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Antioxidant activity of lactic-fermented Chinese cabbage

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ABSTRACT

Lactic-fermented cabbage, similar to Kimchi in Korea, is a very popular fermented vegetable product in Taiwan and China. In this study, fermented cabbage prepared by a dry-salt method was first extracted with water and methanol. Antioxidant activity such as DPPH radical scavenging effects, reducing power and Fe^{2+}-chelating ability of the solvent extracts of fermented cabbage was determined and the effect of fermentation on the change of antioxidant activity, total phenolic and total flavonoid content was also investigated. Results revealed that antioxidant activity observed on the Chinese cabbage mixture may vary with extraction solvents and fermentation. Generally, the methanol extract of the cabbage mixture showed a higher DPPH radical scavenging activity and reducing activity than the water extract. Although, fermentation did not alter the Fe^{2+}-chelating ability and reducing activity of the methanol extract of the cabbage mixture, it reduced these same antioxidant activities in the water extract. Amongst the various extracts examined, the methanol extract of fermented cabbage showed the highest DPPH radical scavenging effect. On the other hand, the highest Fe^{2+}-ion chelating and reducing activities were exerted by the methanol extracts of both the cabbage mixture and the fermented cabbage, which showed no significant difference ($p < 0.05$). Additionally, the type of solvent and fermentation were also found to affect the total phenolic and flavonoid content of the extracts. Fermentation increased the total phenolic content of the methanol extract, whilst reducing the total flavonoid content of the water extract. Furthermore, changes in the antioxidant activity observed on the extracts of the cabbage mixture and fermented cabbage did not coincide exactly with the total phenolic and total flavonoid content.

1. Introduction

Environment pollution, UV radiation and several normal metabolic processes have been linked to the generation of potentially harmful reactive oxygen species such as superoxide anion radicals, hydroxyl radicals and hydrogen peroxide (Ames, Shigenaga, & Hagen, 1993; Halliwell & Gutteridge, 1999; Lenaz, 1998). Surpassing the antioxidant capacity of a biological system gives rise to oxidative stress which may cause oxidative damage by oxidising biomolecules such as proteins, enzymes, lipids, DNA and RNA, and thus lead to cell death and tissue damage (Ames et al., 1993; Halliwell, Murcia, Chirco, & Aruoma, 1995). Various diseases including arthritis, cirrhosis, emphysema, atherosclerosis and cancer are believed to be correlated with the oxidative damage induced by these free radicals (Halliwell et al., 1995; Jacob, 1994; Kehrer, 1993). Therefore, oxidative damage plays a significant pathological role in human disease. However, the ingestion of antioxidative supplements or foods containing antioxidants is now widely considered an effective strategy to reduce oxidative damage and exert a beneficial effect on human health (Aruoma, 2003; Ismail, Marjan, & Foong, 2004; Lin & Yen, 1999; Ramarathnam, Osawa, Ochi, & Kawakishi, 1995; Wang, Yu, & Chou, 2006).

Vegetables are good sources of natural antioxidants such as carotenoids, vitamins, flavonoids, and other phenolics compounds (Bunea et al., 2008; Ismail et al., 2004; Kim, Brecht, & Talcott, 2007; Podsedek, 2007; Sikora, Cieślik, Leszczyńska, Filipiak-Florkiewicz, & Pisulewski, 2008). Frequent intake of cruciferous vegetables, such as broccoli, cauliflower, leaf mustard, cabbage, and Chinese cabbage, which possess antioxidant activity, could be helpful to human health (Podsedek, 2007; Sikora et al., 2008; Wachtel-Galor, Wong, & Benzie, 2008). Recently, Kusznierewicz, Śmiechowska, Bartoszek, and Namieśnik (2008) observed that fermentation processes increased the initial values of antioxidant activity of cabbage. They indicated that the antioxidant capacity of sauerkraut probably combines effects of wounding and chemical processes incurred by lactic bacteria.

Lactic fermented Chinese cabbage, similar to Kimchi in Korea, is a rather popular fermented vegetable product in Taiwan. It is frequently included as a favourite dish in people's daily diet. The preparation of this fermented vegetable product here in Taiwan generally resembles that of Kimchi in Korea with the exception of fewer species added to the Chinese cabbage substrate before fermentation. During the preparation of this fermented vegetable

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product, Chinese cabbage is first soaked in a brine solution containing ca. 10–18% for 24 h then drained. The brined Chinese cabbage is further combined with ingredients such as garlic, pepper, ginger, and carrots and natural fermentation is allowed to proceed with lactic acid bacteria. Usually, a large quantity of waste brine containing a high salt content is also produced during the preparation of fermented Chinese cabbage and this byproduct has contributed to environmental pollution. Likewise in Taiwan, the preparation of Kimchi in Korea has had a destructive environmental impact (Choi & Park, 2003; Choi et al., 2002). In an attempt to relieve the waste brine problem and to improve the quality of fermented cabbage products, we have conducted a series of experiments. We found that the quantities of waste brine produced could be reduced by mixing the Chinese cabbage substrate with 3% dried salt instead of soaking it in high salt brine solution (Sun & Yu, 2003). In addition, the resulting waste brine byproduct had a lower overall salt content. Most importantly, with this dried-salt method, a fermented cabbage product possessing a pleasant sour flavour and the characteristic texture could be obtained at 25 °C after 48 h of natural fermentation with a predominant microbial flora of *Leuconostoc mesenteroides* and *Lactobacillus plantarum* (Sun & Yu, 2003). In this study, we have further explored the antioxidant activity including DPPH radical scavenging activity, Fe^{2+}-chelating ability, and reducing power of the extracts of this fermented vegetable using methanol or water as the solvent. Furthermore, the effect of fermentation on the change of antioxidant activity, and total phenolic and flavonoid content, was investigated.

2. Materials and methods

2.1. Preparation of fermented Chinese cabbage

Chinese cabbage (*Brassica pekinensis* Skeels), the main vegetable substrate used to prepare fermented cabbage, was obtained from a local market. After washing and trimming, it was cut into ca. 7 cm length and was mixed with 3% dry salt (w/w) then placed in 5 L-glass bottles without headspace, under room temperature (ca. 25 °C) for 24 h. After draining the salted Chinese cabbage, of excess water, it was mixed with various spices and ingredients including (g/100 g salted cabbage): sugar 1 g, red pepper powder 5 g, minced garlic 2 g, sliced carrot 4 g. This cabbage mixture was then tightly packed into 5 L-glass bottles and fermented at 25 °C for 2 days. Finally, it was stored in a freezer at −20 °C.

2.2. Preparation of solvent extracts

To prepare the solvent extracts, samples were first dried by a freeze-dryer (Free Dry System/Freezone®4.5, Labconco, Missouri, USA) and homogenised. The ground powder of the samples was then extracted with methanol or distilled water (1:20, w/v) by shaking at room temperature for 24 h. After filtering through Whatman No. 41 filter paper (Whatman, Maidstone, UK), the extract was vacuum concentrated and freeze-dried.

2.3. Measurements of the total phenolics and total flavonoids

The procedures described by Cheung, Cheung, and Ooi (2003) with minor modification were followed in order to determine the total phenolics of the samples. Briefly, an aliquot of 150 μl extract was mixed with 150 μl Folin–Ciocalteau phenol reagent (Sigma, St. Louis, MO, USA) and allowed to react for 3 min. Then 300 μl of 1 N Na_2CO_3 was added and allowed to react for 90 min at room temperature. Absorbance was measured at 725 nm using an automated microplate reader (VersaMax™ Tunable microplate reader, Molecular Devices Co., Sunuyvale, CA, USA) The results were expressed as μg gallic acid/mg extract.

The total flavonoid content of the samples was determined using a modified colourimetric method described previously by Jia, Tang, and Wu (1999), and using quercetin (Sigma, St. Louis, MO, USA) as a standard. Extracts or standard solutions (250 μl) were mixed with distilled water (1 ml) and 75 μl of 5% $NaNO_2$ solution. 75 μl of 10% $AlCl_3$ solution was added to the mixture 5 min later. After 6 min, 0.5 ml of 1 M NaOH and 0.6 ml distilled water was added. The solutions were then mixed and absorbance was measured at 510 nm. The results were expressed as μg quercetin/mg extract.

2.4. Measurement of antioxidative activity

Antioxidative activity, in terms of DPPH radical scavenging activity, Fe^{2+}-chelating ability and reducing power, was examined in the present study.

The DPPH free radical scavenging activity of samples was measured according to the method described by Shimada, Fujikawa, Yahara, and Nakamura (1992) with minor modifications. Briefly, 400 μM DPPH solution in methanol was prepared and 150 μl of this solution was added to 50 μl of the samples at various concentrations. After a 30 min incubation period at ambient temperature, the absorbance was read at 517 nm. The inhibitory percentage of DPPH was calculated according to the following equation:

$$\text{Scavenging effect}(\%) = (1 - \text{absorbance}_{\text{sample}}/\text{absorbance}_{\text{control}}) \times 100\%.$$

Essentially the method described by Oyaizu (1986) was followed to measure the reducing activity of samples. A sample (0.2 ml) containing various amounts of extract was mixed with phosphate buffer (0.2 ml, 0.2 M, pH 6.6) and 1% potassium ferric cyanide (0.2 ml). The mixture was incubated at 50 °C for 20 min and then 10% trichloroacetic acetic acid (0.2 ml) was added. The mixture was centrifuged (700×*g*) at 4 °C for 10 min. The upper layer (0.5 ml) was mixed with 0.1% ferric chloride (0.1 ml) and deionised water (0.5 ml). After 10 min of mixing, the absorbance of the mixture was then measured at 700 nm. A higher absorbance of the reaction mixture indicates a higher reducing power.

Fe^{2+}-chelating ability of the samples was determined according to the method of Dinis, Madeira, and Almeida (1994) with minor modifications. Essentially, a 0.2 ml of sample was mixed with methanol (0.74 ml), 2 mM ferrous chloride (0.02 ml) and 5 mM ferrozine (0.02 ml). The mixture was shaken and left standing at room temperature for 10 min. The absorbance of the mixture was then determined at 562 nm. The ability to chelate the ferrous ion was calculated as follows:

$$\text{Chelating ability}(\%) = (1 - \text{absorbance}_{\text{sample}}/\text{absorbance}_{\text{blank}}) \times 100\%.$$

Additionally, EDTA (ethylenediaminetetraacetic acid, Sigma, St. Louis, MO, USA) and BHA (butylated hydroxyanisole, Sigma, St. Louis, MO, USA) were used as the positive control for the determination of DPPH free radical scavenging activity and Fe^{2+}-chelating ability, respectively.

2.5. Statistical analysis

The results are presented as the average and standard deviation. The statistical analysis was performed using SAS 9.1 software package (SAS Institute, Cary, NC, USA) of the Statistical Analysis System. Statistical data were obtained by Student's *t* test procedure.

3. Results and discussion

3.1. Extraction yield

No single solvent could extract all the antioxidants with different polarities and solubilities from the foodstuff. Methanol is a widely used and effective solvent for the extraction of antioxidants, and methanol residue extracts often exhibited the highest activity and most stable antioxidative activity (García-Alonso, de Pascual-Teresand, Santos-Buelga, & Rivas-Gonzalo, 2004; Iqbal & Bhanger, 2007). Water is also a widely used solvent which can extract the major components in vegetables and plants. In this study, methanol and water were thus employed as the solvents to extract the antioxidants in the cabbage mixture before and after fermentation. Phenolics, as well as non-phenolic compounds, such as organic acid, proteins, pigments and sugars may be present in the solvent extract (Sun & Ho, 2005). As shown in Table 1, the extraction yield varied from 46% to 66%, largely depending on the extraction solvent. With a similar solvent, the extraction yields found with the cabbage mixture before and after fermentation were rather close. On the other hand, the yields of the water extract was significantly higher ($p < 0.05$) than that of methanol extract, regardless of fermentation. The phenomenon of variation in the solvent extraction yields observed was also reported by Sun and Ho (2005) and Iqbal and Bhanger (2007). Furthermore, the data of extraction yields revealed that water and methanol could solubilise ca. half or more of the materials from the cabbage mixture, regardless of fermentation. This indicated that most of the soluble compounds in the vegetables were high in polarity.

3.2. Antioxidant activity of the cabbage mixture before and after fermentation

The scavenging of hydrogen radicals is one of the important mechanisms of antioxidation. In this study, DPPH was used to determine the free radical scavenging activity of the various solvent extracts of the cabbage mixture before and after fermentation. The dose–response curve for the various solvent extracts is shown in Fig. 1. It was generally observed that the DPPH radical scavenging effect increased as the concentration of the solvent extract increased to a certain extent, and then leveled off even with further increases in the concentration. For example, the methanol extract of fermented cabbage at a concentration of 10–80 mg/ml exhibited 21.24–96.99% scavenging activity of DPPH radical. No significant increase in the DPPH scavenging effect was observed with further increases in dosage.

The half-inhibition concentration, IC_{50}, which is the efficient concentration required to reduce initial DPPH concentration by 50% of various extracts examined is summarised in Table 2. The IC_{50} value was obtained by interpolation from linear regression analysis of the data shown in Fig. 1. As shown in Fig. 1 and Table 2, the methanol extract of cabbage mixture showed a higher DPPH radical scavenging activity than the water extract, regardless of fermentation. Among the various extracts examined, the methanol extract of fermented cabbage, with the lowest IC_{50} of 26.69 mg/ml, exhibited the highest DPPH radical scavenging effect whilst the water extract of fermented cabbage, with the highest IC_{50}, exhibited the lowest DPPH radical scavenging effect. Meanwhile, BHA, serving as the positive control, exhibited an IC_{50} of 41.44 μg/ml.

In addition to initiating lipid peroxidation, which leads to the deterioration of food (Gordon, 1990), metal ions possessing catalytic ability have been correlated with incidence of arthritis and cancer (Halliwell et al., 1995). Additionally, ferrous ions, commonly found in the food system, are considered to be the most effective pro-oxidants (Yamaguchi, Takamura, Matoba, & Terao, 1998). In this study, the chelating ability of the extract of the cabbage mixture before and after fermentation toward ferrous ions was examined. As shown in Fig. 2, all the solvent extracts examined showed Fe^{2+}-ion chelating effect. The Fe^{2+}-ion chelating activity of all the solvent extracts increased as their concentrations increased. At the highest dosage level of 20 mg/ml examined, the extract showed a chelating of ca. 41.32% or more. IC_{50} of the solvent extracts in chelating Fe^{2+}-ion ability varied between 4.02 and 7.04 mg/ml, depending on solvents used and whether they were

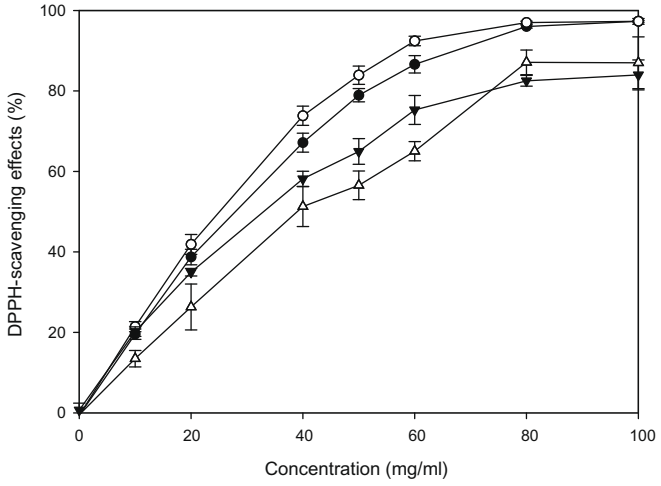

Fig. 1. DPPH radical scavenging effects of the methanol extracts and water extracts of the cabbage mixture and fermented cabbage. Symbols: ●, methanol extract of cabbage mixture; ○, methanol extract of fermented cabbage; ▼, water extract of cabbage mixture; △, water extract of fermented cabbage. Values are presented as means ± standard deviation ($n = 3$).

Table 1
Extraction yield from cabbage mixture and fermented cabbage.

Extract	Yield of extraction[a]	
	Cabbage mixture	Fermented cabbage
Methanol	A 0.48 ± 0.03 b[b]	A 0.46 ± 0.04 b
Water	A 0.66 ± 0.03 a	A 0.62 ± 0.07 a

[a] Yield of extraction expressed as g extract/g (dried wt.) cabbage mixture and fermented cabbage.
[b] Values are mean ± standard deviation ($n = 3$). Means in the same row with different upper case letters (A and B) or in the same column with different lower case letters (a and b) were significantly different ($p < 0.05$).

Table 2
DPPH radical scavenging activity and Fe^{2+}-chelating ability of the methanol extracts and water extracts from cabbage mixture and fermented cabbage.

Treatment	IC_{50} (mg/ml) of extracts[a]		Positive control (μg/ml)
	Methanol	Water	
DPPH radical scavenging activity			
Cabbage mixture	B 28.41 ± 0.20 a[b]	A 33.21 ± 0.47 b	
Fermented cabbage	B 26.69 ± 0.60 b	A 42.18 ± 5.39 a	
BHA			41.44 ± 1.63
Fe^{2+}-chelating ability			
Cabbage mixture	B 4.07 ± 0.32 a	A 7.04 ± 0.11	
Fermented cabbage	4.02 ± 0.1 a	>20	
EDTA			52.60 ± 0.21

[a] IC_{50} is the efficient concentration of the test samples that decreases 50% initial DPPH radical or Fe^{2+} concentration. IC_{50} was obtained by interpolation from linear regression analysis.
[b] Values are mean ± standard deviation ($n = 3$). Means in the same row with different upper case letters (A and B) or in the same column with different lower case letters (a and b) were significantly different ($p < 0.05$).

fermented or not (Table 2). EDTA showed an excellent chelating agent for ferrous ions and the value of IC_{50} was 52.60 μg/ml. The IC_{50} of the methanol extract of fermented cabbage and cabbage mixture without fermentation, showed no significant difference ($p > 0.05$), and was the lowest among the various solvent extracts examined. This finding implied that the methanol extract exhibiting the highest Fe^{2+}-ion chelating ability and fermentation did not alter the Fe^{2+}-chelating ability of the vegetable. On the other hand, a reduced Fe^{2+}-ion chelating ability was noted with the water extract of vegetable after fermentation when compared with that of the non-fermented vegetable.

In this study, reducing activity was determined based on the ability of solvent extracts to reduce a Fe^{3+}/ferricyanide complex to form Fe^{3+} ferrous complex. The Fe^{2+} was then monitored by measuring the formation of Perl's Prussian blue at 700 nm (Oyaizu, 1986). The dose–response curve for reducing activity of various solvent extracts of vegetable with or without fermentation is shown in Fig. 3. Although, lactic fermentation was reported to enhance the reducing activity of soymilk (Wang et al., 2006), such an effect was not observed on the lactic-fermented vegetable as examined in this study. As shown in Fig. 3, the reducing activity of the methanol extract of the fermented and unfermented vegetable showed no significant difference ($p > 0.05$) whilst the water extract of unfermented vegetable exhibited a higher reducing activity than the extract fermented vegetable. Besides, it was noted that the methanol extract of the vegetable, regardless of fermentation, in general, showed a higher reducing activity than the respective water extract.

The components of cabbage, such as phenolic compounds, especially phenolic acid and flavonoid derivatives, carotenoids, tocopherol and vitamin C, possess antioxidant activity (Bunea et al., 2008; Ismail et al., 2004; Kim et al., 2007; Sikora et al., 2008). In addition, the other ingredients in fermented cabbage, such as garlic and carrots, have also been reported to show antioxidant activity (Pedraza-Chaverrí, Medina-Campos, & Segoviano-Murillo, 2007; Sungnoon, Shinlapawittayatorn, Chattipakorn, & Chattiparorn, 2008; Yen, Shih, & Chang, 2008). They may all contribute to the antioxidant activity of the cabbage mixture before and after fermentation observed in this study. Additionally, the lactic acid bacteria present in the fermented cabbage in addition to being capable of balancing intestinal microflora and stimulating the immune system, may further affect the antioxidant activity exhibited by the fermented cabbage (Silvi, Verdenelli, Orpianesi, & Cresci, 2003; Wang, Yu, Yang, & Chou, 2003). On the other hand, fermentation may alter the composition, structure and polarity of antioxidant biofactors in the fermented cabbage and may thus have led to the variation in the antioxidant activity observed in the cabbage mixture with and without fermentation. Nevertheless, the variation of these antioxidant biofactors present in water and methanol and due to fermentation remained to be further investigated.

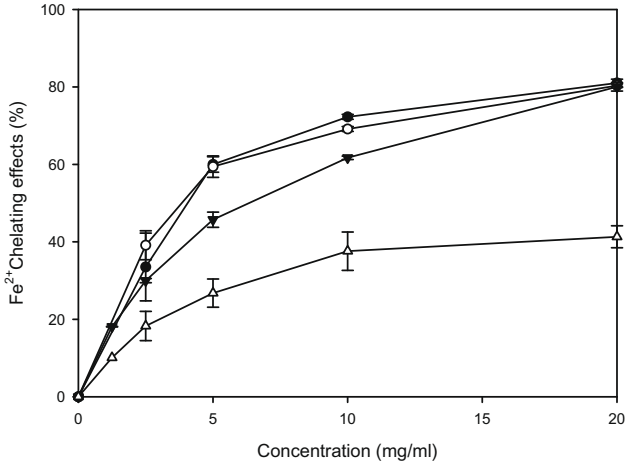

Fig. 2. Fe^{2+}-chelating ability of the methanol extracts and water extracts of cabbage mixture and fermented cabbage. Symbols: ●, methanol extract of cabbage mixture; ○, methanol extract of fermented cabbage; ▼, water extract of cabbage mixture; △, water extract of fermented cabbage. Values are presented as means ± standard deviation ($n = 3$).

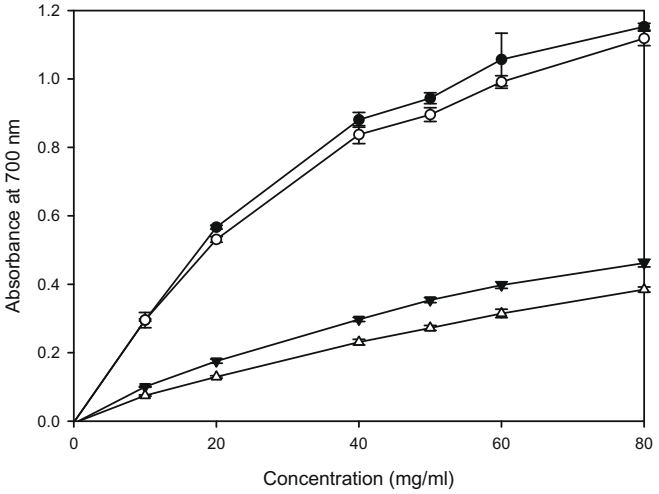

Fig. 3. Reducing power of the methanol extracts and water extracts of cabbage mixture and fermented cabbage. Symbols: ●, methanol extract of cabbage mixture; ○, methanol extract of fermented cabbage; ▼, water extract of cabbage mixture; △, water extract of fermented cabbage. Values are presented as means ± standard deviation ($n = 3$).

3.3. Total phenolic and total flavonoid content

Table 3 shows the contents of total phenolics and total flavonoids expressed as μg of gallic acid and quercetin, respectively, per mg of extract. Generally, a significantly ($p < 0.05$) higher content of total phenolics was found in the fermented-cabbage extract rather than the unfermented-cabbage extract. The total phenolic content of the methanol extract was not significantly different from that of the water extract respectively ($p > 0.05$), whilst the water extract of the cabbage mixture or fermented cabbage mixture contained significantly less ($p < 0.05$) than that of the respective methanol extract.

It has been suggested that phenolics are secondary metabolites, and in part, are produced as a result of the plant's interaction with the environment (Snyder & Nicholson, 1990). Phenolics in plants are usually found in conjugated forms through hydroxyl groups with sugar as glycosides (Robbins, 1980). Lactic acid bacteria may be capable of producing β-glucosidase, which catalyses the release of total phenolics during fermentation (Stechell, 2000; Tsangalis, Ashton, Mcgill, & Shah, 2002). Furthermore, fermentation may change the texture of cabbage and render phenolics more accessible to the extraction solvent. Therefore, these processes may all lead to the increase in total phenolic content observed in the cabbage mixture after fermentation (Table 3).

It has frequently been reported that phenolic compounds are closely associated with antioxidant activity (Velioglu, Mazza, Gao, & Oomah, 1998). However, the change in antioxidant activity observed in the extracts of cabbage mixture and fermented cabbage mixture did not coincide exactly with the total phenolic content. For example, the water extract of fermented cabbage showed a higher total phenolic content than the similar solvent extract of the cabbage mixture without fermentation (Table 3). In contrast, based on the IC_{50} value, the water extract of fermented cabbage exhibited a lower DPPH radical scavenging effect than the

Table 3
Total phenolic and total flavonoid content of the methanol and water extracts from the cabbage mixture and fermented cabbage.

Extract	Total phenolic content (μg gallic acid/mg extract)		Total flavonoid content (μg quercetin/mg extract)	
	Cabbage mixture	Fermented cabbage	Cabbage mixture	Fermented cabbage
Methanol	B 3.25 ± 0.29 a[a]	A 4.93 ± 0.48 a	A 0.29 ± 0.00 a	A 0.30 ± 0.01 a
Water	B 3.18 ± 0.24 a	A 4.38 ± 0.02 a	A 0.13 ± 0.01 b	B 0.10 ± 0.01 b

[a] Values are mean ± standard deviation ($n = 3$). Means in the same row with different upper case letters (A and B) or in the same column with different lower case letters (a and b) were significantly different ($p < 0.05$).

methanol extract (Table 2). These results suggested that both kinds of phenolic compounds in addition to their quantity influence the antioxidant activity observed. Additionally, the possibility that bioactive components other than phenolic compounds may also contribute to the antioxidant activity observed can not be ruled out.

4. Conclusion

This is the first report concerning the antioxidant activity of lactic fermented cabbage, a popular fermented vegetable product in Taiwan and China. Variation in the antioxidant activity including the DPPH radical scavenging effect, Fe^{2+}-ion chelating effect and reducing activity was noted in the extracts prepared with different solvents. Generally, the methanol extract exerted a higher antioxidant activity than did the respective water extract. In addition, lactic fermentation altered the antioxidant activity of the extract of the cabbage mixture. Despite the variation of antioxidant activity observed in the solvent extract, results of this study showed that this probiotic-containing fermented vegetable product possesses antioxidant activity in addition to palatable taste characteristics (Sun & Yu, 2003). As a consequence, not only is this fermented vegetable product popular, its regular consumption has numerous health benefits.

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