Food Sci. Biotechnol. 26(1): 105-112 (2017)
DOI 10.1007/s10068-017-0014-5

Food Science
and
Biotechnology

Changes in the phenolic compounds and antioxidant activities of mustard leaf (*Brassica juncea*) kimchi extracts during different fermentation periods

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Received September 19, 2016
Revised December 13, 2016
Accepted December 13, 2016
Published online February 28, 2017

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pISSN 1226-7708
eISSN 2092-6456

Abstract This study was conducted to investigate the changes in the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities of 80% methanol and water extracts from mustard leaf kimchi during different fermentation periods. The methanol extract exhibited higher TPC and TFC than the water extract. Both extracts from kimchi fermented for two months showed the highest antioxidant effects against the scavenging activities of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radicals and 2,2-azino-bis diammonium salt (ABTS) radicals. Moreover, the methanol extract from kimchi fermented for two months showed the highest nitrite scavenging activity. The highest metal (Fe^{2+}) chelating effect of the methanol extract and water extract was observed after three months and one month, respectively. Caffeic acid showed the highest increase with fermentation. These findings suggest that the antioxidant activities of kimchi depend on the fermentation period. Accordingly, this study provides basic data for improving the antioxidant activity of mustard leaf kimchi through the establishment of their fermentation period.

Keywords: mustard leaf, kimchi, fermentation period, antioxidant activity, phenolic content

Introduction

Fermentation is defined as the breakdown or conversion of carbohydrates into organic acids using bacteria. During fermentation, many biochemical changes that remove antinutritive components and enhance the indicators of product quality, such as bioactivity and digestibility, occur (1). Sandhu *et al.* (2) reported that wheat cultivars fermented with *Aspergillus awamorinakazawa* were effective in increasing antioxidant activity and the amount of bioactive compounds, e.g., phenolic content. Another study reported that extracts of the noble starter culture *Doenjang* showed considerable antioxidant, α-glucosidase inhibitory, and tyrosinase inhibitory activities (3).

Mustard leaf (*Brassica juncea*), which belongs to the Cruciferae family, is a vegetable cultivated in Asia and Europe (4). It is widely used as a spice throughout the world as well as in folk medicines such as diuretics, expectorants, and stimulants (5). It is consumed in cooked, raw, salt-preserved, and pickled forms (6). It is a rich source of vitamin A, vitamin C, and phenolic compounds, which are protective against oxidative damages and carcinogenesis (7). It possesses a large group of glucosinolates, which contain sulfur compounds known to have antioxidant, antihypertensive, and cytotoxic activity (7). Moreover, Jo *et al.* (8) demonstrated that it has an antiatherogenic effect, which reduces the level of plasma cholesterol and increases the level of HDL-cholesterol.

Mustard leaf kimchi is a traditional vegetable food widely consumed in Korea. The city of Yeosu, Korea, is famous for mustard leaf kimchi, which is prepared by combining salted mustard leaf with various kinds of vegetables, seasonings, and fish sauces (9). Song *et al.* (10) reported that the chlorophylls and carotenoids in mustard leaf kimchi prevent the autoxidation of lipids. Moreover, it was shown that the growth of lung and gastric cancer cells is inhibited by mustard leaf kimchi treated with lactic acid bacteria (LAB) and that mustard leaf kimchi fermented at 15°C for 5 days can potentially prevent oxidative damage (11,12). However, the changes in the antioxidant effects of mustard leaf kimchi extracts at various fermentation periods have rarely been monitored. Therefore, this study was conducted to monitor the changes in the antioxidant activities and phenolic content of mustard leaf kimchi over a period

of three months. In addition, the effects of fermentation on the phenolic composition of kimchi extracts were analyzed using high performance liquid chromatography (HPLC).

Materials and Methods

Chemicals and reagents Griess reagent, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,4,6-tripyridyl-s-triazine (TPTZ), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p'-disulfonic acid monosodium salt hydrate (ferrozine), trolox, and all phenolic compounds including gallic acid (GA), chlorogenic acid (CLA), caffeic acid (CA), *p*-coumaric acid (*p*CA), ferulic acid (FA), rutin (R), naringin (N), quercetin (Q), epigallocatechin (EGC), catechin (C), epicatechin (EC), epigallocatechin gallate (EGCG), gallocatechin gallate (GCG), epicatechin gallate (ECG), and catechin gallate (CG) were obtained from Sigma-Aldrich (St. Louis, MO, USA). All the other reagents used in this study were of analytical grade.

Materials Mustard leaf kimchi was purchased from a local market called *Yeosunonghyup* in Yeosu, Korea. It was ground in a mixer (Blixer®, Robot Coupe USA, Inc., Jackson, MS, USA) and lyophilized using a freeze-dryer (FD5512; Ilshin Lab Co., Ltd., Daejeon, Korea). All kimchi samples were passed through a 60-mesh sieve and stored at −70°C prior to use.

Preparation of mustard leaf kimchi The mustard leaf was cleaned and soaked in 10% salt water for 4–6 h. After washing with water thrice, it was placed inside a basket in a refrigerator for more than 2 h and the water was allowed to drain. The salted mustard leaf was mixed well with the other ingredients. The composition of mustard leaf kimchi was as follows: salted mustard leaf 71.2%, red pepper powder 9%, garlic 1.4%, ginger 0.6%, fermented fish sauce (*Jeotgal*) 6.6%, sticky rice starch 7.2%, raw pepper 0.8%, and mixed seasoning 3.2%. The prepared mustard leaf kimchi was tightly packed into bottles and fermented at 4°C for 3 months.

Preparation of extracts Dried mustard leaf kimchi powder (20 g) was mixed with 200 mL of 80% methanol and distilled water, and allowed in a shaking incubator (BS-21; Jeio Tech., Daejeon, Korea) at room temperature for 24 h. The extracts were centrifuged at 11,325×*g* for 20 min and then filtered through Whatman No. 1 filter paper. The combined filtrate was evaporated under reduced pressure using a vacuum rotary evaporator (R-210; Buchi, Flawil, Switzerland) at 40°C, and the remaining solvent was removed via lyophilization. The dried samples were stored at −70°C for further use.

Determination of total polyphenol and total flavonoid contents The total phenolic content (TPC) of the mustard leaf kimchi extracts were determined via the Folin–Ciocalteu procedure (13) with minor modifications. Folin–Ciocalteu's reagent (25 μL) was mixed with 50 μL of sample extract and 150 μL of 20% Na_2CO_3. The mixture was allowed to stand for 15 min at room temperature, after which the absorbance values were measured using an enzyme-linked immunosorbent assay (ELISA) plate reader (VersaMax Molecular Devices, Sunnyvale, CA, USA) at 725 nm. Chlorogenic acid was used as a standard (0–200 μg/mL), and the results were expressed as mg of chlorogenic acid equivalents (CAE)/g of the extract powder. The total flavonoid contents (TFC) were estimated using the colorimetric method described by Jung *et al*. (14); i.e., 20 μL of each extract was reacted with 200 μL of diethylene glycol and 20 μL of 1 N NaOH for 1 h at 40°C. After incubation, the absorbance at 420 nm was measured using an ELISA plate reader. Rutin at 0–1,000 μg/mL was used to develop the calibration curve, with the results presented as mg of rutin equivalents (RE)/g of the extract powder.

DPPH radical scavenging activity assay DPPH radical scavenging activity was performed according to the method described by Blois (15); i.e., various concentrations of 0.1 mL samples were mixed with 0.9 mL of 0.1 mM DPPH radical solution. The mixture was then shaken vigorously and allowed to stand for 30 min in the dark. Next, the absorbance was measured using the ELISA plate reader at 517 nm. The percentage inhibition was calculated as: $[1-(A_{sample}/A_{control})]\times100$. The extract concentration providing 50% of the scavenging effect (EC_{50}) was calculated from the graph of inhibition percentage against extract concentration.

ABTS radical scavenging activity assay ABTS radical scavenging activity was measured according to the method described by Re *et al*. (16) with slight modification. ABTS radical was produced by reacting 7 mM ABTS solution (5 mL) with 150 mM potassium persulfate (88 μL) at ambient temperature in the dark for 15 h. Before use, this mixture was diluted with ethanol to obtain an absorbance of 0.7±0.0 at 734 nm. Subsequently, 1 mL of ABTS solution was mixed with 50 μL of the diluted sample solution, and the resulting sample was allowed to stand for 10 min in the dark. The absorbance values were measured at 734 nm using the ELISA plate reader. ABTS radical scavenging was calculated using the same formula as used for calculating the DPPH radical scavenging activity, and the concentration of EC_{50} was calculated from the graph of inhibition percentage against extract concentration.

Nitrite scavenging activity assay Nitrite scavenging activity was determined using the method described by Kato *et al*. (17) with minor modification; i.e., 40 μL of the extracts were mixed with 20 μL of 1 mM $NaNO_2$, and the resulting solution was adjusted to pH 1.2 using 0.1 N HCl. The solution was incubated at 37°C for 1 h. Following incubation, 1 mL of 5% acetic acid (v/v) and 80 μL Greiss reagent were added to the mixture and allowed to stand for 15 min. The absorbance was then measured at 520 nm using the ELISA plate reader. The percentage inhibition of nitrite was calculated using the

same formula mentioned earlier. The concentration of EC_{50} was calculated from the graph of inhibition percentage against extract concentration.

Metal (Fe^{2+}) chelating activity assay Capacity of the extracts to chelate Fe^{2+} was measured according to the method described by Dinis *et al*. (18); i.e., the extracts (1 mL) were mixed with 25 μL of 2 mM ferrous chloride and 25 μL of 5 mM ferrozine. After 10 min of incubation at room temperature, the absorbance was measured at 562 nm. The chelating capacity was calculated using the same formula used for calculating the DPPH radical scavenging activity. Then, the extract concentration providing 50% chelation of metals (EC_{50}) was calculated from the graph of inhibition percentage against extract concentration.

Ferric reducing antioxidant power assay Ferric reducing antioxidant power (FRAP) assay was performed using the Benzie and Strain (19) method with slight modifications. FRAP reagent comprised 30 mM sodium acetate buffer (pH 3.6), 10 mM TPTZ solution, and 20 mM $FeCl_3 \cdot 6H_2O$ solution at a ratio of 10:1:1. An aliquot of 0.1 mL of the sample solution was mixed with 0.7 mL FRAP reagent, and the solution was allowed to react in the dark for 30 min. The absorbance values of the reaction were measured at 590 nm. The FRAP values were expressed as μM trolox equivalents (TE) using the trolox standard curve.

High performance liquid chromatography-diode array detection (HPLC-DAD) analysis The phenolic compounds were analyzed using an HPLC system (Waters 2695; Waters Co., Milford, MA, USA) equipped with a Gemini C_{18} column (3 μm, 150×4.6 mm, Phenomenex, Casalecchio di Reno, Bologna, Italy) and a diode array detector (DAD) at 220 nm. Injection volume was 10 μL, flow rate was 1.0 mL/min, and temperature was set at 30°C. Solvent A (0.2 M phosphoric acid) and solvent B (acetonitrile) were used as mobile phase, and gradient condition (v/v) was as follows: 0–20 min, 15% B; 20–40 min, 35% B; and 40–50 min, 90% B. The phenolic compounds were identified with commercial standards by comparing their retention time and quantified from the peak areas using calibration curves.

Statistical analysis All experiments were performed in triplicate, and the results were expressed as the means±standard deviations. The collected data were analyzed using statistical package for social science (SPSS) ver. 10.0 (SPSS Inc., Chicago, IL, USA). The significant differences among the samples were evaluated using analysis of variance (ANOVA) followed by Duncan's multiple range test (p<0.05).

Results and Discussion

Extraction yield Extraction yields of the mustard leaf kimchi extracts are shown in Table 1. The extraction yields of the 80% methanol extracts ranged from 40.4 to 43.4%, whereas that of the distilled water extracts ranged from 48.4 to 52.0%. It was obvious that the extraction yields of distilled water would be higher than those of 80% methanol. The extraction yields of the distilled water extracts significantly (p<0.05) increased to 52.0% during the fermentation for 1 month and then decreased to 48.4% during the fermentation for 3 month. The yields of the methanol extracts did not differ significantly (p>0.05) during different fermentation periods. These results were in accordance with those of a previous study, reporting that the yield of the water extract from lactic-fermented Chinese cabbage was significantly higher (p<0.05) than that of the methanol extract regardless of fermentation period (20).

TPC and TFC TPC and TFC in the 80% methanol and distilled water extracts are shown in Table 2. TPC of the 80% methanol extracts ranged from 474.8 to 482.4 mg CAE/g, with the highest value observed after two months of fermentation. For the distilled water extracts, TPC varied from 78.3 to 100.3 mg CAE/g, with the maximum value observed after two months of fermentation. These results show that methanol is a more efficient solvent for extraction of phenolic acids from mustard leaf kimchi. For both the extracts, TPC increased significantly (p<0.05) during two months of fermentation and then declined. As reported by Sandhu *et al.* (2), TPC of wheat grains obtained from various cultivars fermented by *A. awamorinakazawa* over a period of six days showed significant (p<0.05) differences, with the highest TPC observed on Day 4; the TPC content decreased subsequently. TFC of the extracts showed a similar tendency as TPC. TFC of the 80% methanol extract ranged from 9.1 to 12.3 mg RE/g, with the highest value observed after two months of fermentation. In the case of the distilled water extracts, TFC values varied from 3.7 to 5.1 mg RE/g, showing the highest value after two months. TFC of both the extracts increased until two months of fermentation and then decreased. Liang *et al.* (21) found

Table 1. Changes in extraction yields of mustard leaf kimchi extracts during fermentation

Extraction yield (%)	Extraction solvent	Fermentation period (months)			
		Control	1	2	3
	80% methanol	40.4±1.3[1)NS2)]	43.4±1.91	43.2±0.2	42.8±0.5
	Distilled water	49.0±0.8[b 3)]	52.0±2.1[a]	50.4±0.9[ab]	48.4±0.5[b]

[1)]Mean±standard deviation (n=3).
[2)]NS, not significant.
[3)]Means with different letters in the same row are significantly different based on Duncan's multiple range test (p<0.05).

Table 2. Changes in total polyphenol and flavonoid contents of mustard leaf kimchi extracts during fermentation

	Extraction solvent	Fermentation period (months)			
		Control	1	2	3
Total polyphenol content (mg CAE/g extract powder)	80% methanol	474.8±2.0[1)c2)]	477.9±0.6[b]	482.4±0.1[a]	475.3±0.7[c]
	Distilled water	78.3±0.4[c]	81.4±0.8[b]	100.3±0.7[a]	83.4±0.8[b]
Total flavonoid content (mg RE/g extract powder)	80% methanol	9.1±0.8[c]	11.1±0.2[b]	12.3±0.8[a]	9.9±0.2[c]
	Distilled water	3.7±0.3[b]	4.2±0.3[b]	5.1±0.4[a]	3.7±0.2[b]

[1)]Mean±standard deviation (n=3).
[2)]Means with different letters in the same row are significantly different based on Duncan's multiple range test (p<0.05).

Table 3. Changes in EC_{50} values (mg/mL) of mustard leaf kimchi extracts during fermentation

	Extraction solvent	Fermentation period (months)			
		Control	1	2	3
DPPH	80% methanol	10.6±0.2[1)a2)]	10.4±0.1[ab]	9.9±0.2[b]	10.3±0.4[b]
	Distilled water	33.3±0.5[a]	28.7±0.7[b]	25.5±0.7[c]	28.0±0.3[b]
ABTS	80% methanol	33.8±0.3[a]	13.5±0.4[b]	9.4±0.4[d]	11.4±0.2[c]
	Distilled water	16.8±0.6[a]	14.1±0.4[b]	10.5±0.5[c]	13.3±0.4[b]
Nitrite	80% methanol	8.5±0.3[a]	8.3±0.2[a]	6.5±0.3[c]	7.5±0.2[b]
	Distilled water	9.8±0.5[a]	8.8±0.3[b]	9.7±0.8[a]	9.4±0.2[ab]
Fe^{2+} chelating	80% methanol	33.6±1.2[a]	5.2±0.1[b]	4.0±0.0[c]	3.5±0.1[c]
	Distilled water	161.3±0.7[a]	38.0±0.7[c]	58.3±1.9[c]	120.3±5.8[b]

[1)]Mean±standard deviation (n=3).
[2)]Means with different letters in the same row are significantly different based on Duncan's multiple range test (p<0.05).

that TFC of ethanol and water extracts from fermented adlay and rice were significantly higher (p<0.05) than those of nonfermented products. The higher values of TPC and TFC obtained by fermentation were attributed to the metabolic activities occurring during the growth of mycelium, which decomposed large molecules into small phenolics (21). The decrease in TPC and TFC after two months of fermentation might be due to the degradation of phenolic compounds by generated polyphenol oxidase (22). These results indicated that the extraction solvent and the fermentation process affect the changes in the TPC and TFC of mustard leaf kimchi.

Antioxidant activities Multiple assays were used to estimate the antioxidant activity of mustard leaf kimchi extracts. EC_{50} was used to express the antioxidant activity (Table 3). Scavenging of hydrogen radicals is one of the important mechanisms of antioxidation. The DPPH and ABTS radical scavenging activities are based on the ability of the extract to transfer electrons or hydrogen atoms (15,16). The 80% methanol extracts showed higher DPPH scavenging activities than the water extract regardless of fermentation. The EC_{50} values of the 80% methanol extracts ranged from 9.9 to 10.6 mg/mL, with the two-month fermentation and control showing the lowest and highest values, respectively. Moreover, the DPPH radical scavenging activity of the distilled water extracts significantly increased until two months of fermentation (EC_{50}=25.5 mg/mL) and then significantly decreased (p<0.05). Similar to the DPPH radical scavenging activity, ABTS radical scavenging activities of the methanol extracts were higher than those of the distilled water extracts, except for the control sample. EC_{50} values for the ABTS radical scavenging activities with the 80% methanol extracts ranged from 9.4 to 33.8 mg/mL, with the lowest value being observed in kimchi fermented for two months and the control showing the highest value. The scavenging effects of the distilled water extracts increased gradually for two months (EC_{50}=10.5 mg/mL) and then decreased significantly (p<0.05). Park *et al.* (22) reported that EC_{50} values of DPPH in over-ripened fermented kimchi extracts were 19.35 and 42.08 mg/mL for 80% methanol and distilled water, respectively; these values are lower than those obtained for short-term fermented kimchi. Choi *et al.* (23) found that RC_{50} (50% reduction of DPPH radicals) values of *Cheonggukjang* methanol extracts ranged from 0.57 to 0.77 mg/mL and then decreased significantly during fermentation (p<0.05). Sandhu *et al.* (2) reported that scavenging effect of wheat cultivars during fermentation increased until five days and that the ABTS radical scavenging activity was positively correlated with TPC (r=0.808, p<0.05). When compared to TPC (Table 2), the tendency of the ABTS radical scavenging activity was similar. These results show that the quantity of phenolic compounds influences antioxidant activity of mustard leaf kimchi.

Nitrite is a toxic substance that induces cell-damaging and mutagenic reactions. Excess consumption of nitrite can increase the risk of cancer and oxidization of hemoglobin (17). When compared with the distilled water extracts, the 80% methanol extracts showed a slightly stronger nitrite scavenging activity, and the EC_{50} values of inhibitory potential identified to be in the following order: control (8.5 mg/mL)<1 month (8.3 mg/mL)<3 months (7.5 mg/mL)<2 months

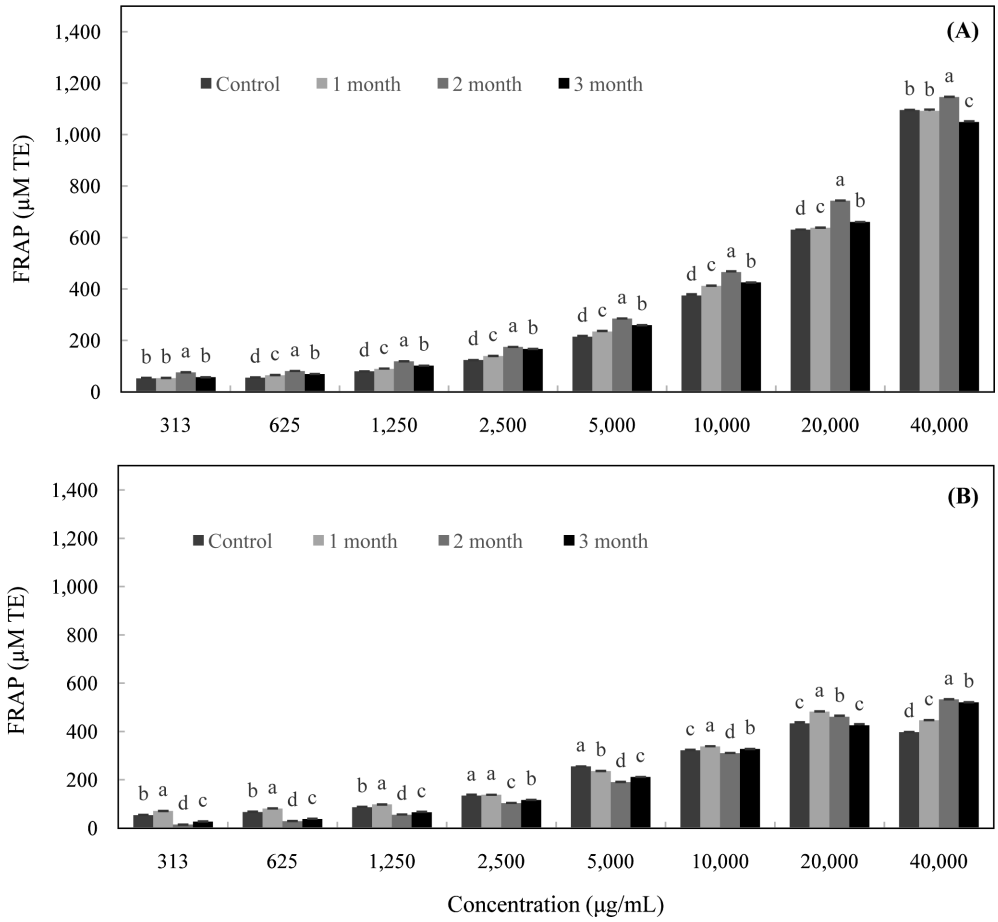

Fig. 1. Changes in FRAP of (A) 80% methanol extracts and (B) distilled water extracts obtained from mustard leaf kimchi during fermentation. The results are presented as means±SD (n=3). The means with different letters in the same concentration are significantly different based on Duncan's multiple range test (p<0.05).

(6.5 mg/mL). EC_{50} values of the distilled water extracts ranged from 8.8 to 9.8 mg/mL, with the extract fermented for one month showing the highest scavenging effect against nitrite. This observation was similar to a previous estimate for cabbage kimchi added to brown rice extract powder, which showed a high nitrite scavenging effect as the fermentation progressed (24). Park *et al.* (22) also reported that the nitrite scavenging ability of fermented kimchi is influenced by the extract solvent (water<ethanol<methanol) and the fermentation period (short term<over-ripening).

Metal ions (Fe^{2+}) lead to lipid peroxidation and food deterioration by initiating the chain reaction; therefore, chelating may render important antioxidant mechanisms by retarding metal-catalyzed oxidation (18). The metal chelating ability of the 80% methanol extracts increased continuously, with the EC_{50} values ranging from 33.6 to 3.5 mg/mL during fermentation. On the other hand, the chelating activity of the distilled water extracts increased remarkably until one month of fermentation (EC_{50}=38.0 mg/mL) and then decreased significantly (p<0.05). Zhang *et al.* (1) demonstrated that metal chelating ability of the water extracts from wheat increased during six day fermentation, with EC_{50} values ranging from 0.8 to 0.4 mg/mL. Huang *et al.* (25) reported that EC_{50} values of the methanol extract from sufu, which is the fermented product of soybean, declined from 19.9 to 3.8 mg/mL as fermentation progressed.

Among the various assays, the 80% methanol extracts showed greater antioxidant activity than the distilled water extracts. Moreover, the antioxidant activity of mustard leaf kimchi increased during the early stages of fermentation and decreased in the later stages. These findings suggest that the antioxidant activity of mustard leaf kimchi depends on the extraction solvents and fermentation periods. Hur *et al.* (26) proposed that antioxidant activity of fermentation foods may be affected by diverse factors such as pH, temperature, solvent, fermentation time, and an aerobic environment.

FRAP value The FRAP values were measured based on the ability of the extracts to convert ferric (Fe^{3+}) ions into ferrous (Fe^{2+}) ions. This donation of electrons enhances termination of free-radical chain reactions (19). Figure 1 shows FRAP values for the mustard leaf kimchi extracts obtained using 80% methanol and distilled water. The FRAP values of these extracts increased in a concentration-dependent manner, and significant differences (p<0.05) were observed during fermentation. The highest FRAP values for the methanol (1,145.9 μM

Table 4. Changes in contents of phenolic compounds in the methanol and water extracts of mustard leaf kimchi

Compounds[1] (μg/g extract powder)	Fermentation period (months)							
	80% Methanol				Distilled water			
	Control	1	2	3	Control	1	2	3
GA	-[2]	-	-	-	-	-	-	-
EGC	-	-	-	-	-	-	-	-
C	-	289.5±16.1[3][a4]	296.6±22.3[a]	-	-	160.4±2.7[c]	191.1±6.2[b]	218.0±9.6[a]
CLA	1,346.2±61.7[a]	1,279.8±23.1[a]	937.9±49.7[b]	689.7±13.9[c]	-	483.8±28.8[b]	845.4±29.7[a]	483.6±19.7[b]
CA	526.1±26.7[c]	937.4±15.7[a]	790.1±6.6[b]	936.9±13.9[a]	367.2±20.5[d]	413.3±11.9[c]	589.9±7.5[b]	766.6±12.3[a]
EC	372.6±19.4[c]	736.5±12.6[a]	467.7±23.5[b]	-	460.9±16.5[a]	247.7±8.5[c]	278.0±16.4[b]	256.5±12.6[bc]
EGCG	255.7±15.0[b]	584.3±7.2[a]	175.9±20.7[c]	191.8±21.3[c]	129.5±26.4[a]	75.0±6.5[b]	138.3±10.4[a]	115.4±8.5[a]
*p*CA	-	215.8±20.5[a]	-	-	-	-	75.3±3.9[b]	96.6±5.9[a]
GCG	-	213.4±33.9[a]	-	-	-	-	89.7±3.2[a]	-
FA	160.7±10.0[c]	268.3±15.7[b]	347.6±20.8[a]	-	68.3±4.2[c]	134.3±9.6[a]	116.3±1.6[b]	103.5±10.8[b]
ECG	369.4±67.0[a]	133.5±17.2[c]	297.9±22.0[b]	90.3±9.9[c]	98.0±5.3[b]	135.2±4.5[a]	132.0±5.7[a]	77.6±3.5[c]
R	119.6±30.3[c]	243.0±20.9[b]	324.7±21.1[a]	-	-	113.3±16.3[a]	126.3±18.8[a]	102.1±7.3[a]
CG	194.2±6.9[c]	238.2±13.9[b]	292.4±22.3[a]	161.7±11.1[d]	59.1±13.3[c]	120.8±8.7[b]	140.8±10.6[ab]	143.7±11.4[a]
N	373.4±17.8[b]	455.8±35.5[a]	504.9±43.9[a]	290.1±29.1[c]	312.3±11.8[b]	310.3±10.8[b]	359.4±12.1[a]	-
Q	-	-	-	-	-	-	-	-

[1]GA, gallic acid; EGC, epigallocatechin; C, catechin; CLA, chlorogenic acid; CA, caffeic acid; EC, epicatechin; EGCG, epigallocatechin gallate; *p*CA, *p*-coumaric acid; GCG, gallocatechin gallate; FA, ferulic acid; ECG, epicatechin gallate; R, rutin; CG, catechin gallate; N, naringin; Q, quercetin.

[2]Not detected.

[3]Mean±standard deviation (n=3).

[4]Means with different letters in the same row are significantly different based on Duncan's multiple range test (p<0.05).

TE) and water (532.9 μM TE) extracts were observed in the kimchi fermented for two months. Lee *et al.* (27) reported that fermented black bean extracts exhibited a higher reducing ability than nonfermented black bean extract. Choi *et al.* (23) also showed that RC_{50} values of FRAP in *Cheonggukjang* tend to decrease significantly (p<0.05) during fermentation. These results indicate that fermentation promotes an increase in FRAP.

Phenolic compounds Phenolic compounds in the 80% methanol and distilled water extracts from mustard leaf kimchi are shown in Table 4; a higher amount of phenolic compounds was observed in the methanol extracts. The methanol extract from mustard leaf kimchi fermented for one month and the water extract from kimchi fermented for two months showed a high diversity of phenolic compounds, which contained the following 12 phenolics: C, CLA, CA, EC, EGCG, *p*CA, GCG, FA, ECG, R, CG, and N. In the methanol extracts, the amounts of phenolic compounds ranged during fermentation from 90.3 (ECG, three months) to 1,346.2 μg/g (CLA, control). CLA gradually decreased from 1,346.2 to 689.7 μg/g during fermentation, but EC significantly (p<0.05) increased until one month of fermentation and then decreased. CA fluctuated during fermentation, and N and FA increased until two months of fermentation and then decreased. This is attributed to an increase in the number of LAB during fermentation which is affecting the microbial oxidation, reduction, or the degradation of the phenolic compounds (28,29). For the distilled water extracts, the amount of detected phenolic compounds ranged from 59.1 (CG, control) to 845.4 μg/g (CLA, two months). CLA and CA increased until two and three months of fermentation, respectively, and the EC ranged from 247.7 to 460.9 μg/g. According to Zhang *et al.* (1), the FA content of fermented wheat was significantly (p<0.05) higher than that of unfermented wheat. Moreover, for fermented oyster mushroom (*Pleurotus ostreatus*) using *Lactobacillus plantarum*, the FA content was found to be significantly (p<0.05) higher than that of unfermented mushrooms (30). Kim and Baik (31) reported that CA content of fermented dandelion beverage was approximately four times higher than that of unfermented dandelion beverage. It is possible that the hydrolytic enzymes produced by LAB during fermentation liberated bound phenolics by decomposing cell-wall materials, thereby increasing the amount of phenolic compounds (32). These findings suggest that proper fermentation can be a useful method for improving the antioxidant activity and the amount of phenolic compounds in mustard leaf kimchi. In addition, the fermentation period is one of the important factors influencing the antioxidative effects of mustard leaf kimchi. Thus, this study provides basic data on improving the antioxidant activity of mustard leaf kimchi by determining appropriate fermentation period.

Acknowledgments This research was conducted with the support of the Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ01084603) of the Rural Development Administration, Republic of Korea.

Disclosure The authors declare no conflict of interest.

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