Journal of Bioscience and Bioengineering
VOL. 112 No. 4, 356–359, 2011

www.elsevier.com/locate/jbiosc

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Effect of antioxidant activity in kimchi during a short-term and over-ripening fermentation period

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Received 18 March 2011; accepted 13 June 2011
Available online 13 July 2011

This study evaluated the antioxidant activities of kimchi at different fermentation times: short-term fermented kimchi (SK; less than 7 days) and over-ripened kimchi (OK; greater than 2 years). In conclusion, antioxidant activity of the OK was significantly higher than the SK. The results of this study suggested that there was an increase in the antioxidant activity of fermented kimchi during the fermentation and ripening processes.

[**Key words:** Chinese cabbage; Kimchi; Short-term fermented kimchi; Over-ripened fermented kimchi; Antioxidant activity]

Chemical antioxidants, such as butylated hydroxytoulene (BHT) and butylated hydroxyanisole (BHA), have been used as food additives to increase the shelf life of food and to preserve food quality. However, these compounds increase reactive oxygen species (ROS) and lipid peroxides, which can cause many diseases, aging, and damage. Recently, there has been an increase in the development and utilization of more effective antioxidants obtained from natural substances. Antioxidants from natural substances can scavenge free radicals found in the human body, delay the progress of some chronic diseases, and reduce lipid oxidative rancidity in foods.

Kimchi is a traditional Korean fermented food made from Chinese cabbage, radishes, onions, red pepper powder, garlic, ginger, and fermented jeotgal (1). Kimchi is a good source of natural antioxidants, such as carotenoids, vitamins, flavonoids, and other phenolic compounds (2). Therefore, kimchi may be used as a protective food (3). Recently, Koreans have started to consume over-ripened kimchi fermented at low temperatures due to its functionality and characteristic flavor. In general, the fermentation period for over-ripened kimchi ranges from 6 months to 3 years. Kimchi fermentation mainly depends on environmental factors, such as temperature, salt concentration, fermentation period, and the microflora species present during the fermentation process (4). Kimchi is especially dependent on the microflora, which dictate the antioxidant properties of kimchi and change during fermentation (5). In a previous study, we have found that *Weisella koreensis* and *Lactobacillus brevis* are the predominant lactic acid bacteria (LAB) in the initial fermentation stage and that *Leuconostoc gelidum* is the predominant LAB in the over-ripened fermentation stage (6).

Several previous studies have developed methods to increase the initial antioxidant activity of cabbage in the fermentation process, and these studies have found that the antioxidant capacity of fermented food may be dependent on the wounding and chemical processes incurred by the lactic bacteria (7). However, there has been no attempt to investigate the antioxidant properties of kimchi as a function of fermentation time. Therefore, this study aimed to evaluate the antioxidant activity, physical properties, and LAB in over-ripened kimchi and to compare the features of short-term fermented kimchi (SK) to those of over-ripened kimchi (OK). The samples were isolated from SK (fermented for less than 7 days) and OK (fermented for more than 2 years at a low temperature of 4°C). The samples were obtained from a retail store in Seoul in the Republic of Korea. To prepare the solvent extract (ethanol, methanol, and water), samples were first dried by a freeze-dryer (EYELA N-1000, Tokyo Rikakikai Co., Tokyo, Japan) and then homogenized. The powdered kimchi samples were then extracted with methanol, ethanol, and water (1:25, w/v) by shaking at room temperature for 24 h. After filtering through Whatman No. 2 filter paper (Whatman, Maidstine, UK), the extract was vacuum-concentrated in each solvent. The dried extract was weighted. The yield of the dried extract was calculated using the follow equation:

$$\%\text{Yield} = (\text{extract (g)} / \text{raw material (g; dry weight)}) \times 100 \quad (1)$$

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doi:10.1016/j.jbiosc.2011.06.003

The total phenolic content was measured by the Folin–Ciocalteau method (8). The results were expressed as gallic acid equivalents (GAE mg of extract powders). The equation of the standard curve with an R^2 value of 0.992 was as follows:

$$\text{Phenolic content} = 0.031 \times \text{OD} + 0.159 \quad (2)$$

The DPPH free radical-scavenging activity of the samples was measured according to the method described by Shimada et al. (9) with minor modifications. The percent of DPPH that was inhibited in the sample was calculated using the following equation:

$$\text{DPPH free radical} - \text{scavenging activity } (\%) = (1 - A_{\text{sample}} / A_{\text{blank}}) \times 100 \quad (3)$$

The IC_{50} values were calculated to express the concentration of antioxidants in the samples necessary to quench 50% of the radicals in the reaction mixture, and they were calculated using a sigmoid dose–response curve. Butylated hydroxyl anisole (BHA) and alpha-tocopherol were used as reference compounds.

Nitrite-scavenging activity was measured using the Griess reagent reaction according to the method described by (10). The nitrite-scavenging activity of the kimchi extracts was determined under different conditions (pH values of 1.2, 3.0, and 6.0) by measuring the absorbance at 520 nm. The nitrite-scavenging activity (%) was then calculated using the following equation:

$$\text{Nitrite scavaging activity}(\%) = \frac{\left[1 - \left(\begin{array}{c}\text{Absorbance of sample containing} \\ \text{1 mM } NaNO_2 \text{ after standing for 1 h}\end{array} - \begin{array}{c}\text{Absorbance of} \\ \text{the control sample}\end{array}\right)\right]}{\text{Absorbance of 1 mM } NaNO_2} \times 100 \quad (4)$$

Antimicrobial tests were then carried out using the disc diffusion method (11) with *Listeria monocytogenes* (ATCC 19111), *Salmonella typhimurium* (ATCC 14028), *Escherichia coli* (KCCM 21052), and *Staphylococcus aureus* (ATCC 6538), which were obtained from the Korea Culture Center of Microorganisms (KCCM, Republic of Korea) and American Type Culture Collection (ATCC, USA).

Identification of microorganisms in the SK and OK samples was performed via DNA extraction experiments. The amplification of the 16S rRNA gene from the SK and OK samples was performed using a thermal cycler (MJ Mini Personal ThermalCycler; Bio-Rad, Hercules, CA, USA) with the 27F and 1492R primer pairs. Ligation was performed using a pGEM-T vector (Promega, Madison, WI, USA). Ligated plasmids were then transformed into competent *E. coli* cells (Promega), and the plasmids were selected according to the standard blue/white screening procedure. Colony PCR was then carried out, and each colony PCR product was digested with *Msp*I (C^CGG) (Fermentas Inc., Glen Burnie, MD, USA) at 37°C for 3 h to determine if specific amplicons contained the 16S rRNA gene. Sequencing was carried out using an automated DNA sequencer (Applied Biosystems, Foster City, CA, USA). The closest known relatives were determined with the partial 16S rRNA sequences and public data libraries (GenBank) using the basic local alignment search tool (BLAST) and RDP programs. All analyses were run in triplicate. Data were analyzed using the Statistical Package for the Social Sciences (SPSS) software for Windows (version 10.0). The statistical probability was considered to be significantly different when p-values were less than 0.05.

The extract yield, total phenol content, and DPPH radical-scavenging activity of the sample extracts using different solvents are shown in Table 1. In general, the extraction yield ranged from 17.48% to 34.75%, and the yield was dependent on the extraction solvent and fermentation period. Among the various extracts examined, the methanol extract of OK exhibited the highest extraction yield. In this study, methanol was the most efficient extraction solvent to extract antioxidants from the SK and OK samples. The total phenol content was affected by the fermentation time and extraction solvents in the following order from high to low: methanol>ethanol>water. A significantly ($p<0.05$) higher total phenol content was found in the OK samples compared to the SK samples. This result may have been due to the increase in the amount of phenolic acids, such as *o*-coumaric acid and ferulic acids, and their interactions with microorganisms during fermentation, which ultimately produce the antioxidant activity (12). Thus, the phenolic content of fermented kimchi was closely associated with the antioxidant activity. These results suggest that fermented kimchi may have high antioxidant properties.

Free radicals are known to be important factors in biological damage, and DPPH has been used to measure the free radical-scavenging activity of natural antioxidants (13). Fig. 1 shows the DPPH radical-scavenging activities of the SK and OK samples extracted with different solvents in order of increasing polarity. The radical-scavenging activity of the various solvent extracts of the SK and OK samples increased in a dose-dependent manner (dose range of 20–100 mg/mL). The DPPH radical-scavenging activity of the OK methanol extract ranged from 53.18% to 88.38%. Many studies have reported that fermented kimchi in various solvent extracts contains active materials, such as phenolic compounds, vitamin C, and phenolic acid, which can scavenge DPPH radicals (14). The IC_{50} values of the samples are shown in Table 1. The IC_{50} value was obtained by sigmoid regression analysis of the data shown in Fig. 1. When compared to the SK samples, the radical-scavenging activity of the OK samples was significantly stronger with IC_{50} values ranging from 19.35 to 40.82 mg/mL, but the radical-scavenging activity of the OK samples was still lower than that of BHA and α-tocopherol with activities of 19.7 μg/mL and 18.4 μg/mL, respectively (Fig. 1 and Table 1).

Overall, the antioxidant activities of the SK and OK samples were highly correlated with their total phenolic contents ($R^2 = 0.992$). These results were similar to previous findings (15). In this study, the DPPH free radical-scavenging activity of the OK methanol extract was superior to any other activities that have been reported to date. Therefore, it has been suggested that the antioxidant activity of kimchi may also be dependent on fermentation time.

TABLE 1. Extraction yield, total phenol content, and DPPH IC_{50} of kimchi according to fermentation time using different solvents.

Extraction solvent	Extraction yield (%)[a]		Total phenolic content (μg of GAEs/mg extract)		DPPH IC_{50} (mg/mL)[d]		Positive control (μg/mL)	
	SK[b]	OK[c]	SK	OK	SK	OK	BHA	α-Tocopherol
Methanol	27.33 ± 1.51Ba [e]	34.75 ± 1.63Aa	22.80 ± 1.01Aa	25.24 ± 2.22Aa	19.49 ± 0.39Ac	19.35 ± 0.46Ac	19.7 ± 0.65	18.4 ± 0.30
Ethanol	23.58 ± 1.25Ab	23.00 ± 2.36Ab	16.83 ± 1.50Bb	21.10 ± 2.10Ab	33.10 ± 1.98Ab	24.99 ± 0.78Bb		
Water	17.48 ± 0.94Ac	19.01 ± 1.00Ab	14.93 ± 0.63Bb	20.86 ± 1.35Ab	42.08 ± 1.22Aa	40.82 ± 0.59Ba		

[a] Yield of extraction expressed as g extract/g (dried weight) of SK and OK.
[b] Short-term fermented kimchi less than 7 days of fermentation.
[c] Over-ripening fermented kimchi after greater than 2 years of fermentation.
[d] IC_{50} is the efficient concentration of the SK and OK samples that 50% initial DPPH radical by interpolation from sigmoid dose–response curve analysis.
[e] Mean ± SD is significantly different in the same row with different upper case letters (A–B) and in the same column with different lower case letters (a–c) ($p<0.05$).