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The Antimutagenic and Antioxidant Effects of Red Pepper Seed and Red Pepper Pericarp (*Capsicum annuum* L.)

– Research Note –

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Abstract

In this study, we examined the antimutagenicity of red pepper seed and red pepper pericarp ethanol extracts using the standard Ames test in the presence and absence of S9 mix. The extracts showed inhibitory effects on both the TA98 and TA100 *Salmenella* Typhimurium strains against the mutagenic activity of promutagen 2-aminoa nthracene, and were also protective against the directly acting mutagens sodium azide and 2-nitrofluorene. The red pepper seed elicited stronger antimutagenicity than the red pepper pericarp. Both the red pepper seed and red pepper pericarp directly quenched nitric oxide to different degrees and the scavenging activities increased with increasing concentrations. Nitric oxide scavenging activity ranged from 22~77% in the red pepper seed, and from 36~49% in the red pepper pericarp. The TEAC values for red pepper seed extract were 47.89±1.64 mg g^{-1} in the ABTS radical scavenging assays, while those of red pepper pericarp extract were 94.18±1.61 mg g^{-1}. Therefore, we conclude that red pepper seed and red pepper pericarp have antimutagenic activities as well as antioxidant activity.

Key words: mutagenicity, ames test, nitirc oxide scavenging activity, ABTS radical scavenging activity

INTRODUCTION

The red pepper (*Capsicum annuum* L.) has been used since ancient times as a food coloring pigment, thus making foods more attractive and acceptable for consumers. Red pepper is a vegetable known for its rich antioxidant content and antimutagenic capabilities. Its attractive red color is due to various carotenoid pigments, including β-carotene with pro-vitamin A activity, and oxygenated carotenoids such as capsanthin, capsorubin, and cryptocapsin, which are distinct to this genus and are shown to be effective free radical scavengers (1).

One of the most highly valued compounds is the high carotenoids, as ultimately, the commercial value of paprika depends directly on its relative pigment richness resulting from them (2). Red peppers also contain high levels of flavonoids which are also natural phenolics, more specifically, quercetin, luteolin, and capsaicinoids (3). Capsicum cultivars have been identified as vegetables with potentially high antioxidant capacity (4).

Red pepper fruits can be divided into stem, pericarp, placenta, and seed. Pericarp has many carotenoids including capsanthin and carotenoids are very important for their pro-vitamin A activity. Other than the pro-vitamin A activity, β-carotene has antioxidative activity, pigment function, and important physiological activities including anticarcinogenic and anti-aging. In the placenta, lots of capsaicin is present, providing characteristically hot taste of pepper. Capsaicin also has functions in appetite improvement, expansion and constriction of blood vessels, lowering of cholesterol, acceleration of energy metabolism, improvement of blood lipid profiles, and maintains blood homeostasis maintenance of antioxidative vitamins. Essential fatty acids are present in red pepper seeds, and the ω-6 unsaturated fatty acid of linoleic acid makes up 64% of the fat content, with much of the remainder being palmitic and oleic acids. Especially red pepper seed has antimutagenic effects against the aflatoxin B1 and MNNG, and the anticarcinogenic effects in human cancer cells have been reported to be high (5-7). The antioxidant effects of red pepper seed and red pepper pericarp powder have been evaluated by various antioxidant assays. These both show strong antioxidant activities based on the test methods that were used. Yet, higher scavenging activity was exhibited by red pepper pericarp powder than red pepper seed powder (8).

Since mutation is one of the mechanisms of cancer development, an antimutagenic substance is likely to prevent carcinogenesis. Work in this area has indicated that turmeric can protect DNA against lipid peroxide-

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induced damage (9) and against fuel smoke condensate-induced damage (10). Importantly, capsaicin exhibits no mutagenicity or comutagenicity (11). In addition, the methanol extract of red pepper powder exhibited no mutagenicity or comutagenicity induced by Aflatoxin B1 (AFB_1) (11), but rather exerted antimutagenicity toward AFB_1. Both hot red pepper and plain red pepper showed the same activity, and the first fraction inhibited 70% of the mutagenicity.

Research in our laboratory has focused on the biological effects of red pepper, such as its influence on lipid metabolism, action as a digestive accelerant, its beneficial hypocholesterolemic effects on cholesterol gallstone disease and diabetic nephropathy, and the influence of red pepper antioxidants on inflammatory disease (12). However, limited information is available on the antioxidant and antimutagenic effects of red pepper with regional differences such as pericap and seed. Therefore, we added different concentrations of red pepper seed and pricarp to examine the antioxidative and antimutagenic effects. In this study, we examined the antioxidant activities of red pepper pericarp and red pepper seed extracts, in which antioxidant activity was examined using nitric oxide scavenging and ABTS radical scavenging activities. The antimutagenic activity of the extracts of both red pepper pericarp and red pepper seed were examined by using the Ames test in *Salmonella* Typhimurium strains.

MATERIALS AND METHODS

Materials and chemiclas

2,2'-Azinobis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) was obtained from Fluka Chemical Co. and 6-hydroxy-2,5,7,8-tetramethylchroman- 2-carboxylic acid (trolox) from Sigma-Aldrich Chemical Co. Other reagents were of analytical grade. The TA98 and TA100 *Salmenella* Typhimurium strains were purchased from the Korean Collection for Type Culture (KCTC, Daejeon, Korean). For the experiment, the dried red pepper was prepared by purchasing clean pepper (Banhemaru Co., Ltd., Chungbuk, Korea) around October and November, 2006. The peppers were separated into pericarp and seed fractions and ground for the experiments.

Extracts preparation

Air dried red pepper pericarp and processed seed samples were crushed in a grinder for 2 min, but at 15 s intervals the process was stopped for 15 s to avoid heating the samples. Air dried red pepper pericarp and processed ground red pepper seed samples (100 g) were extracted by stirring with 500 mL of 70% ethanol at 80 ^{o}C for 3 hr two times, and then filtering through Whatman No. 2 filter paper. The extracts were filtered and evaporated using a rotary vacuum-evaporator at 50^{o}C, and the remaining water was removed by lyophilisation. The freeze-dried extracts were used in assessments for antimutagenicity and their nitric oxide scavenging effects.

Antimutagenic assay

The *Salmonella* mutagenicity assay (13) was performed with *Salmonella* Typhimurium strains TA100 and TA98. The S9 mix was freshly prepared before each test using an Aroclor-1254-induced rat liver fraction (lyophilized) purchased from Moltox (Molecular Toxicology Inc., Boone, NC, USA). The red pepper pericarp and red pepper seed extracts were dissolved in DMSO before being added to the medium. Then, an experiment including treatments with two doses of each extract at 250 and 125 μg/plate, positive controls, and a negative control with DMSO both in the presence and absence of S9, was carried out. In the absence of S9, 2-nitrofluorene (2 μg/plate) was used as a positive control for the TA98 strain, and sodium azide (1 μg/plate) was used for the TA100 strain. In the prescence of S9, 2-aminoanthracene (2 μg/plate) was used for both the TA98 and TA100 strains. In brief, 0.1 mL of freshly prepared test strain culture ($1\sim2\times10^{9}$ cells/mL), 0.1 mL of fresh sample solution, and 0.5 mL of PBS or S9 were added to capped culture tubes containing 2 mL of top agar (0.6% agar, 0.5% NaCl, 50 μM L-histidine, and 50 μM biotin) held at 45^{o}C. The tubes were then vortexed gently and poured onto minimal glucose agar plates. The plates were wrapped and incubated at 37^{o}C for 48 hr. The colonies were counted manually. All treatments were performed in triplicate.

Nitric oxide scavenging activity

Nitric oxide (NO) scavenging activity was determined according to the method described by Kato et al. (14). Here, nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction as described previously. Sodium nitroprusside, in aqueous solution at physiological pH, spontaneously generates NO (15), which interacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. Scavengers of NO compete with oxygen, leading to a reduced production of nitrite ions. Nitric oxide scavenging activites of red pepper seed and pericarp extracts (1.0 mg/mL) were determined under different conditions (pH 1.2) by measuring the absorbance at 520 nm. Sodium nitrite (1 mM) was mixed with different concentrations of the var-

ious plant extracts dissolved in suitable solvent systems, which were then incubated at 37°C for 1 hr and reacted with Greiss reagent (1% sulphanilamidein in 30% acetic acid, and 0.1% naphthylethylenediamine dihydrochloride in 30% acetic acid). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamme was read at 520 nm, and was referenced to the absorbances of standard solutions of potassium nitrite treated in the same way with Griess reagent. Thus, the nitric oxide scavenging activity (%) was calculated with the following equation:

Nitric oxide scavenging activity (%) = [1 − (absorbance of sample with 1 mM $NaNO_2$ addition, after standing for 1 hr − absorbance of control) / absorbance of 1 mM $NaNO_2$] × 100

ABTS radical scavenging activity

The total antioxidant activities of the red pepper seed and red pepper pericarp extracts were measured by the $ABTS^{+}$ radical cation decolorization assay involving the preformed $ABTS^{\cdot+}$ radical cation (16). ABTS was dissolved in water to a 7 mM concentration, and the ABTS radical cation ($ABTS^{\cdot+}$) was produced by reacting the ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12～16 hr before use. Oxidation of the ABTS commenced immediately, but the absorbance was not maximal and stable until more than 6 hrs had elapsed. The radical cation was stable in this form for more than 2 days of storage in the dark at room temperature. After the mixture was kept in the dark at room temperature for 16 hr, to allow the completion of radical generation, it was diluted with ethanol (99.5%) so that its absorbance was adjusted to 0.70±0.02 at 734 nm. To determine the scavenging activity, 0.9 mL of ABTS reagent was mixed with 0.1 mL of extracts and the absorbance was measured at 734 nm after 6 min of reaction at room temperature, using ethanol as a control. The antioxidant activities of red pepper seed and pericarp extracts were compared by Trolox equivalent content in 1.0 g extracts.

Statistical analysis

The treatment results are expressed as means±standard deviations (SD). To determine the effects of the red pepper seed and red pepper pericarp extract concentrations, the data were analyzed by one-way analyses of variance (ANOVA) using SPSS (Statistical Analysis Program, version 12.0). Significance differences among treatment means were determined by Duncan's multiple range tests ($p<0.05$).

RESULTS AND DISCUSSION

Antimutagenic effect

Table 1 shows the antimutagenic effects of the red pepper pericarp and red pepper seed extracts on 2-nitrofluorene, sodium azide, and 2-aminoanthracene induced mutagenesis. The foremost result was that both the red pepper pericarp and red pepper seed extracts were able to induce evident decreases in mutagenicity for the indirectly acting mutagen 2-aminoanthracene, which acts as a genotoxic compound through a liver S9 fraction. The mutagenicity of 2-aminoanthracene was reduced by more than 100% in both cases. These results are consistently better than those reported by Choi et al. (17) based on the analogue antimutagenicity of red pepper seed and whole red pepper, where at the same concentration of extract per plate, the number of revertants was decreased by 50%. Antimutagenicity was higher in the red pepper seed than the red pepper pericarp. The revertants of the *Slamonella* Typhimurium TA98 strain, induced by 2-nitrofluorene, were significantly reduced when the red pepper pericarp and red pepper seed ex-

Table 1. Anti-mutagenicity of red pepper seed and red pepper pericarp by Ames tests with *S*. Typhimurium strains TA98 and TA100

Treatments	Dosage (μg/plate)	-S9		+S9	
		TA98[1)]	TA100[2)]	TA98[3)]	TA100[3)]
Red pepper pericarp	250	288±31[4)d]	699±73[D]	94±10[a]	144±14[A]
	125	399±10[f]	833±49[E]	266±14[d]	169±19[A]
	0	453±31[g]	1779±51[H]	294±22[d]	419±30[C]
Red pepper seed	250	94±4[a]	877±38[E]	80±4[a]	92±2[A]
	125	223±3[c]	1007±29[F]	137±22[b]	105±7[A]
	0	359±47[e]	1279±84[G]	224±16[d]	312±28[B]
Control		167±5	171±8	199±6	212±9

[1)]2-Nitrofluorene (2 μg/plate). [2)]Sodium azide (1 μg/plate). [3)]2-Aminoanthracene (2 μg/plate). [4)]The values are means±SD.
[a-g]Values with different letters within a column differ significantly ($p<0.001$).
[A-H]Values with different letters within a column differ significantly ($p<0.001$).

tracts were added to the test system. Similarly, the revertants of the *Slamonella* Typhimurium TA100 strain, induced by sodium azide, were significantly reduced when the red pepper pericarp and red pepper seed extracts were added to the test system. However, antimutagenicity was different according to the red pepper part. The seed had a strong inhibitory effect on mutagen-induced mutagenicity, whereas the pericarp showed a low inhibition rate in the Ames test. A preceding study suggesting the cancer prevention effects of capsicum, showed mutagenicity inhibition to vinyl carbamate and nitrosodimethylamine (18). These results indicate that the various parts of red pepper differ in their degrees of antimutagenicity. The red pepper seed rather than the red pepper pericarp had strong antimutagenicity.

Nitric oxide scavenging activity

Nitric oxide reactive nitrogen species such as NO_2, N_2O_4, N_3O_4, NO_3^-, and NO_2^-, which form during reactions with oxygen or superoxide, are very reactive. These compounds are responsible for altering the structural and functional behavior of many cellular components (19).

From Fig. 1, nitric oxide scavenging activities of red pepper seed and peicap were concentration dependent, and all the samples seemed to show nitric oxide scavenging activity. Both the red pepper seed and red pepper pericarp reacted directly with and quenched nitric oxide to different degrees, with increased activities at higher concentrations. Nitric oxide scavenging activity ranged from 22～77% in the red pepper seed, and from 36～49% in the red pepper pericarp. The results showed there was a concentration dependency in the nitric oxide scavenging activity for all the red pepper seed and red pepper

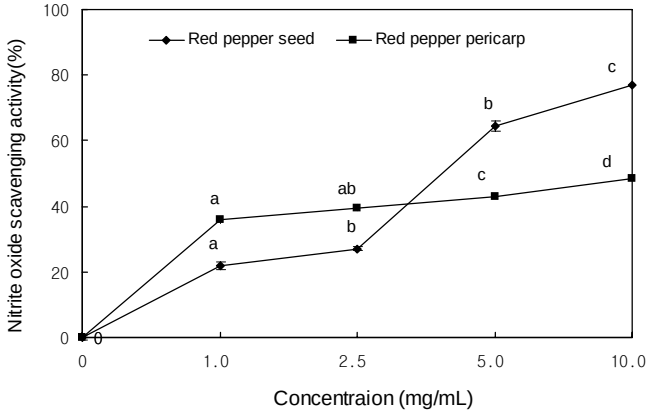

Fig. 1. Nitric oxide scavenging activities of red pepper seed and red pepper pericarp extracts at different concentrations. Each value represents mean±SD (n=3). [a-d]Values with different letters at the same concentration differ significantly ($p<0.001$).

pericarp extracts. The activities of the red pepper pericarp and red pepper seed differed, and the red pepper seed rather than red pepper pericarp generally showed higher activity. It seems that this activity is mostly related to the presence of the phenolic compounds such as flavonoids and other phenolic compounds in the red pepper. Manjeshwar et al. (14) reported that red pepper contains various carotenoid pigments that include β-carotene with pro-vitamin A activity, and other carotenoids such as capsanthin, capsorubin, and cryptocapsin, which are shown to be effective free radical scavengers. Red pepper also contains high levels of natural phenolics and flavonoids like quarcetin, luteolin, and capsaicinoids (3). The role of phenolic compounds as scavengers of free radicals is emphasized in previous reports (20,21). These results suggest that the red pepper seed has greater nitric oxide scavenging effects than the red pepper pericarp.

The roles of NO in numerous disease states have generated considerable discussion over the past several years, ever since the journal *Science* named it the molecule of the year in 1992. NO is an essential bioregulatory molecule required for several physiological processes such as neural signal transmission, immune response, vasodilatation control, and blood pressure; however, the elevation of NO results in several pathological conditions, including cancer. Spices may have properties which counteract the effects of NO formation and in turn may be of considerable interest for preventing the negative effects of excessive NO generation *in vivo* (15).

Antioxidant radical scavenging activity

The ABTS [2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid)] radical scavenging activity is based on antioxidant inhibition of the absorbance of the radical cation 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonate), which has a characteristic long-wavelength absorption spectrum showing its main absorption at 734 nm. The original method was based on the activation of metmyoglobin, acting as peroxidase, with H_2O_2 via the formation of the ferrylmyoglobin radical, which then oxidizes the phenothiazine compound ABTS, forming the $ABTS^{+}$ radical cation. In terms of assay design, several different analytical strategies use decolorization and inhibition strategies, in which the absorbance of the reaction mixture is read when the color of the incubation mixture is stable or at a fixed time point, respectively. For the lag phase strategy, the length of the lag phase is measured before the antioxidant reaction starts (23, 24).

The antioxidant activity and Trolox contents of red peppers were significantly affected by the red pepper parts. The TEAC values for red pepper seed extract were

47.89±1.64 mg g^{-1} in the ABTS radical scavenging assays, while those of red pepper pericarp extract were 94.18±1.61 mg g^{-1} (Fig. 2). Thus, TEAC values of red pepper seed extracts were higher than for the red pepper pericarp extracts. The data obtained revealed that certain compounds in red pepper seed and pericarp are free radical scavenger and primary antioxidants that react with free radicals, which may be attributed their proton donating ability. Kim et al. (25) recently showed that the AAEAC values for red pepper dried by the modified drying methods were 34.87±1.18 mg g^{-1} in the ABTS radical-scavenging assays, while those of red pepper dried by the convention drying method were 10.22± 0.16 mg g^{-1}. Navarro et al. (26) reported that the red pepper extracts exhibited the highest TEAC value compared to the green pepper extracts.

Extensive investigations on the antiradical and antioxidant activities of small phenolics, including flavonoids and phenolic acids, have been reported (27). In addition to these, others have reported that high molecular weight phenolics (tannins) have a greater ability to quench free radicals ($ABTS^{+}$) and that their effectiveness depends more on the molecular weight, the number of aromatic rings, and the nature of hydroxyl group substitutions than on the specific functional groups (28). On the other hand, the formation of tannin-protein complexes, both insoluble and soluble, as the result of conventional food and seed processing, are shown to be potential free radical scavengers and radical sinks. Moreover, such complexes could also be suggested as nutraceutical contributors to prevent free radical mediated diseases that occur in the gastrointestinal tract (29).

In conclusion, the antimutagenic effect, nitric oxide scavenging activity, and ABTS radical scavenging activity of extracts of red pepper seed and red pepper pericarp were evaluated in this study. The antimutegenic effects of red pepper seed and red pepper pericarp extracts increased markedly with increasing concentration. The red pepper seed rather than the red pepper pericarp had stronger antimutagenicity. Overall, the red pepper seed extracts showed higher nitric oxide scavenging activity than the red pepper pericarp extracts. The ABTS radical scavenging activity in the red pepper pericarp extracts were higher than red pepper seed extracts. The DPPH radical scavenging activity and phenol content of linoleic acid in red pepper are high, which increases the antioxidant effect, and the secretion of acidic and neutral steroids is increased to lower cholesterol content in tissue and serum, and has antimutagenic effects against mutagens (30). However, the present studies reported an increase in capsaicin content with aging in red pepper, and several food components including vitamin C have been assumed to be changed into different compositions depend upon red pepper types or species (31). So, the more comprehensive studies are necessary to reveal the varied antimutagenic effects of different red pepper species, and to clarify what component conversions are responsible for such effects. Therefore, summarizing overall antimutagenic effects and antioxidant effect of red pepper, not removing seed from red pepper during red pepper powder process is thought to be more advantageous for antimutagenic and antioxidant effects.

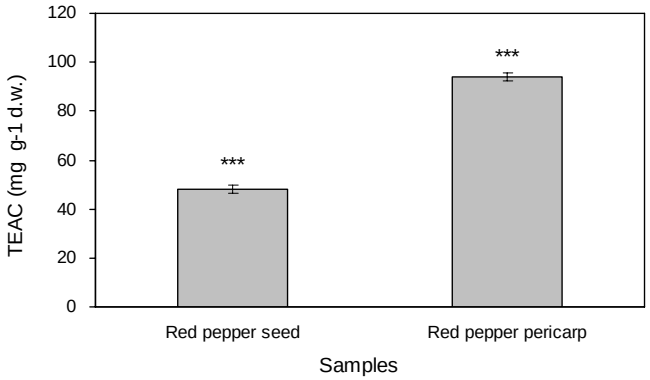

Fig. 2. ABTS radical cation scavenging activities of red pepper seed and red pepper pericarp extracts expressed as Trolox equivalent. Each value represents mean±SD (n=3). ***Significant differences between means at the 0.001% level of probability, respectively.

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