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Changes of SDS-PAGE Pattern of Pork Myofibrillar Proteins Induced by Electron Beam Irradiation

- Research Note -

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Abstract

Actin and myosin solutions and fresh ground pork were irradiated with the electron beam (e-beam) at a dose of 0, 1.5, 3.0, 5.0 and 10 kGy. The changes in SDS-PAGE pattern of 2 proteins and the salt-soluble proteins extracted from ground pork after e-beam irradiation were monitored. When the myosin solution was irradiated with e-beam, myosin was degraded completely. Complete myosin degradations were observed even with the lowest dose (1.5 kGy) of e-beam treatment. Actin was degraded with the irradiation, but to a less extent than myosin was. The degradation of actin increased as the e-beam treatment increased from 1.5 to 10.0 kGy. Among the salt-soluble proteins extracted from ground pork, myosin was degraded gradually when the e-beam dose increased from 1.5 up to 10.0 kGy. Similar gradual increase in the degradation of actin also occurred with the increase of irradiation. Increases of 2 low molecular weight compounds (<29 kDa) were observed when the irradiation dose increased from 1.5 to 10.0 kGy. These 2 molecules are thought to be the breakdown products produced from the degradation of major salt-soluble proteins, myosin and actin. The salt-soluble protein content of ground pork did not change with the e-beam irradiation.

Key words: actin, myosin, salt-soluble protein, degradation, electron beam irradiation

INTRODUCTION

Researches concerning the effect of electron beam (e-beam) irradiation on the changes in quality of food are increasing. Unlike the γ-ray irradiation, e-beam irradiation does not induce radioactivity and requires a few seconds for irradiation. Temperature change does not occur during the treatment and this method is a non-destructive, environment-friendly mean to accomplish the purposes (1-5). The major beneficial effect of e-beam irradiation on the quality changes in food is pasteurization (1,2,6-12). Other effects include destruction of insects, inactivation of parasites, delaying of ripening and prevention of sprouting. Studies on the utilization of e-beam have been conducted in pork (13-20), beef (9,14,17,20,21), turkey (12,17,20,22,23), chicken (6,24-26) and cooked sausages (27). Recent studies of e-beam irradiation expanded to soybean paste (11), ginseng powder (9) and powdered food colorants (28). Since December 1999 when the Food Safety & Inspection Service (FSIS) of USDA allowed the use of ionizing radiation in meat, growing number of industrial application of e-beam irradiation on food has been recorded. Its application was successfully performed in beef patty, poultry meat, papaya and other precooked processed products (29).

Most of the studies of e-beam irradiation on food were focused in pasteurization. During the process of performing this purpose, many studies reported increases in the development of lipid oxidation (3-5,12,13,15,16, 19,20,22,24,25). However, the studies on the effect of e-beam irradiation on the changes of protein structure were limited. This study was carried out in order to find out the changes of electrophoretic pattern of pork myofibrillar protein induced by e-beam irradiation.

MATERIALS AND METHODS

Preparation of actin, myosin model systems and ground pork

Standard proteins, actin and myosin were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and solubilized in 0.6 N NaCl solution. Fresh ground pork was obtained from a local meat market. Ground pork was purchased before 48 hrs postmortem and the visible fat and connective tissue were removed. Samples of ground pork were wrapped with polyethylene film and stored at 4°C before and after the electron beam irradiation. Less than 6 hours were elapsed until the electron beam

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treatment after purchase.

Electron beam irradiation

Actin, myosin solutions and wrapped ground pork were 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, 3.0, 5.0 and 10 KGy and the beam currents were 0.15, 0.30, 0.50 and 1.0 mA, respectively. Conveyer speed was controlled at 10 Hz (2.87 cm/s). For each irradiation, it took 10 to 15 min to calibrate the instrument, but the actual length of time taken for irradiating the samples was less than 10 seconds. There were no temperature changes after the e-beam treatment and the irradiated samples were stored at 4°C until further analyses.

Effect of electron beam irradiation on the changes of electrophoretic patterns of actin and myosin model systems

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed with a slight modification of Laemmli method (30). Standard proteins, e-beam irradiated actin and myosin were mixed with sample buffer (60 mM Tris-HCl, 25% Glycerol, 2% SDS, 14.4 mM mercaptoethanol, 0.1% bromophenol blue, 10 mL distilled water) and resolved on a 12.5% separating gel (Mini-Protean, Bio-Rad Lab. Inc., USA) for 2.5 hours. The buffer solution used for separation was 0.025 M Tris base-0.192 M glycine (pH 8.3) containing 0.1% (w/v) SDS. The electrophoretic patterns of protein samples were stained with Coomassie brilliant blue R-250 (Bio-Rad. Richmond, Calif., USA) followed by destaining with solution of ethanol and acetic acid. The protein bands after various e-beam treatments were compared with the standard protein pattern.

Effect of electron beam irradiation on the changes of electrophoretic pattern of pork salt-soluble myofirillar protein

Pork myofibrillar proteins are mainly salt-soluble. Salt-soluble protein was extracted from muscle as follows: Twenty five gram of ground pork was homogenized (Nihonseiki kaisha Ltd, Japan) in 0.6 N NaCl solution (1:3, w/v) for 3 minutes and left still at 4°C for 1 hour. The homogenate was centrifuged (VS-3000i, Vision Scientific Co. Ltd., Korea) at 12,000×g for 30 minutes. The supernatant contains salt-soluble myofibrillar protein including actin and myosin. Supernatant fraction was mixed with sample buffer in a 1:1 ratio and the electrophoresis was performed in the same manner described in actin and myosin model system. The electrophoretic patterns of pork salt-soluble myofibrillar protein with different doses of e-beam were compared.

Effect of electron beam irradiation on the changes of salt-soluble protein content of ground pork

The contents of salt-soluble protein of pork samples irradiated with different doses were measured with Lowry method (31). The 2.5 mL of assay solution ($CuSO_4 \cdot 5H_2O$, $Na_3C_6H_5O_7 \cdot 2H_2O$, Na_2CO_3, NaOH in distilled water) were mixed with 0.5 mL of salt-soluble protein solution (supernatant) and left for 10 minutes. To the mixture, 0.25 mL of Folin-Ciocalteau phenol reagent was added and left for 30 minutes followed by measuring the absorbance at 750 nm with the spectrophotometer (UVICON 922, Kontron Instrument, Italy). The absorbances were calculated into protein content.

RESULTS AND DISCUSSION

Effect of electron beam irradiation on the changes of electrophoretic pattern of actin and myosin model systems

The SDS-PAGE patterns of myosin are shown in Fig. 1, when the protein was irradiated with various doses. There were apparent myosin degradations when the myosin solutions were irradiated with e-beam. Myosin degradation was clear even when the lowest dose (1.5 KGy) was treated to the solution. With the doses of e-beam irradiation exceeding 1.5 KGy up to 10 KGy, myosin bands disappeared and myosin was degraded completely.

Actin was also degraded with irradiation (Fig. 2), but to a less degree than myosin was (Fig. 1). As shown in the electrophoresis pattern in Fig. 2, the degradation of actin increased when the doses increased from 1.5 to 10 KGy. The protein degradation upon the exposure to

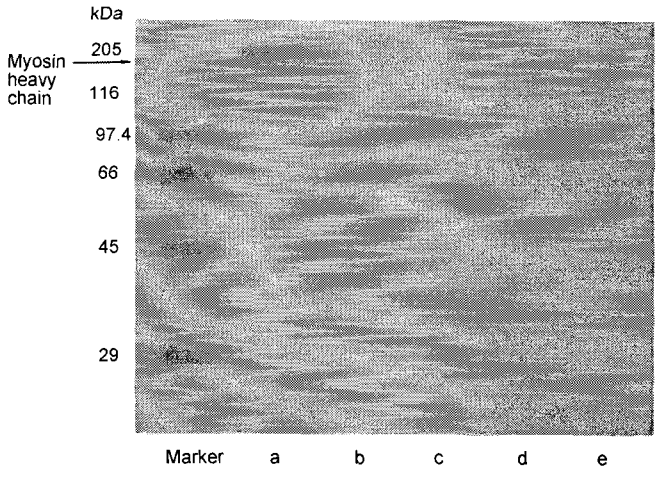

Fig. 1. SDS-PAGE profile of myosin irradiated with various doses of e-beam. a: 0, b: 1.5, c: 3.0, d: 5.0, e: 10.0 kGy.

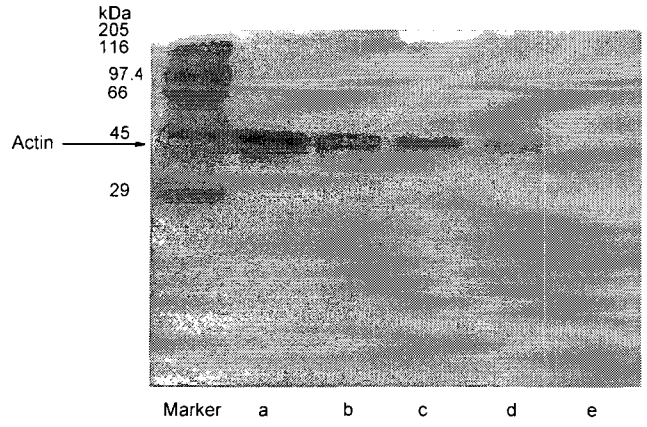

Fig. 2. SDS-PAGE profile of actin irradiated with various doses of e-beam. a: 0, b: 1.5, c: 3.0, d: 5.0, e: 10.0 kGy.

radiation was due to the fragmentation by oxygen radical (32). It was reported that hydroxy (OH·) and superoxide (O_2^-) radicals formed by radiolysis of water could modify the primary structure of proteins. It was proposed that the oxidation of proline residues are main reason for chain cleavage by irradiation (33).

Effect of electron beam irradiation on the changes of electrophoretic pattern of pork salt-soluble myofibrillar proteins

The changes in the SDS-PAGE pattern of pork myofibrillar proteins by irradiation were shown in Fig. 3. A typical pork myofibrillar protein band profile was noted in the figure. Two major myofibrillar proteins monitored were myosin (205 kDa) and actin (43 kDa). Myosin was degraded with irradiation. Myosin band

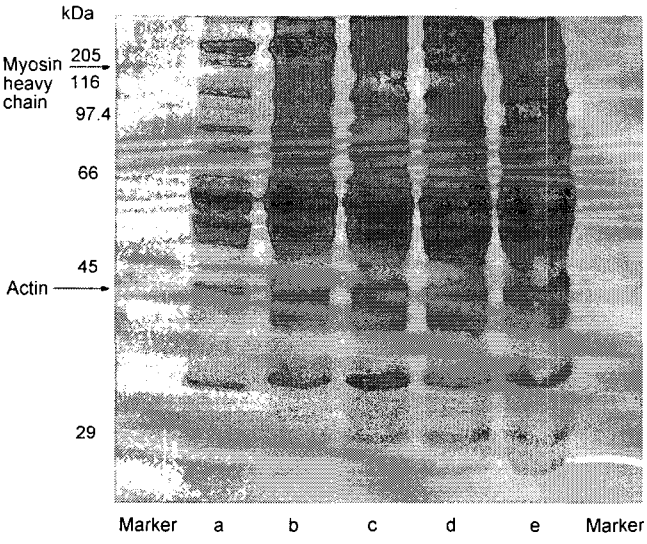

Fig. 3. SDS-PAGE profile of salt-soluble proteins extracted from ground pork irradiated with various doses of e-beam. a: 0, b: 1.5, c: 3.0, d: 5.0, e: 10.0 kGy.

Table 1. Contents of salt-soluble protein of ground pork irradiated with different doses of e-beam (%)

Treatments	Protein content
Control	15.39±0.32[1)a2)]
1.5 kGy	15.87±0.24[a]
3.0 kGy	15.64±0.54[a]
5.0 kGy	15.45±0.28[a]
10.0 kGy	15.91±0.33[a]

[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 ($p<0.05$).

became gradually smeared as the dosage increased from 1.5 to 10 kGy (lane b~e). Actin was also degraded with the increase in the irradiation dose as shown by the smearing pattern of actin band from lane b through e (Fig. 3). It is noteworthy that with the irradiations, 2 low molecular weight molecules (less than 29 kDa) appeared and these molecules became more clear, when the dosage increased from 1.5 to 10 kGy (lane b~e, Fig. 3). It could be explained that high molecular weight proteins (possibly myosin and actin) were degraded with irradiation to lower molecular weight molecules. It means that there existed a breakdown of protein molecule upon irradiation. Lee et al. (32) also observed the appearance of new low molecular weight bands below the major protein band as a result of breakdown of this protein when the bovine and porcine plasma protein solutions were irradiated with low dose of γ-ray. The degree of breakdown of myosin and actin was less than that observed in the model systems (Fig. 1, 2).

Effect of electron beam irradiation on the changes of salt-soluble protein content of ground pork

The contents of salt-soluble protein of ground pork with different doses of electron beam irradiation were shown in Table 1. Electron beam treatment did not influence the salt-soluble protein content of ground pork.

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