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FERMENTATION OF BITTER MUSTARD GREENS (*Brassica juncea* (L.) Czern.: PHYTOCHEMICAL SCREENING, PROXIMATE COMPOSITION, AND ISOLATION OF LACTIC ACID BACTERIA

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ABSTRACT

Fermented vegetables are widely consumed functional foods, but scientific data on traditionally fermented bitter mustard greens are still limited. This study aimed to evaluate the phytochemical profile and proximate composition of *Brassica juncea* after spontaneous fermentation and to isolate lactic acid bacteria (LAB) formed during the process. A laboratory experimental design was applied; bitter mustard greens were spontaneously fermented in brine for seven days at room temperature. Daily changes in pH and organoleptic characteristics were recorded. Phytochemical screening was performed on fresh and fermented samples (leaves, stems, brine). Proximate analysis included moisture, ash, acid-insoluble ash, crude fat, protein, and carbohydrates using AOAC methods. LAB were isolated on MRS–$CaCO_3$ agar and characterized phenotypically. Fermentation produced a progressive decrease in pH (from 5.0 to 2.9–3.3) accompanied by sour aroma and yellowish discoloration, indicating active lactic acid fermentation. Flavonoids, phenolics, and terpenoids were present in both fresh and fermented samples, although weaker reactions occurred in stems and brine. Proximate analysis revealed very high moisture content (88.76–95.20%), low protein (0.53–0.88%), low fat (5.03–5.50%), and low residual carbohydrates. LAB isolates (ASP-CF, ASP-B, ASP-D) were Gram-positive, catalase-negative, acid-producing, and non-H_2S-forming. In conclusion, traditional spontaneous fermentation of Brassica juncea produces a LAB-dominated fermented product that retains major phytochemical groups and exhibits proximate characteristics typical of fermented vegetables, supporting its potential as a functional food. Further molecular identification of LAB and quantitative metabolite profiling are recommended.

Keyword: *Brassica juncea*; spontaneous fermentation; proximate composition; phytochemical screening; lactic acid bacteria

ABSTRAK

Sayuran fermentasi merupakan pangan fungsional yang banyak dikonsumsi, namun data ilmiah mengenai asinan sawi pahit tradisional masih terbatas. Penelitian ini bertujuan mengevaluasi profil fitokimia dan komposisi proksimat sawi pahit (Brassica juncea) setelah fermentasi spontan serta mengisolasi bakteri asam laktat (BAL) yang terbentuk selama proses tersebut. Rancangan eksperimental laboratorium digunakan; fermentasi spontan dilakukan selama tujuh hari pada suhu kamar dalam larutan garam. Perubahan pH dan karakteristik organoleptik dicatat setiap hari. Skrining fitokimia dilakukan pada bahan segar dan terfermentasi (daun, batang, air fermentasi). Analisis proksimat meliputi kadar air, abu, abu tidak larut asam, lemak kasar, protein, dan karbohidrat menggunakan metode AOAC. BAL diisolasi pada media MRS–$CaCO_3$ dan dikarakterisasi secara fenotipik. Fermentasi menghasilkan penurunan pH progresif (5,0 menjadi 2,9–3,3) disertai aroma asam dan perubahan warna kekuningan, menunjukkan fermentasi asam laktat yang aktif. Flavonoid, fenolik, dan terpenoid terdeteksi pada semua

sampel segar maupun terfermentasi, dengan reaksi lebih lemah pada batang dan air fermentasi. Analisis proksimat menunjukkan kadar air sangat tinggi (88,76–95,20%), protein rendah (0,53–0,88%), lemak rendah (5,03–5,50%), serta karbohidrat sisa rendah. Isolat BAL (ASP-CF, ASP-B, ASP-D) bersifat Gram positif, katalase negatif, menghasilkan asam, dan tidak membentuk H_2S. Sebagai kesimpulan, fermentasi spontan sawi pahit menghasilkan produk fermentasi yang didominasi BAL, mempertahankan golongan fitokimia utama, dan memiliki komposisi proksimat khas sayuran terfermentasi, sehingga berpotensi sebagai pangan fungsional. Penelitian lanjutan dengan identifikasi molekuler BAL dan penetapan kuantitatif metabolit direkomendasikan.

Kata kunci: *Brassica juncea*; fermentasi spontan; komposisi proksimat; skrining fitokimia; bakteri asam laktat

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1. Introduction

Fermented vegetables are widely consumed in many Asian countries. They are recognized not only as preserved foods but also as functional foods due to their content of bioactive compounds and beneficial microorganisms. Among fermented vegetables, mustard greens (*Brassica juncea*) are one of the most commonly processed raw materials [1]. Traditional fermentation of mustard greens yields pickled products with a characteristic sour aroma and taste, formed through spontaneous lactic acid fermentation dominated by lactic acid bacteria (LAB). This spontaneous fermentation is typically carried out without the addition of starter cultures, relying solely on indigenous microorganisms from the raw materials, utensils, and the environment [2].

Previous studies have demonstrated that fermentation can modify the nutritional and phytochemical composition of vegetables. During fermentation, enzymatic and microbial metabolism may lead to hydrolysis, transformation, and release of bound phytochemicals [3]. These processes can enhance the bioavailability of phenolic compounds, flavonoids, and other secondary metabolites, which may contribute to antioxidant, antimicrobial, and anti-inflammatory activities. In addition, fermentation typically reduces the carbohydrate content as microbes utilise sugars as substrates. At the same time, organic acids, such as lactic acid, accumulate, decreasing the pH and thereby improving food safety and shelf life [4].

Mustard greens (*Brassica juncea*) are known to contain glucosinolates, flavonoids, phenolic acids, and terpenoid compounds. Several of these constituents are reported to possess promising biological activities, including antioxidant and chemopreventive effects [5]. However, these phytochemicals are labile and may undergo structural modifications during the fermentation process. Such transformations may result in degradation or, conversely, in the formation of new bioactive derivatives. Therefore, evaluating phytochemical profiles before and after fermentation is crucial for understanding the biochemical dynamics that occur in fermented mustard greens [6].

In addition to phytochemical changes, fermentation affects proximate composition parameters, including moisture content, ash, crude fat, crude protein, and carbohydrates. Moisture and ash contents reflect the physical and mineral characteristics of fermented food, while protein and fat composition indicate the nutritional contribution of the product [7]. The decrease in pH and modulation of nutrient profile during fermentation have implications for both nutritional value and consumer acceptance. Comprehensive proximate analysis is thus required to support the development of fermented mustard greens as a safe and functional food product [8].

Lactic acid bacteria play a central role in vegetable fermentation. These microorganisms ferment carbohydrates to produce lactic acid, decrease the pH, inhibit the growth of spoilage and pathogenic bacteria, and contribute to the formation of flavour and texture [9]. LAB are also of increasing interest in pharmaceutical and clinical research due to their potential probiotic properties, including modulation of gut microbiota, enhancement of immune responses, and improvement of gastrointestinal health. The isolation and characterisation of LAB from traditional fermented foods represent an important step toward identifying promising strains for functional food or probiotic applications [10].

Several studies have successfully isolated LAB from fermented plant-based products such as kimchi, sauerkraut, and pickled vegetables. However, the diversity and characteristics of LAB are influenced by raw materials, salt concentration, fermentation conditions, and local practices [11]. Therefore, LAB isolated from traditionally fermented mustard greens may exhibit unique phenotypic properties and carbohydrate fermentation profiles. Nonetheless, systematic studies specifically focusing on LAB from traditionally fermented Brassica juncea pickles are still limited [12].

In Indonesia, mustard greens are widely consumed and processed into various traditional foods, including pickled products prepared using household-scale spontaneous fermentation. Despite their popularity,

scientific reports on the chemical characteristics, phytochemical changes, and LAB profile of traditionally fermented bitter mustard greens remain scarce [13]. Most existing studies tend to focus on sensory quality or general microbiological safety without integrating aspects of phytochemicals, proximate composition, and LAB isolation in a single investigation [14].

Understanding the chemical and microbiological characteristics of fermented bitter mustard greens is important from pharmaceutical, nutraceutical, and public health perspectives. The identification of LAB and bioactive compounds in fermented products may contribute to the development of functional foods with added health benefits [8], [15]. Furthermore, scientific evidence regarding the composition of traditionally fermented foods may support product standardization, safety evaluation, and further clinical research. Therefore, this study was conducted to evaluate the changes in the phytochemical profile and proximate composition of bitter mustard greens during traditional fermentation and to isolate and characterise the lactic acid bacteria formed during this process.

2. Methods

2.1 Chemicals and reagents

All reagents were used without further purification. Media and solutions were prepared according to the manufacturer's instructions, and the pH was adjusted as required using standardised acid or base solutions. Glassware was washed with detergent, thoroughly rinsed with distilled water, and dried prior to use to prevent contamination. All phytochemical tests were performed using freshly prepared reagents.

Where applicable, standard reference reagents and positive controls were employed to confirm test validity (e.g., quercetin for flavonoids, gallic acid for phenolics, cholesterol for terpenoids). Calibration of analytical instruments (pH meter, oven, balance) was performed prior to measurements according to the corresponding operating procedures. All experiments were conducted in triplicate, and results were expressed as mean values to minimize random error.

2.2 Study design and sample preparation

An experimental laboratory design was employed in this study, in which the fermentation of bitter mustard greens was conducted, followed by phytochemical screening, proximate analysis, and isolation of lactic acid bacteria (LAB). Fresh bitter mustard greens (*Brassica juncea*) were cleaned and washed under running water, after which excess water was allowed to drain. The plant material was then used immediately for the fermentation experiments to minimise post-harvest biochemical changes [16].

2.3 Sample identification

The plant material was taxonomically authenticated at Herbarium Medanense (MEDA), Universitas Sumatera Utara. The identification was performed using standard morphological keys, and the specimen was confirmed as *Brassica juncea* (L.) Czern. of the family Brassicaceae. The voucher number and date were recorded in accordance with the official identification letter, and the authenticated voucher specimen was deposited in the Herbarium collection for future reference.

2.4 Fermentation procedure and monitoring

Traditional spontaneous fermentation was performed by immersing chopped bitter mustard greens (leaves and stems) in a brine solution in closed containers and maintaining them at room temperature (27–30 °C) for seven days. The progress of fermentation was monitored daily. Measurements of pH were taken using a digital pH meter, while the colour of the leaves and stems, the colour of the brine, and odour characteristics were visually observed. The pH was recorded from day 1 to day 7, and organoleptic changes were documented descriptively. The advancement of fermentation was interpreted based on pH reduction together with organoleptic changes that were consistent with lactic acid fermentation [17], [18].

2.5 Phytochemical screening

Phytochemical screening was conducted on fresh bitter mustard greens, fermented leaves, fermented stems, and the fermentation brine. The screening was conducted according to classical phytochemical procedures described by Harborne, Farnsworth, and the WHO monographs. The Shinoda test was applied for the detection of flavonoids, the ferric chloride ($FeCl_3$) test was used to identify phenolic compounds, and the Liebermann–Burchard reaction was employed for the detection of terpenoids. During each assay, the development of characteristic colour changes and the formation of precipitates were observed, and the results were recorded qualitatively. The intensity of reactions was classified as positive (+), weakly positive (±), or negative (−) [19], [20].

2.6 Proximate composition analysis

Proximate composition was determined using standard AOAC procedures. Moisture content was measured by oven drying at 105 °C until a constant weight was achieved [21]. Total ash content was determined by furnace incineration at 550 °C, and acid-insoluble ash was obtained by treating the ash with 2N HCl, followed by filtration and drying of the remaining residue [22]. Crude fat content was determined by Soxhlet extraction using n-hexane as the solvent [23]. Protein content was analyzed using the Kjeldahl method, and total nitrogen values were converted to protein using a conversion factor of N × 6.25. Carbohydrate content was evaluated both qualitatively and quantitatively. Qualitative assessment was conducted using the Molisch test for general carbohydrates and the iodine test for starch detection [24]. Quantitative estimation of carbohydrate content was calculated by difference, using the formula 100 − (% moisture + % ash + % fat + % protein) [25], [26]. The absence of a blue–black colouration in the iodine test was interpreted as the absence of starch.

2.7 Isolation and characterization of lactic acid bacteria

Fermented samples were serially diluted in sterile saline and spread onto de Man–Rogosa–Sharpe (MRS) agar supplemented with $CaCO_3$, followed by incubation at 37 °C. Colonies producing clear halo zones as a result of $CaCO_3$ dissolution were considered presumptive lactic acid bacteria (LAB) and were subsequently subcultured and purified through repeated streaking to obtain single colonies [27]. The isolates were characterised based on colony morphology, Gram staining, catalase activity, Triple Sugar Iron Agar (TSIA) reactions, and carbohydrate fermentation profiles (glucose, lactose, and sucrose) [28]. Gram staining was performed to determine cell morphology and Gram reaction, while the catalase test using 3% H_2O_2 was conducted to detect the presence of the catalase enzyme. TSIA medium was used to assess acid production and H_2S formation, while carbohydrate fermentation tests were used to evaluate sugar utilization accompanied by acid production. Isolates fulfilling the criteria of being Gram-positive, catalase-negative, capable of producing acid from carbohydrates, negative for H_2S production, and forming halo zones on $CaCO_3$-containing MRS agar were classified as presumptive LAB [29]. Representative isolates that fulfilled these criteria were coded ASP-CF, ASP-B, and ASP-D.

2.8 Data analysis

All experimental procedures were carried out in triplicate. Data were expressed as mean ± standard deviation. Qualitative observations from phytochemical screening and microbial characterization were presented descriptively, while quantitative proximate data were tabulated and compared between fresh and fermented samples.

3. Results

3.1 Sample Identification

The plant material used in this study was taxonomically authenticated at Herbarium Medanense (MEDA), Universitas Sumatera Utara. Based on the official identification letter No. 1272/MEDA/2025 dated 19 November 2025, the specimen was identified as bitter mustard greens (“sawi pahit”) with the following taxonomic classification:

Kingdom : Plantae
Division : Spermatophyta
Class : Dicotyledoneae
Orde : Brassicales
Family : Brassicaceae
Genus : *Brassica*
Species : *Brassica juncea* (L.) Czern.

This authentication confirms that all plant materials used in the present study were accurately identified as *Brassica juncea* (L.) Czern., ensuring the validity of subsequent analyses.

3.2 Fermentation Process

Traditional spontaneous fermentation of bitter mustard greens was performed for seven days at room temperature. Daily observations demonstrated progressive changes in the pH, aroma, and colour of both plant tissues and fermentation brine. The initial pH on days 1–2 was 5.0, accompanied by a fresh-vegetable odour and green leaves with white stems, while the brine still resembled rice-washing water.

On day 3, a slight sour odour began to appear; the pH remained at 5.0, and the leaf colour became darker, with a yellowish tint and a turbid brine. On day 4, the pH level decreased to 4.0, and the sour odour became more pronounced. The most notable changes occurred on days 5–6, with the lowest pH values of 3.0 and 2.9, respectively. At this stage, the aroma of typical lactic acid fermentation was clearly perceived, and both leaves and stems turned yellow.

On day 7, the pH level increased slightly to 3.3, while a strong and dominant sour aroma was observed. The leaves turned yellow–brown, stems remained yellowish, and the brine was yellowish in colour. These findings indicate an active lactic acid fermentation process, characterised by acid production, pH reduction, and distinct organoleptic changes consistent with spontaneous vegetable fermentation dominated by lactic acid bacteria. The detailed daily changes in pH, aroma, leaf and stem colour, and brine appearance throughout the fermentation period are presented in Table 1.

Table 1. Changes in pH and organoleptic characteristics of fermented bitter mustard greens during seven days of fermentation

Day of fermentation	pH	Aroma	Colour of leaves and stems	Colour of fermentation brine
1–2	5.0	Fresh vegetable odour with rice-wash note	Fresh green leaves, white stems	Original/milky rice-wash appearance
3	5.0	A slight sour odour appeared	Darker yellowish	Turbid, rice-wash-like
4	4.0	Slight sour odour became more apparent	Darker yellowish	Turbid
5	3.0	Sour odour became typical	Yellow leaves and stems	Yellowish
6	2.9	Typical sour odour	Yellow leaves and stems	Yellowish
7	3.3	Strong and dominant sour odour	Yellow–brown leaves, yellowish stems	Yellowish

3.3 Phytochemical screening

Phytochemical screening was performed on both fresh and fermented bitter mustard greens, including samples of fermented leaves, stems, and fermentation brine. The analysis demonstrated the presence of flavonoids, phenolic compounds, and terpenoids in both fresh and fermented materials.

Flavonoids were clearly positive in fresh samples and fermented leaves, while weaker positive reactions were observed in fermented stems and fermentation brine. A positive flavonoid reaction in the Shinoda test was indicated by the development of orange to red colouration after the addition of magnesium powder, followed by concentrated HCl. In contrast, weakly positive samples showed only faint yellow–orange discolouration.

Phenolic compounds yielded a consistently positive reaction in all samples. In the ferric chloride assay ($FeCl_3$), the formation of green to dark green colouration confirmed the presence of phenolic hydroxyl groups. Fresh samples and fermented leaves produced more intense colour than fermented stems and brine.

Terpenoids were also detected in fresh samples and fermented leaves, while fermented stems showed weaker reactions. A positive Liebermann–Burchard reaction was characterized by the appearance of a reddish-brown to greenish colouration following the addition of acetic anhydride and concentrated sulfuric acid. In contrast, weak reactions produced only a pale brownish colouration. These findings indicate that major classes of secondary metabolites were retained after fermentation. However, the reaction intensity was generally weaker in stems and fermentation brine, which is consistent with the possible redistribution and dilution of metabolites during the fermentation process. The results were in agreement with the characteristic colour changes documented in laboratory records for the Shinoda, $FeCl_3$, and Liebermann–Burchard assays, and the overall phytochemical profile of each sample is summarized in Table 2.

Table 2. Phytochemical screening results of fresh and fermented bitter mustard greens

Sample	Flavonoids	Phenolics	Terpenoids	Notes
Fresh bitter mustard greens	+	+	+	clear positive reactions
Fermented leaves	+	+	+	reactions clearly observed
Fermented stems	± (weak)	+	± (weak)	weaker colour intensity
Fermentation brine	± (weak)	+	+	dissolved metabolites present

3.4 Proximate composition

The proximate composition analysis of fermented bitter mustard greens showed a very high moisture content. The mean moisture content reached 95.20% in stems and 88.76% in leaves, indicating high water retention after fermentation. Total ash content ranged between 0.83–0.86%, while acid-insoluble ash ranged between 0.21–0.27%, reflecting the presence of inorganic minerals and non-digestible residues.

Crude fat content obtained by Soxhlet extraction was 5.50% in leaves and 5.03% in stems, whereas protein content was relatively low, measuring 0.88% in leaves and 0.53% in stems. Carbohydrates calculated by difference accounted for 4.0% in leaves, while stems contained trace amounts, likely due to very high moisture levels and metabolic utilization of sugars during fermentation.

Qualitative carbohydrate testing supported these findings. The Molisch test was positive in leaves, stems, and fermentation brine, confirming the presence of carbohydrates. In contrast, the iodine test was negative, indicating the absence of starch and suggesting a predominance of simple sugars and fermentation-derived products over polysaccharides.

Overall, these data demonstrate that fermentation results in products characterised by a very high moisture content, low protein, low fat, and low residual carbohydrate content, which is consistent with the proximate composition profile of fermented leafy vegetables, and the detailed proximate composition values are presented in Table 3.

Table 3. Proximate composition of fermented bitter mustard greens (leaves and stems)

Parameter	Leaves (%)	Stems (%)
Moisture	88.76	95.20
Total ash	0.86	0.83
Acid-insoluble ash	0.21	0.27
Crude fat	5.50	5.03
Protein	0.88	0.53
Carbohydrate (by difference)	4.00	≈0 (trace)
Qualitative carbohydrate (Molisch)	Positive	Positive
Starch (iodine test)	Negative	Negative

3.5 Isolation and characterization of lactic acid bacteria (LAB)

LAB colonies were successfully isolated from fermented bitter mustard greens using MRS agar supplemented with calcium carbonate ($CaCO_3$). Colonies producing the largest clear zones surrounding the growth area were selected, as dissolution of $CaCO_3$ indicates lactic acid production. Three representative isolates were obtained and coded ASP-CF, ASP-B, and ASP-D. The detailed colony morphology of these isolates is presented in Table 4.

All isolates exhibited typical LAB colony morphology, characterised by a circular shape, convex elevation, smooth surface, and a milky-white colour. Clear halo zones were clearly visible around colonies, supporting their classification as acid-producing bacteria. The isolates were subsequently purified by repeated streaking to obtain single colonies.

Microscopically, Gram staining showed Gram-positive cells in all isolates. Morphology consisted mainly of short rods and ovoid cells. Catalase testing revealed adverse reactions, with the absence of gas bubbles upon the addition of hydrogen peroxide, indicating a lack of catalase enzyme activity. Triple sugar iron agar and carbohydrate fermentation assays demonstrated the ability to utilise several carbohydrates, including glucose, lactose, and sucrose, producing acid without gas or H_2S, which supports their identification as presumptive lactic acid bacteria. The biochemical and physiological characteristics of the isolates are summarised in Table 5.

Table 4. Colony morphology of lactic acid bacteria isolated from fermented bitter mustard greens

Isolate code	Colony shape	Elevation	Surface	Color	Clear zone on $CaCO_3$
ASP-CF	Circular	Convex	Smooth	Milky-white	Present (large)
ASP-B	Circular	Convex	Smooth	Milky-white	Present
ASP-D	Circular	Convex	Smooth	Milky-white	Present

Table 5. Biochemical and physiological characteristics of lactic acid bacteria isolates

Test	ASP-CF	ASP-B	ASP-D	Interpretation
Gram staining	Positive	Positive	Positive	LAB character
Cell morphology	Short rods	Ovoid/Coccus-like	Short rods	Lactobacillus–like
Catalase	Negative	Negative	Negative	No catalase
TSIA acid reaction	Positive	Positive	Positive	Carbohydrate fermentation

H_2S production	Negative	Negative	Negative	Non-H_2S producer
Glucose fermentation	Positive	Positive	Positive	Acid producer
Lactose fermentation	Positive	Positive	Positive	Acid producer
Sucrose fermentation	Positive	Positive	Positive	Acid producer

4. Discussion

The present study confirmed that the plant material used for fermentation was accurately identified as Brassica juncea (L.) Czern., ensuring the botanical validity of the sample analysed. Correct species authentication is essential because the phytochemical content and microbial ecology of fermented vegetables are strongly species-dependent within the Brassicaceae family [30]. Previous studies have demonstrated that Brassica species possess abundant phenolics, glucosinolates, and flavonoids, which influence both the nutritional value and microbial selection during fermentation. Therefore, authentication provides a crucial foundation for interpreting the subsequent chemical and microbiological changes observed in this work [31].

Spontaneous fermentation resulted in transparent and progressive physicochemical and organoleptic changes over the seven days. The decline in pH from around 5.0 to below 3.3 indicates intense lactic acid production [32]. A similar pH pattern has been widely reported in fermented vegetables such as kimchi, sauerkraut, and fermented mustard greens, where rapid acidification is associated with the dominance of lactic acid bacteria. The observed yellowing of leaves and brine turbidity is consistent with chlorophyll degradation, pigment oxidation, and leaching of soluble components into the fermentation brine [33]. The strong sour aroma detected after day 5 reflects the accumulation of organic acids, mainly lactic acid, accompanied by minor volatiles such as acetic acid, which is typically produced by heterofermentative lactic acid bacteria [34].

Phytochemical screening demonstrated that flavonoids, phenolic compounds, and terpenoids were retained following fermentation, although reaction intensity was reduced in stems and fermentation brine [35]. The persistence of phenolics after fermentation has also been reported in fermented brassica vegetables. It is attributed to the stability of phenolic acids and the formation of smaller phenolic derivatives through enzymatic hydrolysis [36]. In some studies, fermentation has even been shown to increase phenolic bioavailability, as microbial enzymes release bound phenolics from cell walls. The weaker reactions in stems and brine observed in the present study may be explained by dilution into the fermentation liquor, redistribution between tissues, and microbial metabolism of certain phytoconstituents [37].

The flavonoid reactions observed in both fresh and fermented samples suggest that these compounds are relatively stable under mild lactic acid fermentation conditions. This finding agrees with other works showing that fermentation does not eliminate flavonoids but may convert glycosides to aglycones through microbial β-glucosidase activity, potentially increasing bioactivity [38], [39]. Terpenoid reactions were detectable but weaker in stems than in leaves, suggesting uneven distribution of secondary metabolites within plant tissues. Terpenoids in Brassicaceae play crucial roles in plant defence and flavour development, and their presence after fermentation may contribute to the characteristic aroma and potential functional properties of the product [40].

Proximate analysis revealed very high moisture content and low protein and fat levels, a composition typical of fermented leafy vegetables. The high water content in stems compared with leaves reflects anatomical differences, where stems contain larger parenchyma with higher water storage capacity [41]. The trace level of residual carbohydrate in stems and low carbohydrate in leaves is consistent with microbial utilisation of sugars as substrates for lactic acid production. Comparable findings have been reported in fermented mustard and cabbage products, where simple sugars decrease markedly during fermentation as they are metabolised by lactic acid bacteria into organic acids [42]. The positive Molisch test and adverse iodine reaction in this study corroborate that remaining carbohydrates were mainly simple sugars and fermentation products rather than starch [43].

The successful isolation of lactic acid bacteria on MRS–$CaCO_3$ media further supports the biochemical findings. Clear halo formation surrounding colonies indicates calcium carbonate dissolution due to acid production, a classic indicator of lactic acid–producing microorganisms. The isolates exhibited characteristics typical of LAB, including Gram positivity, catalase negativity, and acid production from several carbohydrates [29]. These traits align with general descriptions of genera such as Lactobacillus, Leuconostoc, and Pediococcus that are commonly found in spontaneously fermented vegetables [44].

The fermentation pattern observed in this study, characterised by rapid acidification, carbohydrate utilisation, and the domination of LAB, is consistent with the ecological succession described in recent literature on vegetable fermentation. Initially, diverse microbial populations are gradually replaced by acid-tolerant lactic acid bacteria as pH declines [45]. LAB not only drive acidification but also influences

phytochemical stability and transformation through enzymatic activities. This interaction explains why secondary metabolites are retained yet modified during fermentation [46].

Overall, the present findings support the view that traditional fermentation of Brassica juncea produces a food product with high moisture content, preserved phenolic and flavonoid constituents, and a microbiota dominated by presumptive lactic acid bacteria. These compositional changes have nutritional and functional implications, including potential antioxidant activity and improved digestibility. Future work should include molecular identification of isolates, quantification of organic acid profiles, and targeted analysis of individual phenolic compounds further to elucidate the health-related properties of fermented bitter mustard greens.

5. Conclusion

This study demonstrated that traditional spontaneous fermentation of bitter mustard greens (Brassica juncea (L.) Czern.) resulted in characteristic physicochemical and microbiological changes indicative of lactic acid fermentation, as evidenced by a marked decrease in pH and progressive organoleptic changes. Phytochemical screening confirmed the persistence of major secondary metabolites, including flavonoids, phenolics, and terpenoids, in both fresh and fermented samples, although variations in reaction intensity were observed among leaves, stems, and brine. Proximate analysis showed very high moisture content with low levels of protein, fat, and residual carbohydrates, reflecting sugar utilisation during fermentation. Lactic acid bacteria were successfully isolated and exhibited typical characteristics of presumptive LAB, including Gram-positive reaction, catalase negativity, acid production, and absence of H_2S formation. Collectively, these findings indicate that fermented bitter mustard greens represent a LAB-dominated fermented vegetable retaining key phytochemical constituents, supporting their potential as a functional food; however, further studies involving molecular identification of isolates and quantitative profiling of metabolites are warranted.

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7. Conflict of Interest

All authors declare that they have no conflict of interest related to this study and its publication.

References

[1] A. Thierry *et al.*, ‘Lactofermentation of vegetables: An ancient method of preservation matching new trends’, *Trends in Food Science & Technology*, vol. 139, p. 104112, Sept. 2023, doi: 10.1016/j.tifs.2023.07.009.

[2] X. Kong *et al.*, ‘Screening, Identification, and Fermentation Characteristics of Lactic Acid Bacteria from Pickled Potherb Mustard and Potential Applications’, *Foods*, vol. 14, no. 8, p. 1431, Apr. 2025, doi: 10.3390/foods14081431.

[3] R. Aleman, I. Montero-Fernández, J. Marcía, S. Saravia Maldonado, and D. Martín-Vertedor, ‘Application of Fermentation as a Strategy for the Transformation and Valorization of Vegetable Matrices’, *fermentation*, vol. 10, no. 3, p. 124, Feb. 2024, doi: 10.3390/fermentation10030124.

[4] S. Saud, T. Xiaojuan, and S. Fahad, ‘The consequences of fermentation metabolism on the qualitative qualities and biological activity of fermented fruit and vegetable juices’, *Food Chemistry: X*, vol. 21, p. 101209, Mar. 2024, doi: 10.1016/j.fochx.2024.101209.

[5] M. Rahman, A. Khatun, L. Liu, and B. J. Barkla, ‘Brassicaceae Mustards: Phytochemical Constituents, Pharmacological Effects, and Mechanisms of Action against Human Disease’, *IJMS*, vol. 25, no. 16, p. 9039, Aug. 2024, doi: 10.3390/ijms25169039.

[6] W. Qinghang *et al.*, ‘Variation in glucosinolates and the formation of functional degradation products in two Brassica species during spontaneous fermentation’, *Current Research in Food Science*, vol. 6, p. 100493, 2023.

[7] S. Upadhyaya and R. Kumar, ‘Fermentation as a Method of Food Processing and Fermented Food as Probiotics: A Review’, *FSN*, vol. 10, no. 5, pp. 1–11, Dec. 2024, doi: 10.24966/FSN-1076/100203.

[8] K. Zhu *et al.*, ‘Effects of different fermentation conditions on physicochemical properties, volatile compounds and antioxidant capacity of rape moss’, *Food Bioscience*, vol. 58, p. 103682, Apr. 2024, doi: 10.1016/j.fbio.2024.103682.

[9] D. Amenu, A. Nugusa, and T. Tafesse, 'Preservative Effectiveness of Lactic Acid Bacteria on Fruits and Vegetables', *International Journal of Cell Biology*, vol. 2025, no. 1, p. 5833236, Jan. 2025, doi: 10.1155/ijcb/5833236.
[10] M. Kowalczyk *et al.*, 'Screening for probiotic properties and potential immunogenic effects of lactobacilli strains isolated from various food products', *Front. Microbiol.*, vol. 15, p. 1430582, Oct. 2024, doi: 10.3389/fmicb.2024.1430582.
[11] X. Yang *et al.*, 'Effect of Lactic Acid Bacteria Fermentation on Plant-Based Products', *Fermentation*, vol. 10, no. 1, p. 48, Jan. 2024, doi: 10.3390/fermentation10010048.
[12] M. Michalak, 'Plant-derived antioxidants: Significance in skin health and the ageing process', *International journal of molecular sciences*, vol. 23, no. 2, p. 585, 2022.
[13] Sarengaowa *et al.*, 'Evaluation of Quality and Microbial Communities in Fermented Chinese Mustard Greens from Guangdong Province, China', *Horticulturae*, vol. 10, no. 4, p. 399, 2024.
[14] A. I. Joy and O. Olubukola, 'Probiotic Lactobacillus Species isolated from Fermented Beverages for Antibacterial Activity and Shelf Life Extension against Spoilage Bacteria', in *2024 International Conference on Science, Engineering and Business for Driving Sustainable Development Goals (SEB4SDG)*, Omu-Aran, Nigeria: IEEE, Apr. 2024, pp. 1–5. doi: 10.1109/SEB4SDG60871.2024.10629730.
[15] Y. Yuan, Y. Yang, L. Xiao, L. Qu, X. Zhang, and Y. Wei, 'Advancing Insights into Probiotics during Vegetable Fermentation', *Foods*, vol. 12, no. 20, p. 3789, Oct. 2023, doi: 10.3390/foods12203789.
[16] J. Lingjuan, C. Yu, D. Zeyuan, Z. Bing, and L. Hongyan, 'Evaluation and comparison of physicochemical properties, volatile substances, and microbial communities of leaf mustard (*Brassica juncea var. multiceps*) under natural and inoculated fermentation', *Journal of Food Science*, vol. 88, no. 8, pp. 3255–3273, Aug. 2023, doi: 10.1111/1750-3841.16687.
[17] X. Chen, M. Zheng, J. Liu, Z. Deng, B. Zhang, and H. Li, 'Selection and use of indigenous mixed starter cultures for mustard leaves fermentation and the improvement of cuocai characteristics', *J Sci Food Agric*, vol. 98, no. 5, pp. 1773–1786, Mar. 2018, doi: 10.1002/jsfa.8652.
[18] S. Vatansever, A. Vegi, J. Garden-Robinson, and C. A. Hall Iii, 'The Effect of Fermentation on the Physicochemical Characteristics of Dry-Salted Vegetables', *JFR*, vol. 6, no. 5, p. 32, Aug. 2017, doi: 10.5539/jfr.v6n5p32.
[19] E. Fachrizal, 'Phytochemical of Ethanolic Extract of Cabbage (Brassica oleracea) Collected from Medan, Indonesia', *JCNaR*, vol. 4, no. 1, pp. 37–41, Aug. 2022, doi: 10.32734/jcnar.v4i1.9356.
[20] Drashti Patel, Jyoti Chauhan, and Bharat Maitreya, 'Analysis of Phytochemical and Antioxidant Variation between Selected Phenophase of Raphanus sativus L. leaves', *Int J Sci Res Sci & Technol*, vol. 12, no. 2, pp. 1162–1171, Apr. 2025, doi: 10.32628/IJSRST251222747.
[21] R. Arezou, P. Maria, and R. Mehrdad, 'Assessment of Soil Moisture Content Measurement Methods: Conventional Laboratory Oven versus Halogen Moisture Analyzer', *J Soil Water Sci*, vol. 4, no. 1, Dec. 2020, doi: 10.36959/624/440.
[22] B. P. Ismail, 'Ash Content Determination', in *Food Analysis Laboratory Manual*, in Food Science Text Series. , Cham: Springer International Publishing, 2017, pp. 117–119. doi: 10.1007/978-3-319-44127-6_11.
[23] S. S. Nielsen, 'Protein Nitrogen Determination', in *Food Analysis Laboratory Manual*, S. S. Nielsen, Ed., in Food Science Texts Series. , Boston, MA: Springer US, 2010, pp. 39–45. doi: 10.1007/978-1-4419-1463-7_5.
[24] M. Sarkar, S. Hait, and S. Joshi, 'Qualitative Tests for Carbohydrate Detection', in *Practical Biochemistry*, P. Jha, Ed., BENTHAM SCIENCE PUBLISHERS, 2024, pp. 8–31. doi: 10.2174/9789815165852124010005.
[25] J. M. Richey, H. G. Richey, and R. Schraer, 'Quantitative analysis of carbohydrates using gas-liquid chromatography', *Analytical Biochemistry*, vol. 9, no. 3, pp. 272–280, Nov. 1964, doi: 10.1016/0003-2697(64)90185-X.
[26] P. Thangaraj, 'Proximate Composition Analysis', in *Pharmacological Assays of Plant-Based Natural Products*, vol. 71, in Progress in Drug Research, vol. 71. , Cham: Springer International Publishing, 2016, pp. 21–31. doi: 10.1007/978-3-319-26811-8_5.
[27] N. Nasri, U. Harahap, J. Silalahi, and D. Satria, 'Antibacterial activity of lactic acid bacteria isolated from Dengke Naniura of Carp (Cyprinus carpio) against diarrhea-causing pathogenic bacteria', *Biodiversitas Journal of Biological Diversity*, vol. 22, no. 8, 2021, Accessed: Sept. 17, 2025. [Online]. Available: https://smujo.id/biodiv/article/view/8697

[28] G. Haro, I. Iksen, and N. Nasri, 'Identification, characterization and antibacterial potential of probiotic lactic acid bacteria isolated from naniura (A traditional batak fermented food from carp) against Salmonella typhi', *Rasayan Journal of Chemistry*, vol. 13, no. 1, pp. 464–468, 2020.
[29] H. Hanasia, N. S. Martani, O. N. Turnip, D. V. Putri Welmart, and D. M. Puspita Sari, 'ISOLATION AND IDENTIFICATION OF LACTIC ACID BACTERIA FROM GAMBUT TAPE', *M*, vol. 13, no. 1, pp. 86–94, June 2025, doi: 10.33992/meditory.v13i1.4040.
[30] D. Liu, C. Zhang, J. Zhang, X. Xin, and Q. Wu, 'Dynamics of the glucosinolate–myrosinase system in tuber mustard (Brassica juncea var. tumida) during pickling and its relationship with bacterial communities and fermentation characteristics', *Food Research International*, vol. 161, p. 111879, Nov. 2022, doi: 10.1016/j.foodres.2022.111879.
[31] A. Šalić and D. Šamec, 'Changes in the content of glucosinolates, polyphenols and carotenoids during lactic-acid fermentation of cruciferous vegetables: A mini review', *Food Chemistry: X*, vol. 16, p. 100457, Dec. 2022, doi: 10.1016/j.fochx.2022.100457.
[32] X. Zhou, Q. Guan, Y. Qin, Z. Qin, B. Du, and D. Lin, 'Dynamic changes in physic-chemical properties and bacterial community during natural fermentation of tomatoes', *Food Sci. Technol*, vol. 42, p. e63520, 2022, doi: 10.1590/fst.63520.
[33] J. Bautista-Gallego, E. Medina, B. Sánchez, A. Benítez-Cabello, and F. N. Arroyo-López, 'Role of lactic acid bacteria in fermented vegetables', *Grasas aceites*, vol. 71, no. 2, p. e358, June 2020, doi: 10.3989/gya.0344191.
[34] S. Sah, S. Putatunda, R. Majumder, and A. Das, 'Changes of volatile flavored compounds during lactic acid fermentation in cereal-based products', *FFHD*, vol. 14, no. 11, pp. 871–882, Nov. 2024, doi: 10.31989/ffhd.v14i11.1503.
[35] H.-C. Huang *et al.*, 'Phytochemicals levels and biological activities in Hibiscus sabdariffa L. were enhanced using microbial fermentation', *Industrial Crops and Products*, vol. 176, p. 114408, Feb. 2022, doi: 10.1016/j.indcrop.2021.114408.
[36] W. Leonard, P. Zhang, D. Ying, B. Adhikari, and Z. Fang, 'Fermentation transforms the phenolic profiles and bioactivities of plant-based foods', *Biotechnology Advances*, vol. 49, p. 107763, July 2021, doi: 10.1016/j.biotechadv.2021.107763.
[37] D. Khayatan *et al.*, 'Plant-Derived Fermented Products: An Interesting Concept for Human Health', *Current Developments in Nutrition*, vol. 8, no. 5, p. 102162, May 2024, doi: 10.1016/j.cdnut.2024.102162.
[38] Y. Wang, C. Wang, J. Shi, and Y. Zhang, 'Effects of derivatization and probiotic transformation on the antioxidative activity of fruit polyphenols', *Food Chemistry: X*, vol. 23, p. 101776, Oct. 2024, doi: 10.1016/j.fochx.2024.101776.
[39] J. Zhang, N. Xia, J. Teng, B. Wei, and L. Huang, 'Effect of lactic acid bacteria fermentation on extractable and non-extractable polyphenols of soybean milk: Influence of β-glucosidase and okara', *Food Bioscience*, vol. 56, p. 103326, Dec. 2023, doi: 10.1016/j.fbio.2023.103326.
[40] H. Qin, G. J. King, P. Borpatragohain, and J. Zou, 'Developing multifunctional crops by engineering Brassicaceae glucosinolate pathways', *Plant Communications*, vol. 4, no. 4, p. 100565, July 2023, doi: 10.1016/j.xplc.2023.100565.
[41] G. O. Ogbuozobe, A. C. Azubuike, I. N. Clara, and A. C. Eziamaka, 'Proximate and Mineral Compositions of the Stem and Leaf of Cnidoscolus aconitifolius', *AJRiB*, vol. 7, no. 1, pp. 44–50, Dec. 2024, doi: 10.56557/ajrib/2024/v7i137.
[42] C. Little *et al.*, 'Vegetable fermentations brined with low salt for reclaiming food waste', *Journal of Food Science*, vol. 87, no. 5, pp. 2121–2132, May 2022, doi: 10.1111/1750-3841.16084.
[43] D. H. Sb, I. Inayah, D. E. Puspaningtyas, P. M. Sari, and N. H. Kusuma, 'Diversity of Traditional Fermented Foods: Sucrose and Reducing Sugar Analysis of Various Fermented-Cassava', *cpmhs*, vol. 2, no. 1, pp. 24–32, Mar. 2023, doi: 10.31098/cpmhs.v2i1.627.
[44] S. Torres, H. Verón, L. Contreras, and M. I. Isla, 'An overview of plant-autochthonous microorganisms and fermented vegetable foods', *Food Science and Human Wellness*, vol. 9, no. 2, pp. 112–123, June 2020, doi: 10.1016/j.fshw.2020.02.006.
[45] X. Lin, S. Bakyrbay, L. Liu, X. Tang, and Y. Liu, 'Microbiota Succession and Chemical Composition Involved in Lactic Acid Bacteria-Fermented Pickles', *fermentation*, vol. 9, no. 4, p. 330, Mar. 2023, doi: 10.3390/fermentation9040330.
[46] X. Yang *et al.*, 'Effect of Lactic Acid Bacteria Fermentation on Plant-Based Products', *Fermentation*, vol. 10, no. 1, p. 48, Jan. 2024, doi: 10.3390/fermentation10010048.