JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Kinetic Changes in Glucosinolate-Derived Volatiles by Heat-Treatment and Myrosinase Activity in Nakajimana (*Brassica rapa* L. cv. *nakajimana*)

Mika Kato,[†,‡] Yuriko Imayoshi,[‡] Hisakatsu Iwabuchi,[‡] and Koichiro Shimomura*[,†,§]

[†]Graduate School of Life Sciences, Toyo University, 1-1-1 Izumino, Itakura-machi, Oura-gun, Gunma 374-0193, Japan

[§]Plant Regulation Research Center, Toyo University, 1-1-1 Izumino, Itakura-machi, Oura-gun, Gunma 374-0193, Japan

[‡]San-Ei Gen F.F.I. Inc., 1-1-11 Sanwa-cho, Toyonaka, Osaka 561-8588, Japan

ABSTRACT: Nakajimana (*Brassica rapa* L. cv. *nakajimana*), of the family Brassicaceae, is a traditional vegetable in Japan. Three isothiocyanates and five cyanides in the leaves of nakajimana were identified by gas chromatography (GC) and GC−mass spectrometry (GC-MS), and their kinetic changes using heat-treatment (temperature and time) were investigated. In addition, myrosinase activity of extracts prepared from fresh nakajimana leaf was determined. In crushed heat-treated leaves of nakajimana (70 °C for 30 s), formation of isothiocyanates and myrosinase activity increased, whereas formation of 1-cyano-3,4-epithiobutane and 1-cyano-4,5-epithiopentane decreased. Heat-treatment can significantly alter the content of potentially beneficial compounds in nakajimana, and ingestion of suitable isothiocyanates for human health may be better facilitated by mild boiling.

KEYWORDS: Nakajimana, *Brassica rapa* L. cv. *nakajimana*, isothiocyanate, cyano-epithioalkane, heat-treatment, myrosinase activity

INTRODUCTION

Brassica rapa L. cv. *nakajimana* (nakajimana, as is known in Japan), belonging to Brassicaceae, is a traditional vegetable eaten in Ishikawa prefecture, Japan. Edible parts such as the leaves and flower buds are normally eaten pickled in salt or as boiled greens with soy sauce dressing after blanching for a very short time. Organoleptic feel and taste of nakajimana boiled for more than 1 min are unpalatable. The leaves cooked for a suitable time are characterized by being particularly bitter and slightly spicy in taste. This differentiates them from other *Brassica* vegetables, such as broccoli, cabbage, and radish. There are a few reports on the bioactivity of this edible plant, such as angiotensin I converting enzyme (ACE) inhibition, antioxidant activity, reduction of blood cholesterol levels, and peroxidized lipids.[1]

Brassica plants contain glucosinolates. When the tissue of these plants is damaged, glucosinolates are converted to isothiocyanates, thiocyanates, cyanides, cyano-epithioalkanes, and oxazolidines by myrosinase β-thioglucoside glucohydrolase enzyme (EC 3.2.3.1) and released from the vacuoles of myrosin cells (Figure 1).[2,3] In addition, isothiocyanates have antibacterial and antifungal activities in plants and provide important protection from insect and herbivore attacks.[4,5] Furthermore, isothiocyanates are considered to be very potent inducers of phase II detoxifying enzymes such as glutathione S-transferase, while phase I enzymes such as cytochrome P450 enzymes are reduced.[3] In this way, the initial stage of the carcinogenic sequence associated with DNA damage is blocked and hence these substances are good blocking agents.[3]

It is thought that cooking mostly affects the contents of glucosinolate and isothiocyanate in the *Brassica* vegetables. Glucosinolates and isothiocyanates are primarily lost from *Brassica* vegetables tissue through leaching into the cooking water, but the rate and extent of loss depend on the type of cooking used, such as the cooking time and/or the amount of water.[6,7] These studies have demonstrated that microwaving and boiling are the cooking methods that cause the largest losses of glucosinolates from broccoli and cabbage.[8−11] Song and Thornalley have reported that boiling of *Brassica* vegetables (brussels sprouts, cauliflower, green cabbage) for up to 30 min gave no detectable isothiocyanates.[7]

Cooking may also be an important factor in determining whether isothiocyanate production dominated over nitrile production. When the florets and sprouts of broccoli were crushed raw at room temperature, sulforaphane nitrile (SFN) was the principal breakdown product of glucoraphanin, due to the action of the epithiospecifier protein (ESP).[9,12−14] It has recently been shown that SFN does not possess the same anticarcinogenic properties as sulforaphane (SF).[12,15] SF production increased, however, after broccoli was heat-treated at 60 °C for 5 or 10 min, and the ESP activity was significantly decreased at a heating temperature of 50 °C.[14] Other *Brassica* plants, including crambe seed (*Crambe abyssinica*), garden cress (*Lepidium sativum*), and other members of the *Brassica* species such as rapeseed (*Brassica napus*) and white cabbage (*Brassica oleracea*) have all been shown to form cyanides.[6,16]

Nakajimana is a traditional vegetable, that is eaten after blanching for a very short time in Japan. However, there are no reports providing a mechanistic understanding on the effects of a very short cooking time (Japanese cooking method) on

Received: April 26, 2011
Accepted: September 13, 2011
Revised: September 7, 2011
Published: September 13, 2011

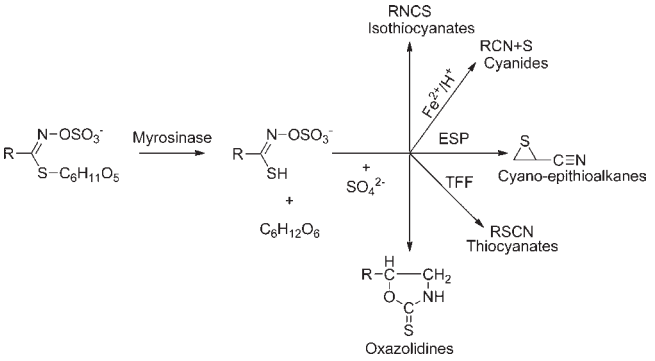

Figure 1. Diagram showing the conversion of glucosinolate. TFF: thiocyanate-forming factor; ESP: epithiospecifier protein. Adapted from ref 6.

concentrations of the isothiocyanate, cyano-epithioalkane, and/or myrosinase activity in *Brassica* vegetables .

This paper describes the identification of isothiocyanates and cyanides in leaves of *Brassica rapa* L. cv. *nakajimana* using gas chromatography (GC) and GC−mass spectrometry (GC-MS). The temperature and duration of heat-treatment was found to affect the formation of the compounds mentioned above. In addition, the kinetic changes of these compounds by heat-treatment and myrosinase activity of nakajimana leaves were investigated.

MATERIALS AND METHODS

Chemicals. Commercially available isothiocyanates and cyanides for identification were purchased from Tokyo Chemical Industry (Tokyo, Japan). 1-Cyano-3,4-epithiobutane and 1-cyano-4,5-epithiopentane were synthesized as reported in the literature for compound identifications.[17] All other reagents and solvents were of analytical grade and were purchased from Wako Chemical Industries (Osaka, Japan). Extraction and dilution solvents for volatile compounds were distilled prior to use.

Plant Materials. The fresh nakajimana used in the present study was purchased from an Agricultural Association in Ishikawa prefecture, Japan.

Heat Treatment of Fresh Leaves. Nakajimana leaves (ca. 30 g, fresh weight) were heat-treated in distilled water for 3 or 30 s at 50, 60, 70, 80, 90, or 100 °C. The samples treated at different time and temperature were immediately cooled in ice−water. Three replicates were performed for the experiments.

Extraction of the Volatile Compounds. The untreated and heat-treated leaves (each ca. 30 g fresh weight) were crushed using a blender for 30 s in 200 mL of 30% calcium chloride solution, containing 5 μg of 3-heptanol as an internal standard (IS), to inhibit enzymatic reactions.[18] The resultant mixture was extracted with 150 mL of dichloromethane by shaking for 30 min at room temperature. The organic layer was separated, dried over anhydrous sodium sulfate, and distilled using a solvent-assisted flavor evaporation (SAFE) apparatus (Kiriyama Glass Co. Ltd., Tokyo, Japan) to carefully isolate a distillate containing volatiles.[19] The SAFE apparatus was thermostatted at 30 °C with a circulating water bath. Distillation was carried out under vacuum (2×10^{-2} Pa), and distillate was trapped by liquid nitrogen. The distillate was concentrated ca. 50-fold in vacuo. The aliquot of the concentrate thus obtained was taken for GC and GC-MS analyses to determine volatile compounds. Extractions of the volatiles were conducted in triplicate.

GC-MS Analyses. Analyses were performed on an Agilent GC-MS system (GC model 6890N with a mass selective detector model 5975 inert) using two columns with different polarities of stationary phases: DB-Wax (60 m × 0.25 mm i.d., film thickness 0.25 μm) and DB-5MS column (30 m × 0.25 mm i.d., film thickness 0.25 μm). GC operating conditions were as follows: DB-Wax: oven temperature programmed, 50 °C (held for 2 min) to 220 C at 3 °C/min, held isothermal (30 min) at 220 °C; DB-5MS: oven temperature programmed, 60 to 246 °C at 3 °C/min, held isothermal (20 min) at 246 °C; carrier gas, helium; constant pressure (for DB-Wax), 25 psi, constant flow (for DB-5MS) 0.9 mL/min; injector temperature, 230 °C; volume injected, 5 μL; split ratio, 1:20. MS conditions were as follows: capillary direct interface temperature, 230 °C; ionization energy, 70 eV; mass range, 30−300 amu; scan rate: 5.1 scans/s.

Nuclear Magnetic Resonance (NMR) Spectra. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a JEOL ECA600 spectrometer at 600 and 125 MHz, respectively, with tetramethylsilane as an internal standard.

Compound Identification. Identifications were based on comparison or linear retention indices (RIs) and mass spectra with those of authentic standard compounds. RIs were calculated using *n*-paraffins C_5−C_{25} as external references.[20]

Internal Calibration. Internal calibration was carried out on an Agilent 6890N GC equipped with a DB-Wax (60 m × 0.25 mm i.d., film thickness 0.25 μm) and a flame ionization detector (FID). Operating conditions were as follows: oven temperature programmed, 50 °C (held for 2 min) to 220 C at 3 °C/min, held isothermal (30 min) at 220 °C; carrier gas, helium; constant pressure, 30 psi; injector temperature, 230 °C; volume injected, 3 μL; split ratio, 1:25; FID temperature, 250 °C; hydrogen flow, 30 mL/min; air flow, 400 mL/min. Internal calibration was carried out on a column with the same stationary phase. Contents of isothiocyanates and cyanides of the plants were calculated by mean values of GC peak area using the internal standard method. The FID response factors for 1-cyano-3,4-epithiobutane and 1-cyano-4,5-epithiopentane relative to 3-heptanol (IS) were taken as one. Actual response factors were measured for commercially available isothiocyanates and cyanides.

Synthesis of 1-Cyano-3,4-epithiobutane and 1-Cyano-4,5-epithiopentane for Compound Identifications. The procedure essentially followed the method reported.[17] 3-Butenyl cyanide (0.50 g) was reacted with *m*-chloroperbenzoic acid (1.28 g) in dry dichloromethane (15 mL) with stirring in an ice bath for 1 h, and the reaction mixture was heated to gentle reflux for 1.5 h and then cooled overnight at room temperature. The reaction mixture was further cooled at 5 °C to precipitate chlorobenzoic acid and then filtered. The filtrate was washed twice with saturated sodium carbonate solution, extracted with distilled diethyl ether, and then dried over anhydrous sodium sulfate. After removal of the solvent in vacuo, the residue was chromatographed on a silica gel and eluted with hexane to afford pure 1-cyano-3,4-epoxybutane (170 mg). Thus-obtained cyanide (160 mg) was reacted with thiourea (125 mg) and benzoic acid (201 mg) in acetone (5 mL) at room temperature with stirring. After the resultant mixture was treated as reported [17] and extracted with distilled diethyl ether, the ether extract was washed twice with saturated sodium carbonate solution and dried over anhydrous sodium sulfate. After removal of the solvent in vacuo, the residue was chromatographed on a silica gel and eluted with hexane:ethyl acetate (4:1, v/v) to afford pure 1-cyano-3,4-epithiobutane (45 mg). 1-Cyano-4,5-epithiopentane was synthesized from 4-pentyl cyanide in the same manner as mentioned above. 1-Cyano-3,4-epithiobutane: ^{1}H NMR δ 1.63−1.70 (m, 1H), 2.27 (dd, J = 1.4, 4.1 Hz), 2.30−2.36 (m, 2H), 2.53−2.57 (m, 2H), 2.59 (dd, J = 1.4, 4.1 Hz), 2.97−3.02 (m, 1H); ^{13}C NMR δ 17.2, 25.6, 32.3, 33.7, 118.9. 1-Cyano-4,5-epithiopentane: ^{1}H NMR δ 1.43−1.49 (m, 1H), 1.85−1.93 (m, 2H), 2.13−2.18 (m, 1H), 2.20 (dd, J = 1.4, 5.5 Hz), 2.39−2.48 (m, 2H), 2.53−2.54 (m, 1H), 2.85−2.89 (m, 1H); ^{13}C NMR δ 16.9, 25.2, 25.6, 34.5, 35.2, 119.3. Mass spectra of these cyanides were in good agreement with those reported data.[21]

Measurement of Myrosinase Activity. The level of myrosinase activity was measured by the method of Hara et al. [22] with a slight

Table 1. Isothiocyanates, Cyanides, and Cyano-epithioalkanes Identified from Nakajinama Leaves (*Brassica rapa* L. cv. *nakajinama*)

		RI[a]		concentration (mean ± SD, ppm)[b]		
no.	compound	DB-Wax	DB-5MS	fresh	heat-treated (100 °C, 3 s)	identification
1	3-butenyl cyanide	1271	758	0.07 ± 0.01	0.13 ± 0.06	MS, RI[c]
2	4-pentenyl cyanide	1347	855	0.06 ± 0.01	0.04 ± 0.01	MS, RI[c]
3	3-butenyl isothiocyanate	1458	977	0.03 ± 0.02	0.06 ± 0.02	MS, RI[c]
4	4-pentenyl isothiocyanate	1542	1077	0.05 ± 0.03	0.08 ± 0.03	MS, RI[c]
5	3,4-epithiobutyl cyanide	1950	1119	0.23 ± 0.08	0.13 ± 0.07	MS, RI[d]
6	2-phenylethyl cyanide	2047	1233	0.17 ± 0.09	0.34 ± 0.17	MS, RI[c]
7	4,5-epithiopentyl cyanide	2096	1239	0.29 ± 0.17	0.10 ± 0.06	MS, RI[d]
8	2-phenylethyl isothiocyante	2235	1456	0.03 ± 0.02	0.06 ± 0.04	MS, RI[c]

[a] RI: Linear retention index based on a series of *n*-hydrocarbons. [b] Concentration: results are the means of three repetitions as mg/kg. [c] Identification using commercial available compounds. [d] Identification using synthetic compounds in this study.

modification. The treated leaf sample (30 g fresh weight) was crushed for 30 s in a blender with 100 mL of cooled reaction buffer (100 mM potassium phosphate buffer, pH 7.0) containing 5 mM dithiothreitol. The homogenate was centrifuged at 12 000*g* at 4 °C for 15 min. The supernatant was adjusted to 80% saturation with ammonium sulfate and the proteins were collected by centrifugation (12 000*g* at 4 °C for 15 min). The precipitate was dissolved in a minimum volume of the reaction buffer and subjected to a NAP-5 column, equilibrated with the reaction buffer. The eluate from the column was used for the myrosinase assay. The assay mixture (950 μL) containing the reaction buffer and 100 μM sinigrin (allylglucosinolate) was preincubated at 37 °C for 5 min. The reaction was started by adding 50 μL of eluate to the mixture. After incubation at 37 °C for 30 min, the reaction was stopped by boiling for 5 min. The reaction solution was centrifuged at 12 000*g* at room temperature for 15 min, and the glucose content in the supernatant was determined using the enzymatic Glucose (GO) Assay Kit (Sigma-Aldrich, Japan). The protein concentration was determined by the Bio-Rad Protein Assay (Bio-Rad, Japan) with bovine serum albumin as a standard. Myrosinase activity (1 U) was defined as the enzymatic hydrolysis of 1 nmol sinigrin per min. Three replicates were performed for the experiment.

Statistical Analysis. All tests were conducted in triplicate, and the data were expressed as means with standard deviation.

RESULTS AND DISCUSSION

Extraction and Identification of Glucosinolate-Derived Volatiles. The volatiles from fresh and heat-treated (100 °C, 3 s, commonly used cooking condition) leaves of nakajinama were prepared using the SAFE method from the corresponding solvent extracts. As a result of GC-MS analyses, three isothiocyanates and five cyanides were identified as contributing to the typical pungent, bitter, and hot flavor of *Brassica* vegetables in both volatile extracts by comparing the MS fragmentation patterns and retention indices with those of commercial and synthesized compounds.[21,23,24] That is, the major components of the fresh leaves were 1-cyano-3,4-epithiobutane and 1-cyano-4,5-epithiopentane. In contract, cyanides such as 3-butenyl and 2-phenylethyl cyanides and isothiocyanates such as 3-butenyl, 4-pentenyl, and 2-phenylethyl isothiocyanates were found as the predominant compounds of the heat-treated leaves (Table 1).

Regarding studies on the heart-treatment and glucosinolate-derived compounds, the kinetic changes on sulforaphane (SF) and sulforaphane nitrile (SFN) contents in broccoli florets and sprouts have been reported.[14] It was also reported recently that heat-treatment methods, steam, microwave, and boiling, significantly reduced SF and SFN contents in broccoli florets compared to the unheated control.[11] These studies reported that the heat-treatments decreased the formations of SF and SFN in broccoli florets. In broccoli sprouts, however, the increase of SF and decrease of SFN by the heat-treatments at 100 °C for more than 5 min were found.[11,14] Relatively long heat-treatments affected the production of SF and SFN in different materials even in plant species. The reason for high SF production in broccoli sprouts preheat-treated at 100 °C was not described. These findings do not necessarily correspond to our results of short heat treatments. On the other hand, decreased levels of two cyano-epithioalkanes found in nakajinama by heat-treatment (100 °C, 3 s) were consistent with the results of 1-cyano-2,3-epithiopropane in cabbage.[25] It can be said that the heat-treatment, of high temperature and short duration, is important for the formation of isothiocyanate, cyano-epithioalkanes, and cyanides.

Effect of Heat-Treatment on Isothiocyanate and Cyano-epithioalkane Productions. Nakajimana is commonly eaten after boiling for a very short time. Boiling for 3 s is the traditional cooking method for nakajimana in Ishikawa prefecture. Nakajimana was treated at 50, 60, 70, 80, 90, 100 °C for 3 and 30 s, and isothiocyanate contents were analyzed by GC (FID). Heat-treatment at 70−100 °C for 3 s and at 50−80 °C for 30 s significantly increased the formation of isothiocyanates compared to the untreated control (Figure 2). The isothiocyanate contents of leaves heat-treated for 30 s was higher than those heat-treated for 3 s. Heat-treatments at 70 °C for 30 s gave significantly greater induction of isothiocyanate contents compared to the other temperature treatments. Isothiocyanate contents for the heat-treatment at 70 °C for 30 s were increased compared to those at 65 and 75 °C for 30 s (data not shown). Heat-treatments at 60 °C for 30 s gave significantly greater 1-cyano-3,4,-epithiobutane and 1-cyano-4,5-epithiopentane contents compared to the other combinations of time and temperature treatments. However, these cyano-epithioalkanes were decreased by heat-treatments at 70 °C for 30 s (Figure 3). In contrast, the content changes of 3-butenyl, 4-pentenyl, and 2-phenylethyl cyanides listed in Table 1 were very small compared with the results shown in Figures 2, and 3 (data not shown). Especially 4-pentenyl cyanide showed almost constant value regardless of the heat-treatment of temperature and duration. This result implied that the heat-treatment led

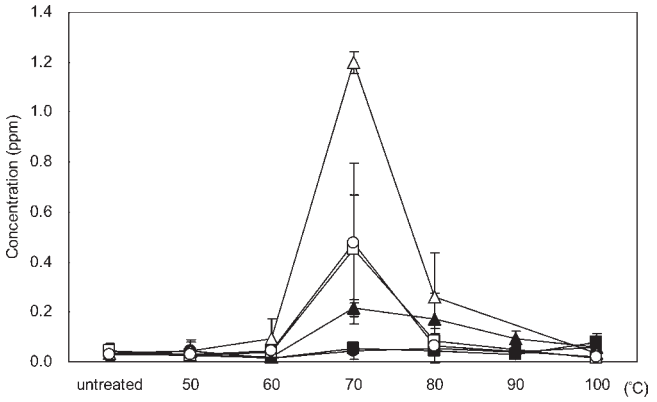

Figure 2. Effects of heat-treatment temperature on isothiocyanate changes.▲ = 3-Butenyl isothiocyanate, ■ = 4-pentenyl isothiocyanate, ● = 2-phenylethyl isothiocyanates, △ = 3-butenyl isothiocyanate, □ = 4-pentenyl isothiocyanate, ○ = 2-phenylethyl isothiocyanates. Black, heat-treatment time for 3 s; white, heat-treatment time for 30 s. Vertical bars represent standard deviation of the mean.

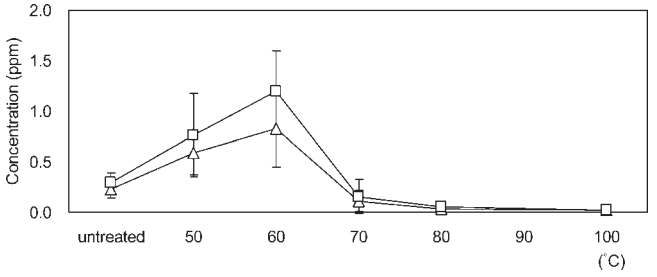

Figure 3. Effects of heat-treatment temperature (for 30 s) on cyano-epithioalkane production. △ = 1-Cyano-3,4,-epithiobutane, □ = 1-cyano-4, 5 epithiopentane. Vertical bars represent standard deviation of the mean.

to a drastic increase in isothiocyanates and cyano-epithioalkanes from glucosinolate but did not affect the cyanides in nakajimana.

Matusheski et al. reported that SF content of broccoli sprouts was significantly increased by preheating sprouts at 60 °C for 10 min or at 50 °C over 20 min. The SF formation in broccoli florets was significantly increased by preheating at 60 °C for 5 and 10 min.[14] It was reported that SFN decreased when SF content increased in broccoli sprouts and florets.[14] Some *Brassica* plants formed cyanides as principal glucosinolates hydrolysis products while others produced primarily isothiocyanates products.[26−28] Nakajimana and other members of the *Brassica rapa* species are cyanide-forming plants. Daikon radish, horseradish, and white mustard, however, have been shown to form only isothiocyanates from their glucosinolates.[29−32] A protein, called epithiospecifier protein (ESP), that appears responsible for the formation of cyano-epithioalkanes has been identified in some *Brassica* vegetables.[31,32] This protein does not catalyze glucosinolate hydrolysis by itself but instead directs the products of glucosinolate hydrolysis toward cyano-epithioalkanes, rather than isothiocyanates. ESP requires iron for its activity, and heat-treatment has been shown to decrease the formation of cyano-epithioalkanes in seeds of turnip rape (*Brassica campestris*).[33]

On the basis of the results mentioned above, the heat-treatment conditions affect the production of isothiocyanates, cyano-epithioalkanes, and cyanides, depending on the plant species.

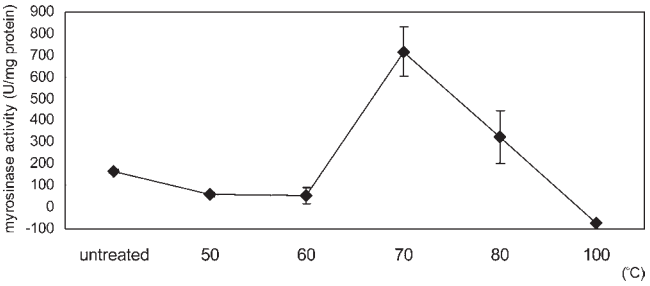

Figure 4. Myrosinase activity of nakajimana leaves heat-treated at different temperatures. Vertical bars represent standard deviation of the mean. Vertical bars represent standard deviation of the mean.

In nakajimana isothiocyanate content was significantly increased by the heat-treatment at 70 °C for 30 s.

Effect of Heat-Treatment on Myrosinase Activity. Nakajimana leaves were heat-treated at 50, 60, 70, 80, 90, and 100 °C for 30 s, and myrosinase activity was analyzed. Myrosinase activity was significantly increased in nakajimana leaves heated at 70 °C for 30 s and significantly decreased when leaves were heated over 70 °C. Myrosinase activity of the leaves heat-treated at 70 °C for 30 s was 4-fold higher than that of untreated control (Figure 4). These results suggest that an increase of myrosinase activity by heat-treatment may allow for greater formation of isothiocyanates from glucosinolates. Myrosinase from broccoli vegetable tissues has been reported to be heat-labile, with loss of approximately 90% of activity after only 3 min of heat treatment at 60 °C.[34] Verkerk and Dekker have shown that microwaving cabbage for 4 min and 48 s effectively activated myrosinase.[35] Myrosinase activity from the seeds of several *Brassica* vegetables has been reported to be relatively more heat stable.[36,37] It is possible that the temperature resistance of myrosinase activity in the sprouts may be a result of its content of a specific seed myrosinase family, as has been reported in white mustard seedlings.[38] However, nakajimana used for this study was grown in the field. Myrosinase activity is completely lost with normal commercial or domestic cooking procedures. Glucosinolates are hydrolyzed by myrosinase to isothiocyanates and cyano-epithioalkanes, depending on the presence or level of ESP and metal ions.[2,3,6] Matusheski et al. reported that ESP activity of broccoli florets was significantly decreased by a heating temperature of more than 50 °C for 10 min and decreased in sprouts heated at a temperature of 40 °C for 20 min.[14]

We have shown that mild heat-treatment of nakajimana increases the myrosinase activity and formation of isothiocyanates. In addition, it has been reported that 3-butenyl isothiocyanate, 4-pentenyl isothiocyanate, and 2-phenetyl isothiocyanate possessed antimicrobial action,[39] and 3-butenyl isothiocyanate and 2-phenethyl isothiocyanate have anticarcinogenic action.[40,41]

There are many *Brassica* plants in the world. However, the anticancer substance or isothiocyanates in other crucifers besides broccoli have not been well investigated. We have identified the isothiocyanates of nakajimana, a traditional vegetable in Japan, and showed that a mild heat-treatment of nakajimana increases the formation of the anticarcinogenic isothiocyanate. Although it has been reported that nakajimana possesses a potent of ACE inhibitor, so far it has not been well investigated yet. We found additional bioactivity and expect that more attention should be given to this and other traditional vegetables in the future.

AUTHOR INFORMATION

Corresponding Author

*E-mail: shimomur@toyo.jp; phone: +81-276-82-9208, fax: +81-276-82-9208.

ACKNOWLEDGMENT

We thank Dr. Mineko Omoto, San-Ei Gen F.F.I., Inc., for syntheses and NMR measurements of 1-cyano-3,4-epithiobutane and 1-cyano-4,5-epithiopentane.

REFERENCES

(1) Yoshimura, K. Characteristics of Angiotensin I-Converting Enzyme Inhibiting Activity of "Nakajimana. *Bull. Ishikawa Agric. Res. Center* **1998**, *21*, 45–51.

(2) Halkier, B. A.; Gershonzen, J. Biology and biochemistry of glucosinolates. *Ann. Rev. Plant Biol.* **2006**, *57*, 303–333.

(3) Fahey, J. W.; Zalcmann, A. T.; Talalay, P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* **2001**, *56*, 5–51.

(4) Bending, G. D.; Lincoln, S. D. Inhibition of soil nitrifying bacteria communities and their activities by glucosinolate hydrolysis products. *Soil Biol. Biochem.* **2000**, *32*, 1261–1269.

(5) Lucianoa, F. B.; Holley, R. A. Enzymatic inhibition by allyl isothiocyanate and factors affecting its antimicrobial action against Escherichia coli O157:H7. *Int. J. Food Microbiol.* **2009**, *31*, 240–245.

(6) Jones, R. B.; Faragher, J. D.; Winkler, S. A review of the influence of postharvest treatments on quality and glucosinolate content in broccoli (Brassica oleracea var. italica) heads. *Postharvest Biol. Technol.* **2006**, *41*, 1–8.

(7) Song, L.; Thornalley, P. J. Effect of storage, processing and cooking on glucosinolate content of Brassica vegetables. *Food Chem. Toxicol.* **2007**, *45*, 216–224.

(8) Rosa, E. A. S.; Heaney, R. K. The effect of cooking and processing on the glucosinolate content: studies on four varieties of Portuguese cabbage and hybrid white cabbage. *J. Sci. Food Agric.* **1993**, *62*, 259–265.

(9) Howard, L. A.; Jeffery, E. H.; Wallig, M. A.; Klein, B. P. Retention of phytochemicals in fresh and processed broccoli. *J. Food Sci.* **1997**, *62*, 1098–1100.

(10) Vallejo, F.; Tomas-Barberan, F. A.; Garcia-Viguera, C. Glucosinolates and vitamin C content in edible parts of broccoli florets after domestic cooking. *Eur. Food Res. Technol.* **2002**, *215*, 310–316.

(11) Jones, R. B.; Frisina, C. L.; Winkler, S.; Imsic, M.; Tomkins, R. B. Cooking method significantly effects glucosinolate content and sulforaphane production in broccoli florets. *Food Chem.* **2010**, *123*, 237–242.

(12) Matusheski, N. V.; Jeffery, E. H. Comparison of the bioactivity of two glucoraphanin hydrolysis products found in broccoli, sulforaphane and sulforaphane nitrile. *J. Agric. Food Chem.* **2001**, *49*, 5743–5749.

(13) Mithen, R.; Faulkner, K.; Magrath, R.; Rose, P.; Williamson, G.; Marquez, J. Development of isothiocyanate enriched broccoli and its enhanced ability to induce phase II detoxification enzymes in mammalian cells. *Theor. Appl. Genet.* **2003**, *106*, 727–734.

(14) Matusheski, N. V.; Juvik, J. A.; Jeffery, E. H. Heating decreases epithiospecifier protein activity and increases sulforaphane formation in broccoli. *Phytochemistry* **2004**, *65*, 1273–1281.

(15) Basten, G. P.; Bao, Y.; Williamson, G. Sulforaphane and its glutathione conjugate but not sulforaphane nitrile induce UDPglucuronosyl transferase (UGT1A1) and glutathione transferase (GSTA1) in cultured cells. *Carcinogenesis* **2002**, *23*, 1399–1404.

(16) Daxenbichler, M. E.; Van Etten, C. H.; Spencer, G. F. Glucosinolates and derived products in cruciferous vegetables. Identification of organic nitriles from cabbage. *J. Agric. Food Chem.* **1977**, *25*, 121–124.

(17) Dietz, H. M.; Panigrahi, S.; Harris, R. V. Toxicity of hydrolysis products from 3-butenyl glucosinolate in rats. *J. Agric. Food Chem.* **1991**, *39*, 311–315.

(18) Buttery, R. G.; Teranishi, R.; Ling, L. C. Fresh tomato aroma volatiles: A quantitative study. *J. Agric. Food Chem.* **1987**, *35*, 540–544.

(19) Engel, W.; Bahr, W.; Schieberle, P. Solvent assisted flavour evaporation — a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. *Eur. Food Res. Technol.* **1999**, *209*, 237–241.

(20) Van den Dool, H.; Kratz, P. D. A generalisation of the retention index system including linear temperature programmed gas-liquid partition chromatography. *J. Chromatogr.* **1963**, *11*, 463–471.

(21) Spencer, G. F.; Daxenbichler, M. E. Gas chromatography-mass sepctrometry of nitriles, isothiocyanates and oxazolidinethiones derived from cruciferous glucosinolates. *J. Sci. Food Agric.* **1980**, *31*, 359–367.

(22) Hara, M.; Eto, H.; Kuboi, T. Tissue printing for myrosinase activity in roots of turnip and Japanese radish and horseradish: a technique for localizing myrosinases. *Plant Sci.* **2001**, *160*, 425–431.

(23) Fenwick, G. R.; Heaney, R. K.; Mullin, W. J. Glucosinolates and their breakdown products in food and food plants. *Crit. Rev. Food Sci. Nutr.* **1983**, *18*, 123–201.

(24) Uda, Y.; Yoshida, S.; Goto, M.; Egashira, H. Glucosinolate-derived volatiles in raw vegetables, Fujisawana and Yukina, and their pickled or salted products. *Nippon Shokuhin Kagaku Kougaku Kaishi* **2007**, *54*, 559–562.

(25) Rungapamestry, V.; Duncan, A. J.; Fuller, Z.; Ratcliffe, B. Changes in glucosinolate concentrations, myrosinase activity, and production of metabolites of glucosinolates in cabbage (Brassica oleracea var. capita) cooked for different durations. *J. Agric. Food Chem.* **2006**, *54*, 7628–7634.

(26) Gil, V.; MacLeod, A. J. Degradation of glucosinolates of Nasturtium officinale seeds. *Phytochemistry* **1980a**, *19*, 1657–1660.

(27) Hasapis, X.; MacLeod, A. J. Effects of pH and ascorbate on benzylglucosinolate degradation in seed extracts of Lepidium sativum. *Phytochemistry* **1982b**, *21*, 291–296.

(28) Lambrix, V.; Reichelt, M.; Mitchell-Olds, T.; Kliebenstein, D. J.; Gershenzon, J. The Arabidopsis epithiospecifier protein promotes the hydrolysis of glucosinolates to nitriles and influences Trichoplusia in herbivory. *Plant Cell* **2001**, *13*, 2793–2807.

(29) Cole, R. A. Isothiocyanates, nitriles and thiocyanates as products of autolysis of glucosinolates in Cruciferae. *Phytochemistry* **1976**, *15*, 759–762.

(30) Cole, R. A. Volatile components produced during ontogeny of some cultivated crucifers. *J. Sci. Food Agric.* **1980**, *31*, 549–557.

(31) Petroski, R. J.; Tookey, H. L. Interactions of thioglucoside glucohydrolase and epithiospecifier protein of cruciferous plants to form 1-cyanoepithioalkanes. *Phytochemistry* **1982**, *21*, 1903–1905.

(32) Tookey, H. L. Crambe thioglucoside glucohydrolase (EC 3.2.3.1): Separation of a protein required for epithiobutane formation. *Can. J. Biochem.* **1973**, *51*, 1654–1660.

(33) Kirk, J. T. O.; Macdonald, C. G. 1-Cyano-3,4-epithiobutane: a major product of glucosinolate hydrolysis in seeds from certain varieties of Brassica campestris. *Phytochemistry* **1974**, *13*, 2611–2615.

(34) Ludikhuyze, L.; Ooms, V.; Weemaes, C.; Hendrickx, M. Kinetic study of the irreversible thermal and pressure inactivation of myrosinase from broccoli (*Brassica oleracea* L. cv. italica). *J. Agric. Food Chem.* **1999**, *47*, 1794–1800.

(35) Verkery, R.; Dekker, M. Glucosinolates and Myrosinase Activity in Red Cabbage (*Brassica oleracea* L. Var. *Capitata* f. *rubra* DC.) after Various Microwave Treatments. *J. Agric. Food Chem.* **2004**, *52*, 7318–7323.

(36) Van Etten, C. H.; Daxenbichler, J. E.; Peters, J. E.; Tookey, H. L. Variation in enzymatic degradation products from the major thioglucosides in *Crambe abyssinica* and *Brassica napus* seed meals. *J. Agric. Food Chem.* **1966**, *14*, 426–430.

(37) Bones, A.; Slupphaug, G. Purification, characterization and partial amino-acid sequencing of β-thioglucosidase from Brassica napus L. *J. Plant Physiol.* **1989**, *134*, 722–729.

(38) Eriksson, S.; Ek, B.; Xue, J.; Rask, L.; Meijer, J. Identification and characterization of soluble and insoluble myrosinase isoenzymes in different organs of Sinapis alba. *Physiol. Plant.* **2001**, *111*, 353–364.

(39) Jang, M; Hong, E; Kim, G. H. Evaluation of antibacterial activity of 3-butenyl, 4-pentenyl, 2-phenylethyl, and benzyl isothiocyanates in *Brassica* vegetables. *J. Food Sci.* **2010**, *75*, 412–416.

(40) Hong, E; Kim, G. Anticancer and antimicrobial activities of β-phenylethyl isothiocyanates in *Brassica rapa* L. *Food Sci. Technol. Res.* **2008**, *14*, 377–382.

(41) Verhoeven, D. T. H.; Verhagen, H; Goldbohm, R. A.; Brandt, P. A.V. D.; Poppel, G. V. A review of mechanisms underling anticarcinogenicity by brassica vegetables. *Chem.-Biol. Interac.* **1997**, *103*, 79–129.