

Beneficial Outcomes of Kimchi Prepared with *Amtak* Baechu Cabbage and Salting in Brine Solution: Anticancer Effects in Pancreatic and Hepatic Cancer Cells

Gyl-Hoon Song,^a Eui-Seong Park,^a Seung-Min Lee,^a Dong-Bok Park,^b & Kun-Young Park^{c,*}

^aDepartment of Food and Nutrition, College of Human Ecology, Yonsei University, Seodaemun-gu, Seoul, 03722, Republic of Korea; ^bJeilseed Bio Co., Ltd., Jeungpyeong-gun, Chungcheongbuk-do, 27906, Republic of Korea;

^cDepartment of Food Science and Biotechnology, College of Life Sciences, Cha University, Bundang-gu, Seongnam-si, Gyeonggi-do, 13488, Republic of Korea

*Address all correspondence to: Kun-Young Park, E-mail: kunypark@cha.ac.kr

ABSTRACT: This study investigated the anticancer effects of kimchi prepared using the standard Baechu cabbage and *Amtak* Baechu cabbage; salting was achieved by either the dry salt treatment or brine solution. Four different kimchi samples were prepared for this study: (1) the standard Baechu cabbage and dry salt treatment method (SK-D); (2) the *Amtak* Baechu cabbage and dry salt treatment method (AK-D); (3) the standard Baechu cabbage and brine solution treatment method (SK-B); and (4) the *Amtak* Baechu cabbage and brine solution treatment method (AK-B). The anticancer effects of kimchi were evaluated using human pancreas cancer cells (Capan-2) and human liver cancer cells (HepG2). Both cancer cells showed a significant increase in cell growth inhibition when exposed to AK-D and AK-B compared with SK-D and SK-B ($p < 0.05$). At 2.0 mg/mL, the inhibition of Capan-2 cells was more than doubled after treatment with AK-B and AK-D compared with SK-B and SK-D, but was only 1.2 times in HepG2 cells. Assessment of mRNA and proteins related to apoptosis and cell cycle arrest revealed a significant increase of p21 levels after AK-D and AK-B treatment compared with SK-D and SK-B. In addition, the ongoing cytotoxic effects were significantly higher with AK-B compared with AK-D ($p < 0.05$). In Western blot analysis, the anticancer effects were more apparent in Capan-2 cells than in the HepG2 cells. Overall, these results indicate that kimchi made with *Amtak* Baechu cabbage and treated with brine solution (AK-B) had a superior anticancer potential against both types of cancer cells, with the effects being greater in pancreatic cancer cells compared with liver cancer cells.

KEY WORDS: *Amtak* Baechu cabbage, brine solution treatment method, kimchi, Capan-2 pancreas cancer cells, HepG2 liver cancer cells

I. INTRODUCTION

Kimchi, one of the traditional fermented foods in Korea, is made by mixing and fermenting the main ingredients (cabbage and radish) with other ingredients (salt, garlic, ginger, and red pepper powder). Lactic acid bacteria are involved in the main fermentation process and organic acid, CO₂, and free amino acid are the byproducts generated.¹ Due to various beneficial effects in intestinal regulation,² anti-hyperlipidemia and anti-obesity,^{3,4} anti-oxidation and anti-cancer,^{5,6} and immunity enhancement,^{5,7} kimchi is now considered a functional food worldwide.⁸

Amtak Baechu cabbage is produced by interspecific hybridization of *Brassica rapa* subsp. *rapa* and *Brassica rapa* subsp. *pekinensis*. The content of β -carotene and gluconasturtiin is more abundant in the *Amtak* Baechu cabbage than in standard Baechu cabbage. β -carotene is effective in preventing cardiovascular disease⁹ and has anticancer properties.¹⁰ Gluconasturtiin is converted to phenethyl isothiocyanate (PEITC) by myrosinase during fermentation and is known to have a highly anti-cancerous effect.¹¹ Because of this nutritional difference, *Amtak* Baechu cabbage is well known as the most health-functional Baechu cabbage.

In the manufacturing of kimchi, the salting process has an important influence on the fermentation and quality of the final product.¹² There are two methods of salting Baechu cabbages: treatment with brine solution containing salt (equivalent to twice the weight of the Baechu cabbage) dissolved in water and treatment with dry salt, which treats 4% of the Baechu cabbage weight in the Baechu cabbage stem.¹³ Brine solution treatment is a simple salting process characterized by hardened tissue structure after salting, whereas dry salt treatment is more complicated and is characterized by tissue softening. Therefore, kimchi production using the dry salting method is expected to have some nutritional loss due to the soft texture.

According to the national cancer statistics of South Korea in 2010, pancreatic cancer had the 9th highest incidence with a 5-year survival rate of 8.0%.¹⁴ Because early detection of pancreatic cancer is difficult, many cases are already in the advanced stages when the disease is determined. Due to this, the mortality of pancreatic cancer is the 5th highest in the world. Hepatic cancer is relatively easier to detect in the early stages, but according to a World Health Organization report, more than 500,000 new cases of liver cancer occur each year, making it the 3rd leading cause of cancer deaths.¹⁵ Although both pancreatic and liver cancers are largely affected by environmental factors such as genetic factors and stress, diet accounts for a large part of their occurrence.

Apoptosis is the process of programmed cell death in multicellular organisms.¹⁶ In cancer cells, apoptosis is inefficient because of genetic damage and mutation. Therefore, to prevent proliferation of the cancer cells, apoptosis must be induced by regulating the mechanisms involved. Apoptosis is induced by apoptosis-inducing factors such as the B-cell lymphoma-2 associated X protein (Bax), B-cell lymphoma-2-interacting mediator of death (Bim), and cysteinyl aspartate-specific proteases (Caspase) families and is inhibited by apoptosis survival factors such as B-cell lymphoma-2 (Bcl-2).¹⁶ By examining the expression levels of apoptosis-inducing and survival factors of cancer cells, it is therefore possible to confirm the level of inhibition in the growth of these cells.

Cell cycle arrest of proliferating cancer cells aids in preventing cancer cell proliferation. p21 activated by p53 inhibits the activity of cyclins/CDKs.¹⁷ Cyclins/CDKs activate cyclin D1, a factor that continuously progresses the cell cycle. The inactivation of cyclins/CDKs by p21 results in inhibition of cyclin D1, resulting in cell cycle arrest. Therefore, by examining the expression levels of p21, it is possible to confirm the cell cycle arrest of cancer cells.

Kimchi is abundant in phytochemicals and metabolites produced by lactic acid bacteria and many studies have shown that kimchi has excellent anticancer effects.¹⁸ However, there is no research on the anticancer effect of kimchi prepared by different salting methods and using the *Amtak* Baechu cabbage. We, therefore, undertook this study to investigate the anticancer effects on Capan-2 (a human pancreatic cancer cell line) and HepG2 (a human hepatic cancer cell line) using kimchi prepared with different types of Baechu cabbage and salting methods.

II. MATERIALS AND METHODS

A. Kimchi Sample Preparation

Standard Baechu cabbages and *Amtak* Baechu cabbages were purchased from Jeil Seedbio Co. (Jeungpyeong-gun, Chungcheongbuk-do, Korea). The cabbages were washed, cut into halves, and salted using two different methods: dry salt treatment and brine solution treatment. In the dry salt treatment method, salt equivalent to 4% of the cabbage weight was treated on the side of the Baechu cabbage stem. The cabbage was then washed 3 times and drained for 3 hours. In the brine solution treatment method, the Baechu cabbage was treated with brine consisting of salt equivalent to 10% of the cabbage weight. After brine treatment, the cabbage was washed 3 times and drained for 3 hours. The drained cabbages from both treatments were blended and mixed with other kimchi ingredients in the following ratios, considering the Baechu cabbage to be 100: 4.0 solar salt, 8.0 radish, 2.0 green onion, 4.0 kelp juice, 4.0 glutinous rice paste, 4.0 red pepper powder, 2.0 crushed garlic, 0.4 crushed ginger, 0.4 anchovy powder, 3.4 anchovy juice,

and 0.8 salted shrimp.¹⁹ The mixture was preserved at 4°C for 3 weeks to allow fermentation.²⁰

When kimchi reached the optimum fermentation period (3 weeks and pH 4.3), it was frozen at -20°C and dried using a freeze dryer (FD5512; Ilshin BioBase Co., Seoul, Korea). The dried kimchi was blended into a powder using a blender. Methanol, corresponding to 20 times (w/v) of the powder, was added and the resultant mixture was stirred for 24 hours. The same amount of methanol was added again with continued stirring. The mixture of methanol and kimchi was filtered to obtain the methanol extract, which was further concentrated using an evaporator (EYELA; Tokyo Rikakikai Co., Tokyo, Japan). Concentrated kimchi extracts were weighed and dimethylsulfoxide (DMSO) (four times the weight of the kimchi samples) was added.²¹

B. Physicochemical Characteristic Analysis of Kimchi

After juicing the prepared kimchi, 100 µl was evaluated every week to assess the number of lactic acid bacteria and total bacteria by culturing on MRS agar and PCA agar, respectively. The pH was measured using a pH meter (M220, Corning, MA, USA). To measure acidity, kimchi samples were diluted 20× and 0.1 N NaOH was added until a pH of 8.4 was quantified. Sensory testing was performed by comparing the appearance, taste, and smell after the 3-week fermentation period (pH 4.3).²²

C. Cytotoxicity Analysis in RAW 264.7 Macrophage

RAW 264.7 murine macrophage cells used in the experiments were purchased from the Korean Cell Line Bank (KCLB, Seoul, Korea). The cells were cultivated in Dulbecco's modified Eagle's medium supplemented with 10% heat inactivated fetal bovine serum (FBS) and 1% penicillin-streptomycin solution. The cultured cells were passaged 2-3 times a week. Cells were counted using a cell counter (Luna automated cell counter; Logos Biosystems, Gyunggi, Korea) and plated at a density of 1.0×10^5 cells/mL in a 96-well plate and incubated for 24 hours at 37°C in a 5% CO₂ incubator. After in-

cubation, kimchi samples were diluted in media at concentrations of 1, 2, and 2.5 mg/mL and incubated for a further 48 hours at 37°C in a 5% CO₂ incubator. After 48 hours, 100 µl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) solution diluted to 5 mg/mL with the medium was added per well and incubated for 4 hours under the same conditions. DMSO was added to each well to dissolve the formed formazan and plates were put on a shaker for 30 minutes in a dark room. The absorbance was measured at 540 nm with a Wallac Victor3 1420 Multilabel Counter (Perkin-Elmer, Wellesley, MA).²³

D. In Vitro Anti-Cancer Effects of Kimchi

1. MTT Assay

Capan-2 (human pancreatic cancer cells) and HepG2 (human hepatic cancer cells) were procured from the Korean Cell Line Bank (KCLB, Seoul, Korea). The cells were cultivated in RPMI supplemented with 10% heat-inactivated FBS and 1% penicillin-streptomycin solution, with regular passaging 2-3 times a week. The cells were then subjected to the MTT assay, as described above, to evaluate the inhibitory effects of kimchi.

2. qRT-PCR

To determine the mRNA expression levels of cancer related genes in cancer cell lines, we performed quantitative reverse transcription polymerase chain reaction (qRT-PCR). Cultured Capan-2 and HepG2 cells were counted with a cell counter (Luna automated cell counter; Logos Biosystems, Gyunggi, Korea), seeded at a density of 1.0×10^6 cells/mL in 6-well plates, and incubated for 24 hours. After the incubation, 2.0 mg/mL of kimchi extract samples were added for 48 hours and total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The isolated total RNA was dissolved in 0.1% diethylpyrocarbonate (DEPC) water and quantified using a NanoDrop ND-1000 (NanoDrop Technologies Inc., Wilmington, DE, USA). Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA) was used to synthesize the first-strand cDNA.

Synthesized cDNA was amplified using a thermal cycler (BioRad CFX-96 real time system; BioRad, USA). FIREPol PCR Mix (Solis BioDyne, Tartu, Estonia) was used to detect the amplified products and measured using fluorescence.

Primers for 18s rRNA, Bax, Bim, Caspase-3, Caspase-9, p21 and p53 were purchased from Bioneer Corporation (Daejeon, Korea). PCR was performed using the following primers: 18s rRNA forward 5'-CAGCCACCCGAGATTGAGCA-3' and reverse 5'-TAGTAGCGACGGGCGGTGTG-3'; Bax forward 5'-TGCTTCAGGGTTTCATCCAG-3' and reverse 5'-GGCGGCAATCATCCTCTG-3'; Bad forward 5'-CGGAGGATGAGTGACGAGTT-3' and reverse 5'-GATGTGGAGCGAAGGTCACT-3'; Bim forward 5'-AGATCCCCGCTTTTCATCTT-3' and reverse 5'-TCTTGGGCGATCCATATCTC-3'; p21 forward 5'-ATGTCAGAACCGGCTGGGG-3' and reverse 5'-GCCGGGGCCCCGTGGGA-3'; p53 forward 5'-ATGGAGGAGCCGCAGTCAGA-3' and reverse 5'-TGCAGGGGCCCCGGGTGTAG-3'; Caspase-3 forward 5'-TTTTTCAGAGGGGATGTTG-3' and reverse 5'-CGGCCTCCACTGGTATTTTA-3'; and Caspase-9 forward 5'-CTAGTTTGCCACACCAGT-3' and reverse 5'-CTGCTCAAAGATGTCGTCCA-3'.

3. Western Blotting

Cultured Capan-2 and HepG2 cells were counted (Luna automated cell counter; Logos Biosystems, Gyeonggi, Korea), seeded at a density of 1.0×10^6 cells/mL in 6-well plates, and incubated for 24 hours. After incubation, cells were exposed to 2.0 mg/mL of kimchi extract samples for 48 hours and total cell lysates were isolated using radioimmunoprecipitation assay buffer (Invitrogen, Carlsbad, CA, USA). The cell lysates were separated by 12% SDS-PAGE and transferred to a polyvinylidene fluoride (BioRad, USA) membrane, blocked with 5% skim milk, and probed with primary antibodies against actin, Bax, Caspase-3, Caspase-9, and p21 (Santa Cruz Biotechnology, CA, USA). Membranes were then exposed to horseradish peroxidase-conjugated secondary antibodies and the bands were quantified using an LAS-4000 luminescent image analyzer (Fujifilm Life Science, Tokyo, Japan).

E. Statistical Analysis

qRT-PCR experimental results are presented as means \pm standard error (SE) and other results are presented as means \pm standard deviation (SD). Duncan's multiple range tests and one-way analysis of variance (ANOVA) were used to determine the significant differences within the group. Student's *t*-test determined the significant differences between two groups. The significance of the experimental results was considered at $p < 0.05$.

III. RESULTS

A. Physicochemical Characteristic Analysis of Kimchi

To assess the different physicochemical characteristics of the kimchi samples, we evaluated the pH, acidity, lactic acid bacterial count, total bacterial count, and sensory attributes of all groups. Considering the different Baechu cabbages and salting methods used, no significant differences were observed among the groups (data not shown). These results indicate that, despite the *Amtak* Baechu cabbage being produced through interspecific hybridization, the resultant variety is reliable for the production of kimchi.

B. Cytotoxicity of Kimchi Samples on RAW 264.7 Macrophage

RAW 264.7 cells are frequently used to determine the cytotoxicity of a sample.²⁴ The toxicity of the kimchi extracts was therefore evaluated by exposing the RAW 264.7 cells to different concentrations of the extracts for 48 h and determining the resultant cell viability using the MTT assay. We observed no inhibition in the proliferation of RAW 264.7 cells at any concentration evaluated from 1.0 to 2.5 mg/mL (Fig. 1). Therefore, these results suggest that kimchi exerted no specific cytotoxicity in cells up to a 2.5 mg/mL concentration.

C. Anticancer Effects of Kimchi Samples in Capan-2 Human Pancreatic Cancer Cells

1. MTT Assay

At all concentrations tested, there was significant inhibition of cancer cell growth in the AK-D group

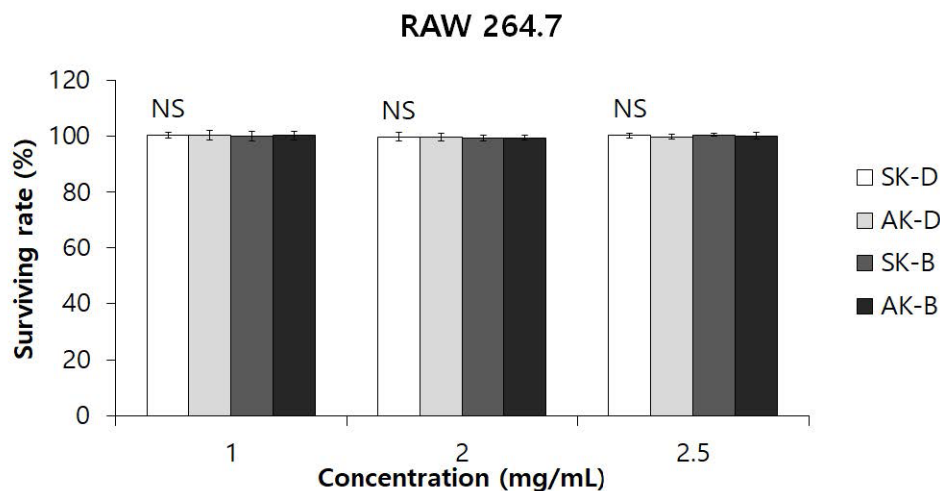


FIG. 1: MTT assay of RAW 264.7 murine macrophages in kimchi extracts. NS, Not significantly different.

compared with the SK-D group and in the AK-B group compared with the SK-B group ($p < 0.05$) (Fig. 2). The cell growth inhibition in AK-B was 1.9 ± 0.7 times more than in SK-B at 2.0 mg/mL and 1.8 ± 0.3 times more at 2.5 mg/mL. These results suggest that Capan-2 growth inhibition rates were markedly increased in the kimchi prepared with *Amtak* Baechu cabbage compared with kimchi prepared with standard Baechu cabbage and the kimchi made with brine solution was significantly more inhibitory than kimchi prepared by dry salt treatment.

2. Gene Expression Analysis of Apoptosis-Related Genes

Apoptosis is a form of cell death controlled by genes; abnormal cells, damaged cells, and aged cells die by themselves.^{16,24} In Capan-2, mRNA and protein expression levels of Bax, Caspase-3, and Caspase-9, all apoptosis-inducing factors, were significantly increased in the AK-D and AK-B groups compared with the SK-D and SK-B groups (Fig. 3). Significant differences were greater using protein expression

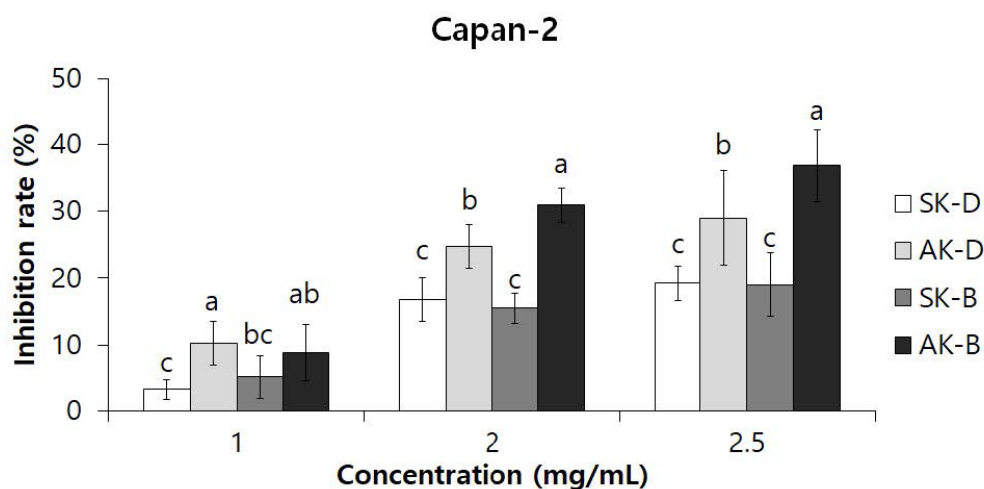


FIG. 2: MTT assay of Capan-2 pancreas cancer cells in kimchi extracts. Means with different letters indicate significant differences at the same storage period ($p < 0.05$) as assessed by Duncan's multiple range tests.

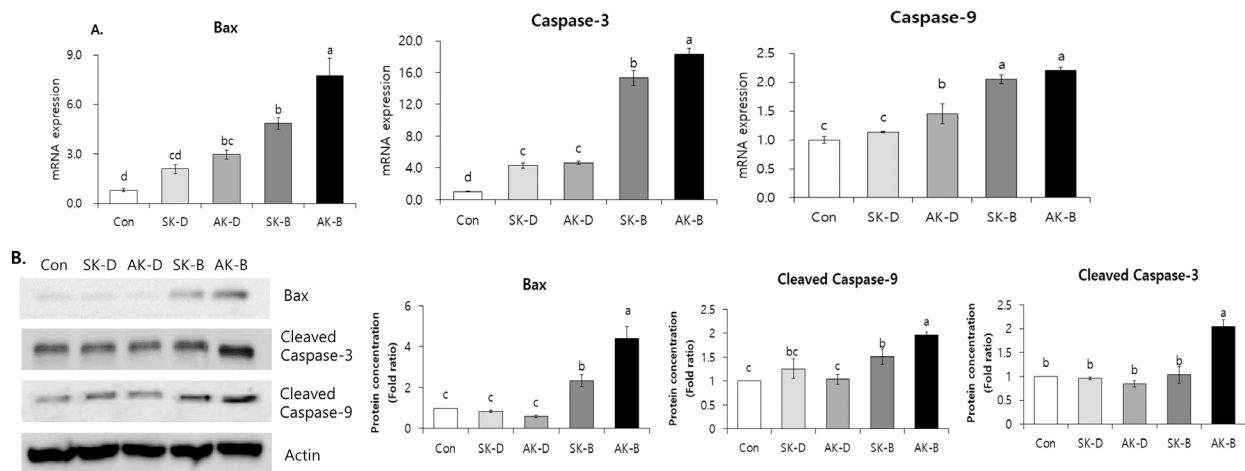


FIG. 3: mRNA (A) and protein (B) expression levels of apoptosis related factors from the Kimchi extracts (2 mg/mL) in Capan-2 human pancreatic cancer cells. Means with different letters at the 2 mg/mL concentration (3 weeks at 4°C) are significantly different ($p < 0.05$) by Duncan's multiple range tests.

analysis than mRNA expression analysis. Results of Western blotting revealed that Bax was 1.8 ± 0.2 times increased in AK-B-treated groups compared with the SK-B-treated groups ($p < 0.05$).

3. Gene Expression Analysis of p21

p21 is involved in cell cycle arrest throughout the G_1/S and G_2/M phases and is involved in blocking cancer cell division.²⁵ In Capan-2 human pancreatic cancer cells, the mRNA and protein expression levels of p21 were significantly increased in the AK-D and AK-B groups compared with the SK-D and SK-B groups (Fig. 4). Western blotting revealed

higher protein expression levels after AK-B exposure ($\sim 2.1 \pm 0.2$ times more) compared with SK-B exposure ($p < 0.05$).

D. Anticancer Effects of Kimchi Samples in HepG2 Human Hepatic Cancer Cells

1. MTT Assay

The inhibitory effect of kimchi on the growth of liver cancer cells is presented in Fig. 5. Few significant differences were observed between kimchi prepared with *Amtak* Baechu cabbage and that prepared with standard Baechu cabbage at all concentrations.

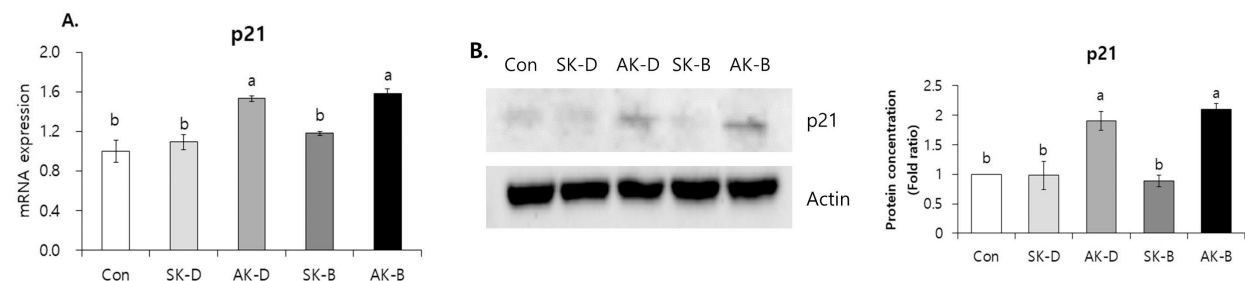


FIG. 4: mRNA (A) and protein (B) expression levels of p21 from the Kimchi extracts (2 mg/mL) in Capan-2 human pancreatic cancer cells. Means with different letters at the 2 mg/mL concentration (3 weeks at 4°C) are significantly different ($p < 0.05$) by Duncan's multiple range tests.

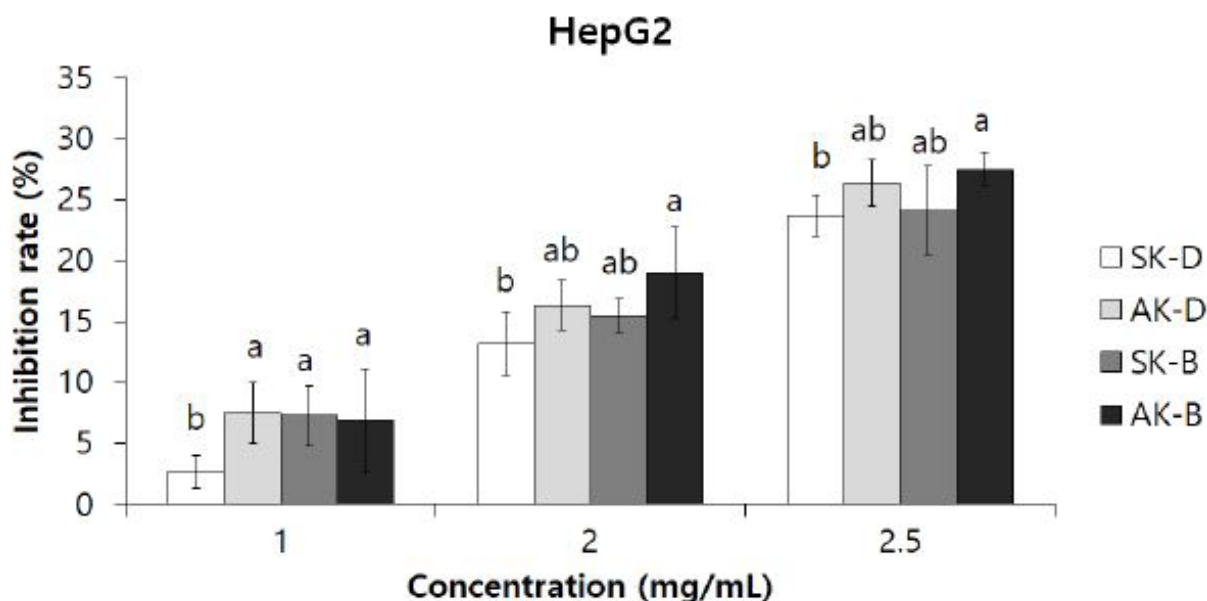


FIG. 5: MTT assay of HepG2 human liver cancer cells in kimchi extracts. Means with different letters at the same storage period are significantly different ($p < 0.05$) by Duncan's multiple range tests.

2. Gene Expression Analysis of Apoptosis-Related Genes

mRNA and protein expression levels of apoptosis-related genes were examined (Fig. 6A,B). The mRNA expression levels of Bax, Caspase-3, and Caspase-9 were increased in the AK-B group compared with the SK-B group. Unlike mRNA expression, protein expression did not differ between the groups. These results were contrary to the results obtained in Capan-2.

3. Gene Expression Analysis of p21

Evaluation of the mRNA and protein expression levels of p21 in the HepG2 cells (Fig. 7) revealed slight differences between the two varieties of Baechu cabbages. The protein expression was also not significantly different between SK-B and AK-B.

IV. DISCUSSION

In this study, we investigated the anticancer effects of kimchi samples prepared using different varieties

of Baechu cabbage and salting methods. In the Capan-2 and HepG2 cancer cell lines, the anticancer effects were increased in the kimchi prepared with *Amtak* Baechu cabbage compared with the standard Baechu cabbage. In addition, kimchi salted with brine solution was associated with higher anticancer effects than the dry salt treatment.

The cytotoxicity of kimchi was confirmed on RAW 264.7 cells (0–2.5 mg/mL) and no growth-inhibitory effects were observed.²³ Jeong et al.²⁶ examined the anticancer effects of kimchi samples in AGS gastric cancer cells and found selective toxicity against cancer cells but not in RGM-1 normal gastric cells (0–7.5 mg/mL). Similar to these results, this study showed that kimchi was selectively toxic to the cancer cells compared with normal cells.

Among the kimchi samples assessed, the pH, acidity, lactic acid bacterial count, total bacterial count, and sensory test were not significantly different (data not shown), thereby establishing that consumption of kimchi made from standard Baechu cabbage or *Amtak* Baechu cabbage has no significant nutritional difference.

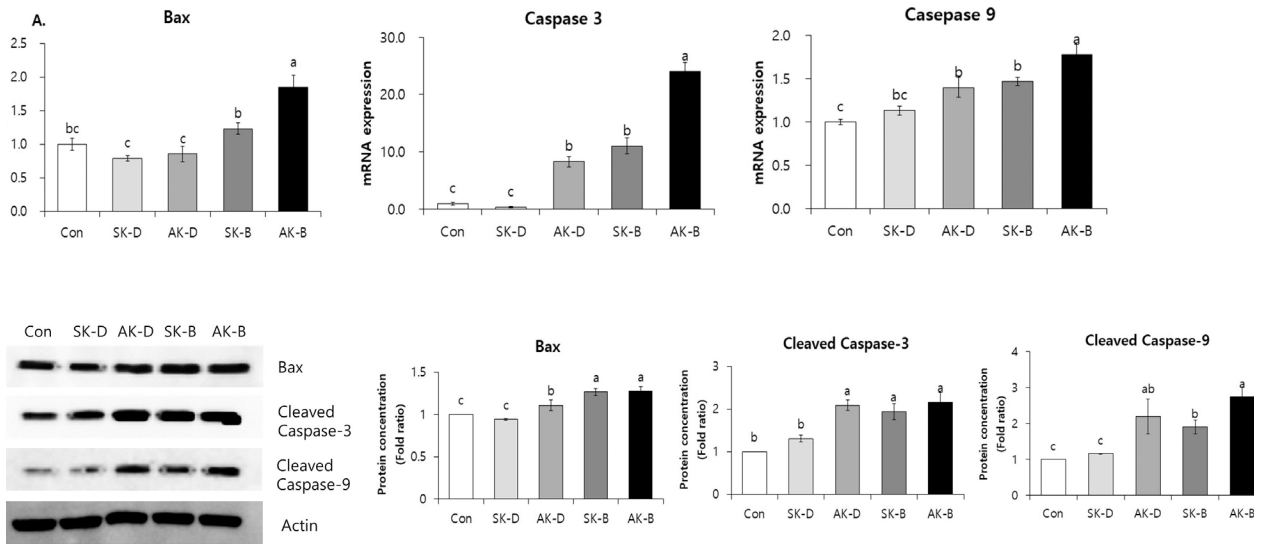


FIG. 6: mRNA (A) and protein (B) expression levels of apoptosis related factors from the Kimchi extracts (2 mg/mL) in HepG2 human liver cancer cells. Means with different letters at the 2 mg/mL concentration (3 wks at 4°C) are significantly different ($p < 0.05$) by Duncun’s multiple range tests.

It is well established that kimchi exerts an anti-cancer effect in various cancer cells such as HL-60 promyelocytic leukemia cells,²⁷ AGS gastric cancer cells,²⁸ MG-63 osteosarcoma cells,²⁹ and HCT-116 and HT-29 colon cancer cells.⁵ Furthermore, Son²⁷ and Bong et al.⁵ reported that, in cancer cells treated with kimchi samples, the gene expression levels of Bax, Bad, Bim, Caspase-3, and Caspase-9 (apoptosis-inducing factors) and p21 and p53 (cell cycle arrest-related factors) were significantly increased. In the current study, the Capan-2 cells treated with kimchi samples showed a similar

increase in the gene expression levels of Bax, Caspase-3, Caspase-9, and p21. This study also shows that, among the various mechanisms inhibiting the growth of cancer cells, apoptosis and cell cycle arrest seem to play a crucial role in inhibiting cell growth. Pancreatic cancer is difficult to detect in the early stages,³⁰ which makes treatment more challenging.³¹ Few studies have evaluated the effect of kimchi on pancreatic cancer. In this study, the kimchi prepared with *Amtak* Baechu cabbage inhibited cell growth by increasing apoptosis in pancreatic cancer cells, thereby exerting its anti-



FIG. 7: mRNA (A) and protein (B) expression levels of p21 from the Kimchi extracts (2 mg/mL) in HepG2 human liver cancer cells. Means with different letters at the 2 mg/mL concentration (3 weeks at 4°C) are significantly different ($p < 0.05$) by Duncan’s multiple range tests.

cancer effect. Due to a lack of medications available for pancreatic cancer, we propose that kimchi made with *Amtak* Baechu cabbage may be a potential therapy in suppressing cancer growth.

A previous study by Park et al.³² reported that, in rats induced with liver cancer using 2-AAF and administered kimchi orally, the liver tumors were reduced by 2.9 ± 0.4 -fold. In addition, *in vitro* studies have shown that growth was significantly lowered in Hep3B liver cancer cells.^{33,34} These studies also reported a significant increase in the gene expression levels of Bax, Caspase-3, Caspase-9, p21, and p53. In this study, kimchi exerted its anticancer effects in the cancer cells by regulating the apoptosis and cell-cycle-arrest genes. However, there was no significant difference between groups with respect to the type of Baechu cabbage used. These results therefore indicate that kimchi prepared with *Amtak* Baechu cabbage is specific on pancreatic cancer cells rather than liver cancer cells.

In the case of kimchi prepared with dry salt treatment, there is considerable water loss during the draining process and the tissue becomes soft and tender when made into kimchi.³⁵ Therefore, it seemed that the nutritional components may have leaked out, resulting in relatively low anti-cancer effects.

Amtak Baechu cabbages have high concentrations of gluconasturtiin and β -carotene, being 33 and 34.5 times higher than standard Baechu cabbage, respectively (data not shown). During fermentation in Baechu cabbage, the gluconasturtiin is converted into isothiocyanate. Isothiocyanates are reported to have anticarcinogenic effects on liver cancer³⁶ and colorectal cancer.³⁷ β -carotene is also known to have anticancer effects.¹⁰ We presume that the increased anticancer effect of *Amtak* Baechu cabbage may be due to the high levels of these specific substances.

At the optimal fermentation period of kimchi (pH 4.0–4.3), metabolites were analyzed using ultra-performance liquid chromatography–tandem mass spectrometry and gas chromatography–mass spectrometry. The levels of pyropheophorbide A and 2-isopropyl-5-methyl-1-heptanol were significantly higher in the kimchi prepared with *Amtak* Baechu cabbage compared with the standard Baechu cab-

bage preparation.¹⁹ Pyropheophorbide (a methyl ester), an analog of pyropheophorbide A, is known to induce apoptosis of colon cancer cells.³⁸ Therefore, it seems that the specific anticancer effect of Capan-2 is enhanced by the chemical present in the *Amtak* Baechu cabbage and the resultant metabolic compound produced during fermentation. However, there are no studies on 2-isopropyl-5-methyl-1-heptanol and future detailed studies will be required.

In conclusion, these results indicate that kimchi prepared with *Amtak* Baechu cabbage and brine treatment has increased anticancer effects in pancreatic cancer cells due to the presence of specific substances such as gluconasturtiin, β -carotene, pyropheophorbide A, etc. Brine solution treatment is less damaging to the cabbage tissue, with less loss of nutrients compared with dry salt treatment. We therefore propose that consuming kimchi prepared with *Amtak* Baechu cabbage is more beneficial for inhibiting pancreatic cancer than kimchi prepared using the standard Baechu cabbage.

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