

Effect of Processing Methods on the Micronutrient Profile, Colour, and Anti-nutritive Components of *Justicia Heterocarpa* (mwidu)

ABSTRACT

Justicia Heterocarpa (mwidu) is a popular indigenous leafy vegetable picked wild in Morogoro, Tanzania, during the rainy season. This research examined how processing affects its micronutrient and anti-nutrient content. The fresh leaves (FL) were treated with un-blanching direct shade drying (UBLDDR), blanching shade drying (BLDR), oven drying (BLDO), fermentation (FFL), and gas and microwave cooking (FLCO5, FLCO10, and FLMCO2). Vitamins, chlorophyll, minerals, and anti-nutrients were tested. All lab experiments followed protocols. The pH dropped more significantly to <3.5 in a 3% salt-3% sugar brine solution with 1.328 mg/100 g of lactic acid compared to its counterpart. Blanching leaves dried in 5 days, but un-blanching leaves took 15 days. Compared to previous processing procedures, FFL samples reduced total chlorophyll concentration to 0.0964 mg/g. Nutritional and anti-nutritional content of *Justicia Heterocarpa* varied significantly ($p < 0.05$) according to processing methods. Compared to 23.21 mg/100g FL, FFL (5.88 mg/100g) and FLCO (108.90 mg/100g) reduced vitamin C levels by 74.57% and 61.64%, respectively. More than twofold higher beta-carotene levels in FLMCO2 (107.4%, 11.29 mg/100 g) and FLCO5 (86.26%, 10.51 mg/100 g) than FL (3.67 mg/100 g). Compared to other processing methods, FLCO10 had high minerals leaching while UBLDDR retained minerals. FFL (532.83 GAE/100g) and UBLDDR (352.83 GAE/100g) samples had 64.19% and 45.9% higher Total Phenolic Compounds than FL (190.83 GAE/100g). Tannins increased 68.1% to 254.44 GAE/100g in FFL. FLCO10 (17.81 mg/100 g) and FFL (40.28 mg/100 g) had lowest phytates. Cooked samples (FLCO5 and FLCO10) considerably reduced oxalate content. *Justicia Heterocarpa* could be a sustainable food source in micronutrient-deficient Regions in Tanzania.

Keywords: Indigenous Leafy Vegetables, Anti-nutrients, *Justicia Heterocarpa*, Processing and micronutrients

1.0. INTRODUCTION

Micronutrient deficiencies are prevalent in many underdeveloped nations, where populations often depend on staple cereals like maize and rice, resulting in minimal dietary diversification (1–3). Micronutrient deficiency or hidden hunger arises when the intake and absorption of essential vitamins and minerals, including vitamins A, C, zinc, and iron, are inadequate to support optimal health and development (4). Vitamin A deficiency accounts for approximately 1.7% of deaths among children under five in Africa (5) In Tanzania, the prevalence of anemia is 59% among children aged 6-59 months (6,7), 37.8% among pregnant women in the Mbeya region(8) and 23% among adolescents in boarding schools in Kilimanjaro (Nicholaus et

al., 2020). The prevalence of stunting and underweight in Morogoro is notably elevated as a result of nutritional deficiencies. A 24-hour dietary recall indicates that the prevalence rates of anemia, vitamin A deficiency, and zinc deficiency in children are 42.9%, 29.3%, and 24.9%, respectively (9). The World Health Organization recommends a daily intake of 400 grams of fruits and vegetables for adults to combat hidden hunger(10–12). Due to challenging economic conditions, numerous rural regions in Sub-Saharan Africa, including Tanzania, lack the financial capacity to afford such quantities (6,10,12).

Indigenous leafy vegetables (ILVs) represent a valuable resource for enhancing dietary diversity and ensuring food security at the household level in developing countries (13–16). Recent studies have identified ILVs as a significant source of nutrition(15–17), economic potential (18) and adaptability to climatic conditions (15,16,19). Indigenous leafy vegetables, while beneficial, experience considerable post-harvest losses during the rainy season (15,20) and are often scarce in the dry season due to their wild growth and reliance on natural rainfall(3). Furthermore, ILVs contain anti-nutrients that hinder the body's capacity to digest and absorb essential nutrients (7,21). Phytates and oxalates are capable of chelating metal ions, including iron, zinc, and calcium, resulting in the formation of insoluble complexes (22). Wide research has been conducted on the processing and preservation of indigenous leafy vegetables (ILVs)(1,7,10,23). Additionally, the effects of processing on the reduction of anti-nutrients in specific ILVs have also been examined (7,10,24). *Justicia heterocarpa*, referred to as "mwidu," is a popular indigenous leafy vegetable among the residents of the Morogoro Region in Tanzania (25). This plant flourishes in rainy seasons and grows abundantly in fields and around family compounds (25). The fresh leaves exhibit a notable micronutrient profile(26). *Justicia heterocarpa* is significantly impacted by drought during arid seasons. Furthermore, the restricted preservation methods impede its accessibility and availability in dry seasons. This study examined the impact of processing methods (blanching, drying, fermenting, and cooking) on the retention of nutrients and the reduction of anti-nutritional components in *Justicia Heterocarpa*.

Comment [2]: The referencing style is different it needs to be consistent through the document

2.0. MATERIALS AND METHODS

2.1. Samples collection

Justicia Heterocarpa leaves were collected from a farmer's plots at Kiroka ward, Morogoro Rural District, Tanzania. Tender leaves were plucked with a sharp knife from each plot during the early morning hours, packaged in polyethylene bags and immediately transported to Food Science and Agro-processing laboratory. The leaves were trimmed, sorted, washed, and placed in a perforated vessel for water drainage. The vegetables were thoroughly mixed to obtain a representative sample

2.2. Sample Preparation

Fresh leaves were washed, drained, vacuum-packaged in polyethylene bags, and freeze-dried until analysis, whereas the room-dried leaves were washed, drained, and dried on racks at ambient temperature until achieving a crispy texture over 15 days.

Blanched dried leaves were washed, drained, and secured in net cloth, subsequently immersed in boiling water at 100°C for one minute. They were immediately rinsed in ice water for one minute, followed by drying on racks at room temperature for five days. The blanched leaves were subsequently dried in an oven at 40°C for 12 hours. The moisture content was measured using a moisture meter (Denver, IR35M-0002300V1-GERMANY). After attaining a crispy texture, the leaves were vacuum packaged and then freeze-dried before analysis.

For fermented leaves, previously trimmed and sorted vegetables were washed with distilled water and placed in the perforated tray with a clean paper towel to enhance the removal of excess water. Fermentation was done in submerged brine (1:3 vegetables: water). Two distinct concentrations of salt to sugar ratios were employed (3%:3% and 4%:2%) as previously outlined by (24) and (11) with certain modifications. Fermenting vessels utilized were 800ml plastic containers with secure seals. Locally produced crock pots, consisting of polyethylene bags filled with water, were utilized to submerge the vegetables, preventing the leaves from floating to the surface and thereby mitigating mold growth during fermentation. The experimental setup was incubated at 25°C, which is the optimal temperature for the growth of fermenting bacteria. Samples were collected at 0, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, and 144 hours. The efficacy of fermentation was evaluated by monitoring and recording pH and lactic acid levels in triplicate. Subsequent to pH measurement, the samples were promptly refrigerated, pending laboratory examination of lactic acid using spectrophotometer (JENWAY 6715 UV/Vis U.K)

Regarding cooked leaves, the cooking time for *J Heterocarpa* leaves varied from 3 to 20 minutes, according to pilot data collected from the study area. 500 grams of leaves were boiled in a pot with three hundred milliliters of water on a gas cooker for 5 and 10 minutes, respectively. Furthermore, 500 grams of the identical vegetables were subjected to microwave cooking for a duration of 2 minutes. Finally, the vegetables were cooled to room temperature and transferred, along with the cooking water, into plastic jars with lids for freeze-drying prior to laboratory analysis.

2.3. Chemical Analysis

2.3.1 Determination of nutritional composition

Beta-carotene

Total Beta-carotene was done as per (27) with slight modification. Frozen samples were thawed to room temperature ($25 \pm 2^\circ\text{C}$) and homogenized using a blender. A total of 2.000 g of sample was weighed in duplicate in 15 ml screw-capped glass tubes, followed by the addition of

10 ml of acetone (Sigma-Aldrich, USA). The samples were vortexed for a minute, then incubated in a water bath (SW23GB, JULABO) set at 85°C for 10 min. After that, 120 µl of 80% (w/v) potassium hydroxide (Sigma-Aldrich, USA) was added to the tubes, vortexed for 1 min, and incubated further at 85°C for 5 min. The tubes were transferred in ice to cool before the addition of 4 ml deionized water. The tubes were then vortexed for 1 min, and 5 ml of hexane (Sigma-Aldrich, HPLC grade, USA) was added to each tube and further vortexed for 1 min before centrifugation at 3000 rpm for 5 minutes. The upper phase of hexane was pipetted into a separate 25 ml labeled flask. Extraction from the pellet was repeated four times using 4×3×3×3 ml hexane, and the extract was combined in the 25 ml labeled flask. Leaves containing higher concentrations of beta carotene were further extracted by carotenoids. Sometimes, more extraction was necessary. 5ml deionized water was added into the extracts, mixed with vortex for 1 minute and centrifuged for 5 mins at 3000 rpm. The hexane layer in each was recovered into a clean test tube and evaporated the hexane under nitrogen in the N-Evaporator machine (Organomation, Model OA-8125) with its water bath set at 40°C to dry. Dried extracts in tubes were reconstituted by adding 10 ml of methanol (HPLC grade Sigma Aldrich) to the tubes, mixing homogeneously for 1 min by a vortex. The samples were transferred in 1 ml to HPLC vials. A volume of 30 µl was injected into the HPLC (SPD-20A SHIMADZU CORPORATION, Japan) with the type and conditions explained above. The method's limit of detection and limits of quantification (LOD and LOQ) were 3.1µg/ml and 5.2 µg/ml, whilst the percentage recovery was 103%, respectively.

Vitamin C (Ascorbic Acid)

The quantification of *Justicia Heterocarpa* ascorbic acid was conducted utilizing the 2,4-dinitrophenylhydrazine (2,4-DNPH) colorimetric assay method as previously described by (28). Each sample was finely chopped, blended, and homogenized using an electric blender. A total of 50 mL of a 3% metaphosphoric acid and 8% acetic acid solution was mixed with 10 mL of the blended sample. The resulting mixture was then diluted to the desired concentration by adding the same metaphosphoric-acetic acid solution to a 100 mL volumetric flask. After filtration, the clear filtrate was collected for vitamin C determination. A few drops of bromine water were added to the filtered sample solution to oxidize the ascorbic acid to dehydroascorbic acid. Excess bromine was neutralized with 10% thiourea, yielding a clear solution. Standard ascorbic acid solutions were prepared by diluting the 500 ppm stock to final concentrations of 5, 10, 15, 20, and 25 ppm. For both the oxidized sample and standard solutions, 1 mL of 2,4-DNPH solution was added. These were incubated in a 37°C water bath for 3 hours. After incubation, the solutions were cooled in an ice bath for 30 minutes and then treated with 5 mL of 85% H₂SO₄, resulting in a colored solution. The absorbance was measured at 530 nm, and ascorbic acid content was quantified using a standard calibration curve.

Minerals

The mineral content (Fe, Zn, Ca, and Mg) was analyzed through microwave-assisted acid digestion and ICP-OES, as outlined by (29) with some modifications. Approximately 0.5 g of the pulverized plant material sample was weighed into a digestion flask. A 10 mL mixture of concentrated nitric acid (HNO₃) and hydrogen peroxide (H₂O₂) (typically in a 3:1 ratio) was added to the flask, which was then allowed to stand for 4 hours in the fume hood. The flask was placed on a microwave digestion system, and the mixture was heated in a hot block (Hot block 150 models: SC-154-240) at 95°C and boiled for 30 minutes until it reached 85°C and became apparent, which took 1-2 hours. After digestion, the flask was allowed to cool, and the solution was diluted to a known volume of 50 mL with distilled water in a volumetric flask following filtration. The machine was turned on and allowed to warm up. The digested samples in the test tubes were introduced into the Inductively Coupled Plasma Optical Emission Spectroscopy machine (ICP-OES-5900 Agilent) with the model number (Agilent 5900 SVDV ICP-OES), Serial

number MY2215CP04, Software version 7.6.0.12121, and firmware version 5590). they were using an auto-sampler. The emission signals from the samples were monitored, and the instrument parameters (e.g., plasma conditions and nebulization rate) were optimized for the analysis. The instrument outputted the concentration of the minerals, which were displayed on the computer.

2.3.2. Determination of anti-nutrients composition

Total Phenol

The total phenol for the *Justicia Heterocarpa* sample was determined by using Folin-Ciocalteu method as described by (30) with some modifications. 80 mg of the sample was added to 2 mL of 50% methanol and extracted by maceration for 2 hours. The resulting mixture was centrifuged at 10,000 rpm for 10 minutes to separate the supernatant, and the volume was adjusted to 5 mL with 50% methanol in a volumetric flask. A 1 mL aliquot of the sample extract was transferred into a test tube, followed by the addition of 9 mL of distilled water and 1.5 mL of Folin-Ciocalteu's reagent. The mixture was incubated at room temperature for 5 minutes. Afterwards, 4 mL of 35% (w/w) Na_2CO_3 was added, and the volume was adjusted to 25 mL with distilled water. The mixture was agitated and left to stand for 30 minutes at room temperature. The absorbance of the sample was measured at 765 nm using a UV-Vis spectrophotometer. Gallic acid was used as a standard for the calibration curve. Total phenolic content was expressed as mg Gallic acid equivalents per gram of sample (mg/g).

Comment [3]: Total phenols are antioxidants not anti-nutrients

Tannins

The tannin content in each sample was determined using insoluble polyvinyl-polyrrolidone (PVPP), as described by (31). Specifically, 1.0 mL of the extract dissolved in methanol (1%) was mixed with 100 mg of PVPP. The mixture was vortexed and then incubated for 15 minutes at 4°C. The sample was centrifuged for 10 minutes at 3,000 rpm to separate the precipitated tannins. The clear supernatant obtained after centrifugation contained non-tannin phenolic, which was subsequently analyzed using the Folin-Ciocalteu method to determine total phenolic content. The difference in absorbance values before and after the removal of tannins was regarded as the absorbance value for total tannins in the sample.

Comment [4]: Tannins are anti-nutrients

Phytates

The phytates content was assessed through the iron-precipitation colorimetric method utilizing thiocyanate detection, following the approach outlined by(21) with certain modifications. Each powdered sample, weighing approximately 0.2 g, was carefully measured into a 125 ml Erlenmeyer flask. To extract the phytic acid, 50 ml of 3% trichloroacetic acid (TCA) was added, and the mixture was swirled by hand for 45 minutes with occasional swirling during the first 30 minutes. After centrifugation, 10 ml of the supernatant was transferred to a 50 ml conical flask. Next, 4 ml of FeCl_3 solution was rapidly added to the aliquot, and the content was heated in a boiling water bath for 45 minutes. After 30 minutes of heating, two drops of 3% sodium sulfate were added to the extract, and the heating process continued. The resulting supernatant was centrifuged for 15 minutes and carefully decanted. The precipitate was washed twice with 20 to 25 ml of 3% TCA by thoroughly dispensing, followed by heating in a boiling water bath for 10 minutes and centrifugation. This washing process was repeated with water. The precipitate was then dispersed in 27 ml of water and mixed with 3 ml of 1.5 N NaOH. The volume was adjusted to approximately 30 ml with water and heated in a boiling water bath for 30 minutes. The precipitate was filtered through moderately retentive filter paper, such as Whatman No. 2. The precipitate was washed with 70 ml of hot water, and the filtrate was discarded. The precipitate was then dissolved from the filter paper using 40 ml of 3.2 N HNO_3 into a 100 ml volumetric flask. The filter paper was thoroughly washed with multiple portions of water, and the washings were collected in the same flask without exceeding the 100 ml volume. The flask was allowed to

cool to room temperature and diluted to volume with water. A 5 ml aliquot was transferred to a 100 ml volumetric flask and diluted to approximately 70 ml. Subsequently, 20 ml of 1.5 M KSCN was added, and the solution was diluted to volume. The resulting color was measured immediately within 1 minute at 480 nm using a spectrophotometer. A reagent blank was included for each set of samples to ensure accuracy. The standard stock solution was prepared by taking 1000 mg/L of phytate (phytic acid) from potassium phytate ($K_3C_6H_6O_{18}P_3$). Precisely, 2.064589 g of potassium phytate was weighed and dissolved in a 1000 mL volumetric flask to prepare a working solution of different concentrations. The standard solutions were then subjected to coloration, and the absorbance was measured at 480 nm. A standard plot of concentration against standard absorbance was created, and a linear regression equation was obtained. The concentration of phytates was calculated using the Beer-Lambert equation.

Oxalates

The oxalic acid content was quantified utilizing the UV-Vis spectrophotometric approach, employing iron Ferron reagent detection, as outlined by(32), with modifications. A 1.00 g sample of dried ground compost was extracted in a 100-ml conical flask with 10 ml of water and 10 ml of citric acid reagent. The flask was heated on a hot plate, allowing it to simmer for 30 minutes with a small glass funnel placed in the neck of the flask as a condenser. The mixture was filtered through No. 30 Whatman filter paper and thoroughly washed with cold water. The flask was drained, and the filter paper and the precipitate were partially dried at 100°C for one hour before both were returned to the flask. Based on the expected calcium oxalate concentration, 25 ml or 50 ml of 0.4 N hydrochloric acid was added using a pipette (25 ml if the expected concentration was below 1% and 50 ml between 1% and 3%). The solution was heated to near the boiling point and maintained at this temperature for 5 minutes with occasional swirling. The solution was then filtered through a small, dry filter paper. The initial filtrate was discarded, and 4 ml of the remaining filtrate was pipetted into a dry beaker. To the filtrate, 5 ml of iron ferron reagent was added. The solution was protected from direct sunlight, and the absorbance was measured at 525 nm using a UV-Vis spectrophotometer (JENWAY 6715 UV/Vis U.K) with the instrument zeroed against a reagent blank containing 4 ml of 0.4 N hydrochloric acid and 5 ml of iron Ferron reagent. A standard curve was constructed using solutions with calcium oxalate ($CaC_2O_4 \cdot H_2O$) concentrations ranging from 0 to 4 mg, dissolved in 4 ml of 0.4 N hydrochloric acid.

2.3.3 Determination of physico-chemical parameters

Chlorophyll Content

The chlorophyll content was assessed using acetone extraction and UV-Vis spectrophotometry, following the methodology outlined by(24), with modifications. Use a mortar and pestle to crush 1.0 g of the material into a fine pulp with 10 ml of 80% acetone (LOBA, India). The pulp was centrifuged at 4000 rpm at room temperature for 5 minutes, then the green supernatant was transferred to a 50 ml volumetric flask for chlorophyll analysis. The centrifuge tube sediment was scraped and pulverized with the same mortar and pestle and a little 80% acetone to remove the remaining chlorophyll. The supernatant was mixed with the previous supernatant in the volumetric flask after 5 minutes of centrifugation at 4000 rpm at room temperature. Re-extraction was repeated until the residue was green-free. A 50 ml volumetric flask with 80% acetone received the supernatant. The extract was chilled for 10 minutes. With 80% acetone as the blank, a Spectrophotometer (JENWAY 6715 UV/Vis U.K) assessed the extract's absorbance at 663 and 645 nm. Chlorophyll was calculated using the empirical formula;

$$\text{Chlorophyll a, mg/g tissue} = 12.7 (A_{663}) - 2.69 (A_{645}) \times V / 100 \times W.$$

$$\text{Chlorophyll b, mg/g} = 22.9 (A_{645}) - 4.68(A_{663}) \times V/100XW$$

$$\text{Total Chlorophyll} = \text{Chlorophyll a} + \text{Chlorophyll b}$$

A represents absorbance at designated wavelengths; V is the final volume of the chlorophyll extract; W indicates the fresh weight of the extracted tissue.

Lactic acid

Colorimetric Method for Lactic Acid Quantification Using Ferric Chloride was used as outlined by (33), incorporating specific modifications. Five grams of dried leaf sample were placed in a beaker, to which boiled distilled water was added at a ratio of approximately 1:20 to 1:50, and allowed to cool. Following heating, the extract was allowed to cool to room temperature. The extract was then filtered through filter paper (Whatman No. 1) to remove the solid leaf residue, the filtrate test solution (50 μ L) containing lactic acid and 2 mL of a 0.1% iron (III) chloride solution in a cuvette and stirred was conducted at $25 \pm 5^\circ\text{C}$. The reaction was allowed to proceed at room temperature (26.2°C) for 10–15 minutes to ensure the lactic acid fully reacted with the ferric ions, forming a yellow-coloured complex. The absorbance was measured at 390 nm. To create the calibration curve, the absorbance of the lactic acid (1.2 g) with a known concentration of 89% (density = 1.2 g/mL) was measured; a stock solution containing 89 g/L of lactic acid was prepared. From this stock solution, a series of lactic acid solutions with different concentrations was prepared by dilution. A plot of absorbance against concentration was generated. The absorbance of the samples was then measured, and the lactic acid concentration was calculated using the calibration curve based on the Beer-Lambert law.

2.4. Statistical Analysis

All data were subjected to Statistical Package for Social Sciences (SPSS) version 27. A one-way analysis of variance (ANOVA) test was performed following a post-hoc Turkey test, with significant differences determined at a 5% level ($p < 0.05$). All results were expressed as mean \pm SD of triplicate values except for beta carotene and minerals, where the values were duplicate.

3.0 RESULTS AND DISCUSSION

3.1 Effect of processing on Physicochemical quality parameter of *Justicia Heterocarpa*

3.1.1. Dynamic trend in pH and lactic acid during spontaneous fermentation

The results of dynamic trend in pH and lactic acid of *Justicia Heterocarpa* leaves are presented in Table 1. Findings suggest significant trends at $p < 0.05$ for pH and lactic acid during the spontaneous fermentation of *Justicia heterocarpa* leaves exposed to two distinct doses of salt-sugar brine solution. The pH drops from neutral to acidic, measuring 3.89 and 3.52 for 4%salt-2%sugar and 3%salt-3%sugar, respectively, within 48 hours, with 3%salt-3%sugar brine concentration showed a more significant pH reduction than its counterpart. The variation could be due to the increased sugar content, which promoted bacterial growth by giving fermentation additional substrate. The quick pH decrease establishes acidic environment that suppresses spoiling organisms which cannot tolerate low pH and promotes beneficial bacteria (10). The study on the fermentation of pumpkin leaves in Kenya reported the spontaneous fermenting microbes present in pumpkin leaves when treated with 3%salt-3%sugar brine solution were *Leuconostoc citreum* at time zero, after 48 hours *Weissella cibaria* prevail the fermentation process, and *Lactiplantibacillus plantarum* finalize the fermentation process (3). The work conducted in Tanzania by (24), demonstrated successful spontaneous fermentation of *Solanum* species using a 4%salt-2%sugar brine solution, resulting in a pH reduction to 3.7 within 48 hours. Acidification of the fermented vegetables is very essential for preservation of the vegetable and extend its shelf life also achieving the desirable flavor (34).

Table 1: Lactic acid and pH dynamics in spontaneous fermentation (SF)

| TIME | Lactic Acid (mg/100ml) | | p ^H | |
|----------|-----------------------------|----------------------------|-----------------------------|----------------------------|
| | 4%salt-2%sugar | 3%salt-3%sugar | 4%salt-2%sugar | 3%salt-3%sugar |
| 0 hrs. | 0.099 ± 0.011 ^a | 0.06 ± 0.011 ^a | 6.68 ± 0.006 ^a | 6.62 ± 0.021 ^a |
| 12 hrs. | 0.125 ± 0.006 ^{ab} | 0.12 ± 0.011 ^b | 5.23 ± 0.01 ^{ab} | 5.66 ± 0.023 ^a |
| 24 hrs. | 0.177 ± 0.011 ^{b*} | 0.22 ± 0.006 ^c | 4.45 ± 0.044 ^{ab} | 4.35 ± 0.031 ^{ab} |
| 36 hrs. | 0.26 ± 0.023 ^{c*} | 0.35 ± 0.006 ^d | 4.0 ± 0.02 ^{abc} | 3.87 ± 0.02 ^b |
| 48 hrs. | 0.28 ± 0.042 ^{cd} | 0.36 ± 0.011 ^d | 3.89 ± 0.012 ^{bcd} | 3.53 ± 0.023 ^b |
| 60 hrs. | 0.29 ± 0.006 ^{cd} | 0.42 ± 0.040 ^e | 3.82 ± 0.02 ^{cde} | 3.4 ± 0.012 ^c |
| 72 hrs. | 0.33 ± 0.006 ^{de} | 0.55 ± 0.022 ^f | 3.78 ± 0.02 ^{de} | 3.2 ± 0.015 ^c |
| 84 hrs. | 0.35 ± 0.011 ^e | 0.65 ± 0.006 ^g | 3.7 ± 0.0 ^{ef} | 3.31 ± 0.006 ^c |
| 96 hrs. | 0.36 ± 0.017 ^e | 0.75 ± 0.011 ^h | 3.77 ± 0.026 ^f | 3.40 ± 0.015 ^d |
| 108 hrs. | 0.42 ± 0.028 ^e | 0.89 ± 0.006 ⁱ | 3.68 ± 0.025 ^g | 3.39 ± 0.012 ^e |
| 120 hrs. | 0.52 ± 0.011 ^f | 0.96 ± 0.011 ^j | 3.66 ± 0.057 ^h | 3.27 ± 0.058 ^f |
| 132 hrs. | 0.69 ± 0.017 ^g | 1.20 ± 0.011 ^k | 3.72 ± 0.012 ⁱ | 3.30 ± 0.006 ^g |
| 144 hrs. | 0.78 ± 0.028 ^h | 1.328 ± 0.006 ^l | 3.64 ± 0.032 ^j | 3.21 ± 0.023 ^h |

Values are means ± SD and values within the same column with different superscript letters are significantly different from each other at $p < 0.05$.

In comparison to a 4%salt-2%sugar brine solution, a 3%sugar-3%salt brine solution exhibited a considerable rise in lactic acid content over time during spontaneous fermentation, indicating enhanced efficacy in producing an acceptable quantity of lactic acid. Upon completion of fermentation, a 3%sugar-3%salt brine solution yielded 1.3288 mg/100ml of lactic acid, which was substantially greater ($p < 0.05$) than the 4%salt-2%sugar brine solution, which produced 0.7877 mg/100ml (Table 1). The lactic acid produced acts as a preservative of the vegetables because spoilage and pathogenic microbes cannot tolerate high acid. However, it contributes to flavor and aroma development, improving fermented vegetables' sensory appeal (10). The study conducted by (24) on the fermentation of nightshade leaves in 4% and 2% salt sugar

brine solution, utilizing the titration method, yielded results comparable to those of the current study.

3.1.2. Moisture Content (MC) of dried *Justicia Heterocarpa*

The moisture content changes with time for un-branched leaves directly dried in the room (UNLDDR), blanched leaves dried in room (BLDR) and blanched leaves dried in oven (BLDO) during drying of *Justicia Heterocarpa* are displayed in Table 2. All three methods displayed substantial moisture reduction of moisture content in which moisture was reduced from 87.83 to 12.38% for UBLDDR, 85.4 to 10.29 for BLDR, and 86.11 to 9.89%. Samples of blanched leaves dried at room (BLDR) temperature for 48 hours exhibited a significant higher moisture content ($p < 0.05$) compared to those un-branched leaves dried directly at room temperature (UBLDDR). The study by (35), indicated a reduction in moisture content to 11.82% in black nightshades and amaranth when dried in shade. This finding is consistent with the current study, which observed that *J. Heterocarpa* achieved a moisture content of 12.38% under similar drying conditions. While oven drying proved to be the most effective drying method (9.89%, 12 hrs) in current study due to its efficiency, it is less feasible in resource-limited settings as it necessitates electricity and specialized equipment. Blanching facilitates moisture reduction by disrupting cell walls, thereby enhancing the rate of moisture escape. Achieving the desired moisture content in unbranched vegetables presents greater challenges. The process required 15 days to achieve a moisture content of 12.38%, rendering it less suitable for rapid processing, though viable under resource constraints. Other studies have highlighted the importance of blanching before drying, which leads to minimal nutrient leaching and preserves more nutrients (36).

Table 02. Moisture Content of Different Drying Methods

| Time | Moisture% | | |
|-----------|------------------|-----------------|---------------|
| | UBLDDR | BLDR | BLDO |
| 0 hours | 87.83 ± 1.444g | 84.33 ± 1.624e | 86.11 ± 0.596 |
| 12 hours | 73.35 ± 2.024f | 43.86 ± 3.879d | 9.89 ± 0.157 |
| 24 hours | 51.00 ± 2.036e | 28.07 ± 1.356c | |
| 48 hours | 19.08 ± 0.251d | 15.41 ± 1.181b | |
| 72 hours | 16.58 ± 0.592cd | 13.06 ± 0.648ab | |
| 96 h0urs | 15.05 ± 0.196abc | 11.95 ± 0.205ab | |
| 120 hours | 15.15 ± 0.933abc | 10.29 ± 0.121a | |
| 144 hours | 15.73 ± 0.529bc | | |
| 168 hours | 14.21 ± 0.754abc | | |
| Day 8 | 13.68 ± 0.7abc | | |
| day 12 | 14.04 ± 0.072abc | | |
| day 13 | 13.44 ± 0.516ab | | |
| day 14 | 12.77 ± 0.252a | | |
| day 15 | 12.38 ± 0.27 | | |

Values are means ± SD and values within the same column with different superscript letters are significantly different from each other at $p < 0.05$. Abbreviations: BLDR: Blanched leaves dried in room, BLDO: Blanched leaves dried in oven, UBLDDR: un-branched leaves directly dried in the room

3.1.4 Effects of processing on chlorophyll content

Chlorophyll content of fresh and processed leaves of *Justicia Heterocarpa* are presented in Table 03. The total chlorophyll content of the leaves subjected to different processing method varied and were significantly reduced to 0.0964 ± 0.075 mg/g in fermented samples and 6.7688 ± 0.448 mg/g for the ten minutes cooked leaf samples (Table 3). Total chlorophyll levels were highest in fresh leaves (FL), measuring 13.429 ± 0.522 mg/g. Leaves dried at room temperature

(BLDR) and those microwaved for 2 minutes (FLMCO2) maintained total chlorophyll levels of approximately 12.92 and 12.15 mg/g, respectively, and did not show a significant difference ($p>0.05$) compared to fresh leaves. Leaves dried at room temperature retained a highest chlorophyll level than all other processing methods, which suggests that blanching before drying can control chlorophyll degradation. Fermented freshly leaves (FFL) samples exhibited significantly reduced chlorophyll levels of 0.0964 ± 0.075 mg/g (table 03), indicating substantial degradation of chlorophyll during acidic fermentation, resulting in the transformation into non-green compounds and observable color changes. Numerous studies indicate a significant decrease in chlorophyll levels in fermented ILVs. The study conducted by (24) on the impact of fermentation on chlorophyll reduction in nightshade species indicated a gradual decrease in chlorophyll a, b, and total chlorophyll levels. (34) reported that the accumulation of acids such as acetic and lactic is the primary factor contributing to the reduction of chlorophyll levels in fermented nightshades. Chlorophyll present in green leafy vegetables serves as a significant antioxidant, aiding in the mitigation of oxidative stress and the prevention of cellular damage. Due to its anti-inflammatory properties, it may reduce chronic inflammation and enhance overall health (37).

Table 3. Chlorophyll Content in raw and processed *Justicia Heterocarpa*

| Sample | Chlorophyll a (mg/g) | Chlorophyll b (mg/g) | Total Chlorophyll (mg/g) |
|--------|-------------------------|-------------------------|---------------------------|
| FL | 8.019 ± 0.104^d | $5.414 \pm 0.422d$ | 13.429 ± 0.522^j |
| BLDR | 7.927 ± 0.108^d | 4.995 ± 0.795^{cd} | 12.918 ± 0.836^{ef} |
| BLDO | 6.403 ± 0.956^c | 4.316 ± 0.117^{cd} | 10.715 ± 0.942^{cd} |
| UBLDDR | 7.35 ± 0.36624^{cd} | 4.0759 ± 0.151^c | 11.4228 ± 0.381^{cde} |
| FFL | 0.1065 ± 0.126^a | -0.01 ± 0.0530^a | 0.0964 ± 0.075^a |
| FLCO5 | 6.3389 ± 0.551^c | 3.8569 ± 0.790^{bc} | 10.193 ± 1.066^c |
| FLCO10 | 3.945 ± 0.5236^b | 2.8258 ± 0.173^b | 6.7688 ± 0.448^b |
| FLMCO2 | 7.7804 ± 0.294^d | 4.3774 ± 0.235^{cd} | 12.1545 ± 0.527^{def} |

Values are means \pm SD and values within the same column with different superscript letters are significantly different from each other at $p<0.05$. Abbreviations: FL: freshly leaves, BLDR: Blanched leaves dried in room, BLDO: Blanched leaves dried in oven, UBLDDR: un-branched leaves directly dried in the room, FFL: fermented fresh leaves; FLCO5:5 min cooked sample in gas cooker, FLCO10:10 min cooked sample in gas cooker, FLMCO2: 2 min microwaving cooked samples

3.2 Effect of processing on *Justicia Heterocarpa* nutritional composition

Ascorbic Acid

Table 4 presents the beta carotene and ascorbic acid content of processed *Justicia heterocarpa* leaves. Unprocessed fresh leaves (FL) exhibited a high concentration of ascorbic acid (23.21 mg/100g), which significantly decreased ($p<0.05$) following various processing methods.

Table 4. Beta carotene and Ascorbic acid of *Justicia Heterocarpa* processed leaves (FW)

| | Ascorbic acid (mg/100g) | % Reduction | Beta carotene (mg/100g) | % Reduction |
|--------|--|-------------|----------------------------|-------------|
| FL | 23.21 ± 0.742 ^e | - | 3.67 ± 0.072 ^c | - |
| BLDR | 18.62 ± 0.557 ^d | 19.79 | 2.06 ± 0.038 ^b | 43.82 |
| BLDO | 16.52 ± 0.185 ^{c^d} | 28.84 | 5.72 ± 0.156 ^d | -55.79 |
| UBLDDR | 19.41 ± 0.186 ^d | 16.40 | 1.55 ± 0.013 ^a | 57.78 |
| FFL | 5.88 ± 0.743 ^a | 74.66 | 1.29 ± 0.053 ^a | 64.69 |
| FLCO5 | 14.02 ± 1.114 ^c | 39.59 | 10.51 ± 0.002 ^f | -186.26 |
| FLCO10 | 8.90 ± 1.299 ^b | 61.65 | 9.14 ± 0.035 ^e | -148.77 |
| FLMCO2 | 18.22 ± 0.371 ^d | 21.49 | 11.29 ± 0.030 ^g | -207.42 |

Values are means ± SD and values within the same column with different superscript letters are significantly different from each other at $p < 0.05$. % Reduction: the percentage ascorbic acid and beta carotene (%). Abbreviations: FL: freshly leaves, BLDR: Blanched leaves dried in room, BLDO: Blanched leaves dried in oven, UBLDDR: un-branched leaves directly dried in the room, FFL: fermented freshly leaves; FLCO5:5 min cooked sample in gas cooker, FLCO10:10 min cooked sample in gas cooker, FLMCO2: 2 min microwaving cooked samples

The fermentation of flesh leaves and gas cooking for ten minutes resulted in a significant reduction of ascorbic acid content, approximately by 74.66% and 61.65%, respectively. Various processing methods resulted in a moderate decrease in ascorbic acid levels. Specifically, BLDO decreased ascorbic acid by 28.84%, FLCO5 by 39.59%, and FLMCO2 by 21.49%. In contrast, BLDR and UBLDDR led to reductions of only 19.79% and 16.40%, respectively. Despite the lack of significant difference ($p > 0.05$), the loss of ascorbic acid in blanched dried in oven leave samples (BLDO) was greater (28.84%) compared to the blanched leaves dried at room temperature (BLDR; 19.79%). Blanching prior to drying enhances the retention of ascorbic acid by inactivating oxidative enzymes, such as ascorbic acid oxidase, responsible for its degradation. Oven drying is a rapid process; however, it results in a significant degradation of ascorbic acid when compared to room drying (BLDR). This degradation may be attributed to elevated thermal temperatures, which enhance the rates of oxidation. The current study achieved a reduction of approximately 75% in ascorbic acid through the fermentation process of *Justicia Heterocarpa*. This reduction may be attributed to the solubility of ascorbic acid (vitamin C) in brine solution, as ascorbic acid is soluble in water(26). The findings align with those reported by (24) in their study conducted in Tanzania, which indicated a significant loss of vitamin C, ranging from 88.33% to 95%, during the fermentation of *solanum species*. Furthermore, the gas cooking of flesh leaves for ten minutes resulted in approximately a 61% reduction in ascorbic acid in this study, likely due to leaching and thermal degradation. A prior study indicated that pasteurizing yellow and red tomatoes at 100°C for 10 minutes resulted in an up to 80% reduction in vitamin C, while cooking broccoli for 5 minutes was associated with a loss of approximately 66% of this vitamin (38).The findings of vitamin C content of *J. Heterocarpa* from the present study are less

than those of daily recommended value for adult men and women (110mg/day and 95mg/day respectively) (38). Traditionally, *Justicia Heterocarpa* is cooked as a composite dish mixed with legumes (cowpea, pigeon peas, beans) and okra (author's unpublished surveyed data). This combination can boost the level of vitamin C to meet the required amount in a day. *Justicia heterocarpa* was reported to be similar with African nightshade (*Solanum nigrum complex*) in taste (24) and that incorporating *Solanum species* or *Justicia heterocarpa* into meat, other vegetables, and legumes such as cowpea improves taste, appetite, and increase carotenoids and vitamin C (39).

Beta-carotene

Beta-carotene levels differed substantially across all processing processes at $p < 0.05$, with the cooked samples FLCO5 and FLCO10 having the greatest quantities (10.5115 mg/100g and 11.2885 mg/100g, respectively) (Table 4). The FFL and IUBLDD samples exhibited the lowest concentrations of beta carotene, with values of 1.30 mg/100g and 1.55 mg/100g, respectively, and were not statistically different from one another. The cooked samples shown an increase in beta-carotene content, with FLCO5 exhibiting an 86.26% increase, FLCO10 a 48.77% increase, and FLMCO2 a 107.42% increase. Additionally, the blanched oven-dried vegetables (BLDO) showed a 55% increase in beta-carotene content. The substantial increase in beta-carotene levels in cooked samples may be attributed to the relative heat stability of beta-carotene, as the plant matrix facilitates its release through cell wall breakdown, thereby enhancing its bioavailability (26). This study demonstrated a reduction of approximately 65% in beta-carotene via the fermentation process of *Justicia Heterocarpa*. This reduction can be attributed to the sensitivity of beta-carotene to oxidation, which affects its stability over time. (40) reported that submerged fermentation of vegetables reduces approximately 20% of total beta-carotene content. However, the study by (24) highlighted an increase in beta-carotene during the fermentation of nightshade species. Findings from the present study showed that microwaving cooking resulted in more than a threefold increase in this vitamin. Several studies have reported similar outcomes, with (41) (2017) noting that microwaving of selected ILVs retained more beta-carotene compared to their fresh counterparts. The observed reduction of beta-carotene in room temperature dried samples (UBLDDR and BLDR) may be attributed to the oxidation of beta-carotene. Research by (41) similarly indicated a reduction in beta-carotene levels in certain vegetables dried at 65°C using a hot air dryer. The study by (42) reported an increase in beta-carotene content in African nightshade and cowpea leaves subjected to various sun drying treatments.

Minerals:

Macro minerals (calcium, magnesium) and trace minerals (iron, zinc) exhibited significant differences across all processing techniques at $p < 0.05$. Considerable concentrations of Fe (9.46-16.74 mg/100g), Zn (3.041-4.39 mg/100g), Ca (255.2-348 mg/100g), and Mg (71.779-159.9 mg/100g) were recorded (Table 5).

Table 5: Mineral Content of raw and processed *Justicia Heterocarpa* in Fresh Weight

| Sample | Minerals (mg/100g) | | | |
|--------|----------------------------|----------------------------|----------------------------|-----------------------------|
| | Iron | Zinc | Calcium | Magnesium |
| FL | 16.74 ± 0.353 ^d | 4.39 ± 0.1718 ^d | 348.73 ± 0.57 ^f | 159.91 ± 0.04 ^g |
| BLDR | 13.40 ± 0.472 ^c | 3.58 ± 0.0665 ^b | 312.74 ± 0.9 ^d | 123.86 ± 0.04 ^b |
| BLDO | 13.84 ± 0.182 ^c | 3.4 ± 0.087 ^b | 338.30 ± 1.34 ^e | 135.30 ± 0.02 ^c |
| UBLDDR | 15.85 ± 0.088 ^d | 3.96 ± 0.0411 ^c | 314.17 ± 0.1 ^d | 149.4 ± 0.66 ^f |
| FFL | 14.25 ± 0.266 ^c | 3.53 ± 0.0553 ^b | 262.36 ± 0.28 ^b | 71.78 ± 0.08 ^a |
| FLCO5 | 11.32 ± 0.242 ^b | 3.28 ± 0.0661 ^a | 286.46 ± 2.12 ^c | 140.18 ± 0.023 ^e |
| FLCO10 | 9.46 ± 0.354 ^a | 3.04 ± 0.0738 ^a | 255.20 ± 1.57 ^a | 139.09 ± 0.04 ^d |
| FLMCO2 | 13.46 ± 0.512 ^c | 4.04 ± 0.0019 ^c | 339.85 ± 1.51 ^e | 138.92 ± 0.06 ^d |

Values are means ± SD and values within the same column with different superscript letters are significantly different from each other at p<0.05. Abbreviations: FL: freshly leaves, BLDR: Blanched leaves dried in room, BLDO: Blanched leaves dried in oven, UBLDDR: un-branched leaves directly dried in the room, FFL: fermented freshly leaves; FLCO5:5 min cooked sample in gas cooker, FLCO10:10 min cooked sample in gas cooker, FLMCO2: 2 min microwaving cooked samples

Iron: Unprocessed fresh leaves (FL) demonstrated a high iron concentration of 16.74 mg/100g, which significantly decreased (p<0.05) after certain processing methods of *Justicia Heterocarpa*. The lowest iron content was observed in samples cooked for ten minutes (FLCO10, 9.46 mg/100g), indicating significant iron leaching during prolonged cooking. The iron content among the samples (BLDO, BLDR, FFL, and FLCO) exhibited no significant variation, indicating the stability of iron levels across certain processing methods. (10) reported a decrease in iron content in fermented African spider plant and African black nightshade, which aligns with the current study. Conversely, the study by (24) utilizing Energy-Dispersive X-ray Fluorescence equipment reported an increase in iron content in fermented *Solanum species*. This study corroborates findings indicating that iron is susceptible to leaching during cooking and blanching(43). Iron is essential in the human body for hemoglobin-mediated oxygen transport, energy synthesis, immune function, and muscle performance. Additionally, it contributes to DNA synthesis and cognitive development (44). The intake of 100 grams of *Justicia Heterocarpa* provides the daily iron requirement for the body, supplying 18 mg for adult women and 6-12 mg for adult men (45).

Zinc: Unprocessed fresh leaves (FL) exhibited a zinc concentration of 4.39 mg/100g, which significantly decreased (p<0.05) following particular processing methods of *Justicia Heterocarpa*, as indicated in Table 5. The study indicated no significant difference in zinc content between blanched dried and fermented samples, likely due to the relative stability of zinc across various processing methods. Furthermore, there is no significant difference between gas-cooked samples subjected to different durations (FLCO5 and FLCO10). A minimal reduction of zinc was observed in microwaved cooked and un-blanched room-dried samples, likely due to the absence of liquid, which reduces mineral loss. The zinc content of the fresh leaves in this study is comparable to that reported by Gowele *et al.* (2019) for the same vegetable (3.9 mg/100 g, FW). The research by Marynurce (2023) indicated a decrease in zinc levels across various drying techniques of nightshade species, consistent with the current study. However, the study by(46) reported a contrasting finding, indicating that zinc content increased more than threefold in selected indigenous leafy vegetables (ILVs) dried using sun, oven, and

shade methods. Furthermore, the research by (47) indicated a reduction in zinc content in blanched sun-dried selected indigenous leafy vegetables by as much as 50%. (48) reported a decrease in zinc content in the selected cooked indigenous vegetables. (24) reported an increase in zinc content in fermented nightshade species, which contradicts the findings of the current study. The zinc content in fresh leaves of *Justicia heterocarpa* exceeds that found in soy (3.7 mg/100 g), indicating a significant mineral concentration in this vegetable (49). Zinc is an essential trace element involved in numerous physiological processes in the body. Its participation in over 300 enzymatic activities and metabolic processes underscores its essential role in maintaining overall health and wellbeing. (50). Excessive zinc consumption has been associated with health risks, including zinc-induced copper deficiency, symptoms of anemia, and neutropenia (50).

Calcium

Unprocessed fresh leaves (FL) demonstrated a calcium concentration of 348.73 mg/100g, which significantly decreased ($p < 0.05$) after specific processing methods of *Justicia Heterocarpa*, as shown in Table 5. There was no significant variation ($p < 0.05$) in calcium content was observed between shade-dried samples (BLDR and UBLDDR) and high thermal-treated samples (BLDO and FLMCO2). This stability can be attributed to calcium's resistance to changes during various processing methods, as it is not sensitive to heat, light, or oxygen. The research conducted by (46) revealed a significant increase in calcium content, exceeding threefold, in selected indigenous leafy vegetables (ILVs) dried through sun, oven, and shade methods. The study by (47) demonstrated a reduction in calcium content in blanched sun-dried selected indigenous leafy vegetables by up to 50%. (48) observed a reduction in calcium content in the chosen cooked indigenous vegetables, consistent with the findings of the current study. (10) reported a decrease in calcium content in fermented African black nightshade and African spider plant, which supports the findings of the current research. (24) reported an increase in calcium content in fermented nightshade species, which contradicts the findings of this study. Numerous studies indicate that the calcium content in ILVs is higher than that observed in the current study (51,52). Calcium is vital for bone and dental health, regulates blood coagulation, influences muscle and nerve function, and activates various enzymes, such as ATPases, crucial for energy production (52). Daily calcium intake should be between 1000 and 1300 mg (44).

Magnesium

Unprocessed fresh leaves (FL) exhibited a magnesium concentration of 159.9 mg/100g, which significantly decreased ($p < 0.05$) following specific processing methods of *Justicia Heterocarpa*, as indicated in Table 5. No significant variation was observed between samples cooked in a microwave and those cooked with gas for ten minutes (FLMCO2 and FLCO10), as magnesium remains stable and does not degrade or volatilize at standard cooking temperatures. Fermented samples exhibit a notably low magnesium content of 71.78 mg/100g, likely due to magnesium leaching into the brine solution. (48) reported a slight reduction in magnesium content in blanched and sautéed indigenous vegetables, which is consistent with the findings of the current study. Factors influencing magnesium bioavailability include dietary fat content, the presence of anti-nutrients, and the individual's age and sex (26). Magnesium facilitates energy production, the transport of calcium and potassium ions, nerve conduction, muscular contraction, vasomotor tone, and the maintenance of regular heart rhythm. It serves as a crucial cofactor in over 300 enzymatic systems (53). The daily recommended intake of magnesium is between 300 and 429 mg (53).

3.4 Effect of processing on the ant-nutrient composition of *Justicia Heterocarpa*.

The anti-nutrient profiles of *Justicia heterocarpa* exhibited significant variation under different processing methods, with $p < 0.05$, as indicated in Table 6. The reduction of oxalates, tannins, and phytates following processing improves the bioavailability of minerals, thereby supporting its potential application in addressing micronutrient deficiency.

Table 06: Anti-Nutrient Profiles in raw and processed *Justicia Heterocarpa*

| Sample | Ant-nutrient (FW) | | | |
|--------|-----------------------------|----------------------------|----------------------------|------------------------------|
| | Total phenol(GAE/100g) | Tannins (GAE/100g) | Phytates (mg/100g) | Oxalates (mg/100g) |
| FL | 190.83 ± 14.91 ^c | 81.17 ± 3.56 ^d | 103.56 ± 4.68 ^d | 560.46 ± 19.16 ^f |
| BLDR | 142.95 ± 5.92 ^b | 59.51 ± 2.96 ^c | 76.24 ± 7.83 ^c | 351.39 ± 7.41 ^d |
| BLDO | 130.42 ± 5.22 ^b | 53.24 ± 2.61 ^c | 73.82 ± 5.99 ^c | 242.44 ± 4.50 ^c |
| UBLDDR | 352.71 ± 12.01 ^d | 164.39 ± 6.01 ^e | 94.92 ± 1.80 ^d | 417.16 ± 4.50 ^e |
| FFL | 532.83 ± 14.91 ^e | 254.44 ± 7.45 ^f | 40.28 ± 3.34 ^b | 225.76 ± 10.34 ^{bc} |
| FLCO5 | 117.19 ± 7.90 ^b | 21.32 ± 2.61 ^b | 50.66 ± 6.25 ^b | 171.77 ± 50.34 ^{ab} |
| FLCO10 | 64.75 ± 7.90 ^a | 4.22 ± 1.98 ^a | 17.81 ± 3.17 ^a | 151.16 ± 6.13 ^a |
| FLMCO2 | 149.11 ± 29.81 ^b | 25.31 ± 7.45 ^b | 75.9 ± 1.59 ^c | 219.87 ± 1.7 ^{bc} |

Values are means ± SD and values within the same column with different superscript letters are significantly different from each other at $p < 0.05$. Abbreviations: FL: freshly leaves, BLDR: Blanched leaves dried in room, BLDO: Blanched leaves dried in oven, UBLDDR: un-branched leaves directly dried in the room, FFL: fermented freshly leaves; FLCO5: 5 min cooked sample in gas cooker, FLCO10: 10 min cooked sample in gas cooker, FLMCO2: 2 min microwaving cooked samples

Total phenol:

The concentration of Total Phenolic Compounds (TPC) in unprocessed leaves of *Justicia Heterocarpa* varied significantly across different processing methods as indicated in Table 06. No significant variation in TPC was observed in the BLDR, BLDO, and FLCO5 samples. The highest levels of TPC were observed in FFL, which exhibited an increase of 64.19% from the unprocessed leaves, and in UBLDDR, where the increase was 45.9%. The increase in Total Phenolic Compounds (TPC) in un-blanching leaves dried at room temperature may be attributed to the concentration of TPC during the drying process. The increase in TPC in the FFL sample may result from the liberation of bound phenols, enhancing their concentration and bioavailability (54). The low pH stabilizes phenolic compounds, which can be detected in significant quantities (10,34). Reduced phenol levels following blanching (BLDR) and extended cooking (FLC10) suggest heat sensitivity and potential loss of phenolic components due to oxidation (31)(Mohankumar *et al.*, 2018). Total phenolic compounds exhibit various health benefits, such as antioxidant activity, anti-inflammatory properties, and antimicrobial effects (55).

Tannins:

The concentration of total tannins in unprocessed leaves of *Justicia Heterocarpa* exhibited significant variation across various processing methods as shown in Table 06. No significant variation was observed between the short time cooked samples (FLCO5 and FLMCO2), likely due to their comparable cooking times, which resulted in similar tannin binding to the food matrix, as well as between the blanched dried samples (BLDR and BLDO). The fermented leaves (FFL) and UBLDDR exhibited the highest tannin concentrations, measuring 254.44 GAE/100g and 164.39 GAE/100g, respectively. An increase of 68.1% in the total tannins in the

FFL sample compared to the fresh sample may result from spontaneous fermentation of *Justicia Heterocarpa* leaves. During this process, microbial activity and enzymatic actions break down complex tannin compounds and degrade cell walls, thereby releasing bound tannins into a more measurable and soluble form. The ten-minute cooked samples exhibited the lowest tannin levels at 4.22 mg/100g, reflecting a reduction of 94.6% compared to the two- and five-minute cooking durations. This demonstrates the efficacy of extended cooking in diminishing tannin levels. (10) reported comparable findings, indicating that tannin content was significantly elevated in spontaneously fermented African black nightshade and African spider plants. In contrast to this study, the research conducted by (24) indicated a reduction in total tannins in fermented *nightshade species*. A study by (43) reported reduced tannin levels in false sesame and common bean leaves when exposed to varying blanching times and drying techniques, corroborating the findings of the present study. Tannins, due to their pronounced properties and capacity to bind with proteins, can influence nutrient absorption efficiency. Tannins can interact with proteins via covalent and hydrogen bonding, resulting in the formation of a complex protein precipitate that inhibits absorption (49).

Phytates

The levels of phytates in unprocessed leaves of *Justicia Heterocarpa* demonstrated considerable significant variation at ($p < 0.050$) among different processing method as indicated in Table 06. No significant difference was observed between the blanched dried and microwaved cooked samples (BLDR, BLDO, and FLMCO2), indicating a moderate reduction of phytates across all methods. Furthermore, the absence of a significant difference between the unprocessed fresh leaves and the un-blanched leaves dried at room temperature may be attributed to Shade drying entails limited processing, thereby retaining certain compounds similar to those found in fresh leaves. The lowest total tannin content was observed in the fermented and ten-minute cooked samples, measuring 40.28 mg/100g and 17.8077 mg/100g, respectively. In spontaneous fermentation, phytase enzymes generated by fermenting microorganisms hydrolyze phytic acid, the storage form of phosphorus in plants, into inositol and free phosphate, resulting in a significant reduction of phytate levels in FFL samples (56). (22) reported a significant reduction of phytates in fermented spinach. Furthermore, researches conducted by (10,24) indicated a decrease in phytates in fermented nightshade species, corroborating the results of the present study. Several studies indicate that blanching and boiling vegetables decrease the phytate content in ILVs (7,21,23), which is consistent with the findings of the current study. Phytates bind essential minerals, forming insoluble complexes that decrease their bioavailability (57). Phytic acid consumption (4-9 mg/100 g) has been shown to decrease iron absorption by 4 to 5 times (58).

Oxalates:

The concentration of oxalates in unprocessed leaves of *Justicia Heterocarpa* exhibited significant variation ($p < 0.05$) across different processing methods, as shown in Table 06. The concentrations of oxalates did not significantly vary between the cooked samples (FLMCO2,FLCO10 and FLCO5), indicating a substantial reduction of oxalates in these methods relative to other processing techniques. The minimum reduction of oxalates was observed in un-blanched samples dried directly in the room, consistent with the findings of (49), which indicated that direct drying and storage did not significantly alter the reduction of oxalic acid in ILVs. Numerous studies indicate that oxalates can be effectively reduced through boiling and blanching (7,21,49) corroborating the findings of the current research. The total oxalic acid content in fresh leaves of *Justicia Heterocarpa* is lower than that found in other green leafy vegetables, including spinach (329.6-2350 mg/100g FW) and rhubarb (1235 mg/100g FW) (32). Soybeans have been reported to contain lower levels of oxalates (124-497 mg/100 g FW) (32), compared to the fresh leaves of *Justicia Heterocarpa*. This suggests that certain indigenous

leafy vegetables (ILVs) may have higher concentrations of anti-nutrients than legumes, which are typically recognized for their anti-nutrient content. The lethal dose of oxalate in adults has been documented to range from 10 to 15 grams per body weight(7).

4.0 CONCLUSION AND RECOMMENDATION

The present study examined how processing affects *Justicia Heterocarpa*'s nutritional and anti-nutritional characteristics. Cooking for ten minutes and blanching before drying reduced anti-nutrients more than un-blanching samples. Beta-carotene, vitamin C, and minerals were moderately preserved in microwaved cooked and un-blanching shade-dried samples. Fermented leaves had highest Total Phenolic Compounds (TPC), which protect against non-communicable diseases. The findings are important for Tanzanian communities with dietary inadequacies. To promote *Justicia Heterocarpa* as a functional food across various dietary patterns, further research is necessary to ascertain its phytochemical composition and antioxidant activity, as well as the role these compounds may play in the prevention of chronic diseases such as cancer and heart disease.

7.0 Data availability

The data produced and examined in this study can be obtained from the corresponding author upon a reasonable request.

REFERENCES

1. Heaney D, Padilla-Zakour OI, Chen C. Processing and preservation technologies to enhance indigenous food sovereignty, nutrition security and health equity in North America. *Front Nutr.* 2024;11(June):1–8.
2. Li X, Yadav R, Siddique KHM. Neglected and Underutilized Crop Species: The Key to

Improving Dietary Diversity and Fighting Hunger and Malnutrition in Asia and the Pacific. *Front Nutr.* 2020;7(November):1–12.

3. Misci C, Taskin E, Dall'Asta M, Fontanella MC, Bandini F, Imathiu S, et al. Fermentation as a tool for increasing food security and nutritional quality of indigenous African leafy vegetables: the case of Cucurbita sp. *Food Microbiol* [Internet]. 2021;99(December 2020):103820. Available from: <https://doi.org/10.1016/j.fm.2021.103820>
4. Jeje OA, G.I. O, O.A OT et. al. Mitigation of Hidden Hunger through Biofortification : An Appraisal. *Int J Recent Innov Food Sci Nutr Innov Food Sci Nutr.* 2023;6(1):13–29.
5. Irakoze ML, Wafula EN, Owaga E. Potential Role of African Fermented Indigenous Vegetables in Maternal and Child Nutrition in Sub-Saharan Africa. *Int J Food Sci.* 2021;2021(Sdg 2).
6. Bukenya R, Laborde JEA, Mamiro P, Mugabi R, Kinabo J. Assessment of nutrient adequacy of complementary foods for infants and young children in Morogoro, Tanzania. *Sci African.* 2023;19.
7. Ngungulu TP, Wenaty A, Chove B, Suleiman R, Mbwana H. Effect of Different Processing Methods on the Anti-nutritive Components of Selected Indigenous Vegetables. *Asian Food Sci J.* 2024;23(8):80–94.
8. John SE, Azizi K, Hancy A, Twin'omujuni A, Katana D, Shine J, et al. The prevalence and risk factors associated with Iron, vitamin B12 and folate deficiencies in pregnant women: A cross-sectional study in Mbeya, Tanzania. *PLOS Glob Public Heal* [Internet]. 2023;3(4 April):1–15. Available from: <http://dx.doi.org/10.1371/journal.pgph.0001828>
9. Stuetz W, Gowele V, Kinabo J, Bundala N, Mbwana H, Rybak C, et al. Consumption of dark green leafy vegetables predicts vitamin a and iron intake and status among female small-scale farmers in Tanzania. *Nutrients.* 2019;11(5):1–18.
10. Irakoze ML, Owaga EE, Wafula EN. Effect of lactic acid bacteria fermentation on nutrients and anti-nutrients of African black nightshade and African spider plant. *Sci African* [Internet]. 2023;21:e01762. Available from: <https://doi.org/10.1016/j.sciaf.2023.e01762>
11. Stoll DA, Wafula EN, Mathara JM, Trierweiler B, Kulling SE, Huch M. Fermentation of African nightshade leaves with lactic acid bacterial starter cultures. *Int J Food Microbiol* [Internet]. 2021;342(January 2020):109056. Available from: <https://doi.org/10.1016/j.ijfoodmicro.2021.109056>
12. Sarfo J, Pawelzik E, Keding GB. Are processed fruits and vegetables able to reduce diet costs and address micronutrient deficiencies? Evidence from rural Tanzania. *Public Health Nutr.* 2022;25(9):2637–50.
13. Atuna RA, Djah J, Achaglinkame MA, Bakker S, Dari L, Osei-Kwarteng M, et al. Types of indigenous vegetables consumed, preparation, preferences and perceived benefits in Ghana. *J Ethn Foods* [Internet]. 2022;9(1). Available from: <https://doi.org/10.1186/s42779-022-00154-3>
14. Chacha JS, Laswai HS. Traditional Practices and Consumer Habits regarding Consumption of Underutilised Vegetables in Kilimanjaro and Morogoro Regions, Tanzania. *Int J Food Sci.* 2020;2020.
15. Imathiu S. Neglected and Underutilized Cultivated Crops with Respect to Indigenous African Leafy Vegetables for Food and Nutrition Security. 2021;9(3):115–25.
16. Mungofa N, Sibanyoni JJ, Mashau ME, Beswa D. Prospective Role of Indigenous Leafy Vegetables as Functional Food Ingredients. *Molecules.* 2022;27(22):1–23.
17. Issa-Zacharia A, Majaliwa NK, Nyamete FA, Chove LM. Diversity of Underutilised Vegetables in Africa and Their Potential in the Reduction of Micronutrient Deficiency: A Review. *World J Food Sci Technol.* 2024;8(1):1–13.
18. Henze J, Abukutsa-Onyango M, Opiyo AM. Production and Marketing of African Indigenous Leafy Vegetables. Training Manual for Extension Officers and Practitioners

[Internet]. 2020. 1–93 p. Available from: https://edoc.hu-berlin.de/bitstream/handle/18452/23783/Hortinlea2020_Production_and_Marketing_of_African_Indigenous-Leafy_Vegetables.pdf?sequence=1

19. Bokelmann W, Huyskens-Keil S, Ferenczi Z, Stöber S. The Role of Indigenous Vegetables to Improve Food and Nutrition Security: Experiences From the Project HORTINLEA in Kenya (2014–2018). *Front Sustain Food Syst.* 2022;6(April):1–19.
20. Kiringia D. African Leafy Vegetables Pre-harvest and Post-harvest constraints and. Researchgate [Internet]. 2019;(May 2018). Available from: <https://www.researchgate.net/publication/325323882>
21. Mwanri AW, Mamboleo TF, Msuya JM, Gowele VF. Oxalate, phytate and nitrate content in African nightshade, spider plant and amaranths at different stages of maturity. *African J Food Sci.* 2018;12(11):316–22.
22. Naseem A, Akhtar S, Ismail T, Qamar M, Sattar DES, Saeed W, et al. Effect of Growth Stages and Lactic Acid Fermentation on Anti-Nutrients and Nutritional Attributes of Spinach (*Spinacia oleracea*). *Microorganisms.* 2023;11(9):1–15.
23. Tiisekwa AB, Chove BE, Mongi R, Rybak C, Stuetz W. Carotenoids, tocopherols, minerals and phytate in fresh and carotenoids and tocopherol contents in sun-dried dark green leafy vegetable varieties from the Lindi region, Tanzania. *Technol Hortic.* 2024;4(1):0–0.
24. Sangija F, Martin H, Matemu A. Effect of lactic acid fermentation on the nutritional quality and consumer acceptability of African nightshade. *Food Sci Nutr.* 2022;10(9):3128–42.
25. Sangija F, Kazosi M, Martin M, Matemu A. TRENDS AND CONSTRAINTS IN THE UTILIZATION OF AFRICAN NIGHTSHADE (*Solanum nigrum* complex) IN TANZANIA: A CASE STUDY OF KILIMANJARO AND MOROGORO REGIONS. *African J Food, Agric Nutr Dev.* 2022;22(6):20623–45.
26. Gowele VF, Kinabo J, Jumbe T, Kirschmann C, Frank J, Stuetz W. Provitamin a carotenoids, tocopherols, ascorbic acid and minerals in indigenous leafy vegetables from Tanzania. *Foods.* 2019;8(1):7–10.
27. Kimura M, Rodriguez-Amaya DB. A scheme for obtaining standards and HPLC quantification of leafy vegetable carotenoids. *Food Chem.* 2002;78(3):389–98.
28. Khasanah NU, Riyanto R. Analysis of Ascorbic Acid Content in Pineapples and Melons Using the Uv-Vis Spectrophotometry Method. *Indones J Appl Sci Eng.* 2023;1(1):1–6.
29. Jachimowicz K, Winiarska-mieczan A, Baranowska-wójcik E, Bąkowski M. Pasta as a source of minerals in the diets of poles; effect of culinary processing of pasta on the content of minerals. *Foods.* 2021;10(9):1–18.
30. Sánchez-Rangel JC, Benavides J, Heredia JB, Cisneros-Zevallos L, Jacobo-Velázquez DA. The Folin-Ciocalteu assay revisited: Improvement of its specificity for total phenolic content determination. *Anal Methods.* 2013;5(21):5990–9.
31. Mohankumar JB, Uthira L, Su M. Total phenolic content of organic and conventional green leafy vegetables . 2018;2(1).
32. Salgado N, Silva MA, Figueira ME, Costa HS, Albuquerque TG. Oxalate in Foods: Extraction Conditions, Analytical Methods, Occurrence, and Health Implications. *Foods.* 2023;12(17).
33. Borshchevskaya LN, Gordeeva TL, Kalinina AN, Sineokii SP. Spectrophotometric determination of lactic acid. *J Anal Chem.* 2016;71(8):755–8.
34. Degrain A, Manhivi V, Remize F, Garcia C, Sivakumar D. Effect of lactic acid fermentation on color, phenolic compounds and antioxidant activity in african nightshade. *Microorganisms.* 2020;8(9):1–12.
35. Traoré K, Parkouda C, Savadogo A, Ba/Hama F, Kamga R, Traoré Y. Effect of processing methods on the nutritional content of three traditional vegetables leaves: Amaranth, black nightshade and jute mallow. *Food Sci Nutr.* 2017;5(6):1139–44.

36. Fombang EN, Mbofung CMFMF. The Effect of Steam Blanching and Drying Method on Nutrients, Phytochemicals and Antioxidant Activity of Moringa (*Moringa oleifera* L.) Leaves. *Am J Food Sci Technol* [Internet]. 2017;5(2):53–60. Available from: <http://pubs.sciepub.com/ajfst/5/2/4>
37. Martins T, Barros AN, Rosa E, Antunes L. Enhancing Health Benefits through Chlorophylls and Chlorophyll-Rich Agro-Food: A Comprehensive Review. *Molecules*. 2023;28(14).
38. Mieszczakowska-Frać M, Celejewska K, Płocharski W. Impact of innovative technologies on the content of vitamin C and its bioavailability from processed fruit and vegetable products. *Antioxidants*. 2021;10(1):1–19.
39. Oluoch MO, Habwe F. Food preparation and processing methods on nutrient retention and accessibility in selected indigenous vegetables from East Africa Promoting Sustainable Agriculture in Borno State View project Vegetable Breeding and Seed Systems for Poverty Reduction in A. 2012;(January). Available from: <https://www.researchgate.net/publication/316990800>
40. Zong C, Wu Q, Wu A, Chen S, Dong D, Zhao J, et al. Exploring the diversity mechanism of fatty acids and the loss mechanisms of polyunsaturated fatty acids and fat-soluble vitamins in alfalfa silage using different additives. *Anim Feed Sci Technol* [Internet]. 2021;280(January):115044. Available from: <https://doi.org/10.1016/j.anifeedsci.2021.115044>
41. Piyarach K, Nipawan K, Chadapon C, Daluwan S, Kunjana R. Effect of Drying on β -Carotene, α Carotene, Lutein and Zeaxanthin Content in Vegetables and Its Application for Vegetable Seasoning. *E3S Web Conf*. 2020;141:1–6.
42. Ahmed A, Almas S, Kissaka K, Suleiman R. Effect of sun-dry on nutritional and sensory acceptability of wilted African leafy vegetables: a case study of Morogoro Region, Tanzania. *Front Sustain Food Syst*. 2023;7(June):1–9.
43. Kandonga A. Effect of blanching and drying on micronutrients and anti-nutritional factors in false sesame and common bean leaves. Master Thesis. Nelson Mandela African Institution of Science and Technology. 2019;
44. F.C O, N.A U, G,H A, L.O O. Inductively Coupled Plasma Optical Electro Spectroscopic Characterisation of Some Indigenous Nigerian Leafy Vegetables as Prospective Mineral Sources In Human Diets. *Niger J Pure Appl Sci*. 2024;37(1):4772–9.
45. Stevens GA, Beal T, Mbuya MNN, Luo H, Neufeld LM, Addo OY, et al. Micronutrient deficiencies among preschool-aged children and women of reproductive age worldwide: a pooled analysis of individual-level data from population-representative surveys. *Lancet Glob Heal*. 2022;10(11):e1590–9.
46. Alassane CT, Abdoulaye T, Fabrice ZA, Ahmont K, Claude L. Effect of Three Drying Modes on Nutritive and Antinutritive Properties of Leafy Vegetables Consumed in Northern Côte d'Ivoire. 2022;1873(4):102–11.
47. Bighaghire R, Okidi L, Muggaga C, Ongeng D. Traditional vegetable preservation technologies practiced in Acholi subregion of Uganda improves mineral bioavailability but impacts negatively on the contribution of vegetables to household needs for micronutrients. *Food Sci Nutr*. 2021;9(2):589–604.
48. Ukom A, Albert M, Ojmelukwe P, Offia-Olua B, Nwanagba L. Impact of cooking methods on the chemical and antioxidant composition of some indigenous vegetables used in different food dishes in Southeast Nigeria. *J Ethn Foods* [Internet]. 2023;10(1). Available from: <https://doi.org/10.1186/s42779-023-00170-x>
49. Natesh N H, SK A, L A. An overview of nutritional and anti nutritional factors in green leafy vegetables. *Hortic Int Journal* . 2017;1(2):58–65.
50. Schoofs H, Schmit J, Rink L. Zinc Toxicity: Understanding the Limits. *Molecules*. 2024;29(13).

51. Duguma HT. Wild Edible Plant Nutritional Contribution and Consumer Perception in Ethiopia. *Int J Food Sci*. 2020;2020.
52. Tanor EB, Ntlatlapa T, Magama S. Assessment of the Nutritional Value of Selected Wild Leafy Vegetables Growing in the Roma Valley, Lesotho. *Eur J Nutr Food Saf*. 2020;12(10):32–45.
53. Farag MA, Abib B, Qin Z, Ze X, Ali SE. Dietary macrominerals: Updated review of their role and orchestration in human nutrition throughout the life cycle with sex differences. *Curr Res Food Sci* [Internet]. 2023;6(January):100450. Available from: <https://doi.org/10.1016/j.crfs.2023.100450>
54. Verardo V, Gómez-Caravaca AM, Tabanelli G. Bioactive components in fermented foods and food by-products. *Foods*. 2020;9(2):4–6.
55. Leonard W, Zhang P, Ying D, Adhikari B, Fang Z. Fermentation transforms the phenolic profiles and bioactivities of plant-based foods. *Biotechnol Adv* [Internet]. 2021;49(October 2020):107763. Available from: <https://doi.org/10.1016/j.biotechadv.2021.107763>
56. Rollán GC, Gerez CL, Leblanc JG. Lactic fermentation as a strategy to improve the nutritional and functional values of pseudocereals. *Front Nutr*. 2019;6(July).
57. Aderibigbe OR, Ezekiel OO, Owolade SO, Korese JK, Sturm B, Hensel O. Exploring the potentials of underutilized grain amaranth (*Amaranthus* spp.) along the value chain for food and nutrition security: A review. *Crit Rev Food Sci Nutr* [Internet]. 2022;62(3):656–69. Available from: <https://doi.org/10.1080/10408398.2020.1825323>
58. Loukou AL, Anvoh KYB, Kouakou KH, Brou K. Nutritional composition and bioavailability prediction calcium, iron, zinc and magnesium in *Justicia galeopsis* leaves in Côte d'Ivoire. *Int J Biol Chem Sci*. 2019;12(6):2615.