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Brining Property and Antimutagenic Effects of Organic Chinese Cabbage Kimchi

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Abstract

Brining property and antimutagenic effects of organically cultivated Chinese cabbage kimchi (OC kimchi) and common Chinese cabbage kimchi (CC kimchi) were studied. The salt absorption rate of leaves was faster than that of stems of the Chinese cabbages. Due to the large portion of leaf in organic Chinese cabbage, organic Chinese cabbage (OC) was much faster in terms of salt absorption rate than common Chinese cabbage (CC). The antimutagenic effects of methanol extracts of CC kimchi and OC kimchi were studied against aflatoxin B_1 (AFB_1) using Ames test on *Salmonella typhimurium* TA100 and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) using SOS chromotest. Methanol extract from 6-day fermented OC kimchi at 15°C showed 80% inhibition rate against the indirect mutagen, aflatoxin B_1 induced mutagenicity where as that from 6-day fermented CC kimchi at 15°C showed 54% inhibition rate in the Ames test. Methanol extracts from 6-day fermented CC kimchi and OC kimchi showed 27% and 58% inhibition rate against direct mutagen, N-methyl-N'-nitro-N-nitrosoguanidine induced mutagenicity, respectively in SOS chromotest, thus OC kimchi exhibited higher antimutagenic activity than CC kimchi.

Key words: organic Chinese cabbage, kimchi, AFB_1, MNNG, antimutagenicity

INTRODUCTION

Kimchi is a major Korean traditional fermented food, as a supplying source of vitamins and minerals for the winter season, which is prepared with various vegetables, condiments, etc. Kimchi is the harmonious food with fresh flavor from fermentation, the peculiar texture of vegetables and the rich taste of seasonings (1).

Vegetables are major ingredients of kimchi and their farming methods are classified into two ways, conventional agriculture and organic one. Conventional agriculture uses manufactured or chemical fertilizers which are easy and cheap to handle, but in its soil the contents of humus is very little. Then, the role of humus is to hold nutrients needed by plants and to serve as an effective buffer regulating the balance between acid and base in the soil solution. So, the nutrient and water holding capacity of soil with chemical fertilizers may be less than that of soil with organic fertilizers (2,3). Whereas organic agriculture has a general rejection of chemical pesticides and an emphasis on building a healthy soil having abundant humus and ions, an important measure of fertility. Thus, consumer health is achieved by eating whole, fresh and organically raised food (2).

In this study, to investigate the basic study for developing chemopreventive kimchi, organic Chinese cabbage having high contents of dietary fiber and strong texture (4) and Gueun salt having antimutagenic activity (5) were used. Organic Chinese cabbage was harvested using fertilizer containing raw materials of high-quality organic substance burned at 60~80°C and fermented over 90 days. To determine brining time, salt absorption rates of common Chinese cabbage (CC) and organic Chinese cabbage (OC) were studied. The antimutagenic effects of methanol extracts of both common Chinese cabbage kimchi as control kimchi and organic chinese cabbage kimchi were studied in Ames test and SOS chromotest systems.

MATERIALS AND METHODS

Brining of Chinese cabbage and salt concentration

To determine the proper salt type for kimchi prepared with common Chinese cabbage, test for salt type dependent-absorption rate was carried out. Common Chinese cabbage were cut into 4 pieces and soaked in 10% brines using Chunil salt and Gueun salt (Sannaedle Co., Seoul). Before making kimchi, salt absorption rates of CC and OC was determined. 3 kinds of samples were prepared ; the whole pieces, leaves, and stems of Chinese cabbages. The samples of CC were soaked in 10% brine with Chuil salt and those of OC were in 10% brine with Gueun salt over wide time ranges (2~12 hrs) at 10°C.

Preparation of kimchi

The Chinese cabbage was cut into 8 pieces and soaked in 10% brine at 10°C and then rinsed with tap water. The ratios of ingredients for kimchi were 13.0 of radish, 2.0 of green onion, 2.5 of red pepper powder, 1.4 of garlic, 0.6 of ginger, 2.2 of anchovy juice, 1.0 of sugar in the proportion of 100 salted Chinese cabbage, and the final salt concentration of the kimchi was 2.5% (6). Chungbang Chinese

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cabbage (from Kimhae, Korea), garlic, radish, spring onion, ginger, red pepper powder, anchovy juice (Miwon, Co.) and salt (CC kimchi for Chunil salt, OC kimchi for Gueun salt from Sannaedle, Co., Seoul) were purchased from Bujeon market in Pusan, Korea. For OC kimchi, Chinese cabbage, garlic, radish, spring onion and red pepper were organically cultivated at Kanglim Natural Farm (Milyang, Kyungnam).

The prepared kimchi (0 day) was put into the pint jars and then fermented for 0, 3, 6, and 9 days at 15°C. After fermentation, each kimchi samples was freeze dried. 20 folds of methanol was added to the dried and powdered samples and extracted 3 times. The methanol extracts were concentrated under a vacuum rotary evaporator and then dissolved in dimethyl sulfoxide (DMSO).

Determination of pH, acidity and the level of 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 (7). 0.1% phenolphthalein was dropped to the kimchi filtrate and titrated with 0.1 N NaOH, and then the lactic acid content was calculated and expressed as the acidity (%). In order to analyze the content of reducing sugar, 100 ml of distilled water was added to the filtrate, added the Fehling solution, heated and then cooled. The amount of reducing sugar was determined by the method of Schoorl (8) using titration with 0.1 N $Na_2S_2O_3$ standard solution.

Ames test

Aflatoxin B_1 (AFB_1) was purchased from Sigma chemical Co., St. Louis, Mo., USA and dissloved in DMSO. *Salmonella typhimurium* TA100 bacterial strain, histidine requiring mutant, was provided by Dr. B.N. Ames, Univ. of California, Berkerly, USA and was maintained as described by Maron and Ames (9). The genotype of 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 mixture to activate the indirect mutagen, AFB_1, was also prepared by the method of Maron and Ames. Mutagenicity test (10) was carried out by a modified plate incorporation test (liquid preincubation of the organism with the test compound). In preincubation test, 0.5 ml of S9 mixture, 0.1 ml of cell suspension of *Salmonella typhimurium* TA100, each sample with appropriate aliquot doses of AFB_1 were prepared (9). The prepared sample mixture was preincubated at 37°C for 20 min, and then 2 ml of molten top agar supplemented with L-histidine and D-biotin at 45°C was added to the mixtures, gently vortexed, and then poured onto minimal glucose agar plates (11). The plates were inverted and incubated at 37°C for 48hrs and then the revertant bacterial colonies were counted.

SOS chromotest

N-Methy-N' nitro-N-nitrosoguanidine (MNNG) was obtained from Aldrich Chemical Co., Milwaukee, WI, USA and dissolved in distilled water. The modified assay method described by Quillardet and Hofnung (12), and Baik and Ham (13) 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 at 37 °C for 2 hrs until the absorbance at 660 nm reached 0.3~0.4, the active culture. The obtained active culture was diluted to 10 folds with L medium. 100 μl of the diluted culture distributed to the 2 series in the wells of 96 well plate. 20 μl of methanol extracts of kimchi that was treated with mutagen (10 μl methanol extracts of kimchi + 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 $Na_2 \cdot CO_3$ 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 to neutralize the HCl and then determine the SOS responses at 420 nm. The SOS responses of the samples were calculated by the method of Miller (14).

RESULTS AND DISCUSSION

To determine the proper salt type for kimchi, common Chinese cabbage were cut into 4 pieces and soaked in 10% brines using Chunil salt and Gueun salt at 10°C. Gueun salt, one of processed salts, is made from chunil salt burned four times at ceramic pot over 800°C to remove impurities, bittern and detrimental. Chunil salt is made from seawater by solar evaporation process that seawater is evaporated by the sun at the plain place like paddy and has been used traditionally in Korea. Fig. 1 showed that salt absorption rate of Gueun salt was faster than that of Chunil salt in the common Chinese cabbages. About the salt absorption rate of CC, Ha (5) also reported that OC was faster than that of CC in the same kind of brine. It is interpreted that types of salt and Chinese cabbage can affect the salt absorption speed. In making kimchi, Gueun salt and organic Chinese cabbage were paired and so were Chunil salt and common Chinese cabbage to determine brining time (Fig. 2). They were soaked in 10% brine over wide time ranges (2~12 hrs) at 10°C. The salt absorption rate of leaf was faster than that of stem. Because OC is lighter in the weight and has less moisture content and larger portion of leaf than CC (4), OC was much faster than CC in terms of salt absorption rate. Fig. 2 also showed that the salt absorption rate of leaf + stem is similar to that of stem. It can be interpreted that the salt absorption rate of Chinese cabbage is mostly dependent on that of the stem. Thus the good pickling method of Chinese cabbage is thought to scatter salt to stem area and then pickle with brine

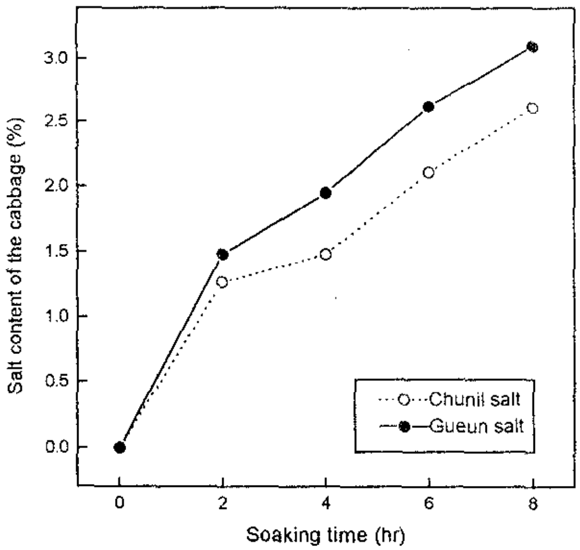

Fig. 1. Change in salt content of the common Chinese cabbage (CC) during soaking in 10% brine with Chunil salt and Gueun salt according to salting time at 10°C.

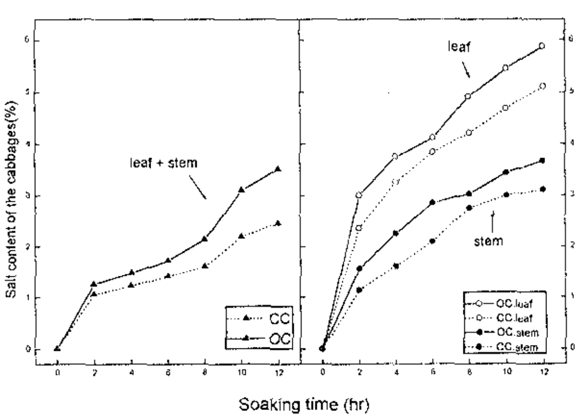

Fig. 2. Salting speed of common Chinese cabbage (CC) and organic Chinese cabbage (OC) when soaked in 10% brine from Gueun salt at 10°C.

to shorten the salting time and sustain same salt concentration in the cabbage. Brining reduces the moisture content and internal void space of the cabbage. These changes affect the physical properties of the vegetable, especially the flexibility and firmness of the tissue, which give a typical textural property to the final product (15,16). The longer soaking time is, the tougher texture of Chinese cabbage is (17). Brining also reduces the content of water- or salt-soluble compounds (15). Thus, using organic Chinese cabbage and Gueun salt for kimchi is desirable to reduce salting time, the loss of water soluble compounds and to give good physical property.

The kimchi fermentation pattern is affected by several factors such as salt concentration, temperaure, pH and related microorganisms, etc (18). The fermentation patterns of CC kimchi and OC kimchi exhibited the typical aspects as shown in Fig. 3. The initial pHs of the CC kimchi and OC kimchi were 5.35 and 5.36, and then decreased to 4.23 and 4.31 after 6-day fermentation at 15°C, respectively as they were properly ripened. The initial acidity expressed as lactic acid content in CC kimchi and OC kimchi were the same as 0.26%, which increased to 0.75% and 0.72% after 6 days, respectively. The initial contents of reducing sugar of CC kimchi and OC kimchi were 2.50 g% and 3.64 g%, respectively, however they were decreased to 1.20 g% and 2.34 g% after 6-day fermentation, respectively. The above data suggest that OC kimchi was slower in the reduction of pH and the increase of the acidity and was higher in the reducing sugar content than CC kimchi. Therefore, the fermentation took place slowly in OC kimchi and it can be interpreted that OC kimchi has high preservative property. The difference of reducing sugar contents was thought to be vegetable types and the content of water-soluble carbohydrate dependent on the strength of temperature and sunlight (19).

The methanol extracts from the CC kimchi and OC kimchi showed antimutagenic activity against AFB_1 in *Salmonella typhimurium* TA100. In this study, the inhibition rates of 0-day and 6-day fermented CC kimchi showed 3% and 42% at 1.25 mg/plate, while inhibition rates of OC kimchi showed 32% and 74%, respectively ($p<0.05$) and at the addition level of 2.5 mg/plate the inhibition rates of 0-day and 6-day fermented CC kimchi showed 29% and 54%, while 35% and 80% of inhibition rate at 0-day and 6-day fermented OC kimchi (Table 1). It means OC kimchi has higher antimutagenic effect than CC kimchi as they were propely ripened. Park et al. (20) also reported that CC kimchi has antimutagenic effect and properly ripened kimchi has higher antimutagenicity than fresh kimchi. When enzyme activities of β-galactosidase and alkaline phosphatase were assayed, the activity of β-galactosidase had to be compared with alkaline phosphatase as a parameter by a given dose, respectively. As shown in Table 2, the kimchi samples had the inhibitory effect on SOS response. Especially, 58% of SOS response induced by MNNG was blocked by adding 125 μg/assay of 6-day fermented OC kimchi but 6-day fermented CC kimchi showed 27% inhibition rate ($p<0.05$). In this study, the strong

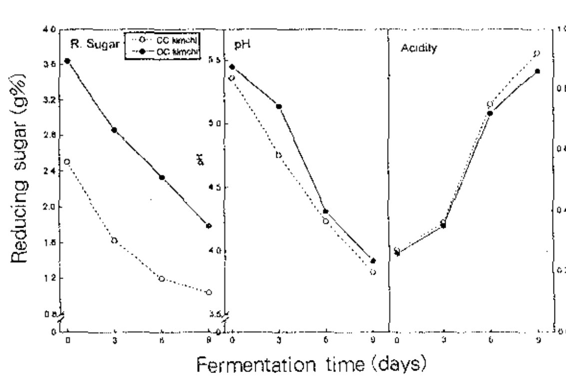

Fig. 3. Changes in reducing sugar, pH and acidity of common Chinese cabbage kimchi (CC kimchi) and organic Chinese cabbage kimchi (OC kimchi) during fermentation at 15°C.

Table 1. Antimutagenic effect of methanol extracts from common Chinese cabbage (CC) kimchi and organic Chinese cabbage (OC) kimchi[1)] on the mutagenicity induced by aflatoxin B_1 (AFB_1, 1 μg/plate) in *Salmonella typhimurium* TA100

Treatment	Revertants/plate	
	1.25mg/plate	2.5mg/plate
Spontaneous	107±10	
Control	1123±43	
CC kimchi		
0 days	1094±47(3)[2)]	825±4(29)
3 days	896±36(22)	711±27(41)
6 days	699±26(42)	575±44(54)
9 days	782±28(34)	656±25(46)
OC kimchi		
0 days	798±32*(32)	766±31*(35)
3 days	685±53*(43)	505±38*(61)
6 days	473±26*(74)	311±28*(80)
9 days	510±8*(60)	301±5*(81)

[1)]Kimchi samples were fermented at 15°C

[2)]The values in the paranthesis are inhibition rate (%)

*Each same day's sample of OC kimchi was significantly different from CC kimchi at the $p<0.05$ level by Student's *t* test

inhibitory effects of 6-day and 9-day fermented OC kimchi in the Ames test and SOS response were showed. Ha (13) carried out the antimutagenic effects of kimchi using the same type of cabbage and 2 kinds of salts (Chunil salt and Gueun salt) in the Ames assay and SOS chromotest systems. It also showed that the highest inhibition rate was appeared in the 6-day fermented kimchi with Gueun salt. Therefore, the reason of high inhibition rate in the OC kimchi may be due to the different kinds of salt and Chinese cabbage. Organic Chinese cabbage has been reported to have higher contents of active compounds such as ascorbic acid, carotenoids and chrolophylls than common Chinese cabbage (4) and Gueun salt also has antimutagenic activity (5).

Thus, organic Chinese cabbage and Gueun salt can be used for basal ingredients of developing chemopreventive kimchi. Further studies about anticancer effect and the identification and purification of active compounds in kimchi are still remained.

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Table 2. SOS response of methanol extract from common Chinese cabbage (CC) kimchi and organic Chinese cabbage (OC) kimchi fermented at 15°C (125 μg/assay) against N-methyl-N'-nitro-N-nitrosoguanidine (MNNG, 50 ng/assay) in *E. coli* PQ37

Sample	β-Glactosidase(β)		Alkaline phosphatase(ρ)		(β)/(ρ)[1)]	SOS induction factor	Inhibition rate(%)
	OD_{420}	Unit[2)]	OD_{420}	Unit			
Spon.	0.695±0.05	23.2	0.509±0.02	18.7	1.2	1.0	
Control	1.796±0.07	59.0	0.553±0.01	18.4	3.2	2.6	
CC Kimchi							
0 days	1.518±0.06	50.6	0.512±0.03	17.1	3.0	2.4	12
3 days	1.598±0.12	53.3	0.539±0.02	18.0	3.0	2.4	12
6 days	1.511±0.06	50.4	0.566±0.02	18.9	2.7	2.2	27
9 days	1.560±0.01	52.0	0.587±0.04	19.6	2.7	2.2	27
OC Kimchi							
0 days	1.350±0.07	45.0	0.521±0.01	17.4	2.6*	2.1	31
3 days	1.137±0.02	37.9	0.485±0.02	16.2	2.3*	1.9	43
6 days	0.982±0.02	32.7	0.478±0.02	15.9	2.1*	1.7	58
9 days	1.001±0.03	33.4	0.472±0.01	15.8	2.1*	1.7	55

[1)]β-Galactosidase/Alkaline phosphatase

[2)]Enzyme unit $= \frac{1000 \times A_{420}}{t}$

*Each same day's sample of OC kimchi was significantly different from CC kimchi at the $p<0.05$ level by Student's *t*-test.

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