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Hepatoprotective effects of ethnic cabbage dishes: a comparison study on kimchi and pao cai

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Abstract

Consuming ethnic vegetable dishes, that has been an integral part in the food culture of many countries, is suggested to bring health benefits to humans. Kimchi from Korea and pao cai from China are two distinct vegetable dishes made from Chinese cabbage (*Brassica rapa*) through different processes. While kimchi is a fermented food, pao cai is produced by pickling in brine or vinegar. The present study aimed to investigate and compare the hepatoprotective effects of kimchi and pao cai in vivo using animal model and in vitro using a cell line. Despite having similar nutritional profiles, kimchi and pao cai exhibited different chemical and microbiological properties. Compared to pao cai, the pH during fermentation of kimchi dropped more rapidly and the antioxidant activity of kimchi was also stronger. In addition, total microorganisms and lactic acid bacteria were consistently higher in kimchi than in pao cai. In vivo, the hepatoprotective properties of kimchi and pao cai were associated with the increase in expression and activity of major liver antioxidant enzymes, particularly glutathione reductase, glutathione peroxidase, glutathione *S*-transferase, catalase, and superoxide dismutase. In vitro, both kimchi and pao cai promoted the formation of glutathione. Upon exposure to chemically induced oxidative stress, kimchi protected liver cells by inhibiting glutathione depletion and limiting lipid peroxidation. In general, kimchi demonstrated stronger hepatoprotective properties compared to pao cai. Thus, the present study provides promising insights into the development of ethnic foods, particularly kimchi and pao cai, as functional foods beneficial for human health.

Keywords Antioxidant, Oxidative stress, Kimchi, Pao cai, Functional food

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Introduction

Food fermentation is defined as a food processing technology that utilizes the growth and metabolic activity of live microorganisms (typically bacteria, yeasts or mold) to result in desirable characteristics [1]. Fermented ethnic foods have been widely consumed throughout human history by different cultures around the world [2]. Interestingly, consuming fermented food has been reported to promote human health [3]. Cabbages are cruciferous vegetables that contain antioxidants such as sulforaphane, kaempferol, quercetin, and apigenin [4]. Kimchi (김치) is an example of a unique fermented food from Korea made from cabbage [5].

Kimchi is a broad term for traditional lactic acid-fermented vegetables originating from Korea. The most popular variant of kimchi is *baechu* kimchi made from Chinese cabbage (*Brassica rapa* subsp. *pekinensis*) fermented in a mixture of seasonings (red chili powder, ginger, garlic, soy sauce, fish sauce, etc.) [6]. In Korea, kimchi is an integral part of the people's food culture and is always present in the diet on daily basis. Korean is estimated to eat 40–57 pounds of kimchi annually [7] or about 50–70 g kimchi daily. Nowadays, kimchi is globally appreciated as a healthy food rich in essential nutrients, dietary fiber, phytochemicals, probiotics and other compounds suggested to promote human health [8]. Previous studies have reported that kimchi exerts health-promoting properties, including antioxidant, anticancer, antimutagenic, antiobesity, antiaging, antiatherogenic, and antidiabetic activities [8].

In contrast to fermentation, pickling is the process of preserving or extending the shelf life of food by immersion in acidic brine or vinegar [9]. It is noteworthy that fermentation and pickling are two distinct types of food processing technology since both processes are often misconceived as similar or overlapping. The fundamental difference between fermentation and pickling lies through the process through which the foods acquire acidity. Fermented foods are sour because of microbial activity that results in the formation of organic acids while pickled foods obtain their acidity mainly by absorbing the acids in the surrounding solution due to difference in osmotic pressure between the foods and the brine or vinegar [10]. In addition, the acidic environment created in pickling aims to kill pathogens and minimize microbial growth [10]. Such a concept is contradictory with fermentation that promotes microbial growth and activity to improve food quality. An example of pickled food is pao cai (泡菜) [11].

Pao cai refers to pickled vegetables found particularly in Sichuan cuisine from China [11]. Chinese cabbage is often used as raw material for pao cai. It is made by soaking vegetables in an anaerobic jar filled with vinegar or brine seasoned by spices like chili pepper, ginger, garlic, and Sichuan pepper [11]. Pao cai has been reported to contain fiber and beneficial microorganisms that contribute to human gut health [12]. In some Sichuan areas, pao cai brine is traditionally used to treat diarrhea, colds, and other diseases [12]. From the perspectives of food processing, kimchi and pao cai are two distinct foods despite both being made from Chinese cabbage (Fig. 1) [13]. They also exhibit different characteristics, particularly in terms of number of lactic acid bacteria population [13]. Nevertheless, history has noted that kimchi and pao cai had been involved in a different series of culture wars and controversies between Korea and China since 2001 [5]. In some cases in the past, both foods were even addressed as the same foods and the same international standards were once applied for both foods [5]. It appears then primordial to emphasize the difference between kimchi and pao cai to avoid misunderstanding among the public.

Oxidative stress, occurring from the imbalance between free radicals and antioxidant defenses, has been strongly associated with premature aging and the development of various diseases, including diabetes, cardiovascular diseases, neurodegenerative diseases, chronic obstructive pulmonary disease, Alzheimer disease, and cancer [14]. Liver as the primary organ for detoxification exerts antioxidative activities that could help protect the cells in our body by counterbalancing the oxidative insults resulting from both internal and external processes, thus preventing the development of oxidation-related diseases [15]. Liver cells possess an integrated antioxidant defense system through the expression of antioxidant enzymes, including catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione S-transferase (GST), and glutathione reductase (GR) [16]. These enzymes play essential roles in regulating the cellular oxidative/antioxidative balance and ensure effective detoxification processes [16]. Improving the antioxidant status of liver could be a strategy to maintain health and help in the prevention of some oxidative-related disorders [17].

Consuming antioxidant compounds through human diet has been shown to enhance the biological antioxidant mechanisms, particularly in the liver [16]. Many natural products, mainly vegetables and fruits, contain bioactive compounds with potent antioxidant properties that support liver detoxification [18]. In previous studies, the consumption of cabbage has been associated with elevated liver health [19]. Plausibly, consuming kimchi and pao cai could also support liver health by boosting liver antioxidant status and expression of antioxidant enzymes which are crucial for detoxification process in the liver [16]. Further processing technology applied in cabbage (fermentation in kimchi and pickling in pao

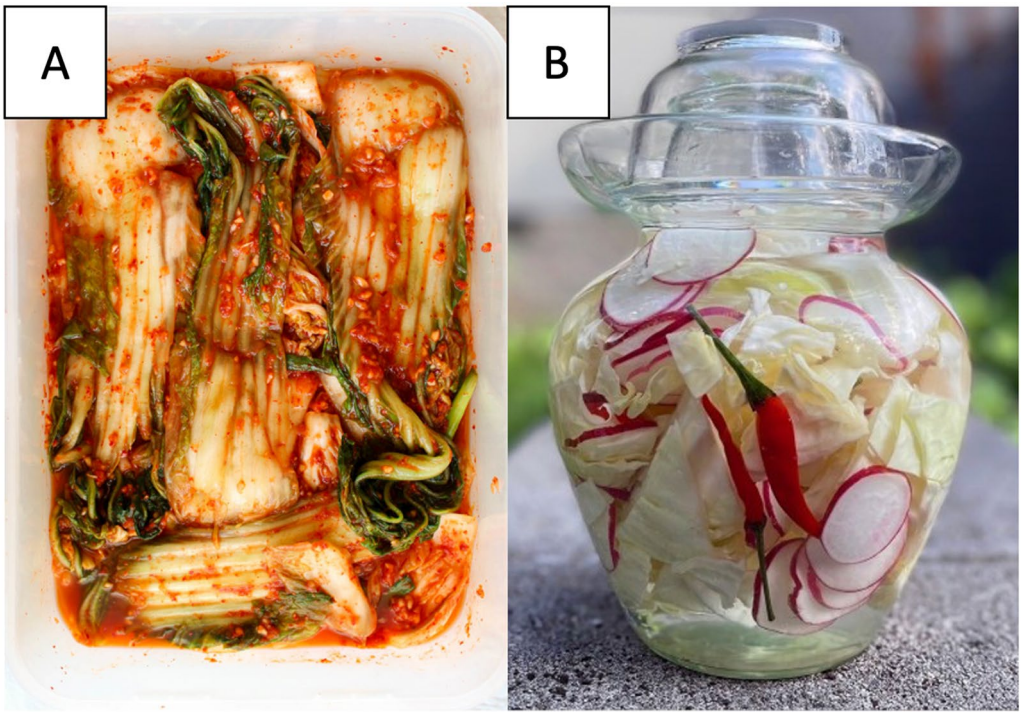

Fig. 1 Two different kinds of ethnic cabbage dishes: **A** kimchi from Korea made through fermentation and **B** pao cai from China made through pickling

cai) could improve the functionality of cabbage for liver health.

The present study aimed to explore and compare the potential of kimchi and pao cai in improving the liver antioxidant status in mice. Firstly, we investigated different profiles during fermentation of kimchi and pao cai in terms of acidity, antioxidant activity, and microbial population, particularly lactic acid bacteria. Secondly, in vivo, we fed mice with standard diet supplemented with freeze-dried kimchi or freeze-dried pao cai and then, we analyzed different blood and hepatic parameters related to antioxidant status. Furthermore, through gene expression analysis, we investigated the expression of several antioxidant enzymes in the liver. Finally, to assess the effects of kimchi and pao cai extract on antioxidant status at the cellular level, we conducted an in vitro study using an immortalized human liver cell line exposed to a chemical inducer of oxidative stress (*tert*-butyl hydroperoxide, TBHP). The originality of this study relied on our focus on the role of kimchi and pao cai in improving the liver antioxidant status in vivo and in vitro. To our knowledge, such a topic has not been previously studied or published. In addition, this is also the first study highlighting the difference between kimchi and pao cai with regard to their biological functions in vivo. The findings of this study would allow to support the development of kimchi and pao cai as functional foods beneficial for human health, in particular regarding their hepatoprotective and antioxidant activity.

Methodology

Preparation of kimchi and pao cai

Kimchi and pao cai were prepared according to previously published recipes [20, 21] with some modifications. Chinese cabbage was bought from a local supermarket in Jakarta, Indonesia. For kimchi, the cabbage was washed and cut lengthwise through the stem into quarters prior to salting with coarse salt (cabbage/salt = 10:1 (w/w)) for 2 h and draining. The seasoning paste was prepared by cooking glutinous rice flour (10 g), anchovy sauce (10 mL), red chili powder (10 g), garlic powder (5 g), ginger powder (5 g), and caster sugar (3 g) in 100 mL water until a homogenous paste was formed. The paste was applied thoroughly to the pre-salted cabbage prior to fermentation in an enclosed glass jar at 35 °C. Usually, kimchi is fermented at room temperature for about half day and stored under 10 °C or in kimchi refrigerator (1–3 °C). However, in this study, pao cai and kimchi were fermented at 35 °C. Microorganisms and metabolites in kimchi may differ with fermentation temperature. For pao cai, the cabbage was washed and cut into smaller uniform pieces (4 cm × 4 cm). Brine solution was prepared

by boiling 100 mL water with coarse salt (8 g), garlic powder (5 g), ginger power (5 g), and caster sugar (3 g). After cooling down, Chinese *baijiu* (20 mL, brand Jiangxiaobai containing 40% (w/v) alcohol) was added into the brine solution. A glass pao cai jar was filled with the cabbage pieces and brine solution with a mass ratio of 2:3, and the mixture was left fermented at 35 °C for 5 days. For animal experiments, kimchi or pao cai at the fifth day of fermentation were crushed using a blender and the filtrate was then freeze-dried (0.10 mbar, −25 °C, 48 h) and ground to form powder.

Analysis of pH, DPPH scavenging activity, lactic acid bacteria, and nutrients

The pH of kimchi paste or pao cai brine solution during fermentation was measured using a pH meter (Mettler Toledo FiveEasy F20, Fisher Scientific) according to the manufacturer's instructions. The DPPH (1,2-diphenyl-1-picrylhydrazyl) scavenging activity assay was done as previously described, and the results were expressed as percentage of scavenged DPPH [22]. The lactic acid bacteria count was done using MRS (de Man, Rogosa, and Sharpe) agar (GranuCult, Merck Millipore) following a 5-day incubation at 35 °C as previously described [23]. The proximate analysis for nutrients was performed using the standardized methods established by the Association of Official Analytical Chemists (AOAC) and the American Oil Chemists Society (AOCS) as previously described [24]. The analyzed nutrients included moisture, ash, fat, protein, fiber, and carbohydrate obtained by difference. The results were expressed in dry basis.

Animal experiments

Forty-five male C57BL/6 mice aged 3–4 weeks old were purchased from the Central Animal House of IPB University (Bogor, West Java, Indonesia). They were housed with a 12-h light/dark cycle at room temperature and had access to food and water ad libitum. The mice were given modified AIN-76 diet [25] with freeze-dried kimchi or freeze-dried pao cai obtained on the fifth day of fermentation as source of fiber (5%). The rats were then randomly divided into 3 groups, each of which consisted of 15 mice: group 1 (fed with standard AIN-76 diet), group 2 (fed with kimchi-enriched diet) and group 3 (fed with pao cai-enriched diet). All the mice consumed, on average, 3.38 ± 0.45 g diet on daily basis for 100 days, and there was no significant difference in terms of diet consumption observed among the 3 groups during the feeding duration ($p > 0.05$). All the mice were euthanized by CO_2 asphyxiation on the 100^{th} day, and their liver were collected for further analyses. The protocol for our animal study has been approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia (Jakarta, Indonesia).

Analysis of blood and hepatic parameters

Blood samples were collected on the day of sacrifice. The plasma alanine aminotransferase (ALT) activity was measured using the Alanine Aminotransferase Activity Assay Kit MAK052 (Sigma-Aldrich) according to the manufacturer's instructions and was reported as unit per liter of plasma (U/L), where 1 mU is defined as the amount of ALT generating 1 nmol of pyruvate per minute at 37 °C. The isolation of hepatocytes was performed by collagenase perfusion as previously described [26]. The glutathione reductase (GR) activity, glutathione S-transferase (GST) activity, and glutathione GSH/GSSG ratio were analyzed using commercial kits (product No. GRSA, MAK453, and MAK440, respectively, Sigma-Aldrich) according to the manufacturer's instructions. The GR activity was expressed as nmol of NADPH oxidized per minute per mg protein (mU/mg). The GST activity was expressed as nmol of glutathione conjugate with 1-chloro-2,4-dinitrobenzene (CDNB) per minute per mg protein (mU/mg). The GSH/GSSG ratio expresses the concentration ratio of cellular reduced glutathione (GSH) with its oxidized form (GSSG).

RT-qPCR analysis

The mRNA expression of several genes in the suspension of mice hepatic tissue was quantified using RT-qPCR following tissue lysis and mRNA extraction using mRNA isolation kit (Roche, Mannheim, Germany) as previously described [27] according to manufacturer's instructions. The synthesis of cDNA was done using High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, MA, USA) with 1 µg RNA, and the qPCR was conducted using AriaMx Real-Time PCR systems (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's instructions. The thermocycling conditions were set as follows: initial denaturation at 95 °C (5 min), denaturation at 94 °C (30 s), annealing at 56 °C (30 s), extension at 72 °C (40 s) and final extension at 72 °C (5 min). The sequences of the primers (5'-3') used are listed below: catalase (S-CGA CCA GAT GAA GCA GTG GA, AS-CCA CTC TCT AGG AAT CCG C), SOD1 (S-GGT GAA CCA GTT GTG TTG TCA GG, AS-ATG AGG TCC TGC ACT GGT ACA G), SOD2 (S-TAA CGC GCA GAT CAT GCA GCT G, AS-AGG CTG AAG AGC GAC CTG AGT T), GPx1 (S-CGC TCT TTA CCT TCC TGC GGA A, AS-AGT TCC AGG CAA TGT CGT TGC G), GSTA1 (S-CTG CCT TGG CAA AAG ATA GGA CC, AS-CTT CCA GTA GGT GGA TGT CCA C), GSTM1 (S-TGT TTG AGC CCA AGT GCC TGG A, AS-TAG GTG TTG CGA TGT AGC GGC T),

GSTT1 (S-TAT CCC GTT CCA GAT GCA CAC G, AS-CCA GGT AGA GCA AGA TAG CCA C), GR (S-GTT TAC CGC TCC ACA CAT CCT G, AS-GCT GAA AGA AGC CAT CAC TGG TG), and housekeeping gene TBP (S-CTA CCG TGA ATC TTG GCT GTA AAC, AS-AAT CAA CGC AGT TGT CCG TGG C).

Cell culture and cellular assays

The immortalized human normal hepatocytes Fa2N-4 cell line was provided by XenoTech (Kansas City, KS, USA) and grown in Cell Biologics' culture complete epithelial cell medium according to the manufacturer's instructions prior to assays. The freeze-dried extract of kimchi or pao cai from the fifth day of fermentation was incorporated into the medium with a ratio of 1:50 (w/v). The cells were incubated either in the medium (for control group) or in the medium containing kimchi or pao chai (for kimchi and pao cai groups) for 12 h prior to exposure to oxidant tert-Butyl hydroperoxide (TBHP, Sigma-Aldrich, 500 μM). The cellular assays, including reactive oxygen species (ROS) assay (4 h-treatment), thiobarbituric acid reactive substances (TBARS) assay (6 h-treatment), glutathione (GSH) assay (6 h-treatment), and cell viability assay (12 h-treatment), were done as previously described [28] using a fluorescence spectrometer (VersaFluor, Bio-Rad Laboratories). Cellular ROS were analyzed using a fluorogenic dye DCFDA/H2DCFDA-Cellular ROS detection kit (Abcam). Cellular TBARS were analyzed using OxiSelect TBARS assay kit (Cell Biolabs). Cellular GSH was analyzed using Glutathione Assay Kit CS0260 (Sigma-Aldrich). Cell viability was quantified with resazurin cell viability kit (Cell Signaling Technology). The RFU (relative fluorescence units) of all the samples were normalized to control (untreated cells at t = 0 h).

Statistical analysis

All data were analyzed using the SPSS (Statistical Package for the Social Sciences) version 25 software by implementing either student's *t*-test if only two groups were compared or analysis of variance (ANOVA) if more than two groups were compared. When ANOVA showed significant differences among the samples ($p < 0.05$), the test was followed by Tukey's post hoc test. The heatmap illustrating the induction or repression of gene expression was generated using online application Displayr.

Results and discussion

Different chemical, microbiological, and nutritional profiles between kimchi and pao cai

Kimchi and pao cai exhibited different chemical and microbiological profiles as shown in Fig. 2. These different profiles are suggested to be due to different types of food processing applied in both foods, which are fermentation in kimchi and pickling in pao cai. In the present study, we opted for fermentation at 35 °C for 5 days to accelerate chemical reaction and microbial growth. In practice, both kimchi and pao cai are usually incubated at room temperature (25 °C) for 2–3 days prior to keeping in a refrigerator (0–4 °C) to allow longer preservation [29].

The drop in pH was observed to be more drastic in kimchi compared to pao cai (Fig. 2A). From a similar initial pH at the day 0 of fermentation (6.15 ± 0.21 for kimchi vs. 6.13 ± 0.23 for pao cai), the pH of kimchi decreased to 4.33 ± 0.18 on day 2 and was stabilized until day 5. In contrast, the pH of pao cai showed a gradual decrease and reached the value of 4.22 ± 0.12 on day 5. These results suggest a faster formation of organic acids in kimchi compared to pao cai. The synthesis of organic acids is associated with microbial growth and activity.

With regard to antioxidant activity, kimchi demonstrated a significantly higher capacity in scavenging free radicals (DPPH) compared to pao cai as shown in Fig. 2B. Such a superiority was maintained during the whole fermentation process, with the overall DPPH scavenging activity of kimchi being generally 2–4 times as strong as pao cai. Previously, kimchi has also been shown to exert antioxidative effects on radical-induced oxidative stress in vitro and over ripened kimchi had the highest antioxidant activity compared to fresh and optimally ripened kimchi [30]. We suggested that the presence of red chili powder (*gochugaru*) in kimchi could be the main reason behind its strong antioxidant activity since such an ingredient was not incorporated in pao cai. Red chili powder contains capsanthin and capsaicin, the two bioactive compounds present in red chili (*Capsicum annuum*) with potent antioxidant activity [31]. While capsanthin is responsible for the red color of chili, capsaicin gives pungency and spiciness to chili [31]. These molecules are also known to exert an anti-microbial activity, promote the growth of lactic acid bacteria, and prevent other spoilage bacteria from growing and damaging the tissues. At the same time, lactic acid bacteria produce bacteriocins such as nisin and pediocin, which destroy the cell membranes of spoilage bacteria, thereby preventing spoilage [5]. The increase in antioxidant activity during fermentation of kimchi could be due to the microbial activity that stimulated various biochemical reactions resulting in the formation of organic acids with antioxidant activity [8]. In addition, microbial activity would also allow the transformation of cellulose, pectin, or sucrose into monosaccharides such as glucose and fructose that are known as reducing sugars able to exert antioxidant activity [8].

Figure 2C, D demonstrates the microbial succession during fermentation of kimchi and pickling of pao

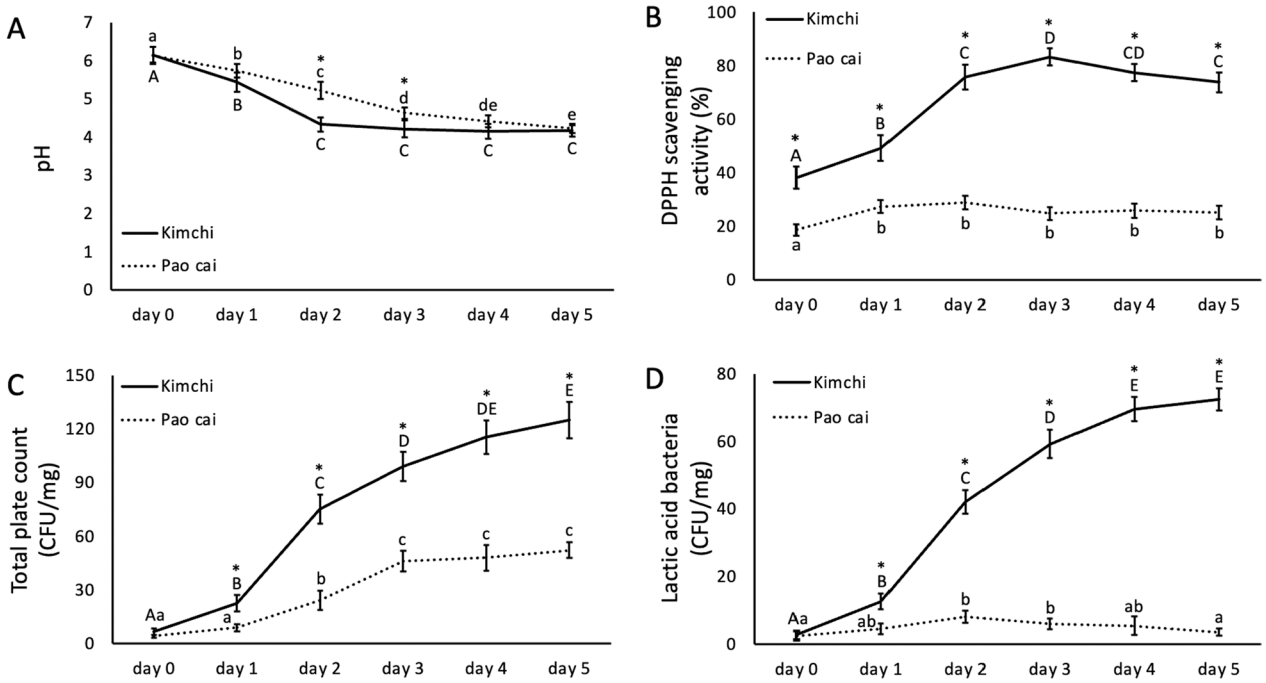

Fig. 2 Chemical and microbiological characteristics of kimchi and pao cai during fermentation, including **A** pH value, **B** DPPH scavenging activity, **C** total plate count, and **D** lactic acid bacteria count. Data ($n=5$) are expressed in mean ± SD. Different lettering indicates statistically significant difference among different fermentation times in kimchi (uppercase) or pao cai (lowercase) according to one-way ANOVA test followed by post hoc Tukey HSD test ($p<0.05$). Asterisk sign (*) indicates statistically significant difference between kimchi and pao cai at the same fermentation time according to student's t-test ($p<0.05$). DPPH: 2,2-diphenyl-1-picrylhydrazyl. CFU: colony forming unit

cai. The increase in total microorganisms was observed in both cases, but it appeared to be higher during fermentation of kimchi compared to pao cai (Fig. 2C). The presence of alcohol (from Chinese *baijiu*) in the brine solution at a relatively high amount might be the factor inhibiting microbial growth during pickling of pao cai. Interestingly, the number of lactic acid bacteria multiplied drastically during fermentation of kimchi from (2.8 ± 1.3) CFU/mg on day 0 to (72.6 ± 4.8) CFU/mg on day 5 (26× increase) (Fig. 2D) and this phenomenon could explain the drastic pH decrease during fermentation of kimchi shown in Fig. 2A. During pickling of pao cai, the number of lactic acid bacteria showed a little increase from (2.5 ± 1.4) CFU/mg on day 0 to its peak of (8.2 ± 1.9) CFU/mg on day 2 and decreased until reaching (3.6 ± 1.1) CFU/mg on day 5 (Fig. 2D). These findings suggested that lactic acid bacteria were the main microorganisms in the fermentation of kimchi while they were not the dominant microorganisms in the fermentation of pao cai, as reported in a previous study [32]. Another study showed that *Pseudomonas* spp. and *Enterobacter* spp. were the most abundant bacteria during the early fermentation of pao cai while lactic acid bacteria, particularly *Lactobacillus* spp., appeared and became dominant at the later stages of fermentation [33]. Since kimchi is rich in lactic acid bacteria, it has been suggested to be developed as a probiotic food that contains live microorganisms intended to provide health benefits when consumed [34].

Kimchi and pao cai possess similar nutritional profile as demonstrated in Table 1. They provide 245.4–259.6 kkal per 100 g dry matter. Carbohydrate is the main constituent of kimchi and pao cai (around 80% of dry matter) with fiber contributing to 1/3 of the present carbohydrate. Indeed, kimchi and pao cai are good sources of fiber since their main ingredients are vegetables. The recommended daily consumption of fiber is 30 g [35],

Table 1 Nutrition profile of kimchi and pao cai (dry basis)

	Kimchi	Pao cai
Energy (kkal/100 g)	259.6	245.4
Carbohydrate (%(w/w))	79.7 ± 6.6	79.4 ± 7.2
Fiber (%(w/w))	26.4 ± 2.8	28.3 ± 3.5
Protein (%(w/w))	11.6 ± 1.3	11.3 ± 1.4
Mineral (%(w/w))	8.6 ± 0.6	9.3 ± 0.8
Fat (%(w/w))	0	0

No significant difference observed between samples ($p \geq 0.05$). Data ($n=5$) are expressed in mean ± SD and were analyzed using the student's t-test

which can be fulfilled from consuming 106–114 g dry matter of kimchi or pao cai. Kimchi and pao cai contain no fat and little protein. In some cases, animal protein is incorporated in kimchi recipes to increase its protein content besides giving flavor, such as fermented seafood (*jeotgal*) [36]. However, it is not common to introduce animal-based ingredients into pao cai [37]. The most abundant mineral in kimchi and pao cai is likely to be sodium since salting is the first step in preparing kimchi and pao cai is pickled in brine solution containing salt (NaCl). The American Heart Association recommends limiting sodium consumption to no more than 2300 mg a day to prevent hypertension and cardiovascular diseases [38]. Interestingly, the regular consumption of kimchi in the daily diet of Koreans was not associated with the prevalence of hypertension [39]. In contrast, high kimchi intake (210 g per day) was associated with a better lipid blood profile related to lower risk of cardiovascular diseases [40].

Kimchi improves the antioxidant status in the liver of mice in a stronger manner compared to pao cai

To investigate the hepatoprotective potential of kimchi and pao cai, we fed mice with diet enriched in freeze-dried kimchi or pao cai for 100 days. Table 2 represents the blood and hepatic parameters of mice following consumption of kimchi- or pao cai-enriched diet. The introduction of kimchi and pao cai in the mice diet was safe for the liver, as indicated by the normal alanine aminotransferase (ALT) activity between 7 and 3 U/L [41]. ALT is an enzyme concentrated primarily in the liver, and its levels can increase in the blood when liver cells are damaged [41]. In this study, the consumption of kimchi or pao cai did not alter the plasma ALT activity in mice and, therefore, did not damage the liver. As an essential organ with an important role in detoxification, the activity of liver depends on the presence of cellular enzymes responsible for antioxidant defense and detoxification of toxic substances [42]. Furthermore, we investigated the activity of two important hepatic enzymes, glutathione reductase (GR) and glutathione *S*-transferase (GST) that are major enzymes in detoxification. The activity of both enzymes along with the GSH/GSSG ratio was stimulated in the liver of mice fed with kimchi-enriched diet but not in the liver of mice consuming pao cai-enriched diet. GR is the enzyme responsible for reducing the oxidized glutathione (GSSG) into its reduced form (GSH) that is able to scavenge free radicals [42]. The GSH:GSSG ratio is therefore an important biomarker of oxidative stress [42].

To verify whether the consumption of kimchi or pao cai could enhance the cellular antioxidant defense system by up-regulating the expression of cellular antioxidant/detoxification enzymes, we conducted RT-qPCR on the hepatic cells of mice. Figure 3 shows that overall, the consumption of kimchi up-regulated the expression of cellular antioxidant enzymes in the mice liver in a stronger manner compared to pao cai. GR, GPx1 (glutathione peroxidase 1), SOD1 (superoxide dismutase 1), and catalase appeared to be the four main enzymes whose expression was strongly stimulated by kimchi consumption. Indeed, the higher activity of GR and GST in the liver of mice fed with kimchi-enriched diet (Table 2) was correlated with the higher expression of antioxidant enzymes regulated by kimchi (Fig. 3).

We suspected that the stronger stimulation of hepatic antioxidant activity by kimchi compared to pao cai would be associated with the presence of bioactive compounds from red chili powder and probiotics (lactic acid bacteria) in kimchi. Bioactive compounds in red chili pepper, such as capsaicin and capsanthin, were reported to

Table 2 Blood and hepatic parameters of mice consuming standard diet, kimchi-, and pao cai-enriched diet on day 100

	Standard diet	Kimchi	Pao cai
ALT (U/L)	28.2 ± 1.8[a]	27.8 ± 1.7[a]	28.3 ± 2.0[a]
GR (mU/mg)	18.6 ± 0.6[a]	20.5 ± 0.4[b]	18.8 ± 0.6[a]
GST (mU/mg)	103.7 ± 4.8[a]	116.4 ± 5.1[b]	107.3 ± 4.2[a]
GSH:GSSG ratio	25.6 ± 1.1[a]	28.4 ± 1.5[b]	25.8 ± 1.2[a]

Different lettering indicates significant difference of a parameter among samples ($p < 0.05$). Data ($n = 15$) are expressed in mean ± SD and were analyzed using ANOVA followed by Tukey's post hoc test

ALT alanine amino transferase, *GR* glutathione reductase, *GST* glutathione S-transferase, *GSH* glutathione, *GSSG* oxidized glutathione

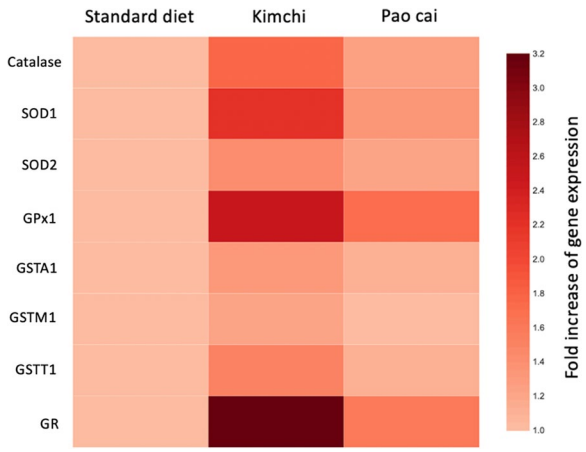

Fig. 3 Heatmap representing relative expression level of genes in the liver of mice consuming standard diet, kimchi-, and pao cai-enriched diet on day 30 ($n = 15$). Gene expression in all groups was normalized relatively to the standard diet group. SOD: superoxide dismutase. GPx: glutathione peroxidase. GST: glutathione S-transferase. GR: glutathione reductase

activate the nuclear factor erythroid 2-related factor 2 (Nrf2), a transcription factor that regulates the expression of antioxidant enzymes [43, 44]. In addition, a recent study has also demonstrated that probiotics activate antioxidant mechanisms and suppress extensive oxidative stress via their ability to activate Nrf2 [45]. *Lactobacillus plantarum* Ln1 isolated from kimchi was suggested to be a useful antioxidant probiotic due to its significant antioxidant activity [46].

Kimchi provides a stronger protection toward hepatic cells against TBHP-induced oxidative stress compared to pao cai

We applied an in vitro approach using a human immortalized liver cell line to study whether the exposure toward kimchi or pao cai extract could protect the cells from oxidative stress. Tert-Butyl hydroperoxide (TBHP) was incubated with the cells to induce the formation of free radicals. Figure 4 demonstrates the antioxidant and protective effects of kimchi and pao cai in the hepatocytes upon exposure to TBHP. This latter induced the formation of reactive oxygen species (ROS) in the hepatocytes resulting in the increase in cellular thiobarbituric acid reactive substances (TBARS) and glutathione (GSH) depletion. Interestingly, pretreatment with kimchi extract and not pao cai extract reduced the amount of basal ROS and TBARS, as well as increasing the amount of GSH in the cells. Upon exposure to TBHP, kimchi extract inhibited the formation of ROS and TBARS, minimalized GSH depletion, and supported cell survival (Fig. 4A–D). Such protective effects were not observed in the cells pre-treated with pao cai extract.

The hepatoprotective effects of kimchi extract toward cells against oxidative stress are suggested to be strongly associated with the up-regulation of the expression of antioxidant enzymes as previously shown in vivo (Fig. 3). Previously, *baechu* kimchi (cabbage kimchi) and *kkakdugi* kimchi (radish kimchi) were reported to exert hepatoprotective activity against liver carcinogenesis induced by diethylnitrosamine and D-galactosamine [47]. A study has also demonstrated that the supplementation of kimchi fermented with black raspberry protected against oxidative stress associated with liver cirrhosis in rats [48]. In addition, lactic acid powder derived from kimchi was shown to protect liver by reducing lipid absorption in the intestine of

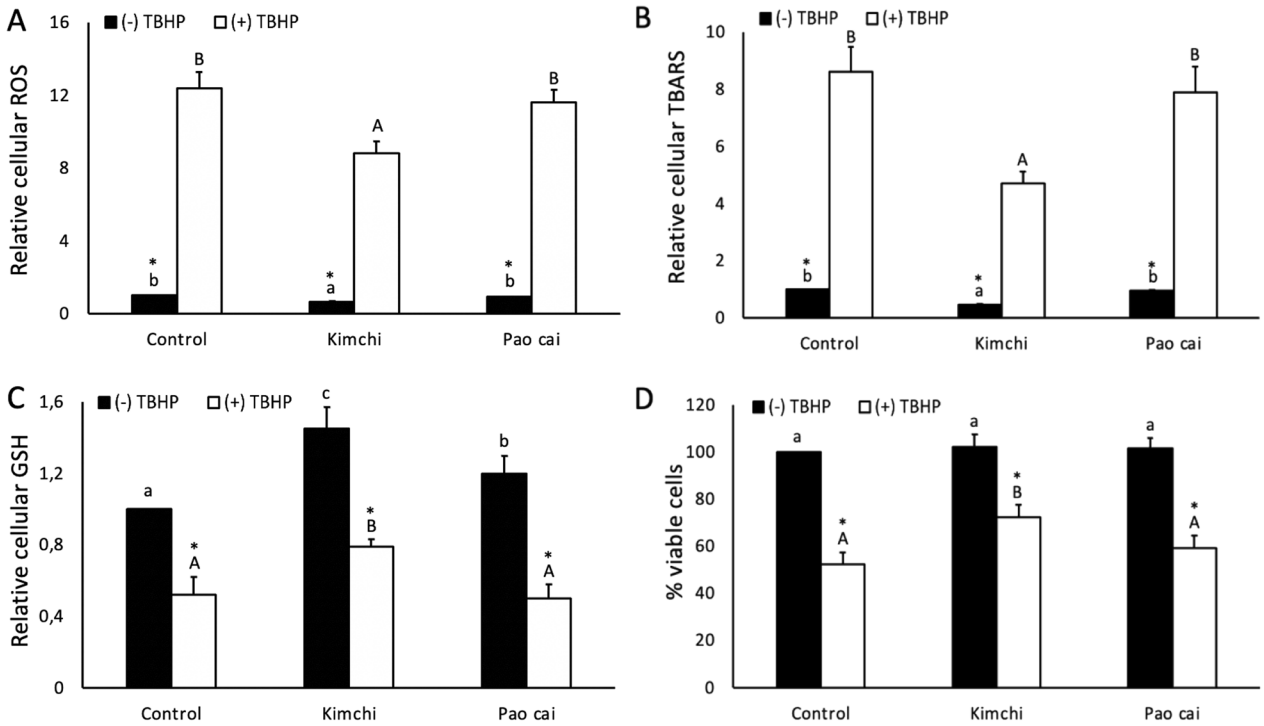

Fig. 4 Antioxidant and protective effects of kimchi and pao cai in cultured human hepatocytes upon exposure to tert-Butyl hydroperoxide (TBHP), an oxidative stress generator. The observed effects included **A** relative cellular reactive oxygen species (ROS), **B** relative cellular thiobarbituric acid reactive species (TBARS), **C** relative cellular glutathione (GSH), and **D** percentage of viable cells. Data ($n=5$) are expressed in mean ± SD. Asterisk sign (*) indicates statistically significant difference between a treatment with and without TBHP as shown by the student's *t*-test ($p<0.05$). Different lettering indicates statistically significant difference among samples treated without TBHP (lower-case letters) and with TBHP (upper-case letters) as shown by one-way ANOVA followed by Tukey's post hoc test. ($p<0.05$)

high fat diet-fed rats [49]. However, there has been no studies reporting the role of pao cai in liver health.

Conclusions

Taken together, our findings in the present study highlighted the potential of ethnic cabbage dishes (kimchi and pao cai) in delivering hepatoprotective properties, particularly by improving liver antioxidant status in mice. In all cases, kimchi appeared to enhance liver antioxidant status in a stronger manner compared to pao cai. This study provides novel insights into the health benefits of kimchi, particularly with regard to liver health and effective detoxification processes. For medical practitioners, this study suggests a possibility for public health recommendation regarding regular kimchi consumption to support liver health and in a long term, prevent liver cancer. For food practitioners, this study gives strong evidence for further development of kimchi as functional food. For further studies, we recommend an animal study on the protection of liver against common oxidative stress inducers (such as alcohol consumption) using kimchi extract. Such a study would lead to a deeper exploration regarding the health benefits of kimchi and in the prevention of chronic diseases related to oxidative stress. Moreover, other studies could also be conducted to investigate the chemopreventive potential of kimchi against liver cancer using animal model. It would also be interesting to investigate whether the probiotics present in kimchi are involved in the hepatoprotective properties of kimchi. In addition, since the type of growing microorganisms and their functional metabolites depend strongly on the temperature at which the fermentation of kimchi takes place, it is recommended to study the hepatoprotective properties of kimchi accordingly at different temperatures: the actual temperature (35 °C), room temperature (25 °C), 10 °C, and kimchi refrigerator temperature (1–3 °C).

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Author contributions
RS, ES, JSO, and RB collaborated in the project and conceptualized the study. DN, RS, FN, and AT were involved in data collection and data analysis. RB and JSO helped in manuscript review and editing. DN and RS were the principal writers of this manuscript.

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Availability of data and materials
The data and materials related to this study are available upon request.

Declarations

Ethics approval and consent to participate
The protocol for animal experiments in this study has been approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia (Jakarta, Indonesia).

Consent for publication
All the authors have read and approved the content of this manuscript for a publication.

Competing interests
The authors declared no competing interests.

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