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# Effect of Irradiation on the Mixture of Egg White Proteins Responsible for Foaming Property

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**ABSTRACT**

Irradiation of egg white increased foaming ability significantly. To investigate the protein modification by irradiation responsible for the increase of foaming ability, 3 major egg white proteins were purchased and mixed (7.7 g/L ovalbumin, 1.8 g/L ovotransferrin, 0.5 g/L lysozyme) as a model system and irradiated at 0, 2.5, and 5 kGy. The different protein expressions were evaluated using 2-D electrophoresis and it was found that ovotransferrin was cleaved by irradiation and molecular weight and isoelectric point were changed. In addition, many uncharacterized proteins were found and it indicated that irradiation modified proteins randomly but mainly fragmentation was observed. Therefore, it can be concluded that protein fragmentation of 3 major egg white proteins responsible for foaming ability may be the main reason for the improvement of foaming ability.
(**Key words :** Irradiation, Egg white protein mixture, Foaming ability, 2-D electrophoresis)

## INTRODUCTION

Egg white is traditionally used as an ingredient by food industry because of its exceptional functional properties: foaming, emulsifying, and gelling properties (Lechevalier et al, 2003). Among them, the foaming ability is one of the most important functions of egg white protein in the manufacturing of bakery and confectionary products. Several studies have been reported that heating and high pressure treatment may improve foaming ability of egg white. Because moderate heat or higher than 450 MPa of high pressure treatment induce denaturation of egg white protein and results a loss of secondary structure, and this change can improve foaming ability of egg white (Donovan et al, 1975; Van der Planken et al., 2005). However, egg white proteins are quite sensitive to heat treatment inducing insolubility, especially for ovotransferrin. High pressure treatment also has several limitations in a mass production and an increased cost (Song et al., 2009).

Several studies indicated that irradiation improved the foaming ability and foam stability of egg white and more stable viscosity than heated egg white (Ball and Gardner, 1968; Ma et al., 1990; Wong et al., 1996 Song et al., 2009). Irradiation generates hydroxyl radical and superoxide anion radical and these radicals induce chemical changes such as fragmentation, aggregation, and cross-linking between peptides and proteins. This change may improve functional properties of egg white (Moon and Song, 2001). Recently, Song et al. (2009) reported that irradiation improved foaming ability and this improved ability maintained after processing of an angel cake. However, the mechanism of this phenomenon is unclear so far.

Three major egg white proteins known to be responsible for foaming property are ovalbumin, ovotransferrin, and lysozyme and they account for 54, 13, and 3.5% of the egg white proteins, respectively (Stadelman and Cotterill, 1977). Therefore, the objective of this study was to investigate the irradiation effect on foaming ability of egg white and the protein modification of 3 major egg white proteins including ovalbumin, ovotransferrin, and lysozyme, which are known to be responsible for foaming property of egg white.

## MATERIALS AND METHODS

### 1. Sample preparation and irradiation

Three major egg white proteins including ovalbumin, ovotransferrin, and lysozyme were purchased (Sigma Co., St. Louis, MO, USA) and mixed (ovalbumin : ovotransferrin : lysozyme = 7.7 : 1.8 : 0.5 g/L) with the same ratio of an ordinary egg as a model system.

The egg white protein mixture was irradiated by cobalt-60

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gamma irradiator at doses of 0, 2.5, and 5 kGy at Advanced Radiation Technology Institute (Jeongeup, Korea). The source strength was approximately 42 kCi with a dose rate of 20 kGy/h at 12±0.5℃. Dosimetry was performed using 5 mm diameter alanine dosimeters (Bruker Instruments, Rheinstetten, Germany), and the free-radical signal was measured using a Bruker EMS 104 EPR Analyzer. The dosimeters were calibrated against an international standard set by the International Atomic Energy Agency (Vienna, Austria).

## 2. Foaming ability and foam stability

The foaming ability and foam stability measurements were conducted using 1-d-old chicken egg from Lohman Brown hens. Egg white was separated and measured using the modified method of Philips et al. (1990). Egg white (25 mL) was mixed with 25 mL deionized distilled water (DDW) in a 100 mL graduated cylinder and then homogenized at 24,200 × *g* for 30 sec using a homogenizer (T25B, IKA, Staufen, Germany). Foam height was measured as foaming ability. Foam stability was determined by measuring water content in a graduated cylinder after 30 min of foaming at room temperature. Higher number indicates lower foam stability.

## 3. Extraction of solubilized proteins for 2-D analysis

For 2-D PAGE, soluble proteins were extracted as described by Han et al. (2007). Sodium dodecyl sulfate (SDS), phenylmethanesulfonyl fluoride (PMSF), urea, thiourea, 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesul-fonate (CHAPS), dithiothreitol (DTT), isopropanol, Tris-HCl, $NH_4HCO_3$, a-ciano-hydroxycinnamic acid, tributylphosphine (TBP), trifluoroacetic acid (TFA), and trypsin were obtained from Sigma Co. (St. Louis, MO, USA). Acrylamide was obtained from Amresco (Solon, Ohio, USA). An equal volume of lysis buffer A containing 1% SDS, 1 mM PMSF, protease inhibitor cocktail (Roche, Indianapolis, IN, USA), 100 mM Tris-HCl, (pH 7.0) for pH 3-10 was added to the egg white. Samples were sonicated for 5 sec and placed in chilled ice water, and then mixed with an equal volume of lysis buffer B (7 M urea, 2 M thiourea, 4% CHAPS, 0.1 M DTT, 1 mM PMSF, protease inhibitor 40 mM Tris-HCl, pH 7.0). The samples were shaken gently for 1 hr at room temperature, and then centrifuged at 15,000 × *g* for 20 min. The solubilized protein extracts were quantified by Bradford protein assay (Bio-Rad, Hercules, CA, USA). Three different samples per single irradiation dose were used for 2-D analysis.

## 4. 2-D gel electrophoresis

Precast 18 cm IPG strips (dry polyacrylamide gel strip with an immobilized pH gradient) with pH 3-10 range were obtained from GE Healthcare Bioscience (Uppsala, Sweden). Preparative egg white protein mixture (1 mg) was used for isoelectric focusing (IEF). The sample was mixed with modified rehydration buffer (7 M urea, 2 M thiourea, 4% CHAPS, 2.5% DTT, 10% isopropanol, 5% glycerol, 2% v/v IPG buffer pH 3-10) to total volume of 350 μL. A mixture of sample was loaded onto an IPG strips (pH 3-10; 180 × 3 × 0.5 mm). The strip was allowed to rehydrate overnight in swelling tray. After rehydration, first adednsion, IEF, was performed using an Amersham Pharminga Multiphor II IEF unit. Automatic isoelectric focusing was carried out for with $1.5 \times 10^5$ Vh. Voltage was started at 100 V and gradually increased to a final voltage of 8000 V. After the first dimensional IEF, IPG gel strip were placed in an equilibration solution (6 M urea, 2% SDS, 50% v/v glycerol, 2.5% acrylamide, 1.5 M Tris-HCl, pH 8.8) containing 5 mM TBP for 20 min with gentle shaking. The second dimensional separation was performed on 8-16% linear gradient SDS polyacrylamide gels. The gels were placed into an ISO-DALT system (Hoefer Scientific Instruments, San Francisco, CA, USA). The gels (200 × 250 × 1.0 mm) were run overnight at 10-15 mA per gel until the bromophenol blue marker dye (Amersham Biosciences, Piscataway, NJ, USA) had disappeared at the bottom of the gel.

## 5. Staining and image analysis of 2-D-gels

After 2-DE, gels were stained with colloidal coomassie brilliant blue G-250 (CBB, Amersham Biosciences, Piscataway, NJ, USA). The gels were fixed for 1 hr in fixation solution (30% v/v methanol, 10% v/v acetic acid) and stained with colloidal CBB G250 for 24 hr, and then destained with 1% acetic acid. The gels were analyzed by ImageMaster V software (GE Healthcare Bioscience, Uppsala, Sweden). The calculations were applied to the percent volume parameter representative of the protein expression. Variations in abundance were calculated as the ratio of average values (% vol) between the two classes.

## 6. Sample preparation for MALDI-TOF mass spectrometry analysis

For analysis of coomassie-stained proteins, the pieces of gel slab were destained by 120 μL wash solution (50% v/v acetonitrile, 25 mM $NH_4HCO_3$, pH 7.8). The washed gel pieces

were dehydrated with 50 μL of acetonitrile, and then dried for 30 min with a vacuum centrifuge. The dried gel pieces were rehydrated with 5 μL of trypsin solution (trypsin at a concentration of 0.0012 μg/μL in 25 mM $NH_4HCO_3$, pH 7.8). The gel pieces were completely covered with additional ammonium bicarbonate buffer if needed, and then placed at 37℃ overnight. The supernatants were discarded when digestion was completed. The gel pieces were added with extraction buffer (50% acetonitrile/ 0.5% TFA), and then sonication was applied for 30 min to extract residual peptides. The extracted peptides were used for MALDI-TOF analysis. Mass spectrometric analysis of peptide mass fingerprinting (PMF) was performed using a Voyager-DE STR MALDI-TOF-MS (PerSeptive Biosystems, Framingham, MA, USA). The extracted peptide solution (1 μL) was mixed with the same volume of matrix solution (10 mg/mL α-ciano-4-hydroxicinnamic acid, 0.1% v/v TFA, and 50% v/v acetonitrile), loaded onto a MALDI sample plate (96 well) and crystallized. For each sample, average 500 spectra were obtained and scans were performed twice. Spectra were calibrated upon acquisition automatically using an external 3-point calibration. Peak assignment was performed manually using Data Explorer™ software that is part of the Voyager-DE STR MALDI-TOF-MS software package (PerSeptive Biosystems, Framingham, MA, USA) and spectra were searched against non-redundant protein sequence database on SWISS-PROT and/or NCBInr (2008/10/01, Data Bank).

### 7. Statistical analysis

One way ANOVA was performed by using SAS software (SAS, 2002) and when the significance was found, Student-Newman-Keuls' multiple range tests was carried out to differentiate among the mean values at $P<0.05$ level.

## RESULTS AND DISCUSSION

To investigate the irradiation effect on the foaming ability and foam stability of egg white, the separated egg white was irradiated at 0, 2.5, and 5 kGy. The foaming ability was increased by 3.2 folds after 2.5 kGy of irradiation while the foam stability was decreased (Table 1). However, no difference was found between irradiation doses at 2.5 and 5 kGy for both foaming ability and foam stability. Similarly, Ma et al. (1990) and Liu et al. (2009) reported that the foaming ability was improved by irradiation dose due to the conformational changes of protein in egg white and increased surface hydrophobicity. Clark et al. (1992) also reported improved functional properties in spray-dried egg white irradiated at 2 kGy or above because irradiation caused small changes in secondary structure from α-helix to random structure enhancing some functional properties. Song et al. (2008) recently reported that irradiation of egg white offered advantages in increasing foaming ability and improving quality of final bakery products. They demonstrated that irradiation dose up to 5 kGy decreased batter density and increased cake height and cake volume in both liquid egg white and egg white powder (Song et al., 2008). It means that the decrease of foam stability by irradiation presented in the present study may not affect significantly when applied for the processing of food products.

Table 1. Foaming ability and foam stability of irradiated egg white

| Irradiation dose (kGy) | Foaming ability (cm) | Foam stability (cm) |
|---|---|---|
| 0 | 18.0[b] | 5.9[b] |
| 2.5 | 58.1[a] | 18.8[a] |
| 5.0 | 60.4[a] | 20.2[a] |
| SEM[1)] | 1.76 | 2.21 |

Values with different letters (a, b) within the same column differ significantly ($P<0.05$).
[1)] SEM: Standard error of the mean (n = 9).

Different protein expressions were evaluated using 2-D electrophoresis image (Fig. 1). The expression levels of variant protein spots were statistically analyzed and represented by the mean of % volume histograms and the differently expressed protein spots were shown in Figs. 2 and 3. Totally 16 spots showed different protein expressions. Among them, the protein spots cs385 and cu243 showed decrease in their expression while TS310 and TS343 were not identified in non-irradiated samples but appeared in the samples after irradiation of 2.5 and 5 kGy. The protein spot TS392 also showed increase of its intensity.

The protein spots with different expression by irradiation treatment were excised from gels and identified after tryptic digestion by MALDI-TOF MS and identified as the known protein from SWISS-PROT and NCBInr database searching. The search results were evaluated on the source of accepted standard that take into account the number of peptides matched to the candidate protein, the coverage of the candidate protein's sequence by the matching peptide, and agreement of the experimental and theoretical isoelectric point (pI) and molecular weight with values. Table 2 shows the identified proteins. Spots TS310 and TS343 were identified to ovotransferrin, according to the 2-D analysis. The molecular weight and pI of original

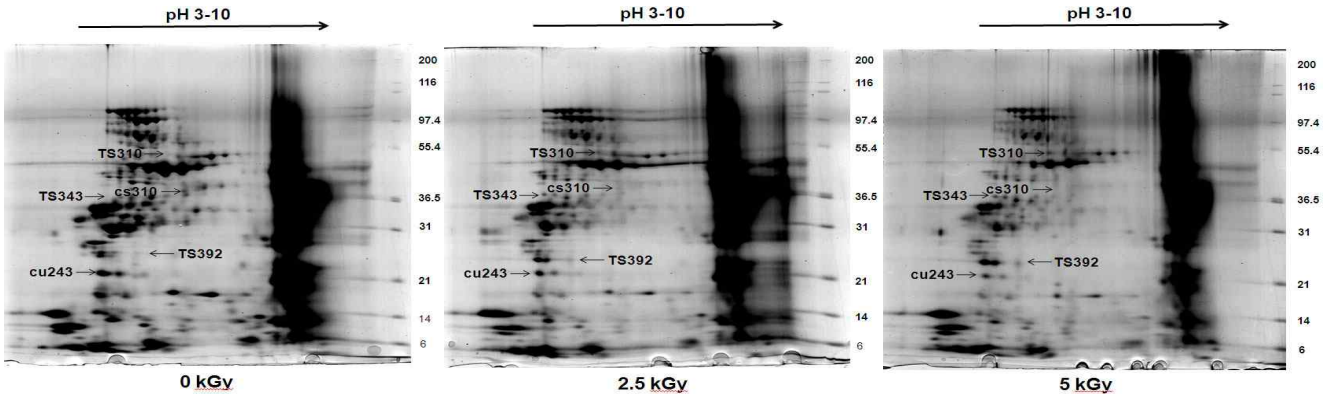


Fig. 1. 2-D PAGE protein separation of irradiated egg white protein mixture (ovalbumin:ovotransferrin:lysozyme = 7.7:1.8:0.5 g/L) as visualized by coomassie brilliant blue staining.

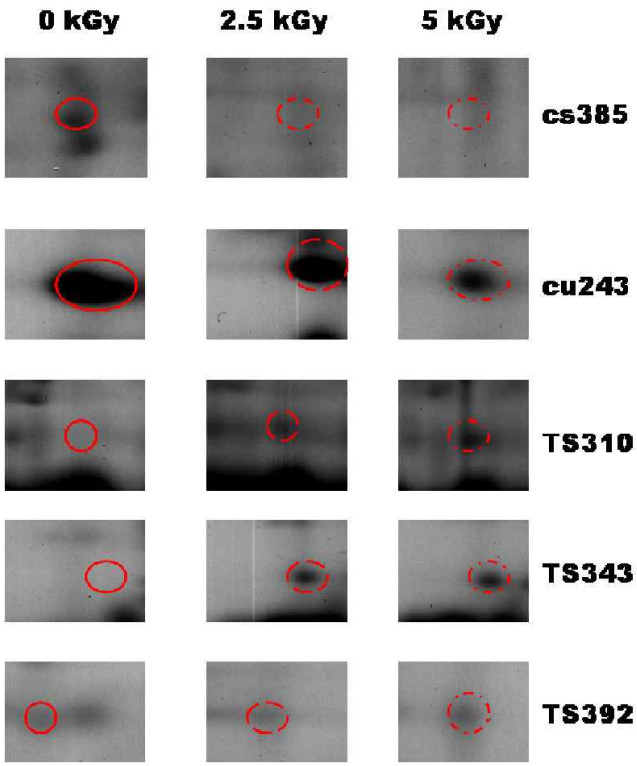


Fig. 2. Differently expressed protein spots of irradiated egg white protein mixture (ovalbumin:ovotransferrin:lysozyme = 7.7:1.8:0.5 g/L).

ovotransferrin are known as 80.03 kDa and 6.9, respectively, but those of TS310 were identified as 57.3 kDa and 5.9. The result of the spot TS343 also showed similar to TS310, which had changed molecular weight and pI. It can be explained that irradiation caused fragmentation of ovotransferrin and induced the changes in molecular weight and pI. In addition, cs385, cu243, and TS392 were identified as T-complex protein 1, transient receptor potential cation channel subfamily C member 1 isoform alpha, similar to dishevelled-associated activator of morphogenesis, respectively. This indicated that irradiation modified proteins randomly with fragmentation in addition to the result of many uncharacterized proteins. Regarding the radiation damage to proteins, two types of damages are commonly observed: fragmentation and aggregation (Filali Mouhim et al., 1997). Moon and Song (2001) indicated that irradiation at low dose caused break down of polypeptide chains and resulted in the formation of degraded small molecular weight molecules. Other reports suggested that irradiation caused agglomeration as well as breakdown when different doses of irradiation were applied (Marija et al., 2005). However, it is confirmed that the fragmentation of protein was main event

Foam consists of an aqueous continuous phase and a gaseous (air) dispersed phase. When a protein performs effectively as a foaming agent or an emulsifier it must meet the following basic requirements able to rapidly adsorb to the air-water interface, readily unfold and rearrange at the interface, and able to form a viscous cohesive film through intermolecular interactions (Damodaran, 2007). The foaming properties are affected by molecular flexibility, charge density and distribution, and hydrophobicity (Damodaran, 2007). Molecular flexibility plays a role in foaming ability of protein, which is related to lowering of interfacial tension. Irradiation caused disruption of ordered structure of protein molecules to random-coil-type protein, which lowered interfacial tension (Moon & Song, 2001).

The 2-D analysis in this study showed that irradiation induced the change of protein structure mainly by fragmentation of the mixture of 3 major egg white proteins. Denatured egg white protein by irradiation may possess higher foaming ability by enhancing the rate surface tension decrease than native egg white protein, because the denatured proteins adsorb more easily to the air bubble surface. The changes of protein structure cause protein

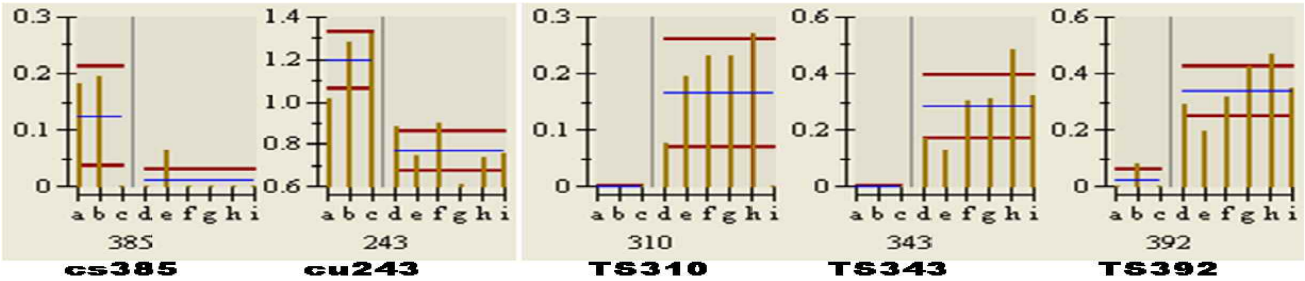


Fig. 3. Differentially expressed protein spots by the mean of % volume histograms of irradiated egg white protein mixture (ovalbumin:ovotransferrin:lysozyme = 7.7:1.8:0.5 g/L). a-c, 0 kGy; d-f, 2.5 kGy; g-i, 5 kGy.

Table 2. Up-expressed egg white protein mixture[1)] identified by MALDI-TOF

| | Estd'z | Protein information | % | pI[2)] | kDa[2)] | pI[3)] | kDa[3)] |
|---|---|---|---|---|---|---|---|
| cs385 | 1.45 | T-complex protein 1, alpha subunit (IPI00584300.2) | 36 | 5.4 | 61.79 | 6.2 | 38.5 |
| cu243 | 1.4 | Transient receptor potential cation channel subfamily C member 1 isoform alpha ( IPI00587728.1) | 15 | 8.9 | 91.41 | 4.7 | 22.5 |
| TS310 | 1.75 | Ovotransferrin precursor (IPI00683271.1) | 38 | 6.9 | 80.03 | 5.9 | 57.3 |
| TS343 | 1.58 | Ovotransferrin precursor (IPI00683271.1) | 38 | 6.9 | 80.03 | 4.8 | 37.5 |
| TS392 | 1.31 | Similar to dishevelled-associated activator of morphogenesis 1 (IPI00592529.3) | 11 | 7.2 | 122.7 | 5.2 | 23.8 |

[1)] Egg white protein mixture was composed of ovalbumin : ovotransferrin : lysozyme = 7.7 : 1.8 : 0.5 g/L
[2)] The pI and kDa values of appeared spots were identified as the known protein from SWISS-PROT and NCBInr database searching.
[3)] The pI and kDa values of appeared spots were estimated proportionally from gel.

unfolding at the interface, thereby improvement of their foaming properties exhibiting enhanced adsorption capacity and efficiently decreasing the surface tension.

In conclusion, irradiation up to 5 kGy caused mainly scission of 3 major egg white proteins, which provided the modification of egg white protein structure with improved foaming ability, thereby improving the quality and functional property of food products.

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