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ARTICLE

Antioxidant Activity of Gamma-Irradiated *Asparagus cochinchinensis* (Asparagi radix) (Lour.) Merr. Extract and Inhibition Effect on Lipid Oxidation of Emulsion-Type Pork Sausage

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Abstract The objective of this study was to determine the antioxidant activity of gamma-irradiated *Asparagus cochinchinensis* (Asparagi radix) (Lour.) Merr. Extract (ARE) and its inhibition effect on food lipid oxidation using emulsion-type pork sausage as a model. ARE was prepared from dried Asparagi radix root and ARE solution (1.0 g/mL) was gamma-irradiated with designated doses at 5, 10, and 20 kGy. Antioxidant activity of ARE solution was determined by measuring 1,1-diphenyl-e-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-9-sulphonic acid) (ABTS) radicals. Activities of DPPH and ABTS radicals were decreased, whereas total phenolic contents increased after gamma irradiation with a dose dependence. Addition of gamma-irradiated ARE dose-dependently retarded lipid oxidation of emulsion-type pork sausage during storage at 4°C. These results indicated that gamma-irradiated ARE might have antioxidant activity more than non-irradiated ARE due to increase of the content of polyphenolic compounds by ionizing radiation.

Keywords *Asparagus cochinchinensis* (Asparagi radix), Asparagi radix extract, emulsion type sausage, antioxidant effects, gamma irradiation

Introduction

Asparagus cochinchinensis (Lour.) Merr. (Asparagi radix) is a perennial herb belonging to family of Liliaceae. It is widely distributed in China, Japan, and Korea (Lee et al., 2008). Asparagi radix has various substances, including asparagine, β-sitosterol, 5-methoxy-methylfurfural, mucopolysaccharide, steroidal saponin, furostanol saponin, and phenol compounds (Shen et al., 2011; Zhu et al., 2014). It has been shown that Asparagi radix is efficacious against inflammation diseases due to its high antioxidant activity (Koo et al., 2016; Lee et al., 2009a; Xiong et al., 2011). Rye et al. (2003) have reported that extract from Asparagi radix can inhibit browning reaction

of mushroom, similar to ascorbic acid. Therefore, Asparagi radix might be used as a natural antioxidant.

Several studies have reported that herbal and plant resources can inhibit lipid oxidation and lead to quality improvement of meat products, including rosemary and citron peel powder (Lee et al., 2005), green tea extract (Yang et al., 2006), *Bokbunja* (*Rubus coreanus*) extract (Park and Chin, 2007), and medicinal herb extract mix (Choe et al., 2008). Herbal resources have also been applied to substitute nitrite, inhibit the generation of nitrosamines, and reduce artificial antioxidants (Ha et al., 2001).

Meanwhile, several studies have reported that irradiation of extracts from natural resources could increase their antioxidant activities, including green tea extract (Byun et al., 2004), cumin seed extract (Kim et al., 2009), seaweeds (Choi et al., 2009), and tamarind juice (Lee et al., 2009b). Increase of the contents of compounds with antioxidant ability such as polyphenols in irradiated solutions of plant extracts is considered due to the breakdown of bind of compounds by radicals generated by ionization of water when irradiating. However, the effect of irradiation on antioxidant activity of Asparagi radix extract (ARE) is currently unknown. Therefore, the objective of this study was to determine the antioxidant activity of gamma-irradiated ARE and its retardation effect on lipid oxidation and color change using emulsion-type pork sausage as a model.

Materials and Methods

Preparation of Asparagi radix extract

Dried Asparagi radix of 8 year old was purchased from Jung Dam A Co. Ltd. (Kyungbuk, Korea). Asparagi radix was milled using a micro hammer cutter mill (Type 3, Culatti Co., Zurich, Switzerland) with 50 mesh. Powder of Asparagi radix of 100 g was put into a beaker containing 1 L of 70% ethanol, stirred for 12 h at 25℃, and centrifuged at 10,000×g for 20 min at 4℃. The supernatant was filtered with a filter paper (Whatman No. 2, Camlab, Frankfurt, UK). The precipitate was extract with 70% ethanol (1 L) as described above twice. Collected supernatants were concentrated using an evaporator (EYELA N-1100 series, Tokyo Rikakikai Co., Ltd., Tokyo, Japan). The concentrate was lyophilized. Lyophilized extract of Asparagi radix was dissolved in deionized distilled water (DDW) to obtain a concentration of 1.0 g/mL. The extract solution was divided into cap tubes and gamma-irradiated at designated doses.

Gamma irradiation

Samples in tightly capped containers were irradiated with a cobalt-60 irradiator (point source, AECL, IR-79, Nordion, Ottawa, Canada) launched into Greenpia Technology Inc. (Kyunggi, Korea) at 0, 5, 10, and 20 kGy of absorbed doses. The source strength was approximately 100 kCi with a dose rate of 70 Gy/min at 20±0.5℃. Dosimetry was performed using 5 mm diameter alanine dosimeters (Bruker Instruments, Rheinestetten, Germany). Free radical signal was measured using a Bruker EMS 104 EPR Analyzer. The actual dose was within±0.02 kGy of the target dose. Samples were turned 360° continuously during the irradiation process to achieve uniform target doses. Non-irradiated ARE (Control) was placed outside the irradiation chamber to have the same environmental temperature effect as the irradiating sample. Irradiated ARE sample solutions were lyophilized and stored in a refrigerator of 4℃.

Scavenging effects against DPPH and ABTS radical

Free radical scavenging effect of ARE was estimated according to the method of Blois (1958). Briefly, 0.1 mL of each non-irradiated and irradiated AREs was added to 0.9 mL of DDW and 1.0 mL of 0.2 mM DPPH radical. The mixture was shaken

at room temperature for 30 min. The reaction mixture was measured at wavelength of 517 nm using a spectrophotometer (UV1600PC, Shimadzu Co. Ltd., Kyoto, Japan).

ABTS was dissolved in ethanol: water (5:1) solvent mixture to prepare a 7 mM ABTS stock solution. ABTS in the stock solution was reacted with 2.45 mM potassium persulfate in ethanol: water (1:3) and allowed to stand in the dark at room temperature for 16-20 h to prepare ABTS•+ radical. After addition of ABTS•+ solution to 10 μL of each sample, measurements were recorded at wavelength of 734 nm. The 50% radical-scavenging concentration is expressed as SC_{50}.

Total phenolic contents

Total phenolic contents were measured using the Folin-Ciocalteau colorimetric method (Gao et al., 2000). Briefly, samples (0.9 mL) of non-irradiated and irradiated AREs were mixed with 0.1 mL of 50 units/mL of ascorbic oxidase and then incubated at 23℃ for 90 min to remove the ascorbic acid. Ascorbic acid-free samples (0.1 mL) were mixed with 0.2 mL of Folin-Ciocalteau reagent (Sigma Chemical Co., MO, USA) and incubated at 23℃ for 1 min. Then 3 mL of 5% of Na_2CO_3 was added. Absorbance at wavelength of 765 nm was then recorded for the mixture after 2 h of incubation at 23℃. Phenolic contents were expressed as garlic acid equivalents.

Manufacture of emulsion type sausage

Emulsion-type cooked pork sausages were prepared with published method (Ahn et al., 2002). Briefly, vacuum packaged, refrigerated lean pork, and frozen pork back fat were obtained from a local meat packer within 48 h of slaughter. They were ground (Model 160, Fatosa, Barcelona, Spain) twice through 9 mm and a 3 mm plates, respectively. All ingredients were purchased from Sewoo Co. Ltd. (Kyunggi, Korea). Spice mix contained coriander, glucose, red pepper, and onion powder. Lean pork (59.38%), salt (1.39), and polyphosphate (0.24) were placed in a silent cutter (C-75, Fatosa) and mixed with meat for about 1 min. Then 50% of ice (9.5%) was added and mixed at a high speed. When the temperature of the mixture decreased by about 1℃–2℃ (approximately 2 min), ground pork back fat (19.0%) was added and mixed until the temperature of the mixture reached 10℃ (about 8 min). The remainder (50%) of ice (9.5%), spices (0.4%), and 0.02% ARE powder (C: non-irradiated, T-5: 5 kGy-irradiated, T-10: 10 kGy-irradiated, or T-20: 20 kGy-irradiated) or 0.02% sodium ascorbate (Positive control, P) were added and mixed until the temperature of the mixture reached 11℃. Total mixing time was about 10 min. The processing room temperature was about 15℃. These sausages were stuffed (Patron Sausage Filler MWF 591, MADO, Oud-Ade, the Netherlands) into collagen casing (2.5 cm of diameter, Woosung Co. Ltd., Seoul, Korea), dried (45℃ for 30 min), smoked (55℃ for 40 min) by sawdust, and cooked to 70℃ of internal temperature (about 1 h) using a smokehouse (Fracomat 1200, Franke GmbH & Co., Koln, Germany). The cooked sausage was cooled by water-spray for 5 min, dried at room temperature for 30 min, and cut into pieces (about 100 g each). Cut sausages were vacuum-packaged (75 cmHg pulled) in oxygen-impermeable nylon bags (2 mL O^2 m^{-2} 24 h^{-1} at 0℃; 20 cm×30 cm using Sunkyung Co. Ltd, Seoul, Korea) using a vacuum packaging machine (Leepack, Hanguk Electronic, Kyunggi, Korea). Samples were stored at a 4℃ refrigerator.

2-Thiobarbituric acid values

Using the method of Turner et al. (1954), thiobarbituric acid (TBA) values of these emulsion-type pork sausages mixed with gamma-irradiated AREs were measured during storage. A 0.5 g sample was homogenized in a 50 mL centrifuge tube with 15 mL extracting solution (5 mL of 20% trichloroacetic acid in 2 M of phosphoric acid, and 10 mL of 0.01 M 2-TBA).

The tube containing the homogenate was heated in boiling water of 100℃ for 30 min with occasional stirring. After chilling in an ice bath for 10 min, 15 mL of solvent mixture of isoamyl alcohol : pyridine (2:1) was added to the tube. The tube was then vigorously shaken for 2 min and centrifuged at 4,800×g for 15 min. The clear solvent extract of the upper layer was obtained and its absorbance at wavelength of 538 nm was measured with a spectrophotometer (UV 1600 PC, Shimadzu, Kyoto, Japan). The concentration (mg/kg sample on the basis of wet weight) of malondialdehyde was calculated by using a determination curve.

Statistical analysis

Data from antioxidant effects and total phenolic contents were subjected to the analysis of variance (ANOVA) using general linear model procedure of SPSS 18.0 software (SPSS Inc., NY, USA) with three replications. The Duncan's multiple range was applied for comparisons of means, differences were considered significant at $p<0.05$. The effects of lipid oxidation of emulsion-type pork sausage were also analyzed by two-way ANOVA. Correlations between variables were determined by correlation analyses using Pearson's linear correlation coefficient with the above statistical software package.

Results and Discussion

Changes in antioxidant effects by gamma irradiation

Gamma irradiation to ARE solution appeared to have radical scavenging effect (Table 1). The activities of DPPH or ABTS radicals decreased by irradiation in a dose-dependent manner. Although gamma-irradiated ARE had not enough as much as antioxidant activity of ascorbic acid, the result indicated that gamma irradiation increased the radical scavenging effect of ARE. Increase of irradiation dose also increased the inhibition effect of ARE on the generation of ABTS radicals, similar to results obtained for DPPH radicals. These results were similar to those of previous reports (Kim et al., 2009; Lee et al., 2009b; Sung et al., 2009), suggesting that contents of polyphenols and other low molecular weight compounds with antioxidant effect might increase in the ARE solution due to the cleavage of bind among organic small compounds by free radicals with powerful reactivity generated by ionizing radiation.

Changes of total polyphenol contents by gamma irradiation

Total polyphenol contents were measured in order to observe correlations between increases of antioxidant effects and total

Table 1. DPPH and ABTS radical scavenging activities of *Asparagus cochinchinensis* (Lour.) Merr. (Asparigi radix) extracts after gamma-irradiation at designated doses

Irradiation dose	DPPH radical	ABTS radical
0 kGy	77.5±3.5[a]	32.5±3.5[a]
5 kGy	50.6±2.6[a]	25.2±2.1[a]
10 kGy	42.4±2.1[b]	17.5±1.6[b]
20 kGy	35.7±1.2[b]	12.5±1.3[c]
Ascorbic acid	1.2±0.5	-
Trolox	-	0.3±0.4

[a–c] Different letters within the same column differ significantly ($p<0.05$).

polyphenol contents in ARE solutions (Table 2). Total polyphenol contents of ARE were increased by gamma-irradiation in a dose-dependent manner.

Increase of total polyphenol contents seemed to be correlated with increase of inhibition effects on the generation of DPPH and ABTS radicals in gamma-irradiated ARE solutions as shown in Table 1. Byun et al. (2004) have reported that ionizing radiation does not affect polyphenol contents in medicinal plants. However, it can increase the release of polyphenols bound with other compounds in the extract solution. The release reaction could depend on irradiation conditions such as irradiation dose, pH and ionic strength of the solution, temperature of irradiation chamber, and radiation type (gamma or electron beam). Previous studies (Choi et al., 2009; Kim et al., 2009; Lee et al., 2009b; Sung et al., 2013) have also shown that ionizing radiation can increase total polyphenol contents in several plants extracts. These results indicate that the increase of antioxidant activities of ARE by gamma irradiation could be due to increase of total polyphenol contents in the extract solution caused by gamma irradiation.

Changes of Thiobarbituric acid reactive subtances (TBARS) of emulsion-type pork sausage with gamma-irradiated ARE

Antioxidant effects of gamma-irradiated ARE were determined using emulsion-type pork sausage as a model food during storage at 4℃ (Table 3). All sausage samples did not show significant differences on the generation of malondialdehyde at 0 day storage. Amounts of malondialdehyde generated in samples were increased during storage. C appeared to have the highest content of malondialdehyde at 3.26 mg/kg while P showed the lowest content at 1.49 mg/kg after 28 day for storage. Lipid

Table 2. Contents (mg/mL) of total polyphenolic compounds in *Asparagus cochinchinensis* (Lour.) Merr. (Asparigi radix) extracts after gamma-irradiation at designated doses

Irradiation dose	Content of total polyphenolic compounds
Control	0.61±0.09[a]
5 kGy	0.74±0.11[ab]
10 kGy	0.87±0.12[b]
20 kGy	1.42±0.08[c]

[a–c] Different letters within the same column differ significantly ($p<0.05$).

Table 3. Changes of TBARS (mg malondialdehyde/kg sample) in emulsified pork sausages manufactured with powder of *Asparagus cochinchinensis* (Lour.) Merr. (Asparigi radix) root extracts gamma-irradiated at designated doses during storage at 4℃ for 28 d

Sample	Storage period (d)				
	0	7	14	21	28
C	0.43±0.04	0.87±0.04[d]	1.56±0.13[d]	2.49±0.18[d]	3.26±0.14[d]
T-5	0.41±0.02	0.81±0.04[d]	1.19±0.05[c]	2.03±0.24[c]	2.97±0.12[c]
T-10	0.36±0.05	0.64±0.03[c]	1.06±0.04[b]	1.55±0.08[b]	2.18±0.05[b]
T-20	0.34±0.03	0.51±0.02[b]	0.81±0.08[a]	1.24±0.05[a]	1.62±0.12[a]
P	0.35±0.06	0.45±0.04[a]	0.69±0.07[a]	1.21±0.08[a]	1.49±0.24[a]

[a–d] Different letters within the same column differ significantly ($p<0.05$).
C, Sample with Non-irradiated ARE; T-5, Sample with 5.0 kGy-irradiated ARE; T-10, Sample with 10.0 kGy-irradiated ARE; T-20, Sample with 20 kGy-irradiated ARE; P, Sodium ascorbate 0.02% only; ARE, Asparigi radix extract.

oxidation was retarded more in samples mixed with gamma-irradiated ARE powder than that in sample mixed with non-irradiated ARE powder (C). The retardation effect showed a dose-dependent manner: T-20 (1.62 mg/kg) > T- 10 (2.18 mg/kg) > T-5 (2.97 mg/kg). The retardation effect of T-20 was similar to that of P from 14-day storage. This result indicated that gamma irradiation increased the antioxidant efficiency of ARE in emulsion-type pork sausage. Similar results have been reported previously (Kim et al., 2009; Sung et al., 2013), showing that antioxidant effect increased by gamma-irradiation can retard lipid oxidation by increase of the contents of polyphenolic compounds compared with non-irradiated samples. Therefore, increase of antioxidant effect in gamma-irradiated ARE could be shown by increase of the content of polyphenols and other organic compounds with low molecular weight rather than by the change of the structure of polyphenols.

Conclusion

This study was conducted to evaluate change of antioxidant activity of ARE by gamma irradiation and application of irradiated ARE to a food additive. Gamma-irradiated ARE showed higher radical scavenging abilities and content of polyphenolic compounds than those of non-irradiated one. In the application study with emulsion-type pork sausage, samples with gamma-irradiated ARE appeared to retard the lipid oxidation dose-dependently during storage. The results indicated that antioxidant activity of ARE can increase by ionizing radiation and the application of irradiated ARE should be considered in the meat processing in order to retard and/or inhibit lipid oxidation of meat products.

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