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Inhibitory Effect of *Kochujang* Extracts on Chemically Induced Mutagenesis

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Abstract

Antimutagenic effects of 5 kinds of *kochujang* (Korean red pepper soybean paste) samples compared with *doenjang* (Korean soy paste) were studied using the Ames test with *Salmonella typhimurium* TA100 and the SOS chromotest with *E. coli* PQ37. The antimutagenic effects of methanol extracts from red pepper powder and *meju* (fermented soybean) powder, the major ingredients of the *kochujang*, were also evaluated for the mutagenicity of aflatoxin B_1 (AFB_1) in the Ames assay. The methanol extracts from the *kochujang* samples showed lower antimutagenicities than those of *doenjang* against AFB_1 and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in the Ames assay. Traditional *kochujang* I and II exhibited strong antimutagenic activity against AFB_1 and MNNG. The traditional *kochujang* III also effectively reduced the mutagenicity induced by MNNG. The antimutagenic effects of the *kochujang* samples against MNNG were also observed in the SOS chromotest system with the same fashions as shown in the Ames mutagenicity test. The methanol extracts from *meju* powder had the strongest inhibitory effects on mutagenicity induced by AFB_1, however, those from red pepper powder showed lower inhibition rate than *kochujang*. These results suggest that traditional *kochujang* exhibit higher antimutagenic activity than the commercial variety, and that *meju* powder seems to be one of the major antimutagenic components in *kochujang*.

Key words: *kochujang*, Ames test, SOS chromotest, antimutagenicity

INTRODUCTION

Kochujang (red pepper soybean paste) is a traditional Korean fermented food, which has been eaten with *doenjang* (soy paste) for a long time in Korea. It has played an important role in providing the specific taste and flavor in foods. Various tastes of *kochujang* have originated from the fermentation of raw materials such as soybean, starch sources and red pepper powder (1-5). The compound primarily responsible for the pungency of *kochujang* is capsaicin in red pepper powder. Kawada et al. (6,7) reported that capsaicin enhanced lipid and energy metabolism. In humans, Lim et al. (8) found that the ingestion of red pepper powder stimulated carbohydrate oxidation at rest and during exercise.

Kochujang can be classified into two groups, traditional *kochujang* using *meju* (by the conventional method) and commercial one (by the convenient method) using *koji* or bacterial enzyme. Generally traditional *kochujang* is made of glutinous rice, *meju* (naturally fermented soy paste), red pepper powder and salt, which is fermented by enzymatic reaction of bacteria or yeast. The malt is an optional ingredient that may be used to saccharify glutinous rice. *Meju* makes the difference between traditional *kochujang* and the commercial variety. In commercial *kochujang*, saccharogenic amylase or *koji* from glutinous rice which had already been incubated with *Aspergillus oryzae* is added. Differences in quality of *kochujang* is due to the ratio of raw material, fermentation time, and meshing method. Also it varies with different localities and manufacturers.

Kochujang is fermented with red pepper powder and *meju* etc. that are known to have antimutagenic and the anticancer properties (9-16). Several studies indicated that red pepper powder revealed antimutagenic activity(9-11). It was reported that some components of soybean, especially trypsin inhibitor, isoflavones, saponin and phytic acid etc., showed anticancer effects (12-16). There was a report that *meju* which was fermented soybean cake showed antimutagenic effect against AFB_1 (17).

In this study, to determine the antimutagenic effects of methanol extracts from 5 varieties of *kochujang* (3 traditional and 2 commercial product), compared with *doenjang*, the Ames test and the SOS chromotest were carried out. The effects of methanol extracts from red pepper powder and *meju* powder, the major ingredients of *kochujang*, were also evaluated on AFB_1-mediated mutagenicity in the Ames assay.

MATERIALS AND METHODS

Samples

Traditional *kochujang* I, II, *doenjang*, red pepper powder and *meju* were obtained from Sunchang traditional *kochujang* village (Dookeobi Food Co., Sunchang, Choenbuk). Traditional *kochujang* III were obtained from Andong. Commercial *kochujang* I (chungjungwon from Miwon, Co., Sunchang, Cheonbuk) and Commercial *kochujang* II (Hapyo, Co., Chungnam)

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were purchased from local market in pusan, Korea.

Kochujang and *doenjang* samples were freeze dried and powdered, and *meju* was powdered. 20-folds of methanol was added to the powdered samples and extracted by shaking with 3 times. The methanol extracts were evaporated using a vacuum evaporator, concentrated, then dissolved in dimethyl sulfoxide (DMSO, Sigma Chemical Co., USA).

Mutagens

AFB_1 was purchased from Sigma Chemical Co., St. Louis, Mo. (USA) and dissolved in DMSO. MNNG was obtained from Aldrich Chemical Co., Milwaukee, WI (USA) and dissolved in distilled water.

Ames mutagenicity test

The *Salmonella typhimurium* TA100 bacterial strain, a histidine requiring mutant was provided by Dr. B. N. Ames, Univ. of California, Berkerly, CA, USA and was maintained as described by Marons and Ames (18). The genotype of the tester stain was checked routinely for their histidine requirement, deep rough (*rfa*) character, UV sensitivity (*uvr* B mutation) and the presence of R factor.

S9 mixture to activate the indirect mutagen, AFB_1, was also prepared by the method of Maron and Ames (18). The mutagenicity test (19,20) was carried out by a modified plate incorporation test (liquid preincubation of the organism with the test compound). In the preincubation test, 0.5 ml of S9 mixture (or 0.5 ml of phosphate buffer for direct mutagen, MNNG) was distributed into sterilized capped tubes in an ice bath and then 0.1 ml of test strain cultured overnight ($1\sim2\times10^9$ cells/ml), 0.1 ml of test compound (50 µl of mutagens and 50 µl of methanol extracts) were added. The tubes were vortexed gently and preincubated at 37°C for 20 min. 2 ml of the top agar supplemented with L-histidine and D-biotin kept at 45°C were added to each tube and vortexed for 3 seconds. The resulting entire mixture was overlaid on the minimal agar plate. The plates were inverted and incubated at 37°C for 48 hrs and then the revertant bacterial colonies on each plate were counted. Dose response tests (18) of the mutagens on the tester strain were carried out to determine the regions revealing mutagenicity induced by the mutagens. Toxicity tests for the different levels of the methanol extracts were also carried out. The methanol extracts used for antimutagenicity test did not show any toxicity on the test strain.

SOS Chromotest

The modified method of Quillardet and Hofnung (21), and Baik and Ham (22) was employed. 50 µl of frozen stock of *E. coli* PQ37 was added to 5 ml/L medium and incubated in shaking water bath at 37°C overnight, then it was inoculated to the 5 ml/L medium and incubated for 2 hrs at 37°C until absorbance at 660 nm reached 0.3~0.4, in the active culture. The obtained active culture was diluted 10 fold with L medium. 100 µl of the diluted culture was distributed to the 2 series in the wells of 96 well plate. 20 µl of methanol extracts that was treated with mutagen (10 µl methanol extracts + 10 µl mutagen) were added, and then the SOS response was induced at 37°C for 90 min. 100 µl of ONPG (*o*-nitrophenyl-β-D-galactopyranoside) and 100 µl of PNPP (*p*-nitrophenyl phosphate disodium) were added to each set of the wells to determine the activities of β-galactosidase (β-G) and akaline phosphatase (A-P), respectively.

After the color development for 30 min., 100 µl of 1.5 M Na_2CO_3 and 50 µl of 1 M HCl were added to stop the color developments of β-G and A-P, respectively. After 5 min., 50 µl of 2 M Tris buffer was added to the A-P assay system to neutralize the HCl and then the absorbance of each well was read on Elyza reader at 420 nm. Units of enzyme activities were calculated using the formula outlined by Miller (23).

$$Eu = \frac{1000 \times OD_{420}}{t(min)}$$

Statistical analysis

Statistical analysis was performed by analysis of variance. Significant differences between treatment means were determined by using Duncan's multiple range test (24).

RESULTS AND DISCUSSION

The methanol extracts from various kinds of *kochujang* and *doenjang* showed antimutagenic activity against AFB_1 in *Salmonella typhimurium* TA100 (Table 1). *Doenjang* (Korean fermented soy paste) has already demonstrated its strong antimutagenic and anticancer activities (25-34). Compared to the *doenjang* sample, extracts from *kochujang* exhibited lower antimutagenicities. However, AFB_1 induced mutagenesis was especially inhibited by traditional *kochujang* II which inhibited

Table 1. Effect of methanol extracts from various kinds of *kochujang* and *doenjang* on the mutagenicity induced by aflatoxin B_1 (AFB_1, 2.0 µg/plate) in *Salmonella typhimurium* TA100

Treatment	Revertants/plate (level of sample, mg/plate)	
	1.25	2.5
Spontaneous	92 ± 1[6)]	
Control	1245 ± 32^a	
Traditional *kochujang* I[1)]	739 ± 23^d (44)[7)]	640 ± 15^e (52)
Traditional *kochujang* II[2)]	711 ± 15^d (46)	561 ± 17^f (59)
Traditional *kochujang* III[3)]	874 ± 17^c (32)	774 ± 22^d (41)
Commercial *kochujang* I[4)]	918 ± 14^c (28)	826 ± 11^c (36)
Commercial *kochujang* II[5)]	1025 ± 24^b (19)	917 ± 16^b (28)
Doenjang	655 ± 16^e (51)	485 ± 28^g (66)

[1)]Sunchang traditional *kochujang* prepared with glutenous rice powder
[2)]Sunchang traditional *kochujang* prepared with glutenous rice powder and malt
[3)]Andong traditional *kochujang*
[4)]Sunchang chungjungwon
[5)]Haepyo
[6)]The values are means of 3 replicates ± SD
[7)]The values in parentheses are the inhibition rates (%)
[a~f]Means with the different letters beside data are significantly different at the p<0.01 level of significance as determined by Duncan's multiple range test.

the mutagenicity of AFB_1 by 59% at 2.5 mg/plate concentration. The inhibition rates for AFB_1 were 41~59% for the traditional *kochujang* samples while the inhibition rates of commercial *kochujang* extracts were 28~36% on the 2.5 mg/plate. Among the traditional *kochujang* samples, Sunchang traditional *kochujang* had higher antimutagenicity than the Andong variety. Jung et al. (35) reported that *kochujang* had an inhibitory effect similar to *doenjang* on the mutagenicity induced by AFB_1 in *Salmonella typhimurium* TA98 and homemade *kochujang* had higher antimutagenicity than commercial *kochujang*. Therefore, the traditional *kochujang* has higher antimutagenicity than commercial *kochujang*, even though the methanol extract of *kochujang* is less effective than that of *doenjang*. It is thought that the differences in the activity between the traditional *kochujang* and commercial variety depended on the ratio of raw material, fermentation time and meshing method. Table 2 showed the antimutagenicity of the methanol extracts of *kochujang* samples and *doenjang* against MNNG. The methanol extracts from traditional *kochujang* I and II exhibited weaker antimutagenic effects against direct mutagen, MNNG, than against indirect mutagen, AFB_1. However, on the 2.5 mg/plate, both of them inhibited the mutagenicity of MNNG by above 40%.

Table 2. Effect of methanol extracts from various kinds of *kochujang* and *doenjang* on the mutagenicity induced by N-methyl-N′-nitro-N-nitrosoguanidine (MNNG, 0.3 μg/plate) in *Salmonella typhimurium* TA100

Treatment	Revertants/plate (level of sample, mg/plate) 1.25	2.5
Spontaneous	105± 7[6]	
Control	1333±18[a]	
Traditional *kochujang* I [1]	923±25[cd] (33)[7]	807±26[de] (43)
Traditional *kochujang* II [2]	915±28[cd] (34)	767±17[e] (46)
Traditional *kochujang* III [3]	954±15[c] (31)	833±17[d] (41)
Commercial *kochujang* I [4]	1052±21[b] (23)	924±19[c] (33)
Commercial *kochujang* II [5]	1095±21[b] (19)	973±12[b] (29)
Doenjang	896±15[d] (36)	672±35[f] (54)

[1~7]The explanation is the same as shown in Table 1.
[a~f]Means with the different letters beside data are significantly different at the $p<0.01$ level of significance as determined by Duncan's multiple range test.

It is known that NaCl in foods plays a cocarcinogenic role in the presence of MNNG (10, 36-37). Although *kochujang* contained high level (10~15%) of NaCl, its extracts inhibited the mutagenicity of MNNG. These results suggest that *kochujang* samples have antimutagenic effects on both AFB_1 and MNNG. In the SOS chromotest, the *kochujang* extracts also revealed antimutagenic effects on the direct mutagen, MNNG (Table 3). 43~45% of SOS response induced by MNNG were blocked by adding 100 μg/assay of traditional *kochujang* samples but commercial *kochujang* extracts showed 27~33% inhibition rate ($p<0.01$). Patterns of antimutagenic effects were almost the same as shown in the Ames mutagenicity test. From the above studies, the methanol extracts of traditional *kochujang* concluded to reveal higher antimutagenic activities than those of commercial variety against AFB_1 and MNNG in Ames test, and MNNG in SOS chromotest

It is reported that the quality of *kochujang* is influenced by several factors such as ratio of raw material, fermentation time and meshing methods (3). Among the ingredients of *kochujang*, red pepper powder and *meju* (fermented soybean cake) powder that are known for their antimutagenic and anticancer activities (9-11,17) were evaluated on AFB_1-mediated mutagenicity as compared with *kochujang* in the Ames assay (Table 4). At the 2.5 mg/plate concentration, the methanol extract of *meju* powder exhibited 83% antimutagenicity against AFB_1, while that of red pepper powder showed 29% antimutagenicity. *Meju* pow-

Table 4. Effect of methanol extracts from *kochujang*, red pepper powder and *meju* on the mutagenicity induced by aflatoxin B_1 (AFB_1, 2.0 μg/plate) in *Salmonella typhimurium* TA100

Treatment (mg/plate)		Revertants/plate	Inhibition rate (%)
Spon		107± 6	
Control		1246±23	
kochujang	1.25	713±17	47
	2.5	642±15	53
Red pepper powder	1.25	918±45	29
	2.5	1027±21	18
Meju powder	1.25	414±23	73
	2.5	295±23	83

[1]The values are means of 3 replicates±SD

Table 3. SOS response of methanol extracts(100 μg/assay) from various kinds of *kochujang* and *doenjang* against N-methyl-N′-nitro-N-nitrosoguanidine (MNNG, 40 ng/assay) in *E. coli* PQ37

Treatment	β-Glactosidase(β) OD_{420}	Unit	Alkaline phosphatase(ρ) OD_{420}	Unit	(β)/(ρ)	SOS induction factor	Inhibition rate (%)
Spon	0.467±0.003[6]	15.6	0.415±0.010	13.8	1.13	1.00	
Control	1.093±0.048	36.4	0.049±0.004	14.0	2.60[a]	2.30	
Traditional *kochujang* I [1]	0.815±0.016	27.2	0.418±0.003	13.9	1.95[c]	1.73	44
Traditional *kochujang* II [2]	0.804±0.010	26.8	0.414±0.005	13.8	1.94[c]	1.72	45
Traditional *kochujang* III [3]	0.832±0.009	27.7	0.422±0.070	14.1	1.97[c]	1.74	43
Commercial *kochujang* I [4]	0.889±0.010	29.6	0.423±0.007	14.1	2.10[b]	1.86	33
Commercial *kochujang* II [5]	0.926±0.016	30.9	0.421±0.003	14.0	2.20[b]	1.95	27
Doenjang	0.725±0.010	24.2	0.424±0.006	14.1	1.70[d]	1.50	62

[1~6]The explanation is the same as shown in Table 1.
[a~d]Means with the different letters beside data are significantly different at the $p<0.01$ level of significance as determined by Duncan's multiple range test.

der exerted stronger antimutagenicity than *kochujang* and red pepper powder. The above data suggest that the high antimutagenicity of *kochujang* probably results from some end products produced by the action of microorganisms during fermentation of the soybean or soybean itself. There was the report that *meju* showed an antimutagenic effect on AFB_1 (17). Thus, the amount of *meju* in *kochujang* can be one of the important factors that inhibit mutagen-induced mutagenesis.

In conclusion, the methanol extract of *kochujang* is less effective than that of *doenjang* in antimutagenic activity and traditional *kochujang* has higher antimutagenic activity than commercial *kochujang* according to the Ames test and the SOS chromotest. Further study is needed to identify the components and mechanisms for the differences of activity between traditional *kochujang* and commercial *kochujang*.

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