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Antimutagenic Effects of Juices from the Peppers in *Salmonella* Assay System

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Abstract

The antimutagenic effects of juices from green pepper (GP), red pepper leaf (RPL), red pepper (RP) and sweet pepper (SP) were examined by the Ames method using *Salmonella typhimurium* TA100. The juice supernatants of GP, RPL and RP showed antimutagenic activities against aflatoxin B_1 (AFB_1) in *Salmonella typhimurium* TA 100. The juice supernatants of GP and RPL also exhibited the inhibitory effects ($p<0.05$) to the mutagenicities induced by N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) and 4-nitro-quinoline-1-oxide (4-NQO). The juice of RP showed antimutagenic activities against indirect mutagen of AFB_1, however, the activity was reduced at higher concentration (5.0%), furthermore, as the adding concentration of sample increased to 5.0%, it exhibited slight comutagenicity on direct mutagen of MNNG. The antimutagenic activities of GP and RPL juices were reduced significantly after heating at 100° C for 20min, supposing that the antimutagenic compound(s) in the juices were heat labile.

Key words : antimutagenicity, green pepper, red pepper leaf, red pepper, sweet pepper

INTRODUCTION

Human cancer is closely related to daily food (1). Our daily food contains naturally occuring carcinogens and mutagens and artificial ones, such as the pyrolysates of amino acids (2). Fortunately, food also contains anticarcinogenic or antimutagenic substances (3-7). It is known that vegetables and fruit juices inhibit the mutagenic activity of various mutagens or carcinogens (8,9). The antimutagenic substances have been identified from the vegetables and fruits such as vitamin C, vitamin A, chlorophyll, cysteine, polypenols, peroxidase, fibers, and lignin-like compounds (10-18).

Peppers (*Capsicum annum* L.) have long been used as pungent vegetable foods, spices or colorant in several countries including Korea. The genus Capsicum contains over 200 varieties. These range from the very hot peppers to heatless peppers. Peppers are rich in vitamin C and provitamin A contents. Green pepper and red pepper are the major peppers eaten in Korea. The pungency of peppers is due to capsaicin which is a fat soluble, flavorless, odorless and coloreless compound (19). The pigment of red pepper is mainly due to carotenoids, and capsanthin is the major one which comes to 35% of the total carotenoids (20). In Korea, red pepper is used as condiment for various purposes, especially in Kimchi (the Korean traditional fermented food). However, it has little been reported on the physiological effects of peppers including antimutagenic activity.

In this study, the antimutagenic effect of juices from green pepper, red pepper leaf, red pepper and sweet pepper was examined by the Ames method using *Salmonella typhimurium* TA100.

MATERIALS AND METHODS

Material

Green pepper (GP), red pepper leaf (RPL), red pepper (RP) and sweet pepper (SP) were purchased from a local market in Pusan, Korea. RPL was harvested sample on June and July 1992 in Kimhae field area.

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Preparation of juice supernatants from the pepper samples

The samples were washed thoroughly with tap water and crushed by a juicer (Angel Juicer Co., Korea) to obtain the juices. The resulting juices were centrifuged at 9000×g (4° C) for 30min to obtain a clear supernatant. The supernatant solutions were stored at −80° C, and the supernatant was freeze dried at −50 ° C by freeze dryer to get the dried powder. The dried samples was dissolved in autoclaved distilled water with 1% and 5% concentrations. The prepared samples were applied to an antimutagenic assay after sterilizing by filteration through a milipore filter (0.45 μm). To examine the heat stability of antimutagenic compounds in juices, the freeze dried juice supernatants are dissolved in autocalved distilled water and heated them at 100° C for 20min in water bath.

Ames mutagenicity test

Mutagens/Carcinogens

Aflatoxin B_1 (AFB_1), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and 4-nitro-quinoline-1-oxide (4-NQO) were used as mutagens/carcinogens for this study. AFB_1 was purchased from Sigma Chemical Co., St. Louis, Mo, USA, and weighed the appropriate amount and dissolved in DMSO. MNNG and 4-NQO were obtained from Aldrich Co., Milwaukee, WI, USA, and were dissolved in distilled water and 95% ethanol, respectively.

Bacterial strains

Salmonella typhimurium TA100 strain, histidine requiring mutant, were kindly provided by Dr. B. N. Ames, Univ. of Califonia, Berkeley, CA, USA and were maintained as described by Maron and Ames (21). The genotype of the tester strain was checked routinely for the histidine requirement, deep rough (*rfa*) character, UV sensitivity (*uvr* B mutation) and the presence of R factor.

S9 fraction and S9 mix

Sprague-Dawley male rats were injected intraperitioneally with Aroclor 1254 dissolved in corn oil (500 mg/kg of body wt.). Five days after the injections, the rats were sacrified, the livers were removed and minced in 0.15M KCl, and then homogenized with a Potter-Elevhjem apparatus. The homogenates were centrifuged at 9000×g for 10min in a refrigerated centrifuge and the supernatant S9 fraction was distributed in 1.8~2.0ml portion in Nunc tubes, and stored at −80 ° C until used for mutagenic studies. In order to prepare the S9 mix, S9 fraction was thawed immediately before being used for the preparation of S9 mix. Ten percent of S9 fraction in S9 mix was used as S9 mix for the experiment.

Antimutagenicity test

A modified plate incorporation test (22,23) in which 30min liquid preincubation of the organism with the test compounds was employed to determine the antimutagenic effect of the juices on mutagenesis of AFB_1, MNNG and 4-NQO. In the preincubation test, 0.5ml of S9 mix (or 0.5ml phosphate buffer for direct mutagen) distributed in sterile capped tubes in ice bath and then 0.1ml of testers from overnight culture ($1\sim2\times10^9$ cells/ml), 0.1ml of test compound (50μl of mutagen and 50μl of freezed dried juice supernatants) were added. The tubes were vortexed gently and preincubated at 37° C for 30min, 2ml of the top agar kept at 45° C was added to each tube and vortexed 3 seconds. The resulting entire mixture was overlaid on the minimal agar plate. The plates were incubated at 37 ° C for 48hrs and then the revertants bacterial colonies on each plate were counted.

Dose response tests of the mutagens on the tester strain were carried out to determine the regions of revealing mutagenicity induced by mutagens. Toxicity tests for the different juice samples were also carried out (21), and the concentration of the juices for the antimutagenicity test in this study did not show any toxicity to the tester strain.

Statistical analysis

Statistical analysis was performed by analysis of variance. Significant differences between treatment means were determined by using the Student's t test (24).

RESULTS AND DISCUSSION

The juices of GP, RPL and RP showed antimutagenic activity ($p<0.01$) against AFB1 though the activity was low in the juice of SP (Table 1). As the adding concentration increased, the antimutagenic activities increased in RPL, however, the activities decreased in case of GP and RP. It has been reported that the methanol extracts of GP, RPL, RP and SP decreased the mutagenicities induced by various mutagens (25,26). Morita *et al.* (9) reported that juices prepared from cabbage, broccoli, green pepper, egg plant, apple, burdock, shallot, ginger, pineapples and mint were found to possess strong capacities of inactivating the mutagenicity of tryptophane pyrolysis products. Shinohara *et al.* (27) had also shown that the dialyzates of broccoli, burdok, cucumber and green pepper exhibited a high antimutagenic activity.

Table 2 shows the antimutagenic effects of the juices on MNNG. Most of the juices exerted antimutagenicity but RP juice showed a weak effect and slight comutagenicity with 5% level. RPL exhibited the highest inhibition rate of the mutagenicity of MNNG. The antimutagenic effect of the juices on the mutagenicity of 4-NQO in *Salmonella typhimurium* TA100 revealed in Table 3. The juices of GP, RPL and SP inhibited the mutagenicity mediated by 4-NQO significantly ($p<0.01$) in *Salmonella typhimurium* TA100.

From the results, the juices of GP and RPL concluded to inhibit the mutagenicities induced by both indi-

Table 1. Antimutagenic effect of the juices from green pepper, red pepper leaf, red pepper and sweet pepper on the mutagenicity of aflatoxin B1 (AFB1, 0.15μg/plate) in *Salmonella typhimurium* TA100[1]

Treatment	Reverants/plate	Inhibition (%)
AFB1 (Control)	807±25[2]	
AFB1 + Green pepper		
2.5%	473±38**	41
5.0%	576±16**	29
AFB1 + Red pepper leaf		
2.5%	456±76**	44
5.0%	286±39**	65
AFB1 + Red pepper		
2.5%	371±37**	45
5.0%	484± 7**	40
AFB1 + Sweet pepper		
2.5%	710±15	12
5.0%	689±19	17

[1] Spontaneous revertant number was 127±3
[2] The values were 3 replicates±SD and were adjusted by subtracting spontaneous revertants from each revertants/plate
**Significantly different from the control at the $p<0.01$ level

Table 2. Antimutagenic effect of the juices from green pepper, red pepper leaf, red pepper and sweet pepper on the mutagenicity of N-methyl-N'-nitro-N-nitrosoguanidine(MNNG, 0.45μg/plate) in *Salmonella typhimurium* TA100[1]

Treatment	Reverants/plate	Inhibition (%)
MNNG (Control)	1080±44[2]	
MNNG + Green pepper		
2.5%	607±21**	44
5.0%	633±36**	41
MNNG + Red pepper leaf		
2.5%	439±19**	59
5.0%	331±15**	69
MNNG + Red pepper		
2.5 %	940±59*	13
5.0 %	1208±76	−12
MNNG + Sweet pepper		
2.5%	723±59**	17
5.0%	988±94	9

[1] Spontaneous revertant number was 138±48
[2] The values were 3 replicates±SD and were adjusted by subtracting spontaneous revertants from each revertants/plate
*Significantly different from the control at the $p<0.05$ level
**Significantly different from the control at the $p<0.01$ level

Table 3. Antimutagenic effect of the juices from green pepper, red pepper leaf, red pepper and sweet pepper on the mutagenicity induced by 4-nitro-quinoline-1-oxide (4-NQO, 0.15μg/plate) in *Salmonella typhimurium* TA100[1]

Treatment	Reverants/plate	Inhibition (%)
4-NQO (Control)	859± 1[2]	
4-NQO + Green pepper		
2.5%	500± 8**	42
5.0%	578±29**	33
4-NQO + Red pepper leaf		
2.5%	437±54**	49
5.0%	496±58**	42
4-NQO + Red pepper		
2.5%	733± 2**	15
5.0%	822± 4**	4
4-NQO + Sweet pepper		
2.5%	578±35	33
5.0%	680± 9	21

[1] Spontaneous revertant number was 163±1
[2] The values were 3 replicates±SD and were adjusted by subtracting spontaneous revertants from each revertants/plate
**Significantly different from the control at the $p<0.01$ level

rect mutagen of AFB1 and direct mutagens of MNNG and 4-NQO. However, the juice from RP exhibited antimutagenic effect on indirect mutagen (AFB1) and showed a weak antimutagenicity at low concentration (2.5%) on direct mutagen (4-NQO, MNNG) but as the concentration of the sample increased, the activity decreased, and it showed slight comutagenic activity on MNNG. It seems that the active compound (s) of RP juice inhibited the activation of mutagen by S9 mix to the ultimate carcinogen but, they might not affect the direct mutagen.

Kim *et al.* (28) reported that the extracts of red pepper powder did not show any mutagenicity with or without S9 mix in *Salmonella typhimurium* TA100 and TA98. These extracts showed antimutagenicity against AFB1 and did not show comutagenicity on MNNG at 2% level. It seems that juice supernatant of RP contains both comutagenic and antimutagenic substance (s) on MNNG, however, Kweon (29) reported that methanol extract of RP did not show comutagenic effect on MNNG though the antimutagenicity was low compared to other peppers.

The juices of GP, RPL, RP and SP were boiled to examine a changes of antimutagenic effect after heating them at 100° C for 20min. The numbers of histidine requring revertants induced by AFB1, MNNG and 4-NQO were generally reduced by the treatment with GP, RP and RPL juices (Figs. 1~3). As shown in Fig. 1, the antimutagenic activities against AFB1 by RPL and RP reduced significantly ($p<0.05$) after heating. The antimutagenic effects of the juices from GP, RPL and RP on MNNG and 4-NQO also significantly reduced ($p<0.05$) with the heating (Figs. 1 and 2). The antimutagenic effects might be abolished by the boiling of the juices. RP juice again showed reduced antimutagenicity and induced slight comutagenicity especially on MNNG after the heating (Fig. 2). Shinohara *et al.* (27) reported that the antimutagenic activity of the dialyzates of broccoli, burdock, komatsuma, eggplant, carrot and spinach were still retained even after heating them at 100° C for 20min whereas the antimutagenic effects of onion, cabbage, cucumber, green pepper, radish and tomato decreased after heating. It has already been demonstrated that one of the antimutagens in the juice of cabbage was enzyme such as peroxidase which possessed NAD PH-oxidase activity (10). This enzyme (s) can be expected to be unstable when heated and it is possible that the decrease in antimutagenic activity of GP, RPL may have been due to the inactivation of such enzy-

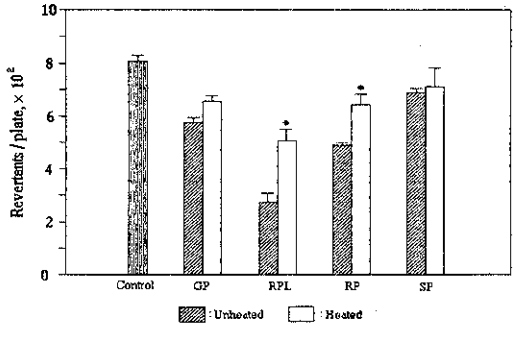

Fig. 1. The effect of heat treatment (100° C for 20min) on the antimutagenicities of green pepper (GP), red pepper leaf (RPL), red pepper (RP) and sweet pepper (SP) against aflatoxin B1 (AFB1, 0.2μg / plate) in *Salmonella typhimurium* TA100[1].

[1] Spontaneous revertant number was 127±3.
The values were 3 replicates±SD and were adjusted by subtracting spontaneous revertants from each revertants / plate.
*Significantly different from the unheated juice sample at the $p<0.05$ level.

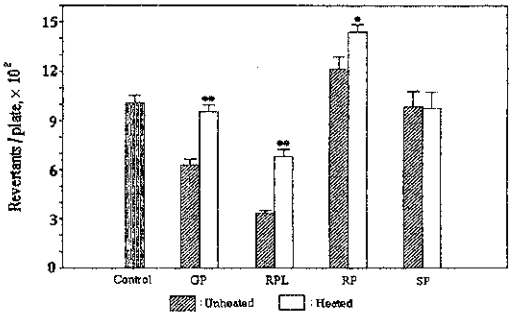

Fig. 2. The effect of heat treatment (100° C for 20min) on the antimutagenicities of green pepper (GP), red pepper leaf (RPL), red pepper (RP) and sweet pepper (SP) against N-methyl-N′-nitro-N-nitrosoguanidine (MNNG, 0.45μg / plate) in *Salmonella typhimurium* TA100[1].

[1] Spontaneous revertant number was 138±48.
The values were 3 replicates±SD and were adjusted by subtracting spontaneous revertants from each revertants / plate.
*Significantly different from the unheated juice sample at the $p<0.05$ level.
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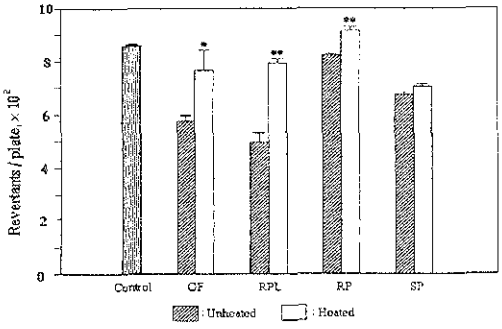

Fig. 3. The effect of heat treatment (100° C for 20min) on the antimutagenicities of green pepper (GP), red pepper leaf (RPL), red pepper (RP) and sweet pepper (SP) against 4-nitro-quinoline-1-oxide (4-NQO, 0.15μg / plate) in *Salmonella typhimurium* TA100[1].

[1] Spontaneous revertant number was 163±1.
The values were 3 replicates±SD and were adjusted by subtracting spontaneous revertants from each revertants/plate.
*Significantly different from the unheated juice sample at the $p<0.05$ level.
**Significantly different from the unheated juice sample at the $p<0.01$ level.

mes by heating. Further study is needed on the identification of the active antimutagenic compounds, the mechanism of the antimutagenicity and *in vivo* experiment in detail. It also need the further study on finding the major compound(s) that lower the antimutagenicity or causing a weak comutagenicity in the juice of RP.

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살모네라 실험계에서 고추류 즙액의 항돌연변이 효과

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요 약

풋고추, 고추잎, 붉은고추 및 피망의 즙액은 아플라톡신 B1(AFB1)에 대해서 항돌연변이 효과가 나타났는데, 첨가 농도 2.5와 5%에서 특히 고추잎의 즙액이 가장 현저한 항돌연변이 효과(44~65%)가 있었으며, 붉은고추 역시 40~45% 정도의 돌연변이 억제 효과가 관찰되었다. 그리고 N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)와 4-nitro-quinoline-1-oxide (4-NQO)에 대한 각 시료 즙액들의 항돌연변이 효과를 실험한 결과 붉은고추를 제외한 나머지 시료들에서는 항돌연변이 효과를 나타내었다. 붉은고추의 즙액은 사용한 돌연변이원의 종류에 따라서 그 항돌연변이 활성에 큰 차이를 보였으며 직접 돌연변이원인 MNNG와 4-NQO에 대해서는 AFB1에서와는 달리 약한 항돌연변이원성을 나타내거나 농도가 증가됨에 따라 약간의 보돌연변이원성이 관찰되었다. 또한 각 시료 즙액들의 가열(100°C, 20분)에 의한 항돌연변이 효과의 변화를 살펴보면 피망을 제외한 풋고추, 붉은고추, 고추잎은 가열에 의해 항돌연변이 효과가 현저히 감소되어 고추류 즙액의 항돌연변이 활성 물질은 열에 불안정한 것으로 나타났다.