Eur Food Res Technol (2015) 240:559–565
DOI 10.1007/s00217-014-2355-0

ORIGINAL PAPER

Retention of glucosinolates during fermentation of *Brassica juncea*: a case study on production of *sayur asin*

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Received: 18 June 2014 / Revised: 9 September 2014 / Accepted: 5 October 2014 / Published online: 17 October 2014

Abstract Fermentation can reduce the concentration of health-promoting glucosinolates in *Brassica* vegetables. The endogenous enzyme myrosinase is hypothesised to mainly responsible for the degradation of glucosinolates during fermentation. In order to retain glucosinolates in the final fermented product, the role of myrosinase activity during the production of *sayur asin* was investigated. *Sayur asin* is a traditionally fermented product of Indian mustard (*Brassica juncea*) commonly consumed in Indonesia. It is prepared by a spontaneous fermentation of withered (sun-dried) *B. juncea* leaves. The leaves of *B. juncea* contain a substantial amount of the aliphatic glucosinolate sinigrin. Three withering methods were investigated to obtain *B. juncea* leaves with different myrosinase activities prior to fermentation. Results show that withering by oven at 35 °C for 2.5 h and by microwave at 180 W for 4.5 min reduced myrosinase activity by 84 and 74 %, respectively. Subsequently, sinigrin was not detectable in the leaves after 24 h of incubation in the fermentation medium. However, withering by microwave for 2 min at 900 W inactivated myrosinase completely and produced *sayur asin* with a sinigrin concentration of 11.4 μmol/10 g dry matter after 7 days of fermentation. This high power-short time pretreatment of *B. juncea* leaves contributes to the production of *sayur asin* containing significant levels of health-promoting glucosinolate. In this study, the effect of myrosinase activity during *Brassica* fermentation was quantified, and optimised production methods were investigated to retain glucosinolate in the final product.

Keywords Glucosinolate · Myrosinase activity · Fermentation · *Brassica juncea*

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Introduction

Glucosinolates (GLSs) are a group of β-thioglucoside *N*-hydroxysulphates with a sulphur-linked β-D-glucopyranose moiety and side-chain group. GLSs are secondary metabolites derived mainly from one of certain amino acids, such as methionine, tryptophan, and phenylalanine. Depending on side-chain group, glucosinolate (GLS) can be classified either as aliphatic, aromatic, or indolyl [1–3].

Reviews of epidemiological studies reported inverse associations between intake of *Brassica* vegetables and the risk of certain cancers [4, 5]. Isothiocyanates, one of the GLS breakdown products, are assumed to lower the risk of cancer by inhibiting phase 1 and inducing phase 2 enzymes during carcinogen metabolism [6]. Upon damage of plant tissue, GLSs are highly prone to hydrolysis catalysed by myrosinase (β-thioglucosidase EC 3.2.1.147), an enzyme that occurs in *Brassica* vegetables [2, 7].

Fermentation is a processing method commonly performed on milks, meats, and vegetables [8]. Fermentation of *Brassica* vegetables involves the growth and metabolic activity of lactic acid bacteria (LAB), either spontaneously or starter-induced to produce fermented products [9]. Previous studies reported that fermentation considerably reduces

the concentration of GLSs in *sauerkraut* [10, 11] and radish *kimchi* [12].

The mechanisms underlying GLS changes in *Brassica* vegetables during fermentation are less investigated than other processing methods, such as boiling, steaming, and microwave (MW) processing [13, 14]. Several factors can influence the loss of GLS during fermentation, such as the myrosinase activity, LAB growth and activity, low pH, and sodium chloride concentration [9, 11, 15]. *Sayur asin* is a traditional fermented product of Indian mustard (*Brassica juncea*) commonly produced in some regions in Indonesia [16] and other Asian countries, in different local names [17]. The product is prepared by a spontaneous fermentation for 7 days of withered (sun-dried) Indian mustard leaves in the addition of salt followed by rubbing [16]. Either starch water of boiling rice or coconut water can be used as the fermentation medium. In the preliminary study, it was shown that during the production of *sayur asin* sinigrin concentration was reduced to 1.3 % after 3 days of fermentation [18]. The loss of GLS is probably attributed to hydrolytic activity of myrosinase. The myrosinase activity is mainly ascribed to the endogenous myrosinase present in the leaves, although microorganisms can also show a myrosinase-like activity [19, 20].

This study aims to retain the GLS in Indian mustard during the *sayur asin* production and to study how the myrosinase activity affects the GLS concentration during the fermentation. For this purpose, three withering methods were applied to produce Indian mustard leaves with different myrosinase activities prior to fermentation, namely by oven at 35 °C and by MW at 180 and 900 W. Upon withering, the samples were fermented for 7 days, and the GLS concentration and the myrosinase activity were monitored. Previous studies reported the diversity of microbes in traditionally fermented sayur asin [16] and mustard products [21]. Since the present study modifies the production method of *sayur asin*, the microbiological profile of sayur asin was also investigated.

Materials and methods

Preparation of inoculum and fermentation medium for *sayur asin*

Since the withering treatments will greatly reduce the number of natural LAB on the leaves, a standard inoculum was prepared for all three treatments, by taking an aliquot of coconut water of a 5 days fermented *sayur asin* previously withered in the oven at 35 °C. The aliquot was inoculated in the coconut water (Omega & More, Witsenburg Natural Products BV) at 1 % (v/v) to achieve about similar number of total LAB that is reached at the beginning of fermentation of a traditionally withered *sayur asin* (i.e. approximately 6 log CFU/ml).

Preparation of *sayur asin*

Raw Indian mustard was obtained from a local supplier (Oriental Webshop, Duiven, The Netherlands). Indian mustard leaves were separated, washed, drained and randomly divided into portions of 200 g (3–4 leaves). There were three withering methods performed, i.e. by an oven at 35 °C for 2.5 h to mimic the traditional process, by MW at a power of 180 W for 4.5 min to reduce the myrosinase activity, and by MW at 900 W for 2 min to inactivate the myrosinase activity. Hence, the withered leaves containing different levels of myrosinase activity were obtained. Then, salt was added to the withered vegetables (2.5 %, w/w) and rubbed gently by hand, similar to the traditional process. Subsequently, the leaves were immersed in coconut water containing 1 % (v/v) of the standard inoculum, at a ratio of 1:5 (w/v). Each product was packed in 360-mL glass jar and incubated in the dark at a room temperature of 22 °C for 7 days. Each treatment was carried out in triplicate.

Sample preparation

Half of the amount of the vegetable and of the medium in the jar were taken aseptically followed by a quick freezing in liquid nitrogen, while the remaining vegetable and medium of the sample was mixed thoroughly with a stomacher for further microbiological analysis. The raw, withered, and fermented vegetables as well as the fermentation medium were frozen in liquid nitrogen followed by lyophilisation. Subsequently, the vegetables were grinded and stored at −22 °C until further analyses of GLS and myrosinase activity. These analyses were also performed to the fermentation medium.

Myrosinase activity analysis

The myrosinase activity was determined by a coupled enzymatic procedure followed by spectrophotometry measurement [22], in which the produced glucose, from the reaction of native myrosinase and a standard GLS, was measured. A 50 mg lyophilised sample was mixed overnight in 140 mL of 50 mM potassium phosphate buffer (pH 7.0) at 15 °C. On the second day, the plant matrix was removed from the myrosinase solution by centrifugation at 2,670*g* for 10 min followed by clarification (folded filters Grade 595 ½—4–7 µm, Whatman). Subsequently, 2 mL of this solution was filtered (cut-off 30 kD Amicon Ultra-4 Millipore) from the molecules smaller than myrosinase with the aid of centrifugation at 4,000*g* for 10 min. The concentrated enzyme was dissolved in 500 µL of potassium phosphate buffer. Eventually, the myrosinase activity was measured by a D-glucose enzyme

kit (Enzyplus EZS 781+). The reaction mixture consisted of a water solution containing 0.05 g/L of magnesium chloride and 1 g/L ascorbic acid, a ATP/$NADP^+$ solution, buffer solution, hexokinase/glucose-6-*P*-dehydrogenase solution and 50 µL sample solution containing the myrosinase, and sinigrin 30 mg/mL solution. The formation of NADPH was followed by a spectrophotometer (Cary UV 50) at 340 nm for 7 min. To quantify myrosinase activity, an external calibration was determined by following the same procedure for the samples analysis. In the reaction mixture of 50 µL, a standard myrosinase solution instead of the myrosinase sample extract was added. Activity was expressed as unit/g dry material (DM) where one unit produces 1.0 µmol glucose per minute from sinigrin at pH 6.0 at 25 °C.

Glucosinolate analysis

Extraction of GLSs with hot methanol (70 %), containing an internal standard of glucotropaeolin, followed by on-column desulphation and analysed by high-performance liquid chromatography was done in duplicate according to the procedure described by Sarvan et al. [15]. The de-sulpho-GLSs were separated by using the Lichrospher100 column (Merck RP-18, 5 µm) with an attached LiChroCART guard column (RP-18, 4 × 4 mm) (Merck, Darmstadt) at a flow rate of 1 mL/min. The elution was performed by the gradient system of water and acetonitrile. Detection was performed with a DAD detector (Spectra System UV 6000 LP) at a wavelength of 229 nm. The GLSs were identified by the spectra as well as comparing with the external standard of sinigrin and internal standard of glucotropaeolin.

Microbiological analyses

Samples of the fermenting liquid and vegetables were collected from the jars for counting of total bacteria, LAB, yeasts, and *Bacillus cereus* by the standard spread plate method. Total colony counts, LAB, *B. cereus*, and yeasts were counted on Plate Count agar (PCA, Oxoid), deMan-Rogosa-Sharpe agar (MRSA, Merck), Mannitol Yolk Polymyxin agar base (MYP, Merck) plus egg yolk emulsion and *B. cereus* selective supplement, and Oxytetracycline Glucose Yeast Extract agar (OGYE, Oxoid), respectively. Plates of PCA, MYP, and OGYE were aerobically incubated at 35 °C for 2 days, at 32 °C for 1 day, and at 25 °C for 5 days, respectively. Plates of MRS were anaerobically incubated at 35 °C for 3 days. The colonies that developed on each plate were counted.

Statistical analysis

Effects of withering methods, namely the oven, MW 180, and MW 900, on GLS concentration, total LAB, and total colony count during the production of *sayur asin* were analysed by one-way ANOVA and Duncan's multiple range test at the 5 % significance level (IBM SPSS Statistics version 20, U.S.). Each sample was produced in triplicate, and the GLS analysis was performed in duplicate.

Results and discussion

Myrosinase activity

The effect of withering methods on myrosinase activity

Raw leaves of Indian mustard showed a myrosinase activity of 162 ± 56 units/g DM. The effect of the three withering methods on myrosinase activity in the leaves can be seen in the Table 1. Withering by oven at 35 °C and by MW at 180 W retained the myrosinase activity at 16 and 26 %, respectively. While, after a MW treatment at 900 W no myrosinase activity was detected in the leaves. Accordingly, various studies reported that microwaving is an efficient treatment for partial or complete inactivation of myrosinase in *Brassicas,* by varying the time–temperature and power [23–25]. The MW conditions and amount and type of vegetables determine largely the degree of enzyme inactivation. Moreover, thermal inactivation of myrosinase depends also on the plant source, with reported inactivation temperatures in the range from 30 to 60 °C [26, 27].

Myrosinase activity during the fermentation

The present study shows that the remaining myrosinase activity in the oven and MW at 180 W treated samples was almost completely lost after one day of fermentation (Table 1). Further fermentation led to complete loss of myrosinase activity in all samples. Myrosinase activity was also not detected in the fermentation medium (data not shown). In accordance, Kim and Rhee [12] reported a decrease of myrosinase activity from the radish tissue after 3 days of fermentation until less than 3 %. They explained this by reduced stability due

Table 1 Myrosinase activity (units/g DM) of withered and fermented Indian mustard leaves ($n = 3$)

	Withering treatment		
	Oven	MW 180	MW 900
After withering	26.5 ± 1.9	41.9 ± 4.7	n.d.
Fermentation day-1	0.043 ± 0.004	0.109 ± 0.018	n.d.
Fermentation day-3–7	n.d.	n.d.	n.d.

Oven, MW 180, and MW 900 = oven at 35 °C for 2.5 h, MW at 180 W for 4.5 min, and at 900 W for 2 min, respectively

n.d. not detectable

Values are presented as mean ± SD

Table 2 Glucosinolate concentration (μmol/10 g DM) in Indian mustard leaves treated by three withering methods ($n = 6$)

Glucosinolate	Raw leaves	Withering treatment		
		Oven	MW 180	MW 900
Sinigrin	178.9 ± 51.6^{c}	53.3 ± 19.0^{a}	85.1 ± 8.2^{b}	86.0 ± 21.2^{b}
4-Hydroxy-glucobrassicin	1.1 ± 0.6^{a}	1.0 ± 0.4^{a} ($n = 5$)	1.3 ± 0.5^{a}	1.5 ± 1.3^{a}
Glucobrassicin	1.4 ± 0.6^{b}	0.5 ± 0.4^{a} ($n = 5$)	0.4 ± 0.08^{a} ($n = 5$)	0.5 ± 0.3^{a}
4-Methoxy-glucobrassicin	3.3 ± 0.9^{c}	1.1 ± 0.4^{a}	2.3 ± 0.4^{b}	1.9 ± 0.9^{b}
Total glucosinolates	184.7	55.9	89.1	89.9

Oven, MW 180, and MW 900 = oven at 35 °C for 2.5 h, MW at 180 W for 4.5 min, and at 900 W for 2 min, respectively

Mean reported of duplicate analyses from 3 treatment replications

Values are presented as mean ± SD

Values with superscript letters on the same row, which are different are significantly different ($P < 0.05$)

to the decrease of pH and the possible production of microbial proteolytic enzyme during fermentation. The initial pH of the fermentation medium in the present study was 4.6 and decreased after 1 day and during fermentation of *sayur asin* of all treatments to the range of 3.3–3.7. Moreover, the high water activity of the sayur asin may affect the myrosinase stability. A previous study reported that the inactivation rate of myrosinase in broccoli increases with the increase of water activity of the sample [28].

Glucosinolates concentration

Glucosinolates in raw Indian mustard

Sinigrin was found to be the predominant aliphatic GLS in Indian mustard accounting for more than 95 % of the total GLSs with a concentration of 179 ± 52 μmol/10 g DM (Table 2). Many studies on the corresponding isothiocyanate of the sinigrin, the allyl isothiocyanate, show that the isothiocyanate inhibits proliferation of human prostate and bladder cancer cells [29, 30].

Three indolyl GLSs, i.e. 4-hydroxy-glucobrassicin, glucobrassicin, and 4-methoxy-glucobrassicin, were detected in the Indian mustard leaves in total accounting for about 5.8 μmol/10 g DM. In general, the profile of the main GLSs is in accordance with previous studies on Indian mustard [31–33]. However, other minor GLSs, such as progoitrin, glucobrassicanapin, gluconapin, neoglucobrassicin, and gluconasturtiin, have been reported [33, 34]. Differences in the GLS concentration and profile could be due to the variations in cultivars, growing conditions, environmental factors, and the analytical methods [2, 11, 33–35].

The effect of withering methods on glucosinolates

Withering Indian mustard leaves caused a considerable reduction of total GLSs ranging from about 50 % by MW treatments to 70 % by oven treatment (Table 2). Decrease of GLSs during withering could be caused by thermal degradation and myrosinase hydrolysis. Previous studies reported a lower GLS loss upon MW treatment on cabbage [23, 25] than the present study. The different size and shape as well as structural matrix between Indian mustard and cabbage could have led to a different loss of GLSs in Indian mustard. Moreover, the damage of the cell membranes in the leaves containing active myrosinase, followed by diffusion, may have allowed myrosinase to hydrolyse the GLSs, which can occur at a different rate for different vegetables.

Glucosinolates during fermentation

After the first day of fermentation, there was a marked drop of total GLSs in all samples (Table 3). The MW at 180 W treated sample led to the greatest loss of up to 95 %, followed by up to 90 % for the oven-treated sample, and up to 70 % for the MW at 900 W treated sample, relative to the total GLS concentration upon withering. Furthermore, GLSs were not detectable after the third day of fermentation in the *sayur asin* previously withered in the oven and in the MW at 180 W. The withering by MW at 900 W resulted in a retention of sinigrin of 13 % upon fermentation when compared to the concentration after withering (Table 3), while negligible amount of sinigrin was found in the fermentation medium (data not shown).

Accordingly, previous studies reported that fermentation can significantly reduce the amount of GLSs in vegetables [9–11, 36]. During *sauerkraut* production, Tolonen et al. [9] observed a very low quantity of 4-methoxy-glucobrassicin, while others reported traces or even no detectable GLSs in the fermented product [10, 11]. Kim and Rhee [12] also reported a 25 % loss of total GLSs in radish (*Raphanus sativus* L.) compared to the total GLSs in raw radish on the third day of radish *kimchi* fermentation. Moreover, Suzuki et al. [36] reported smaller quantities of the GLSs in

Table 3 Glucosinolate concentration (μmol/10 g DM) in Indian mustard leaves during the production of *sayur asin* ($n = 6$)

Glucosinolate	Withering treatment	Fermentation (days)			
		1	3	5	7
Sinigrin	Oven	4.5 ± 2.6^{a}	n.d.	n.d.	n.d.
	MW 180	3.3 ± 0.7^{a}	n.d.	n.d.	n.d.
	MW 900	$22.6 \pm 5.8^{b,2}$	12.3 ± 2.9^{1}	11.3 ± 1.8^{1}	11.4 ± 2.5^{1}
4-Hydroxy-glucobrassicin	Oven	0.6 ± 0.3^{ab}	n.d.	n.d.	n.d.
	MW 180	0.3 ± 0.1^{a}	n.d.	n.d.	n.d.
	MW 900	$1.2 \pm 0.7^{b,2}$	1.4 ± 0.5^{2}	1.7 ± 1.1^{2}	0.4 ± 0.2^{1}
Glucobrassicin	Oven	n.d.	n.d.	n.d.	n.d.
	MW 180	n.d.	n.d.	n.d.	n.d.
	MW 900	n.d.	n.d.	n.d.	n.d.
4-Methoxy-glucobrassicin	Oven	0.2 ± 0.1^{ab}	n.d.	n.d.	n.d.
	MW 180	0.2 ± 0.05^{a}	n.d.	n.d.	n.d.
	MW 900	0.4 ± 0.1^{b}	0.2 ± 0.06	n.d.	n.d.

Oven, MW 180, and MW 900 = oven at 35 °C for 2.5 h, MW at 180 W for 4.5 min, and at 900 W for 2 min, respectively

n.d. not detectable

Mean reported of duplicate analyses from 3 treatment replications

Values are presented as mean ± SD

Values with superscript letters on the same column and superscript number on the same row, which are different are significantly different ($P < 0.05$)

nozawana-zuke, a fermented product of *Brassica rapa* L. in Japan, relative to the raw *nozawana*. It could be that the cell and vacuole walls were damaged during fermentation due to the increase of osmotic pressure as a result of difference concentration of salt and water activity. Consequently, hydrolysis of GLSs by active myrosinase could have occurred.

The sample that was withered by MW at 900 W does not contain active myrosinase showed a much lower reduction of GLSs during fermentation. Accordingly, Sarvan et al. [15] observed that GLSs were better retained during fermentation in *sauerkraut* where myrosinase was inactivated (by blanching) prior to fermentation. However, the GLSs were reduced (about 50 % reduction for sinigrin) in the pre-blanched upon 71 h fermentation samples [15]. In the present study, about 80 % of GLSs were lost during the fermentation even when the myrosinase was inactivated upon withering. Therefore, it might be that during fermentation of the *sayur asin* some myrosinase-like activity was produced by bacteria that catalysed the GLS hydrolysis [19, 20].

The present study shows that inactivation of myrosinase in Indian mustard by using a MW heat treatment can lead to a partial retention of GLS upon fermentation in the product. Ingestion of GLS-containing products without active plant myrosinase can still result in the formation and bioavailability of bioactive breakdown products by enzymes from the gut flora, although the catalytic efficiency of the plant endogenous myrosinase is higher [6, 19, 37–39].

The GLSs breakdown product formation upon withering and fermentation should be also analysed to better understand the mechanisms that lead to different breakdown profiles in the final product. Among many other factors, the pH plays an important role to influence the breakdown product profile [2]. Moreover, the activity of epithiospecifier proteins may also affects the further conversions upon enzymatic GLS hydrolysis, i.e. the nitriles formation is higher when such proteins are active [2].

LAB and colony count during *sayur asin* fermentation

The natural LAB on the leaves were not detected after MW withering. Therefore, a standard inoculum was added into coconut water prior to fermentation. The total LAB and total colony count in *sayur asin* during fermentation are shown in Table 4. Total LAB increased only until the first to third day of fermentation followed by a steady decline into 7.1–7.3 log CFU/mL. There is no difference in effect of withering methods on the total LAB in the samples. It is likely that in each sample, there were similar conditions that led to similar LAB growth. A previous study on microbial ecology of *sayur asin* reported a successional development of species within the LAB group during *sayur asin* fermentation. Fermentation was initiated by *Leuconostoc mesenteroides* and *Lactobacillus confusus* and completed by *Lac. curvatus, Pediococcus pentosaceus* and *Lac. plantarum* [16].

Another study on microbial community of *fu-tsai* and *suan-tsai*, traditional fermented mustard (*B. juncea* Coss.) products of Taiwan, reported a high level of diversity in

Table 4 Total LAB and total colony count (log CFU/mL) during *sayur asin* fermentation ($n = 3$)

	LAB	COL	Withering treatment					
			Oven		MW 180		MW 900	
			LAB	COL	LAB	COL	LAB	COL
Raw leaves	4.4 ± 0.0	7.2 ± 0.2						
After withering			n.d.	3.4 ± 0.1^{a}	n.d.	5.7 ± 0.5^{b}	n.d.	6.4 ± 0.5^{b}
Fermentation day-1			8.9 ± 0.2^{1}	8.8 ± 0.1^{a}	9.0 ± 0.3^{1}	9.2 ± 0.1^{b}	9.1 ± 0.1^{1}	9.1 ± 0.1^{b}
Fermentation day-3			9.1 ± 0.2^{1}	9.0 ± 0.1^{a}	9.3 ± 0.1^{1}	8.9 ± 0.1^{a}	9.0 ± 0.2^{1}	9.0 ± 0.2^{a}
Fermentation day-5			8.0 ± 0.3^{1}	7.9 ± 0.4^{a}	7.9 ± 0.2^{1}	7.9 ± 0.1^{a}	7.8 ± 0.2^{1}	7.7 ± 0.2^{a}
Fermentation day-7			7.2 ± 0.2^{1}	6.7 ± 0.2^{a}	7.1 ± 0.3^{1}	6.9 ± 0.3^{ab}	7.3 ± 0.2^{1}	7.3 ± 0.2^{b}

Oven, MW 180, and MW 900 = oven at 35 °C for 2.5 h, MW at 180 W for 4.5 min, and at 900 W for 2 min, respectively

LAB, COL total lactic acid bacteria, total colony count

n.d. not detectable

Values are presented as mean ± SD

Values with superscript letters or numbers on the same row, which are different are significantly different ($P < 0.05$)

LAB at the different stages of fermentation, including *Enterococcus faecalis*, *Lac. alimentarius*, *Lac. brevis, Lac. plantarum, Leu. citreum*, *Leu. mesenteroides*, *Ped. pentosaceus*, *Weissella cibaria*, and others [21]. The diversity of LAB was also reported during fermentation of cabbage *kimchi* [17] and *sauerkraut* [40].

The present study also found that total colony in *sayur asin* increased to 8.8–9.2 log CFU/mL after the first day of fermentation as compared to the ones in the withered leaves, followed by a decline into about 7.7–7.9 and 6.7–7.3 log CFU/mL after the fifth and seventh day of fermentation, respectively. Accordingly, Puspito and Fleet [16] reported the total bacterial count is similar with the total LAB count; therefore, LAB are mainly responsible for the fermentation of *sayur asin* fermentation. Additionally, total yeast was detected at 2.6 ± 0.4 log CFU/mL at day 0, but no yeast colonies were detected after the seventh day of fermentation (data not shown). At day 0, total *B. cereus* was also detected, but uncounted due to interference of other microbes. While at the seventh day of fermentation, the total *B. cereus* was not detected (data not shown).

Conclusions

Sinigrin is the most dominant GLS in raw Indian mustard, accounting for about 95 % of the total GLSs with a concentration of 179 ± 52 μmol/10 g DM. Both withering and fermentation substantially reduced the GLS concentration in the final *sayur asin* product. The myrosinase activity contributed strongly to the remaining GLS in the vegetable upon fermentation. A withering treatment that fully inactivates myrosinase results in a fermented product containing substantial amounts of GLS, whereas treatments with residual myrosinase activity result in products void of GLS. The total LAB and total colony counts were not affected by the preparation methods. Lactic acid bacteria are mainly responsible for the fermentation of *sayur asin*.

The present study revealed the importance of myrosinase activity on GLS concentration of Indian mustard during fermentation. This finding can contribute to design the *sayur asin* production in order to obtain a final product that has higher GLS concentration plus the probiotics of a fermented product. Nevertheless, further studies are needed to identify the factors, i.e. pH, salinity, substrate, and LAB, and the breakdown products of the GLS hydrolysis to better understand the mechanisms underlying the GLS degradation during fermentation. Moreover, other withering methods can be investigated such as blanching and steaming, to better retain GLSs upon *sayur asin* production.

Acknowledgments The project entitled *Glucosinolates Behaviour throughout the Production of "Sayur Asin"* was funded by the International Foundation for Science (IFS, Sweden), Grant No. E/5360-1. The authors thank Xandra Bakker-de Haan for the microbiological analysis assistance.

Conflict of interest None.

Compliance with Ethics Requirements This article does not contain any studies with human or animal subjects.

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