Antioxidant Activity of Various Fractions Extracted from Mustard Leaf (*Brassica juncea*) and Their Kimchi

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The antioxidant activities of the various fractions extracted from mustard leaf (*Brassica juncea*) and mustard leaf kimchi were determined by the radical scavenging effect on 1,1-diphenyl-2- picryl-hydrazyl (DPPH) radical. The fractions from dried mustard leaf, and fermented mustard leaf kimchi for 0 day and fermented mustard leaf kimchi for 5 days at 15℃ were screened for the scavenging effects by using DPPH assay. The fractions prepared by the systematic extraction procedure with the solvents such as hexane, CH_2Cl_2, EtOAc, BuOH and H_2O were used for the determination of free radical scavenging effects. The antioxidant activity of EtOAc and n-BuOH soluble fraction from mustard leaf and mustard leaf kimchi for 0 day had stronger than the others. During fermentation at 15℃ for 5 days, the antioxidant activity was changed. CH_2Cl_2 and EtOAc soluble fraction showed more potent radical scavenger effects than the others. The difference of results were to the various supplements and fermentation process.

Key words – antioxidant, mustard leaf, mustard leaf kimchi, free radical

Mustard leaf (*Brassica juncea*) is one of the major main ingredient for kimchi, such as Korean cabbage, radish, cucumber and green onion which used for the preparation of various type of *kimchi* in Korea. It belongs to *Cruciferae* and the varieties of these species, a green vegetables are grow up for one and 2 years rapidly. Mustard leaf and stem are edible and mustard seed is used as spices. In particular, kimchi is made from fermented leaf of use for good in Korea. The selection for the ingredient other than vegetables is various, so kimchi can be the major sources for various kind of nutrients. The fermentation process accompanies with complicated reaction mechanism in which bacteria, fungi and yeast are involved by producing aroma and taste (1). And also kimchi contains chlorophylls, cartenoids, β-carotene, L-ascorbic acid, phenolic and other compounds.

The interest about dietary supplement has increased, kimchi is considered as functional food which has antioxidant, antimutational and anticarcinogenic effects as well as its nutritional benefits. Since mustard leaf is abundant in Korea, it is frequently consumed as the main ingredients of kimchi. In the past study, the important researches about mustard leaf are focused on the cartenoid and chlorophyll (2), antibiotics activity and screening of antimicrobial activity (3), changes in chlorophylls and cartenoids of mustard leaf kimchi (4). The antioxidant activity of mustard leaf and their kimchi has not yet been investigated for the MeOH extract and their fraction of mustard leaf, mustard leaf kimchi for 0 day and fermented at 15℃ for 5 days on 1,1-dimethyl-2-picryhydrazyl (DPPH) radical. DPPH assay would be useful in a short time as well as a numerous sample and is detective at low concentrations, Thus we used this method for primary screening of antioxidant activity of 3 samples of material, freezing dried mustard leaf, mustard leaf kimchi at 0 day fermented mustard leaf kimchi for 5 days at 15℃.

This study was conducted with freeze dried mustard, mustard leaf kimchi and fermented mustard leaf kimchi for the antioxidant activities of mustard leaf and mustard leaf kimchi by measuring radical scavening effect on DPPH radical (5,6).

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Material and Methods

Mustard leaf material

Mustard leaf were collected in August 1998, Dolsando,

in Chonnam, South Korea. The ingredients of mustard leaf kimchi were described in Table 1. Mustard leaf was brined with salt, and blended with diverse spices and other materials, and then fermented. Respectively, Mustard leaf and their kimchi were used for freeze-dried at -40℃ for 24 hours by freeze-drier (Freeze Dryer-5, Ilsin Engineering, Korea).

Reagent

1,1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma Chemical Co., USA. The highest grade of other chemicals were purchased commercially.

Extraction and fractionations

Methanol (MeOH), hexane (C_6H_{14}), dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), buthanol (BuOH) and water (H_2O) was used in order to extract active components. After three kinds of mustard leaf and their kimchi were freeze-dried, mustard leaf (1,650 g), mustard leaf kimchi (2,440 g) and fermented mustard leaf kimchi (2,230 g) were extracted with methanol by three times, respectively. mustard leaf was yielded hexane (62.4 g), dichloromethane (2.8 g), ethyl acetate (4.5 g), butanol (75.2 g) and water soluble fractions (123.9 g), respectively. In mustard leaf kimchi, hexane (54.6 g), dichloromethane (4.2 g), ethyl acetate (5.8 g), butanol (77.2 g) and water soluble fractions (301.4 g). In fermented mustard leaf kimchi, hexane (39.4 g), dichloromethane (6.1 g), ethyl acetate (19.5 g), butanol (134.0 g) and water soluble fractions (340.1 g) (Fig. 1).

Scavening effect of DPPH radical

The scavening effect of DPPH radical of methanol extract and all fractions of mustard leaf and their kimchi was determined by the method of Blois (7). Four milliliters of MeOH solution of diversified sample concentrations was added to 1.0 ml DPPH methanol solution (1.5×10^{-4} M) and

Table 1. Ingredients ratio for the preparation of mustard leaf *Kimchi*

Ingredients (Scientific name)	Ratio (%)
Fresh mustard leaf (*Brassica juncea*)	77.22
Salted and fermented anchovy	7.72
Red pepper powder (*Capsicum annnum*)	6.18
Garlic (*Allium sativum*)	2.32
Ginger (*Zingiber officinale*)	1.16
Paste of glutinous rice powder	4.63
Sugar	0.77

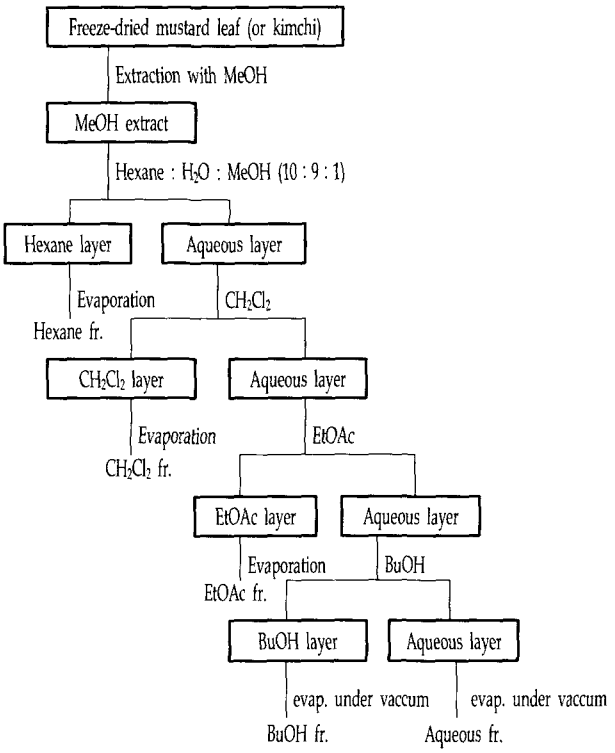

Fig. 1. Flow chart for extraction and fractionation of freeze-dried raw mustard leaf and their *kimchi*.

reacted at room temperature for 30 min. The absorbance of the solution was determined at 520 nm. UV visible spectrophotometer was used and measured for the remained DPPH (8,9). The result was calculated by the log-dose inhibition of triplicated values.

Results

The scavenging effects of methanol extract and their fractions from mustard leaf and their kimchi are shown in Table 2. Excepted for the water fraction, the scavenging activity of DPPH radical was observed in all case. The different activity of all fraction dependent on concentration was observed. Mustard leaf at both EtOAc and CH_2Cl_2 fractions were stronger than other fractions. IC_{50} of these fractions, the scavening effect of DPPH radical were 35.44 μg/ml and 45.34 μg/ml, respectively. In mustard leaf kimchi for 0 days, also EtOAc and CH_2Cl_2 fractions were stronger than oother fraction. IC_{50} of these fractions were 48.88 μg/ml and 73.06 μg/ml, respectively. However, in fermented mustard leaf kimchi at for 5 day, CH_2Cl_2 fraction was stronger than other fractions. The second stronger fractions was EtOAc fraction. IC_{50} of these fractions were 20.76 μg/ml and 92.92 μg/ml, respectively. The

Table 2. The scavenging effect of the methanol extract and its fractions obtained from mustard leaf, mustard leaf *kimchi* for 0 day and fermented mustard leaf *kimchi* at 15℃ for 5 days on 1,1-diphenyl -2-picrylhydrazyl (DPPH) radical

Material	Fractions	Concentration (μg/ml)								IC50
		480	320	160	80	40	20	10	5	(μg/ml)
Mustard leaf	MeOH extract	26.79	26.79	12.77	-*	-	-	-	-	>120
	hexane fr.	61.05	39.25	24.92	6.54	1.09	-	-	-	99.73
	CH_2Cl_2 fr.	90.65	74.30	46.26	23.68	12.21	3.42	-	-	45.34
	EtOAc fr.	93.94	85.20	56.39	28.35	12.77	8.10	3.42	-	34.44
	n-BuOH fr.	52.49	26.48	14.33	6.54	3.42	-	-	-	116.17
	H_2O fr.	15.26	26.01	17.76	8.88	3.42	-	-	-	>120
	L-Ascorbic acid					90.65	92.99	93.77	94.54	<0.625
Mustard leaf *kimchi*	MeOH extract	29.58	21.51	11.78	8.20	2.74	2.74	1.37	0.68	>120
	hexane fr.	41.51	29.72	17.12	6.16	5.21	2.47	2.05	1.37	>120
	CH_2Cl_2 fr.	72.19	53.42	33.70	17.81	7.53	4.10	0.68	-*	73.06
	EtOAc fr.	90.41	76.84	45.34	23.75	16.43	8.22	5.07	3.42	48.88
	n-BuOH fr.	37.67	26.03	13.70	6.85	4.10	4.10	2.74	1.37	>120
	H_2O fr.	20.00	13.01	9.86	4.79	2.74	1.37	1.37	1.37	>120
	L-Ascorbic acid					92.47	93.15	94.52	95.48	<0.16
Fermented mustard leaf *kimchi*	MeOH extract	28.77	18.77	9.59	6.85	1.37	-*	-	-	>120
	hexane fr.	32.88	23.01	11.78	2.05	1.37	-	-	-	>120
	CH_2Cl_2 fr.	100.00	100.00	84.93	48.63	28.77	20.55	10.27	6.16	20.76
	EtOAc fr.	61.78	44.38	23.97	11.64	6.85	2.05	1.37	2.05	92.92
	n-BuOH fr.	21.23	15.07	6.85	3.42	1.10	-	-	-	>120
	H_2O fr.	30.82	21.92	11.64	4.66	0.68	-	-	-	>120
	L-Ascorbic acid					92.47				

*No radical scavenging effect was shown.

differences of results from those were to the various components and fermentation process.

Discussion

Kimchi is a Korean traditional food which is fermented properly from salted Korean cabbage or raddish and mustard leaf with other various supplement. Kimchi is effective for the antimutagenic and anticancer (10). Kimchi, has large amounts of ascorbic acid, carotenoid, chlorophyll, lactic acid bacteria and dietary fiber which have antimutagenic or anticarcinogenic effects (11-13). And also kimchi is known to improve digestion, prevent constipation, control intestinal flora and other pharmaceutical functions (14,15).

Mustard leaf kimchi was prepared to preserve the organoleptic and nutritional qualities of fresh vegetables during the winter in Korea. Recently, it has changed the whole year food because fresh vegetables are available for four seasons. Mustard leaf contains sinigrin, which is a predominant glucosinolate in mustard leaf and is mainly degraded upon the enzymatic action of myrosinase under normal condition to give allyl isothiocyanate (16). This is well-known for its aromatic flavor and functional component ascorbic acid, carotene and dietary fiber in green vegetables such as red pepper, garlic, ginger and onion are reported to inhibit mutagenicities induced by several carcinogens and mutagens (17,18). It was reported that nucleoside identified in muatard leaf and their kimchi, had a function to prevent of arteriosclerosis (24). Song, E. S. et al was reported that mustard leaf kimchi, had a strong antioxidative effect (4). The radical scavenging effect of the MeOH extract and their fraction were tested for the freeze-dried mustard leaf, mustard leaf kimchi for 0 days and mustard leaf kimchi at 15℃ for 5 days. Especially, the methanol extract was more important than that of other fraction, however, further isolated fraction was stronger than that of other fraction. Owing to an odd number of its electron, the scavenging effect of DPPH was used to evaluate free radical scavenger (19). Slater et al. reported that active oxygen species such as superoxide radicals, hydrogen radical and hydrogen peroxide have been familiar as the principle agent responsible for the deterioration of polyunsaturated fatty acids or lipid containinng food when exposed to air (20). The capacity of the measured substance to donate electrons can be determined

from the extent of their loss of color (21,22).

The order of the radical scavenging effect at IC_{50} was EtOAc fr.〉 CH_2Cl_2 fr. 〉 hexane fr. 〉 *n*-BuOH fr.〉 H_2O fr. from mustard leaf. The order of the effect at IC_{50} was EtOAc fr.〉 CH_2Cl_2 fr. from mustard leaf kimchi. During fermentation at 15℃ for 5 days, the antioxidant activity was changed. CH_2Cl_2 and EtOAc soluble fraction showed more potent radical scavenger effects than the others. The order of the effect at IC_{50} was CH_2Cl_2 fr.〉 EtOAc fr. from fermented mustard leaf kimchi. It showed the strong inhibitory activity of low concentration range for the scavenging effect of DPPH radical.

Above all, radical scavenging effect of phenolic compounds isolated from natural sources has been reported (23). It was considered that these compounds react with the free radicals during oxidation, and a new radical is stabilized by the resonrance effect of the arometic nucleus (24). The radical scavenging property of phenolic acids is perhaps concerned with superior stability of radical derived from the adjacent additional presence of hydroxyl group which hydrogen bonds with the keto group, compared to that of phenoxyl radical (25,26).

In previous research the active compounds of mustard leaf were reported as sinigrin, allyl isothiocyanate, nucleotides (27), flavonoid (28) and so on. As EtOAc fraction and BuOH fraction of mustard leaf and their kimchi were presented flavonol glycoside reaction of pink with Mg-HCl test and positive Molisch test, it seemed that comprehended among them. Mustard leaf is plentiful as flavonoid of phenolic compounds, because of EtOAc fraction and BuOH fraction are seemed that it contained flavonoid which conduct a chemical experiments by TLC plate confirmed from qualitative analysis. Also, it seemed that mustard leaf kimchi estimate contained a great deal of flavonoids as phenolic compounds, Flavonoid is carry out at specific scavenging of hydroxy (29), superoxide (30), peroxyl radical (31). EtOAc fraction and BuOH fraction from mustard leaf is antioxidant effect for quanching of DPPH radical. The present research suggests that the methanol extract and their fractions from mustard leaf, mustard leaf kimchi for 0 day, fermented mustard leaf kimchi at 15℃ for 5 days, may be desirable substances for the prevention of oxidative injury.

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초록 : 청갓과 청갓김치의 용매별 추출물의 항산화성

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동결건조한 청갓과 담금당일 청갓김치, 15℃에서 5일간 발효시킨 청갓김치로부터 극성이 다른 용매들을 사용하여 각각의 용매별 추출물을 얻고 methanol(MeOH), hexane(C_6H_{14}), dichlorometane(CH_2Cl_2), ethyl acetate(EtOAc), butanol(BuOH), water(H_2O)의 각각의 용매분획별(DPPH) 1,1-diphenyl-2-picrylhydrazyl assay에 의한 항산화성을 측정하였고 항산화효과를 상호비교하였다. 청갓과 담금당일 청갓김치에서 EtOAc와 n-BuOH에서 얻은분획층이 다른 분획층보다 항상화효과가 높게 나타났으며 15℃ 5일간 발효시킨 청갓김치에서는 CH_2Cl_2와 EtOAc분획층이 다른 분획층에 비하여 radical scavenger 효과가 있음을 확인할 수 있었다. 결과의 차이는 다양한 재료에 의한 발효공정에 기인된다고 판단된다.