# Antioxidant effects of kimchi supplemented with black raspberry during fermentation protect against liver cirrhosis-induced oxidative stress in rats

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**BACKGROUND/OBJECTIVES:** Oxidative stress is a major effector of various diseases; accordingly, antioxidants are frequently ingested in order to prevent or alleviate disease symptoms. Kimchi contains various natural antioxidants, and it is known that the functional activity varies depending on the ingredients and fermentation state. Black raspberries (BR) contain various bioactive compounds with antioxidant effects. This study investigated the antioxidant and liver-protection effects of kimchi supplemented with black raspberry juice powder (BJP).

**MATERIALS/METHODS:** BJP-added kimchi (BAK; at 0.5%, 1%, and 2% concentrations of BJP) and control (without BJP) were prepared and fermented at 4°C for 4 weeks. Changes in the antioxidant effects of BAK during fermentation were investigated. In addition, the protective activity of BAK against oxidative stress was investigated in a liver cirrhosis-induced animal model *in vivo*.

**RESULTS:** BAK groups showed the acidity and pH of optimally ripened (OR) kimchi at 2 weeks of fermentation along with the highest lactic acid bacterial counts. Additionally, BAK groups displayed a higher content of phenolic compounds and elevated antioxidant activities relative to the control, with the highest antioxidant effect observed at 2 weeks of fermentation of OR 1% BAK. After feeding the OR 1% BAK to thioacetamide-induced liver cirrhosis rats, we observed decreased glutamate oxaloacetate transaminase and glutamate pyruvate transaminase activities and elevated superoxide dismutase activity.

**CONCLUSIONS:** These findings showed that the antioxidant effects of OR BAK and feeding of OR 1% BAK resulted in liver-protective effects against oxidative stress.

## INTRODUCTION

Oxidative stress results from an imbalance between the production of reactive oxygen species (ROS) and antioxidant reactions that remove them, which increases intracellular ROS and causes damage to DNA, proteins, and lipids. Therefore, ROS are potentially harmful and directly impact various disease pathologies, including those associated with arteriosclerosis, diabetes, hypertension, and liver disease [1]. Antioxidants play various biological roles, including prevention of ROS-mediated cellular damage [2]. Synthetic phenolic antioxidant compounds can increase the shelf life and preserve the quality of food; however, synthetic antioxidants can be carcinogenic and reportedly cause various diseases [3]. Therefore, there is increasing interest in developing more effective antioxidants derived from natural substances that can replace synthetic antioxidants for application in functional foods [4].

Kimchi is prepared from brined kimchi cabbage using various seasonings, such as radishes, red pepper powder, garlic, ginger, onions, and fermented fish sauce [5]. Kimchi has various functional effects, including antioxidant, anti-mutagenic, and anti-obesity effects [6-8]. Previous studies of the antioxidant activities associated with kimchi, which resulted in increased cell viability through inhibition of lipid peroxidation in different cell lines, highlighted their relation to fermentative lactic acid bacteria (LAB) [7,9]. Although the antioxidant activities of kimchi change depending on the ingredients used and fermentation state, scientific evidence of these changes is insufficient.

Black raspberries (BR) belong to the *Rosaceae* family and are cultivated in the United States, Japan, and southern parts of the Korean peninsula [10]. BR contain various bioactive anthocyanins, flavonoids, phenolic acids, and organic acids with

antioxidant effects [11]. BR are used for jam, liquor, beverages, or in freeze-dried powder form as a food additive due to their short shelf life [12]. Studies of BR-related antioxidant activity revealed the properties of BR juice and freeze-dried BR extract, as well as their ability to improve the stability of anthocyanins contained in BR [13,14]. However, few studies have focused on the *in vivo* antioxidant properties and ROS-moderating effects of foods containing freeze-dried BR powder.

This study investigated changes in phenolic compounds (total phenolic and total flavonoid contents) and the antioxidant activities [2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) cation radical scavenging/reducing activity] during the fermentation period of kimchi in the presence of various concentrations of freeze-dried BR juice powder (BJP). Additionally, the serum and antioxidant enzyme activity of liver cirrhosis (LC)-induced rats fed kimchi supplemented with BJP were investigated to confirm the antioxidative effects *in vivo*.

## MATERIALS AND METHODS

### Materials

BR and kimchi ingredients (kimchi cabbage, red pepper powder, garlic, ginger, carrots, dropworts, sesame, white sugar, glutinous rice powder, white radishes, and salted anchovy sauce) were purchased in a local market. Folin-Ciocalteu reagent, sodium carbonate, gallic acid, sodium nitrite, aluminum chloride, sodium hydroxide, catechin, DPPH, ABTS, potassium ferricyanide, trichloroacetic acid, and thioacetamide were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other reagents used in this study were of analytical grade.

### Sample preparation

BR were stored at -40°C before processing. Frozen BR were thawed at room temperature for 12 h and then pulverized using a blender (Hurom slow juicer; Quezon City, Philippines). The extracted juices were freeze-dried using a PVTFD 500R device (ilShinBioBase, Gyeonggi-do, Korea) and then powdered using an electric mixer (HMF-3260S; Hanil Co., Seoul, Korea). White radishes, carrots, and dropworts were cut into small pieces, and garlic and ginger were ground together using an electric mixer. Glutinous rice powder and water were mixed (1:10, w/w) and heated in a saucepan until gelatinization, after which the cooled gelatinized rice powder was mixed with the other kimchi ingredients. The composition (%, w/w) of kimchi seasoning (KS) was as follows: 27.0% white radish, 23.0% glutinous rice paste, 12.2% red pepper powder, 9.0% salted shrimp sauce, 5.4% garlic, 5.4% dropwort, 4.5% salted anchovy sauce, 4.0% chives, 4.0% carrot, 2.7% ginger, 1.4% sesame, and 1.4% white sugar. To prepare BJP-added kimchi (BAK), 1 kg of brined kimchi cabbage was combined with 370 g of KS, and BJP was added at 0.5% (BAK-0.5), 1% (BAK-1), and 2% (BAK-2) (w/w) concentrations relative to the total weight of the brined kimchi cabbage. As a control, the same method was used, except in the absence of BJP. Kimchi fermentation proceeded at 4°C for 4 weeks. Characteristics of the fermented kimchi samples were investigated by measuring the pH, acidity, and microbial count in half of each sample, while the other half was freeze-dried, powdered,

and stored at -70°C for subsequent measurement of antioxidant effects. The kimchi powders (30 g) used for antioxidant analysis were extracted with boiled distilled water (600 mL) for 2 h and 20 min. The extracts were centrifuged at 13,000 g for 20 min and filtered through Whatman No. 1 filter paper (GE Life Sciences, Pittsburgh, PA, USA). The extraction step was repeated twice. The filtrate was concentrated in a rotary evaporator (R-210; Buchi, Flawil, Switzerland), and the residual water was removed via lyophilization. The dried samples were stored at -70°C until further use.

### Chemical and microbial analyses

The BAK samples (300 g each) were blended using a hand blender (HHM-800; Hanil, Gyeonggi-do, Korea), filtered through sterilized gauze, and used as kimchi filtrates. The pH values of the kimchi filtrates were determined with a pH meter (MP120; Mettler Toledo, Greifensee, Switzerland). Diluted BAK samples were further diluted 2-fold, and the total acidity was titrated to pH 8.3 with 0.1 N NaOH and expressed as the percentage of lactic acid. To determine total bacterial counts, samples were serially diluted in saline (0.85% NaCl), spread onto plate-count agar (Merck, Darmstadt, Germany), and incubated at 37°C for 24 h. LAB were plated on MRS agar (Difco, Detroit, MI, USA) and incubated at 30°C for 24 h. Microbial counts were determined for each population as the mean values from triplicate measurements and expressed as the log colony forming units (CFU)/mL.

### Total phenolic content (TPC) and total flavonoid content (TFC)

The TPC and TFC were measured using a modification of a previously described method [15,16]. The samples (200 μL) were oxidized with 5 mL of Folin-Ciocalteu reagent, and the mixtures were neutralized with 3 mL of 5% sodium carbonate. After a 30 min incubation at room temperature in the dark, the absorbance of each sample at 725 nm was measured in a 96-well plate using a plate reader (SpectraMax i3x; Molecular Devices, Wals-Siezenheim, Austria). The TPC in each sample was determined using a standard curve for gallic acid, and the results are expressed as the μg of gallic acid equivalents (GAE) per mg of BAK extract powder. The TFC was measured by performing an aluminum chloride-colorimetric assay. Briefly, 500 μL of each sample was mixed with 75 μL of 5% sodium nitrite and incubated for 5 min at room temperature, after which 150 μL of 10% aluminum chloride was added to the mixture, followed by 0.5 mL of 1 M NaOH and 275 μL of deionized water. The absorbance of each sample was measured at 510 nm in a 96-well plate using the plate reader. The TFC was expressed as the μg of catechin equivalents (CE) per mg of BAK extract powder.

### DPPH free radical and ABTS cation radical scavenging

DPPH free radical scavenging activity [DPPH half maximal effective concentration ($EC_{50}$)] was measured according to a previously described method [17], with slight modifications. Briefly, 0.2 mL of each diluted BAK sample was added to 2.8 mL of 60 μM DPPH solution, and the mixture was shaken and allowed to stand for 30 min in the dark, followed by measurement of the absorbance at 515 nm in a 96-well plate using

a plate reader. ABTS cation radical scavenging activity (ABTS $EC_{50}$) was measured using a modified version of the method described by Re *et al.* [18]. The stock solution was prepared by stirring 7 mM ABTS (5 μL) and 150 mM potassium persulfate (88 μL) at room temperature for 12 h to 15 h, after which the ABTS solution was diluted with ethanol to achieve an absorbance of 0.70 at 734 nm. The ABTS solution (1 mL) was then added to 0.1 mL of the diluted sample solution, which was vortexed and incubated for 10 min in the dark. ABTS $EC_{50}$ values were measured at 734 nm using the plate reader. The percentage of radical scavenging was calculated from the following formula: [1 - (A sample/A control) × 100]. The $EC_{50}$ value was calculated from a curve of the radical-scavenging percentage versus the extract concentration.

*Reductive-potential test*

Reducing power was determined according to the method described by Oyaizu [19], with slight modifications. BAK sample extracts (1 mL) were mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6), after which 2.5 mL of 1% potassium ferricyanide was added, and the mixture was incubated at 50℃ for 20 min. After cooling, 2.5 mL of 10% trichloroacetic acid (w/w) was added, and the mixture was centrifuged at 3,000 rpm for 10 min. A 2.5 mL sample of the supernatant was mixed with 500 μL of 0.1% ferric chloride. The absorbance of the sample was measured at 700 nm. The reducing power was presented as the difference in absorbance between samples with and without extract addition. The $EC_{50}$ was calculated as described for radical scavenging activity.

*Animals and experimental diets*

Six-week-old male Sprague-Dawley rats were purchased from Koatech (Pyeongtaek, Korea). After 1 week of adaptation, the rats were divided into 4 groups (n = 9/group; average weight: ~150 g) and separately housed in stainless-steel-bottomed cages. All animals were housed at room temperature (22 ± 2℃) and 50 ± 5% humidity, with a 12-h light/dark cycle (7:00-19:00). To induce LC in rats, thioacetamide (TAA) was dissolved in physiological saline and administered intraperitoneally twice weekly based on a dose of 200 mg/kg body weight for 4 weeks. The 4 groups included a normal group (rats fed a normal chow diet), the TAA group (LC rats fed a normal chow diet), the TAA + CTL group (LC rats fed chow diets containing 0.1% kimchi without BJP), and the TAA + BAK-1 group (LC rats fed chow diets containing 0.1% kimchi with 1% added BJP). The study was designed to provide 210 mg wet weight of kimchi to each rat daily, which is equivalent to the amount of kimchi consumed by healthy Korean adults (96.3 g/day). Chow was provided daily for 4 weeks, and body weights were measured once weekly. Dietary intake was measured at a fixed time daily, and dietary intake efficiency was calculated by dividing the weight gain over the entire period by the dietary intake during the same period. The experimental protocols in this study were approved by the institutional animal care and use committee (IACUC) of Berry & Biofood Research Institute (approval number: BBRI-IACUC-15002).

*Serum analysis and determination of antioxidant enzyme activity*

Rats were fasted for 12 h before being sacrificed by anesthetization with diethyl ether. Blood was collected from the abdominal vein immediately after laparotomy, and serum was obtained by centrifuging the heparin tube at 3,000 rpm for 15 min in order to separate the supernatant. Serum glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) enzyme levels were analyzed using a Fuji DRI-CHEM 4000 instrument (Fuji Film, Tokyo, Japan) to identify liver damage. The activity of superoxide dismutase (SOD), an antioxidant enzyme, was measured using a colorimetric assay kit (BioVision, Milpitas, CA, USA).

*Statistical analysis*

All data are expressed as the mean ± standard deviation (SD) of 3 replicates. Statistical analysis was performed using SPSS software (v23.0 for windows; IBM Corp., Armonk, NY, USA). Data were subjected to Student's *t* test, one-way analysis of variance (ANOVA), and two-way ANOVA, and the mean values were separated using Duncan's multiple-range test, with statistical significance defined as $P < 0.05$. The differences in the antioxidant activities of kimchi according to the addition of BJP (same fermentation state) and fermentation state (same BJP amount) were verified using one-way ANOVA. The effects of BJP addition and fermentation period on kimchi antioxidant effects were evaluated by two-way ANOVA. Differences in serum levels and antioxidant enzyme activity between BAK and control groups were evaluated using Student's *t* test.

## RESULTS

*Changes in chemical and microbial properties*

BAK with various concentrations of BJP was fermented at 4℃ for 4 weeks, and changes in the chemical (pH and acidity) and microbiological (total bacterial and LAB counts) properties of the 4 groups were monitored (Fig. 1 and 2). During the fermentation period, the pH and acidity changes in the 4 groups showed similar patterns. The pH decreased from a range of 5.14-5.74 to 3.95-4.11, and the acidity increased from a range of 0.28%-0.39% to 0.93%-0.99%. As the BJP content of the BAK groups increased, the pH decreased and the acidity increased ($P < 0.05$). The total bacterial and LAB counts in the BAK groups ranged from 9.11 log CFU/mL to 9.53 log CFU/mL and 9.11 log CFU/mL to 9.60 log CFU/mL, respectively, with the highest levels observed at 2 weeks of fermentation ($P < 0.05$). However, there was no significant difference according to the amount of BJP added.

*Changes in TPC and TFC*

Changes in the TPC and TFC during fermentation of the 4 groups were monitored (Table 1). BAK groups showed higher TPC and TFC relative to those of the control group, regardless of fermentation time. During the fermentation period, the TPC and TFC in the BAK groups ranged from 4.82 μg GAE/mg to 6.63 μg GAE/mg and 3.61 μg GAE/mg to 5.36 μg CE/mg, respectively, which were higher than those in the control group ($P < 0.05$). In the BAK groups, TPC and TFC increased along with the concentration of BJP until the first week of fermentation. However, there was no significant difference in TPC or TFC
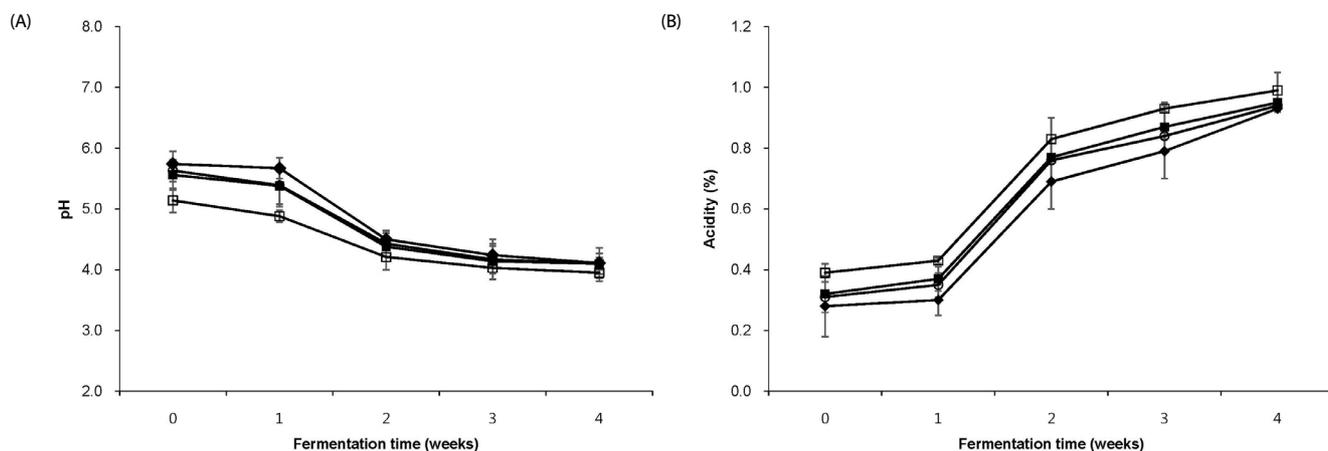
(A)



(B)



**Fig. 1.** Changes in pH (A) and acidity (B) of kimchi supplemented with BJP during fermentation for 4 weeks at 4℃. (♦) control, kimchi without BJP; (○) BAK-0.5, kimchi with 0.5% BJP; (■) BAK-1, kimchi with 1% BJP; (□) BAK-2, kimchi with 2% BJP. Data are shown as the mean ± SD (n = 3). BAK, kimchi with added BJP; BJP, freeze-dried black raspberry juice powder.
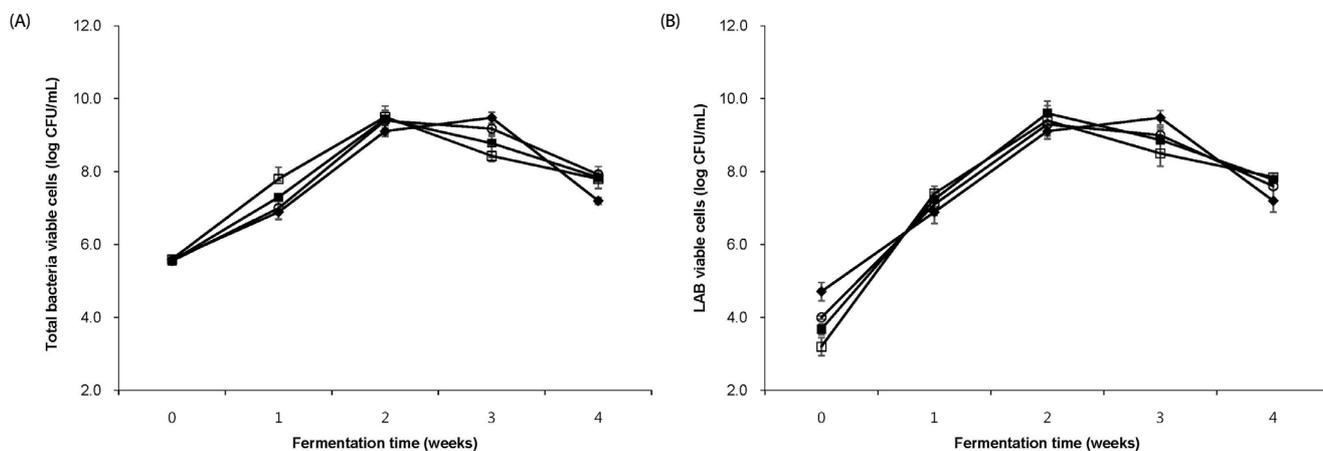
(A)



(B)



**Fig. 2.** Changes in total viable bacteria (A) and viable LAB (B) cells in kimchi supplemented with BJP during fermentation for 4 weeks at 4℃. (♦) control, kimchi without BJP; (○) BAK-0.5, kimchi with 0.5% BJP; (■) BAK-1, kimchi with 1% BJP; (□) BAK-2, kimchi with 2% BJP. Data are shown as the mean ± SD (n = 3). BAK, kimchi with added BJP; BJP, freeze-dried black raspberry juice powder; LAB, lactic acid bacteria.

according to the amount of BJP added after 2 weeks of fermentation. The TPC and TFC in the BAK groups increased up to 2 weeks of fermentation, reaching their highest levels, followed by decreases. The BAK-1 group exhibited the highest TPC (6.63 μg GAE/mg) and TFC (5.36 μg CE/mg) levels at 2 weeks of fermentation.

**Table 1.** Changes in the total phenolic and total flavonoid contents of kimchi supplemented with BJP during fermentation

| | Samples[1] | Fermentation period (wk) | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 |
| Total phenolic content (μg GAE[2]/mg) | Control | 4.77 ± 0.11[cC] | 5.22 ± 0.22[cB] | 5.58 ± 0.86[cA] | 5.77 ± 0.28[dA] | 5.05 ± 0.38[B] |
| | BAK-0.5 | 4.82 ± 0.10[cE] | 5.41 ± 0.04[cC] | 6.22 ± 0.13[bA] | 5.87 ± 0.02[cB] | 5.25 ± 0.19[D] |
| | BAK-1 | 5.01 ± 0.23[bD] | 5.97 ± 0.97[bB] | 6.63 ± 0.28[aA] | 6.11 ± 0.11[aB] | 5.39 ± 0.17[C] |
| | BAK-2 | 5.17 ± 0.34[aD] | 6.28 ± 0.23[aB] | 6.31 ± 0.15[bA] | 5.99 ± 0.05[bC] | 5.56 ± 0.65[D] |
| Total flavonoid content (μg CE[3]/mg) | Control | 3.27 ± 0.26[dE] | 3.95 ± 0.18[cC] | 4.17 ± 0.14[cB] | 4.79 ± 0.26[bA] | 3.57 ± 0.22[cD] |
| | BAK-0.5 | 3.61 ± 0.45[cE] | 4.43 ± 0.20[bcC] | 4.94 ± 0.17[bA] | 4.69 ± 0.34[abB] | 3.89 ± 0.17[bD] |
| | BAK-1 | 4.15 ± 0.17[bC] | 4.85 ± 0.10[abB] | 5.36 ± 0.34[aA] | 4.77 ± 0.29[aB] | 4.37 ± 0.19[aC] |
| | BAK-2 | 4.34 ± 0.42[aD] | 5.08 ± 0.30[aB] | 5.34 ± 0.16[aA] | 4.73 ± 0.31[abBC] | 4.34 ± 0.19[aC] |

Data are shown as the mean ± SD (n = 3).
Means with different letters in a column (a-d) and a row (A-E) are significantly different based on one-way ANOVA, followed by Duncan's multiple-range test (P < 0.05).
[1] Control, kimchi without BJP; BAK-0.5, kimchi with 0.5% BJP; BAK-1, kimchi with 1% BJP; BAK-2, kimchi with 2% BJP.
[2] Presented as the number of μg GAE.
[3] Presented as the number of μg CE.
Abbreviations: BAK, kimchi with added BJP; BJP, freeze-dried black raspberry juice powder; CE, catechin equivalents; GAE, gallic acid equivalents.

**Table 2.** Changes in DPPH and ABTS radical scavenging and reducing ability of kimchi supplemented with BJP during fermentation

| | Samples[1] | Fermentation period (wk) | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 |
| DPPH EC$_{50}$ (mg/mL) | Control | $42.26 \pm 2.50^{aA}$ | $40.30 \pm 1.55^{aAB}$ | $38.89 \pm 1.85^{aB}$ | $37.23 \pm 3.54^{aB}$ | $39.34 \pm 2.00^{aAB}$ |
| | BAK-0.5 | $30.00 \pm 3.15^{bA}$ | $26.91 \pm 3.04^{bA}$ | $21.54 \pm 2.54^{bC}$ | $22.90 \pm 1.59^{bBC}$ | $26.30 \pm 2.48^{bAB}$ |
| | BAK-1 | $15.13 \pm 2.49^{cA}$ | $12.27 \pm 3.74^{cB}$ | $9.41 \pm 3.11^{cC}$ | $11.15 \pm 2.20^{cBC}$ | $12.59 \pm 3.04^{cB}$ |
| | BAK-2 | $12.89 \pm 1.15^{cA}$ | $10.50 \pm 3.61^{cBC}$ | $9.52 \pm 2.98^{cC}$ | $12.59 \pm 3.19^{cBC}$ | $11.80 \pm 1.98^{cAB}$ |
| ABTS EC$_{50}$ (mg/mL) | Control | $32.40 \pm 1.53^{aA}$ | $29.26 \pm 1.71^{aB}$ | $28.56 \pm 2.85^{aB}$ | $27.58 \pm 2.60^{aB}$ | $30.10 \pm 3.14^{aAB}$ |
| | BAK-0.5 | $23.52 \pm 3.24^{bA}$ | $19.88 \pm 3.52^{bB}$ | $16.21 \pm 2.14^{bC}$ | $16.88 \pm 3.37^{bC}$ | $20.84 \pm 1.25^{bB}$ |
| | BAK-1 | $12.63 \pm 2.18^{cA}$ | $10.08 \pm 1.14^{cAB}$ | $8.26 \pm 0.45^{cC}$ | $8.87 \pm 1.26^{cBC}$ | $9.53 \pm 1.08^{cBC}$ |
| | BAK-2 | $10.18 \pm 2.30^{cA}$ | $8.87 \pm 2.20^{dB}$ | $8.27 \pm 0.75^{cB}$ | $8.54 \pm 1.14^{cB}$ | $9.76 \pm 0.61^{cA}$ |
| Reducing power EC$_{50}$ (mg/mL) | Control | $6.95 \pm 0.55^{aA}$ | $6.08 \pm 0.23^{aB}$ | $5.50 \pm 0.39^{aC}$ | $5.11 \pm 1.27^{aD}$ | $5.09 \pm 0.98^{aD}$ |
| | BAK-0.5 | $6.01 \pm 0.54^{bA}$ | $5.52 \pm 0.56^{bB}$ | $4.28 \pm 0.74^{bE}$ | $4.43 \pm 0.45^{bD}$ | $4.91 \pm 1.02^{bC}$ |
| | BAK-1 | $5.52 \pm 0.37^{cA}$ | $4.77 \pm 1.04^{cB}$ | $3.71 \pm 0.23^{cE}$ | $3.99 \pm 0.37^{cD}$ | $4.35 \pm 0.88^{cC}$ |
| | BAK-2 | $5.01 \pm 0.50^{dA}$ | $4.21 \pm 1.15^{dB}$ | $3.51 \pm 0.50^{dD}$ | $3.78 \pm 0.59^{dC}$ | $4.23 \pm 1.01^{dB}$ |

Data are shown as the mean ± SD (n = 3).
Means with different letters in a column (a-d) and a row (A-E) are significantly different based on one-way ANOVA, followed by Duncan's multiple-range test (*P* < 0.05).
[1] Control, kimchi without BJP; BAK-0.5, kimchi with 0.5% BJP; BAK-1, kimchi with 1% BJP; BAK-2, kimchi with 2% BJP.
Abbreviations: ABTS, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; BAK, kimchi with added BJP; BJP, freeze-dried black raspberry juice powder; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EC$_{50}$, half maximal effective concentration.
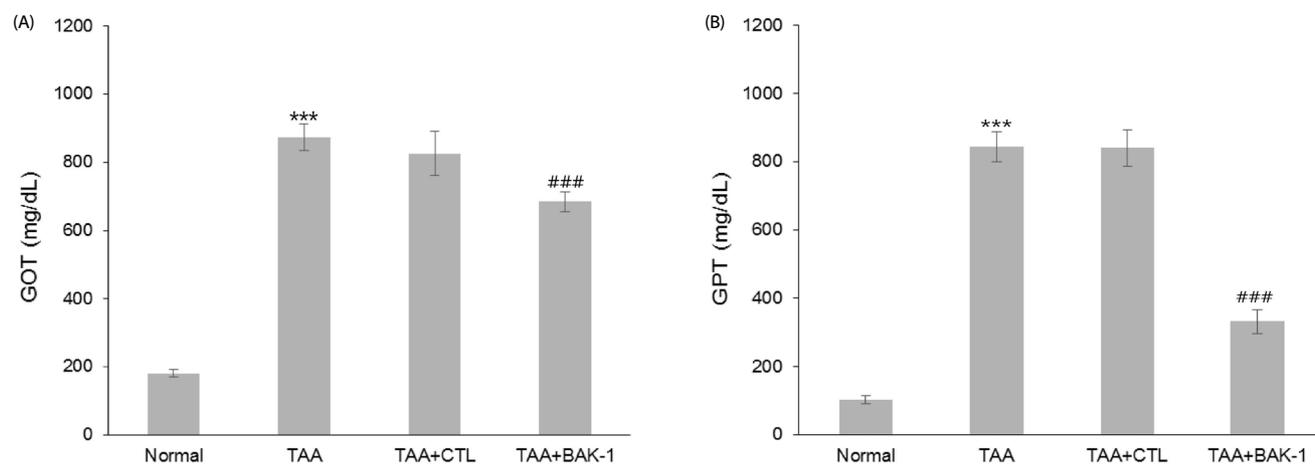


**Fig. 3.** Determination of the GOT and GPT activities of kimchi supplemented with BJP. The effects of kimchi on GOT (A) and GPT (B) levels in the livers of rats fed the indicated diets for 4 weeks are shown. Data reflect the mean ± SD (n = 9/group). *** *P* < 0.001 compared with the normal group; ### *P* < 0.001 compared with the TAA group. Normal, rats fed a normal chow diet; TAA, LC rats fed a normal chow diet; TAA + CTL, LC rats fed a chow diet containing 0.1% kimchi without BJP; TAA + BAK-1, LC rats fed a chow diet containing 0.1% kimchi with 1% BJP. Abbreviations: BAK, kimchi with added BJP; BJP, freeze-dried black raspberry juice powder; CTL, control; GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; LC, liver cirrhosis; TAA, thioacetamide.

*Changes in antioxidant activities*

The changes in DPPH free radical and ABTS cation radical scavenging activities and reducing power observed during the fermentation period in each of the 4 groups are shown in Table 2. The changes in DPPH and ABTS scavenging activities in the BAK groups were similar during fermentation, although the ABTS EC$_{50}$ values were lower. The DPPH and ABTS scavenging activities of the BAK groups were significantly higher than that of the control group, regardless of fermentation time (*P* < 0.05). The DPPH and ABTS EC$_{50}$ values of the BAK groups ranged from 9.41 mg/mL to 30.00 mg/mL and 8.26 mg/mL to 23.52 mg/mL, respectively, with the lowest and highest values observed in the BAK-1 group fermented for 2 weeks and the BAK-0.5 group immediately after creation (0 weeks).

Similar to the DPPH and ABTS scavenging activities, the reducing power observed in the BAK groups was significantly

higher than that of the control group, showing the highest activity at 2 weeks of fermentation (*P* < 0.05). The EC$_{50}$ values of the BAK groups ranged from 3.54 mg/mL to 6.01 mg/mL, with the highest reducing power observed in the BAK-2 group. In contrast to the DPPH and ABTS scavenging activities, the reducing power significantly increased depending on the amount of BJP added (*P* < 0.05). Based on these results, the BAK-1 group exhibiting excellent antioxidant activities was selected for analysis of antioxidant activity *in vivo*.

*Serum GOT and GPT activities*

Changes in serum GOT and GPT activities following ingestion of BAK-1 are shown in Fig. 3. GOT and GPT activities were significantly elevated in the liver of rats treated with TAA as compared with the normal group (*P* < 0.001). The TAA + BAK-1 group displayed significantly reduced GOT and GPT activities
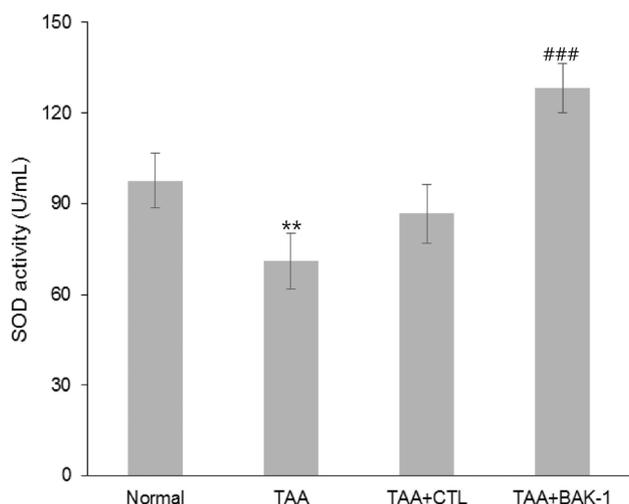
**Fig. 4.** SOD activity of kimchi supplemented with BJP. The effects of kimchi on SOD activity in the livers of rats fed the indicated diets for 4 weeks. Data represent the mean ± SD (n = 9/group). ** $P < 0.01$ compared with the normal group; ### $P < 0.001$ compared with the TAA group. Normal, rats fed a normal chow diet; TAA, LC rats fed a normal chow diet; TAA + CTL, LC rats fed a chow diet containing 0.1% kimchi without BJP; TAA + BAK-1, LC rats fed a chow diet containing 0.1% kimchi with 1% BJP. Abbreviations: BAK, kimchi with added BJP; BJP, freeze-dried black raspberry juice powder; CTL, control; LC, liver cirrhosis; SOD, superoxide dismutase; TAA, thioacetamide.

compared with those of the TAA group ($P < 0.001$), whereas the TAA + CTL group showed no significant difference. Compared with the TAA + CTL group, the GOT and GPT activities of the TAA + BAK-1 group were significantly decreased by 14.1% and 60.5%, respectively ($P < 0.01$).

*Effect on antioxidant enzyme activity*

The effects of dietary supplementation with BAK-1 on hepatic SOD activity reduced by TAA treatment were assessed. As shown in Fig. 4, hepatic SOD activity was significantly decreased in the TAA-treated group as compared with that observed in the normal group ($P < 0.01$). BAK-1 supplementation remarkably restored the hepatic SOD activity reduced by TAA ($P < 0.001$), and these effects were more pronounced than those of kimchi without BJP. Additionally, compared with the TAA + CTL group, SOD activity in the TAA + BAK-1 group was higher ($P < 0.01$), increasing by 48.2%.

## DISCUSSION

Antioxidants perform a variety of functions in biological systems, including prevention of cell damage by ROS [2]. Therefore, characterizing safe and effective natural antioxidants for possible application in functional foods has received increasing attention.

The main types of phenolic compounds in berries include phenolic acids, flavanols, and anthocyanins [20]. BR contain an abundance of phenolic compounds, including gallic acid, ellagic acid, cinnamic acid, protocatechuic acid, sanguiin H-4, and sanguiin H-6, that reportedly exhibit strong antioxidant effects and inhibit oxidation by scavenging free radicals or ROS in living systems [21].

Kimchi is a traditional Korean fermented food that contains

various functional ingredients and is an especially rich source of natural antioxidants, such as vitamins, carotenoids, flavonoids, and phenolic compounds [22]. Various antioxidant effects of kimchi have been reported along with investigations of correlations between fermentation period and changes in antioxidant activity [7,9,16]. Although there is a lack of rigorous scientific evidence, the antioxidant effects of kimchi appear to vary depending on supplementation and fermentation state. Therefore, in this study, the antioxidant effects of kimchi were compared and analyzed according to BJP supplementation and fermentation period.

Comparison of pH and acidity values with those of the control group (kimchi without BJP) showed that the fermentation patterns of the BAK groups were ~1 week faster, reaching OR at 2 weeks (the control group reached OR at 3 weeks). Additionally, the total bacterial and LAB counts in the BAK groups exhibited similar patterns, reaching maximum values at 2 weeks. These results indicated that the organic acids contained in BJP, in addition to the organic acids produced during fermentation, affected kimchi pH and acidity. During kimchi fermentation, carbohydrates are decomposed or converted by LAB into various organic acids [16]. Therefore, pH and acidity are important indicators of kimchi quality and are influenced by organic acids [23]. The optimal pH and acidity for the best kimchi ripening range from 4.2 to 4.5 and 0.6% to 0.9%, respectively, with a pH < 4.0 or acidity > 1.0 indicating over-fermentation [24].

Measurement of TPC and TFC, as well as DPPH and ABTS radical scavenging and reducing power, showed that the BAK groups exhibited increased antioxidant effects relative to those of the control group. These results suggested that various antioxidants, such as the phenolic compounds contained in BJP, affected the antioxidant activity of kimchi. These findings were similar to those reported previously, which showed that the antioxidant effects of kimchi and KS were enhanced by adding antioxidant ingredients. Specifically, the DPPH-scavenging ability of kimchi supplemented with cultured wild ginseng roots improved, as did the phenolic compound, DPPH, and nitric oxide scavenging activities of tofu supplemented with KS [25-27].

On the other hand, the antioxidant effects of kimchi supplemented with BJP differed depending on the fermentation period, with the antioxidant activities of the BAK groups and control group changing over the 4-week fermentation period. Additionally, the highest levels of TPC, TFC, and antioxidant activities were observed in the OR kimchi. Several studies demonstrated correlations between fermentation period and the antioxidant effects of kimchi. Kim *et al.* [9] reported that the TPC and DPPH and nitrite scavenging activities of over-ripened kimchi (> 2 years) were higher than those of short-term fermented kimchi (< 7 days), and Park *et al.* [16] found that the TFC of mustard leaf kimchi extracts during the ripening period was higher than that at the beginning and end of fermentation. Additionally, studies reported that fermentation using microorganisms such as LAB and yeast promotes antioxidant activities involving natural compounds [28,29].

We performed two-way ANOVA of the effects of BJP supplementation and fermentation period on TPC, TFC, and the

antioxidant activities of kimchi. TPC and TFC levels were significantly influenced by BJP addition (TPC: F value = 129.64; TFC: F value = 141.34; $P < 0.001$) and fermentation period (TPC: F value = 360.41; TFC: F value = 131.26; $P < 0.001$). Additionally, there was a significant relationship (TPC: F value = 13.74; TFC: F value = 6.11; $P < 0.001$) between BJP addition and fermentation period, which affected the TPC and TFC levels of the kimchi. Moreover, DPPH and ABTS radical scavenging and reducing power were significantly influenced by BJP addition (DPPH: F value = 1,152.76; ABTS: F value = 827.79; reducing power: F value = 2,607.05; $P < 0.001$) and fermentation period (DPPH: F value = 19.91; ABTS: F value = 23.31; reducing power: F value = 1,925.29; $P < 0.001$) as well as TPC and TFC. However, the relationship (DPPH: F value = 2.58; $P < 0.05$; reducing power: F value = 58.34; $P < 0.001$) between these factors affected DPPH, but not ABTS. Thus, our results showed that the antioxidant effect of kimchi was influenced by fermentation period, BJP content, and their interactions. Park and Rhee [30] suggested that the antioxidant effect of kimchi could be influenced by both the primary and supplementary ingredients as well as their respective levels and the fermentation period.

On the other hand, we conducted a sensory evaluation of kimchi prepared with various concentrations (0, 0.5, 1, and 2%) of BJP in consideration of its practical application (Supplementary Table 1). The test was conducted in a blinded manner with 20 trained panelists. The appearance, flavor, taste, and overall acceptance were significantly highest for BAK-1 ($P < 0.05$). *In vitro*, BAK-1 fermented for 2 weeks showed the best antioxidant effect based on TPC, TFC, and antioxidant activities, except in the reducing power test. Based on these results, we selected BAK-1 fermented for 2 weeks for animal experiments.

The animal experiment in this study was performed to confirm the antioxidant effects of the kimchi preparations based on enzyme antioxidant activity, which regulates ROS levels within the body. TAA is a hepatotoxic agent used in experimental models of chronic liver disease (chronic infection, LC, and liver cancer). TAA causes hepatotoxicity characterized by hepatocyte necrosis and proliferation as well as ROS-induced oxidative stress in experimental models of chronic liver disease [31]. Additionally, TAA induces ROS production through $H_2S$ hydrolysis and reduction of antioxidant enzyme levels inside hepatocytes [32]. Therefore, we selected the TAA-induced LC model to verify the antioxidant effect of the kimchi prepared with freeze-dried BR powder. GOT and GPT activities are closely related to liver damage, including symptoms associated with increased cellular activity and secretion into the circulatory system following cell damage and apoptosis [33]. In particular, when abnormalities in cells or tissues are caused by liver disease, such as fatty liver disease and hepatitis, GOT and GPT are released into the blood and can be used as indicators of liver damage according to their elevated serum concentrations [34]. SOD is an important antioxidant enzyme, representing an antioxidant defense mechanism *in vivo* through its ability to convert superoxide radicals into less reactive molecules (*i.e.*, hydrogen peroxide) [35]. Following our *in vitro* antioxidant studies, we confirmed the presence of *in vivo* antioxidant effects in the BAK-1 group. In the TAA-induced LC model, BAK-1 dietary supplementation significantly reduced elevated GOT and GPT activities and

increased SOD activity, indicating recovery of liver function. These results suggested that kimchi supplemented with BJP could serve as a protective agent against hepatic injury.

Previous studies showed that liver protection is achieved through the administration of berries in animal models. Similar to our results, Choi *et al.* [36] reported that oral administration of Korean BR seed extract resulted in increased antioxidant enzyme activity, such as that of SOD, in hepatotoxic mice induced by acetaminophen, thereby preventing hepatotoxicity. Additionally, Chen *et al.* [37] reported that blueberry anthocyanin extract significantly enhanced SOD activity in mice with liver injury induced by CC14.

In conclusion, we showed that the antioxidant effect of kimchi was improved by addition of BJP and fermentation, with our data suggesting that OR kimchi supplemented with BJP represents a promising functional food that can protect against ROS-mediated oxidative stress. Future research on antioxidant activity and mechanisms associated with kimchi supplemented with BJP should be conducted in relation to fermentation stage.

## CONFLICT OF INTEREST

The authors declare no potential conflicts of interests.

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