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Antioxidant Properties of Kombucha Made with Tartary Buckwheat Tea and Burdock Tea

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ABSTRACT: Kombucha is a beverage fermented by SCOBY, which is a symbiotic culture of bacteria and yeast. Recently, kombucha has received significant attention due to its health benefits, which include antioxidant and anti-obesity effects. In this study, we investigated the characteristics of kombucha made with Tartary buckwheat and burdock, both known for their high polyphenols content. First, the total polyphenol content and antioxidant activity were measured by 2,2-diphenyl-1-picrylhydrazyl and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assays, which revealed a polyphenol content of 180 ug/mL in Tartary buckwheat kombucha and a high radical scavenging ability of over 90% in both kombucha preparations. Analysis of the changes in the organic acid content during fermentation revealed increases in various organic acid contents, such as glucuronic acid, lactic acid, and acetic acid. Glucuronic acid, especially, which has many functional properties in health, was found to be produced at a concentration of 4.03 g/L in Tartary buckwheat kombucha. Pancreatic lipase inhibitory ability analysis revealed inhibitory effects of 40.47% and 57.68% for Tartary buckwheat and burdock kombucha, respectively. The results of this study confirmed the antioxidant and anti-obesity effects of kombucha made from Tartary buckwheat and burdock, indicating the potential value of these ingredients as functional kombucha ingredients.

Keywords: burdock, fermentation, functional food, kombucha tea, Tartary buckwheat

INTRODUCTION

Kombucha is a fermented, carbonated beverage with a slightly sour taste. The fermentation of kombucha is achieved through symbiotic culture of bacteria and yeast (SCOBY), and this process leads to the production of various metabolites, such as polyphenols and organic acid (Kumar and Joshi, 2016). Due to the taste and functional health benefits of these materials, kombucha has gained attention as a functional food for health, with the kombucha market predicted to reach 6.14 billion USD worldwide by 2028 (Statista Inc., 2017).

The health benefits of kombucha are derived from various substances, such as polyphenols and vitamins, as well as organic acids, such as acetic acid, glucuronic acid, citric acid, oxalic acid, and lactic acid, produced during fermentation (Martínez Leal et al., 2018). Polyphenols, which are widely recognized for their excellent antioxidant ability, primarily contribute to the antioxidant and anti-obesity capacity of kombucha. These polyphenols tend to increase during the fermentation process because the various yeasts and bacteria present in kombucha increase the phenolic compound content during fermentation by producing hydrolysis enzymes such as invertase, cellulase, and amylase, which convert phenolic compounds into phenolic monomers (Zubaidah et al., 2019). Many studies have demonstrated the capacity of phenol to inhibit lipase activity (Buchholz and Melzig, 2015; Martinez-Gonzalez et al., 2017); this inhibitory effect is attributed to the high affinity between the hydroxyl groups of polyphenols and those of proteins, which are facilitated by hydrogen and hydrophobic bonding (Buchholz and Melzig, 2015; Martinez-Gonzalez et al., 2017). In addition, the bioavailability of polyphenol is enhanced by its interaction with glucuronic acid, which is known as characteristic organic acid in kombucha (Martínez Leal et al., 2018). Glucuronic acid plays a critical role in both anti-inflammation and detoxification, and it also contributes to the taste and flavor of kombucha, providing a lightly refreshing sour taste (Martínez-Leal et al., 2020; Li et al., 2022). Acetic acid, one of the primary organic acids in kombucha, also gives strong sourness. Acetic acid ex-

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hibits antibacterial ability that helps to suppress pathogenic microorganisms such as *Helicobacter pylori*, *Escherichia coli*, and *Staphylococcus aureus* (Steinkraus et al., 1996).

Traditionally, the substrate used for kombucha is green or black tea (Jayabalan et al., 2007; Değirmencioğlu et al., 2021; Woo et al., 2021); however, due to the increased interest in kombucha and its potential health benefits, the range of ingredients and fermentation methods has now expanded to include a variety of food materials, such as apple, spinach, yuzu, and coffee bean (Aspiyanto et al., 2017; Zubaidah et al., 2018; Bueno et al., 2021; Woo et al., 2021). Among plant sources, Tartary buckwheat and burdock are known for their high concentrations of rutin, a type of flavonoid that is broken down into quercetin in the gut (Sun and Ho, 2005). These sources are also known for their antioxidant and anti-inflammatory effects and their preventative effect against neurodegenerative disorders and cardiovascular disease (Oomah and Mazza, 1996). In this study, we explore the potential application of Tartary buckwheat and burdock as raw ingredients for kombucha fermentation, with a focus on their ability to enhance antioxidant functionality.

MATERIALS AND METHODS

Tea preparation

Tartary buckwheat and burdock (7.5 g each) were separately added to 500 mL of water and extracted at 80°C for 10 min. After cooling at room temperature, sucrose (CJ CheilJedang Corp.) was dissolved in 400 mL of the extracted tea sample until the solution reached 5°Bx. The solution was then fermented at 25°C for 15 days under aerobic conditions using 20 g of SCOBY (DEARBREW Corp.). The solution was covered with a Miracloth (EMD Millipore Corp.) (Zubaidah et al., 2019).

Sugar content, pH, total acidity, and organic acid profiles

The pH of the samples was measured using an electronic pH meter (ORION STAR A215, Thermo Scientific). Total acidity was measured using titration methods. In brief, 10 mL of the samples with added phenolphthalein solution was titrated using 0.1 M NaOH until a clear color appeared. The sugar content (expressed as °Bx) was measured using a refractometer (MASTER-53M, ATAGO).

The metabolite changes, such as organic acid and ethanol contents, during fermentation were analyzed using a high-performance liquid chromatography (HPLC Series 6000, Futecs) column equipped with a refractive index detector (Lee et al., 2021). Samples were taken 0, 3, 7, 10, and 14 days after inoculation of the SCOBY, and each sample was filtered using a 0.22 μL hydrophilic PTFE syringe filter (Futecs). An Aminex HPX-87H organic acid column (Bio-Rad Laboratories) was used and the mobile phase was 0.005 M sulfuric acid. The flow rate was 0.5 mL/min, and the column temperature was 55°C. The concentrations of each material were calculated from standard curves.

Antioxidant activity assay and pancreatic lipase inhibition activities

The antioxidant activities were investigated by measuring the total phenolic content (TPC) and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activities. The TPC of kombucha was measured using the Folin-Denis method (Folin and Denis, 1912). Samples (0.02 mL) were mixed with 0.04 mL of 2 N Folin-Ciocalcu reagent and reacted for 3 min. Then, 0.4 mL of 20% Na_2CO_3 was added, followed by a color reaction at room temperature for 1 h. The absorbance of the solution was then measured at 765 nm using an ultraviolet-visible (UV-Vis) spectrophotometer (EPOCH microplate spectrophotometer, Bio Tek). The TPC of the sample was calculated using gallic acid as the standard.

The DPPH radical scavenging ability of kombucha was measured by referring to Blois (1958). In brief, 800 μL of 0.1 mM DPPH was mixed with 200 μL of sample and reacted for 30 min in the dark. The absorbance of the mixture was measured at 517 nm using a UV-Vis spectrophotometer. The DPPH radical scavenging ability was calculated using the following equation:

$$\text{DPPH antioxidant activity (\%)} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100$$

The ABTS radical scavenging ability of kombucha was measured by referring to the Roberta (1999) method (Re et al., 1999). After mixing a 7 mM ABTS solution with 2.45 mM potassium persulfate, the mixture was left at room temperature in the dark for 12 h to form radicals. Then, the ABTS solution was diluted with PBS (pH 7.4) until the absorbance at 734 nm was around 0.70±0.02. Next, 50 μL of sample was reacted with 950 μL of the diluted ABTS solution for 10 min in the dark, and the absorbance was measured at 734 nm using an UV-Vis spectrophotometer. Ascorbic acid was used as a standard, and the ABTS radical scavenging ability was calculated using the following equation:

$$\text{ABTS antioxidant activity (\%)} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100$$

The pancreatic lipase inhibition activity was measured the procedures described by Ban et al. (2023) with some modifications. The samples were mixed with 20 mg/mL

porcine pancreatic lipase, 20 mM p-nitrophenyl palmitate, and 0.1 M Tris-HCL buffer (pH 8.5). Orlistat was used as the positive control and was mixed with 0.1 M Tris-HCl buffer. After incubating each mixture at 37°C for 30 min, the supernatants from the mixtures were centrifugated. Then, the absorbance was measured at 405 nm using a UV-Vis spectrophotometer. Finally, pancreatic lipase inhibition activity was calculated using following equation:

$$\text{Pancreatic lipase inhibition (\%)} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100$$

Statistical analysis

The statistical analysis of all the experimental data were conducted using SPSS software (Statistics 26, IBM Corp.) and were expressed as the mean±standard deviation. One-way ANOVA was used to detect significant differences at the $P<0.05$ level. Duncan's method was conducted as a post-test.

RESULTS AND DISCUSSION

In this study, the changes in pH, sugar content, and total acidity of kombucha fermented with Tartary buckwheat and burdock were investigated (Table 1). As fermentation progressed, both kombuchas became acidic (i.e., from neutral pH to pH 2.5 in the Tartary buckwheat kombucha and pH 2.65 in the burdock kombucha). This pH reduction was due to the breakdown of sucrose by microorganisms, leading the production of various organic acids (Essawet et al., 2015; Zubaidah et al., 2019). Accordingly, the sugar content also decreased in both types of kombucha, with reductions of 0.8°Bx and 3.4°Bx in Tartary buckwheat and burdock kombucha, respectively. These results indicated that sucrose was converted to other necessary metabolites during fermentation, which was in good agreement with the increase in total acidity. Moreover, in the burdock kombucha, a significant decrease in sugar content and an increase in total acidity compared to buckwheat kombucha were observed, suggesting that fermentation was more active in the burdock kombucha compared to the Tartary buckwheat kombucha, which might affect microbial growth and the structure of phytochemical compounds that contribute to functionalities of kombucha (Villarreal-Soto et al., 2018).

The production of organic acids such as glucuronic acid, malic acid, lactic acid, and acetic acid and the ethanol contents of the kombuchas were explored (Table 2). Overall, organic acids were found to be produced from the beginning of fermentation; however, the specific patterns of organic acid production were considerably different depending on the raw materials: Tartary buckwheat kombucha produced mainly malic acid (up to 181.5 g/L) after 2 weeks of fermentation, while acetic acid was a major metabolite in burdock kombucha. More specifically, at day 3, approximately 0.61 g/L of glucuronic acid, 21.91 g/L of malic acid, 3.32 g/L of lactic acid, 0.58 g/L of acetic acid, and 4.69 g/L of ethanol were produced in the Tartary buckwheat kombucha. As fermentation progressed, glucuronic acid, malic acid, and acetic acid were increased to 4.03 g/L, 181.50 g/L, and 5.31 g/L, respectively, with consumption of lactic acid and ethanol, probably due to the aerobic environment. In particular, the increases in glucuronic acid and malic acid production were significant after 10 days of fermentation, and the reduction of lactic acid and ethanol concentration were significant after 7 days of fermentation. Ethanol can be converted into acetic acid and glucuronic acid by acetic acid bacteria in SCOBY (Chen and Liu, 2000). Similar fermentation patterns, such as increases in glucuronic acid, malic acid, and acetic acid concentration, and decreases in lactic acid and ethanol were also observed in the burdock kombucha. In addition, the quantities of glucuronic acid and malic acid produced in the Tartary buckwheat kombucha were much higher than those reported for green tea (1.86 g/L) and black tea (2.33 g/L) kombucha (Jayabalan et al., 2007), implying the beneficial effects of introducing Tartary buckwheat as a substrate for kombucha.

These organic acids are known to offer numerous health benefits in various aspects. Acetic acid has inhibitory effects against pathogenic bacteria, as well as preventing obesity and metabolic disorders by regulating glucose production in the liver, increasing insulin secretion, and improving lipid metabolism (Valdes et al., 2021). Malic acid, as another substance that provides the sour

Table 1. Total acidity, pH, and sugar content of Tartary buckwheat kombucha and burdock kombucha

Days	pH		Total acidity (%)		Sugar content (°Bx)	
	Tartary buckwheat	Burdock	Tartary buckwheat	Burdock	Tartary buckwheat	Burdock
0	7.30	7.06	0.008	0.009	5.0	5.0
3	2.94	3.40	0.210	0.192	4.6	3.8
7	2.69	2.87	0.660	0.480	4.2	2.4
10	2.85	2.71	0.594	1.122	4.2	2.4
14	2.50	2.65	0.822	1.206	4.2	1.6

Table 2. Organic acids contents in Tartary buckwheat kombucha and burdock kombucha during 14-day fermentation

Organic acid (g/L)	Fermentation days				
	Day 0	Day 3	Day 7	Day 10	Day 14
Tartary buckwheat kombucha					
Glucuronic acid	ND	0.613±0.144^{bc}	0.238±0.008^{cd}	0.996±0.151^{b}	4.027±0.477^{a}
Malic acid	ND	21.910±3.135^{c}	7.735±0.373^{d}	34.657±3.394^{b}	181.501±1.570^{a}
Lactic acid	ND	3.321±0.001^{b}	4.492±0.062^{a}	2.160±0.244^{c}	2.321±0.280^{c}
Acetic acid	ND	0.575±0.019^{d}	3.199±0.009^{c}	4.732±0.153^{b}	5.305±0.143^{a}
Ethanol	ND	4.690±0.090^{b}	7.003±0.120^{a}	2.747±0.475^{c}	2.798±0.006^{c}
Burdock kombucha					
Glucuronic acid	ND	0.117±0.012^{c}	0.110±0.007^{c}	0.193±0.008^{b}	1.006±0.010^{a}
Malic acid	ND	1.793±0.008^{b}	1.075±0.013^{c}	1.681±0.255^{b}	6.631±0.157^{a}
Lactic acid	ND	5.563±0.144^{b}	6.917±0.015^{a}	6.794±0.015^{a}	4.911±0.479^{c}
Acetic acid	ND	0.632±0.031^{d}	6.282±0.007^{c}	12.137±0.519^{a}	8.834±0.361^{b}
Ethanol	ND	8.902±0.498^{b}	14.260±0.065^{a}	8.093±0.532^{c}	5.087±0.147^{d}

Values are presented mean±SD of triplicates.
Mean values with different letters (a-d) in the same row indicate significant differences by Duncan's multiple range test and one-way ANOVA (P<0.05).
ND, not detected.

taste of kombucha, has been researched for its effects in alleviating fibromyalgia and chronic fatigue syndrome and has been reported to have cardioprotective effects when combined with citric acid (Russell et al., 1995; Tang et al., 2013). Lactic acid is actively used in the cosmetic industry due to its moisturizing and pH-regulating effects on the skin, as well as its effectiveness in wrinkle improvement and whitening caused by photoaging (Abd Alsaheb et al., 2015; Huang et al., 2020). Glucuronic acid is known to have excellent nutritional properties, including serving as a precursor for vitamin C synthesis and detoxification functions resulting from its high antioxidant and anti-inflammatory effects (Martínez Leal et al., 2018; Li et al., 2022). With the presence of these diverse and functional organic acids in both Tartary buckwheat and burdock kombucha, it is anticipated that the consumption of these kombuchas can provide beneficial health effects.

As an example of the functionality of kombuchas produced using Tartary buckwheat or burdock, their antioxidant activities and pancreatic lipase inhibition activities were investigated, since Tartary buckwheat is known to contain high levels of the flavonoid "rutin," a powerful antioxidant (Sun and Ho, 2005). Burdock is known for its high free radical scavenging and antioxidant abilities, which provide anti-inflammatory and liver protective effects (Chan et al., 2011). Because phenolic compounds are known to have high antioxidant activities and are reported to have a significant correlation with DPPH and ABTS radical scavenging activities (Srihari and Satyanarayana, 2012), the antioxidant activities were measured via TPC and the DPPH and ABTS radical scavenging activity. The TPC of the Tartary buckwheat and burdock kombucha was approximately 100 μL/mL (Fig. 1A). As fermentation progressed, both Tartary buckwheat and burdock kombucha showed a gradual increase in TPC, followed by a slight decrease on day 14. In the case of the Tartary buckwheat kombucha, the highest content (210.94 μg/mL)

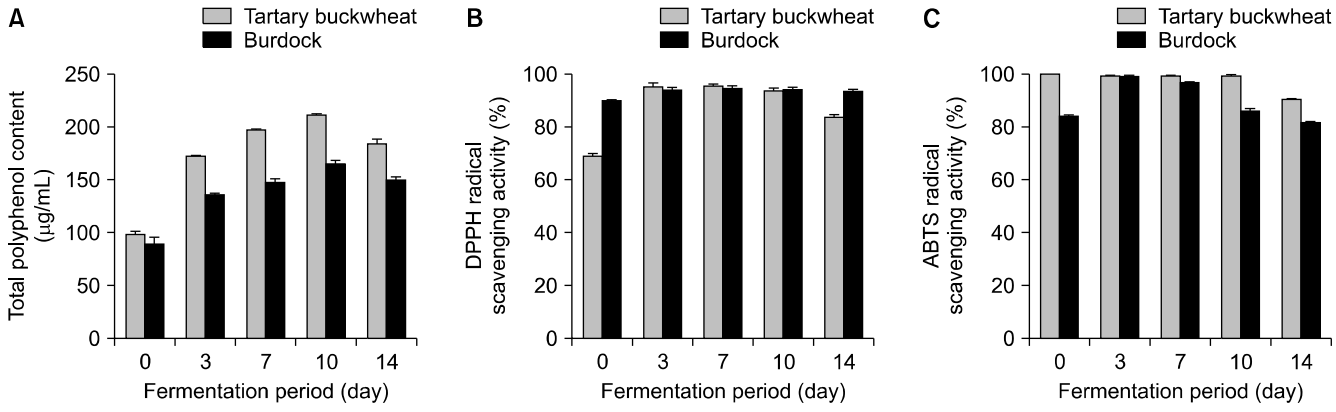

Fig. 1. Antioxidant activities of Tartary buckwheat kombucha and burdock kombucha during 14-day fermentation. (A) Total polyphenol content. (B) 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. (C) 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity.

was observed on day 10, while in the burdock kombucha, the highest polyphenol content was 165.11 μg/mL. The TPC of Tartary buckwheat kombucha was slightly higher than that of the burdock kombucha. Regardless of the changes in TPC, the DPPH and ABTS radical scavenging activities of the Tartary buckwheat kombucha and the burdock kombucha were maintained at a high level (>80%) throughout the fermentation period (Fig. 1B and 1C), with the Tartary buckwheat and burdock kombucha showing 95.62% and 94.64% for DPPH and 99.78% and 99.23% for ABTS radical scavenging activities, respectively. Both kombucha preparations showed similar or higher radical scavenging abilities than black tea kombucha, which is the most typical ingredient for kombucha production (Lobo et al., 2017; Değirmencioğlu et al., 2021).

Pancreatic lipase is a fat hydrolyzing enzyme that break down triglycerides into glycerol and fatty acids; inhibiting this enzyme can help prevent excessive fat accumulation by promoting the excretion of fats outside the body (Kwon et al., 2014). Since one of the main functionalities of kombucha is aiding body weight loss, the pancreatic lipase inhibition activity of the Tartary buckwheat and burdock kombucha at different fermentation days was investigated by following the procedures described by Ban et al. (2023) (Fig. 2). Orlistat was used as the positive control, and its inhibitory activity was 98.49%. The results showed that the burdock kombucha exhibited a relatively strong inhibitory activity at 56.68% on the third day of fermentation, and the activity decreased as fermentation progressed. The Tartary buckwheat tea showed the highest activity (40%) on day 7 and then decreased. Polyphenols are known to inhibit the activity of pancreatic lipase enzyme by binding to it. In particular, rutin and flavonoids, which are abundant in buckwheat, have been found to significantly inhibit pancreatic lipase activity (Li et al., 2011).

As fermentation progressed, both kombuchas showed good antioxidant and anti-obesity functionality due to the various organic acids produced by diverse microorganisms within the SCOBY and the presence of polyphenols derived from the substrates, which are abundant in antioxidants. In particular, Tartary buckwheat kombucha exhibited high radical scavenging activity and significant production of glucuronic and malic acid, while burdock kombucha was characterized by its high acetic acid content and anti-obesity activity. These results suggest that Tartary buckwheat and burdock could serve as potential commercial ingredients for kombucha production. To better understand the effectiveness of kombucha as a functional food, the mechanisms of the functionalities and the microbial physiology during kombucha fermentation should be explored further in the future.

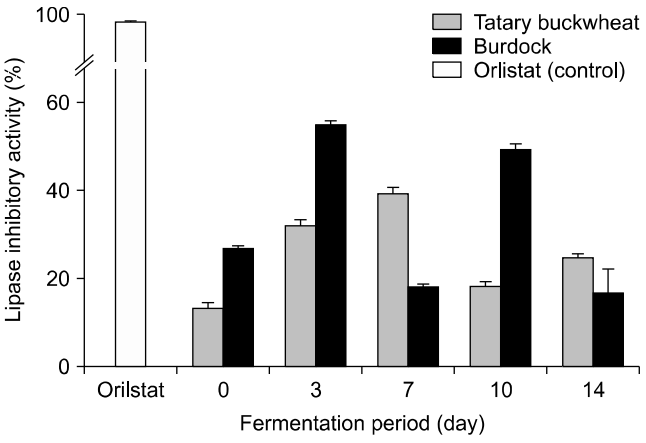

Fig. 2. Changes in porcine pancreatic lipase inhibition (%) in Tartary buckwheat kombucha and burdock kombucha during 14-day fermentation.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: SHY, YHJ. Analysis and interpretation: YJL. Data collection: HJK. Writing the article: YJL. Critical revision of the article: YHJ. Final approval of the article: all authors. Statistical analysis: HJK. Overall responsibility: YHJ.

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