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연구논문

저장 기간 중 돌산갓 피클의 폴리페놀, 플라보노이드 및 항산화 활성에 대한 변화

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Total Polyphenol, Flavonoid Contents and Antioxidant Activities of Dolsan Leaf Mustard Pickle during Storage

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Abstract: Dolsan leaf mustard has various biological functions, including those in the immune system and cancer prevention. It contains high amounts of nutritional and medicinal compounds, which are important for maintaining optimum health. The objective of this study was to determine the total phenolics contents and antioxidant activity in Dolsan leaf mustard pickle (DLMP) during storage. DLMP methanol extracts had the highest total polyphenol and flavonoid contents of 35.56±0.01 GAE mg/g and 4.54±0.03 QE mg/g, respectively. The DPPH and ABTS radical scavenging activities in the DLMP methanol extracts showed the highest activities of 79.4 and 85.36%, respectively. The ferric reducing antioxidant power (FRAP) assay showed 2.24-3.82 mM $FeSO_4$ eq. (p<0.05) in DLMP extracts. Overall, storage at day 14 showed the highest antioxidant activity.

Keywords: Dolsan leaf mustard, Pickle, Antioxidant activity, Polyphenol, Flavonoid

1. INTRODUCTION

Pickles are produced for the long-term storage of many seasonal and local vegetables. Pickled vegetables are prepared using edible acid (which is added or formed *in situ* by fermentation) and vegetable preservation is due, at least partially, to the presence of this acid [1]. Leaf mustard (*Brassica juncea*) is a member of the Brassicaceae family, and mustard seeds are used as a spice. Dolsan leaf mustard is larger than traditional leaf mustard, and has abundant glucosinolates, polyphenols, and sulfur compounds [2]. Leaf mustard on the surface of pickles influences released of isothiocyanate by the action of myrosinase [3]. Other released components such as yeasts, molds, and various bacteria have antimicrobial activities; therefore, food prepared with leaf mustard has increased storability. In addition, leaf mustard is a rich source of iron, phosphorus, calcium, potassium, proteins, and minerals [4]. Dolsan leaf mustard strengthens the immune system and helps to prevent cancer [3]. Phenolic contents in plants play an important role as primary antioxidants or free radical terminators, suggesting their use in preventing the oxidative breakdown of lipids and in preventing chronic diseases resulting from reactive oxygen species (ROS). Enzymatic ROS scavenging includes superoxide dismutase (SOD), ascorbate peroxidase (APX), and glutathione peroxidase (GPX) activities. In contrast, non-enzymatic ROS scavenging includes cellular redox buffers, ascorbate and glutathione (GSH), tocopherol, flavonoids, and carotenoids [5]. The potential antioxidant activity of food and medicinal plants is associated with phenolic contents, type, and structure [6]. Recently, extensive progress has been made in studying the

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antioxidant activity during the storage of pickles using tea leaves [1], garlic [6], bitter melon [7], and mountain herbs [8]; however, to the best of our knowledge, antioxidant activity during storage of Dolsan leaf mustard has not been researched. Hence, this study aims to examine the total phenolic and flavonoid contents and antioxidant activities of Dolsan leaf mustard pickles (DLMP) during storage.

2. MATERIALS AND METHODS

2.1. Materials

Dolsan leaf mustard was produced at Yeosu-si, Dolsan-eup, Korea and harvested in October 2015. Dolsan leaf mustard was blanched at 80°C for 10 s. DLMP were prepared using Dolsan leaf mustard (65%), soy (10%), apple vinegar (7%), water (12 %), garlic (1%) and condiment sauce (5%), and stored at 4°C for 28 days after preparation. DLMP was used for the experiment after a 7 day interval.

2.2. Reagents

Folin-Ciocalteu's phenol reagent, 2,20-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, quercetin, BHA (2 [3]-t-Butyl-4-hydroxyanisole), ascorbic acid, ferrous and ferric chloride were purchased from Sigma Chemical Co. (St, Louis, MO, USA). All other reagents were of the highest grade commercially available.

2.3. Preparation of extracts from DLMP

DLMP were ground to a particle size of 5-10 mm using a high speed mixer (Blender, Hanil, HNF-340, Seoul, Korea). DLMP samples (10 g) were placed in a Soxhlet extractor with 200 mL 50% (v/v) acetonitrile in a round flask extracted at 93-94°C and 80% (v/v) methanol, 80% (v/v) ethanol in a round flask extracted at 60°C for 24 h. After cooling at room temperature (RT) of 25°C, the extracts were filtered with Whatman No. 2. The filtrate was evaporated by an evaporator (EYELA, Tokyo, Japan) at 60°C, the extracts were transferred to a freeze-drying tube and lyophilized. The dried extracts were weighed and stored at 0°C prior to analysis. The absorbance of all extracts (300 μL) was analyzed in a 96-well plate (SPL Lifescience Co., Pocheon, Korea) using a Microplate Reader (UVM340, Biochrom, Cambridge, UK).

2.4. Determination of total polyphenol contents

Total phenolic contents were determined using a Folin-Ciocalteau assay [9]. The DLMP extracts were dissolved in methanol (1 mg/mL) and aliquot (100 μL) mixed with 20 mL of 2% aqueous sodium carbonate solution. After 3 min, 100 μL of 50% (v/v) Folin-Ciocalteau reagent was added to the mixture. After 30 min of standing, the extract was measured at 750 nm. Gallic acid was used as a standard to construct the calibration curve and the total polyphenol contents of DLMP extracts were expressed in milligram gallic acid equivalents (GAE) per gram of extract (dry weight).

2.5. Determination of total flavonoid contents

Total flavonoid contents were determined using the aluminum chloride colorimetric method [10]. DLMP extracts were dissolved in methanol (1 mg/mL) and solution (0.5 mL) and were mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. The mixture was then incubate at 25°C for 30 min. After 30 min of standing, the extract was measured at 750 nm. Quercetin as a standard was used to construct the calibration curve and the total flavonoid contents were expressed in milligram quercetin equivalents (QE) per gram of extract (dry weight).

2.6. DPPH radical scavenging activity

The electron donation ability of the obtained methanol extracts was measured by bleaching the purple-colored solution of DPPH radicals based on the method of Blois [11]. Briefly, the DLMP extracts were dissolved in methanol (1 mg/mL) and were added to a 1 mL DPPH radical solution in methanol (the final concentration of DPPH was 0.2 mM). The solution was incubated for 30 min in the dark at RT, and the mixture absorbance was measured using at 517 nm. The control absorbance was measured 0.02% BHA (v/v). The DPPH radical scavenging activity was calculated according to the following equation:

$$\text{DPPH scavenging activity (\%)} = (1 - \text{sample absorbance}/\text{control absorbance}) \times 100$$

2.7. ABTS radical scavenging activity

The total antioxidant activities of the DLMP extracts dissolved in methanol (1 mg/mL) were measured by the $ABTS^{\cdot+}$ radical cation decolorization assay, which involves the preformed $ABTS^{\cdot+}$ radical cation [12,13]. ABTS was dissolved in water to a 7 mM concentration, and the ABTS radical action ($ABTS^{\cdot+}$) was produced by mixing the ABTS stock solution with 2.45 mM potassium persulfate ($K_2S_2O_8$), and allowing the mixture to stand in the dark at RT. for 14 h before use. Oxidation of ABTS commenced immediately, but absorbance was not maximal and stable in this form for more than 2 days of storage in the dark at R.T. After the mixture was kept in the dark at R.T for 14 h, to allow the completion of radical generation, it was diluted with ethanol to an absorbance of 0.70±0.05 at 734 nm. To deter-

mine the scavenging activity, 0.9 mL ABTS reagent was mixed with 0.1 mL extract and the absorbance was measured at 734 nm after 6 min of reaction at R.T, using ethanol as a control [14].The control absorbance was measured at 0.02% ascorbic acid (v/v). The ABTS radical scavenging activity was calculated according to the following equation:

ABTS scavenging activity (%) = (1 − sample absorbance/ control absorbance) × 100

2.8. Ferric reducing antioxidant power (FRAP) assay

To obtain the antioxidant capacity, the FRAP method was conducted, following the previously described [15]. For the assay, a 3 mL FRAP reagent aliquot, a mixture of 0.3 M acetate buffer, 10 mM TPTZ in 40 mM HCl, and 20 mM ferric chloride [10:1:1 (v/v/v)], were combined with 1 mL DLMP extracts dissolved in methanol (1 mg/mL). The antioxidant capacity values were expressed as mM $FeSO_4$ equivalents per gram extract (mM $FeSO_4$ eq.) per gram of extract (dry weight).

2.9. Statistical analysis

All tests and analyses were repeated at least three times. The results are expressed as mean±standard deviation (SD). A one way analysis of variance (ANOVA) and Duncan's test were used for multiple comparisons using SPSS version 21.0 (SPSS Institute, Chicago, IL, USA). The values were considered to differ significantly if the P value was less than 0.05.

3. RESULTS AND DISCUSSION

3.1. Total polyphenol and total flavonoid contents

The total polyphenol contents of DLMP extracts are shown in Table 1. Total polyphenol contents were significantly different (p<0.05) among the different DLMP extracts, ranged 7.96 to 35.56 GAE mg/g. DLMP Methanol extracts had the highest total polyphenol content of 35.56±0.01 GAE mg/g at day 14 and DLMP ethanol extracts had the lowest total polyphenol content of 7.96±0.01 GAE mg/g at day 0. The results were consistent with those from a previous report [16], which saw that total polyphenol content of Dolsan leaf mustard was 0.42 mg/g and DLMP extracts had the higher polyphenol contents than Dolsan leaf mustard. Total flavonoid contents of DLMP extracts are shown in Table 2. Total flavonoid contents were significantly different (p<0.05) among the different DLMP extracts, ranged from 1.96 to 4.54 QE mg/g. DLMP methanol extracts had the highest total flavonoid content of 4.54±0.03 QE mg/g at day 14 and DLMP ethanol extracts had the lowest total flavonoid content of 1.96±0.01 QE mg/g at day 0. Solvent extraction is a useful technique for the isolation of plant antioxidant compounds. However, resulting antioxidant activities and extract yields of plant materials depend on the extraction solvent, due to different chemical characteristics and polarities that may or may not be soluble in a particular solvent. Polar solvents, such as aqueous mixtures containing ethanol, methanol, acetone, and ethyl acetate, have been used frequently to extract phenolics from a plant matrixes [5]. In the present study, methanol extract was the most suitable solvent for extracting phenolic compounds from DLMP. Analysis of the physiological activities of *Yangha* [17] showed the highest of phenolic compounds at day 15 of storage; these results showed a similar trend.

3.2. Antioxidant activities

The DPPH radical scavenging activity was measured to determine antioxidant activities of DLMP extracts (Fig. 1), which were 34.15-79.4% (p<0.05). The antioxidant activities measured

Table 1. Total polyphenol contents of Dolsan leaf mustard pickle (DLMP) extracts

Extract	Total polyphenol contents (GAE mg/g)				
	0[1)]	7	14	21	28
MeOH	15.06±0.03^{Ba}[2)]	25.66±0.02^{Ca}	35.56±0.01^{Ca}	27.46±0.04^{Aa}	16.56±0.02^{Da}
EtOH	7.96±0.01^{Ab}	9.56±0.01^{Cb}	12.76±0.02^{Bb}	12.66±0.01^{Bb}	8.86±0.01^{Db}
ACN	10.86±0.02^{Bc}	13.06±0.01^{Bc}	14.96±0.03^{Dc}	13.86±0.01^{Cc}	12.36±0.03^{Ac}

[1)]Storage. [2)]All values are mean±SD of the triplicate determination. The mean in row (a-c) and a column (A-E) followed by different superscripts are significantly different at p<0.05 by Duncan's range test.

Table 2. Total flavonoid contents of Dolsan leaf mustard pickle (DLMP) extracts

Extract	Total flavonoid contents (QE mg/g)				
	0[1)]	7	14	21	28
MeOH	2.60±0.01^{Ca}[2)]	3.25±0.02^{Aa}	4.54±0.03^{Ea}	4.09±0.01^{Ca}	3.68±0.01^{Da}
EtOH	1.96±0.01^{Db}	2.50±0.03^{Db}	2.69±0.01^{Ab}	1.81±0.01^{Cb}	1.56±0.02^{Db}
ACN	2.23±0.02^{Ec}	2.72±0.01^{Bc}	3.22±0.02^{Dc}	2.83±0.02^{Cc}	2.56±0.02^{Dc}

[1)]Storage. [2)]All values are mean±SD of the triplicate determination. The mean in row (a-c) and a column (A-E) followed by different superscripts are significantly different at p<0.05 by Duncan's range test.

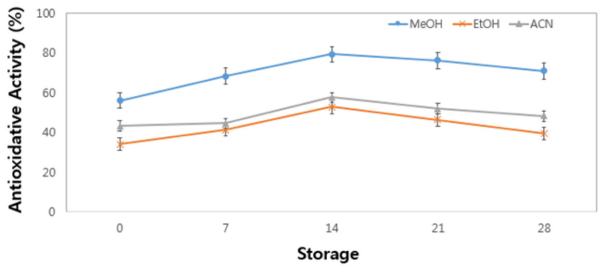

Fig. 1. DPPH radical antioxidant activities of DLMP extracts.

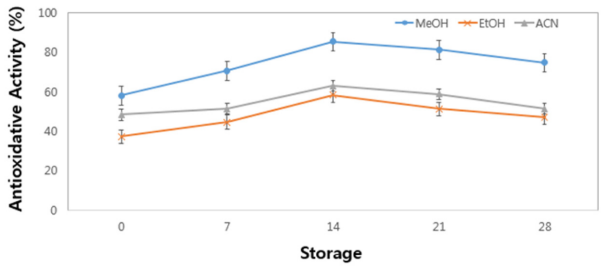

Fig. 2. ABTS radical antioxidant activities of DLMP extracts.

84.5% control in 0.02% BHA (v/v) (data not shown). The antioxidant activities from previous studies measured 11.0% in roots, 11.6% in stems and 80.4% in leaves of Dolsan leaf mustard, respectively [3]. When compared to this result, the antioxidant activity in Dolsan leaf mustard in the present study was higher than it was in the roots and stem, but lower than that observed in the leaves. DLMP methanol extracts at day 14 showed the highest antioxidant activity of 79.4%, whereas the DLMP ethanol extract at day 0 showed the lowest antioxidant activity of 34.15%. The ABTS radical scavenging activity was measured to determine antioxidant activities of DLMP extracts (Fig. 2). The range of antioxidant activities 37.25-85.36%. Moreover, DLMP methanol extracts at day 14 showed the highest antioxidant activity of 85.36%, and the DLMP ethanol extracts at day 0 showed the lowest antioxidant activity of 37.25%. In a previous study [1], tea leaf pickles (10% vinegar content) decreased at day 5 of storage. However, the same vinegar contents used for DLMP increased the antioxidant activity from day 0 to 15. ABTS radical scavenging activity was DPPH radical scavenging activity probably because the ABTS method could measure the ability of hydrogen-donating and chain-breaking antioxidants. Moreover, the ABTS method is advantageous as it can be applied to both water-soluble substances and fat-soluble substances [17]. Therefore, ABTS radical scavenging activity was more highly represented. Boo et al. [18] determined the total polyphenol and flavonoid contents was high based on the ABTS radical scavenging activity. These results suggest that higher polyphenol and flavonoid contents showed higher ABTS radical scavenging activity. The FRAP assay measured

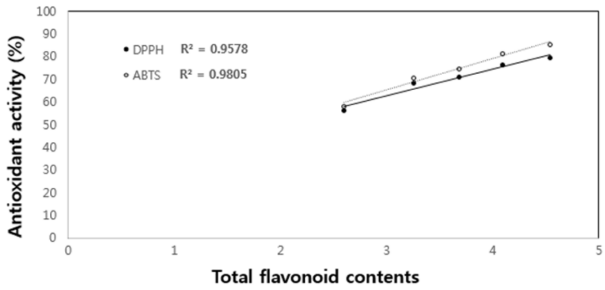

Fig. 3. Relationship between total flavonoid contents and antioxidant activities base on DPPH and ABTS radicals analysis of DLMP extracts.

the antioxidant effect of any substance in the reaction medium as a reducing ability. The antioxidant capacities of the DLMP extract are shown in Table 3, from 2.24-3.82 mM $FeSO_4$ eq. (p<0.05). The antioxidant activity of bitter melon pickle increased only slightly during storage [7]. Similarly, there was no significant difference in the antioxidant activity during storage in the present study. The FRAP measured 1.24-2.65 mM $FeSO_4$ eq. at day 0 and 1.97-2.60 at day 28. The ethanol extract showed lower antioxidant activity then the methanol extract; and overall, storage at day 14 showed the highest antioxidant activity. The could be explained by the antioxidants found in fresh leasves of the Brassicaceae family, including p-hydroxybenzoic, vanillic, protocatechuic, hydroxybenzoic, hydroxycinnamic, p-coumaric, ferulic, caffeic and sinapic acids [19,20]. Phenolic acids such as p-coumaric and ferulic acid have vinyl derivatives through the reaction of microorganisms, increasing the

Table 3. FRAP(ferric reducing antioxidant power) assay of Dolsan leaf mustard pickle (DLMP) extracts

Extract	Total flavonoid contents (QE mg/g)				
	0[1)]	7	14	21	28
MeOH	2.65±0.01^{Da}[2)]	2.54±0.02^{Ea}	3.82±0.01^{Aa}	3.56±0.01^{Aa}	2.59±0.03^{Ba}
EtOH	1.24±0.01^{Bb}	2.05±0.03^{Db}	2.80±0.01^{Cb}	2.59±0.01^{Eb}	1.97±0.01^{Eb}
ACN	2.61±0.02^{Ac}	3.22±0.01^{Bc}	3.46±0.02^{Cc}	3.23±0.02^{Cc}	2.60±0.01^{Ac}

[1)]Storage.

[2)]All values are mean±SD of the triplicate determination. The mean in row (a-c) and a column (A-E) followed by different superscripts are significantly different at p<0.05 by Duncan's range test.

total phenolic content of leafy vegetables during fermentation is increased. Generally, there is a correlation between phenolic content and antioxidant activities. Similar results were found were found in the present study; there was a high correlation between flavonoid contents and DPPH, and ABTS antioxidant activity in the DLMP methanol extracts (Fig. 3).

4. CONCLUSION

It is concluded that various extracts from DLMP, exhibited a wide range of antioxidant capacities, thus making them valuable sources of natural antioxidant, both for preparation of crude extracts and further isolation and purification of antioxidant components. Significant correlations were found between the antioxidant capacities and total phenolic contents indicating that phenolic compounds are the major contributor of antioxidant capacities of these plant samples [21]. Therefore, the results could provide the basis for improving the availability and quality of Dolsan leaf mustard.

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