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A Study on Buchu(Leek, *Allium odorum*) Kimchi

–Changes in Chemical, Microbial and Sensory Properties, and Antimutagenicity of Buchu Kimchi during Fermentation–

Kyeoung-Im Lee*, Keun-Ok Jung, Sook-Hee Rhee†, Myung-Ja Suh and Kun-Young Park

Dept. of Food Science and Nutrition, and Kimchi Research Institute, Pusan National University, Pusan 609-735, Korea
**Dept. of Traditional Food Preparation, Yangsan Junior College, Yangsan 626-800, Korea*

Abstract

This study was conducted to investigate the changes in chemical, microbial and sensory characteristics, and antimutagenicity of buchu(leek, *Allium odorum*) kimchi during fermentation at 15°C. Reducing sugar contents and pH of buchu kimchi were decreased during the fermentation. The reduction rate of reducing sugar of glutinous rice paste and shrimp added buchu kimchi(GSBK) was faster than those of control buchu kimchi(CBK) and glutinous rice paste added buchu kimchi(GBK). Acidity increased rapidly until 4 days, and optimum acidity (0.6%) of buchu kimchi was reached within 2 days. Also total bacterial and lactic acid bacterial counts greatly increased after 4 days of the fermentation. The numbers of lactic acid bacteria after 8 day-fermentation in CBK and GSBK, and 10 day-fermentation in GBK were the highest values, 4.7×10^8CFU/ml, 4.8×10^8CFU/ml and 6.1×10^8CFU/ml, respectively. In the sensory evaluation, appearance of sample was good at 0 day, taste and overall quality of buchu kimchi were acquired the highest values at 6th day. The methanol extracts from buchu kimchi(GBK) showed antimutagenicity against aflatoxin B_1(AFB_1) in *Salmonella typhimurium* TA100. The inhibition ratios were 58~69% with treatment of the 5% methanol extracts, and when the adding concentration increased the effect increased.

Key words: buchu(leek, *Allium odorum*) kimchi, fermentation sensory characteristics, antimutagenicity

INTRODUCTION

Kimchi is a Korean traditional food, which refers to the fermented vegetable product. Major ingredients and various condiments(especially, red pepper, garlic and salted fish) are used in kimchi preparation, and kimchi is fermented as the action of microorganisms from the ingredients(1).

There are many types of kimchi depending on the ingredients and preparation methods used. About 30 items are known as the traditional special kimchi in Kyungsang province. In these various kimchi, buchu kimchi is an important popular kimchi in Pusan and Kyungnam, southern regions of Korea(2,3).

Buchu(Leek, *Allium odorum*) is generally cultivated in Korea and Japan, which is originated in China and India. Presently, leeks mainly cultivated in Kimhae and Pohang in Korea, are used for the kimchi preparation and other dishes, since the amounts of buchu production in the above areas are more than any other regions in Korea and the quality is good. Leeks are available all the year round, however, the leeks cultivated from early spring to summer are more tender and tasty(4). Leeks are rich in vitamin A, B_1, and C(2). The allyl sulfide from the leek is known to remove the odor of fish and meat. Leeks have the medical effects such as helping digestion, intestinal disorders, and treatment of burns(5).

Leek is used as the major ingredient and subingredient(condiment) in the preparation of kimchi because of the unique flavor and taste. Buchu kimchi is prepared with a large quantities of red pepper powder and salted anchovy juice, therefore, it is known as a good side dishes which is because of the unique flavor of leek and hot taste(2).

Although many studies have been conducted on the kimchi, most of them were focused on baechu(Chinese cabbage) kimchi. Quality characteristics of Chinese cabbage kimchi with different ingredients(6,7) and effect of lactic acid bacteria and temperature of kimchi fer-

†Corresponding author

mentation(8,9) were reported. There are studies about the quality changes during fermentation of the kimchi (10-12), especially, added cooked glutinous rice flour and soused shrimp during kimchi fermentation(13-15). There are also several studies on godulbaegi(Korean lettuce) kimchi(16), dongchimi(17,18) and yulmoo kimchi (19,20).

In order to develop and standardize the traditional special kimchi in Kyungsang province buchu kimchi, was chosen through the survey in Pusan and Kyungnam province and using the literatures including cooking books(2,3). The aim of this study is to investigate changes in chemical, microbial and sensory properties, and the antimutagenic effect of buchu kimchi.

MATERIALS AND METHODS

Preparation of buchu kimchi

Leek, garlic, ginger, red pepper powder, fermented anchovy juice, sugar, glutinous rice and crushed small shrimp were purchased from the Agricultural Cooperative Association and Onchun market in Pusan, Korea.

The ingredient ratios and preparation methods of buchu kimchi were employed as described by Han et al.(3). The leeks were cut into 2~3 pieces and soaked in 20% salt solution for 20 minutes at room temperature, then rinsed with water twice. The compositions of the leek and other ingredients are shown in Table 1. The samples employed in the experiments were control buchu kimchi(CBK), glutinous rice paste added buchu kimchi(GBK), and glutinous rice paste and shrimp added buchu kimchi(GSBK). The initial NaCl concentration of the sample was 2.2~2.3%. The prepared kimchi was put into pint jars, pushed the stuffs, down and the lid closed tightly and then fermented for 10 days at 15°C.

Table 1. The ratio of ingredients used for various buchu kimchi preparation (Units : g)

Ingredients	CBK[1]	GBK[2]	GSBK[3]
Leek	1000	1000	1000
Garlic	47	47	47
Ginger	21	21	21
Red pepper powder	67	67	67
Fermented anchovy juice	108	108	108
Sugar	17	17	17
Glutinous rice paste	–	130	130
Shrimp	–	–	100

[1]Control buchu kimchi
[2]Glutinous rice paste added buchu kimchi
[3]Glutinous rice paste and shrimp added buchu kimchi

Determination of pH, acidity and reducing sugar

The blended kimchi samples were filtered with cheese cloths and the pH of the filtrate was determined using pH meter. The acidity was determined by the method of AOAC(21). A drop of phenolphthalein was dropped to the kimchi filtrate and titrated with 0.1N NaOH, then the lactic acid content was calculated and expressed as the acidity(%). In order to analyze the content of reducing sugar, 100ml of distilled water was added to the filtrate, and then Fehling solution was added, heated and then cooled. The amount of reducing sugar was determined by the method of Schoorl(22) using titration with 0.1N $Na_2S_2O_3$ standard solution.

Microbial analysis

Plate count agar(PCA) was used for the determination of total bacterial counts. Lactic acid bacteria was enumerated on MRS agar. All plates were triplicated and incubated at 37°C for 2 days. Viable cell numbers were counted as colony forming units(CFU)/ml(23).

Sensory evaluation

Sensory evaluations were conducted by a panel of 7 experienced judges. Characteristics of buchu kimchi evaluated were appearance and color, carbonated flavor, off-flavor, taste, texture and overall acceptability quality using a five point scale hedonic test(24). Sensory scores were statistically analyzed according to the ANOVA-oneway and Duncan's multiple range test(25).

Antimutagenicity test of buchu kimchi

Methanol extraction of buchu kimchi :

After fermentation at 15°C for 0, 6 and 10 days respectively, kimchi samples(GBK) were freeze dried and powdered, 20-folds of methanol was added to the powdered samples and extracted by shaking 3 times. The methanol extracts were evaporated using vacuum evaporator, concentrated, then dissolved in dimethyl sulfoxide(DMSO, 1.25%, 2.5% and 5.0%)(26).

Mutagen/carcinogens :

Aflatoxin B_1(AFB_1) was purchased from Sigma Chemical Co.(USA) and appropriate amount was dissolved in DMSO.

Mutagenicity/antimutagenicity test :

Mutagenicity/antimutagenicity test of the buchu kimchi extracts were carried out by using Ames assay system(27). *Salmonella typhimurium* TA100, histidine requiring mutants was used as the testing strain. To activate AFB_1, the S9 mixes were prepared from the S9 fraction(microsomal fraction of rat liver) according to the method of Maron and Ames(28). The S9 mix was prepared with 8mM $MgCl_2$, 33mM KCl, 5mM glucose-6-phosphate, 4mM NADP, 100mM sodium phosphate(pH 7.4) and the S9 fraction(10%). In the preincubation test, 0.5ml of S9 mix, 0.1ml of the test strain from an overnight culture($1\sim2\times10^9$cells/ml) and 0.1ml of the test sample(100μl of the diluted kimchi extracts for mutagenicity test, and 50μl of mutagen and 50μl of the kimchi extracts for the antimutagenicity test) were added. The tubes were vortexed gently and preincubated at 37°C for 30min. Two milliliters of the top agar 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 incubated at 37°C for 48 hrs, then the revertant bacterial colonies on each plate were counted.

The toxicity test for the buchu kimchi extracts was also carried out and the concentrations employed for the test in this study did not show any toxicity to the tester strain.

RESULTS AND DISCUSSION

Fig. 1 shows the changes in reducing sugar content of control buchu kimchi(CBK), glutinous rice paste added buchu kimchi(GBK) and glutinous rice paste and shrimp added buchu kimchi(GSBK) during fermentation at 15°C. The initial contents of reducing sugars of CBK, GBK and GSBK were 2.41g%, 2.37g% and 2.41g%. They decreased to 1.82g%, 1.58g% and 1.14g% after 10 days, respectively. The reduction rate of reducing sugar of the GSBK was faster than those of CBK and GBK. This result was in agreement with the report that content of reducing sugar exhibited a higher value as the addition of glutinous rice flour, but a lower value with increasing the soused shrimp concentrations(13,14).

The curve pattern of pH reduction(Fig. 2) was almost similar to that of reducing sugar reduction. The initial pHs of CBK and GBK were 5.03 and 5.17, then decreased to 4.12 and 3.98 after 10 day-fermentation, respectively. The optimum pH(4.2~4.30) of kimchi based on sensory evaluation was reached in 6 days of fermentation in the case of CBK and GBK. The pH of

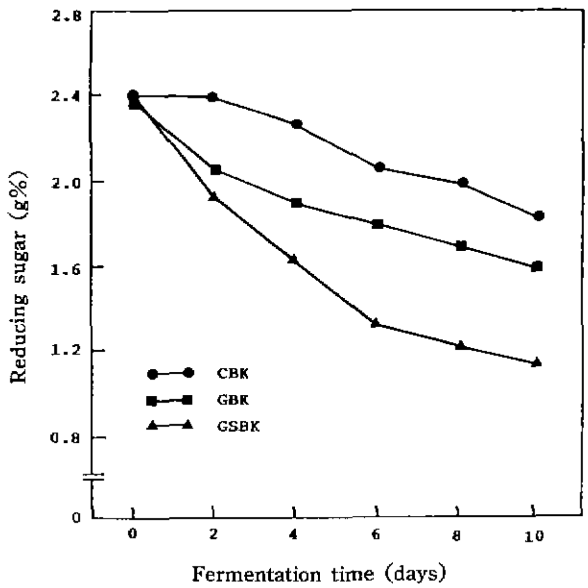

Fig. 1. Changes of reducing sugar during fermentation of buchu kimchi at 15°C.
CBK: Control buchu kimchi
GBK: Glutinous rice paste added buchu kimchi
GSBK: Glutinous rice paste and shrimp added buchu kimchi

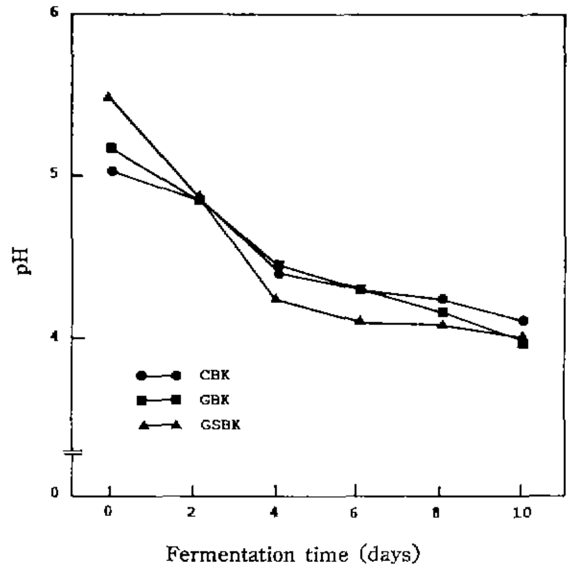

Fig. 2. Changes of pH during fermentation of buchu kimchi at 15°C.
CBK: Control buchu kimchi
GBK: Glutinous rice paste added buchu kimchi
GSBK: Glutinous rice paste and shrimp added buchu kimchi

GSBK sharply dropped within 4 days of fermentation from 5.47 to 4.25 and then decreased slowly. It was considered that pH of GSBK was higher than those of CBK and GBK because of the addition of shrimp at the initial stage, but shrimp accelerated the fermentation of kimchi and then the pH of GSBK decreased rapidly. The pH decreased depending on the storage periods, however, the decreasing rate was not high due to the buffered action of amino acid, etc.(13, 15). Mheen and Kwon(10) had reported that pH of kimchi was 4.2(optimum pH) after 6 days of fermentation at 15°C.

The initial acidity expressed as lactic acid content in CBK and GBK was 0.51% and 0.46% and then rapidly increased to 1.13% in GBK, however, that of CBK slowly increased to 10 days. The initial acidity of GSBK was 0.39%, which drastically increased to 0.90% after 4 days, then increased slowly(Fig. 3). It was reported that the total acidity rapidly increased in kimchi added cooked glutinous rice flour and salted shrimp and the fermentation of kimchi was rapidly progressed(13,14).

Total bacterial and lactic acid bacterial counts increased rapidly to 4 days and then remained constant, and the increase of total bacterial counts was mainly caused by that of lactic acid bacterial counts as shown in Fig. 4 and 5. The initial total bacterial counts of CBK, GBK and GSBK were 2.6~2.8×10^6CFU/ml, and then increased to 4.9~5.6×10^8CFU/ml after 8 days.

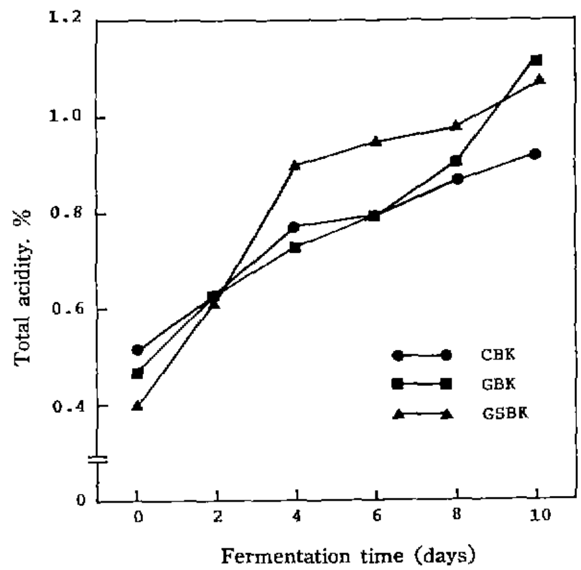

Fig. 3. Changes of total acidity during fermentation of buchu kimchi at 15°C.
CBK: Control buchu kimchi
GBK: Glutinous rice paste added buchu kimchi
GSBK: Glutinous rice paste and shrimp added buchu kimchi

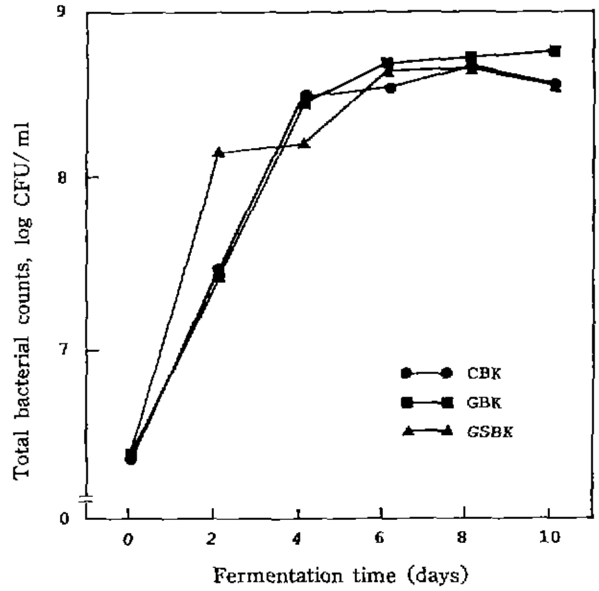

Fig. 4. Changes of total bacterial count during fermentation of buchu kimchi at 15°C.
CBK: Control buchu kimchi
GBK: Glutinous rice paste added buchu kimchi
GSBK: Glutinous rice paste and shrimp added buchu kimchi

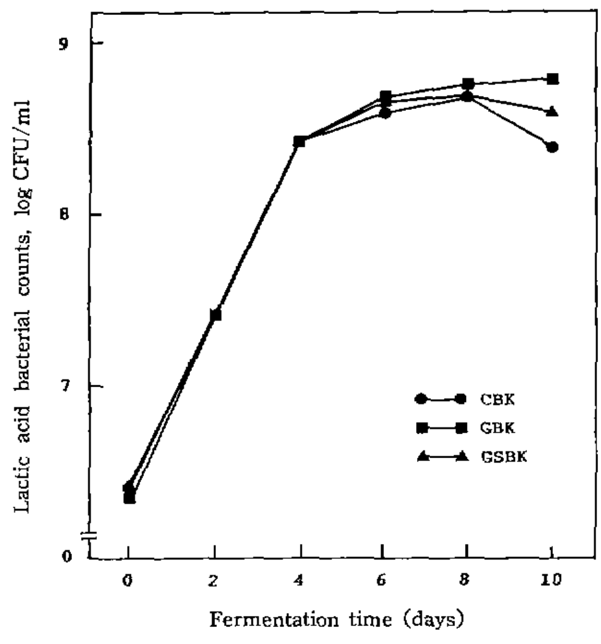

Fig. 5. Changes of lactic acid bacterial count during fermentation of buchu kimchi at 15°C.
CBK: Control buchu kimchi
GBK: Glutinous rice paste added buchu kimchi
GSBK: Glutinous rice paste and shrimp added buchu kimchi

The total bacterial counts of GSBK was higher than those of the other samples on the second day of fermentation. The number of lactic acid bacteria was increased gradually according to the fermentation time of buchu kimchi, and it reached a maximum level of $3.9\sim5.6\times10^{8}$CFU/ml after 6~8 days.

The results of the sensory evaluation in CBK, GBK and GSBK are shown in Table 2. All kimchi samples acquired the highest sensory scores on appearance and color at the first day while during the fermentation processes, they gradually became bad in appearance and color. Experimented buchu kimchi acquired the highest sensory scores on texture, taste and overall acceptability in 6 days of fermentation at 15°C. The glutinous rice paste added kimchi(GBK and GSBK) resulted in better overall acceptability than the control buchu kimchi at 6 days of fermentation($p<0.05$). However, there was not a considerable difference in sensory characteristics among the samples. It was considered that a good taste, flavor and texture of buchu kimchi remained up to 6 days and after that day, it was overripened to eat.

Table 3 shows the result of experiments which was conducted mutagenicity test of the methanol extracts from GBK(6 days fermentation at 15°C) in *Salmonella typhimurium* TA100. The revertant numbers of *Salmonella typhimurium* TA100, in the added concentration of 0.5~2.0%, showed less than that of control, while a little increased when 5% methanol extracts were added. Thus buchu kimchi itself does not induce the mutagenicity in *Salmonella typhimurium* TA100.

As shown in Table 4, the antimutagenic effect against AFB_1 of the methanol extracts from GBK was observed in all different fermentation periods of buchu kimchi. At 1.25% level addition, the inhibition rates of AFB_1 were 36~40%, while the inhibition rates increased 58~69% when the sample added to the test system 5%. Park et al.(29) had also reported that the extracts from Chinese cabbage kimchi inhibited the mutagenicity against AFB_1 in *Salmonella typhimurium* TA100. It seems that the major antimutagenic compounds are from the ingredients of garlic, red pepper powder, and the leeks, etc. The antimutagenicity effect of kimchi(baechu kimchi) was exhibited the highest

Table 2. Sensory score[1)] of buchu kimchi during fermentation at 15°C

Sensory characteristics	Samples	Fermentation periods (days)				
		0	3	6	8	10
Appearance & Color	CBK[2)]	3.6 ± 0.5^{a}	2.9 ± 0.6^{a}	2.6 ± 0.7^{a}	2.3 ± 0.7^{a}	2.4 ± 0.9^{a}
	GBK	3.4 ± 0.7^{a}	3.2 ± 0.7^{a}	3.1 ± 0.4^{a}	2.1 ± 0.4^{a}	1.7 ± 0.5^{a}
	GSBK	3.5 ± 0.5^{a}	2.9 ± 0.6^{a}	2.9 ± 0.4^{a}	2.6 ± 0.7^{a}	2.0 ± 0.8^{a}
Carbonated flavor	CBK	1.4 ± 1.1^{a}	2.6 ± 0.9^{a}	2.7 ± 1.0^{a}	4.0 ± 0.8^{a}	3.6 ± 1.2^{a}
	GBK	1.4 ± 1.1^{a}	2.9 ± 0.8^{a}	2.6 ± 0.5^{a}	3.9 ± 0.6^{a}	3.7 ± 1.3^{a}
	GSBK	1.4 ± 0.1^{a}	3.1 ± 0.8^{a}	3.4 ± 1.1^{a}	4.3 ± 1.2^{a}	3.4 ± 1.2^{a}
Off-flavor	CBK	2.0 ± 1.3^{a}	2.4 ± 0.9^{a}	2.9 ± 1.0^{a}	2.9 ± 0.6^{a}	3.1 ± 0.8^{a}
	GBK	1.9 ± 1.1^{a}	2.1 ± 0.8^{a}	2.3 ± 0.9^{a}	3.4 ± 1.1^{a}	3.6 ± 0.9^{a}
	GSBK	2.0 ± 1.3^{a}	2.4 ± 0.9^{a}	2.3 ± 0.7^{a}	3.2 ± 1.1^{a}	3.3 ± 1.2^{a}
Taste	CBK	2.7 ± 0.7^{a}	2.9 ± 0.6^{a}	2.9 ± 0.8^{a}	2.9 ± 0.9^{a}	2.7 ± 0.9^{a}
	GBK	2.6 ± 0.7^{a}	2.6 ± 0.5^{a}	3.3 ± 0.5^{a}	2.9 ± 0.6^{a}	2.7 ± 0.9^{a}
	GSBK	2.9 ± 0.8^{a}	2.9 ± 0.6^{a}	3.1 ± 0.6^{a}	2.6 ± 0.7^{a}	3.0 ± 0.8^{a}
Texture	CBK	3.0 ± 1.1^{a}	2.9 ± 0.6^{a}	2.6 ± 0.5^{a}	2.3 ± 0.7^{a}	2.3 ± 0.5^{a}
	GBK	2.9 ± 1.0^{a}	2.4 ± 0.5^{a}	3.0 ± 0.5^{a}	2.1 ± 0.6^{a}	2.1 ± 0.4^{a}
	GSBK	3.0 ± 0.9^{a}	2.9 ± 0.6^{a}	3.3 ± 0.7^{a}	2.3 ± 0.9^{a}	2.4 ± 0.7^{a}
Overall acceptability	CBK	3.4 ± 0.7^{a}	2.9 ± 0.6^{a}	2.7 ± 0.7^{a}	2.4 ± 1.1^{a}	2.7 ± 0.7^{a}
	GBK	3.0 ± 0.8^{a}	2.9 ± 0.4^{a}	3.6 ± 0.5^{b}	2.3 ± 0.5^{a}	1.7 ± 0.5^{b}
	GSBK	3.3 ± 1.0^{a}	3.0 ± 0.5^{a}	3.6 ± 0.6^{b}	2.3 ± 0.7^{a}	2.3 ± 0.7^{ab}

[1)]The rating score was: very good, 5 ; good, 4 ; fair, 3 ; poor, 2 ; and very poor, 1

[2)]CBK, GBK and GSBK are control buchu kimchi, glutinous rice paste added buchu kimchi, and glutinous rice paste and shrimp added buchu kimchi, respectively

[abc]Data were analyzed by oneway ANOVA and Duncan's multiple range test carried out to test where the differences are present($\alpha=0.05$)

Table 3. Mutagenicity test of the methanol extracts from buchu kimchi[1)] in *Salmonella typhimurium* TA100

Treatment	Revertants/plate
Control	131± 6
0.5%	105± 1
1.0%	88± 2
1.5%	81± 3
2.0%	85± 2
5.0%	193±18

[1)]Glutinous rice paste added buchu kimchi(GBK) that fermented at 15℃ for 6 days

Table 4. Antimutagenic effect of the methanol extracts from buchu kimchi[1)] against aflatoxin B_1 (AFB_1, 0.5μg/plate) in *Salmonella typhimurium* TA100

Treatment	Buchu kimchi(fermentation period, days)		
	0	6	10
	Revertants/plate		
Spontaneous	119±23		
Control	982±37		
AFB_1+BK 1.25%[2)]	633±4 (40)[3)]	655±18(37)	674±11(36)
AFB_1+BK 2.5%	559±29(49)	555±40(49)	585±10(46)
AFB_1+BK 5.0%	387±18(69)	470±13(59)	483±4 (58)

[1)]Samples used were glutinous rice paste added buchu kimchi(GBK)

[2)]Methanol extract of buchu kimchi ; 1.25%=0.63mg/plate, 2.5%=1.25mg/plate, 5%=2.5mg/plate

[3)]The values in parentheses are the inhibition rate(%)

an the sample of optimally fermented kimchi(29), however, the antimutagenic effect was the highest from the fresh kimchi(0 day fermentation) in the case of buchu kimchi(Table 4). Further studies on this function of buchu kimchi are needed.

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