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Effect of Lactic Acid Fermentation on Phytochemical Content, Antioxidant Capacity, Sensory Acceptability and Microbial Safety of African Black Nightshade and African Spider Plant Vegetables

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Abstract: Traditional preparation of African indigenous vegetables (AIVs) such as African black nightshade (*Solanum nigrum*) and African spiderplant (*Cleome gynandra*) involves either boiling and discarding the first water or lengthy boiling. Fermentation is considered a better alternative processing technique due to the enhanced retention of phytochemical contents and sensory properties. However, little is known about the impact of lactic acid fermentation on the phytochemical content, antioxidant capacity, sensory acceptability and microbial safety of the African black nightshade and African spiderplant. This study aimed to ferment AIVs using combined starter cultures (*Lactobacillus fermentum* and *Lactococcus lactis*) and further determine their effect on the phytochemical content (phenolic compounds and flavonoids), antioxidant capacity, sensory acceptability and microbial safety of the vegetables. There was a marked increase in phenol and flavonoid contents in all fermented vegetables ($p < 0.05$). The highest phenol content was 228.8 mg/g GAE (gallic acid equivalent) in the starter-culture-inoculated African black nightshade, while flavonoid content was 10.6 mg/g QE (quercetin equivalent) in the same. Starter-culture-inoculated AIVs presented significantly higher antioxidant capacity with a 60–80% radical scavenging activity compared to levels in uninoculated batches ($p < 0.05$). Fermented vegetables were more liked than the boiled vegetables and were microbiologically safe. In conclusion, lactic fermentation of AIVs increased phytochemical contents (phenolic compounds and flavonoids), maintained antioxidant capacity and improved product safety and sensory acceptability. Therefore, fermentation and consumption of the African indigenous vegetables are to be encouraged.

Keywords: African black nightshade; African indigenous vegetables; African spiderplant; antioxidant; fermentation; flavonoid; *Lactobacillus fermentum*; *Lactococcus lactis*; phenolic compounds

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1. Introduction

African indigenous vegetables (AIVs) are rich in micronutrients such as vitamins (β-carotene, ascorbic acid, riboflavin and folic acid), minerals and dietary fibre [1]. The wide varieties of AIVs such as *Amaranthus* spp., okra (*Abelmoschus caillei*), cowpea (*Vigna unguica*), jute mallow (*Corchorus olitorius*), black nightshade (*Solanum scabrum* and *Solanum nigrum*), turkey berries (*Solanum torvum*), spiderplant (*Cleome gynandra*), cassava leaves (*Manihot esculenta*), kidney bean leaves (*Phaseolus vulgaris*) and eggplant (*Solanum aethiopicum*) contain nutrients which can help meet micronutrients requirements [2].

The FAO/WHO recommends a daily intake of 400 g of vegetables per individual [3]. However, low intake of fruits and vegetables is prevalent in Sub-Saharan Africa (SSA), particularly in rural areas among vulnerable families [4]. AIVs are climate resilient, nutritious and locally available, and hence are considered sustainable food strategies for

the mitigation of food insecurity and micronutrient deficiency [2,5]. AIVs are produced and consumed mainly in rural areas where they are a source of income and are used in medicinal and religious practices [6].

The African black nightshade (*Solanum nigrum*) is common in Africa. It is eaten cooked or fermented (although still by few) [7,8]. Its fruits are also eaten. These vegetables have a high content of nutrients, namely iron, proteins and ascorbic acid, and phytochemicals, namely phenolic compounds and carotenoids [8,9]. The ethanolic and aqueous extracts of African black nightshade can protect the body from DNA damaging aflatoxin B_1 and Reactive Oxygen Species [7].

The African spiderplant (*Cleome gynandra*) is a climate-resilient vegetable. It grows in regions of 15 to 25 °C under complete sun exposure. It contains minerals and vitamins, therefore it has huge potential in reducing micronutrient deficiencies [10]. The African spiderplant is used as food and herbal medicine due to its high levels of β-carotene, α-tocopherols, folic acid, ascorbic acid, iron and calcium. In addition to the phytochemical content, it has exhibited antifungal, anti-inflammatory, anti-cancer, anti-diabetic and immunomodulator properties in vitro [11–13].

Both the African black nightshade and African spiderplant are adapted to the SSA environment and can help to achieve food security, the second Sustainable Development Goal (SDG) [2,9]. However, these AIVs are underutilized because they are considered to be for the poor. Their limited consumption can be attributed to the lack of nutritional information and the dietary shift from indigenous foods to exotic ones due to rapid urbanization [2,14]. Like most AIVs, African black nightshade and African spiderplant are mostly available only during rainy seasons due to inadequate preservation techniques [8,9].

These two vegetables contain phytochemicals such as phytates, oxalates, lectins, tannins, alkaloids and saponins [8]. These phytochemicals contribute to the sensory properties of these vegetables and their antioxidant capacity [15]. Normally, they are boiled for a long time or boiled and the water is thereafter discarded to eliminate bitterness, thereby reducing the heat-labile and water-soluble nutrients in the end product [16,17]. In addition, these pre-cooking processes are labour-intensive and consume a lot of heating energy when compared with alternative techniques for AIV processing such as fermentation [8,9,18,19].

Lactic acid bacteria (LAB) have been widely associated with beneficial health effects due to their probiotic properties. They have been used for centuries to improve the nutritional and sensory properties of foods and for preservation [20–22]. *Lactobacillus fermentum* is a heterofermenter and is known to produce bacteriocins which can help in food preservation. Further, *Lb fermentum* metabolism contributes to flavour and texture of the product and probiotic properties including modulation of the microbial environment of the host's gut [23]. *Lactococcus lactis* is a homofermenter that produces lactic acid, thus contributing to a lower pH of food. The *Lc lactis* is commonly found in lactic fermented foods such as cheese, yoghurt and sauerkraut and has been observed to prevent microbial growth in foods and prevent gut wounds. With respect to the symbiotic relationship between the two LAB during fermentation, the acidity achieved by *Lc lactis* metabolism helps the *Lb fermentum* to proliferate [24,25].

Fermentation of vegetables is widely practiced in the SSA but with variation in techniques and outcomes [8,26,27]. Products such as *kawal* and *bilkaga* are popular in Sudan and South Sudan, whereas the *okpehe* and *ugba* are widely known in West Africa. The traditional fermentation skills are usually transferred in the family from one generation to another and it can be different even within a community [8,28]. In spontaneously fermented vegetables, lactic acid bacteria like *Lactobacillus plantarum* dominate the microbial population [29,30]. Generally, lactic acid bacteria and mesophilic microorganisms are commonly found in fermented vegetables [31].

LAB fermentation of AIVs has the potential to reduce micronutrient deficiency, protect vegetables against spoilage and increase their sensory acceptance [8,9]. Stoll et al. [18] fermented African indigenous vegetables using *Lactiplantibacillus plantarum* and *Limosilactobacillus fermentum* and observed faster acidification of the vegetables' media. This

fermentation can be performed using readily available materials, and is considered to be a better processing technique than cooking and sun drying [18]. This study used a different combination of *Lc. lactis* and *Lb. fermentum* to analyse their effect on the vegetables. These starter cultures have been successfully used in the fermentation of African kale, an African indigenous vegetable [32].

Additionally, *Lb. fermentum* was previously isolated from fermented African products [33], while *Lc. lactis* was isolated from vegetables and used as a bio-preservative agent against *Listeria monocytogenes* in cheese [34] and in milk [35]. However, there is limited information on the influence of lactic acid fermentation on the phytochemical content, antioxidant capacity and sensory acceptability of African black nightshade and African spiderplant. Therefore, this study aimed to evaluate the effect of lactic bacteria starter culture (*Lc. lactis* and *Lb. fermentum)* fermentation on the phytochemical content (phenolic compounds and flavonoids), antioxidant capacity, sensory acceptability and microbial safety of African black nightshade and African spiderplant.

2. Results and Discussion

2.1. Fermentation Dynamics

The pH values dropped from 6.80 and 6.82 to 3.82 and 3.23 at the end of the fermentation period in the starter-culture-inoculated African spiderplant and African black nightshade, respectively, (Figure 1), while the pH values in the control fermentation (uninoculated batches) were 3.83 and 4.46 in African black nightshade and African spiderplant, respectively. Just within 24 h of the present fermentation, the pH was lower when compared to the pH of 5.20 in the starter-culture-inoculated African black nightshade and African spiderplant observed by Degrain et al. [36] at 72 h. Between 24 h and 48 h, lactic acid bacteria were at the logarithmic phase of growth in all fermented AIVs (Figure 1).

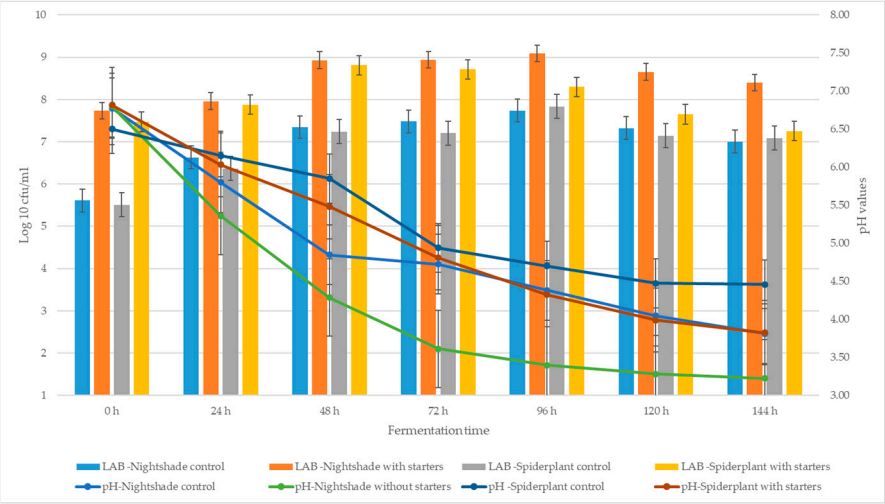

Figure 1. Lactic acid bacteria counts and pH of fermented African black nightshade and African spiderplant. The mean ± SD counts and pH values were determined from duplicate fermentations.

The LAB counts were approximately log 7.70 CFU/mL and 7.83 CFU/mL for the uninoculated African black nightshade and African spiderplant, respectively, whereas log 9.09 CFU/mL and log 8.30 CFU/mL of LAB counts were observed in the starter-culture-inoculated African black nightshade and African spiderplant, respectively, at 96 h (Figure 1). The inoculum contributed to the rapid growth pattern of lactic acid bacteria in starter-culture-inoculated AIVs.

The starter strains produce antimicrobial compounds such as organic acids and bacteriocins that help to promote the fermentation process [18,19]. The organic acids lower the pH of the food medium. The low acidity facilitates the organic acids to diffuse through the cell membrane, collapsing the electrochemical gradient of protons through the cell which

leads to death of the cells of harmful microorganisms. The bacteriocins, on the other hand, destabilize and permeabilize the cell membrane by forming ionic pores or channels, which release molecules such as amino acids and prevent the formation of macromolecules [37]. This ability of lactic acid bacteria to replenish harmful microorganisms has opened their use as bio-preservation agents [34,35,38].

In both starter-culture-inoculated and uninoculated fermentation batches, the enteric bacteria were eliminated after approximately 72 h (Figure 2). It is noticeable that in the uninoculated batches, it took 72 h for their removal, while the use of the starter cultures led to the removal of contaminants just after 48 h (Figure 2). In all batches, there was no more presence of yeast and moulds after 48 h (Figure 3). This is in agreement with the study by Chen et al. [31], who observed up to 96% LAB at the end of fermentation. This unique growth pattern gives safety to fermented foods, as suggested by Oguntoyinbo et al. [32]. The ability of the starter strains to produce antimicrobial compounds such as organic acids and bacteriocins helps to safeguard the fermentation product [19].

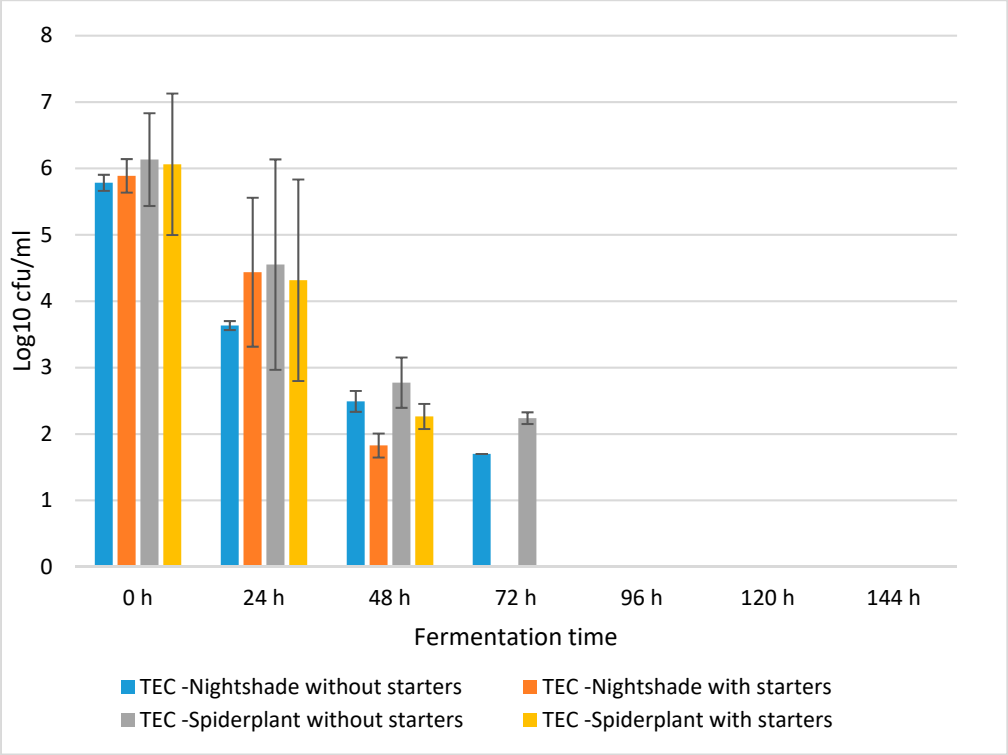

Figure 2. Total enteric count of fermented African black nightshade and African spiderplant. The mean ± SD counts were determined from duplicate fermentations.

Lc. lactis rapidly lowers the pH, hence inhibiting the growth of potential microbial contaminants while at the same time providing a favourable environment for *Lb. fermentum* to grow. At 72 h, there was no more growth of enteric bacteria, yeast and moulds in the starter-culture-inoculated batches (Figures 2 and 3). However, in uninoculated batches, enteric bacteria, yeast and moulds could be detected even after 72 h (Figures 2 and 3). Both *Lb. fermentum* and *Lc. lactis* produce lactic acid, which lowers the pH of the fermented products and concomitantly inhibits the growth of hazardous microorganisms [19]. These results are in agreement with those of Stoll et al. [33], where lactic acid bacteria proliferated in both spontaneous and starter-inoculated vegetables [33]. However, LAB counts were lower in uninoculated AIVs than in the starter-inoculated samples. The same trend was observed by Chen et al. (20) and Stoll et al. [33]. The growth of LAB has been associated with lower pH, which confers to the food safety and quality attributes [23].

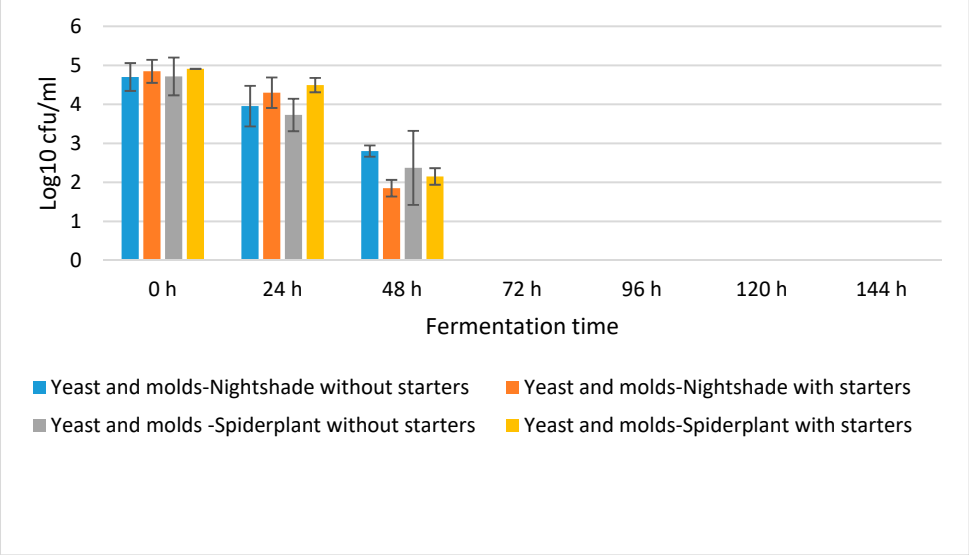

Figure 3. Yeast and mould count of fermented African black nightshade and African spiderplant. The mean ± SD counts were determined from duplicate fermentations.

2.2. Phytochemical Content (Total Phenolic Compounds and Flavonoids)

Fermentation increased flavonoid and phenolic compound contents, especially in starter-culture-inoculated AIVs. Both starter-culture-inoculated African black nightshade and African spiderplant showed significantly higher flavonoids and total phenolic compounds than levels in the uninoculated AIVs (Figures 4 and 5) after 144 h of fermentation. In the inoculated African black nightshade and African spiderplant, flavonoid contents were 10.96 mg/g QE and 6.67 mg/g QE, respectively. The increased flavonoids and total phenolic compounds during the fermentation process are associated with biochemical changes in the food matrix due to increases in phytochemicals and volatile compounds in fermented foods [33,38,39]. Enzymes present in lactic acid bacteria such as *Lb. fermentum* catalyse changes in the food through the cleavage of bonds or hydrolysis of phytochemical molecules such as coumaric acid and vanillic acid [40].

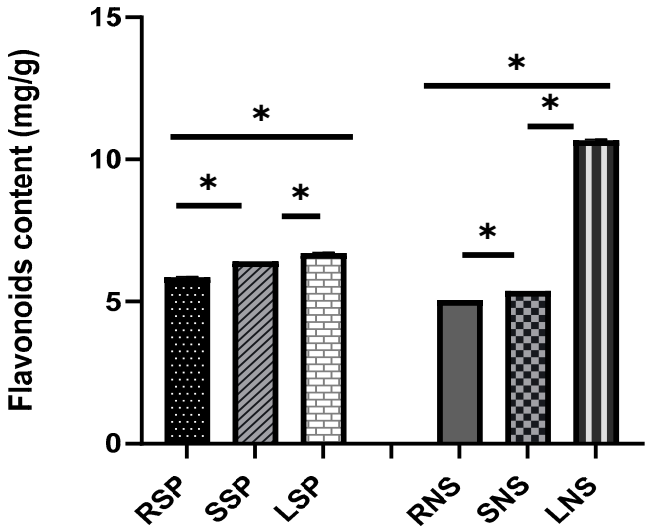

Figure 4. Flavonoid content in mg/g quercetin equivalent (QE) of uninoculated and starter-cultured AIVs. RNS: unfermented African black nightshade, SNS: uninoculated African black nightshade, LNS: starter-culture-fermented African black nightshade, RSP: unfermented African spiderplant, SSP: uninoculated African spiderplant, LSP: starter-culture-fermented African spiderplant. The analysis was carried out in triplicate. Means with * are statistically different; $p > 0.05$ according to Tukey's test.

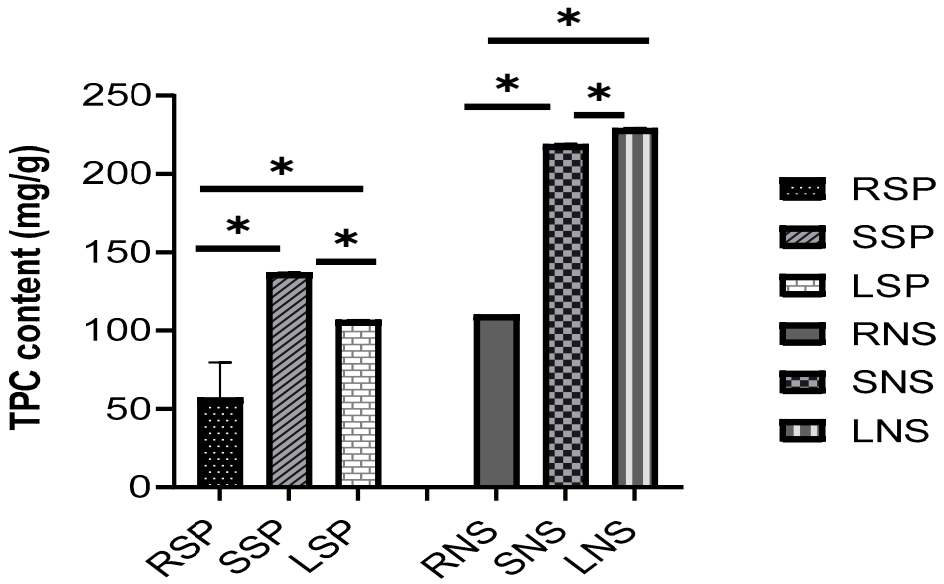

Figure 5. Total phenolic compound (TPC) content in mg/g gallic acid equivalent (GAE) of uninoculated and starter-cultured AIVs. RNS: unfermented African black nightshade, SNS: uninoculated African black nightshade, LNS: starter-culture-fermented African black nightshade, RSP: unfermented African spiderplant, SSP: uninoculated African spiderplant, LSP: starter-culture-fermented African spiderplant. The analysis was carried out in triplicate. Means with * are statistically different; $p > 0.05$ according to Tukey's test.

There was a statistically significant increase ($p < 0.05$) in the total phenolic compounds in all fermented AIVs (Figure 5). In the uninoculated and culture-inoculated African black nightshade, the phenolic compound contents were 218.4 mg/g GAE and 228.8 mg/g GAE, respectively. An increase in phenolic compounds has been observed in the fermentation of African black nightshades and is explained by the ability of lactic fermentation to enhance the phytochemical content of fermented foods through enzyme-catalysed changes [41,42]. De Grain et al. [36] reported the increase in phenolic compounds in a fermented vegetable medium with the increase in acidity. The low pH stabilizes the phenolic compounds, and they can be detected in significant amounts. The enzymatic activity of lactic acid bacteria results in simpler phenolic compounds and increases in the quantity [43].

2.3. Antioxidant Capacity

In the present study, radical scavenging activity (RSA) was observed in unfermented African spiderplant and African black nightshade and was maintained in starter-culture-inoculated vegetables (LNS and LSP) more than in control (uninoculated batch) vegetables (SSP and SNS) (Figure 6). The RSA was between 60 to 80% in the starter-cultured AIVs, and this was higher than levels in the control (uninoculated) vegetables. The findings of this study on antioxidant activity in fermented products are similar to those of Gomez-Zavaglia et al. [41], and also match the high antioxidant activity noted in apple juice fermented with *L. plantarum* [39]. Adebo and Medina-Meza (2020) [42] noted the increase in total phenolic compounds in African fermented products, which potentially increase the antioxidant activity of African fermented products. The increase in phytochemical content during fermentation has been attributed to the increase in antioxidant capacity in fermented foods [41,43]. Overall, fermentation plays a big role in improving and maintaining the antioxidant capacity of food through the production of important phenolic compounds such as quercetin [41].

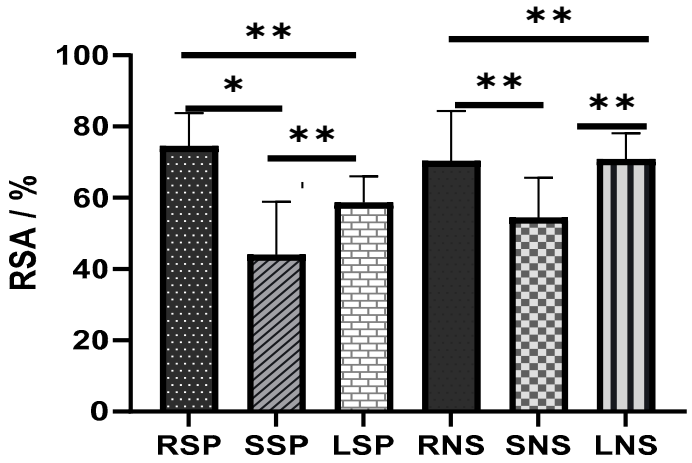

Figure 6. Radical scavenging activity of uninoculated and starter-cultured AIVs. RNS: unfermented African black nightshade, SNS: uninoculated African black nightshade, LNS: starter-culture-fermented African black nightshade, RSP: unfermented African spiderplant, SSP: uninoculated African spiderplant, LSP: starter-culture-fermented African spiderplant. The analysis was carried out in triplicate. Means with * are statistically different, while the means with ** are not significantly statistically different; $p > 0.05$ according to Tukey's test.

2.4. Evaluation of Sensory Acceptability

In this study, fermented vegetables were generally more liked than boiled vegetables for taste and aroma properties (Figure 7). LAB ferment sugar present in the food and produce lactic acid, which acidifies the products, while the enzymes and aroma compounds such as diacetyl and ethyl ester give the product pleasant organoleptic properties [44]. The fermented vegetables were preferred more than those prepared using conventional methods (boiling). Lactic acid fermentation is believed to enhance flavour in fermented products [45,46]. The improved sensory properties of lactic fermentation are likely to be the reason for panellists preferring the fermented ones.

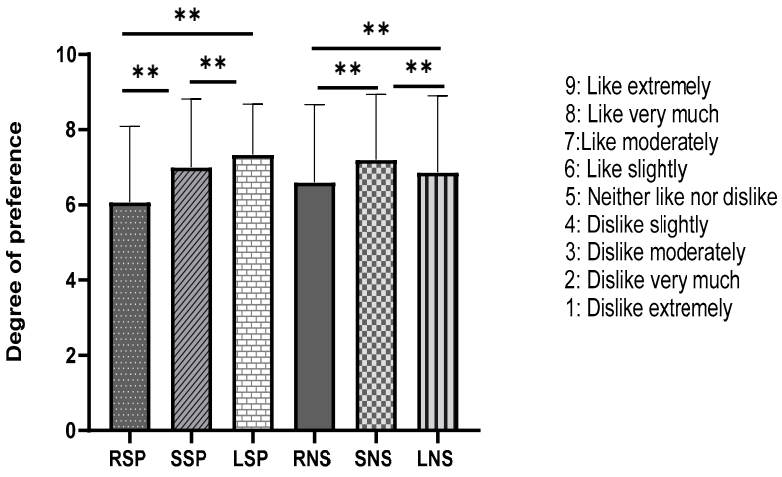

Figure 7. Sensory acceptability evaluation of uninoculated and starter-cultured AIVs. RNS: unfermented (boiled) African black nightshade, SNS: uninoculated African black nightshade, LNS: starter-culture-fermented African black nightshade, RSP: unfermented (boiled) African spiderplant, SSP: uninoculated African spiderplant, LSP: starter-culture-fermented African spiderplant. The analysis was carried out in triplicate. Means with * are statistically different, while the means with two asterisks (**) are not significantly statistically different; $p > 0.05$ according to Tukey's test.

3. Materials and Methods

3.1. Preparation of Materials and African Indigenous Leafy Vegetables

The African black nightshade and African spiderplant used in this experiment were purchased from Musanze agricultural products market in Rwanda. They were grown in the manure fertilized volcanic soil, along the Birunga region, where annual rainfall is between 1300 to 1600 mm. They were grown during the short rainy season and were harvested by hand between 6–8 weeks. They were transported in a cool box to the Food Microbiology laboratory, Institute of Food and Bioresources Technology at Dedan Kimathi University of Technology (DeKUT, Nyeri, Kenya). The leaves were washed with tap water and dried with paper towels and stored for analysis.

3.2. Preparation of Starter Inocula

The starter cultures *Lc lactis* and *Lb fermentum* used in this experiment were obtained from Jomo Kenyatta University of Agriculture and Technology (JKUAT, Juja, Kenya), Department of Food Science and Technology, Food Microbiology laboratory. On arrival at DeKUT Microbiology laboratory, the strains were grown in MRS (De Man, Rogosa and Sharpe medium, Himedia (Mumbai, India)) at 30 °C overnight. The inocula were prepared by centrifuging 2 mL of fresh overnight cultures at 13,000 rpm for 10 min and washed twice with quarter-strength Ringer's solution (Himedia (Mumbai, India)).

3.3. Fermentation of African Black Nightshade and Spider Plant Leaves

Fermentation was performed in 10 L stainless steel buckets with a combined inoculum of both starter cultures (*Lb. fermentum* and *Lc. lactis)* where for each vegetable 1 kg of leaves and 3 L of salt and sugar brine solution were used. The solution consisted of a combination of salt and sugar, 3.0 % each. Common table salt (Kensalt ltd), (Mombasa, Kenya) and retail sugar (Kabras sugar company ltd), (Kakamega, Kenya) were purchased at local stores in Kenya. The brine solution was sterilized by autoclaving for 15 min at 121 °C. Weights were used to hold all plant material below the surface of the liquid. Inoculation and sampling were performed under sterile conditions. The samples were allowed to cool to room temperature, or for the starter batches, they were inoculated with the combined *Lc lactis* and *Lb fermentum* cultures approximately 10^6–10^7 CFU/mL, whereas the uninoculated (control) batch was performed within each fermentation trial [18]. The fermentations were carried out in duplicate at 25 °C. The progress of the fermentation was determined by microbial enumeration on De Man, Rogosa and Sharpe agar (MRS) Himedia (Mumbai, India), and pH determination was as described by Wafula et al. [47], while the total enteric bacteria count and yeast and moulds were enumerated on violet red bile glucose agar (VRBGA) and potato dextrose agar (PDA) Himedia (Mumbai, India), respectively, as described by Oguntoyinbo et al. [32] at 0 h, 24 h, 48 h, 72 h, 96 h, 120 h and 144 h. After fermentation, the leaves were separated from the brine for the analysis of phytochemical content, antioxidant capacity and sensory acceptability.

3.4. Determination of Phytochemical Content

(a) Determination of flavonoid content

Aluminium chloride colorimetric method was used for the determination of flavonoids [36]. The sample (0.25 g) was mixed with 25 mL of methanol in an amber bottle and extracted with a shaker for 30 min. The extracts were kept for 72 h and then filtered through a Whatman Nº 1 filter paper into a 50 mL round flask. The content was topped up by methanol before centrifuging for 10 min at 5000 rpm. Then, 4 mL of distilled water and 1 mL of plant extract were added into a 10 mL volumetric flask. After 3 min, 0.3 mL of 5% sodium nitrite solution was added, and held for 3 min before adding 0.3 mL of 10% aluminium chloride. After 5 min, 2 mL of 1 M sodium hydroxide was added, and the volume was made up to 10 mL with distilled water. Absorbance was measured at 415 nm using a UV-Vis spectrophotometer (Shimadzu model UV–1601 PC, Kyoto, Japan). The amount of total flavonoids was calculated from the calibration curve of standards prepared

from quercetin. The treatments analysed were as follows: RNS, unfermented African black nightshade; SNS, uninoculated African black nightshade; LNS, starter-culture-fermented African black nightshade; RSP, unfermented African spiderplant; SSP, uninoculated African spiderplant; LSP, starter-culture-fermented African spiderplant.

(b) Determination of total phenolic compound content

Total phenolic compounds were determined using the spectrophotometric method described by Matenge et al. [1]. For the total phenolic compound analysis, a 72 h extraction was performed as described above. First, 1 mL of the supernatant was filtered through a 0.45 µ micro-filter into test tubes and mixed with 2 mL of 10% Folin–Ciocalteu's reagent and vortexed. Then, 4 mL of 0.7 M sodium carbonate was added, and the mixture was vortexed again. The mixture was left for 2 h to develop colour before absorbance measurement at 735 nm with a UV-VIS spectrophotometer (Shimadzu model UV–1601 PC, Kyoto, Japan). Gallic acid was used as the standard.

3.5. Determination of Antioxidant Capacity

Antioxidant capacity was determined through evaluation of the radical scavenging activities of the various fermented treatments against 2, 2-Diphenyl-1-picryl hydrazyl (DPPH) radical (Sigma-Aldrich) using the spectrophotometric method described by Degrain et al. [36] and Rahman et al. [48]. Different concentrations were prepared in methanol: 0, 2.5, 5, 10, 20 and 40 mg/mL. Vitamin C was used as the antioxidant standard at concentrations similar to those of the extract concentrations. An amount of 1 mL of the extract was placed in a test tube, and 3 mL of methanol was added, followed by further addition of 0.5 mL of 1 mM DPPH in methanol. A blank solution was prepared containing the same amount of methanol and DPPH. Methanol was used to zero the spectrophotometer and the absorbance was read at 517 nm using a UV-Vis spectrophotometer (Shimadzu model UV–1601 PC, Kyoto, Japan). The radical scavenging activity was calculated using the following formula:

$$\text{Scavenging activity } = \frac{\text{absorbance of control} - \text{absorbance of th sample}}{\text{absorbance of control}} \times 100$$

3.6. Evaluation of Sensory Acceptability

Sensory analysis (preference) of the fermented AIVs was performed by 20 untrained panellists consisting of university students using a 9-scale hedonic scale (9 represented 'extremely like' and 1 represented 'extremely dislike'). The panellists were informed about the study and gave consent to participate in the study. The unfermented leaves were prepared by boiling for 10–15 min before sensory evaluation, while the fermented ones were given to the panellists as they were. Panellists examined their sensory perception of colour, appearance, taste, feel in the mouth and smell and scored the general acceptability based on nine (9)-point hedonic scale. The panellists also provided additional descriptive comments on the fermented AIVs.

3.7. Data Analysis

Statistical analyses of pH values and $\log_{10}$-transformed microbial counts were performed using Microsoft Excel 2021. The results for the phytochemical analyses were presented as the means and standard deviation of two replicates. The data were analysed using a one-way analysis of variance using Sigma plot version 14.0, Stat software Inc., Munich (Germany), at the significance level of $p < 0.05$, followed by post hoc comparison using Tukey's test. The graphs were plotted using GraphPad Prism software Inc. 9.0.0 California (USA). Sensory acceptability results were analysed using SPSS version 21 (IBM, International Business Machines Corporation, New York, NY, USA).

4. Conclusions

In the present study LAB starter culture fermentation increased phytochemical content (phenolic compounds and flavonoids) in both African black nightshade and African spider-

plant. Furthermore, the radical scavenging activity in the LAB starter-culture-fermented AIVs was between 60 to 80%, which was higher than levels in the control spontaneously fermented vegetables. There was no statistically significant difference in sensory acceptability between African black nightshade and African spiderplant fermented with starter culture. From the observations of this study, lactic fermentation could help to improve phytochemical content and antioxidant capacity. However, more studies need to be carried out using other assays such as the Oxygen Radical Absorption Capacity test, the Hydroxyl Radical Antioxidant Capacity test, the Total Peroxyl Radical Trapping Antioxidant Parameter test, the Total Oxyradical Scavenging Capacity test, the Cupric Reducing Antioxidant Power test, the Ferric Reducing Antioxidant Power test, the Folin–Ciocalteu test and the 2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid test to be able to draw conclusive observations.

Author Contributions: Conceptualization, M.L.I., E.N.W. and E.E.O.; methodology, M.L.I., E.N.W. and E.E.O.; software, M.L.I. and E.N.W.; validation, M.L.I., E.N.W. and E.E.O.; formal analysis, M.L.I.; investigation, M.L.I.; resources, M.L.I. and E.N.W.; data curation, E.N.W. and E.E.O.; writing—original draft preparation, M.L.I.; writing—review and editing, E.N.W. and E.E.O.; visualization, E.N.W. and E.E.O.; supervision, E.N.W. and E.E.O.; project administration, M.L.I.; funding acquisition, M.L.I. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Not applicable.

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Conflicts of Interest: The authors declare no conflict of interest.

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