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Effect of lactic acid bacteria fermentation on nutrients and anti-nutrients of African black nightshade and African spider plant

Marie Lys Irakoze[a], Eddy Elkana Owaga[a], Eliud Nalianya Wafula[b,*]

[a] Institute of Food Bioresources Technology, Dedan Kimathi University of Technology, Private Bag, 10143, Dedan Kimathi, Nyeri, Kenya
[b] Department of Public Health, Bomet University, College, P.O. Box 701-20400, Bomet, Kenya

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ABSTRACT

African black nightshade is a leafy vegetable consumed in many parts of Africa. African spider plant is also a very important indigenous leafy vegetable consumed all around Africa. Both the African black nightshade and African spider plant are popular despite their tedious preparation techniques which involve boiling and discarding the first water or a lengthy boiling duration to remove bitterness. However, this preparation technique reduces heat-labile and water-soluble nutrients. Fermentation can be a better alternative processing technique since it has been observed that fermented products have better nutritional content and increased safety. However, there is still limited literature on the effect of fermentation on the nutrients and anti-nutrients content of these vegetables. This study aimed to ferment African black nightshade and African spider plant using *Lactobacillus fermentum* and *Lactococcus lactis* cultures and determined the effect of the fermentation on their nutrients and anti-nutrients content. Both vegetables were submerged in a 3% sugar and 3% salt brine solution. Total ash, crude proteins and crude fibres significantly increased in starter culture inoculated vegetables. However, both β-carotene and ascorbic acid were slightly reduced in all fermented batches. Anti-nutrients were significantly reduced in all fermented batches. In conclusion, lactic acid fermentation of these vegetables demonstrated the ability to maintain or increase nutrients while reducing of anti-nutrients.

Introduction

African indigenous vegetables (AIVs) such as African spiderplant (*Cleome gynandra*) and African black nightshade (*Solanum nigrum*) play an important role as source of food for many African communities [1,2]. The indigenous preparation techniques of AIVs are often passed on from generation to generation and are mainly consumed by the older generation who are interested in their medicinal benefits. On the other hand, the younger generations prefer exotic vegetables, which are more available and require less time and energy to prepare [3]. Nevertheless, as a strategy towards mitigation of food and nutrition insecurity in Sub-Saharan Africa (SSA), there's need to promote the consumption of AIVs because of their comparatively higher nutritional value than found in many exotic vegetables [4].

* Corresponding author.
E-mail address: eliwafula@gmail.com (E.N. Wafula).

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African spider plant and African black nightshade are indigenous to the tropical regions of SSA, where its available as either wild or grown [5]. African spider plant is reported to contain higher ascorbic acid, phosphorus, zinc and iron than amounts found in cabbage and carrots [6,7]. Some studies have reported antimicrobial and other health benefits from the African black nightshade [8,9,10]. Due to its potential economic value, it is increasingly being sold on informal and formal markets [9].

Both African spider plant and African black nightshade are generally bitter and contain anti-nutrients like phytates, oxalates, cyanogenic glycosides, and alkaloids [10,11]. Therefore, their leaves are normally pre-boiled then the first water is discarded before proceeding with various cooking methods preferred by consumers. This pre-boiling preparation technique leads to a loss of water-soluble and heat-labile nutrients [12,13]. Traoré et al. [13]. studied the effect of boiling, sun drying and shade drying on the African black nightshade and observed a substantial decline in β-carotene after boiling. Wafula et al. [14] studied the effect different processing methods (solar drying and lactic fermentation) on the African black nightshade and observed better nutrients retentions in the fermented batches. Further studies show marked polyphenols and vitamin A pre-cursors retention in fermented vegetables [15].

Application of lactic acid fermentation of AIVs has gained a lot of interest due to various beneficial effects. For instance, the action of mixed culture of *Lactococcus lactis* (*Lc. lactis)* and *Lactobacillus fermentum* (*Lb. fermentum)* unbinds minerals from anti-nutrients thus contributing to enhanced bioavailability of the affected nutrients [16]. Fermentation also contributes to improved food safety through inhibition of growth of pathogenic bacteria that cannot thrive under acidic environment [17]. Lactic fermentation increases phytochemical content and sensory acceptability of AIVs [1,18]. Overall, the fermented AIVs have potential to contribute towards food and nutrition security particularly in SSA regions [19].

Previous studies have largely focused on spontaneous fermentation of AIVs with limited information of the influence of specific LAB starter cultures such as *Lb. fermentum* and *Lc. lactis* on the nutrients and anti-nutrient composition. This study aimed to determine the effect of African black nightshade and African spider leaves fermentation using starter cultures *Lb. fermentum* and *Lc. lactis* on the nutrients (β-carotene and ascorbic acid, minerals (calcium, potassium, iron and phosphorus) and anti-nutrients (oxalates, tannins, alkaloids and saponins) contents.

Materials and methods

Preparations of materials and samples

African black nightshade and African spider plant were purchased from Musanze agricultural products market in Rwanda. They were transported in a cool box and transported to Institute of Food and Bioresources Technology, Food Microbiology laboratory at Dedan Kimathi University of Technology (DeKUT), Kenya.

Both *Lc. lactis* and *Lb. fermentum* cultures were provided by the Department of Food Science and Technology, Food Microbiology laboratory, at Jomo Kenyatta University of Agriculture and Technology (JKUAT). The inocula were prepared as described by Irakoze et al. [18].

Fermentation of African black nightshade and African spider plant leaves

The African black nightshade (NS) and African spider plant (SP) leaves were prepared according to methods described by Ibinabo [20] and Stoll et al. [21] placed into sterilized stainless steel buckets. The experimental design comprised four batches: SNS (uninoculated fermented African black nightshade), SSP (spontaneous fermented African spider plant), LNS (Starter culture inoculated African black nightshade) and LSP (Starter culture inoculated African spider plant).

The fermentation set-ups were inoculated with a combination of overnight cell cultures of *Lc. lactis* and *Lb. fermentum* at approximately 10^6–10^7 CFU/ml, whereas the spontaneously fermented batches were uninoculated. The buckets were carefully swirled upon incubation to mix the fermentation brine and AIVs leaves. The fermentation was carried out at room temperature (19–20 ^{0}C). Fermentations were done in duplicates on different occasions from different harvests after fermentation, all the samples were separated from the brine solution and frozen. Then, they were taken in a cool box to Food Chemistry and Food Instrumentation laboratories, Jomo Kenyatta University of Agriculture and Technology (JKUAT) for further analysis (nutrients and anti-nutrients) (proximate composition, β-carotene and C, mineral content, oxalate content, tannin content, alkaloids and saponin presence).

Analysis of nutrients content of AIVs

Proximate composition analysis

Moisture content was determined by the air-oven technique according to AOAC 950.46 method [22]. The Crude ash content was estimated by muffle furnace technique according to AOAC 923.03 method [23]. The crude fiber content was determined by AOAC 985.29 method. Crude fat content was analyzed using the solvent extraction method as described by Matenge et al. [24]. Crude protein was estimated by Kjeldahl method AOAC 955.04 with some modifications as described by Matenge et al. [24]. Carbohydrate content was obtained by subtracting the sum of the moisture, crude fat, crude protein and crude fiber content from a 100.

Determination of ascorbic acid and β-carotene

Ascorbic acid content in the vegetables was estimated using HPLC method as described by Matenge et al. [24]. Analysis was done using Shimadzu UV–VIS detector. The mobile phase was 0.8% Metaphosphoric acid, at 1.2 ml/min flow rate and wavelength of 266.0 nm. Beta carotene content was analyzed by the acetone and petroleum ether extraction method as described by Degrain [25] using column chromatography (cotton + silica in a glass column) and UV Spectrophotometer. The β-carotene elute was made to a volume of 25 ml with petroleum ether and the absorbance was read at 440 nm in a UV–Vis spectrophotometer (Shimadzu model UV–1601 PC, Kyoto, Japan).

Determination of minerals

Minerals (calcium, potassium, iron and phosphorus) were analyzed by dry ashing and atomic absorption spectrophotometer (AAS), according to the method described by Matenge et al. [24]. For phosphorus, the UV–VIS spectrophotometry was used for quantification. The crude ash was dissolved in 100 ml of 0.5 N HNO_3. About 5 ml of samples were placed in 50 ml volumetric flask and p-nitrophenol indicator was added. Then 6 N ammonium hydroxide was added to it drop-wise until the color changed to blue (alkaline). Then, 1 M nitric acid was added drop-wise to neutralize the solution colorless. Mixture of ammonium molybdate (5 ml) and ammonium vanadate (color developer) were added. Then topped up to 50 ml using de-ionized water and followed by mixing. The mixture were kept in the dark for 15 min and the absorption read at wavelength 420 nm in UV–Vis-1800 (Shimadzu, Tokyo, Japan).

Determination of anti-nutrient content of AIVs

Analysis of oxalates content. Sample (0.5 g) was homogenized in 4 ml of 0.5 N HCl and the homogenate was heated at 80 °C for 10 min in a shaking water bath. To the homogenate, distilled water was added up to a volume of 25 ml. About 3 ml of the solution was withdrawn and centrifuged at 50,000 rpm for 10 min. One milliliter of supernatant was passed through a 0.45 µm micro-filter before HPLC analysis. Standards were prepared at varying concentrations of oxalic acid. HPLC analysis was done using Shimadzu UV–VIS detector, Hypsil C18 column (5µ M, 4.6 mm *250 mm) equipped waters 550 was used as the static phase and the mobile phase was a solution 0.01 N H_2SO_4. The flow rate was 0.6 ml min^{-1}, at pressure of 62 kgf and detection wavelength of 221 nm.

Determination of tannin content. Ground sample (0.25 g) was extracted with 10 ml of 4 % HCl in methanol by shaking for 20 min using a shaker (Labortechnik KS 250b, Germany) and separated using centrifuge (Kokusan, Type H-2000C, Japan) at 5000 rpm for 10 min at room temperature. The supernatant was transferred into a 25 ml volumetric flask and the extraction step from the residue was repeated with 5 ml of 1 % HCl in methanol. The second supernatant was combined with the first one and diluted to 25 ml. Standards were prepared using cathechin hydrate at 0, 10, 20, 40, 60, 80 and 100 µg/ml. Duplicate aliquots of 1 ml of sample extracts were put into test tubes where one served as a blank sample. The samples and standard solutions were mixed with 5 ml vanillin-HCl reagent (prepared by mixing just before use, equal volumes of 8 % HCl in methanol and 1 % vanillin in methanol) and allowed to stand for 20 min. About 5 ml of 4 % HCl in methanol were added to the samples blanks. Absorbance for all prepared solutions were read at 500 nm and tannin content calculated as percent cathechin equivalent (CE) using the standard calibration curve.

Determination of alkaloids and saponin content. Two milliliters from the 72 h methanolic extracts were mixed with 3 ml of Mayer's reagent and cream cloudiness is an indication of alkaloids presence [24]. methanolic extract (1 ml) of each sample was placed in a test tube then 3 ml of cold distilled water was added. The tubes were thereafter shaken vigorously and a persistent foam was observed if saponins were present [24].

Data analysis

The results were presented as the means and standard deviation of three replicates. The effect of fermentation on African nightshade and African spider plant leaves was tested using a one-way analysis of variance (Sigma plot version 14.0, Stat software Inc. Munich, Germany) at significance level of $p<0.05$ followed by post hoc comparison using Tukey's test.

Results

Analyses of nutrients content of AIVs

Proximate composition

There was a significant increase in crude fibers and crude ash up to 2.1 % and 2.04 % and crude proteins up to 3.4 % and 4.1 % in starter culture inoculated African black nightshade and African spiderplant, respectively (Table 1). The results further showed that starter culture fermentation of AIVs led to a reduction in crude fat content (Table 1).

Table 1
Proximate composition of African black nightshade and African spiderplant.

Vegetables Proximate content	RNS	SNS	LNS	RSP	SSP	LSP
Crude ash%	1.6 ± 0.40*	2.7±0.35**	2.5 ± 0.10**	2.2 ± 0.10*	2.9 ± 0.07**	2.7 ± 0.01**
Crude fibers%	0.7 ± 0.01*	2.3 ± 0.06	2.1 ± 0.03	0.9 ± 0.005*	2.2 ± 0.005	2.1 ± 0.22
Crude fats%	1.6 ± 0.29	1.9 ± 0.016	0.8 ± 0.035*	0.9 ± 0.017	1.7 ± 0.012*	0.9 ± 0.02
Crude proteins%	2.6 ± 0.25*	2.8 ± 0.08*	3.4 ± 0.03**	2.3 ± 0.06*	2.9 ± 0.09**	4.1 ± 0.10**
Moisture content%	87.2 ± 0.11*	84.5 ± 0.12	84.3 ± 0.31	83.5 ± 0.41	83.4 ± 0.15	83.4 ± 0.41
Carbohydrates%	6.3 ± 0.56*	5.8 ± 0.07**	6.9 ± 0.32*	10.2 ± 0.37**	6.9 ± 0.11*	6.8 ± 0.47*

Means with an asterisk (*) and (**) are statistically significantly different ($p \leq 0.05$) according to Tukey's test.
raw African black nightshade (RNS), raw African spider plant (RSP), uninoculated African black nightshade (SNS), starter culture fermented African black nightshade (LNS), uninoculated African spider plant (SSP) and starter culture fermented African spider plant (LSP).

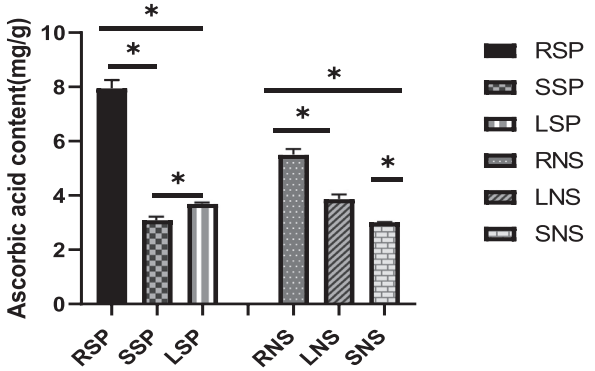

Fig. 1. Ascorbic acid content in African black nightshade and African spiderplant.
Raw African black nightshade (RNS), raw African spider plant (RSP), uninoculated African black nightshade (SNS), starter culture fermented African black nightshade (LNS), uninoculated African spider plant (SSP) and starter culture fermented African spider plant (LSP). Means with an Asterix (*) are statistically significantly different (p>0.05) according to Tukey's test.

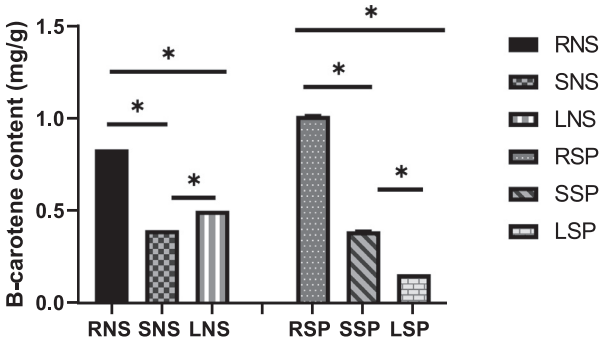

Fig. 2. β-carotene content in African black nightshade and African spiderplant.
Raw African black nightshade (RNS), raw African spider plant (RSP), uninoculated African black nightshade (SNS), starter culture fermented African black nightshade (LNS), uninoculated African spider plant (SSP) and starter culture fermented African spider plant (LSP)]. Means with an asterix (*) are statistically significantly different (p>0.05) according to Tukey's test.

Ascorbic acid and β-carotene content

Fresh African black nightshade and African spiderplant leaves had 9.7 mg/g and 5.4 mg/g of ascorbic acid content, respectively. After fermentation, the total ascorbic acid content reduced to 3.6 mg/g in uninoculated African black nightshade and 3.9 mg/g in starter culture inoculated African black nightshade, respectively (Fig. 1). Additionally, 1.01 mg/g and 0.83 mg/g β-carotene was recorded in fresh African black nightshade and African spider plant respectively. However, they were reduced significantly to 0.15 mg/g and 0.5 mg/g β-carotene in starter culture inoculated African black nightshade and African spiderplant, respectively (Fig. 2).

Table 2
Mineral composition of African black nightshade and African spiderplant.

Minerals Vegetables	Calcium	Potassium	Iron	Phosphorus
RNS	88.32 ± 0.15**	34.32 ± 0.24**	6.04 ± 0.04**	23.90 ± 0.06*
SNS	55.60 ± 0.13**	29.54 ± 0.03*	2.66 ± 0.02*	14.82 ± 0.06
LNS	42.56 ± 0.20**	25.26 ± 0.13*	3.09 ± 0.06*	15.26 ± 0.18
RSP	66.44 ± 0.20**	26.82 ± 0.12**	7.19 ± 0.20*	18.75 ± 0.02**
SSP	44.66 ± 0.40**	21.69 ± 0.21*	2.37 ± 0.25	14.55 ± 0.4*
LSP	58.53 ± 0.30**	18.23 ± 0.11*	2.33 ± 0.20	13.27 ± 0.02*

Means with Asterix (*) and (**) are statistically significantly different ($p \leq 0.05$) according to Tukey's test.
Raw African black nightshade (RNS), raw African spider plant (RSP), uninoculated African black nightshade (SNS), starter culture fermented African black nightshade (LNS), uninoculated African spider plant (SSP) and starter culture fermented African spider plant (LSP).

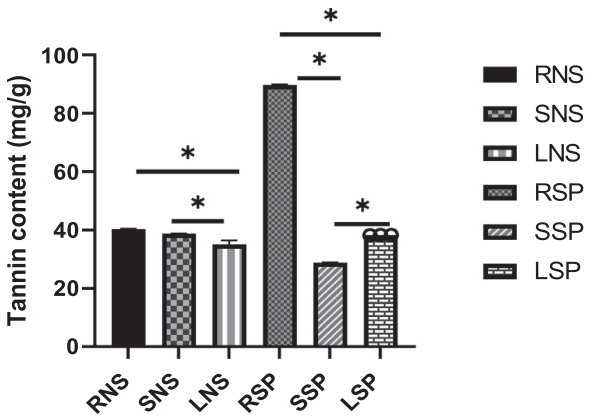

Fig. 3. Tannin content in African black nightshade and African spiderplant.
Raw African black nightshade (RNS), raw African spider plant (RSP), uninoculated African black nightshade (SNS), starter culture fermented African black nightshade (LNS), uninoculated African spider plant (SSP) and starter culture fermented African spider plant (LSP). Means with an Asterix (*) are statistically significantly different, p>0.05 by Tukey's test.

Mineral content

The mineral content, notably calcium, potassium, iron and phosphorus were significantly reduced in all fermented batches (Table 2). Potassium was relatively conserved in fermented African black nightshade; 25.2 mg/g and 29.5 mg/g in uninoculated African black nightshade and culture inoculated African black nightshade, respectively. .

Anti-nutrients content

Fermentation with starter culture reduced tannins in raw African black nightshade from 40.2 mg/g to 34.1 mg/g in culture inoculated African black nightshade and in raw African spiderplant from 89.7 mg/g to 38.2 mg/g in culture inoculated African spiderplant (Fig. 3). Tannin content was significantly higher in spontaneous fermentated AIVs without starter cultures compared to starter culture fermented vegetables (Fig. 3). Oxalate compistion in uninoculated African black nightshade was 25.9 mg/g

Both vegetables contained alkaloids. However, fermentation led to alkaloid reduction (Table 3). Saponins were recorded in small amounts in African spiderplant fermented with starter cultures (Table 3).

Fig. 4.

Discussion

Nutrient content of African black nightshade and African

Proximate composition

Crude fat content reduced in fermented vegetables particularly, in starter culture fermented batches. However, crude protein and ash significantly increased in all fermented batches. This observation agrees with the observation by Josiah et al. [26] on nutrient conservation in fermented vegetables. Traoré et al., [13] also reported reduction of nutrients in cooked vegetables. After fermentation the crude ash content increased, potentially due to the added salt which was critical for the fermentation process. The salt might have induced plasmolysis which in turn caused minerals and proteins to be re-

Table 3
Qualitative analysis of alkaloids and saponins in African black nightshade and African spider plant.

Samples	Alkaloids	Saponins
RNSa	++	–
RNSb	++	–
RNSc	R	r
RSPa	++	+
RSPb	++	+
RSPc	R	r
SNSa	+	–
SNSb	+	–
SNSc	R	r
SSPa	+	+
SSPb	+	+
SSPc	R	R
LNSa	+	–
LNSb	+	–
LNSc	R	R
LSPa	+	–
LSPb	+	–
LSPc	R	R

Key: Raw African black nightshade (RNS), raw African spider plant uninoculated African black nightshade (SNS), starter culture fermented African black nightshade (LNS), uninoculated African spider plant (SSP) and starter culture fermented African spider plant (LSP) ++: Positive, +: partial positive, _: negative, r: reference sample.

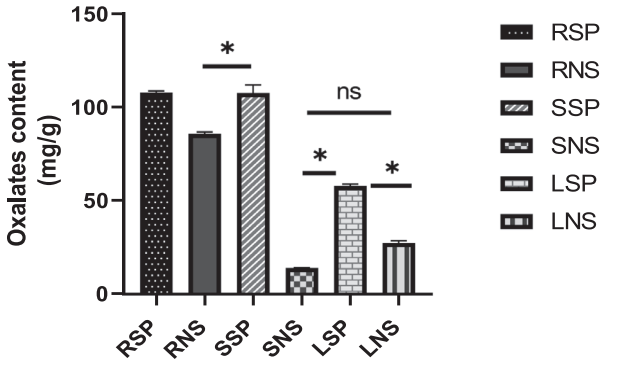

Fig. 4. Oxalates content in African black nightshade and African spiderplant.
Raw African black nightshade (RNS), raw African spider plant (RSP), uninoculated African black nightshade (SNS), starter culture fermented African black nightshade (LNS), uninoculated African spider plant (SSP) and starter culture fermented African spider plant (LSP). Means with an Asterix (*) are statistically significantly different ($p>0.05$) according to Tukey's test.

leased [14]. The change in carbohydrates might have resulted from the utilization of the fermentable carbohydrates by the microorganisms during fermentation.

Ascorbic acid and β-carotene contents

The final content in ascorbic acid and β-carotene (Fig. 1 and Fig. 2) was lower than what other studies [2,26] had reported, i.e. a slight reduction in water-soluble vitamins in fermented vegetables African spider plant retained more ascorbic acid compared to African black nightshade. The ascorbic acid levels observed agreed with the levels < 60 mg/100 g reported by Degrain et al., [25]. Leaching of nutrients in the fermentation solution (submerged fermentation) can also account for the reduction in water-soluble nutrients. Nevertheless, the observed content of ascorbic acid was found sufficient for an adult (19 to 64 years old) to meet the daily recommended intake by NHS- United Kingdom.

Mineral content

Whereas ash content increased in both uninoculated and starter culture inoculated batches (Table 2), there was a decline with the specific mineral elements. This reduction of minerals can be attributed to leaching in the fermentation liquid (only fermented leaves were used for mineral analysis). However, the remaining minerals can be still nutritionally effective and bioavailable. The observed content of minerals (Table 2) in fermented vegetables are higher compared to the levels in normal cooking methods [13].

Anti-nutrients content

Fermented foods are believed to be more nutritious mainly because the fermenting starter culture agents delink the bonds between nutrients and antinutrients and liberate nutrients thus making them bioavailable [8]. The enzymes in both *Lc. lactis* and *Lb fermentum* can unbind the nutrients from anti-nutrients thereby enhancing the bioavailability of nutrients [16]. Also, it has been observed that fermentation detoxifies foods by eliminating certain chemicals or transforming them [24]. In this study, all tested anti-nutrients (oxalates, tannins, alkaloids and saponin) were reduced especially in the culture fermented vegetables (34. This observation agrees with that of the study conducted by Felix and Francis [27] on fermented maize and African locust beans.

Conclusion and recommendations

Conclusion

The use of starter culture in the fermentation led to a significant increase in protein and ash while at the same time reduced ascorbic acid, β-carotene, crude fiber and carbohydrates. However, the fermented product still contained enough ascorbic acid to supplement the daily recommended intake. Also fermentation reduced minerals though the minerals were higher in fermented product than in normally cooked vegetables. The use of starter cultures led to a reduction of all tested anti-nutrients i.e. oxalates, tannins, alkaloids and saponins.

Recommendations

There have been noted that fermented AIVs had a wide range of flavours. It would be worth studying these flavours and identifying the proper time to stop the fermentation. Also, shelf-life studies are needed to establish how long they can be safely conserved. There is an urgent need to encourage agriculture and fermentation of AIVs for they hold the potential in helping to solve the ever-growing problem of food and nutrition insecurity. Fermented AIVs need to be integrated into the food system in order to help the population gain from their nutritional, sensory, phytochemical, shelf life and monetary benefits.

Data availability

The reference data used to support the findings of this study are included in the article.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Marie Lys Irakoze: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Writing – original draft. **Eddy Elkana Owaga:** Conceptualization, Data curation, Methodology, Project administration, Resources, Supervision, Validation, Visualization. **Eliud Nalianya Wafula:** Conceptualization, Data curation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing.

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