}	6.25 ± 0.09^{b}

[1)]Values are means (μmole/g of protein) ± standard deviations. All the values are means of 3 replicates.
[2)]Values in the same column bearing different superscripts are significantly different ($p<0.05$).

Table 3. Disulfide (-S-S) contents of salt-soluble proteins in pork irradiated with different doses of e-beam

Treatments	-S-S-
Control	$2.85\pm0.10^{1)a2)}$
1.5	2.97 ± 0.12^{a}
3	2.81 ± 0.09^{a}
5	2.84 ± 0.11^{a}
10 kGy	2.88 ± 0.12^{a}

[1)]Values are means (μmole/g of protein) ± standard deviations. All the values are means of 3 replicates.
[2)]Values in the same column bearing different superscripts are significantly different ($p<0.05$).

Table 4. Surface hydrophobicities of salt-soluble proteins in pork irradiated with different doses of e-beam

Treatments	Hydrophobicities
Control	381±12[1)a2)]
1.5	384±16[a]
3	398±21[a]
5	431±27[a]
10 kGy	516±23[b]

[1)]Values are means±standard deviations. All the values are means of 3 replicates.
[2)]Values in the same column bearing different superscripts are significantly different ($p<0.05$).

increase (Table 2) with the e-beam irradiation.

Effect of electron beam irradiation on the changes of hydrophobicities of pork salt-soluble proteins

Hydrophobicities of pork salt-soluble proteins increased with increasing doses of e-beam irradiation (Table 4). When the pork was irradiated at a dose of 10 kGy, the hydrophobicity was significantly higher ($p<0.05$) than with other treatments. This suggests that protein degradation or denaturaton induced by e-beam irradiation results in the exposure of hydrophobic moieties (amino acids) buried inside the protein molecule to the outside of the environment increasing the hydrophobicities. UV radiation (42) and heat treatment (43), that are known to increase the hydrophobicities of protein, are presumed to have similar protein denaturating mechanisms as e-beam irradiation.

Effect of electron beam irradiation on the changes in emulsifying capacities of pork

Emulsifying capacity of pork muscular protein is a very important processing property, contributing to the final quality of processed meat such as ham and sausages. It determines the stability of the meat emulsion. Higher emulsifying capacity contributes stabilizing fat in the emulsified sausages, preventing fat pockets (29). As shown in Table 5, emulsifying capacity of ground pork increased when the dose of e-beam irradiation increased from 1.5 to 10 kGy. The emulsifying capacities of ground pork treated with 3, 5 and 10 kGy e-beam all had significantly higher ($p<0.05$) values than those of control, but the differences in values among 1.5, 3, 5 and 10 kGy irradiated and between control and 1.5 kGy irradiated were not significantly different. This increase in the emulsifying capacity of e-beam irradiated pork is mainly thought to be due to the increase in the hydrophobicity of salt-soluble protein (Table 5). Pork salt-soluble proteins, mainly myosin and actin, are natural emulsifying agents (29). The increased hydrophobicity of pork salt-soluble protein could increase the hydrophobic trapping of lipid droplets in meat emulsion.

Table 5. Emulsifying capacities of ground pork irradiated with different doses of e-beam

Treatments	Emulsifying capacity
Control	61.2±0.10[1)a2)]
1.5	62.3±0.11[ab]
3	63.0±0.14[b]
5	63.8±0.15[b]
10 kGy	65.0±0.12[b]

[1)]Values are means (g oil added/g meat)±standard deviations. All the values are means of 3 replicates.
[2)]Values in the same column bearing different superscripts are significantly different ($p<0.05$).

Table 6. Water holding capacities of ground pork irradiated with different doses of e-beam

Treatments	Water holding capacity
Control	63.87±0.12[1)a2)]
1.5	63.81±0.15[a]
3	63.99±0.18[a]
5	63.95±0.24[a]
10 kGy	63.88±0.13[a]

[1)]Values are means (%)±standard deviations. All the values are means of 3 replicates.
[2)]Values in the same column bearing different superscripts are significantly different ($p<0.05$).

Effect of electron beam irradiation on the changes in water holding capacities of pork

Water holding capacity of meat is also an important processing characteristic that determines the final quality of meat and meat products. It has a high correlation with tenderness and juiciness in processed meat (29). Water holding capacities of pork after various doses of e-beam treatment are shown in Table 6. As shown in the Table, e-beam irradiation did not affect the water holding capacity of pork.

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