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Effect of soaking and heating stimulation on the formation of isothiocyanates in *Eruca sativa* Mill. seeds and sprouts

[1,3]Wang, S., [1]Li, X., [2]Wang, Q., [1]Wang, P., [1]Gu, Z. and [1]*Yang, R.

[1]*College of Food Science and Technology, Nanjing Agricultural University, Nanjing, Jiangsu 210095, China*
[2]*Beijing Key Lab of Plant Resource Research and Development, Beijing Technology and Business University, Beijing 100048, China*
[3]*Institute of Food Safety and Nutrition, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China*

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Abstract

The present work investigated the effects of soaking followed by heating on the metabolism of glucosinolates (GLs) in rocket seeds and sprouts. Soaking for 1 h increased myrosinases (MYR) activity of seeds. Heating at 70°C for 10 min after soaking resulted in the highest isothiocyanates (ITCs) formation in seeds. Both immerse-heating and steam-heating decreased the total GLs content in seeds and 2-day old sprouts. However, steam-heating showed minor effect on total GLs content when compared with immerse-heating. Both methods showed a decreased MYR activity in seeds and sprouts. Nevertheless, heating had a positive impact on ITCs formation. Under immerse-heating, ITCs formation was the highest in seeds and sprouts at 70°C heating. However, under steam-heating, ITCs formation in seeds and sprouts was higher at 70 and 60°C, respectively. The results indicated that steam-heating treatment was effective for improving ITCs formation in rocket seeds and 2-day old sprouts.

Introduction

Plant seeds have vitality, and are able to form sprouts by germination under suitable conditions. During germination, endogenous enzymes are activated, and the metabolism level increases significantly (Pérez-Balibrea *et al.,* 2011; Gu *et al.,* 2012). After water absorption, oxygen can easily pass through the seed coat which then changes its condition to swollen and soft, thus enhancing embryonic respiration. As a result, many storage substrates including protein and starch are degraded into amino acids and low molecular sugars. Hence, germination could improve the utilisation and nutrient availability of seeds (Pająk *et al.,* 2014). Moreover, germination could also enhance the degradation and reduction of phytic acid, flatulence factor, protease inhibitor, and other anti-nutritional factors (Shanmugam *et al.,* 2018). In addition, some functional components including bioactive peptides (Wen *et al.,* 2009), γ-aminobutyric acid (Yang *et al.,* 2013), and isothiocyanates (ITCs) (Yang *et al.,* 2016) accumulate in seeds after germination. Therefore, germination is an effective way to improve nutritional and functional quality of plant seeds.

Rocket (*Eruca sativa* Mill.) is one of the *Brassica* vegetables rich in bioactive components including carotene, vitamin C, flavonoids, and glucosinolates (GLs). As a type of secondary metabolites, GLs in rocket seeds could be hydrolysed by myrosinases (MYR) to produce unstable thiohydroxamate-O-sulfonates, which can then rapidly break down to form a range of products including ITCs, thiocyanates, nitriles, cyclic nitriles, and oxazolidinones through molecular rearrangement (Jing *et al.,* 2009). Among these products, ITCs have beneficial functions such as anticancer, anti-tumour, and anti-inflammatory (Traka and Mithen, 2009). The types and concentrations of products are affected by abiotic stresses including salt (Tian *et al.,* 2016), low-temperature (Guo *et al.,* 2015), and heating (Yang *et al.,* 2017). Under salt stress, GLs content increased and ITCs formation shows a relatively higher level in broccoli sprouts (Guo *et al.,* 2014b). Freezing at -20°C increased the ITCs formation in broccoli sprouts (Guo *et al.,* 2015). Heating at 60°C prior to homogenisation improved the sulforaphane formation but decreased sulforaphane nitrile formation in broccoli florets (Matusheski et al., 2004a). It has been observed that there was higher epithiospecifier protein (ESP) activity resulted in the formation of nitrile during GLs hydrolysis, while there was a decrease in the formation of ITCs (Yang *et al.,* 2015). Therefore, reducing ESP activity is critical for more ITCs formation. When

*Corresponding author.
Email: yangrq@njau.edu.cn

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compared with MYR, ESP is more heat-sensitive (Wang *et al.,* 2012), and proper heating could promote the transformation of GLs to ITCs rather than to nitriles (Macleod and Rossiter, 1986).

Soaking can activate MYR in seeds of *Brassica* vegetables, which is beneficial for the hydrolysis of GLs. Simultaneously, ESP activity is higher, and it is unamiable for ITCs formation. Hence, inactivating ESP by heating after soaking might be an effective way to improve ITCs formation during GLs hydrolysis. In the present work, the soaked seeds and 2-day old sprouts of rocket were treated with immerse- and steam-heating to investigate GLs metabolism and ITCs formation in order to seek an efficient way for increasing the functional quality of rocket.

Materials and methods

Materials and reagents

Rocket seeds (cv. Dongsheng) were purchased from Shouguanghuinong Seed Corporation (Shandong, China), and kept at -20°C. Sinigrin and arylsulfatase were purchased from Sigma Aldrich (St. Louis, MO, USA). DEAE Sephadex A-25 and iminazole were purchased from Solarbio Science and Technology Ltd. (Beijing, China). Methanol and acetonitrile were of high-performance liquid chromatography (HPLC) grade. Other chemicals and reagents were of analytical grade, and purchased from Shanghai Institute of Biochemistry (Shanghai, China).

Seed treatment and germination

Dry rocket seeds were surface-sterilised in 1% of sodium hypochlorite for 10 min, washed and soaked in distilled water at 30°C for 0.5, 1, 1.5, 2, 2.5, and 3 h, and subjected to heating treatment. For sprout preparation, the surface-sterilised seeds were soaked with distilled water for 3 h at 30°C, and then evenly sprinkled in the sprouting machine (BX-801, Beixin Hardware Electrical Factory, Zhejiang, China) laid with vermiculite, and placed in the light incubator (PGX-2000A, Saifu Experimental Instrument, Ningbo, Zhejiang, China) with alternate dark and light (8 h dark/16 h light) cycle at 30°C. During germination, seeds were sprayed with distilled water every 12 h. The samples were rapidly collected after 2 d of germination.

Heating treatment

For immerse-heating, the soaked seeds or 2-day old sprouts were immersed in hot water for 10 min at 50, 60, 70, and 80°C, and then drained off quickly for further measurement.

For steam-heating, the soaked seeds or 2-day old sprouts were placed in a closed steriliser filled with hot steam for 10 min at 50, 60, 70, and 80°C, and then collected quickly for further measurement.

Desulfo-glucosinolate analysis

Crude GLs were extracted with 2 mL of 70% (v/v) boiling methanol for 15 min at 80°C, twice. Sinigrin (2-propenyl glucosinolate) was added to each sample before the first extraction as an internal standard. Following centrifugation at 10,000 *g* for 15 min, 2 mL of extract was applied to DEAE Sephadex™ A-25. Then, 500 μL of arylsulfatase solution (12 U/mL) was added, and the columns were kept at 30°C for 16 h in a dark incubator. The desulfo-glucosinolates were rinsed with 4 mL of distilled water, and filtered through 0.45 μm membrane filter. HPLC (Agilent 1200, Agilent Technologies Co. Ltd., Santa Clara, CA, USA) analyses of GLs were carried out according to the procedure of Guo *et al.* (2014a) The GLs content was expressed as nmol/seed or nmol/sprout.

MYR activity analysis

For the sample preparation, ten of soaked seeds or 2-day old sprouts (approximately 2 g) were ice-ground with phosphate buffer (0.1 M, pH 6.5), and centrifuged at 10,000 *g* and 4°C for 15 min, and the supernatants were considered as the crude MYR. The measuring method for MYR activity was reported in detail by Guo *et al.* (2014a). One enzyme unit corresponded to 1 nmol glucose formed per minute. The specific activity was expressed as U/seed or U/sprout.

ITCs formation analysis

The method of Chung *et al.* (1998) with slight modifications was used for determining the level of ITCs. Twenty soaked seeds or 2-day old sprouts were ground and extracted with distilled water (1:5, m/v), and the samples were self-hydrolysed at 37°C for 2 h. Following centrifugation at 10,000 *g* for 15 min, 250 μL of supernatants were mixed with 250 μL of phosphate buffer (0.1 mol/L, pH 8.5) and 500 μL of 2-propanol solution of 1,2-benzenedithiol (10 mmol/L). The mixtures were incubated in a water bath shaker at 65°C for 2 h. The content of ITCs was analysed using an Agilent 1200 Series HPLC (Agilent Technologies, Santa Clara, CA, US) with Eclipse XDB-C_{18} column (4.6 mm × 150 mm × 5 μm) at 365 nm using sulforaphane as an standard. The mobile phase was 70% methanol, at a flow rate of 1.0 mL/min.

Data analysis and statistics

Experimental data were expressed as mean ± standard deviation (SD) of three biological replications ($n = 3$). SPSS 17.0 (SPSS Inc., Chicago, IL, USA) was used for the significant difference test. Variables of replications were compared by Duncan's Multiple-Range Test at $p < 0.05$.

Results

Effect of soaking on GLs metabolism of rocket seeds

As shown in Table 1, the content of total GLs in seeds decreased at first, and then maintained after 2.5 h soaking, which decreased by 32.45% at 3 h when compared with the control. Glucoraphanin (GRA) content showed a shape of parabola, with the highest value at 1.5 h, which was 1.36 times of the initial content. The content of glucoerucin (GER) generally decreased with soaking time, with no significant difference between 0.5 and 1 h, or between 2.5 and 3 h of soaking. Heating (70°C) after soaking led to the reduction of both total and individual GLs contents, and they rapidly decreased during the first 0.5 h of soaking. After 1.5 h of soaking, GLs content reached the lowest level, and maintained for another 1.5 h. GRA and GER showed similar trend with no significant difference during 1 - 3 h of soaking ($p > 0.05$). Under the same soaking time, the total and individual GLs under soaking plus heating treatment lost more than those under soaking treatment. At 3 h of soaking, GRA, GER, and total GLs content decreased by 46.1, 30.7, and 31.4%, respectively, under soaking plus heating treatment when compared with that of under soaking treatment.

The changes of MYR activity and ITCs formation in rocket seeds are shown in Figure 1. At 1 h of soaking, MYR activity showed the peak value (1.93 U/seed), and there was no significant difference among 0.5 - 1.5 h or 2 - 3 h of soaking ($p > 0.05$). When heating at 70°C, MYR activity gradually decreased with soaking time. At 2.5 h of soaking, MYR activity was almost undetectable (Figure 1A). A significant decrease in ITCs formation appeared during soaking, and the decrease was up to 80% when exceeding 2 h of soaking. However, under heating treatment, ITCs formation dramatically increased when compared with that soaking alone, yielding maximum value (68.08 nmol/seed) at 1.0 h of soaking. In summary, 1 h of soaking of seeds followed by 70°C heating for 10 min resulted in higher content of GLs and higher formation of ITCs. Hence, 1 h soaking was selected in the subsequent study.

Table 1. Changes in glucosinolate contents of seeds during soaking.

Treatment	Soaking time (h)	DS-GLs (nmol/seed)		
		GRA	GER	Total GLs
Soaking	0	19.91 ± 0.57^{c}	693.18 ± 0.68^{a}	713.09 ± 0.89^{a}
	0.5	23.92 ± 1.64^{b}	588.92 ± 10.42^{b}	612.84 ± 10.55^{b}
	1	22.43 ± 0.62^{b}	600.05 ± 6.80^{b}	622.48 ± 6.83^{b}
	1.5	27.03 ± 0.25^{a}	524.13 ± 1.17^{c}	551.16 ± 1.20^{c}
	2	23.96 ± 0.25^{b}	488.54 ± 5.67^{d}	512.5 ± 5.68^{d}
	2.5	23.73 ± 1.20^{b}	456.94 ± 18.51^{e}	480.67 ± 18.55^{e}
	3	22.61 ± 0.43^{b}	459.04 ± 12.48^{e}	481.65 ± 12.49^{e}
Soaking plus heating	0	23.15 ± 0.25^{a}	540.45 ± 2.35^{a}	563.60 ± 2.36^{a}
	0.5	16.48 ± 1.43^{b}	390.19 ± 24.93^{b}	406.67 ± 24.97^{b}
	1	13.51 ± 1.16^{c}	404.51 ± 0.69^{b}	418.02 ± 1.35^{b}
	1.5	13.74 ± 0.09^{c}	327.22 ± 3.61^{c}	340.96 ± 3.61^{c}
	2	12.82 ± 0.88^{c}	329.23 ± 3.16^{c}	342.60 ± 3.28^{c}
	2.5	11.50 ± 0.53^{c}	332.23. ± 7.05^{c}	343.73 ± 7.07^{c}
	3	12.19 ± 0.90^{c}	318.44 ± 8.20^{c}	330.63 ± 8.25^{c}

Soaking = surface-sterilised seeds were soaked in distilled water at 30°C for 0, 0.5, 1, 1.5, 2, 2.5, and 3 h. Soaking plus heating treatment = the soaked seeds were treated at 70°C for 10 min after soaking by immersing in distilled water. DS-GLs = desulfo-glucosinolates; GRA = glucoraphanin; and GER = glucoerucin. Values are mean ± SD. Means followed by different superscript letters in the same column under each treatment are significantly different according to Duncan's Multiple-Range Test ($p < 0.05$).

Immerse-heating on GLs metabolism of seeds and sprouts

Table 2 shows that heating treatment increased the content of total GLs in seeds, and no significant difference existed between different temperatures ($p > 0.05$). GRA content also increased with temperature, reaching the highest value at 70°C. Interestingly, GER content continuously decreased with increasing temperature. There was no DMB detected in seeds. In sprouts, total GLs content decreased with temperature; it decreased by 94.97% at 80°C when compared with the control. GER and DMB content showed similar trend. However, GRA content exhibited higher value at 50°C.

Results showed that immerse-heating induced the decrease of MYR activity in seeds and sprouts (Figures 2A and 2B). After treatment at 80°C, MYR activity was only 7.77% on the unheated sample in seeds, but was inactive in sprouts (Figure 2A). The 50 and 60°C heating treatments had no significant effects on the formation of ITCs for seeds and immerse-heating at 70°C significantly increased the ITCs formation (54.46 nmol/seed). However, ITCs formation decreased after heating treatments when compared with the control in sprouts. Nevertheless, 70°C treatment showed relatively higher content of ITCs (Figure 2B).

Steam-heating on GLs metabolism of seeds and sprouts

As shown in Table 3, the content of total and individual GLs in seeds and sprouts were significantly affected by steam-heating. In seeds, GER and total GLs content showed downward trend with increasing temperature. However, heating treatment resulted in the increase of GRA content, and showed higher content at 60°C. In sprouts, GRA, GER, and total GLs content continuously decreased with increasing temperature.

Similar to immerse-heating, steam-heating caused continuous decrease in MYR activity both in seeds and sprouts with increasing temperature (Figures 2C and 2D). MYR activity of sprouts was higher than that of seeds, but was more sensitive to heating treatment. However, steam-heating significantly enhanced the formation of ITCs in seeds and sprouts. ITCs formation increased at first, and then decreased with increasing temperature. The highest level was observed at 70 and 60°C for seeds and sprouts, respectively.

Discussion

With the progress of soaking, GLs content continuously decreased (Table 1). This might be due to the water absorption; some GLs in seeds were dissolved into water. In addition, endogenous enzymes were activated and GLs were hydrolysed

Table 2. Changes in glucosinolate contents of seeds and sprouts under different temperatures.

	Temperature (°C)	DS-GLs (nmol/sprout)			
		GRA	GER	DMB	Total GLs
Seeds	Con.	22.43 ± 0.62^{c}	600.05 ± 6.80^{a}	ND	622.48 ± 6.83^{b}
	50	102.58 ± 4.00^{b}	574.32 ± 5.22^{b}	ND	676.89 ± 6.58^{a}
	60	101.10 ± 1.99^{b}	579.35 ± 16.26^{ab}	ND	$680.45. \pm 16.38^{a}$
	70	137.30 ± 10.30^{a}	549.09 ± 9.50^{c}	ND	686.39 ± 14.01^{a}
	80	126.17 ± 9.67^{a}	538.95 ± 6.81^{c}	ND	665.12 ± 11.83^{a}
Sprouts	Con.	57.98 ± 1.43^{b}	137.49 ± 2.62^{a}	114.95 ± 3.46^{a}	310.42 ± 4.60^{a}
	50	178.52 ± 1.38^{a}	77.37 ± 7.70^{b}	42.04 ± 2.63^{b}	297.93 ± 8.34^{a}
	60	22.88 ± 1.11^{c}	3.68 ± 0.23^{c}	2.53 ± 0.30^{c}	29.10 ± 1.17^{b}
	70	20.84 ± 0.77^{c}	1.94 ± 0.09^{d}	1.93 ± 0.02^{c}	24.71 ± 0.78^{c}
	80	12.72 ± 0.42^{d}	1.47 ± 0.03^{d}	1.52 ± 0.09^{c}	15.61 ± 1.09^{d}

For seeds, after 1 h of soaking, the seeds were immersed in hot water for 10 min at 50, 60, 70, and 80°C. For sprouts, seeds were germinated with distilled water for 2 d, then they were immersed in hot water for 10 min at 50, 60, 70, and 80°C. ND = not detected; DS-GLs = desulfo-glucosinolates; GRA = glucoraphanin; GER = glucoerucin; DMB = dimer-4-mercaptobutyl glucosinolate; and Con. = unheated. Values are mean ± SD. Means followed by different superscript letters in the same column under each treatment are significantly different according to Duncan's Multiple-Range Test ($p < 0.05$).

Table 3. Changes in glucosinolate contents of seeds and sprouts under different steam-heating temperatures.

	Temperature (°C)	DS-GLs (nmol/sprout)			
		GRA	GER	DMB	Total GLs
Seeds	Con.	22.43 ± 0.62^{c}	600.05 ± 6.80^{a}	ND	622.48 ± 6.83^{a}
	50	124.41 ± 8.90^{ab}	327.60 ± 7.17^{b}	ND	452.01 ± 11.43^{b}
	60	130.60 ± 0.89^{a}	318.21 ± 9.01^{b}	ND	448.81. ± 9.06^{b}
	70	120.55 ± 1.48^{ab}	258.13 ± 6.75^{c}	ND	378.68 ± 6.91^{c}
	80	116.67 ± 6.78^{b}	219.53 ± 8.37^{d}	ND	336.20 ± 10.78^{d}
Sprouts	Con.	61.33 ± 4.44^{a}	500.68 ± 10.77^{a}	39.29 ± 2.29^{a}	601.30 ± 11.89^{a}
	50	44.58 ± 4.00^{b}	250.76 ± 8.11^{b}	38.07 ± 0.96^{a}	333.41 ± 9.09^{b}
	60	30.08 ± 0.53^{c}	195.72 ± 18.23^{c}	42.00 ± 2.02^{a}	267.81 ± 18.34^{c}
	70	13.55 ± 1.33^{d}	122.96 ± 4.43^{d}	27.50 ± 1.11^{b}	164.01 ± 4.76^{d}
	80	3.49 ± 0.07^{e}	47.98 ± 3.43^{e}	6.79 ± 0.16^{c}	58.26 ± 3.43^{e}

For seeds, after 1 h of soaking, the seeds were put into steam for 10 min at 50, 60, 70, and 80°C. For sprouts, seeds were germinated with distilled water for 2 d, then the formed sprouts were put into steam for 10 min at 50, 60, 70, and 80°C. ND = not detected; DS-GLs = desulfo-glucosinolates; GRA = glucoraphanin; GER = glucoerucin; DMB = dimer-4-mercaptobutyl glucosinolate; and Con. = unheated. Values are mean ± SD. Means followed by different superscript letters in the same column under each treatment are significantly different according to Duncan's Multiple-Range Test ($p < 0.05$).

partially (Tyagi, 2002).

One hour soaking resulted in relatively higher activity of MYR. Correspondingly, the ITCs also showed higher level (Figure 1) although GLs content continuously decreased (Table 1). This indicated that the higher level of MYR activity contributed to the increased ITCs formation rather than the higher GLs content during soaking of seeds. When compared with soaking, soaking plus heating (70°C) further decreased the GLs content and MYR activity, but increased ITCs formation (Table 1, Figure 1). These results might be attributed to the enhanced dissolution of GLs into water and depressed the activity of MYR by high temperature although this led to the high level of ITCs formation. The results indicated that heating treatment was beneficial for ITCs formation which was not dependent on the high GLs content or MYR activity.

GLs could be hydrolysed by MYR to form the unstable intermediate products. Later, they were degraded into ITCs, nitrile, and etc. (Guo *et al.,* 2014a). The efficiency of GLs converting to ITCs is related to the environmental condition and ESP activity. When ESP exists, GLs were hydrolysed along the direction of the nitrile, thereby reducing the formation of ITCs (Williams *et al.,* 2008). ESP, a thermal sensitive protein, could be inhibited under appropriate heating treatment to improve the formation of ITCs. The present work found that steam-heating at 60°C for 10 min greatly increased ITCs formation in rocket sprouts although MYR

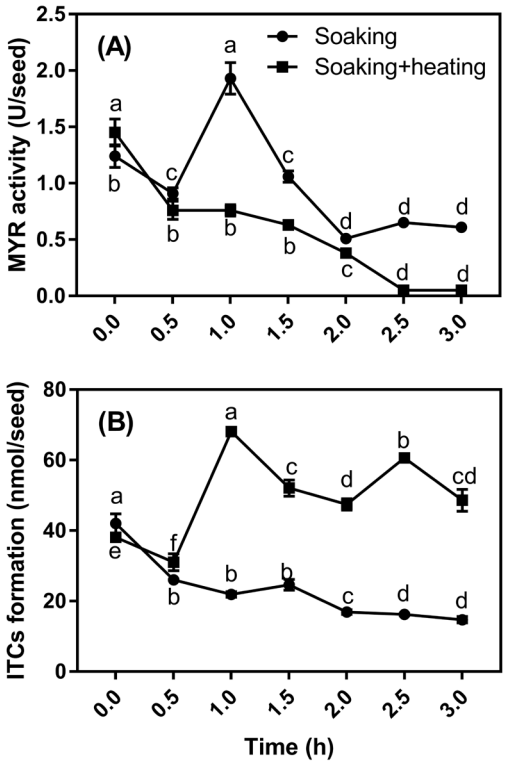

Figure 1. MYR activity (A) and ITCs formation (B) of seeds during soaking. Seeds were soaked in distilled water for 0, 0.5, 1, 1.5, 2, 2.5, and 3 h. Soaking plus heating treatment indicated that the soaked seeds were immerse-heating treated at 70°C for 10 min after soaking. Values are mean ± SD. Means followed by different superscript letters in the same treatment are significantly different according to Duncan's Multiple-Range Test ($p < 0.05$).

activity was partially passivated. Similar finding was also observed by Matusheski *et al.* (2004b) who reported that heating treatment of 5 or 10 min at 60°C resulted in a significant decrease in ESP activity and sulforaphane nitrile formation, but increased the sulforaphane formation in broccoli florets and sprouts.

Both heating methods continuously decreased MYR activity with increasing temperature (Figures 2A and 2C). However, the variation of ITCs formation was different between the two heating methods. Under immerse-heating, the formation of ITCs in seeds or sprouts was higher at 70°C than those at the other heating temperatures. However, ITCs formation in sprouts was lower than the control (Figure 2B). Meanwhile, steam-heating resulted in the higher ITC formation at 70 and 60°C in seeds and sprouts, respectively, than of those at other tested temperatures and the control (Figure 2D). Hence, the heating method significantly affected the formation of ITCs, and steam-heating was highly efficient for ITCs formation when compared with immerse-heating. It was shown that sprouts were more sensitive to heat than seeds under steam-heating treatment. The differences between seeds and sprouts might be due to the difference in the initial GLs content (Table 1) and the heat endurance. Sprouts showed vigorous physiological activity from containing high moisture but without the protection such as seeds which have coat. Hence, its ESP was vulnerable under high temperature. The relatively lower temperature (60°C) was sufficeint to inactivate ESP (Guo *et al.,* 2016).

During soaking, GRA content significantly increased at first, and then decreased, while other GLs content continuously decreased (Table 1). This might be due to the conversion among GLs resulted from the activation of endogenous enzymes related to GLs metabolism (Leng *et al.,* 2019). During immerse-heating, the variation of GLs content was similar with the soaking procedure following the increase in temperature. GRA content showed the peak value in seeds and sprouts at 70 and 50°C, respectively (Table 2). Vallejo *et al.* (2002) reported that GRA was the only existed GL in broccoli florets

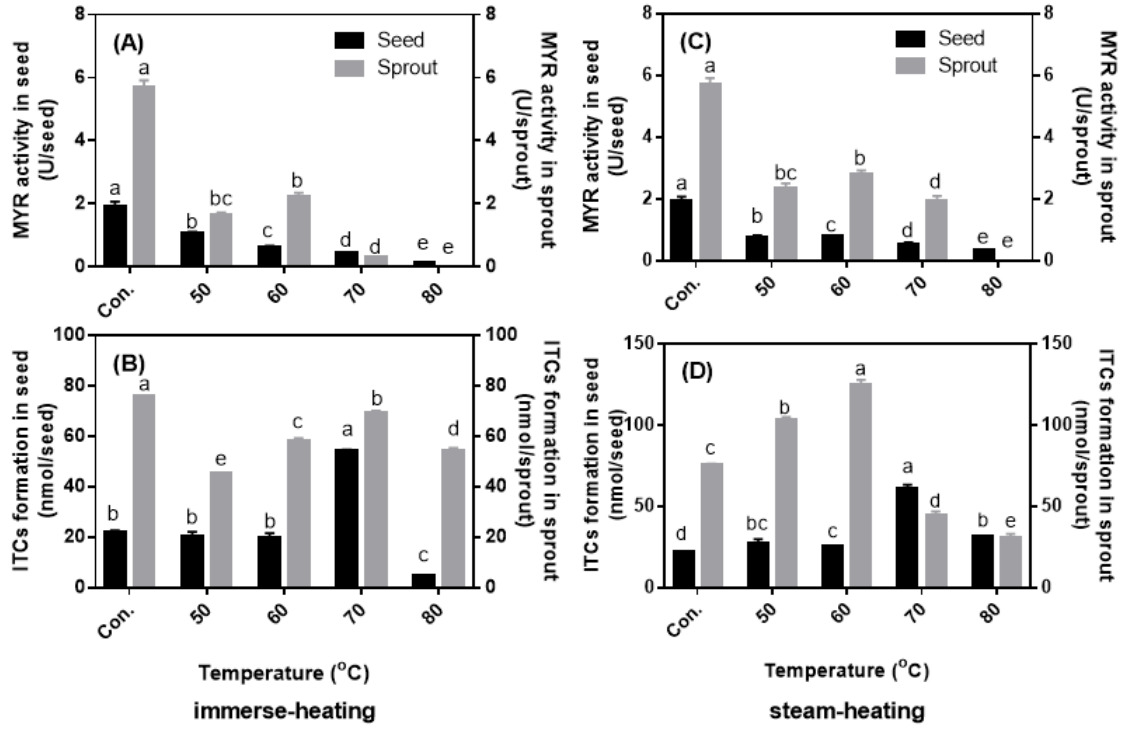

Figure 2. Changes of MYR activity and ITCs formation in seeds and sprouts under different immerse-heating [(A) and (B)], or steam-heating [(C) and (D)] temperatures, respectively. For immerse-heating, the seeds were immersed for 10 min in hot water at 50, 60, 70, and 80°C after 1 h of soaking. For sprouts, seeds were germinated with distilled water for 2 d, then the formed sprouts were immersed in hot water for 10 min at 50, 60, 70, and 80°C. For steam-heating, the seeds were put into steam for 10 min at 50, 60, 70, and 80°C after 1 h of soaking. For sprouts, seeds were germinated with distilled water for 2 d, then the formed sprouts were put into steam for 10 min at 50, 60, 70, and 80°C. Values are mean ± SD. Means followed by different superscript letters in the same column under each treatment are significantly different according to Duncan's Multiple-Range Test ($p < 0.05$).

after cooking, which indicated that GRA was more stable than other aliphatic GLs, and heating was a potential method to improve the GRA content. The effects of heating treatment on the metabolism of GLs are mainly dependent on heating time, method, and type of samples (Dekker *et al.,* 2000). When temperature exceeded 110°C, most of the GLs in purple cabbage degraded. However, in the common domestic cooking, the temperature is usually under 100°C, so the retention of GLs would be relatively high. Commonly, boiling causes great loss of GLs because of the leaching into water due to cell lysis besides enzyme degradation, thermal degradation, or volatilisation (Ciska and Kozłowska, 2001). In the present work, steam-heating was more effective for GLs reservation than immerse-heating (Table 3). This might be due to the fact that seeds and sprouts did not directly come into contact with liquid during steam-heating (Jones *et al.,* 2010; Odland and Eheart, 2010). Meanwhile, immerse-heating accelerated the leakage of GLs for the loose structure of tissue after immerse-heating. Mukherjee *et al.* (2010) reported that steamed broccoli has stronger heart protection function than boiled broccoli. These findings indicated that steam-heating was the efficient way to improve the functional quality of *Brassica* vegetables.

Conclusion

One hour soaking at 30°C was selected as the suitable method for improving ITCs formation in rocket seeds. The followed steam-heating at 70 and 60°C was more beneficial for ITCs formation in soaked seeds and their 2-day sprouts, respectively, although the GLs content and MYR activity decreased. Hence, heating treatment enhanced ITCs formation in rocket seeds and sprouts, and did not rely on the accumulation of GLs and the enhancement of MYR activity.

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