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Enhancing the Nutritional Value and Sensory Appeal of *Cleome gynandra* and *Solanum nigrum* through Controlled Fermentation Using *Lactoplantibacillus plantarum*

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Abstract

This study evaluated the effects of blanching, glucose concentration, and fermentation duration on the nutritional composition and sensory attributes of black nightshade and spider plant vegetables. Blanching at 80˚C for 10 minutes recorded significantly high levels of protein, vitamin A and vitamin C content for both vegetables. In black nightshade, protein, vitamin A, and vitamin C contents were 4.81%, 10.69 mg/100g, and 0.50 g/100g, respectively. Corresponding values in spider plant were 4.03% for protein, 10.18 mg/100g for vitamin A, and 0.58 g/100g for vitamin C. The highest nutrient levels were recorded under fermentation with 3% glucose, yielding protein contents of 5.03% in black nightshade and 4.02% in spider plant, along with vitamin A concentrations of 11.09 mg/100g and 10.66 mg/100g, and vitamin C levels of 0.50 g/100g and 0.56 g/100g, respectively. In black nightshade, protein content increased significantly from 3.89% to 5.14%, vitamin A from 9.64 mg/100g to 10.91 mg/100g, and vitamin C from 0.40 g/100g to 0.50 g/100g by day 21 of fermentation. Comparable improvements were observed in spider plant, with significant increase in protein content from 3.21% to 3.80%, 8.84 mg/100g to 10.58 mg/100g for vitamin A, and 0.54 g/100g to 0.56 g/100g for vitamin C. Sensory evaluation revealed higher acceptability in blanched and fermented samples. Spider plant blanched at 90˚C/5mins scored highest (~4.0), while unblanched, spontaneously fermented samples scored lowest (<3.0). These findings highlight controlled fermentation as a viable method to enhance the nutritional value and sensory appeal of ILVs, supporting their potential for broader dietary adoption in Sub-Saharan Africa.

Keywords

Indigenous Leafy Vegetables, Fermentation, Blanching, Nutrient Retention,

Sensory Evaluation, Black Nightshade, Spider Plant

1. Introduction

Indigenous leafy vegetables (ILVs) refer to plant species integral to traditional diets in specific regions. They hold significant potential for addressing food security challenges and enhancing livelihoods across Sub-Saharan Africa [1] [2]. With the Sub-Saharan region hosting 45,000 plant species, of which 1000 are edible, it is noteworthy that approximately 22.8% of the African population still suffers from malnutrition despite this rich diversity. Researchers have demonstrated the nutritional importance of ILVs, emphasizing their role in addressing malnutrition [3]. Indigenous leafy vegetables are renowned for their health-enhancing properties and are recognized for their abundance of bioactive compounds and micronutrients in African diets. *Cleome gynandra* is a rapidly emerging as a widely utilized leafy vegetable, attributed to its dense nutritional profile and bioactive compounds associated with various health-promoting effects. *C. gynandra* is a rich source of essential micronutrients and antioxidants, containing high levels of α-, β-, and γ-tocopherols, ascorbic acid, α- and β-carotene, lutein, violaxanthin, and β-cryptoxanthin. The abundant nutritional profile of *Cleome gynandra*, particularly its high concentrations of macro- and micronutrients, underscores its potential role in combating malnutrition and addressing the rising prevalence of diet-related obesity and non-communicable diseases. *Solanum nigrum* is a traditional plant that is rich in polyphenols, polysaccharides, glycoproteins, and glycoalkaloids, which makes the plant active against various ailments. Studies have demonstrated that the leaves and berries of *Solanum nigrum* are rich in essential minerals and nutrients that help prevent iron deficiency anaemia. Notably, *S. nigrum* leaves are an excellent source of essential fatty acids, carotenoids, β-carotene, and lycopene, which further enhance their medicinal value and potential role in improving nutritional and health outcomes. Compared to exotic counterparts, ILVs are often more nutritious, boasting higher levels of vitamins, minerals, and other essential nutrients [4]. They are particularly rich in vitamin C and beta-carotene, while also serving as vital sources of fibre, protein, and various phytochemicals that confer health benefits [5].

The widely consumed ILVs in East and West Africa include cowpea leaves (*Vigna unguiculata*), Amaranth (*Amaranthus viridis*), spider plant (*Cleome gynandra*), African night shade (*Solanum scabrum*), cassava leaves (*Manihot esculenta*), pumpkin leaves (*Curcubita* spp.), slender leaves (*Crotalaria ochroleuca*), sweet potato leaves (*Ipomea batatas*), Morenga leaves (*Moringa oleifera*) and the African kale (*Brassica carinata*) [6]. However, despite their nutritional significance, ILVs are susceptible to high post-harvest losses (PHLs), estimated at 50%, largely due to inadequate handling techniques among farmers [7]. Furthermore, the consumption of ILVs remains low primarily because they are abundant during wet seasons

but scarce in dry seasons, which limits their availability [8].

The post-harvest losses of ILVs remain poorly documented due to their high reliance on informal marketing channels and limited availability of effective loss assessment technologies [9]. Furthermore, ILVs are prone to post-harvest losses due to their high moisture content, active metabolism, and tender texture, making them susceptible to senescence, desiccation, physiological disorders, mechanical injury, and microbial spoilage. Despite their nutritional richness, ILVs also contain antinutritional compounds such as phenolic compounds, phytates, tannins, oxalates, alkaloids, glucosinolates, saponins, and protease inhibitors that can bind to specific nutrients, reducing their bioavailability [10] [11].

Traditional treatment methods for ILVs, such as boiling in water followed by sun drying, are commonly employed but face challenges related to hygiene and temperature control, resulting in significant losses of nutritive value and microbial spoilage [8]. Although lactic acid fermentation has been used for the preservation of milk, cereals, and vegetables, its application in Africa often occurs at a small scale, household level, utilizing simple non-sterile equipment and natural inoculum under unregulated conditions, leading to variations in food quality and sensory attributes [3] [12].

The conventional method for preserving ILVs often involves blanching pretreatment, followed by sun-drying [13]. However, this technique poses challenges in ensuring product safety, quality, consistency, and efficiency, leading to undesired sensory fluctuations. Therefore, there is a growing need to explore processing techniques that can extend the storage life of ILVs while retaining their nutritional content. Fermentation has emerged as a promising approach in this regard [3]. Studies have demonstrated that fermentation, when combined with solar drying, can produce ILVs with a reduced antinutrient composition, thereby enhancing their acceptability and potential to address malnutrition by providing essential micronutrients [13]. Additionally, fermentation holds potential value for small-scale producers as it can enhance the nutritional value of ILVs and contribute to reducing post-harvest losses, resulting in more stable product prices and availability [8].

Studies have consistently shown that fermentation can effectively reduce antinutrients such as oxalates and phytates compared to unfermented ILVs [14]. Furthermore, fermentation has been found to decrease the levels of cyanide in cassava leaves intended for use as vegetables [15]. In terms of essential minerals, fermentation has been shown to retain levels of important nutrients such as beta-carotene and alpha tocopherol, which positively impact product quality [16]. Studies [13] [17] focused on cowpea fermentations using spontaneous lactic acid fermentation without inoculation. In both cases, fermentable sugars were added to support lactic acid bacteria (LAB), with sugars like sucrose and glucose added at levels of 2% to 3% being effective in fermenting leafy vegetables [3]. This study explored the effects of blanching methods, glucose concentration, and fermentation duration on the physicochemical, nutritional, and sensory attributes of spider plant and black nightshade vegetables. By assessing the influence of diverse processing techniques

on the preservation and nutritional quality of ILVs, this research aimed to identify practical strategies to promote their availability, reduce antinutrient levels, and enhance overall nutritional content, thereby improving food security and promoting dietary diversity in Kenya.

2. Materials and Methods

2.1. Study Site

The study was conducted at Egerton University, located in Nakuru County, Kenya. Microbial analyses were performed at the Soil Microbiology Laboratory, while protein analyses were conducted in the Food Analysis Laboratory. Vitamin and antinutrient analyses were carried out at the Animal Science Laboratory, all within Egerton University.

2.2. Experimental Design

A completely randomized design (CRD) with a factorial arrangement was employed for the study. The main factors investigated included blanching temperature regimes, fermentation time and glucose concentration. Blanching was conducted at temperatures of 80˚C for 10 minutes and 90˚C for 5 minutes. Fermentation durations studied were 0, 7, 14, and 21 days, while glucose concentrations used were 0%, 1%, 2%, and 3%. The experiment was conducted in triplicates. Samples were analyzed for moisture content, protein content, beta-carotene, ascorbic acid, calcium and iron. The six best-performing samples were selected based on their nutritional performance during fermentation for sensory evaluation.

2.3. Material Collection and Preparation

2.3.1. Lactic Acid Bacteria Preparation

Lactoplantibacillus plantarum, the fermenting microorganism used in this study, was isolated from *Mursik*, a traditionally fermented milk from the Kalenjin community, Kenya [18]-[20]. *Mursik* samples were randomly collected from three households and three commercial vendors in Njoro town, Nakuru County, totalling six samples. Approximately 200 ml per sample was obtained using sterile, autoclaved containers. Farmers provided milk in their original fermentation containers, which were mixed thoroughly before sampling. Three replicates per sample were taken aseptically into labelled containers, sealed, and transported at 4˚C to Egerton University within 3 - 4 hours for lactic acid bacteria isolation.

Isolation of LAB for fermentation and sequencing

Isolation of lactic acid bacteria

Lactic acid bacteria were enumerated by pour-plating on de Man, Rogosa, and Sharpe (MRS) agar (Oxoid Ltd.), incubated anaerobically at 35˚C for 2 days using Anaerocult A packs (Merck, Darmstadt, Germany). Distinct colonies from the highest dilution plates were selected based on morphology (size, shape, gloss, colour) using a sterile loop. Colonies were purified by triple streaking on MRS agar and stored in 0.25 mol/L sucrose solution at −18˚C for further identification.

Phenotypic and Physiological Characterization of Lactic Acid Bacteria

Isolates were first confirmed as lactic acid bacteria by Gram staining and catalase testing. Colonies were described morphologically (shape, size, elevation, surface, edges). Gram-positive bacteria stained blue/purple and were either rod-shaped or cocci, while Gram-negative bacteria-stained pink/red under a light microscope. Catalase test involved adding 3% hydrogen peroxide to colonies; gas production indicated a positive result. Only catalase-negative, Gram-positive isolates proceeded to physiological tests. These isolates were cultured in MRS broth to assess growth at 15˚C and 45˚C by observing turbidity after 72 hours. Gas production from glucose metabolism was also evaluated. Salt tolerance was tested by incubating isolates in MRS broth with 4% and 6.5% NaCl at 37˚C for 4 days; increased turbidity indicated positive growth.

Molecular Characterization of Lactic Acid Bacteria

Genomic DNA was extracted from 12-hour-old MRS broth cultures following the method of Cardinal *et al.* (1997) [21], with cell lysis achieved using lysozyme, SDS, proteinase K, and CTAB/NaCl, followed by purification with chloroform/isoamyl alcohol. DNA was precipitated with isopropanol, washed with 70% ethanol, air-dried, and resuspended in TE buffer containing RNase. DNA quality and concentration were assessed using a Nanodrop spectrophotometer, and 0.8% of the agarose gel electrophoresis was stained with ethidium bromide.

Purified DNA samples were stored at –20˚C and sent to Inqaba Biotech (South Africa) for 16S rRNA gene sequencing using group-specific primers Lac1 and Lac2-GC, and universal primers 907R(C), 1492R, ITS1, and ITS4. Sequences were analyzed using the BLAST algorithm and aligned in MEGA 6.0 software. Phylogenetic trees were constructed using the neighbour-joining method with 100 bootstrap replicates to assess branch reliability.

2.3.2. Black Nightshade and Spider Plant Collection

Black nightshade and spider plant vegetables were collected from Njoro, Nakuru County. Young and tender leaves were selected and transported to Egerton University for analysis using a cool box at 4˚C. Upon arrival, the leaves were sorted, and the decayed and overmature leaves were discarded. After sorting, the leaves were washed with tap water to remove dirt. Using a sharp sterile knife, the leaves were then shredded into 3 mm pieces. Both raw and processed ILVs were analyzed for moisture content, vitamins (vitamin C, beta-carotene content) and minerals (iron and zinc).

2.4. Blanching of ILVs

The tender shoots of ILVs were blanched at 80˚C/10mins and 90˚C/5mins [8]. This was performed by bringing a large pan half full of water to a temperature of 80˚C and 90˚C. The shredded vegetables (500 g) were put into a wire basket and gently lowered into blanching water. Once the blanching time was over, the vegetables were removed from the blanching water and plunged into cold, sterile water at room temperature to halt the blanching process.

2.5. Fermentation

After blanching, the shredded vegetables were divided into 500g batches for fermentation, with glucose added at concentrations of 0%, 1%, 2%, and 3%. Fermentation was carried out in 2.5% brine, as it is commonly recommended for vegetable fermentation due to its ability to inhibit spoilage microorganisms, support lactic acid bacteria growth, and maintain the texture, flavour, and the final product quality. Subsequently, the vegetables were inoculated with LAB isolates at a concentration of approximately 1×10^8 CFU/mL to achieve this inoculum concentration, a 0.5 McFarland standard was used as a reference. This standard was prepared by mixing 0.05 mL of 1.175% barium chloride with 9.95 mL of 1% sulfuric acid. Well-isolated colonies of LAB previously stored under 2% sucrose were carefully picked and suspended in sterile saline to form a turbid bacterial suspension. The turbidity of this suspension was then visually compared to the 0.5 McFarland standard in a well-lit environment against a white background. The suspension was diluted with sterile saline or concentrated by adding more bacterial cells to match the standard. Additionally, the optical density (OD) at 600 nm was measured using a spectrophotometer to confirm that the suspension fell within the expected OD_{600} range of 0.08 to 0.1, corresponding to the desired bacterial concentration [12]. Fermentation was conducted for up to 21 days at 25˚C, with samples analyzed for moisture content, protein, vitamins (vitamin C, beta carotene), and minerals (calcium and iron) on days (0, 21).

2.5.1. Moisture Content Determination

Moisture content was evaluated by oven drying using the Association of Official Analytical Chemists [22] method 970.23. Samples were dried in an oven at 105˚C for 3 hours and then cooled in a desiccator for 10 minutes. Moisture content was calculated as weight loss represented as a percentage of original sample weight.

2.5.2. Crude Protein Determination

Using procedure 991.20 of the Kjeldahl technique [22], the crude protein content was ascertained. Samples (2 grams) were weighed and put into the micro-Kjeldahl digestion tubes. Each tube was then filled with 10 mL of concentrated nitrogen-free sulfuric acid and one selenium tablet, which served as a catalyst. The materials were subsequently digested for three hours at 445 degrees Celsius in a digester. The Kjeldahl distillation apparatus was used to distil the byproducts of digestion. The distillate was collected in 15 millilitres of 0.1M HCl, to which a mixture of methylene blue and methyl red was added as an indicator. The excess HCl was titrated against 0.5 N NaOH. The crude protein content was calculated using Equation (1):

$$\text{Crude protein (g/100g)} = (V_1 - V_2) \times M \times 1.4 \times 6.25 / W \quad (1)$$

where V_2 is the volume of HCl used for the test portion, V_1 is the volume of HCl used for the blank test, M is the molarity of the acid, W is the weight of the test portion, and 6.25 is the conversion factor. The analysis was performed in triplicate, and the results were reported as means ± standard deviations.

2.6. Antioxidants Determination

2.6.1. Determination of Vitamin C

Ascorbic acid was determined by titration with 2,6-dichlorophenolindophenol dye following the AOAC method 990.23 [22]. Samples were homogenized in metaphoric acid solution and the extract filtered, then diluted appropriately to a concentration of 100 mg ascorbic acid/100 mL.

A standard solution of pure ascorbic acid was prepared by dissolving 50 mg in 100 mL of water. Sample filtrate was titrated against the standard solution to a pink end point within 10 seconds and ascorbic acid was calculated as per Equation (2):

$$\text{Concentration of ascorbic acid (mg/g)} = C \times V \times (DF/WT) \quad (2)$$

where C = Milligrams of ascorbic acid per millilitre of dye (mg/mL).

V = Volume of dye used in titrating the sample (mL).

DF = Dilution factor.

WT = Weight of the sample (grams).

2.6.2. Determination of Beta Carotene

Using spectrophotometry (UV-VIS Spectrophotometer, model 2377, India), the vitamin A content was ascertained in accordance with AOAC International Method 2000.10 [22]. After precisely weighing the sample (2 grams), it was put in a mortar and 15 millilitres of acetone were added. To extract the chlorophyll, the mixture was mashed using a mortar and pestle, and then acid-washed sand was added. After that, 15 mL volumes of acetone were used to rewash the residue until the extract lost its color. Acetone was added to the extract to get it up to the 100 mL level. From this, 25 mL of the extract was evaporated in a rotary vacuum evaporator, and roughly one millilitre of petroleum ether was used to wash the residue. After that, the mixture was put into a silica gel-prepared chromatographic column (TLC Silica Gel 60), and to eliminate any remaining water from the sample, a 1 cm layer of anhydrous sodium sulphate was placed on top of the column. Next, the evaporated samples were quantitatively spotted into a column, dissolved in 2 mL of petroleum ether, and eluted with the petroleum ether. Petroleum ether was used to raise the initial yellow elute to the required level after it had been collected in a 25 mL flask. A CE of 440 UV/Vis double-beam scanning spectrophotometer calibrated with standard solutions of pure beta-carotene in petroleum ether was used to measure the beta-carotene at 450 nm.

A solution of 0.001 g of pure beta-carotene in 1000 mL of petroleum ether was used to prepare the standards. This created a 1000 ppm (1000 µg/mL) solution. The standards were 0.5 µg/mL, 1.0 µg/mL, 1.5 µg/mL, 2.0 µg/mL, 2.5 µg/mL, and 3.0 µg/mL, whose absorbances were read at 440 nm.

The concentration of beta-carotene (CX) was calculated using Equation (3):

$$CX\left(\frac{\text{mg}}{100\text{ g}}\right) = \frac{AX \times CS\left(\text{mg/mL}\right) \times \text{Total volume of extract}\left(\text{mL}\right)}{AS \times \text{Sample weight}\left(\text{g}\right)} \quad (3)$$

where CX represents the concentration of β-carotene, AX represents the peak area

of β-carotene, CS represents the concentration of the standard, and AS represents the peak area of the standard.

2.7. Determination of Minerals

Using the Atomic Absorption Spectrophotometer (AAS) and the AOAC International technique 970.30 [22], the two minerals, calcium and iron, were measured. A hollow cathode lamp, a recorder, and an air-acetylene flame were included in the spectrophotometer's setup. Calcium was measured at a wavelength of 248 nm, and iron was measured at 234 nm. In a digester, 10 mL of concentrated HCl and 20 mL of concentrated HNO_3 were used to digest about 0.5 grams of the material. The samples were heated to 250˚C with caution until the strong reaction stopped. After that, the temperature was raised to 450˚C for 45 minutes. Next, filtering was carried out in 60 mL filter bottles using Whatman filter paper no.4 (22 µm pore size) to obtain the distillate. Distilled water was added until the filtrate reached the desired level. Following a comparison of the amounts of each component with the standards, the calcium and iron concentrations in (mg/100g) were reported.

2.8. Consumer Acceptability Test

2.8.1. Subjects

Panelists who use the product were selected for the test. Prior to the test, the study was reviewed and approved by the Egerton University Research Ethics Committee. An informed consent was obtained from each subject before the sensory evaluation test.

2.8.2. Sensory Evaluation

The black nightshade and spider plant vegetables, under different treatments, were conventionally boiled 90˚C/10mins before the sensory test. Spontaneously fermented spider plant and black nightshade vegetables were used as the controls. The six samples were coded and then presented to 30 panelists to evaluate them for appearance, aroma, taste, texture, and overall acceptability. The 5-point hedonic scale was used, with the expressions ranging from "dislike extremely" (1) to "like extremely" (5).

2.9. Data Analysis

The data obtained from the experiments above were subjected to statistical analysis using statistical package SAS 9.4. The results were expressed as mean ± standard deviation. Means were analyzed for significant differences by analysis of variance (ANOVA) at ($p < 0.05$)

3. Results

3.1. Nutritional Composition of Fresh Spider Plant and Black Nightshade Vegetables

The nutrient content of fresh black nightshade (BN) and spider plant (SN) per 100 grams is shown in Table 1. Black nightshade had a statistically higher ($p < 0.05$)

protein content (5.90%), Vitamin A content (11.57 mg/100g), moisture content (87.45%), and Fe (11.33 mg/100g) compared to spider plant (5.61%, 10.97 mg/100g, 83.29%, and 7.12 mg/100g, respectively). Spider plant on its part had statistically higher vitamin C (0.62 g/100g) and calcium (385.69 mg/100g) compared to black night shade (0.52 g/100g and 218.11 mg/100g, respectively). The nutritional values are in the similar range reported in the other studies [23] [24], with variations attributed to climatic and soil conditions.

Table 1. Nutritional composition of fresh spider plants and black nightshade vegetables.

Type of vegetable	Ptn (%)	VitC (g/100g)	VitA (mg/100g)	MC (%)	Ca (mg/100g)	Fe (mg/100g)
BN	5.90 ± 0.03[a]	0.52 ± 0.02[b]	11.57 ± 0.24[a]	87.45 ± 0.67[a]	218.113 ± 0.77[b]	11.33 ± 0.07[a]
SN	5.61 ± 0.03[b]	0.62 ± 0.01[a]	9.97 ± 0.19[b]	83.29 ± 0.09[b]	385.69 ± 0.40[a]	7.12 ± 0.06[b]

Note: BN = Black nightshade; SN = Spider plant; Ptn = Protein; VitC = Vitamin C; VitA = Vitamin A; MC = Moisture content; Ca = Calcium; Fe = Iron. Means followed by the same letter are not significantly different at $p < 0.05$.

3.2. Effect of Blanching Regimes on Protein, Antioxidant (Vitamin C and A), Moisture, Mineral Content (Calcium, Iron) of Fermented Black Nightshade and Spider Plant Vegetables

The effect of different blanching regimens on the nutrient content of fermented black nightshade (BN) and spider plant (SN) is shown in Table 2. In both vegetables, blanching at 80˚C for 10 minutes resulted in significantly higher protein retention (4.81% for BN and 4.03% for SN) compared to blanching at 90˚C for 5 minutes (4.23% for BN and 2.98% for SN) at ($p < 0.05$). Blanching also led to loss of vitamin A but similar to protein, blanching at 80˚C for 10 minutes led to significantly high vitamin A retention in both vegetables (10.69 mg/100g for BN and 10.18 mg/100g for SN) compared to 90˚C for 5 minutes (9.99 mg/100g for BN and 9.28 mg/100g for SN). Significant differences in moisture content between the two treatments for either vegetable were observed (Table 2). Blanching significantly affected vitamin C content within the two treatments for either vegetable at $p > 0.05$. Blanching at higher temperatures, 90˚C/5mins led to significant loss of vitamin C (0.48 g/100g for BN and 0.52 g/100g for SN), while blanching at 80˚C /10mins showed a higher vitamin C retention of (0.50g/100g for BN and 0.58 g/100g for SN). Blanching significantly increased the moisture content of both vegetables compared to the fresh vegetables. The results showed a significant decrease in calcium levels ($p < 0.05$) following blanching, with spider plant exhibiting more pronounced reductions compared to black nightshade. Interestingly, when comparing different blanching conditions within each vegetable type, spider plant blanched at 80˚C for 10 minutes retained significantly high calcium content (241.26 mg/100g) compared to the 90˚C for 5 minutes treatment (229.52 mg/100g) (Table 2). Similarly, blanching led to a significant reduction ($p < 0.05$) in iron content, with spider plant also demonstrating higher iron retention following milder blanching conditions (3.07 mg/100g for 80˚C/10mins vs. 1.93 mg/100g for 90˚C/5mins). Black nightshade exhibited a similar trend, with higher iron content observed

after milder blanching (4.95 mg/100g for 80˚C/10mins vs. 3.53 mg/100g for 90˚C/5mins).

Table 2. Effect of blanching regimes on Ptn, VitC, vita, MC, calcium, iron of fermented black nightshade and spider plant vegetable.

Type of vegetable	Blanching	Ptn (%)	VitC (g/100g)	VitA (mg/100g)	MC (%)	Ca (mg/100g)	Fe (mg/100g)
BN	90˚C/5mins	4.23 ± 0.15^{b}	0.48 ± 0.03^{b}	9.99 ± 0.20^{b}	90.84 ± 0.30^{a}	211.05 ± 5.55a	3.53 ± 0.44^{b}
	80˚C/10mins	4.81 ± 0.23^{a}	0.50 ± 0.22^{a}	10.69 ± 0.21^{a}	90.15 ± 0.33^{b}	188.44 ± 7.12b	4.95 ± 0.62^{a}
SN	90˚C/5mins	2.98 ± 0.08^{b}	0.52 ± 0.02^{b}	9.28 ± 0.40^{b}	86.66 ± 0.29^{b}	229.52 ± 3.98^{b}	1.93 ± 0.36^{b}
	80˚C/10mins	4.03 ± 0.16^{a}	0.58 ± 0.09^{a}	10.18 ± 0.24^{a}	87.46 ± 0.29^{a}	241.26 ± 3.09^{a}	3.07 ± 0.42^{a}

Note: BN = Black nightshade; SN = Spider plant; Ptn = Protein; VitC = Vitamin C; VitA = Vitamin A; MC = Moisture content; Ca = Calcium; Fe = Iron; mins = minutes. Means followed by the same letter are not significantly different at $p < 0.05$.

3.3. Effect of Glucose Concentration on the Nutritional Composition of Fermented Black Nightshade and Spider Plant Vegetables

The effect of glucose concentration on the nutritional composition of fermented spider plant and black nightshade vegetables was investigated and the results are presented in Table 3. Both vegetables displayed a trend of increasing protein content with increasing glucose concentration. Black nightshade showed a significant increase in protein content (from 3.92% at 0% glucose to 5.03% at 3% glucose). Spider plant also exhibited a similar trend (from 3.13% at 0% glucose to 4.02% at 3% glucose). Black nightshade displayed a significant increase in vitamin A content with increasing glucose concentration (from 9.52 mg/100g at 0% glucose to 11.09 mg/100g at 3% glucose). Spider plant also showed a similar trend, where vitamin A increased with increase in glucose concentration (from 8.88 mg/100g at 0% glucose to 10.66 mg/100g at 3% glucose). There were no significant differences in vitamin C content observed between different glucose concentrations (1% - 3%) for spider plant vegetables. This suggests that glucose concentration within the

Table 3. Effect of glucose concentration on protein, vitamin C, vitamin A, moisture content, calcium, iron of fermented black nightshade and spider plant vegetables.

Type of vegetable	Glucose (%)	Ptn (%)	VitC (g/100g)	VitA (mg/100g)	MC (%)	Ca (mg/100g)	Fe (mg/100g)
BN	0.0	3.92 ± 0.06^{d}	0.48 ± 0.02^{b}	9.52 ± 0.12^{c}	91.64 ± 0.12^{a}	209.49 ± 0.73^{a}	5.66 ± 0.38^{a}
	1.0	4.38 ± 0.15^{c}	0.50 ± 0.01^{a}	10.18 ± 0.18^{b}	90.29 ± 0.42^{b}	203.16 ± 1.40^{b}	4.18 ± 0.73^{b}
	2.0	4.74 ± 0.27^{b}	0.50 ± 0.02^{a}	10.31 ± 0.24^{b}	90.28 ± 0.42^{b}	197.87 ± 0.23^{c}	3.82 ± 0.83^{c}
	3.0	5.03 ± 0.42^{a}	0.50 ± 0.01^{a}	11.09 ± 0.45^{a}	89.76 ± 0.58^{c}	188.47 ± 0.62^{d}	3.31 ± 0.97^{d}
SN	0.0	3.13 ± 0.13^{d}	0.53 ± 0.01^{b}	8.88 ± 0.14^{d}	88.03 ± 0.20^{a}	279.74 ± 5.25^{a}	3.19 ± 0.38^{a}
	1.0	3.34 ± 0.17^{c}	0.55 ± 0.01^{a}	9.42 ± 0.22^{c}	87.22 ± 0.34^{b}	233.10 ± 5.77^{b}	2.73 ± 0.48^{b}
	2.0	3.53 ± 0.21^{b}	0.56 ± 0.01^{a}	9.88 ± 0.36^{b}	86.69 ± 0.40^{bc}	222.42 ± 2.98^{c}	2.19 ± 0.63^{c}
	3.0	4.02 ± 0.32^{a}	0.56 ± 0.01^{a}	10.66 ± 0.59^{a}	86.30 ± 0.55^{c}	206.30 ± 5.44^{d}	1.89 ± 0.73^{d}

Note: BN = Black nightshade; SN = Spider plant; Ptn = Protein; VitC = Vitamin C; VitA = Vitamin A; MC = Moisture content; Ca = Calcium; Fe = Iron; mins = minutes. Means followed by the same letter are not significantly different at $p < 0.05$.

tested range (1% - 3%), which influences microbial growth did not significantly affect vitamin C levels. Both vegetables exhibited a slight decrease in moisture content with increasing glucose concentration, with those vegetables with 3% glucose concentration recording significantly low moisture content of 89.76% for BN and 86.30% for SN. Both vegetables showed a significant decrease in Fe^{2+} and Ca^{2+} content with increasing glucose concentration. The lowest iron content of (3.31 mg/100g for BN and 1.89 mg/100g for SN) and calcium content (188.47 mg/100g for BN and 206.30 mg/100g for SN) was recorded for those vegetables with 3% glucose concentration.

3.4. Effect of Fermentation Duration on the Nutritional Composition of Fermented Black Nightshade and Spider Plant Vegetables

The vegetables exhibited a significant increase in protein, vitamin A and vitamin C content with fermentation, as shown in Table 4. On the other hand, moisture content, Fe^{2+} and Ca^{2+} contents decreased significantly. Fermented black nightshade vegetables recorded the highest levels of protein (5.14%), vitamin C (0.51 g/100g), and vitamin A (10.91 mg/100g) on day 21, while the lowest levels of calcium (122.13 mg/100g), iron (2.03 mg/100g), and moisture content (89.38%) were recorded on day 21. Similarly, fermented spider plant vegetables exhibited the highest levels of protein (3.80%), vitamin C (0.56 g/100g), and vitamin A (10.58 mg/100g) on day 21, while day 21 held the record for the lowest moisture (86.12%), calcium (144.59 mg/100g), and iron (0.79 mg/100g).

Table 4. Effect of fermentation time on protein, vitamin C, vitamin A, moisture content, calcium, iron of fermented black nightshade and spider plant vegetables.

Type of vegetable	Fermentation days	Ptn (%)	VitC (g/100g)	VitA (mg/100g)	MC (%)	Ca (mg/100g)	Fe (mg/100g)
BN	0	3.89 ± 0.05^{b}	0.48 ± 0.01^{b}	9.64 ± 0.09^{b}	91.60 ± 0.09^{a}	277.38 ± 2.56^{a}	6.45 ± 0.24^{a}
	21	5.14 ± 0.21^{a}	0.51 ± 0.02^{a}	10.91 ± 0.24^{a}	89.38 ± 0.31^{b}	122.13 ± 3.91^{b}	2.03 ± 0.37^{b}
SN	0	3.21 ± 0.07^{b}	0.54 ± 0.02b	8.84 ± 0.92^{b}	88.00 ± 0.17^{a}	326.18 ± 2.44^{a}	4.22 ± 0.15^{a}
	21	3.80 ± 0.20^{a}	0.56 ± 0.01^{a}	10.58 ± 0.31^{a}	86.12 ± 0.28^{b}	144.59 ± 2.18^{b}	0.79 ± 0.24^{b}

Note: BN = Black nightshade; SN = Spider plant; Ptn = Protein; VitC = Vitamin C; VitA = Vitamin A; MC = Moisture content; Ca = Calcium; Fe = Iron; mins = minutes. Means followed by the same letter are not significantly different at $p < 0.05$.

3.5. Consumer Acceptability of Fermented Black Night Shade and Spider Plant Vegetables

The study also developed and tested the acceptability of the fermented spider plant and black nigh shade vegetables. Generally, vegetables subjected to controlled fermentation scored preferably higher values based on appearance, aroma, texture, taste and overall acceptability. Whereas the controls (spontaneously fermented) scored the lowest values based on all the attributes tested, as shown in Table 5. The samples chosen for sensory evaluation were selected based on their nutritional quality following fermentation. Within each blanching regime, the best sam-

ple was selected for sensory evaluation. Fermented spider plant vegetables blanched at 90˚C/5mins recorded significantly high levels of aroma, taste and overall acceptability. Whereas fermented black night shade vegetables blanched at 90˚C/5mins scored higher values for appearance and texture. Fermented black night shade and spider plant vegetables blanched at 90˚C/5mins were the most preferred vegetables by consumers (Table 5).

Table 5. Acceptability of fermented black nightshade and spider plant as evaluated by consumer sensory panel.

Type of vegetable	Blanching	Appearance	Aroma	Texture	taste	Overall acceptability
BN	90˚C/5mins	4.48 ± 0.57^{a}	3.45 ± 0.77^{bc}	4.48 ± 0.57^{a}	3.83 ± 0.96^{ab}	4.13 ± 0.43^{a}
	80˚C/10mins	3.93 ± 0.57^{b}	3.32 ± 0.74^{c}	4.00 ± 0.73^{ab}	3.55 ± 0.99^{b}	4.00 ± 0.68^{ab}
ST	90˚C/5mins	4.35 ± 0.55^{ab}	4.58 ± 0.72^{a}	3.81 ± 0.79^{b}	4.29 ± 0.69^{a}	4.32 ± 0.54^{a}
	80˚C/10mins	4.00 ± 0.57^{b}	3.97 ± 0.75^{b}	3.16 ± 0.86^{c}	3.19 ± 0.11^{b}	3.61 ± 0.72^{b}
BN S. F	N. B	3.03 ± 0.60^{c}	2.35 ± 0.95^{d}	2.87 ± 0.81^{d}	2.48 ± 0.89^{c}	2.38 ± 0.72^{c}
ST S. F	N. B	2.58 ± 0.81^{c}	1.90 ± 1.01^{e}	2.03 ± 0.71^{e}	1.58 ± 0.72^{d}	1.32 ± 0.54^{d}

Note: BN = Black nightshade; SN = Spider plant; N. B = Not blanched; S. F = Spontaneously fermented. Means followed by the same letter are not significantly different at $p < 0.0$.

4. Discussion

The reduction in protein content during blanching can be attributed to the leaching of water-soluble nitrogen-containing compounds, including free amino acids, nucleic acids, nucleotides, and specific water-soluble vitamins, and the process is severe with increase in temperature [8]. The decrease in vitamin A in both vegetables corresponds to a study done by [25], who found that boiling resulted in the greatest loss of β-carotene in Amaranthus species. Vitamin A is not destroyed by heat, but heat helps to break down cell walls and hence the release. The blanching regimes caused some vitamin C loss compared to fresh vegetables (Table 1). Vitamin C is a heat-sensitive and prone to degradation during blanching [26]. Additionally, its water solubility can lead to leaching into the cooking water [27]-[29]. Since vitamin C loss is often used as an indicator of overall nutrient loss during blanching [26], the minimal vitamin C change suggests the chosen blanching regimes were relatively mild and didn't cause substantial nutrient losses in these vegetables.

Blanching significantly increased the moisture content of both vegetables due to water absorption by damaged cells and water adhesion to the surface of the vegetables [30]. The observed decrease in calcium and iron levels post-blanching may be attributed to the leaching of these minerals into the blanching water due to the heat treatment [31]. Despite the reduction in mineral content, the choice of blanching conditions appeared to influence mineral retention, with milder treatments resulting in higher levels of both calcium and iron in the vegetables. These findings suggest that optimizing blanching parameters could potentially mitigate mineral loss during processing, thereby preserving the nutritional quality of the

vegetables.

Vitamin A content in fermented vegetables can vary depending on the plant material and fermentation conditions. [32] observed a decrease in vitamin A compared to raw materials, other studies have reported increases in vegetables like carrots and peppers following fermentation [33] [34]. This suggests that lactic acid bacteria (LAB) fermentation might enhance vitamin A content in some vegetables. There were no significant differences in vitamin C content observed between different glucose concentrations for spider plant vegetables. This suggests that glucose concentration within the tested range (1% - 3%), which influences microbial growth, did not significantly affect vitamin C levels. This indicates little utilization, although some studies have demonstrated decrease in Vitamin C with fermentation [32].

Both vegetables exhibited a slight decrease in moisture content with increasing glucose concentration. During fermentation, increased glucose concentration can lead to higher microbial activity and acid production. This, combined with the leaching of water-soluble components and potential structural changes in the vegetables, can contribute to a reduction in moisture content [35]. Both vegetables showed a statistically significant decrease in Fe^{2+} and Ca^{2+} content with increasing glucose concentration. Our observed decrease in Fe^{2+} and Ca^{2+} content aligns with findings by [32], who reported lower Fe^{2+} and Ca^{2+} levels in fermented vegetables compared to their raw materials. This reduction likely occurs due to leaching of mineral-rich sap from plant tissues during fermentation [36].

Similar to our results, [37] and [38] reported a significant increase in protein content during the fermentation of vegetables by lactic acid bacteria. Fermentation can enhance the protein content of vegetables in two ways [39] [40]. Firstly, the breakdown of carbohydrates by microbes fuels their growth, resulting in increased microbial biomass rich in protein. Secondly, fermentation can break down complex proteins already present in the vegetables into smaller peptides and amino acids, making them more bioavailable for absorption by the body. The impact of fermentation on vitamin A content varies depending on the vegetable and fermentation process. Some studies, like those on fermented peppers [33] and Chinese cabbage [41], report increases in β-carotene, a precursor to vitamin A. This might be due to improved release of carotenes from the vegetables during fermentation or storage [41]. Additionally, structural changes caused by fermentation could potentially make carotenes more readily extracted [34]. The fermented black night shade and spider plant vegetables recorded minimal changes in vitamin C content, though the change was significant. Some researchers have reported that fermentation caused a significant increase in vitamin C content. [42] and [43] reported significant increase in ascorbic acid content during the fermentation of okra seeds and white cabbage, respectively. This suggests that fermentation duration did not significantly affect vitamin C levels, this could be a result of little utilization by the microbes.

The decrease in moisture content is likely due to microbial growth increasing

dry matter content [44], similar to findings in fermented soy milk [45]. However, some studies report increased moisture content during fermentation [46], suggesting the effect might vary depending on the vegetable and microbes involved. There was significant reduction in Ca^{2+} and Fe^{2+}, as fermentation progressed for both vegetables. Fermentation is associated with the leakage of sap and minerals from plant tissues. Minerals such as Ca^{2+} and Fe^{2+} are essential for proper fermentation, as they facilitate the growth and metabolism of microorganisms. The reduction in Ca^{2+} and Fe^{2+} content suggests their utilization by microorganisms for growth and metabolism, while Fe^{2+} also plays an important role in various physiological and metabolic activities. The decrease could also be attributed to leaching into the fermenting media [32] [36] [47]-[49].

Blanching, starter cultures, and controlled fermentation processes seem to play a crucial role in achieving desirable sensory qualities in these fermented vegetables. Black nightshade fermented with the starter culture received lower scores ($p < 0.05$) for aroma compared to spider plant, but the scores were higher ($p < 0.05$) than those of the spontaneously fermented vegetables. Fermentation of the vegetables using *Lactobacillus plantarum* could have positively influenced the aroma of the vegetables.

The generation of flavour during fermentation is a result of the production of metabolites that alter the flavour profile of the vegetables [50]**.** It has been reported that controlled fermentation of vegetables using *Lactoplantibacillus plantarum* produces various aroma compounds, such as ethyl acetate and isoamyl acetate, that could positively influence the aroma of the vegetables [51]. Furthermore, it has been shown that fermentation improves flavours of fermented foods, but the type of starter culture offers more complex actions [52]. *Lactoplantibacillus plantarum* produces esterases that can generate a wide range of phenolic alcohols, short-chain esters, and fatty acids that positively impact the flavour of fermented foods [53]. [54] reported that controlled fermentation has been shown to improve the consistency and quality of final products.

For appearance, the blanched vegetables had similar scores but significantly higher than the unblanched and spontaneously fermented vegetables, while for taste, spider plant blanched at 90˚C/5mins received higher scores than black nightshade**.** Blanching improves the appearance of some vegetables as it removes intracellular gases from plant tissues, removes surface dust, and alters the wavelength of reflected light, hence brightening the colour of foods [55]. This explains the reason why the panellist preferred blanched fermented vegetables over the spontaneously fermented ones that were not blanched. Fermentation also improves the organoleptic properties of food, such as taste. Although fresh spider plant and black nightshade are known to be bitter, fermentation has been shown to reduce the bitter phenolic compounds. This explains why consumers preferred the fermented vegetables subjected to controlled fermentation. However, the taste of fermented vegetables depends on the microorganism carrying out the fermentation process.

For texture, blanched black nightshade had higher scores ($p < 0.05$) compared to the unblanched spontaneously fermented spider plant. The panelists may have liked the blanched vegetables because they were softer compared to those spontaneously fermented, which had a tougher texture. Therefore, the softer texture gave the consumers a higher preference. [56] reported that a higher blanching temperature of 100˚C increased the softness of vegetable soybeans, resulting in higher sensory attributes. Fermentation has also been shown to improve the organoleptic properties of food, such as texture [57]. The findings suggest that controlled fermentation offers a promising approach to enhancing the palatability of these two vegetables.

5. Conclusions

This study confirms that blanching, glucose supplementation, and controlled fermentation improve the nutritional and sensory properties of spider plant and black nightshade. Blanching at 80˚C for 10 minutes was most effective in preserving protein, vitamin A, and vitamin C compared to 90˚C for 5 minutes. Increasing glucose concentration during fermentation enhanced vitamin A content, vitamin C and protein content. Fermentation for 21 days further improved protein content and vitamin A and vitamin C while decreasing iron and calcium levels.

Sensory evaluation demonstrated that controlled fermentation enhanced consumer acceptability, with blanched and fermented vegetables scoring higher in appearance, texture, aroma, and taste. Spider plant blanched at 90˚C for 5 minutes received the highest overall acceptability (~4.0), while unblanched, spontaneously fermented samples scored the lowest (<3.0). These findings suggest that fermentation not only improves the shelf life and nutritional profile of ILVs, but also enhances their palatability, making them more suitable for widespread consumption and commercialization.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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