

Original Research Article

EFFECT OF DIFFERENT PROCESSING METHODS ON THE ANTI-NUTRITIVE COMPONENTS OF BLACK JACK, BITTER LETTUCE AND CASSAVA LEAVES.

ABSTRACT

Indigenous vegetables are widely utilized due to their high nutritional profiles, easy availability, and accessibility in rural areas. The consumption of indigenous vegetables is limited by their high perishability nature which causes high post-harvest losses. Also, the presence of anti-nutritive components which tend to affect the bioavailability of micronutrients, poses a challenge in availing their full nutritional potential to consumers. This study aimed to determine the effect of boiling, fermentation, solar and sun drying (with and without blanching) on the anti-nutritive components of the leaves of Cassava (*Manhot esculenta Crantz*), Black jack (*Bidens pilosa*) and Bitter lettuce (*Launaeacornuta*). The raw, dried, boiled and fermented vegetables were evaluated directly after being subjected to their corresponding treatments. Total oxalate and hydrogen cyanide (HCN) were analyzed using titrimetric methods and phytate was determined using the UV spectrophotometric method. From the results, oxalic acid ranged from 180.2 ± 1.56 to 26.3 ± 2.34 mg/100g, phytate ranged from 18.8 ± 1.07 to 0.7 ± 1.11 mg/100g and hydrogen cyanide ranged from 357.1 ± 10.56 to 4.1 ± 2.49 mg /100g across the treatments. All processing methods significantly ($P < 0.05$) reduced hydrogen cyanide and oxalic acid content in all vegetable samples. Phytate was significantly reduced ($P < 0.05$) after boiling and fermentation while, all drying treatments resulted in non-significant changes in phytate content. In consequence, all processing methods studied proved to be effective in the reduction of hydrogen cyanide and oxalic acid. Boiling and fermentation showed their effectiveness in the reduction of phytate. Therefore, it is recommended that these plants should be eaten boiled or fermented to increase the bioavailability of micronutrients.

Keywords: Indigenous vegetables, boiling, anti-nutritive components, blanching, fermentation, solar drying, sun drying

INTRODUCTION

Micronutrient deficiency happens when the body is not getting enough of the vital vitamins and minerals it needs to function properly. Common micronutrient deficiencies include a lack of iron, vitamin A, iodine, calcium, and zinc, which can result in several health problems that impact development, immunity, and general human well-being (Conti *et al.*, 2021). The deficiency of micronutrients affects almost half of children under five worldwide and pregnant women, making it a serious health concern (Stevens *et al.*, 2022). Low or middle-income countries bear the disproportionate burden of micronutrient deficiencies. Tanzania suffers a serious problem with

micronutrient deficiencies, which cost the nation more than US\$ 518 million a year, or 2.65% of its GDP in health care and nutritional interventions (Noor, 2019).

According to the Ministry of Health *et al.* (2022), the prevalence of anemia is 59% in 6 to 59 months' children in Tanzania, where 26% have mild anemia, 31% have moderate anemia and 2% have severe anemia, also, the prevalence of anemia is 42% in women aged between 15 to 49 years. Iron deficiency is highest in the Eastern zone of Tanzania, at 70% (Ministry of Health *et al.*, 2022). According to Nicholaus *et al.* (2020), 23.1% of adolescents in boarding high schools in Kilimanjaro Tanzania are anemic. This was associated with diets with inadequate key micronutrients such as iron, zinc, and calcium. Also, the study done by John *et al.* (2023) found a 37.8% prevalence of iron deficiency among pregnant women in the Mbeya Region. This shows that micronutrient deficiency is still a public health concern in Tanzania.

Micronutrient deficiency may result from inadequate consumption of micronutrient-dense foods or problems associated with the absorption of vital minerals and vitamins by the body (Kiani *et al.*, 2022). Poor dietary practices, food insecurity, and poverty are some of the reasons that can lead to an inadequate intake of micronutrient-dense foods, whereas infections, chronic inflammation, and other medical problems that may influence the digestive system can cause decreased absorption and availability of minerals (Lopes *et al.*, 2023; Angeles-Agdeppa *et al.*, 2021). The former cause of micronutrient deficiency may also be caused by the consumption of micronutrient-dense foods that are poorly processed. Foods that have been inappropriately processed, primarily vegetables, may preserve the concentration of anti-nutritive ingredients that bind to minerals and make them difficult for the body to absorb, also, they may lose minerals and phytochemicals.

Micronutrient deficiency can be addressed by the consumption of micronutrient-dense foods. One group of the micronutrient-dense foods cheaply available is indigenous vegetables. Indigenous vegetables are plant species that are native to a specific region or that have been introduced to that area long enough to have evolved through natural processes (Chadha, 2003). In rural areas, indigenous vegetables have a bigger impact on improving food security and nutrition (Bokelmann *et al.*, 2022). Indigenous vegetables are a potent source of nutrients and play an important role in lowering micronutrient deficiencies (Oboh *et al.*, 2017). They contribute to a well-balanced diet, providing important minerals, vitamins and antioxidants. Through the inclusion of a range of indigenous vegetables in their diet, people can increase their consumption of important micronutrients such as magnesium, potassium, zinc, iron, copper, folate (vitamin B9), vitamin A, vitamin C, vitamin E and vitamin K (Sarkar *et al.*, 2023). These support overall health and aid in treating micronutrient deficiencies, particularly in regions where micronutrient deficiencies are prevalent.

The high perishability of indigenous vegetables, however, can limit their availability and intake. Indigenous vegetables have a short shelf life and begin to lose their freshness as soon as they are harvested (Smith and Eyzaguirre, 2007). According to Gogo *et al.*, (2018), vegetable losses during postharvest stages might reach 50%. Vegetables lose their wholesomeness through physical damage, inadequate processing and their high perishability nature due to moisture content. Vegetable losses lead to the loss of nutrients as well, for instance, Gogo *et al.*, (2017) found that along the African nightshade supply chain in Kenya, a loss of micronutrient and macronutrient range from 3.2% -29.4% and for chlorophyll and carotenoid range from 70.9% - 90.9% and 70.4% - 91.9% respectively. This

implies that the nutritional value of vegetables is also reduced in addition to the financial losses due to poor postharvest handling and processing.

Furthermore, indigenous vegetables contain anti-nutritive components such as phytate, oxalates, tannins, hydrocyanic acid, nitrates, saponins and protease inhibitors (Katosh, 2022). These anti-nutritive components frequently combine minerals such as iron, calcium and zinc which as a result, decreases their absorption into the body following digestion. This in turn leads to micronutrient deficiencies (Samtiya *et al.*, 2020). When taken in large quantities, anti-nutritive components have negative health impacts such as decreased nutrition intake, absorption and utilization, at low concentrations, however, they have various health benefits (Ali *et al.*, 2022; Dey *et al.*, 2022).

Fortunately, using appropriate processing techniques like fermentation, boiling and drying may assist in solving issues with anti-nutritive components and post-harvest losses in vegetables. The concentration of anti-nutritive components can be reduced, the shelf life of vegetables may be increased and the optimal intake of important compounds can be guaranteed with the use of appropriate processing techniques (Samtiya *et al.*, 2020). Also, appropriate processing can help ensure that these vegetables preserve their valuable ingredients until consumption because inadequate postharvest handling can cause loss of minerals and retain anti-nutritive components which can negatively impact the nutritional value of the vegetables (Sivakumar *et al.*, 2020).

Therefore, this study aimed to determine the effect of boiling, sun drying and solar drying (with or without blanching), and fermentation on three vegetables (bitter lettuce, black jack and cassava leaves) that are commonly consumed in Mkuranga District, in the coastal region on the eastern part of Tanzania on anti-nutritive components (phytate, hydrogen cyanide and oxalic acid). This study will help to give an insight towards zero hunger and good health and well-being. The results obtained will further inform the respective community on the choice of appropriate processing methods that may reduce anti-nutritive components.

MATERIALS AND METHODS

Description of the study area

This study was conducted in Mkuranga District, which is located in the Pwani Region in the eastern part of Tanzania. Mkuranga District has a total surface area of 2,827 of which 1985 km² is on the mainland and 447 km² is covered by the Mafia channel. Mkuranga is located on the latitude of 7.1219° South and longitude of 39.2° East. It is bordered to the North by Dar es Salaam's Kigamboni, Temeke and Ilala Districts. To the East, it is flanked by the Mafia Channel, to the South by Kibiti District and to the West by Kisarawe District. The area of the mainland that is covered by forest natural reserves is 52 km². 1934 km² of land is suitable for cultivation, whereas 1662.3 km² of the arable land is under cultivation. The Mkuranga District has 25 Wards and among those two (Mkamba and Kisegese) were purposely selected for sample collection.

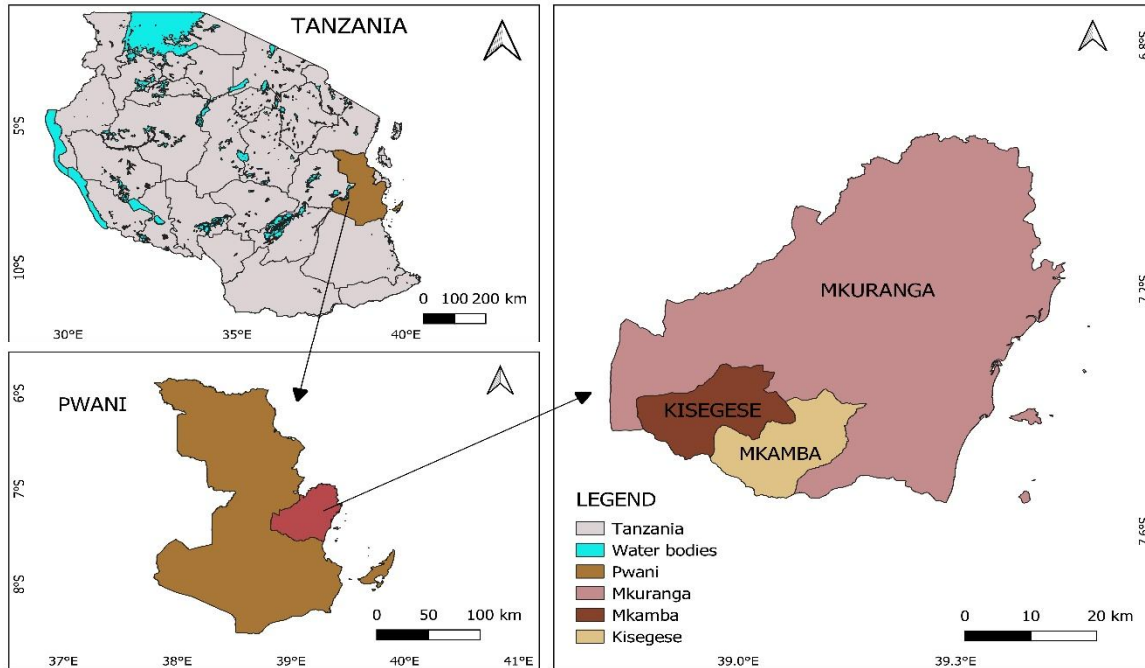


Figure 1: Map of Mkuranga showing the study area, (Source; Author)

Sample collection

Three indigenous vegetables (black jack leaves, cassava leaves and bitter lettuce leaves) that are commonly consumed in Mkuranga District were used in this study. Vegetable samples were purposively selected due to their availability and accessibility during the study period. A total of 4 kg of each vegetable sample was collected from two production areas in each ward (Mkamba and Kisegeze) with the help of the natives. Vegetable samples were harvested, cleaned to remove dust, and put in sealed plastic bags. The samples were then placed in a cool box and transported to the laboratory at Sokoine University of Agriculture, where they were stored at 4°C for one day before further processing and laboratory analysis.



Figure 2: Pictures of the three vegetables taken from the selected farms (A: Bitter lettuce, B: Cassava leaves, C: Black jack)

Sample preparation and treatment

Before processing, vegetable samples were sorted, washed with distilled water and left to drain water. The portions of vegetables were subjected to boiling, fermentation, solar drying with blanching, sun drying with blanching, solar drying without blanching and sun drying without blanching. The processing methods were carried out as follows:

Boiling

Five hundred grams (500 g) of vegetables (black jack, cassava leaves and bitter lettuce) were cut into 4-8 mm pieces and put in 1 liter of distilled water and boiled separately to 100°C for 15 minutes before the analysis of anti-nutritive components (da Silva Santos *et al.*, 2020; Ekpo and Baridia, 2020).

Fermentation

The fermentation process was carried out according to Vatansever *et al.*, (2017) with some modification, five hundred (500) g of raw vegetables were shredded into 2 cm thick pieces and placed into jars. The jars with the vegetables were filled with 15% salt (NaCl). The amount of salt was sufficient to cover the sliced vegetables completely. The jars were then covered with screw caps and kept at room temperature for 14 days. The vegetables were subjected to pH measurement after every one week. The fermented vegetables were analyzed for anti-nutritive components.

Sun drying and solar drying of vegetables

Approximately one kilogram (1 kg) of each vegetable sample was divided into two equal portions and one portion was subjected to blanching at 95°C for 5 minutes and immediately cooled under running distilled water to avoid further cooking and the other portion was left without blanching. The blanched and non-blanched portions of each vegetable were left to dry under the sun for two consecutive days before analyzing anti-nutritive components, after measuring the moisture content. The same procedures were conducted for solar-dried samples, where the two portions were subjected to the walk-in/greenhouse-solar driers of which temperature varied for 5-7 hours in a single day. In both cases, the samples were constantly turned to avert fungal growth (Mongi and Ngoma, 2022; Mepbaet *et al.*, 2007).

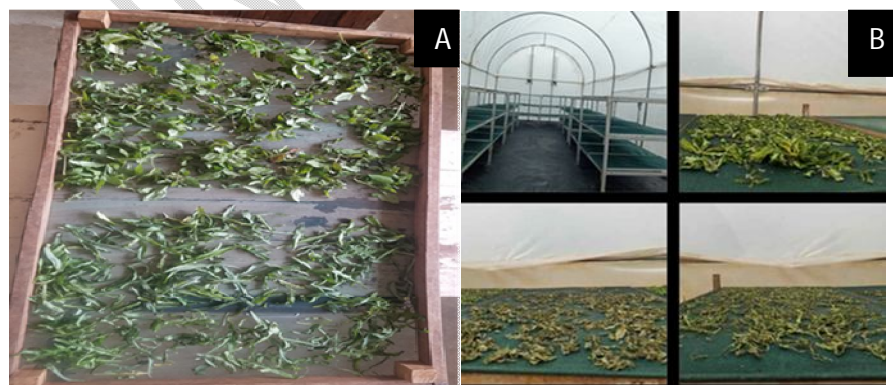


Figure 3: Pictures A and B show Sun drying and solar drying of vegetables respectively

Determination of phytate

Phytate was determined according to Okikiet *al.*, (2015) by adding one gram of the sample to 10ml of 3% of Trichloroacetic acid (TCA). In a solution, 0.1% of Ammonium Ferric sulfate was added to precipitate phytate as ferric phytate. The precipitate was converted to ferric hydroxide and sodium phytate by adding 10ml of 0.5 sodium hydroxide. The residue was boiled and dissolved with the addition of dilute nitric acid. The absorbance of the sample was taken at 519nm and the phytic acid content of the samples was calculated using the standard calibration curve.

Determination of oxalic acid

Oxalate ions were determined by using the AOAC (1998) Official Method, where one gram of sample was measured and placed in a 300ml conical flask, to each flask 250ml of 1M H₂SO₄ (sulphuric acid) was added, the burette was filled with 0.1M KMnO₄ (potassium permanganate) solution and the initial volume was noted. Oxalate mixtures in the flasks were boiled up to 80°C to 90°C and were kept above 70°C throughout the process of titration. The mixture of vegetable samples mixed with sulphuric acid was titrated against potassium permanganate solution while stirring frequently up to the endpoint where an extremely pale pink color persisted in the solution for 15 seconds. Oxalate content was calculated by using the formula:

$$\text{Oxalate in mg/kg} = \frac{(A - B) \times M \times \text{Mwt} \times 100}{\text{Sw} \times 1000 \times A}$$

where;

A = Amount (g) of KMnO₄ used for sample

B = Amount (g) of KMnO₄ used for blank

M = Molarity of KMnO₄ (0.1)

Mwt = Mass (g) of KMnO₄ equivalent to 0.1M

Sw = Amount of sample taken (g) for analyses

Determination of hydrogen cyanide

This was done according to AOAC (2000) Official Method 915.03, in which ten-gram grams of the sample were placed in a Kjeldahl flask, and 200 ml of distilled water was added and let to stand for 2-4 h (autolysis had to be conducted with apparatus completely connected for distillation), 150 ml of distillate was collected in sodium hydroxide solution (0.025 M NaOH). To 100ml distillate (it is preferable to dilute 250 ml and titrate 100 ml aliquot), 8 ml 6 N NH₄OH and 2ml 5% KI solution were added and titrated with 0.02 N AgNO₃ until the end point of faint but permanent turbidity and may easy to be recognized especially against black background observed.

1ml 0.02N AgNO₃=1.08 HCN (equivalent to 2 CN)

Data analysis

All data on anti-nutritive components were analyzed using the Statistical Package for Social Sciences (SPSS) statistical software, version 25. One-way analysis of variance (ANOVA) test was performed to determine if there was a significant difference in assessed parameters among different processing methods. It was then followed by a post hoc test, Turkey (HSD) with significant differences being determined at a 5% level of significance ($P < 0.05$) to identify which processing methods differ from each other. All results were expressed as mean \pm standard variation of triplicate values.

RESULTS AND DISCUSSION

Anti-nutritive components of the selected vegetables

The results of anti-nutritive components of vegetables before and after the following treatments: boiling, fermentation, direct solar drying, direct sun drying, sun drying with pre-treatment blanching, and solar drying with pre-treatment blanching in the three indigenous vegetables are presented in table 1.

Table 1: Anti-nutritive components (mg/100g) of raw and processed cassava leaves, black jack leaves and bitter lettuce leaves

Vegetables	Processing methods	Oxalic acid (mg/100g)	Phytate (mg/100g)	Hydrogen cyanide (mg/100g)
Cassava leaves	Raw	136.5 \pm 0.06 ^d	18.8 \pm 1.07 ^b	357.1 \pm 10.56 ^f
	Boiling	61.3 \pm 3.86 ^{ab}	0.7 \pm 1.11 ^a	146.3 \pm 0.06 ^d
	Fermentation	89.5 \pm 4.31 ^c	4.5 \pm 1.97 ^a	215.7 \pm 0.05 ^e
	Blanching-solar drying	77.4 \pm 4.43 ^{bc}	17.8 \pm 1.18 ^b	40.4 \pm 8.27 ^c
	Blanching-sun drying	64 \pm 5.71 ^{ab}	14.6 \pm 0.95 ^b	4.1 \pm 2.49 ^a
	Direct solar drying	51.1 \pm 3.69 ^a	17.3 \pm 2.38 ^b	12.15 \pm 0.82 ^{ab}
	Direct sun drying	87.5 \pm 4.32 ^c	14.3 \pm 0.58 ^b	26.4 \pm 4.21 ^{bc}
Bitter lettuce	Raw	163.0 \pm 0.08 ^d	16.8 \pm 1.80 ^b	116.4 \pm 0.01 ^f
	Boiling	103.9 \pm 5.41 ^c	2.65 \pm 0.27 ^a	38.7 \pm 0.27 ^d
	Fermentation	95.7 \pm 6.45 ^c	3.7 \pm 0.67 ^a	77.2 \pm 0.84 ^e
	Blanching-solar drying	67.7 \pm 3.78 ^b	15.4 \pm 3.15 ^b	11.6 \pm 0.02 ^{ab}
	Blanching-sun drying	46.5 \pm 1.25 ^a	13.6 \pm 0.93 ^b	19.3 \pm 2.56 ^{bc}
	Direct solar drying	46.9 \pm 4.52 ^a	12.9 \pm 2.21 ^b	28.5 \pm 7.39 ^c
	Direct sun drying	49.8 \pm 1.47 ^a	12.5 \pm 2.46 ^b	9.9 \pm 2.51 ^a
Black jack	Raw	180.2 \pm 1.56 ^e	16.8 \pm 1.23 ^b	84.9 \pm 0.41 ^d
	Boiling	26.3 \pm 2.34 ^a	3.9 \pm 0.47 ^a	42.5 \pm 0.34 ^c
	Fermentation	102.6 \pm 0.02 ^d	2.1 \pm 0.38 ^a	42.4 \pm 0.36 ^c
	Blanching-solar drying	50.2 \pm 4.17 ^b	15.7 \pm 1.38 ^b	11.5 \pm 0.07 ^a
	Blanching-sun drying	31 \pm 4.53 ^a	12.8 \pm 2.33 ^b	8.8 \pm 4.13 ^{bc}

Direct solar drying	64.6 ± 3.74 ^c	16.8 ± 1.73 ^b	14.4 ± 4.15 ^c
Direct sun drying	71.3 ± 1.97 ^c	14.4 ± 2.79 ^b	22.2 ± 1.66 ^b

Values are expressed as mean ± SD (n = 3). Mean values with different superscript letters along the columns are significantly different at P < 0.05

Oxalic acid

The results of oxalic acid ranged from 51.1 ± 3.69 to 136.5 ± 0.06 mg/100g in cassava leaves, 46.5 ± 1.25 to 163.0 ± 0.08 mg/100g in bitter lettuce and 31 ± 4.53 to 180.2 ± 1.56 mg/100g in black jack. The highest content of oxalic acid was observed in raw black jack followed by raw bitter lettuce and lastly raw cassava leaves with 180.2 ± 1.56, 163.0 ± 0.08 and 136.5 ± 0.06 mg/100g respectively. There was a significant reduction (P < 0.05) in oxalic acid in all processed samples in all three vegetables. In cassava leaves, the oxalic content was observed in the following trend: Raw > Fermentation > Direct sun drying > Blanching-solar drying > Blanching-sun drying > Boiling > Direct solar drying. In bitter lettuce the results of oxalic acid contents after different processing operations were observed in the following order: Raw > Boiling > Fermentation > Blanching-solar drying > Direct sun drying > Direct solar drying > Blanching-sun drying. Furthermore, the oxalic acid contents in black jack were observed in the following order: Raw > Fermentation > Direct sun drying > Direct solar drying > Blanching-solar drying > Blanching-sun drying > Boiling. The trend in the reduction of oxalic acid contents after different processing operations varied among vegetable samples and this could be attributed to the differences in the morphology and structure of the vegetable samples used.

Oxalic acid forms oxalates when it is combined with its salts such as calcium. Many green leafy vegetables contain oxalic acid in their cell sap as soluble salts of potassium and sodium and insoluble salts of calcium, magnesium and iron, or as a combination of these two types (Noonan and Savage, 1999). Soluble oxalates combined with dietary calcium and other minerals produce a strong chelate, the complex becomes indigestible and difficult for the body to absorb and assimilate, thus affecting its bioavailability (Radek and Savage, 2008).

The total oxalate content reported in this study for cassava leaves and black jack was below the results reported by Nasaka *et al.* (2022). The results of oxalate for raw bitter lettuce were higher compared to the one reported by Chacha *et al.* (2020). The inconsistency in oxalate content could be attributed to differences in vegetable species, varieties and the growing conditions of the vegetables.

From the findings, oxalate content was less than the compounds' fatal dose (200-500mg/100g), this implies that the vegetable varieties were safe for consumption (Bushway *et al.* 1998).

Because soluble oxalate leached into the cooking water during boiling, the concentration of oxalic acid was dramatically reduced ($P < 0.05$) in the current study (Huynh *et al.*, 2022). Similarly, Kasimala *et al.*, 2018 observed a reduction in oxalate after 15 minutes, based on their investigation of the impact of boiling on oxalate in various vegetables. There have also been reports of oxalates being lost from parsley (28.33%) and spinach (40%) after 15 minutes of boiling (Mohamad *et al.*, 2021). Furthermore, higher temperatures and longer cooking times can break down vegetable tissues and release oxalate (Wang *et al.*, 2018). In their study, it was found that oxalic acid concentrations decreased dramatically after cooking for three minutes and at all treated temperatures. The present study confirms earlier research showing that boiling vegetables and other foods high in oxalate before consuming them can greatly lower the quantity of oxalic acid to safe levels and improve the minerals' bioavailability. Boiling causes, the loss of total oxalate, insoluble oxalates and soluble oxalates (Wanyo *et al.*, 2020). The decrease in oxalic acid in processed vegetables is linked to an increase in the vital minerals' bioavailability, which helps to avert micronutrient deficiencies (Ertop and Bektaş, 2018).

The results of this investigation showed that when compared to fresh vegetable samples, all drying methods considerably ($P < 0.05$) reduced the amount of oxalate in each sample. This might be the result of the intense heat during solar and open drying which breaks down the leaf cells and vaporizes oxalate (Gafar *et al.*, 2012). These results are in line with those of Nasaka *et al.*, (2022), who discovered that sun drying reduces the amount of oxalate. Additionally, they align with the findings published by Kandonga (2019), who observed a drop in oxalic acid following the drying of common bean leaves. Furthermore, Kirakou *et al.*, (2017) demonstrated that cowpea leaves' oxalate can be decreased by solar drying. This demonstrates that drying vegetables is a useful method for lowering the content of ant-nutritive elements and increasing the bioavailability of micronutrients.

The outcome of blanching vegetable samples in advance of solar and sun drying was only significant in bitter lettuce and black jack and varied with vegetable samples. In black jack blanched-dried samples had lower oxalate content than unblanched samples, while blanched-dried bitter lettuce had higher oxalate content than unblanched samples. This is not in line with findings from other writers who discovered that blanching speeds up the removal or decrease of oxalate in the cowpea leaves

(Kirakou et al., 2017; Kandonga, 2019; Virginia *et al.*, 2012). The variations in the vegetable samples as well as the morphological structures of the samples used in the investigation could be the cause of the discrepancy in the results.

Additionally, several studies have found that sun and solar drying, with or without blanching, increases the oxalate level of vegetables. This suggests that the drying procedure has varying impacts on oxalate content. For instance, a study on jute mallow reported an increase in oxalate content after sun drying than other processing methods and suggested it was due to the concentration of existing compounds accelerated by evaporation and the breakdown of certain compounds that contributed to the rise in oxalic acid levels (Igwiolo *et al.*, (2018); Musa and Ogbadoyi, 2012). Nevertheless, Musa and Ogbadoyi, (2012) reported that the extended drying time under the sun can result in the breakdown of organic compounds possibly resulting in higher oxalate concentration in the sun and solar-dried samples. Also, Wanyo *et al.* (2018) and Kondareddy *et al.* (2020) reported a significant increase in oxalate after the drying operation while the study done by Alassane *et al.* (2022) reported a non-significant difference in the levels of oxalate between raw and dried leafy vegetables consumed in Northern cote d'Ivoire. This implies that the effect of drying on oxalate content varies among vegetables.

In the case of fermentation, the oxalate content decreased in both vegetable samples. These results are in line with earlier research that showed a significant ($P < 0.05$) drop in oxalate levels following fermentation. According to a study by Ijeoma and Adeyemi (2020), the oxalate content of fermented fluted pumpkin leaves decreased. The action of microorganisms that convert oxalic acid to formic acid and carbon dioxide during the fermentation process is responsible for the decrease in oxalic acid after fermentation (Li *et al.*, 2022). The amount of oxalate breakdown is greatly influenced by the presence of bacteria and fermentation conditions such as salt content and incubation time (Srihardvastuti *et al.*, 2021). This demonstrates how fermentation harms the amount of oxalate present in vegetable samples. The effect of fermentation on oxalate varies with vegetables as some writers have noted non-significant changes in oxalate after the fermentation of local vegetables in Kenya (John *et al.*, 2014). The variability is due to differences in the vegetable matrix and the specific microbial communities involved in the fermentation process. The decrease in oxalate content after fermentation is of great importance since the presence of oxalate in low concentration tends to spare the available minerals in vegetables that will be required by the body.\

Hydrogen Cyanide

The results of hydrogen cyanide (HCN) ranged from 4.1 ± 2.49 to 357.1 ± 10.56 mg/100g in cassava leaves, 9.9 ± 2.51 to 116.4 ± 0.01 mg/100g in bitter lettuce and 8.8 ± 4.13 to 84.9 ± 0.41 mg/100g in black jack. Among the three raw vegetable samples, the highest HCN content was observed in cassava leaves, followed by bitter lettuce and black jack with 357.1 ± 10.56 , 116.4 ± 0.01 and 84.9 ± 0.41 mg/100g respectively. In cassava leaves, there was a significant reduction ($P < 0.05$) in hydrogen cyanide in all processing methods. The highest reduction in hydrogen cyanide was observed in blanched-sun-dried cassava leaves followed by direct solar drying, direct sun drying, blanching-solar drying, boiling and lastly fermentation. In bitter lettuce, there was a significant reduction ($P < 0.05$) in hydrogen cyanide in all processed samples. Direct sun-dried samples had the lowest content of hydrogen cyanide. The results of hydrogen cyanide were observed in the following order: raw > fermentation > boiling > direct solar drying > blanching-sun drying > blanching-solar drying > direct sun drying. In black jack, there was a significant reduction of hydrogen cyanide in all processing methods. The highest reduction in hydrogen cyanide was observed in blanched-sun-dried black jack followed by blanched-solar dried samples, direct solar-dried samples, direct sun-dried samples, fermented samples and finally boiled samples. The effect of the pretreatment blanching showed a significant difference ($P < 0.05$) in hydrogen cyanide. The trend in the content of hydrogen cyanide was inconsistent among the three vegetable samples and this observation could be attributed to the difference in sample morphology and structure.

Cyanide is a toxic substance that can be lethal to humans and it is naturally present in several plants. It is a secondary plant metabolite stored in cellular vacuoles of plant tissues and tends to release hydrogen cyanide to protect plants from microbial and insect attacks (Kassim *et al.*, 2015). The release of hydrogen cyanide during the hydrolysis of cyanogenic substance is toxic since it reacts with iron ions making it impossible to perform its normal functions in the body (Yu *et al.*, 2015).

This study found that hydrogen cyanide concentrations in raw cassava leaves and black jack leaves were high compared to the results reported by Maitha *et al.* (2022) and Essacket *et al.* (2018) respectively. The inconsistency in the concentration of hydrogen cyanide may be attributed to the differences in the varieties used in the study, plant maturity and the growing conditions. FAO/WHO, (2021) recommends the cyanide level in diet not to exceed 10 mgHCN/kg dry weight, the assessed

vegetables were not within the acceptable level for consumption and hence thorough processing before consumption is recommended to reduce its toxicity.

All processing methods reduced the content of hydrogen cyanide in all three vegetable samples, even though the final concentration was not within the safe levels. Boiling resulted in a significant decrease in hydrogen cyanide because, hydrogen cyanide is soluble in water and tends to leach in water and volatilize to lower levels (Bolarinwa *et al.*, 2018). The same effect of boiling in hydrogen cyanide was reported by Ojiambo *et al.* (2017) who did a study on the effect of boiling on hydrogen cyanide in sweet cassava leaves and found a reduction of hydrogen cyanide by 88.65% after 25 minutes. Perhaps boiling for above 15 minutes could reduce the cyanide content to safe levels for consumption. Also, a study done by Tambalo *et al.* (2023) found that boiling whole cassava leaves resulted in a 32.01% reduction in total hydrogen cyanide. In addition to that, cutting or slicing cyanogenic foods into small pieces tends to disrupt the structure of the plant, following cooking such as boiling water may reduce cyanide content (Samad *et al.*, 2018). This depicts that boiling is among the effective methods in reducing the hydrogen cyanide content in vegetables for human consumption.

The present study also found that hydrogen cyanide significantly decreased ($P < 0.05$) after the fermentation of all vegetable samples. This could be attributed to the bacterial-produced linamarase that activates the hydrolysis of cyanogenic glycosides to hydrogen cyanide (Ahaotu *et al.*, 2013). Most of the previous reports on fermentation are consistent with the findings of this study. Fermentation has been shown to significantly decrease the hydrogen cyanide content in various food products. Studies have demonstrated that the process of fermentation can lead to a substantial reduction in hydrogen cyanide content to safe levels. For example, in the case of cassava tubers, prolonged fermentation (5 to 6 days) was found to effect a tremendous reduction in hydrogen cyanide (Iwuoha *et al.*, 2013). Thus fermentation process can be regarded as an effective way to reduce cyanides in vegetables.

All drying techniques used in this study reduced hydrogen cyanide content in vegetable samples because of the volatility property of cyanide at an ambient temperature (Andama *et al.*, 2017). Also according to Oliveira *et al.* (2016), the combination of blanching as pretreatment before drying has an additional effect in the reduction of anti-nutritive components due to the double treatments the foods are subjected to. In this study, the blanching treatment showed a non-significant difference in

hydrogen cyanide content between the blanched and unblanched samples subjected to solar and sun drying, except for solar-dried bitter lettuce which proved to have an additional effect in reducing hydrogen cyanide content. This is in agreement with the report of Ayele *et al.* (2022) on dried cassava leaves after blanching. The reduction of hydrogen cyanide after different drying operations as per this study shows that drying is also an effective way of reducing anti-nutritive components in vegetables.

Phytate

The results of phytate ranged from 0.7 ± 1.11 to 18.8 ± 1.07 mg/100g in cassava leaves, 2.65 ± 0.27 to 16.8 ± 1.80 mg/100g in bitter lettuce and 3.9 ± 0.47 to 16.8 ± 1.73 mg/100g in black jack. Among the three raw vegetable samples, the highest phytate content was observed in cassava leaves, followed by bitter lettuce and lastly, black jack with 18.8 ± 1.07 , 16.8 ± 1.80 and 16.8 ± 1.23 mg/100g respectively. The effect of the pretreatment blanching showed a non-significant difference in phytate content when compared to the drying treatment without pretreatment blanching in all three vegetable samples. Boiling treatment was observed to be effective in reducing the content of phytate among all processing operations in cassava leaves and bitter lettuce. In all vegetable samples, there was a significant decrease ($P < 0.05$) in phytate after boiling and fermentation processes and there was a non-significant difference in phytate between raw samples and the sun solar dried samples with and without pretreatment blanching. The results of phytate in cassava leaves were observed in the following order: blanching-solar drying > direct solar drying > blanching-sun drying > direct sun drying > raw > fermentation > boiling. Moreover, the results of phytate in bitter lettuce were observed in the following order: raw > blanching-solar drying > blanching-sun drying > direct solar drying > direct sun drying > fermentation > boiling. Furthermore, the following trend was noted regarding the effect of different processing on phytate: direct solar drying > raw > blanching-solar drying > direct sun drying > blanching-sun drying > boiling > fermentation. The effects of pretreatment blanching showed a non-significant difference ($P < 0.05$) in phytate content when compared to the drying treatment with and without pretreatment blanching in all three vegetable samples. The trend of the effect of different processing methods on phytate content varied among the three vegetable samples. This observation may be attributed to the nature of the vegetables analyzed, the initial concentration of phytate in the analyzed vegetables and the morphological structure of the analyzed vegetable could explain the varied effects of processing methods among the three vegetables.

Phytate refers to phytic acid made up of an inositol ring with six phosphate ester groups and its associated salts. Phytate is found in some indigenous vegetables and has diverse effects on the nutritional values of divalent and trivalent metal cations when it exceeds 20 mg/100g – 25 mg/100g (Nicodemus and Magoha, 2019). Phytate hinders protein digestibility but also affects the bioavailability of minerals such as iron, zinc and calcium since it is a negatively charged structure and can bind with positively charged ions such as zinc, iron and calcium to form complexes that are essentially non-absorbable and reduce their bioavailability (Al-hasan *et al.*, 2016). The chelating property of phytate proves that phytate can cause mineral deficiencies in humans (Grases *et al.*, 2017).

In this study, the average total phytate content reported for raw cassava leaves was low compared to the one reported by Tefere *et al.*, (2022) and the phytate content of the raw black jack was higher than the one reported by Chatepa and Masamba, (2020). Moreover, bitter lettuce had a higher phytate level than the one reported by Chacha *et al.*, (2020). The difference in vegetable samples used and growing environments for each vegetable may all have contributed to the inconsistent phytate results in raw vegetable samples. The concentration of phytate in the assessed samples was low compared to the tolerable level of phytate in foods (25mg/100g) (Bushway, 1998). This implies that the vegetable samples were safe for consumption. The phytate content of boiled samples was found to have significantly decreased, this could be explained by phytate leaching into water and the development of insoluble complexes that reduce the amount of free phytate (Shimi and Hasnah, 2013). This observation aligns with research findings on grains and legumes reported by Lopez-Moreno *et al.*, (2020) and on other traditional vegetables consumed in Malawi as reported by Issa *et al.*, (2019). Furthermore, phytate content decreased significantly on heating dietary grains (Gupta *et al.*, 2015). The reduction in phytate due to boiling can boost the bioavailability of minerals like calcium and iron which are otherwise blocked by phytate (Sathe and Venkatachalam, 2001). These findings support previous studies that boiling lowers the phytate content of vegetables, which is important for enhancing their nutritional value.

Additionally, this study found that the fermentation procedure greatly lowered the amount of phytate in vegetables. This may be related to the enzymatic breakdown of phytate during fermentation caused by bacteria secreting phytase enzymes (Wedad *et al.*, 2008). According to the study on phytic acid in rice flour, phytase breaks the hexa form of phytic acid (IP6, Myo-inositol 1,2,3,4,5,6-hexakisphosphate) into lower forms during natural fermentation, which can result in a significant

reduction in phytic acid (Gupta *et al.*, 2015). This could account for the decrease in phytate that occurs during fermentation in vegetable samples. Similar effects of fermentation on phytate have been reported by Terefe and Nduko, (2022) and Etsuyankpa *et al.* (2015). Due to low Ph, the breakdown of phytate by phytase enzymes increases the bioavailability of minerals and other nutrients during fermentation (Jeyakumar and Lawrence, 2022). When phytate is reduced through boiling, it can increase the bioavailability of minerals like iron and calcium, which are otherwise hindered by phytate (Sathe and Venkatachalam, 2001). This is vital for improving the nutritional quality of vegetables.

Furthermore, following drying operations, the present study reports a non-significant difference in phytate concentration. The results contradict the earlier research that indicated a rise in phytate upon drying, this can be related to the concentration impact attributed to the water loss during drying processes (Alassane *et al.*, 2022). Oulai *et al.*, (2015) who researched the impact of sun drying on phytate in several green vegetables, likewise reported a rise in phytate following different drying processes. Also, a study on *Amaranthus Hybridus* and the leafy vegetables consumed in Northern Cote d'Ivoire reported an increase in phytate after drying (Alassane *et al.*, 2022: Okafor and Agbam, 2023).

Moreover, several earlier investigations have documented the drop in phytate following sun exposure and solar drying: (Ekpo and Baridia, 2020: Adegunwaet *al.* 2011: Nasakaet *al.*, 2022). According to Ademiluyiet *al.*, (2018), vegetables lose phytate due to leaching brought on by the vaporization that happens during sun drying.

The effect of blanching before drying operations showed a non-significant difference in phytate in vegetables. The inconsistent outcomes could be ascribed to variations in the vegetable samples utilized in the study as well as variations in their morphological composition. These results show that drying methods do not affect the concentration of phytate in vegetables, and vegetable types and the drying conditions influenced the obtained results.

CONCLUSION

This study demonstrated that boiling, fermentation, solar and sun drying (with and without blanching) have varied effects on the anti-nutritive components in the three indigenous vegetables. Through data analysis, it is obvious that processing methods can significantly decrease or have no effect on anti-

nutritive components in the selected vegetables. Boiling and fermentation treatment was found to be efficient in the reduction of phytate, oxalic acid and hydrogen cyanide, this has a positive impact on the bioavailability of micronutrients in the selected vegetables. On the other hand, solar and sun drying of the selected vegetables have been found to decrease oxalic acid and hydrogen cyanide and have no effect on phytate. Blanching of vegetables before sun or solar drying has shown inconsistent effects among vegetables, but still, it is an important pretreatment as it increases the rate of drying and inactivates polyphenol oxidase enzymes.

Conclusively, this research highlights the effects of different commonly used processing methods on indigenous vegetables in local communities and hence it gives insights into the prevention of micronutrient loss as some of the processing methods assessed have shown how they can effectively reduce the concentration of anti-nutritive components which means enhancing micronutrients availability. Understanding these effects is crucial for optimizing food preparation techniques to preserve the nutritional value of indigenous vegetables.

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