

The impact of fermentation on the antioxidant activity and bioactive components in crucifers

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ABSTRACT

Crucifers have been widely recognized for their chemical components contributing to their pharmacological benefits. This study investigated the changes in glucosinolate (GSL) and isothiocyanate (ITC) levels in five cruciferous vegetables—alugbati (*Basella rubra*), cabbage (*Brassica oleracea* var. *capitata*), mustard (*Brassica juncea*), radish (*Raphanus sativus* var. *longipinnatus*), and Napa cabbage (*Brassica rapa* ssp. *pekinensis*)—following a 14-day fermentation period using high-performance liquid chromatography (HPLC). Furthermore, the effects of fermentation on the antioxidant activity of the vegetables were also determined using the DPPH assay. Results showed that raw extracts had high GSL levels, with concentrations ranging from 19.55 to 384.07 $\mu\text{mol/g}$, while fermented samples obtained lower GSL levels ranging from 6.90 to 49.16 $\mu\text{mol/g}$. An increase in the ITC contents was observed in both raw and fermented samples after subjecting to hydrolysis in the presence of exogenous myrosinase enzyme. DPPH assay revealed that raw vegetables exhibited varying antioxidant activities with $\text{IC}_{50} < 200$ ppm. Raw cabbage showed the strongest antioxidant activity with $\text{IC}_{50} = 25.32 \pm 3.96$ ppm, while the rest of the raw samples only exhibited intermediate activity with IC_{50} values ranging from 50 to 100 ppm. On the other hand, fermented vegetables exhibit IC_{50} values ≥ 200 ppm, indicating weak antioxidant activities. Therefore, the results from both chromatographic and DPPH assays indicate that fermentation further decreases the metabolite concentration and antioxidant capacity of the vegetable samples due to the chemical degradation of bioactive components. Controlled and monitored fermentation conditions play crucial roles in preserving GSL, ITC, and other bioactive components in crucifers.

Keywords: crucifers; fermentation; glucosinolates; isothiocyanates; antioxidant activity

INTRODUCTION

Cruciferous vegetables or crucifers are crops that belong to the family of *Brassicaceae* or *Cruciferae* (Ishida et al., 2014). While they are a genetically diverse group, most species are characterized by having four equal-sized petals that resemble crosses. This pattern is generally observed among the members of the *Brassica* genus, including cabbage, cauliflower, kale, mustard, broccoli, Brussels sprouts, and turnips (Higdon et al., 2007). Other commonly consumed cruciferous vegetables include radish, arugula, wasabi, horseradish, and watercress. They are one of the dominantly cultivated crops and are economically important plants for many reasons, including their vast health and pharmacological implications (Higdon et al., 2007). They contain vitamins and other bioactive components like glucosinolates, phenolic compounds, tocopherols, and fatty acids (McNaughton and Marks, 2007). Incorporating these vegetables into one's diet can significantly improve their health due to their high-nourishing properties. As a result, these vegetables have been subject to numerous studies that aimed to elucidate their potential pharmacological properties (Sikora et al., 2008; Šamec et al., 2016; Favela-González et al., 2020).

Glucosinolates (GSL, Figure 1a) are predominant sulfur-containing phytochemicals that are assumed to be one of the prime sources for brassica vegetables' nutritional and health-related effects. The hydrolysis of this compound, via myrosinase enzymatic activity, generates several biologically active products, including isothiocyanates (ITC, Figure 1b) (Dinkova-Kostova and Kostov, 2012). These hydrolysis products and their glucosinolate precursors have been investigated for several pharmacological purposes but mainly for cancer preventive studies (Sharma et al., 2024). The cancer-preventive properties of these constituents are further being analyzed and judged as to whether they can be alternative sources for chemo-preventive therapies. Brassica vegetables are also rich in phenolic compounds, another bioactive metabolite known for its potent antioxidant properties (Podsedeck, 2007). Because of their high dietary phenolic content, their antioxidant capacities have been observed and investigated in recent years (Jaiswal et al., 2011; Podsedeck et al., 2006; Soengas et al., 2011). The increased attention and popularity for natural sources of antioxidants were mainly attributed to their health properties and capacity to reduce oxidative damage from radical oxygen species. Furthermore, GSLs and ITCs have been evident in non-Brassica vegetables, including Malabar spinach, locally known as alugbati (*Basella rubra*), and drumstick/horseradish tree, locally known as malunggay (*Moringa oleifera* L.), which have also exhibited similar activities with other cruciferous vegetables (Fahey, 2005).

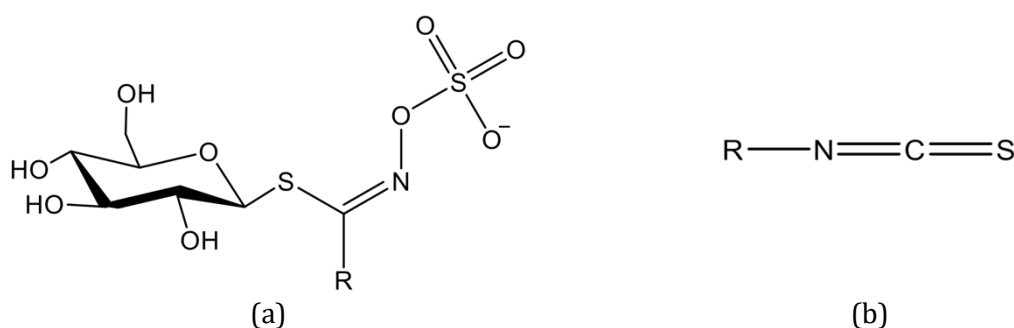


Figure 1. The general structures of glucosinolate (GSL) **(a)** and isothiocyanate (ITC) **(b)**. The structures may vary depending on the R-group attached, which could be alkenyl, aliphatic, aromatic, or indole side chains.

Although most vegetables can be consumed raw, they are still commonly subjected to various processing techniques such as heating, cooling, sun-drying, fermenting, and pickling, which could diminish some nutrients. However, not all nutrients and bioactive compounds are susceptible to changes due to processing and may be retained during preparation. As such, it is crucial to investigate the effects of these methods on the stability and content of cruciferous vegetables, specifically on their effects on the levels of glucosinolates and bioactive isothiocyanates. These studies identify which method can produce desirable results for practical health and nutritional purposes, such as increased or retained glucosinolate and antioxidant levels (Palermo et al., 2014; Rungapamestry et al., 2007).

The study aimed to characterize glucosinolates and isothiocyanates in Malabar spinach or alugbati (*Basella rubra*), cabbage (*Brassica oleracea* var. *capitata*), mustard (*Brassica juncea*), radish (*Raphanus sativus* var. *longipinnatus*), and Napa cabbage (*Brassica rapa* ssp. *pekinensis*) and compare the GSL and ITC contents in raw and fermented samples, using HPLC. The study also investigated the antioxidant properties of the vegetable extracts using a DPPH assay to determine the effects of fermentation on the samples.

METHODS

Materials and Equipment. Vegetable samples were obtained from local markets. Reagent (ACS) grade methanol (Thermo Fisher Scientific) was used as the solvent for GSL extraction. The desulfation utilized sulfatase from *Helix pomatia* (Sigma-Aldrich), DEAE Sephadex A25 ion exchange resin (Sigma-Aldrich), and acetate buffer at a pH of 4.0. Sinigrin (Sigma-Aldrich) was used as the internal standard. HPLC-grade acetonitrile (Duksan Pure Chemical, Co. Ltd.) and distilled water were used as the mobile phase in the HPLC analysis of GSLs. Reagent grade dichloromethane (DCM, Thermo Fisher Scientific) was used for ITC extraction, while myrosinase (Sigma-Aldrich) was used for the hydrolytic conversion of glucosinolates to isothiocyanates. Cyclocondensation of allyl isothiocyanate (Sigma-Aldrich) and hydrolysates was carried out using 1,2-benzene-dithiol (Merck, Inc.) using 80:20 HPLC grade methanol (Duksan Pure Chemical, Co. Ltd.) and water. The antioxidant assay required 80 mM DPPH (2,2-diphenyl-1-picrylhydrazyl, Sigma-Aldrich), while ascorbic acid (Thermo Fisher Scientific) and quercetin (Sigma-Aldrich) were used for positive controls. Samples were lyophilized using a Telstar LyoQuest benchtop freeze dryer. Analyses were run on an Agilent Technologies 1200 Series HPLC equipped with a reversed-phase C18 column for the glucosinolate and isothiocyanate contents. DPPH assay was performed using a FLUOstar Omega multimode microplate reader equipped with a UV/Vis spectrometer for absorbance detection at 517 nm.

Sample Preparation. Vegetable samples were obtained from a local supermarket. The leaf and rib parts of the cabbage, mustard, and Napa cabbage were used, while only the leaf part was used in alugbati. The peels were removed for radish samples, and only the taproot portion was used. The samples were washed, chopped, dried, and weighed into a mass of 100 grams. The raw samples were subjected to lyophilization for 48 hours. While the other samples were subjected to fermentation.

Fermentation of Vegetable Samples. Fresh samples were washed, chopped into small pieces, and dried in a paper towel. The samples were each weighed into a mass of 100 grams. The weighed samples were placed in separate jars and filled with enough 3% (w/v) salt solution prepared from table salt (sodium chloride) in distilled water, ensuring the samples were completely submerged. The jar lids were tightly sealed and stored in the laboratory at room temperature for 14 days. The fermented samples were subjected to lyophilization for 48 hours. The raw and fermented samples were ground to fine powder, placed in separate labeled Ziploc bags, and stored in a cool, dry place.

Glucosinolate Analysis. Ground samples were extracted using hot methanol for 20 minutes to obtain the GSL extracts. The extracts were then desulfated using a DEAE Sephadex ion exchange resin. The extracts were treated with the sulfatase enzyme at pH 4 and incubated for 16 hours at room temperature. The desulfated samples were collected in HPLC vials by eluting the column with 1.25 mL of distilled water. The desulfated extracts were subjected to HPLC analysis to separate and quantify glucosinolates. A 250 x 4.6 mm reversed phase C18 column was used. The mobile phase consists of distilled water and acetonitrile fixed in a gradient system starting with 100% distilled water. The overall elution time per injection is 27.0 minutes. The flow rate of the samples was maintained at 1.5 mL/min with each injection of a 10 µL sample. Sinigrin was used as an internal standard. Samples were read at 234 nm using a UV detector. Only one trial was done per sample due to the length of the analysis time and sample stability.

Isothiocyanate Analysis. The samples were divided for the unhydrolyzed and hydrolyzed treatments. Unhydrolyzed refers to a sample that did not undergo any treatment. On the other hand, hydrolyzed samples are obtained from samples treated with 5 µL myrosinase enzyme, 40 µL 18.75 mM ascorbic acid, and 5 mL distilled water incubated at room temperature for 2 hours. Both unhydrolyzed and hydrolyzed samples were subjected to DCM extraction to obtain ITC. The extracts (100 µL) were treated with 600 µL 10 mM 1,2-benzene-dithiol and 500 µL 0.10 M potassium phosphate buffer (pH 8.5). The mixtures were vortexed for 1 minute and incubated at 65 °C for 2 hours in a heat block to undergo cyclocondensation. The samples were then transferred and filtered into HPLC vials and subjected to analysis using a reverse phase column. An isocratic solvent system of 80% methanol in water was used with a flow rate of 2.0 mL/min for 10 minutes. The eluates were monitored with a UV detector at 365 nm. Triplicate measurements were done for this analysis. The total isothiocyanates were calculated from varying concentrations (0.50 to 5.00 µM) of allyl isothiocyanate (100 µL) treated similarly with 600 µL 10 mM 1,2-benzene-dithiol and 500 µL 0.10 M potassium phosphate buffer (pH 8.5).

DPPH Assay. The samples were extracted using hot methanol for 20 minutes, which was similar to the GSL extraction. The extracts were dried and weighed. The samples were reconstituted in methanol to obtain a stock solution with a concentration of 1 mg/mL. Final concentrations of 12.5 ppm to 200 ppm were used for the assay. Samples were treated with 160 µM DPPH reagent and were incubated in the dark for 30 minutes. The samples were subjected to spectrophotometric analysis at 517 nm using a FLUOstar Omega multimode microplate reader. Quercetin and ascorbic acid were used as positive controls. Triplicate measurements were done for this assay and the DPPH activity was calculated using the formula (Equation 1).

$$\text{DPPH radical scavenging activity} = \left(\frac{A_{517} \text{ blank} - A_{517} \text{ sample}}{A_{517} \text{ blank}} \right) \times 100$$

Equation 1. Formula for DPPH scavenging activity

RESULTS AND DISCUSSION

Effect of Fermentation on GSL. The desulfated glucosinolate content of the freeze-dried vegetable extracts was quantified through HPLC analysis, using sinigrin as an internal standard. The total GSL concentration (µg/mol) was determined by peak area comparison of the sample with the sinigrin standard (Figures 2-6).

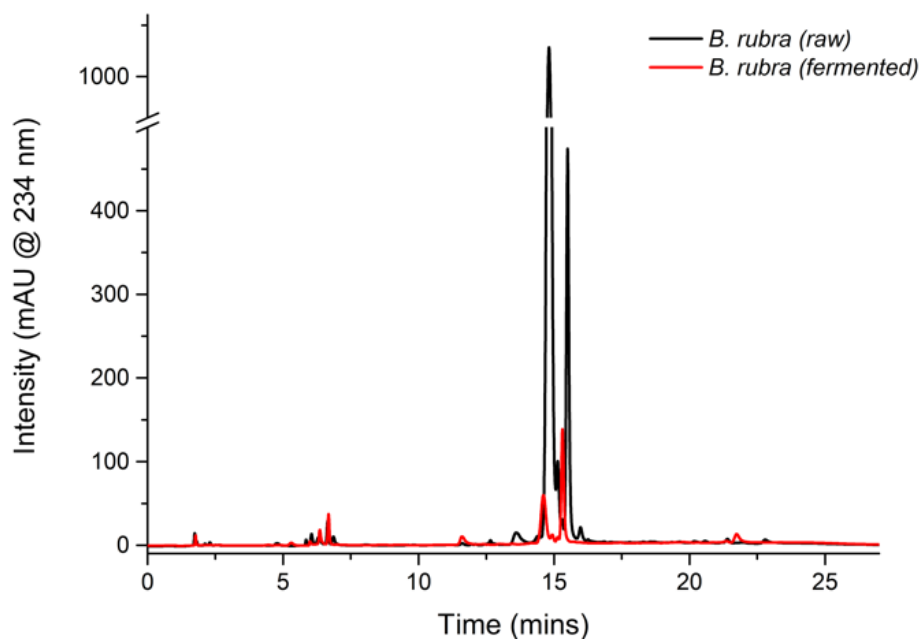


Figure 2. HPLC chromatogram of *B. rubra* (alugbati); raw (*black*), fermented (*red*)

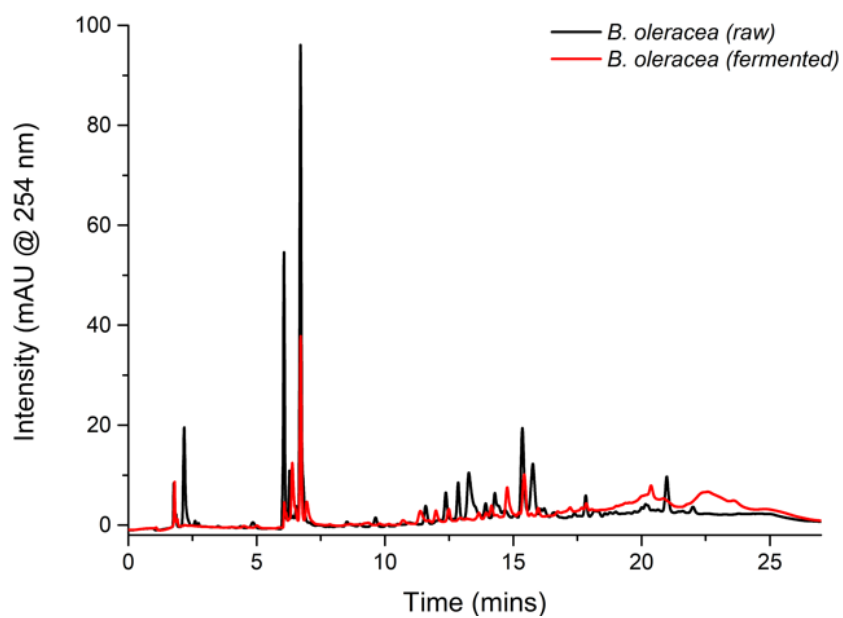


Figure 3. HPLC chromatogram of *B. oleracea* (cabbage); raw (*black*), fermented (*red*)

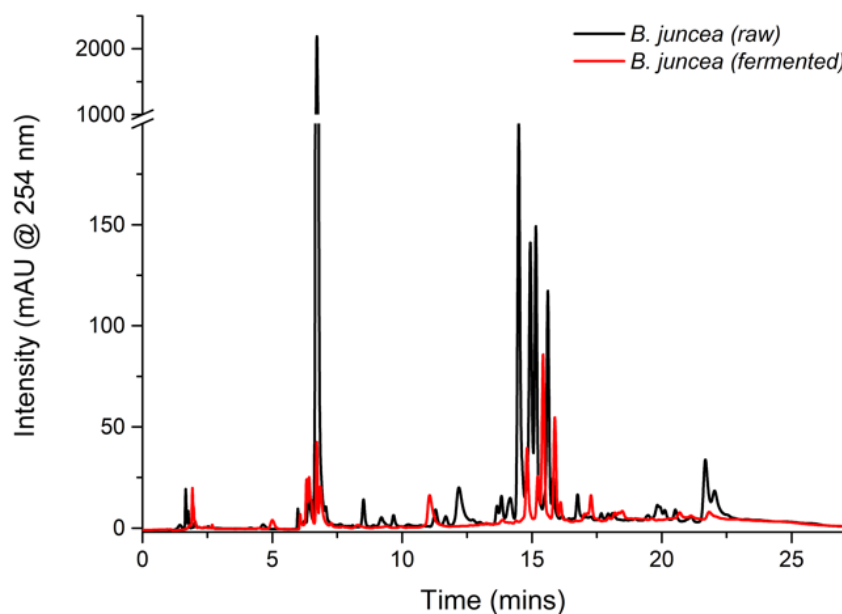


Figure 4. HPLC chromatogram of *B. juncea* (mustard); raw (*black*), fermented (*red*)

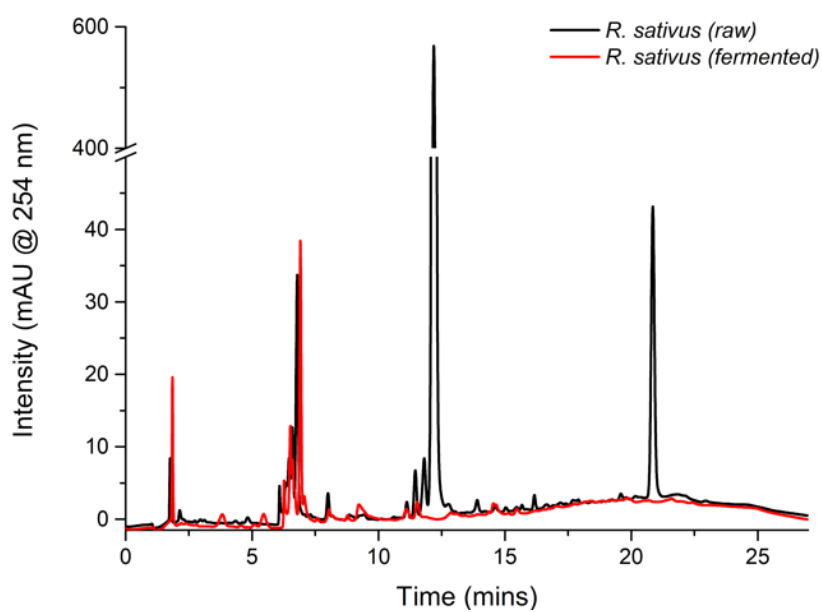


Figure 5. HPLC chromatogram of *R. sativus* (radish); raw (*black*), fermented (*red*)

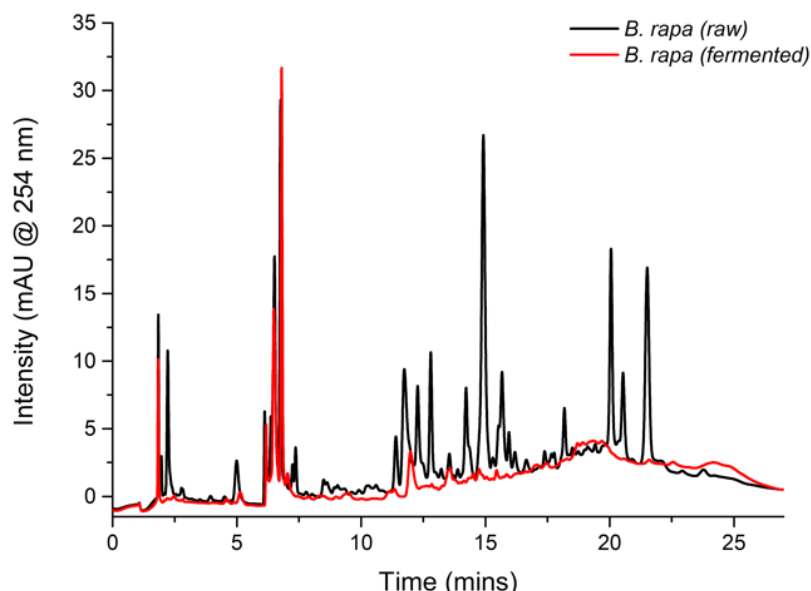


Figure 6. HPLC chromatogram of *B. rapa* (Napa cabbage); raw (*black*), fermented (*red*)

Table 1. Total GSL content of vegetable extracts

Sample	Total GSL ($\mu\text{mol/g}$)		
	Raw	Fermented	%GSL retained
alugbati (<i>B. rubra</i>)	384.07	42.99	11.29%
cabbage (<i>B. oleracea</i>)	24.07	7.03	29.20%
mustard (<i>B. juncea</i>)	342.93	49.16	14.34%
radish (<i>R. sativus</i>)	111.64	7.84	7.02%
Napa cabbage (<i>B. rapa</i>)	19.55	6.90	35.31%

The analysis revealed that all samples consistently experienced a sharp decrease in total GSL content, as shown in Table 1, indicating the loss of glucosinolates after being subjected to fermentation for 14 days. The percentage (%) glucosinolates retained in the fermented samples ranged from 7.02% to 35.31%, suggesting either the degradation or transformation of the metabolite. The overall loss of GSL in the fermented extracts coincided with the findings of Sarvan et al. (2013), who reported that the compound is nearly absent in its native form due to its immediate hydrolysis and further transformations into its respective derivatives. After two weeks of fermentation, Daxenbichler et al. (1980) found that no unhydrolyzed GSL was detected in their cruciferous vegetable extracts.

One primary contributing factor to the reduction of GSL levels during fermentation is the compound's degradation into various bioactive components. This can be attributed to the presence of lactic acid bacteria that thrive in fermentation conditions (Mullaney et al., 2013). As mentioned, plant myrosinase is the principal catalytic agent in the breakdown of glucosinolates. It has been discovered that even without plant-sourced enzymes, some bacteria can induce the same reaction due to their myrosinase-like activity (Elfoul et al., 2001; Lai et al., 2010). Lactic acid bacteria and other fermenting microbes have essential roles in the general fermentation process since they mainly account for converting sugars into lactic acid, thus resulting in the acidic pH environment in fermented products (Mullaney et al., 2013). The involvement of such microbes in the decomposition of glucosinolates has already been previously established in several studies. A study by Palani et al. (2016) validated the direct relationship between colony count and glucosinolate level reduction, wherein the highest colony count occurred at the sharpest decline in glucosinolate levels. With this, it can be presumed that lactic acid bacteria may enhance the

degradation of glucosinolates into their various breakdown products. However, further microbial analysis must be conducted to ascertain the presence and amount of fermenting bacteria in both the vegetable extracts and brine solution. In addition to this, the observable decrease in GSL levels was attributed to the leaching or transferring of glucosinolates from the vegetable to the brine solution due to the softening of the vegetable's tissue fibers (Ciska and Pathak, 2004).

This study revealed that 3% (w/w) of brine solution induced 61.92% to 98.37% GSL loss after 14 days of fermentation. This data agreed with a study by Nugraherdi et al. (2014), where the effect of fermentation on the glucosinolate levels of cruciferous vegetables was examined with 2.5% (w/w) of salt for their brine solution. Their results revealed that approximately 70% to 95% of glucosinolates were lost after fermentation. Subsequently, it was reported that up to 25% of GSLs were degraded after three days of fermentation in radish (Kim and Rhee, 1993). Nugraherdi et al. (2013) described how the preservation process most likely caused significant deterioration to the vacuole and cell walls of the plants, given the difference in salt and water concentrations imparted by the brine solution, thus causing high osmotic pressure. As such, high extracellular salinity can induce further leaching and transfer of glucosinolates into the brine. When compounded by the metabolic activity of lactic acid bacteria, the factors impart greater diminishing effects on glucosinolate levels (Mullaney et al., 2013). These observations further imply that the general biochemical conditions in the fermentation process are unfavorable to the given vegetable samples in terms of retaining optimal glucosinolate levels. Given these results, the preservation technique would not be recommended for maximizing the pharmacological properties of GSL derivatives for one's consumption due to its diminishing effects on the total glucosinolate content.

Effect of Fermentation on ITC. The vegetable extracts were subjected to HPLC analysis to establish the presence of isothiocyanates in the unhydrolyzed and hydrolyzed samples. The extracts were treated with 1,2-benzene-dithiol producing 1,3-benzenedithiol-2-thione, which directly measures the total ITC content present in the samples. Allyl isothiocyanate of varying concentrations was used for standard calibration purposes.

The ITC concentrations (μM) of the hydrolyzed and unhydrolyzed extracts were then quantified and reported in Table 2. It was observed that the isothiocyanate content evidently increased after being subjected to hydrolysis for all raw and fermented vegetables. These findings were consistent with published literature that reported how the presence of water allows myrosinase to hydrolyze glucosinolates and transform them into their respective isothiocyanate compounds (Hanley et al., 1990). Similarly, the lower ITC content in unhydrolyzed samples further validated the breakdown mechanism of GSL since the lack of water prevents the hydrolysis of the thioglucoside bond to produce the aglycone intermediate and their ITC products.

Table 2. ITC concentrations of unhydrolyzed and hydrolyzed samples

	Concentration (μM)	
	Unhydrolyzed	Hydrolyzed
Raw alugbati (<i>B. rubra</i>)	3.48 ± 0.89	127.15 ± 4.33
Fermented alugbati (<i>B. rubra</i>)	3.30 ± 0.92	6.78 ± 1.84
Raw cabbage (<i>B. oleracea</i>)	1.81 ± 0.36	21.12 ± 5.19
Fermented cabbage (<i>B. oleracea</i>)	3.40 ± 0.57	4.81 ± 3.49
Raw mustard (<i>B. juncea</i>)	2.67 ± 0.14	38.45 ± 6.09
Fermented mustard (<i>B. juncea</i>)	3.72 ± 0.21	4.87 ± 1.70
Raw radish (<i>R. sativus</i>)	3.88 ± 1.03	8.38 ± 1.76
Fermented radish (<i>R. sativus</i>)	2.76 ± 0.86	12.11 ± 4.07
Raw Napa cabbage (<i>B. rapa</i>)	4.15 ± 1.23	28.91 ± 5.35
Fermented Napa cabbage (<i>B. rapa</i>)	2.12 ± 0.75	3.25 ± 1.82

The data shows no distinguishable trend among the ITC concentrations of unhydrolyzed extracts. This suggests that ITC levels in raw and fermented samples remain minimal without hydrolysis. The absence of water accounts for limited glucosinolate hydrolysis, denoting glucosinolates in the extracts instead of isothiocyanates. This remains consistent with literature where raw plant materials were found to possess an abundance of native glucosinolates (Šalić & Šamec, 2022). This is further confirmed by the hydrolyzed trend, where an increase in ITC levels signifies that the inherent glucosinolates have been hydrolyzed into isothiocyanates.

However, the fermentation process drives the hydrolysis of glucosinolates to produce their respective degradation products, such as isothiocyanates (Šalić & Šamec, 2022). Hence, it is expected that even before hydrolysis, fermented extracts should display significant levels of ITC, but the trend says otherwise. Minimal ITC levels have been observed for unhydrolyzed extracts, which suggests that glucosinolates may have shifted to a different metabolic pathway upon fermentation producing other derivatives or these native glucosinolates may have completely deteriorated, hence, no ITCs have been formed (Sikorska-Zimny, & Beneduce, 2020). This further explains why no significant change was observed upon hydrolysis.

After validating the implications of water on glucosinolate degradation to isothiocyanates, fermentation's effect was investigated by comparing the ITC content (μM) between raw and fermented hydrolyzed extracts and calculating the percent (%) ITC retained. It was observed in Figure 7 that the hydrolyzed extracts of alugbati (*B. rubra*), cabbage (*B. oleracea*), mustard (*B. juncea*), and radish (*B. rapa*) exhibited a great reduction in isothiocyanate concentration after being subjected to fermentation. These findings suggest that the fermentation process negatively alters the ITC levels of hydrolyzed vegetables based on its diminishing effects. The reduction in ITC levels of hydrolyzed samples was anticipated due to the preliminary observations of the reduction in GSL levels. Since a decrease in GSLs was reported, the consequent reduction in their production of ITCs was proportionally small. The decline in ITC content was then directly attributed to the deteriorating effects of fermentation on their intact precursor compounds. This was similar to the study of Jang & Kim (2017).

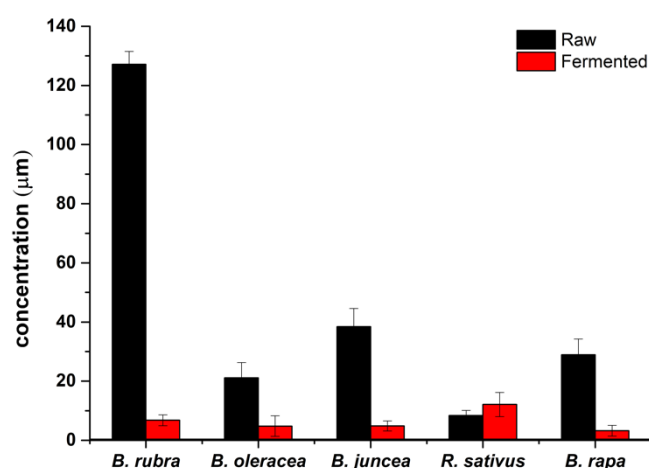


Figure 7. ITC concentration of hydrolyzed extracts

The degradation of glucosinolates yields various products, including isothiocyanates, thiocyanates, nitriles, and epithionitriles (Popović et al., 2020). In addition, different factors can influence the predominance of the hydrolysis product, including temperature, pH, salinity, and microorganisms. In the case of this study, fermenting microorganisms such as lactic acid bacteria (LAB) shifted the mechanism and produced non-ITC reaction products, making them the favored

derivatives. These microorganisms have a myrosinase-like activity that alters the normal pathway of ITC synthesis from GSL degradation (Mullaney et al., 2013).

Similar to the leaching of GSLs, the decline in retained ITC levels was heavily attributed to its leaching from the vegetable tissue, which has drastically softened, and into the brine (Ciska et al., 2021). Fermenting vegetables in brine consequently weakens tissue fibers and releases intact isothiocyanates. As such, the technique would not be recommended if the goal was to retain optimal ITC levels in vegetable tissue. However, in radish (*B. rapa*), an increase in the ITC levels was observed. LAB fermentation could also lead to a high concentration of isothiocyanates. In addition, cutting, shredding, and slicing vegetable samples can initiate the hydrolysis of inherent glucosinolates through their native myrosinase, converting these GSLs into their respective hydrolysis products. Furthermore, individual glucosinolates have varying stability so that other GSLs can predominate during fermentation. For instance, glucoraphasatin, the predominant glucosinolate in radish, and its hydrolysis product are found to be more chemically stable than other aliphatic and indolyl GSLs (Lee et al., 2017). Kim et al. (2015) confirmed in their study that the reaction medium and conditions affected the production and stabilization of hydrolysis products. This could then explain the findings as to why fermentation increased radish ITC content while it decreased for the other vegetable samples.

Effect of Fermentation on Antioxidant Properties. Antioxidants exist naturally in plants, animals, and microorganisms. The abundance of antioxidants in a particular material is correlated to the level of its antioxidant activity (Wang & Zhu, 2006; Kumar et al., 2014) that is defined as the ability to block or inhibit oxidative chain reactions by free radicals (Lobo et al., 2010). With the capacity to limit oxidative stress in cells, antioxidant activity has been a subject of study in the battle against degenerative and chronic diseases, which makes use of a free radical, 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) that is highly reactive in the presence of an antioxidant. The DPPH assay was employed in this analysis because it is an easy, accurate, and economical method. DPPH appears as a violet solution, but in the presence of an antioxidant, it is reduced to a DPPH-H + A[•], diminishing the intensity of the violet color.

Quercetin and ascorbic acid were used as the controls for this analysis because, aside from their availability, they also possess potent antioxidant activity and versatility in ambient conditions (David et al., 2016; Gęgotek & Skrzydlewska, 2022). Both quercetin and ascorbic acid exhibited antioxidant activities at lower concentrations. Similarly, the raw and fermented samples were subjected to DPPH assay to determine the effect of fermentation on the antioxidant activity. Lower IC₅₀ values mean higher antioxidant activity (Olugbami et al., 2014). Furthermore, Phongpaichit et al. (2007) presented specific concentration ranges that categorize the levels of antioxidant activity. The assay revealed that cabbage (*B. oleracea*) has the strongest antioxidant activity with IC₅₀ = 25.32 ± 3.96 ppm. Other raw vegetable samples only exhibited intermediate activity with IC₅₀ values ranging from 50 to 100 ppm. Fermented vegetable samples have IC₅₀ values ≥ 200 ppm, indicating weak antioxidant properties. These results are summarized in Table 3. Figure 8 compares the IC₅₀ values of raw and fermented samples.

Table 3. IC₅₀ values of Raw and Fermented Samples

Sample	IC ₅₀ (ppm)	
	Raw	Fermented
alugbati (<i>B. rubra</i>)	73.91 ± 5.10	> 200
cabbage (<i>B. oleracea</i>)	25.32 ± 3.96	> 200
mustard (<i>B. juncea</i>)	82.56 ± 4.58	194.50 ± 26.76
radish (<i>R. sativus</i>)	54.24 ± 3.14	> 200
Napa cabbage (<i>B. rapa</i>)	98.70 ± 5.42	197.80 ± 16.32

Note: 200 ppm is the highest concentration used for the DPPH assay.

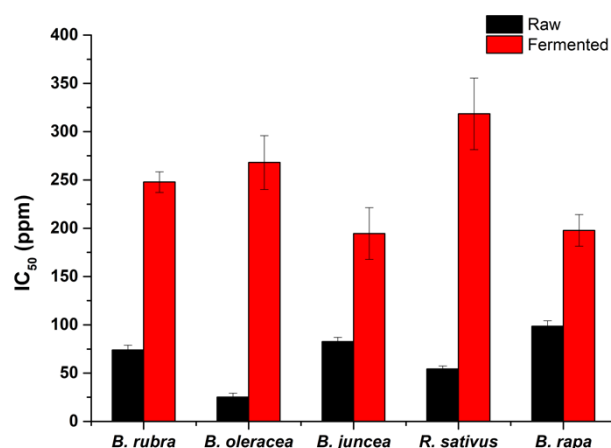


Figure 8. IC₅₀ values of Raw vs. Fermented Samples

Cruciferous vegetables are known for their nutritional profiles that exhibit mechanisms against degenerative and chronic diseases. Among these nutrients, antioxidants are one of the most essential components in vegetables that exhibit these health-related benefits. The major antioxidants in cruciferous vegetables are flavonoids, hydroxycinnamic acids, anthocyanins, and carotenoids (Cartea et al., 2010). Because antioxidant content in plants defines their antioxidant capacities, the degree of degradation of the major antioxidants during fermentation can be accounted for by the significant decrease in antioxidant activities in cruciferous vegetables. During fermentation, structural breakdown and modifications by microbial enzymes impose biotransformation on plant bioactive compounds. Sugar moieties are converted into lactic acid, generating an acidic environment that plays a crucial role in enzymatic activity and antioxidant stability. For instance, lactic acid bacteria facilitate the liberation of bound hydroxycinnamic acids through esterase, reductase, and decarboxylase enzymes (Filannino et al., 2015). Fermentation conditions such as pH and storage periods have been found to affect enzymes responsible for forming major phytochemical compounds and alter metabolic pathways. The results of a study by Odongo et al. (2017) demonstrated a 75% decline in phenolic compounds in fermented Ethiopian kale due to a structure-dependent induced degradation process on more complex flavonoids and hydroxycinnamic acids. Fang et al. (2008) observed a decrease in antioxidant capacity during the fermentation of potherb mustard, which was associated with the decrease of total phenolics and total phenolic acid content caused by phenolic degradation through polyphenol oxidase. Wiczowski et al. (2015) discussed the nutritional profile of fresh and processed red cabbage. They emphasized that fresh red cabbage exhibited stronger scavenging ability than fermented, stored, and stewed red cabbage due to anthocyanin degradation. During long storage periods, oxidative enzymes can be generated to respond to microbial activity. These oxidative enzymes prompt the polymerization of phenolic compounds, reducing levels of measurable antioxidants.

Studies have deliberated food materials' processes during fermentation, affecting their antioxidant capacities. During fermentation, enzymatic hydrolysis is caused by strains that initiate chemical transformations of phytochemicals. In addition, cell-degrading enzymes are generated, which break plant cell walls, releasing bound phenolics. This imposes a phenomenon of decreased antioxidant activity in fermented foods, such as in this study. All five fermented cruciferous vegetables produced lower antioxidant activity than their raw counterparts. Phytochemicals in the actual plant sample may have leached through, getting into the brine solution during fermentation. A study by Sawicki & Wiczowski (2018) indicated stronger activity in the liquid collected from fermented red beetroot than its actual fermented red beet

slices. Enzymatic hydrolysis alongside the maceration of the outer peel of red beet enabled the liberation of phytochemicals, particularly an intense release of betalains, the primary compound responsible for antioxidative potential, from the root matrix to the liquid. The migration of antioxidative compounds to the brine solution may have reduced the antioxidant activity of the actual plant sample during fermentation.

CONCLUSIONS

Cruciferous vegetables have been widely recognized for their abundant bioactive compounds, contributing to their rich nutritional profiles. Bioactive compounds that exist predominantly in cruciferous vegetables are glucosinolates, isothiocyanates, and phytochemicals that contribute to their antioxidant capacities. The total GSL contents of each sample were investigated after subjecting the methanolic extracts to HPLC analysis. Based on the results obtained, fermentation was shown to cause a reduction in GSLs. These findings could be attributed to the leaching and degradation of glucosinolates from the weakened vegetable tissue. Reported literature further ascribes its degradation to the microbial activity of fermenting bacteria that enhance the metabolite's decomposition. Isothiocyanate levels among the vegetable samples were optimized under hydrolyzed conditions due to the enhanced effects of water on the hydrolysis of glucosinolates. Upon fermentation, the isothiocyanate content drastically decreased due to the initial reduction of their precursor glucosinolate compounds. DPPH assay validated the concentration-dependency of % scavenging activity where lower concentrations exhibit lower scavenging levels. It can be deduced that the fermentation process degraded most of the phytochemicals present in the samples, reducing their capacity to scavenge free radicals. Moreover, the massive change in the antioxidant activity of raw and fermented vegetables could be attributed to the tendency of phenolic compounds to leach out of the sample matrix into the brine solution. Thus, fermentation conditions and assays strongly influence fermented cruciferous vegetables' antioxidant capacities.

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