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Fermentation characteristics and radical scavenging capacities of ginseng berry kombucha fermented by *Saccharomyces cerevisiae* and *Gluconobacter oxydans*

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Abstract

Kombucha is a healthy carbonated beverage made by fermenting tea extracts such as green tea and black tea through symbiotic culture of bacteria and yeast. In this study, fermentation characteristics and radical scavenging activity of ginseng berry kombucha (GBK) by *Saccharomyces cerevisiae* M-5 and *Gluconobacter oxydans* were measured. As fermentation time increased, pH decreased and titratable acidity increased. Reducing sugars decreased rapidly on day 3. Alcohol content increased dramatically during this period and then decreased. GBK showed increased radical scavenging activity and increased total flavonoid content on day 18 of fermentation compared to before fermentation. In particular, during GBK fermentation, the content of phenolic compounds such as gallic acid (2.09-fold) and chlorogenic acid (2.11-fold) increased, contributing to antioxidant activity. In addition, the major ginsenosides of GBK were identified as Rg2 (10.1 μg/mg) and Re (6.59 μg/mg), and the content of minor ginsenosides, which are easily absorbed forms, increased 2.19-fold by fermentation. GBK also extended survival in a *Drosophila* model treated with 15% hydrogen peroxide. GBK also reduced reactive oxygen species ($p < 0.001$) through upregulation of gene expression of antioxidant enzymes such as catalase ($p < 0.001$), superoxide dismutase ($p < 0.05$), and glutathione peroxidase ($p < 0.001$). Therefore, GBK can be presented as a functional food that inhibits oxidative stress by increasing radical scavenging activity during fermentation.

Keywords Ginseng berry kombucha, *Saccharomyces cerevisiae*, *Gluconobacter oxydans*, ROS

Introduction

Kombucha is a non- or low-alcohol carbonated beverage that is fermented by adding sugar to tea extracts such as black or green teas and fermenting it using a symbiotic culture of bacteria and yeast (SCOBY), which contains yeast and acetic acid bacteria [1, 16]. As kombucha fermentation progresses, the organisms in the SCOBY produce cellulose to form a flat, soft gel or a microbial biofilm film, such as a mushroom cap [13]. The yeasts in the SCOBY include *Saccharomyces* sp., *Zygosaccharomyces kombuchaensis*, *Torulopsis* sp., *Pichia* sp., *Brettanomyces* sp., *Z. bailii, Schizosaccharomyces pombe*, *S. ludwigii,* and *Candida kefyr*. Acetic acid producing bacteria in SCOBY comprise *Acetobacter xylinum, A.*

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xylinoides, A. aceti, A. pasteurianus, Gluconobacter oxydans, and *Bacterium gluconicum* [8, 32, 33].

In addition to the taste of kombucha, fermentation metabolites and physiological activities are determined by the fermentation substrates, SCOBY constituent microorganisms, additive materials, and fermentation methods. The various types of fermented substrates used in the manufacture of kombucha include green tea, green tea, black tea, lemon balm, oak leaves, tangerines, coffee, *Vitis coignetiae*, wheat sprout juice [16]. Kombucha exhibits various physiological activities such as anti-inflammatory, antioxidant, cholesterol reduction, lowering blood pressure, cancer cell reduction, liver function improvement, immune modulation, intestinal health, antimicrobial, and antidiabetic. Moreover, it has been reported that these physiological activities are due to the presence of polyphenols in the raw material used to manufacture kombucha. Ginsenosides, the active ingredients in ginseng, are found in higher amounts in *Panax ginseng* berries than in the roots and have a unique ginsenoside profile [14, 18]. In addition, the leaves and fruits of ginseng, which contain large amounts of polyphenolic substances, have been reported to have excellent antioxidant activity [6, 28].

The use of ginseng berry (ginseng fruit) as a substrate in this study for the preparation of kombucha has not been reported earlier. In addition, for quality control and industrial production, kombucha was manufactured using only yeast and acetic acid bacteria, which have been procured safely. Therefore, we inoculated *S. cerevisiae* and *G. oxydans* from sugar-preserved ginseng and kombucha to ferment ginseng berry kombucha (GBK). The fermentation characteristics of GBK prepared using ginseng berries and isolates that differed from those of traditional kombucha were evaluated. Further, the effects of GBK on the survival rate and gene expression of enzymes related to reactive oxygen species (ROS) removal were measured in *Drosophila* under hydrogen-peroxide (H_2O_2)-induced oxidative stress.

Methods

Strains

The strains used to produce kombucha in this study was *S. cerevisiae* M-5 and *G. oxydans*, which were isolated and stored at the Nutraceuticals laboratory of Korea University (Seoul, Korea). In a previous study, *S. cerevisiae* M-5, a strain with high β-glucosidase activity, was isolated from sugar-preserved ginseng. Also, *G. oxydans* is an acetic acid-producing bacterium isolated from commercial kombucha. For the preparation of GBK, *S. cerevisiae* M-5 and *G. oxydans* were pre-cultured in potato glucose and mannitol media for 48 h at 30 °C with shaking.

Fermentation of ginseng berry kombucha (GBK)

Ginseng berries purchased from a ginseng farm in Goesan (Chungcheonbuk-do, Republic of Korea) were used to prepare the GBK. For the fermentation, 2% each of the *S. cerevisiae* and *G. oxydans* precultures were inoculated into sterilized ginseng berry-containing broth (GBB) and incubated at 30 °C. The change in components during fermentation was measured using samples collected at regular intervals during the preparation. GBB was prepared using 20.0 g of ginseng berry, 2.0 g of sucrose, 0.02 g of ascorbic acid, and 20 mL of tea infusion dissolved in a total of 200 mL, and adjusted to pH 6.0. A solution containing 5.4 g of black tea leaves per liter of water was extracted at 85 °C for 20 min and used as a tea infusion.

Analysis of component changes during fermentation

pH was measured using a pH meter (Orion Star A211; Thermo Fisher Scientific, Waltham, MA, USA). Titratable acidity was measured using the neutralization titration method [7]. The titratable acidity was converted to citric acid (%) by adding 10 mL of the sample and neutralized with 0.1 N NaOH using phenolphthalein as the indicator. Reducing sugar was analyzed using the 3,5-dinitrosalicylic acid method and was calculated using glucose as a standard [22]. Total polyphenol content was determined using the Folin-Ciocalteu reagent and calculated as gallic acid equivalents [31].

Microbial analysis during fermentation of GBK

Acetogenic bacteria were isolated by plating on a medium containing 3% glucose, 0.5% yeast extract, 1% $CaCO_3$, 3% ethanol, and 2% agar. GBK diluted to an appropriate concentration was spread on an acetic acid bacteria separation medium and cultured at 30 °C for 3 days. The number of acetic acid bacteria was calculated based on the strains that produced a transparent ring [3].

For yeast analysis, 3 M™ Petrifilm™ Rapid Yeast and Mold Count (RYM) (St. Paul, MN, USA) was used. After inoculating the dried films with diluted GBK and incubating at 30 °C for 3 days, pale pink to cyan colonies with clearly distinguished edges were counted [5]. All experiments were repeated three times, and the number of microorganisms was expressed as log colony forming units (CFU)/mL.

Estimation of radical scavenging activity during fermentation

To measure the antioxidant activity of GBK, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities and ferric reducing antioxidant power (FRAP)

were measured as previously described [23]. The scavenging ability of GBK was expressed as the IC_{50} value, which was the sample concentration that reduced the generated radicals by 50%. The FRAP value was extrapolated from a standard curve, using iron (II) sulfate heptahydrate as the standard.

Analysis of flavonoid and ginsenoside composition in GBK extracts

To analyze the flavonoid composition of GBK, 100% ethanol was added to the GBK sample in an amount of 4 times the total volume (v/v = 1:4), followed by extraction under reflux twice for 2 h at 90 °C. The GBK extract was filtered, concentrated using a rotary evaporator, and lyophilized. The flavonoid content of the GBK extract was analyzed using high-performance liquid chromatography (HPLC). The HPLC instrument (Agilent, Waldbronn, Germany) equipped with a UV detector was used. YMC-Triart C 18 (250 × 4.6 mm, 5 μm) was used as the column, and 0.2% formic acid in water for mobile phase A and 0.2% formic acid in acetonitrile for mobile phase B were used for component separation. The gradient conditions of the solvent (A:B) were 95%:5% (0–2 min), 75%:25% (10 min), 60%:40% (30 min), 50%:50% (40 min), 40%:60% (50 min), and 95%:5% (55–60 min). The solvent flow rate was 0.8 mL/min, the column temperature was maintained at 35 °C, and the injection volume was 10 μL. The wavelengths of the UV detector were analyzed at 260, 292, 310 and 365 nm. The ginsenoside content was also measured using a Cadenza CD-C18 (75 × 4.6 mm, 3 μm) column in an HPLC system (Agilent).

For the analysis of ginsenoside, (A) 10% acetonitrile in water and (B) 90% acetonitrile in water were used as mobile phases, and the flow rate was maintained at 1.2 mL/min. The gradient conditions of the solvent were 90–76%:10–24% (0–44 min), 76–60%:24–40% (44–56 min), 60–50%:40–50% (56–79 min), 50–90%:50–10% (79–82 min) and 90%:10% (82–85 min). The wavelength of the UV detector was measured at 203 nm, the column temperature was 40 °C, and the injection amount was 5 μL.

Estimation of survival rate in *Drosophila* model

Canton-S wild-type *Drosophila melanogaster* Meigen was a gift from the Department of Food Science and Nutrition Jeju National University. *Drosophila* were reared at 25 °C with 60% humidity and a 12-h light–dark cycle. Adult fruit flies were reared in a standard corn meal medium. For H_2O_2 treatment, male *Drosophila* were transferred to empty culture tubes and fasted for 2 h, and filter paper soaked in different solutions (200 μL) was placed inside. The control (CON) was fed 5% sucrose (m/m) containing 15% H_2O_2 (m/m). The other two groups (GBK-L and GBK-H) were fed 5% sucrose (m/m) and 2.5 and 5.0 mg GBK, respectively, containing 15% H_2O_2 (m/m). The number of surviving *Drosophila* in each culture tube was recorded for 24 h.

Oxidation level in *Drosophila* (mRNA expression of related oxidative factors)

The *Drosophila* whole body was homogenized with 50 mM Tris–HCl buffer (pH 7.4) in an ice bath and centrifuged at 4 °C (10,000 × *g* for 15 min). Next, the supernatant was collected, and ROS production was measured using the 2,7-dichlorofluorescin diacetate (DCF-DA) method as previously described [2]. Following addition of 10 μM DCF-DA to the supernatant and incubating for 30 min at 37 °C in the dark, the fluorescence (excitation, 485 nm; emission, 535 nm) was measured. Total RNA from *Drosophila* whole bodies was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol [12]. RQ1 RNase-free DNase I (Promega, WI, USA) treated the RNA samples were reverse-transcribed using SuperScript™ III reverse transcriptase (Invitrogen), and the mRNA expression of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) was analyzed by quantitative real-time PCR using the StepOne Plus Real-time PCR system (Applied Biosystems, CA, USA) and a Power TaqMan PCR Master Mix Kit (Applied Biosystems) with RpL32 (NM_001144655.3). The target genes used in qRT-PCR were SOD (NM_057387.5), CAT (NM_080483.3), and GPx (NM_168024.2).

Statistical analysis

The data are presented as the mean ± standard deviation from three repeated measurements. The statistical significance of the experimental group was verified at $P < 0.05$, using Tukey's multiple range test. The analysis was carried out using SPSS (version 12.0; Statistical Analysis System, SPSS, Inc., Chicago, IL, USA).

Results

Component changes during the fermentation of ginseng berry kombucha (GBK)

Changes in pH, titratable acidity, reducing sugars, polyphenols, and flavonoid contents were measured during the fermentation of GBK by *S. cerevisiae* and *G. oxydans* (Fig. 1). During kombucha fermentation, the pH gradually decreased, with a slight change being noted 3 days after fermentation. From pH 3.92 on the 3rd day of fermentation to pH 3.68 on the 18th day, only a marginal decrease was observed. Titratable acidity increased with the incubation period and ranged from 0.05% before fermentation to 0.90% after 18 days (Fig. 1A).

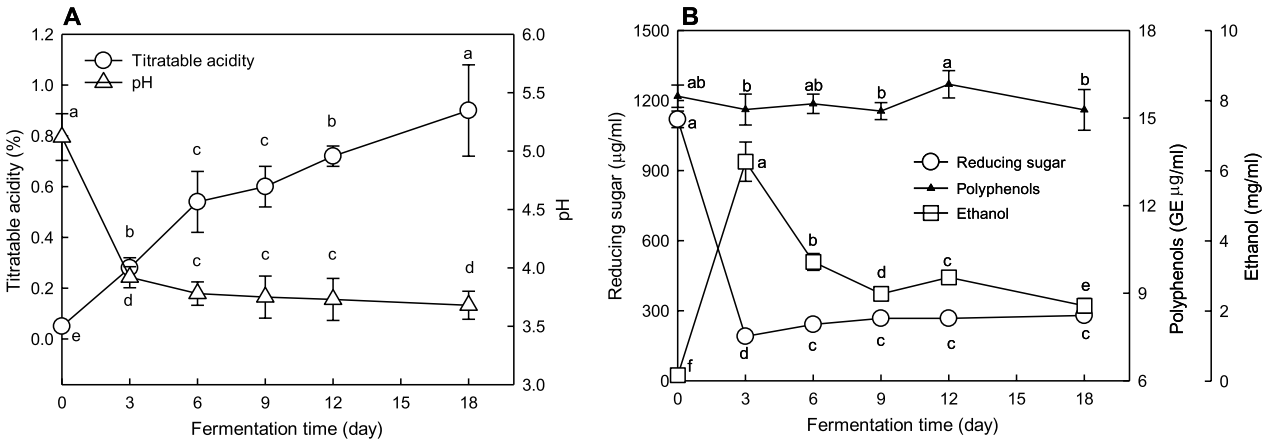

Fig. 1 Changes of titratable acidity and pH (**A**), and components (**B**) during the fermentation of ginseng berry kombucha (GBK). Values are the means ± SD for each group; different letters indicate significant differences at $p < 0.05$ vs. day 0 in each group based on Tukey's test. *GE* gallic acid equivalent

The content of reducing sugars content in GBK rapidly decreased to 190.14 μg/mL on the 3rd day of fermentation and then gradually increased to 241.49–279.85 μg/mL from day 6 to day 18 of fermentation (Fig. 1B). The alcohol content which was 0.17 mg/mL before fermentation, increased rapidly to 6.26 mg/mL on the 3rd day, and eventually declined to 2.15 mg/mL on the 18th day (Fig. 1B). There was no significant change in the polyphenol content of GBK during fermentation, but it was highest on the 12th day of fermentation (Fig. 1B).

As regards the measured changes in microorganisms during kombucha fermentation, acetic acid bacteria showed a tendency to increase as the fermentation progressed, and the yeast numbers increased until the 9th day of fermentation and gradually decreased thereafter (Table 1). The total number of bacteria also increased until the 9th day and then gradually reduced.

Changes in radical scavenging activity during fermentation

The radical scavenging ability and reducing power were measured before and after GBK fermentation (Fig. 2). As compared to before the fermentation, the IC_{50} value for radical scavenging of ABTS and DPPH decreased at the end of the fermentation process, indicating an increase in the scavenging ability after the fermentation. In addition, in comparison to before fermentation, the FRAP value for reducing power increased after the process (34.89 vs. 46.75 mM). Collectively, this implies that during the fermentation of GBK, there is a simultaneous increase in the reducing power of FRAP and radical scavenging activity.

Changes in flavonoid and ginsenoside contents before and after fermentation

The changes in flavonoid contents in the GBK extract, presumed to be the active constituents, before and after fermentation, are shown in Tables 2. The total content of flavonoids, which are active ingredients, before fermentation was 0.78 μg/mg but increased to 1.32 μg/mg after fermentation (Table 2). After fermentation of GBK, the major flavonoids in the extract were gallic acid, 3,4 dihydroxy benzoic acid, and chlorogenic acid, which increased significantly after fermentation.

As shown in Table 3, the main ginsenosides of GBK after fermentation were Rg2 (sum of Rg2s and Rg2r) and Re, with concentrations of 10.10 μg/mg and 6.59 μg/mg, respectively. The total ginsenoside content,

Table 1 Content of different microorganisms during the fermentation of ginseng berry kombucha (GBK)

CFU (×10^7/mL)	Day					
	0	3	6	9	12	18
Acetic-acid producing bacteria	0	1.33 ± 0.03	1.29 ± 0.01	1.98 ± 0.12	2.20 ± 0.01	2.24 ± 0.64
Yeast	0	1.52 ± 0.12	1.87 ± 0.04	3.29 ± 0.11	3.07 ± 0.17	2.81 ± 0.11
Total microorganisms	0	2.89 ± 0.13	3.28 ± 0.23	5.37 ± 0.22	5.06 ± 0.36	5.09 ± 0.51

Values are the means ± SD for each group

CFU colony forming unit

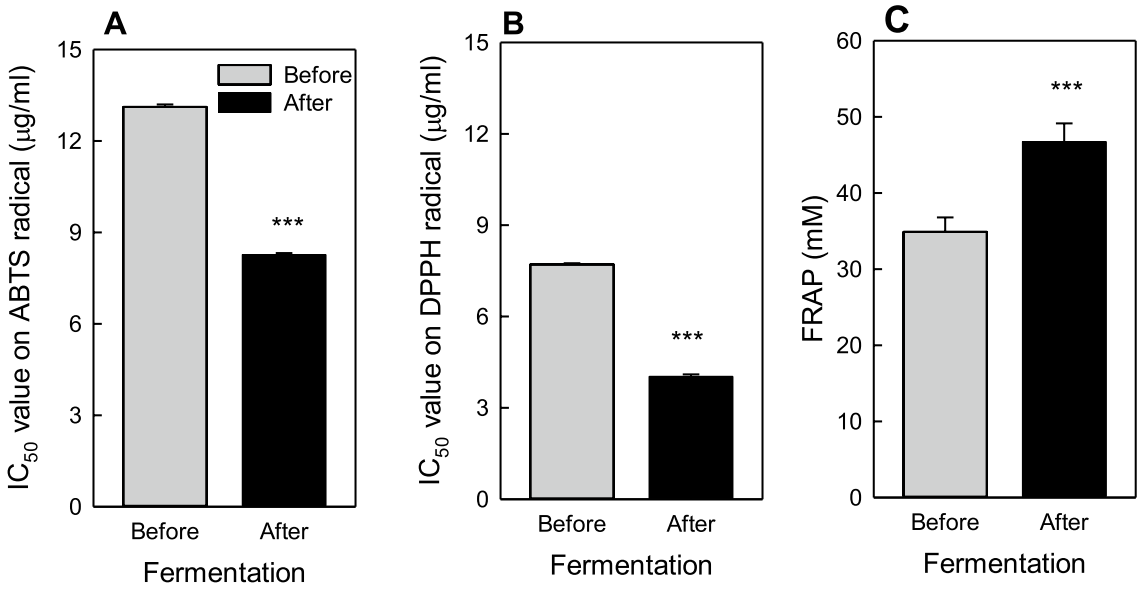

Fig. 2 IC_{50} value on **A** ABTS and **B** DPPH radical and **C** FRAP value before and after fermentation of ginseng berry kombucha (GBK). Values are the means ± SD for each group; *** indicates significant difference ($p < 0.001$) after fermentation as compared to before. *ABTS* 2,2′-azino-bis(3-ethylbenz othiazoline-6-sulfonic acid), *DPPH* 2,2-diphenyl-1-picrylhydrazyl, *FRAP* ferric reducing antioxidant power

Table 2 Changes in contents of flavonoids before and after ginseng berry kombucha (GBK) fermentation

Flavonoids	Contents (μg/mg of extract)	
	0 day	18 day
Gallic acid	0.067 ± 0.004	0.140 ± 0.011
3,4-Dihydroxybenzoic acid	0.120 ± 0.002	0.420 ± 0.032
Rutin	0.050 ± 0.001	0.063 ± 0.002
Quercetin-3-glucuronide	0.023 ± 0.003	0.068 ± 0.002
Chrysin	0.082 ± 0.002	0.092 ± 0.002
Chlorogenic acid	0.095 ± 0.001	0.20 ± 0.006
Caffeic acid	0.042 ± 0.001	0.076 ± 0.003
ρ-Coumaric acid	0.026 ± 0.001	0.028 ± 0.001
Trans ferulic acid	0.037 ± 0.001	0.056 ± 0.001
Apigenin	0.015 ± 0.001	0.011 ± 0.001
Quercetin	0.140 ± 0.001	0.100 ± 0.001
Kaempferol	0.094 ± 0.001	0.072 ± 0.001
Total flavonoids	0.78 ± 0.006	1.32 ± 0.024

Values are the means ± SD for each group

which was 13.34 μg/mg at the beginning of fermentation, increased to 32.58 μg/mg after fermentation. In addition, the content of the major ginsenoside and minor ginsenoside increased from 6.19 to 11.78 μg/mg and from 7.16 to 20.80 μg/mg compared to before fermentation, respectively. Therefore, it was confirmed that the content of easily absorbed ginsenosides in GBK increased through fermentation. In the analysis

Table 3 Changes in content of ginsenosides before and after ginseng berry kombucha (GBK) fermentation

Ginsenoside	Contents (μg/mg of extract)	
	0 day	18 day
Rg1	0.53 ± 0.02	1.04 ± 0.03
Re	3.40 ± 0.08	6.59 ± 0.01
Rf	0.21 ± 0.02	0.37 ± 0.01
Rb1	0.32 ± 0.03	0.79 ± 0.05
Rc	0.59 ± 0.01	1.04 ± 0.05
Rb2	0.36 ± 0.02	0.59 ± 0.01
Rd	0.77 ± 0.01	1.36 ± 0.01
Rh 1 (s)	0.27 ± 0.02	1.03 ± 0.02
Rg2 (s)	0.72 ± 0.10	3.41 ± 0.04
Rg2 (r)	1.46 ± 0.10	6.69 ± 0.04
Rg6	0.140 ± 0.001	0.100 ± 0.001
Rk3	1.02 ± 0.15	2.00 ± 0.03
Rh4	0.22 ± 0.04	0.59 ± 0.01
Rg3 (s)	0.75 ± 0.03	2.28 ± 0.01
Rg3 (r)	0.97 ± 0.03	0.95 ± 0.02
CK	0.35 ± 0.03	0.49 ± 0.03
Rk1	0.24 ± 0.06	0.44 ± 0.02
Rg5	0.52 ± 0.11	1.23 ± 0.04
Rh2 (s)	0.21 ± 0.03	0.31 ± 0.00
Major ginsenosides	6.19 ± 0.19	11.78 ± 0.17
Minor ginsenosides	7.16 ± 0.72	20.80 ± 0.26
Total ginsenosides	13.34 ± 0.91	32.58 ± 0.43

Values are the means ± SD for each group. Major ginsenosides: Rg1, Re, Rf, Rb1, Rc, Rb2, and Rd; minor ginsenosides: Rh1, Rg2, Rk3, Rh4, Rg3, Rk1, and Rh2

of flavonoids and ginsenosides in GBK extracts, compounds that did not show differences were not included in the results.

Effects of ginseng berry kombucha (GBK) on survival rate and relief of oxidative stress in male fruit flies under oxidative stress conditions

The survival rate after treatment with low concentration (2.5%) and high concentration (5%) of GBK was measured in male fruit flies treated with 15% H_2O_2 to induce oxidative stress (Fig. 3). The GBK-administered groups showed an increase in the survival rate compared to the CON group treated with H_2O_2 alone. A high concentration of GBK resulted in a significant increase in the survival rate compared to CON ($p < 0.001$). The low concentration of GBK did not show a significant increase in survival rate compared to the CON group. There was no significant difference in survival rates between high and low GBK concentrations.

Figure 4 shows the effect of GBK on ROS production and gene expression of SOD, CAT, and GPx in the *Drosophila* model, wherein oxidative damage was induced by H_2O_2 treatment. Treatment with low and high concentrations of GBK significantly reduced ROS production by 44.5% and 65.4%, respectively, compared to the control ($p < 0.001$). As the GBK treatment concentration increased, the gene expression of CAT, GPx, and SOD, enzymes related to oxidative stress removal, tended to decrease in a concentration-dependent manner. Both CAT and GPx expression levels were significantly increased compared to CON by both GBK concentrations ($p < 0.001$). Therefore, the inhibitory effect of GBK on the increase in oxidative stress caused by H_2O_2 treatment was attributed to an increase in the expression level of oxidative stress-related enzymes.

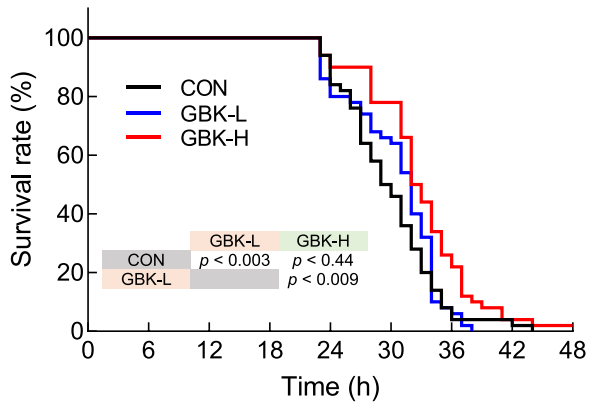

Fig. 3 Effects of ginseng berry kombucha (GBK) on the survival rate in *Drosophila* treated with 15% H_2O_2. Significant differences in percent survival (%) among groups were analyzed by log-rank statistical method (n = 100/group). *CON* control, *15%* H_2O_2-treated group, *GBK-L* Group treated with 2.5 mg/g of GBK and 15% H_2O_2, *GBK-H* Group treated with 5 mg/g of GBK and 15% H_2O_2

Discussion

Despite the various physiological activities of kombucha, its application and utility in the industry are limited. Since the fermentation of kombucha is performed by a microbial community, the species and distribution of microorganisms involved in the process are not confirmed, and there are concerns about the safety and combination of microorganisms, such as SCOBY, using sugar and extracts of green or black tea as fermentation substrates. Quality control and industrial production of kombucha should be carried out using officially recognized microorganisms that are safe for use. To achieve various physiological activities to kombucha, attempts should be made to use substrates other than green or black teas. Therefore, in this study, ginseng berries were used as a fermentation substrate to prepare kombucha using *S. cerevisiae* and *G. oxydans*, and the fermentation characteristics were measured.

There was an increase in acidity and a decrease in pH as a result of the organic acid acetic acid produced during the fermentation process by acetic acid bacteria among the strains used for fermentation of kombucha [29]. Kombucha is known to release organic acids such as acetic, gluconic, glucuronic, citric, L-lactic, malic, tartaric, malonic, oxalic, succinic, and pyruvic acid through fermentation [25]. In this study, the organic acid produced by *G. oxydans* resulted in a decrease in pH and an increase in titratable acidity (Fig. 1). Organic acids are produced due to the metabolism of the yeast and acetic acid bacteria during the fermentation process. These organisms utilize carbohydrates as the main substrate to produce alcohol and organic acids [34]. We found that during the fermentation of GBK, the content of reducing sugars decreased, the ethanol content initially increased and then declined, and the titratable acidity increased continuously (Fig. 1). The ethanol content generated during kombucha fermentation is reported to be 0.7% to 1.3% [4]. When the alcohol content remains above 1%, it is proposed to initiate processes such as oxygen exposure and microfiltration to lower the concentration [15]. However, in this study, because the final alcohol content was 0.25%, an additional process for lowering the alcohol was not required.

Kombucha largely contains polyphenols, organic acids, sugars, and proteins [10], and its composition varies depending on the fermentation substrate used. Kombucha, which uses black tea (black tea kombucha) and green tea (green tea kombucha) as the fermentation substrates for kombucha, contains polyphenols, including

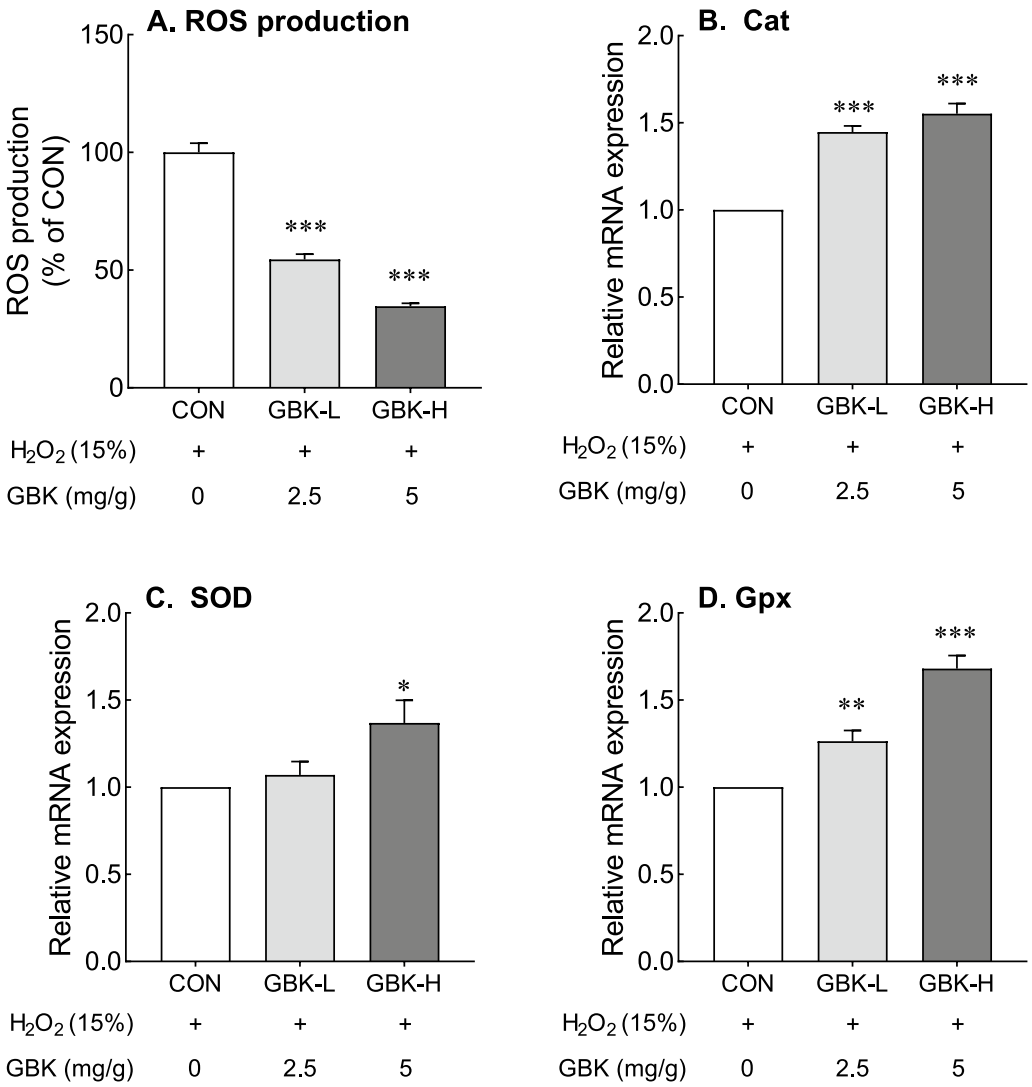

Fig. 4 Effect of ginseng berry kombucha (GBK) on **A** ROS production and **B**–**D** antioxidant enzyme-related mRNA expression in *Drosophila* treated with 15% H_2O_2. Values are the means ± SD for each group; different levels of significance have been indicated *p < 0.05, **p < 0.01, ***p < 0.001 in each group based on Tukey's test. CON: control, 15% H_2O_2-treated group, GBK-L: Group treated with 2.5 mg/g of GBK and 15% H_2O_2, GBK-H: Group treated with 5 mg/g of GBK and 15% H_2O_2; *ROS* reactive oxygen species, *CAT* catalase, *SOD* superoxide dismutase, *GPx* glutathione peroxidase

catechins, as active constituents. It has been reported that in both black and green tea kombucha, the amount of catechin decreased until the 9th day of fermentation and increased 12th day onward [11]. As shown in Table 2, the flavonoid content increased after fermentation, and 3,4-dihydrobenzoic acid and chlorogenic acid were found to be the main flavonoids. In addition, the content of ginsenosides, both major and minor (which are easily absorbed) increased by upon fermentation (Table 3).

The changes described above were due to the β-glucosidase activity of S. cerevisiae used for kombucha fermentation. β-glucosidase cleaves the sugar chain site bound to the glycoside ginsenoside of red ginseng and converts it into a non-glycoside ginsenoside, leading to an improvement in the absorption rate of ginsenoside [30]. Rg2, which is produced by the deglycosylation of ginsenoside Re [26], is abundantly present in ginseng berries [17]. In the GBK, not only was Re increased upon fermentation, but also Rg2. The concentration of Rg6 produced by the dehydration reaction of Rg2 also increased in GBK (Table 3). Ginsenosides Rb1, Rc, Rb2, Rd, Ra1, Ra2, and Ra3 are converted to ginsenoside Rg3 by heating during the manufacturing process of red ginseng [19]. In ginsenoside Rg3, a double bond is formed by dehydration at position 20 of the dammarane backbone and is converted into three

structural isomers of ginsenosides Rk1, Rg5, and Rz1 [20]. Ginseng berry kombucha also exhibited increased levels of Rg3, Rk1, and Rg5. The sugar moiety attached to C-3, C-6, or C-20 ginsenosides is deglycosylated and converted to minor ginsenosides, contributing to an increase in bioavailability and physiological activity [27]. Therefore, minor ginsenosides produced by the fermentation of GBK, increase its bioavailability and contribute to an increase in its physiological functions.

The physiological activity of kombucha is closely related with its antioxidant activity. Through fermentation, kombucha prepared using green or black tea increases polyphenol components, which are known to be associated with an increase in antioxidant activity [8]. When the antioxidant activities were compared before and after fermentation, it was noted that the FRAP activity increased along with ABTS and DPPH after fermentation (Fig. 2). The content of chlorogenic acid [24] and 3,4-dihydroxybenzoic acid [35] increases during GBK fermentation, which is involved in the increase in antioxidant activity.

Free radicals generated in intracellular metabolic processes lead to oxidative damage to DNA, proteins, and lipids, the major cell components. Accumulated oxidative damage affects cellular function and, furthermore, leads to deterioration of tissue function, and this ultimately is responsible for increasing promoting aging, or decreasing an individual's lifespan [9]. The most abundant free radicals are ROS, which are mainly produced in the electron transport chain of the mitochondria. Free radicals, including superoxide (O_2^-), hydroxyl radicals ($^{\cdot}OH$), and hydrogen peroxide (H_2O_2) can be eliminated by the cellular antioxidant defense mechanisms which include the use of enzymes such as SOD and CAT and a process that involves antioxidants such as flavonoids, vitamins, and ginsenosides [21]. The radical-scavenging activity of GBK can be attributed to the presence of various flavonoids, in addition to the minor ginsenosides. Administration of GBK to *Drosophila* induced to undergo oxidative stress by treatment with H_2O_2 appears to decrease ROS production due to increased gene expression of SOD, CAT, and GPx, enzymes involved in oxidative stress removal (Figs. 3 & 4).

Oxidative stress, a product of the interaction of genetic, environmental, and lifestyle factors, increases the risk of aging, disease, and death. Thus, it is necessary to identify favorable foods that have the ability to suppress or eliminate the generation of ROS and halt the acceleration of aging in the human body and lead to numerous diseases. The quality control of ginseng berry kombucha can be easily achieved, and its safety can be ensured by using strains isolated from food. In addition, the use of GBK, which has improved radical-scavenging capabilities along with consumer preferences, can aid in improving physical health.

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Author contributions
Data curation: EJC, HHS, and KYK, formal analysis: HHS, and KBH, visualization: KYK, and KBH, methodology: HHS, and KYK, investigation: KYK, and KBH, validation: HHS, and KBH, conceptualization: HJS, and YA, project administration: HJS, and YA, supervision: HJS, and YA, writing—original draft: EJC, HJS, and YA. writing—review and editing: EJC, HHS, KYK, KBH, HJS, and Y A. All authors read and approved the final manuscript.

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Availability of data and materials
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Declarations

Competing interests
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