Korean Society for Biotechnology and Bioengineering Journal 31(1): 20-26 (2016)
http://dx.doi.org/10.7841/ksbbj.2016.31.1.20 ISSN 1225-7117 / eISSN 2288-8268

총설

다양한 데침조건에서 돌산갓의 이화학적 특성 및 LC-PDA/MS/MS 분석

손혜련[1], 오선경[1], Chigen Tsukamoto[2], 최명락[1]*

Quality Characteristics of Dolsan Leaf Mustard according to Various Blanching Conditions and Liquid Chromatography with Photodiode Array and Tandem Mass Spectrometry Analysis

Hae-Reon Son[1], Sun-Kyung Oh[1], Chigen Tsukamoto[2], and Myeong-Rak Choi[1]*

Received: 13 October 2015 / Revised: 7 January 2016 / Accepted: 14 January 2016
© 2016 The Korean Society for Biotechnology and Bioengineering

Abstract: This study aims to examine the optimum blanching conditions as a pretreatment condition to improve the storage stability of Dolsan leaf mustard pickle. The effects of the blan- ching temperature and time were investigated at a temperature range of 80-100°C. Sampling was done for 1 month after a 5 days interval. The L value of the Dolsan leaf mustard was found to be the highest at 80°C. The cutting force increased as the blanching temperature increased. The tensile strength decreased at 95°C and 100°C. In addition, the sensory evaluation scores were the best at 80°C. The storage stability was assessed at various blanching temperatures to increase the sinigrin content during storage. Liquid chromatography with photodiode array and tandem mass spectrometry (LC-PDA/MS/MS) analysis was conducted to identify and quantify the sinigrin content in the Dolsan leaf mustard. Sinigrin as an internal standard was co-injected into each sample solution. The sample was monitored by recording the ultraviolet absorbance at 228 nm and by electrospray ionization (ESI) positive ion mode in the m/z 50-1,500 range. Blanching the sample at 80°C showed the highest sinigrin concentration during storage among various temperatures and the maximum concentration was 350 ppm at 15 days storage. Study on utilization of vegetable from food processing of leaf mustard and preservation conservation results suggest that blanching at 80°C is expected to improve the palatability of the pickle.

Keywords: Leaf mustard, LC-PDA/MS/MS, Sinigrin, Glucosinolates, Blanching

1. INTRODUCTION

Leaf mustard (*Brassica juncea*) is a member of the Brassicaceae family, and mustard seeds are used as a spice. The Dolsan leaf mustard is larger in size than traditional leaf mustard, and is abundant in glucosinolate, polyphenols, and sulfur compounds [1]. Which play critical roles in the pharmacological actions of the body as physiologically active substance. Moreover, Dolsan leaf mustard strengthens the immune system and helps in preventing cancer [2]. Dolsan leaf mustard kimchi is abundant in vitamins, minerals, and sinigrin, a glucosinolate produced during fermentation [3]. Glucosinolates are found in *Brassica*

[1]전남대학교 바이오전자메디컬협동과정
[1]Department of Biomedical and Electronic Engineering, Chonnam National University, Yeosu 550-749, Korea
Tel: +82-61-659-7303, Fax: +82-61-659-7309
e-mail: mrchoe@chonnam.ac.kr

[2]일본 이와테대학교 농학부 응용생물화학과정
[2]Department of Applied Biological Chemistry, Iwate University, Morioka 020-8550, Japan

crops and are known to decrease cancer risk. Glucosinolates are characterized by a core sulfated isothiocyanate group, which is conjugated to thioglucose and a further R-group. R-group are sulfur containing side chains, aliphatic-straight chains, aliphatic-branched chain, olefins, aromatics, benzoates, indoles, glycosylated, benzyl glycosides, cinnamic glycosides and seleno. Both the glucose and central carbon of the isothiocyanate group are often further modified [4]. Dolsan leaf mustard is known to contain higher sinigrin content among other glucosinolate components [5]. Sinigrin, one of the glucosinolates, is a bioactive compound that has an olefinic glucosinolate structure [6]. Till date, the blanching condition of onion, *Aster scaber*, and dandelion has progressed extensively, but blanching condition of the leaf mustard is yet to be explored. Hence, this study aims to examine the optimum blanching condition as a pretreatment to improve storage stability of Dolsan leaf mustard using Dolsan leaf mustard pickle. This process can also be used as a supplementary data for the production of Dolsan leaf mustard pickles.

2. MATERIALS AND METHODS

2.1. Materials

Dolsan leaf mustard (DLM) was produced at Yeosu-si Dolsan-eup, Korea and harvested in October 2014. Sinigrin standard was purchased from Sigma chemical Co. (St. Louis, MO, USA) for analysis.

2.2. DLM blanching sample preparation

DLM was blanched at different temperatures 80, 90, 95, and 100°C for 10 s and were washed three times with cold water. Excess water was removed during 3 min, and 350 g sample was packed in food grade polyethylene bags. They were stored at 0°C for 30 days after preparation (30 DAP) were and used for the experiment after a 5 day interval.

2.3. Color measurement

Color measurement was performed using a Color Reader (JC 801S, Color Techno System Co., Japan) with an 8 mm diameter measuring area. The instrument was calibrated with a standard white plate. Measured L, a, and b values were used as indicators of lightness, greenness, and yellowness, respectively. All samples were measured three times.

2.4. Hardness analysis

DLM samples size of 4×1 cm² were cut from the central part of the DLM at each stage of storage. The hardness of the DLM were measured using a rheometer (CR-500DX, Osaka, Japan). A 10.00 kg load cell was installed at the rheometer cross-head and chart speeds were 5 and 1.0 mm/s, respectively. Hardness analysis is a type of compression test that was used to determine the hardness of the sample. Moreover, compression elasticity test jig and cutting force test jig were used to determine the tensile strength and cutting force of the materials, respectively. Samples were stored at room temperature for 30 min before analysis. All samples were measured in three times.

2.5. Sensory evaluation

For the sensory evaluation, stored DLM were periodically taken out (0, 5, 10, 15, 20, 25, and 30 days). The sensory evaluation was performed by 10-trained panelists, who were graduate students at the Chonnam National University, and were familiar with DLM consumption. The panelists evaluated the DLM randomly. The appearance, color, flavor, texture and overall acceptability were evaluated using a 5-point scale (1 = very weak, 3 = moderate, 5 = very strong).

2.6. Sinigrin extraction

DLM sample (1 g) was subjected to a soxhlet extractor with 200 mL of 50% (v/v) acetonitrile in a round flask and extracted at 93-94°C for 24 h. After cooling at room temperature, the extract was analyzed.

2.7. Quantification of the sinigrin concentration using HPLC analysis

Sinigrin was extracted from DLM and was analyzed by HPLC

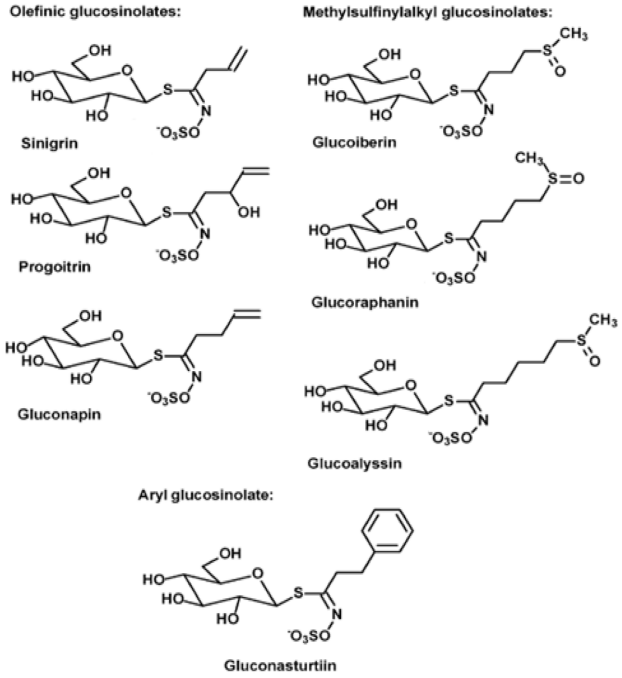

Fig. 1. Molecular structures of glucosinolates.

using an Inertsil Ph-3 column (250 mm × 4.6 i.d.; GL Science, Tokyo, Japan). The mobile phase was consisted of water (A) and acetonitrile (B) using gradient elution according to the following program; 0-7 min 10% B; 7-16 min 35% B; 16-18 min 100% B; 18-35 min 0% B. The total flow rate was 0.94 mL/min and was monitored at an ultraviolet (UV) wavelength was of 228 nm. The oven temperature was set at 40°C.

2.8. Analysis of sinigrin using LC-PDA/MS/MS

Liquid chromatography with photodiode array and tandem mass spectrometry (LC-PDA/MS/MS) analysis was performed by Shimadzu Prominence UPLC with a Thermo Orbitrap XL system (Thermo Fisher Scientific, Bremen, Germany). The LC column used was Inertsil 03 Ph-3 (250×4.6mm i.d.)(GL Science, Tokyo, Japan), and the mobile phase used was solvent A (water) and solvent B (acetonitrile) with a gradient of 0 -35% for 35 min, and the data acquisition time was 18 min. Total flow rate was 0.94 mL/min and oven temperature was 40°C. Sinigrin concentration (ppm) was quantified using a photodiode array (PDA) detector by monitoring the UV absorbance at 228 nm. Sinigrin (5% solution) as an internal standard was co-injected into each sample solution. MS and MS/MS data were obtained by electrospray ionization (ESI) positive ion mode using the following parameters: m/z range 50-1,500, source voltage 5.40 kV, capillary voltage 48.00 V, capillary temperature 275°C, sheath gas flow 50 L/min, aux gas flow 10 L/min, source current 1,000 mA, and tube lens 100 V.

2.9. Statistical analysis

All tests and analyses were repeated at least three times. The results are expressed as mean± standard deviation (SD). One way analysis of variance (ANOVA) and Duncan's test were used for multiple comparisons using the 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 DISSCUSSION

3.1 Color measurement

Change in color of the DLM samples caused by different blanching treatments at 10 s and of DLM samples stored at 0°C for 30 days are presented in Table 1. The L value, which indicates the lightness, was highest at 80°C in 17.69±0.51 and was the lowest at 100°C in 13.48±0.38 at 30 days storage (p<0.05). Hence, the L value result show that a tendency significantly decreased at high temperature and with longer storage period. The a value, which indicates the greenness , was the highest at 90°C in -4.50±0.37, and was the lowest at 80°C in 6.52±0.35 at 30 days storage (p<0.05). This result is similar to the previous reports of Choi et al. [7], Lee at al. [8]. The b value indicates yellowness was the highest at 100°C in 26.52±0.33, and was the lowest at 90°C in 18.85±0.38 at 30 days storage (p<0.05). Previous studies have suggested that [8,9], this phenomenon involved removal of air around the fine hairs on the surface of plant tissues as well as between the cells, which are responsible for the alteration of the reflecting properties of the surfaces. The results show the effect of blanching temperature on the L, a and b values.

3.2. Hardness analysis

The hardness of the DLM samples of different blanching treatments at 10 and stored at 0°C for 30 days are presented in Table 2. The tensile strength ranged from 10.81-10.73 kgf/cm^2

Table 1. Changes in the color of blanched Dolsan leaf mustard in various water temperatures

C[1)]	T (°C)[2)]	Storage days						
		0	5	10	15	20	25	30
L	80	18.68±0.25^{Ca3}	19.31±0.26^{Ca}	19.25±0.25^{Ca}	19.18±0.26^{Ba}	20.50±0.36^{Ca}	19.69±0.51^{Ba}	17.69±0.51^{Aa}
	90	18.47±0.47^{Cb}	19.14±0.88^{Cb}	18.73±0.26^{Cb}	18.35±0.29^{Bb}	19.87±0.28^{Cb}	17.72±0.15^{Bb}	16.29±0.14^{Ab}
	95	18.37±0.25^{Cb}	17.73±0.09^{Cb}	19.47±0.02^{Cb}	18.25±0.24^{Bb}	18.82±0.26^{Cb}	18.40±0.20^{Bb}	15.04±0.23^{Ab}
	100	19.54±0.10^{Cc}	18.30±0.20^{Cc}	18.05±0.20^{Cc}	16.89±0.38^{Bc}	19.39±0.31^{Cc}	15.71±0.18^{Bc}	13.48±0.38^{Ac}
a	80	-12.66±0.44^{Ab}	-8.01±0.38^{Bb}	-10.10±0.48^{Cb}	-9.96±0.37^{Cb}	-9.91±0.39^{Db}	-8.49±0.24^{Eb}	-6.52±0.35^{Fb}
	90	-12.450.36^{Ab}	-11.92±0.44^{Bb}	-10.21±0.20^{Cb}	-10.51±0.34^{Cb}	-8.36±0.18^{Db}	-7.42±0.39^{Eb}	-4.50±0.37^{Fb}
	95	-14.34±0.22^{Aa}	-12.62±0.11^{Ba}	-10.87±0.27^{Ca}	-9.93±0.44^{Ca}	-9.82±0.36^{Da}	-7.86±0.15^{Ea}	-4.92±0.57^{Fa}
	100	-13.02±0.86^{Ac}	-11.23±1.98^{Bc}	-10.03±0.78^{Cc}	-9.99±0.48^{Cc}	-9.05±0.92^{Dc}	-7.16±1.46^{Ec}	-5.38±0.87^{Fc}
b	80	16.89±0.36^{Ds}	11.17±0.32^{Cs}	16.75±0.26^{Aa}	15.84±0.10^{Aa}	14.30±0.05^{Ba}	16.41±0.37^{Ea}	18.96±0.16^{Fa}
	90	15.73±0.25^{Db}	17.59±0.17^{Cb}	15.33±0.11^{Ab}	17.18±0.27^{Ab}	15.47±0.43^{Bb}	17.58±0.31^{Eb}	18.85±0.38^{Fb}
	95	20.28±0.22^{Dc}	17.52±0.23^{Cc}	15.67±0.09^{Ac}	12.61±0.22^{Ac}	13.63±0.23^{Bc}	19.54±0.09^{Ec}	23.12±0.33^{Fc}
	100	16.86±0.36^{Dd}	17.30±0.23^{Cd}	12.69±0.21^{Ad}	14.48±0.44^{Ad}	18.54±0.38^{Bd}	22.59±.012^{Ed}	26.52±0.33^{Fd}

[1)]Color value. [2)]Blanching temperature. [3)]All values are mean±SD of the triplicate determination. The mean in row (a-d) and a column (A-G) followed by different superscripts are significantly different at p<0.05 by Duncan's range test.

Table 2. Changes in the tensile strength at hardness of blanched Dolsan leaf mustard in various water temperatures

H[1)]	T (°C)[2)]	Storage days						
		0	5	10	15	20	25	30
TS[4)]	80	10.81±0.01^{Ed3}	10.81±0.01^{Ed}	10.80±0.01^{Dd}	10.75±0.05^{Cd}	10.82±0.07^{Bd}	10.70±0.20^{Ad}	10.41±0.04^{Cd}
	90	10.82±0.04^{Ec}	10.73±0.13^{Ec}	10.81±0.01^{Dc}	10.90±0.01^{Cc}	10.79±0.01^{Bc}	10.08±0.04^{Ac}	10.06±0.01^{Cc}
	95	10.83±0.04^{Eb}	10.81±0.06^{Eb}	10.78±0.03^{Db}	10.36±0.05^{Cb}	10.26±0.05^{Bb}	10.06±0.10^{Ab}	9.59±0.09^{Cb}
	100	10.73±0.04^{Ea}	10.84±0.04^{Ea}	10.54±0.04^{Dz}	10.22±0.08^{Ca}	9.49±0.07^{Ba}	9.63±0.03^{Aa}	9.45±0.07^{Ca}
CF[5)]	80	974.9±2.8^{Aa}	1008.4±7.4^{Bs}	1163.1±8.5^{Ca}	1415.0±3.0^{Da}	1858.6±14.1^{Ea}	1252.6±10.9^{Ea}	1489.6±5.2^{Fa}
	90	951.5±15.7^{Ab}	1153.0±8.5^{Bb}	1223.0±3.8^{Cb}	1125.4±10.3^{Db}	1790.2±8.9^{Eb}	1964.3±16.6^{Eb}	1837.7±22.3^{Fb}
	95	947.8±2.4^{Ac}	1244.66±7.5^{Bc}	1333.7±2.4^{Cc}	1518.6±14.5^{Dc}	1784.6±8.6^{Ec}	1624.0±21.5^{Ec}	2421.6±9.8^{Fc}
	100	926.8±5.3^{Ad}	1347.6±3.0^{Bd}	2642.3±9.5^{Cd}	2793.0±8.1^{Dd}	3499.6±16.6^{Ed}	3342.6±16.6^{Ed}	3586.3±47.1^{Fd}

[1)]Hardness. [2)]Blanching temperature. [3)]All values are mean±SD of the triplicate determination. The mean in row (a-d) and a column (A-G) followed by different superscripts are significantly different at $p<0.05$ by Duncan's range test. [4)]Tensile strength(kgf/cm^2) in hardness. [5)]Cutting force(gf/cm^2) in hardness.

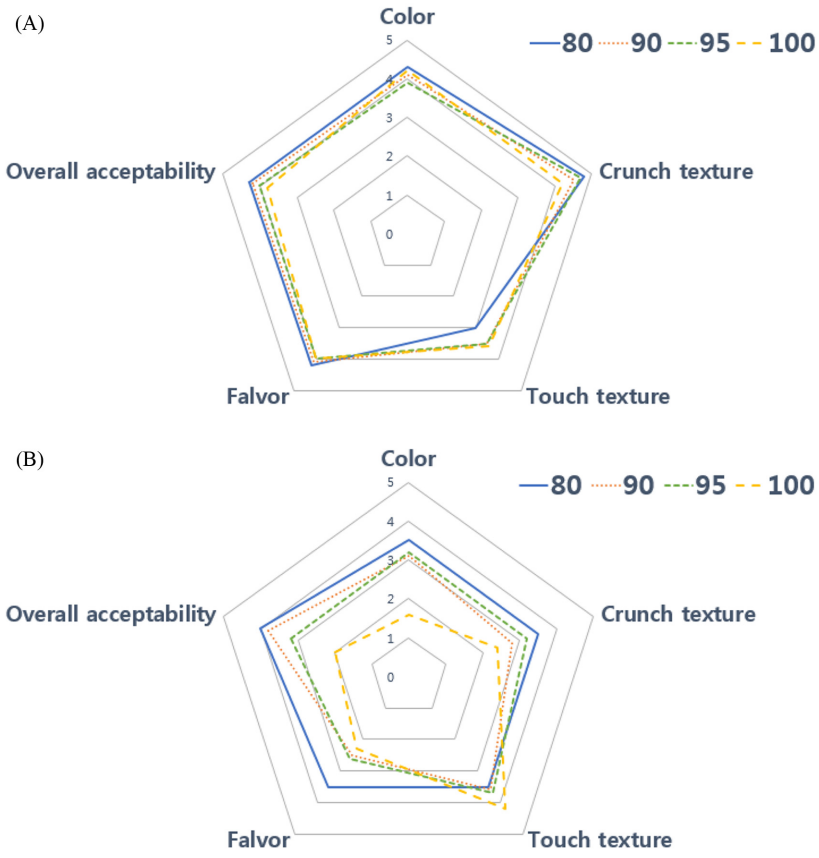

Fig. 2. (A) Sensory characteristics of blanched Dolsan leaf mustard at 0 day, (B) Sensory characteristics of blanched Dolsan leaf mustard at 30 days.

at 0 days storage and then the duration of the storage was significantly decreased. Least hardness was recorded at 100°C at 30 days storage in 9.45±0.07 kgf/cm^2. Lee at al. [8] reported that decrease the hardness of onion at a higher blanching treatment. Cutting force ranged from 974.9~926.8 gf/cm^2 at 0 days storage, and then the storage period was significantly increased. Out of that, 100°C at 30 days storage increased in 3,586.3±47.1 gf/cm^2 ($p<0.05$). Thus, in the blanching process, the volume of plant tissues by heat treatment, the density and the change in weight as well as changes in the cell structure, are due to a difference in texture [11].

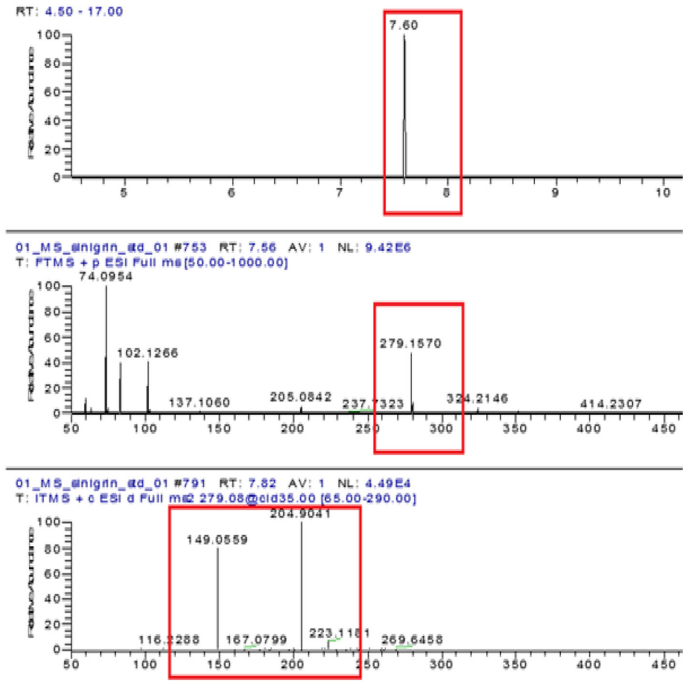

Fig. 3. MS and MS/MS analysis of standard sinigrin. Selected ion chromatography screened by m/z 279.2 (upper) and MS spectrum (middle) of 7.6 min component, and MS/MS of the m/z 279.2 component (bottom).

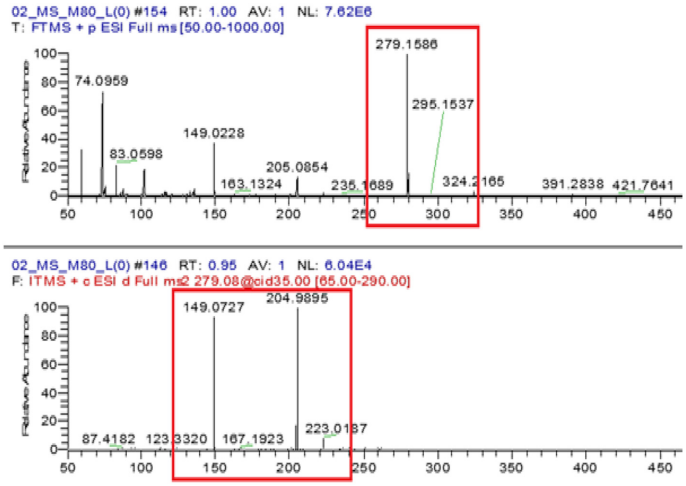

Fig. 4. MS and MS/MS analysis of the sample extract of blanching at 80°C. MS spectrum (upper) of 7.6 min component and MS/MS of the m/z 279.2 component (bottom). These fragment patterns completely corresponded to those of standard sinigrin (desulfo-sinigrin).

3.3. Sensory evaluation

The sensory evaluation of DLM samples using different blanching treatment at 10 s stored at 0°C in 0 days and 30 days are presented in Fig 2A and 2B. All values were significantly increased or decreased (data not shown) with longer storage period. The color ranged from of 4.3 to 3.9 at 0 days storage, and 80°C at 30 days storage increased to 3.5±0.12 (p<0.05). The crunch texture significantly decreased with time, and 30 days storage was the lowest at 2.4±0.31 at 100°C (p<0.05). The touch texture significantly increased with time and was the highest at 30 days to 4.2±0.41 at 100°C and the lowest to 3.5±0.01 at 100 °C (p<0.05). The flavor ranged from 4-4.2 at 0 days storage; 80 °C at 30 days storage significantly to decreased to 2.3±0.07. The overall acceptability was highest at 30 days at 4.3±0.17 at 80°C

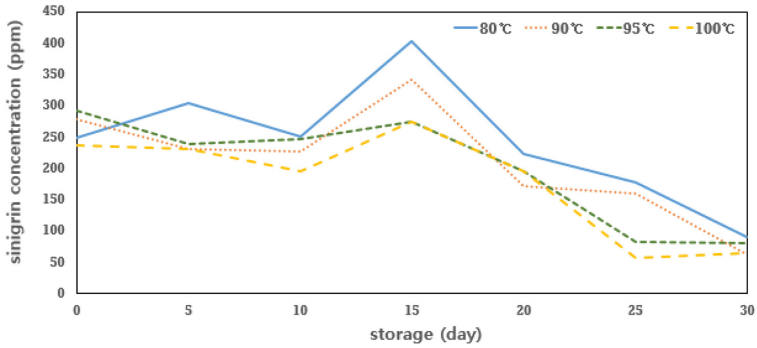

Fig. 5. Effect of blanching temperature on sinigrin concentration of DLM during storage.

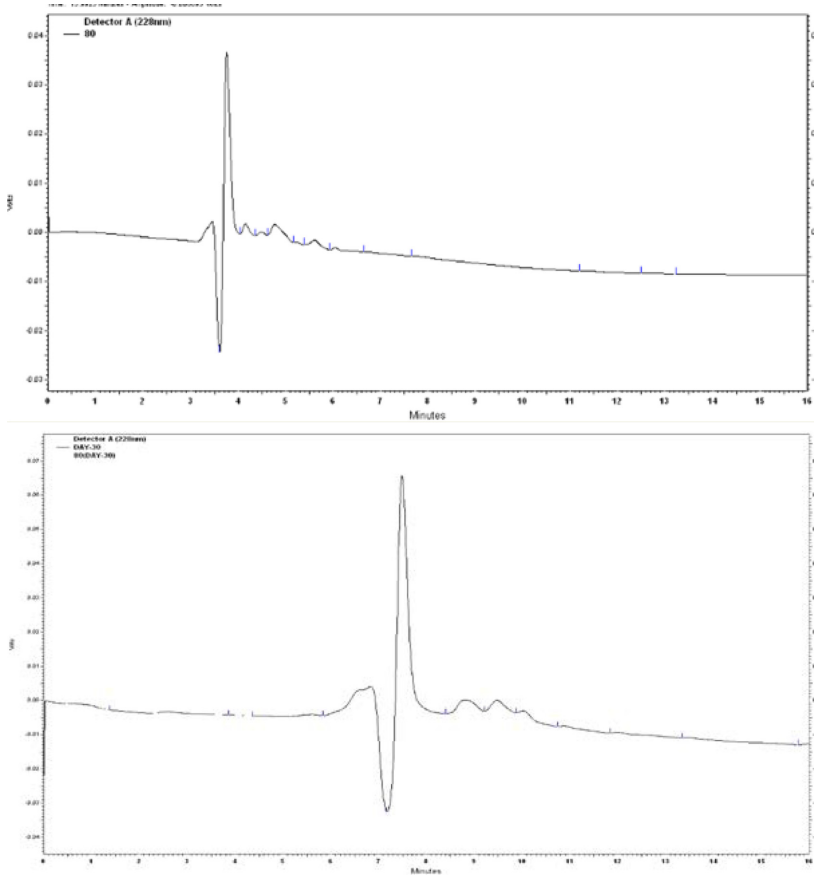

Fig. 6. HPLC chromatograms (UV 228 nm) of 80°C blanching and at 15 day after preparation (upper) and 30 day after preparation (bottom).

and the lowest at 2.0±0.81 at 100°C (p<0.05). These results suggest that the blanching at 80°C is expected to improve the comprehensive palatability of the pickle.

3.4 Analysis of sinigrin by LC-PDA/MS/MS

Sinigrin as a standard material eluted at 7.60 min and MS analysis of the peak component showed that the molecular mass was m/z 279.1570 and that fragment ions gave m/z 204 and 149.06 (Fig. 3). These data show that sinigrin formed desulfoglucosinolates $[M\text{-}SO_3+H]^+$. Sinigrin, which was confirmed by LC-MS/MS analysis (Fig. 4), was detected from blanched DLM. Although blanching temperature affected the sinigrin concent-

ration during storage at 0°C (Fig. 5), sinigrin concentration increased until 15 day after preparation (DAP) and decreased after 15 DAP. Blanching at 80°C showed constantly higher sinigrin concentration than the other temperature in the whole storage period (30 days). It seems that sinigrin produced by enzyme activity remained in DLM for the first 15 days but degraded during storage. Unknown components, whose basic structure was similar to glucosinolates, were detected from 30 DAP samples (Fig. 6). Decreased sinigrin concentration might have increased the other components. Common blanching condition of DLM to prepare pickle is 94°C for 13s. If blanching temperature was lower than 80°C, the physical properties and permeability of ingredients would be drastically changed. Blanching at 80°C must be the lowest to prepare DLM pickle, and thus. blanching at 80°C will be the best condition for sinigrin production.

4. CONCLUSION

The color, hardness and sensory evaluation showed that DLM blanched at 80°C for 10 s is highly preferred ($p<0.05$). Blanching at 80°C showed constantly higher sinigrin concentration than other temperatures during the storage period. These results suggest that blanching at 80°C is expected to improve the palatability of the pickle. We were able to optimize the blanching condition for 10 s of DLM at 80°C.

ACKNOWLEDGEMENTS

This work was supported by the BK21 program.

REFERENCES

1. Cho, Y. S., B. S. Ha, S. K. Park, and S. S. Chun (1993) Contents of carotenoids and chlorophylls in Dolsan leaf mustard (*Brassica juncea*). *Korean J. Diet. Cult.* 8: 153-157.
2. Lee, S. M. and S. H. Rhee (1997) Inhibitory effect of various cruciferous vegetables on the growth of human cancer cells. *Korean J. Life Sci.* 7: 234-240.
3. Diplock, T. A (1991) Antioxidants nutrient and disease prevention. *Am. J. Clin. Nutr.* 53: 189-193.
4. Clarke, D. B (2010) Glucosinolates, structures and analysis in food. *Anal. Methods*. 2: 310-325
5. Choi, M. R., E. J. Yoo, S. H. Song, D. S. Kang, J. C. Park, and H. S. Lim (2001) Comparison of physiological activity in different parts of Dolsan leaf mustard. *Korean J. Soc. Food Nutr.* 30: 721-725.
6. Lijiang, S., J. M. John, P. B. Nigel, and J. T. Paul (2005) Analysis of glucosinolates, isothiocyanates, and amine degradation products in vegetable extracts and blood plasma by LC-MS/MS. *Anal. Biochem.* 347: 234-243.
7. Choi, N. S., S. S. Oh, and J. M. Lee (2001) Change of biologically functional compounds and quality properties of *Aster scaber* (*Chamchwi*) by blanching condition. *Korean J. Food Sci. Technol.* 33: 745-752.
8. Lee, H. O., Y. J. Lee, J. Y. Kim, D. H. Yoon, and B. S. Kim (2011) Quality characteristics of frozen welsh onion (*Allium fistulosum L.*) according to various blanching treatment conditions. *Korean J. Food Sci. Technol.* 43: 426-431.
9. MacKinney, G. and C. A. Weast (1940) Colors changes in green vegetables frozen-pack peas and string beans. *Industri. Engi. Chem*. 32: 392-395.
10. Tijskens, L. M. M., E. P. H. M. Schijvens, and E. S. A. Biekman (2001) Modelling the change in colour of broccoli and green beans during blanching. *Innovative Food Sci. Emerg. Technol.* 2: 303-313.
11. Arpaia, M. L., F. G. Mitchell, A. A. Kader, and G. Mayer (1986) Ethylene and temperature effects of softening and white core inclusion of kiwifruit stored in air or controlled atmospheres. *J. Amer. Soc. Hort. Sci.* 111: 149-153.