Radiation Physics and Chemistry 80 (2011) 1258–1262

Contents lists available at ScienceDirect

Radiation Physics and Chemistry

journal homepage: www.elsevier.com/locate/radphyschem

Changes in antigenicity of porcine serum albumin in gamma-irradiated sausage extract by treatment with pepsin and trypsin

Koth-Bong-Woo-Ri Kim [a], Eu-Jin Song [a], So-Young Lee [b], Jin-Gyu Park [c], Ju-Woon Lee [d], Myung-Woo Byun [e], Dong-Hyun Ahn [a,*]

[a] Department of Food Science and Technology/Institute of Food Science, Pukyong National University, Busan 608-737, Republic of Korea
[b] Traditional Food Research Group, Korea Food Research Institute, Seongnam 463-746, Republic of Korea
[c] Radiation Food Science and Biotechnology, Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, Jeongup 580-185, Republic of Korea
[d] National Federation of Fisheries Cooperatives, Fisheries Economic Institute, Seoul 138-827, Republic of Korea
[e] Department of Culinary Nutrition, Woosong University, Daejon 300-718, Republic of Korea

ARTICLE INFO

Article history:
Received 23 June 2010
Accepted 30 May 2011
Available online 23 June 2011

Keywords:
Porcine serum albumin
Antigenicity
Gamma irradiation
Sausage
Pepsin
Trypsin

ABSTRACT

Pork is known as an allergenic food with porcine serum albumin (PSA, 66 kDa) representing the major allergen. This study was conducted to investigate the change in antigenicity of PSA in gamma-irradiated sausage extract treated with pepsin and trypsin. Sausage products (A and B) were irradiated at 1, 3, 10, and 20 kGy. After irradiation, sausage proteins were extracted and digested with pepsin (1:200, 30 min) and trypsin (1:300, 5, 30, 60, 90, and 120 min). The binding ability of PSA in extracts of the irradiated sausages (A and B) decreased by over 3 kGy relative to the binding ability of PSA in extracts of intact sausages and showed no notable differences when the dose of radiation ranged from 3 to 20 kGy. After treatment with pepsin and trypsin, the binding ability of PSA in extracts of the irradiated sausages was decreased more relative to that of intact sausages and showed no significant differences when the period of trypsin treatment is increased or when the dose of irradiation is increased. The sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) results indicated that there was no visible change in the intensity of the PSA band in extracts of the irradiated sausages. After pepsin and trypsin treatment, the intensity of PSA band faded with increasing doses of irradiation. In conclusion, antigenicity of PSA in pork sausages could be reduced by gamma irradiation.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

The allergenic potential of food is altered by various manufacturing processes. Food processing steps such as heating, pressurizing, autoclaving, and fermentation are commonly used in production of hypoallergenic food products (Maier et al., 2009). In recent years, a few reports have indicated that gamma irradiation can alter the allergenicity of certain foods (Kume and Matsuda, 1995; Lee et al., 2000). Modification of the protein structure by gamma irradiation is caused by free radical formed by radiolysis of water (Acasandrei et al., 2007). Protein modifications produced by ionizing radiation include deamination, decarboxylation, reduction of disulfide linkages, oxidation of sulfhydryl groups, breakage of peptide bonds, and changes in valence states of the coordinated metal ions in enzymes (Garrison, 1987; Nawar, 1986).

The allergenicity of an allergenic protein may be decreased or even increased by food processing steps as a result of destruction of epitope structures or the formation of the new epitopes (Besler et al., 2001). In the last few decades, most investigations of methods for reducing the allergenicity of foods have focused on purified allergens or allergen extracts of native foods. Serum albumin is a major allergen of various meats, including beef, pork, and chicken. Purified serum albumin (66 kDa) or extracts of serum albumin obtained from unprocessed meats were mainly used not only for the evaluation of allergenic potential, but also to study the feasibility of reducing allergenicity by means of heat processes. On the other hand, the residual allergenicity of finished meat products has not been investigated. The gelation of meat batter of sausage stars at 40–50 °C (Patana-Anake and Foegeding, 1985). Its thermal transition occurs at 54–57 °C and further consolidation of the gel structure takes place at 72–83 °C (Quinn et al., 1980). Thus, sausage products are manufactured using mild heat processing with temperatures ranging from 75 to 80 °C. In previous studies (Kim et al., 2011), the antigenicity of the heated PSA at 80 °C for 20 min showed a slight decrease. In the result of pork meat treated with heat, antigenic activity of the heated pork meat at 80 °C for 20 min was not completely suppressed (Kim et al., 2009). Hence, at the heat conditions, allergenicity of serum albumin may not be sufficiently eliminated.

Food allergens have a significant extent of structural stability and are generally stable to acid and resistant to degradation by proteases (Sampson, 1999). Porcine serum albumin (PSA) has a

* Corresponding author. Tel.: +82516295831; fax: +82516295824.
E-mail address: dhahn@pknu.ac.kr (D.-H. Ahn).

0969-806X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.radphyschem.2011.05.014

stable structure consisting of 9 separate disulfide-bonded loops (http://www.ncbi.nlm.nih.gov/protein/NP_001005208.1). While reduction of allergenicity of serum albumin of different species by treatment with digestive enzymes has been reported (Fiocchi et al., 1995), there have been no specific reports regarding the effects of digestive enzymes on altering the allergenicity of PSA.

Therefore, the aim of this study is to evaluate the residual antigenicity of PSA in extracts of sausage products and to investigate the possibility of reducing the residual antigenicity by means of gamma irradiation. The digestibility of proteins in food can be affected by food processing. Since little is known about the correlation between digestibility and antigenicity as a result of food processing, we also investigated the combined effect of gamma irradiation and treatment with digestive enzymes on the antigenicity of PSA in extracts of pork sausages.

2. Materials and methods

2.1. Antigen and antibodies

Porcine serum albumin (PSA) and anti-goat IgG rabbit-IgG horseradish peroxidase conjugate were purchased from Sigma Chemical Co. (St Louis, MO, USA). Anti-pig serum albumin goat IgG was obtained from Bethyl Laboratories Inc. (Montgomery, TX, USA).

2.2. Preparation of sausage and raw pork extracts

The sausages (products A and B containing around 90% and 85% pork meat, type: vienna sausage) were purchased from a market and were cut and homogenized at 10,000 rpm for 1 min with 0.01 M PBS (phosphate buffered saline, pH 7.3). Homogenates were centrifuged at 16,000*g* for 30 min. The supernatant was filtered and stored at 4 °C. The protein concentration was adjusted to 1 mg/mL for the competitive inhibition enzyme-linked immunosorbent assay (CI-ELISA) experiment and to 3 mg/mL for the SDS-PAGE experiment, which employed a BSA (bovine serum albumin) protein assay kit (Pierce, Rockford, IL, USA). Fresh pork loins were purchased on the day after slaughter from a local retail store. The method of extraction of raw pork was the same as sausage extracts.

2.3. CI-ELISA

Each well was coated with 100 μL of 10 μg/mL of PSA solution in a 0.2 M bicarbonate buffer (pH 9.6) at 4 °C, overnight. The well was then blocked with 1% gelatin in 0.01 M PBS (pH 7.3) for 2 h at 37 °C. A volume of 50 μL of diluted anti-PSA Gt-IgG (1:500) in 0.01 M PBS and a volume of 50 μL of irradiated and digested sausage extract were added to each well followed by incubation for 2 h at 37 °C. A volume of 100 μL of diluted anti-Gt-IgG rabbit-IgG-HRP secondary antibody conjugate (1:40,000) was added into the well, followed by incubation for 2 h at 37 °C. Phosphate citrate buffer (pH 5) containing 0.05% o-phenylenediamine and 0.04% H_2O_2 was added to the well, followed by incubation for 30 min at 37 °C. The reaction was stopped by addition of 50 μL of 2 M H_2SO_4. Absorbance was measured at 490 nm using an ELISA reader (Model 550, Bio-Rad, CA, USA). After incubation, the wells were washed 3 times with 0.01 M PBS containing 0.1% (v/v) Tween 20. For 100% binding (C_{max}) between the coated PSA solution and anti-PSA Gt-IgG without competitive antigens (raw meat extract and sausage extracts), 50 μL of each primary antibody and 0.01 M PBS (pH 7.3) were added to the well. A volume of 100 μL of 0.01 M PBS (pH 7.3) was used as the blank. The binding ability of PSA for anti-PSA Gt-IgG was calculated using the following equation:

$$\text{binding ability (\%)} = \frac{\text{absorbance of } C_{max} - \text{absorbance of irradiated/digested sausage extract}}{\text{absorbance of } C_{max} - \text{absorbance of non-treated meat extract}} \times 100$$

2.4. SDS–PAGE

SDS–PAGE was carried out according to the method of Laemmli (1970) to determine the degree of PSA modification by gamma irradiation. The concentration of the sample was 3 mg/mL. The SDS–PAGE of the sample was performed on a 15–20% separating gel and 4.5% stacking gel. The gels were stained with CBB (coomassie brilliant blue R250) solution and destained with a solution containing 5% methanol and 7% acetic acid. Molecular weight (Mw) markers were purchased from New England BioLabs (P7702S, Beverly, Massachusetts, USA). The Mw standards were insulin A and B chains (2.3 and 3.4 kDa, respectively), aprotinin (6.5 kDa), lysozyme (14 kDa), trypsin inhibitor (20 kDa), triose-phosphate isomerase (26 kDa), lactate dehydrogenase (36 kDa), MBP2 (42 kDa), glutamic dehydrogenase (55 kDa), serum albumin (66 kDa), phosphorylase b (97 kDa), ß-galactosidase (116 kDa), MBP-β-galactosidase (158 kDa), and myosin (212 kDa). A scanner (Power Look III, Amersham Pharmacia Biotech Company, Piscataway, New Jersey, USA) was used to analyze the gel.

2.5. Gamma irradiation treatment

The finished sausage products were transferred into a cobalt-60 irradiator (IR-79, Nordion International Ltd., Ontario, Canada) with 100-kCi activity at 10 ± 0.5 °C and irradiated at a dose rate of 10 kGy/h. The applied dose levels were 1, 3, 10, and 20 kGy.

2.6. Pepsin and trypsin treatments

Extracts from gamma-irradiated sausages were adjusted with 0.1 M HCl at pH 2 and a pepsin (Sigma Co.) solution in 0.01 M HCl was added to sausage extracts to obtain a pepsin to soluble protein ratio of 1:200 (enzyme:substrate, w/w). The sausage extracts were incubated for 30 min at 37 °C. To inactivate pepsin, 0.1 M NaOH was added to the mixture (pepsin and sausage extracts). Trypsin (Sigma Co.) solution in 0.1 M ammonium bicarbonate was added to the digested sausage extract, at a ratio of 1:300 (enzyme:substrate, w/w). The digestions were incubated for 5, 30, 60, 90, and 120 min at 37 °C. To inactivate trypsin, trypsin inhibitor (Sigma Co.) solution in KH_2PO_4 buffer was added at the same concentration as that of trypsin and the solution was boiled for 10 min. The sample was then stored at 4 °C.

2.7. Statistical analysis

Data were analyzed by analysis of variance and mean comparison according to Duncan's multiple range test at the $P < 0.05$ level using SAS software (SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

3.1. Effect of gamma irradiation on the antigenicity of PSA in extracts of pork sausages

Sausages were extracted after gamma irradiation and the extracts were used to analyze the antigenicity of PSA protein in the sausage extracts. CI-ELISA was performed for measurement of changes in antigenicity induced by gamma irradiation (1, 3, 10,

Download English Version:

https://daneshyari.com/en/article/1891591

Download Persian Version:

https://daneshyari.com/article/1891591

Daneshyari.com