Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.37921

Minerals, antinutrients content and the bioaccessibility of iron and zinc in cooked, spontaneously fermented-dried, and blanched-dried dark green leafy vegetables commonly consumed in Sub-Saharan Africa

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Abstract

Iron and zinc deficiencies remain a significant problem in sub-Saharan Africa. This study aimed at determining mineral, antinutrient contents of differently processed ALVs, and the bioaccessibility of iron and zinc from cooked, blanched-dried, as well as spontaneously fermented-dried ALVs. Mineral and antinutrient contents of ALVs were analysed and the bioaccessibility of iron and zinc from ALVs were analysed by in vitro dialysability assay. Iron, zinc, magnesium, and calcium contents of the ALVs were high. Compared to cooked vegetables, both spontaneously fermented-dried and blanched-dried vegetables had significantly lower levels of condensed tannins, while significantly lower total phenolic contents were found in blanched-dried vegetables. Compared to cooked counterparts, percentage iron and zinc bioaccessibilities in spontaneously fermented-dried and blanched-dried ALVs showed no significant ($p > 0.05$) differences except for iron in Ethiopian kale and zinc in amaranth. Based on data from this study, it is recommended that ALVS should be consumed concurrently with iron and zinc enhancers to enhance bioaccessibility.

Keywords: traditional leafy vegetables; dialysability; mineral; preservation; nutritive value.

Practical Application: Improving in vitro bioaccessibility of vegetables through blanching and fermentation.

1 Introduction

The prevalence of anaemia is estimated at 22.8% globally with sub-Saharan Africa as one of the regions bearing the greatest burden (Gardner & Kassebaum, 2020). Iron deficiency anaemia (IDA) is associated with inadequate dietary intake, increase in iron demand from the body, chronic loss of blood, and poor iron absorption (Chaparro & Suchdev, 2019; Gardner & Kassebaum, 2020). The prevalence of inadequate zinc intake was estimated to exceed 25% in the sub-Saharan Africa region (Wessells & Brown, 2012). Iron and zinc deficiencies in sub-Saharan Africa are mainly caused by inadequate dietary intake of animal sources of iron and zinc due to most households relying mainly on cereal-based staple foods, which have low iron and zinc bioavailability (Fanzo, 2012). Consumption of African leafy vegetables (ALVs) can be effective in alleviating iron and zinc deficiencies by increasing dietary diversification and improving household nutrition security (Yang et al., 2013; Elisha et al., 2016). ALVs are mostly utilized by households in rural areas, cooked fresh and consumed as a relish accompanying staple foods (Uusiku et al., 2010; van der Hoeven et al., 2013; Bvenura & Afolayan, 2015). Major challenges in the sustainable incorporation of ALVs into diets are their seasonality and post-harvest losses due to their high perishability (Uusiku et al., 2010; Gogo et al., 2018).

However, traditional processing methods such as blanching and spontaneous fermentation in combination with drying have been previously used to reduce postharvest losses (Wafula et al., 2016; Masarirambi et al., 2010; Natesh et al., 2017). ALVs also contains high levels of antinutrients (Kruger et al., 2015; Essack et al., 2017). Blanching and spontaneous fermentation have been reported to be effective in reducing antinutrients levels in ALVs (Wafula et al., 2016; Kirakou et al., 2017). To the best of our knowledge, no research has been published on the bioaccessibility of iron and zinc from blanched-dried and spontaneously fermented-dried ALVs compared to their cooked counterparts. In this sense, the two processing methods may present a possibility of better bioaccessibility of iron and zinc from ALVs hence this study aimed to determine the mineral and antinutrient contents of processed ALVs, and the iron and zinc bioaccessibility of processed ALVs.

2 Materials and methods

2.1 Plant materials

Five commonly consumed ALVs *Cleome gynandra* (spider plant), *Amaranthus hybridus* (amaranth), *Corchorus olitorius*

Received 08 Dec., 2020
Accepted 19 Aug., 2021
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(wild jute) and *Vigna unguiculata* (cowpea leaves) and *Brassica Carinata A. Baun* (Ethiopian kale) were procured in Botswana. Two separate samples from each ALV were processed. All reagents were of analytical grade and were purchased from Sigma-Aldrich, Johannesburg, South Africa unless stated otherwise.

2.2 Vegetable sorting and washing

ALVs were inspected for fungal contamination, surface contaminants, debris as well as defective colour and appearance as outlined in the world vegetable centre manual (Acedo, 2010). Approximately 5 kg of each vegetable was washed twice in deionized water to remove any impurities and was drained after which it was divided into three equal batches for cooking, blanching, and spontaneous fermentation. The ALVs were prepared the way in which they are normally prepared in households when in season and out of season (Figure 1).

2.3 Cooking

ALVs (500 g) were boiled in 100 ml of deionized water for 20 minutes as per households methods previously described (Matlhare et al., 1999), cooled to room temperature, and transferred along with the cooking water into plastic jars with lids and stored at –20 °C for a month until laboratory analysis. To prepare for laboratory analysis, the cooked samples were then freeze-dried and milled to a fine powder using a laboratory miller (IKA A11, Staufen, Germany) and stored in zip-lock bags for two to three days at -20 °C before analysis. Cooking was used as a control in this study.

2.4 Blanching

For blanching, 1 kg of ALVs were submerged into deionized boiling water (4L) at 97 °C for five minutes as previously described (Mosha et al., 1995). The vegetables were then transferred into oven drying trays and dried in a conventional oven dryer at 50 °C with 0% humidity for 24 hours (ClimaPlus Combi, Germany). The dried samples were stored at 5 °C in the brown craft paper bags for a month. For laboratory analysis, samples were milled and stored as described for cooked samples.

2.5 Spontaneous fermentation

The third batch of ALVs were fermented according to the method by (Ifesan et al., 2014). Each vegetable (1 kg) was fermented with 30 g of salt as per the method described by the World Vegetable Centre (Acedo, 2010). An additional 30 g of sugar was added as adopted from the method used by (Kasangi et al., 2010) with some modifications. The ALVs were placed in a 2.5 cm layer and sprinkled with the 50:50 salt-sugar mixture, on to which another layer of leaves was added and sprinkled with the salt-sugar mixture. This was repeated until the whole 1 kg of leaves and 60 g of the 50:50 salt-sugar mixture was used. A cheesecloth was placed over the ALVs and weight was added to compress the vegetable and to assist with the formation of brine. The vegetables were left to ferment for seven days at room temperature. Temperature and pH of the fermented ALVs were monitored and measured daily. A Crison basic 20, Barcelona, Spain pH meter and a handheld digital thermometer (-50 to +200 °C, P300 Temp, France) were used. After fermentation, the vegetables were weighed and placed in drying trays and the

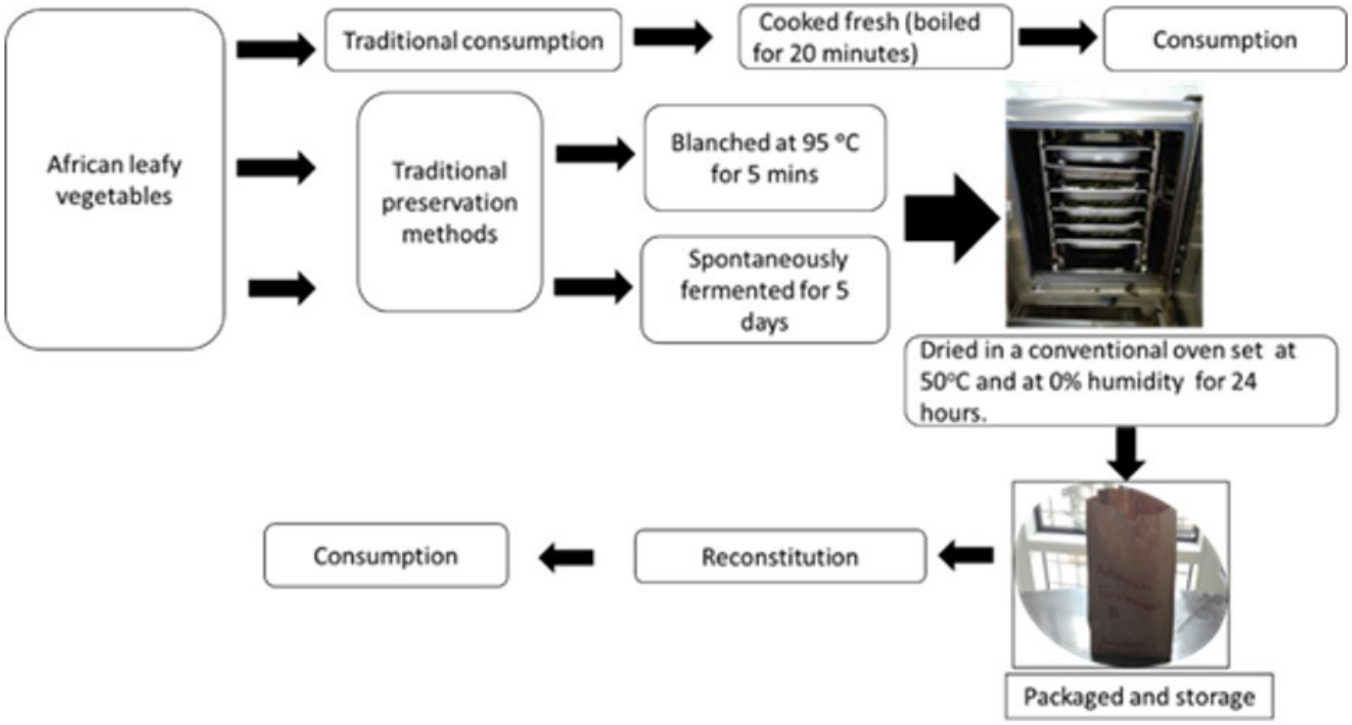

Figure 1. Experimental design illustrating traditional processing methods of African leafy vegetables.

fermentation liquid was discarded. Fermented ALVs were dried as described for blanched samples. After drying, the vegetables were weighed and stored at 5 °C in sealed, brown craft paper bags for a month. For laboratory analysis, samples were milled and stored as described for cooked samples.

2.6 Mineral contents

Iron (Fe), zinc (Zn), magnesium (Mg), calcium (Ca), aluminium (Al), and phosphorus (P) contents of the ALVs were analysed at the Central Analytical Facilities (CAF) at Stellenbosch University, which is accredited by the South African National Accreditation System (SANAS). The ALV samples were digested by concentrated nitric acid and hydrogen peroxide by adopting the USEPA - United Nations Environment Protection Agency (2007) method 3051A. The concentration of minerals in the digested samples were determined by using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) (ICAP 6000 series, Thermo Fisher Scientific, Waltham, USA). Elements were analysed in the following wavelengths, 239.5nm for iron, 206.7nm for zinc, 315.8nm for calcium, 285.2nm for magnesium, 214.9nm for phosphorus, and 308.2nm for aluminium. For quality assurance, samples were analysed against National Institute for Standards and Technology traceable standards and independent quality controls. An internal standard technique was established, and a calibration acceptance criterion of $R^2 > 0.9995$ was used to ascertain the accuracy of the result.

2.7 Total phenolic content, condensed tannins, phytates, and oxalate contents

Total phenolic content (TPC) was determined using a modified Folin-Ciocalteu method (Kaluza et al., 1980). Condensed tannins were determined by the Vanillin- HCl method with slight modifications of the protocol (Price et al., 1978). For both TPC and tannin contents, blank samples without the addition of the reagent were analysed for each sample to correct for the colour of the extracts. The absorbance for TPC was measured at 760nm and for condensed tannins at 500nm. Phytate content was determined by anion exchange chromatography, an indirect quantitative analysis by measuring the organic phytate-phosphorus (inositol -1 to 6- phosphate) (Frühbeck et al., 1995). Purification of the extracts was conducted using Dowex1-anion-exchange resin-AG 1 x 4 (4% Cross-linkage, chloride form, 100-200 mesh (74-149 µm) in a glass barrel Econo-column (Poly-Prep®Chromatography columns (Bio-Rad, Hercules, California, United States). Purified extracts were reacted with the Wade reagent and the absorbance was measured at 500nm. Oxalates were determined using the titration method and calculated assuming that 1 ml of 0.1 M $KMnO_4$ was equivalent to 2.2 mg of oxalate (Association of Official Analytical Chemists, 1999).

2.8 Iron and zinc bioaccessibility

The *in vitro* dialysability method described by (Miller et al., 1981) was used, with some minor modifications. The dialysates were acidified with 0.002 ml 65% nitric acid/ml dialysate when decanted to avoid potential precipitation of minerals (Kruger et al., 2012). The following enzymes were used: pepsin (P-7000), bile extract (P-8631) and pancreatin (P-1750) (Sigma-Aldrich, Johannesburg, South Africa). In the intestinal phase, a dialysis tubing of Spectra/Por 7 (Ø = 20.4 mm) with a molecular mass cut-off (MMCO) of 10000 Da (Labretoria) was used (GIC Scientific, Johannesburg, South Africa). Mineral contents of the dialysates were determined by the ICP-OES (ICAP 6000 series, Thermo Fisher Scientific, Waltham, USA) as described above under mineral contents analysis but without the digestion step.

2.9 The potential contribution of the vegetables towards total absolute requirements

Though iron and zinc deficiency are also a problem for other aged groups e.g children and adolescents, this study based its calculation on women who are at stage of menstruation and childbearing who are also a risk group in need of iron and zinc. The contribution that each processed ALV could make towards iron and zinc AR of women aged 19 to 50 years were calculated using bioaccessibility data assuming that a standard portion size of ALV (300 g wet basis) was consumed (van der Hoeven et al., 2013). For women aged 19 to 50 years, the AR for iron and zinc is 1.46 mg/day and 1.75 mg/day, respectively (Sandstead, 2015; Food Agriculture Organization, 2001). The contributions were expressed as a percentage.

$$\%contribution = \frac{\text{absolute accessible mineral}\left(\text{mg}/100\text{g}\right)}{\text{absolute requirements}\left(\text{mg}/\text{day}\right)} X\,100 \quad (1)$$

2.10 Statistical analysis

The composition analyses on the ALVs were conducted in duplicates with each antinutrient analysis repeated on two separate days. Independent dialysability experiments were conducted in duplicates with the intestinal digestion step for each time performed in triplicates. IBM SPSS Statistics 25.0, Armonk, USA was used for data analysis and Analysis of Variance (ANOVA) and Tukey's HSD Post-hoc test was applied to determine significant differences between the means. Values of $p \leq 0.05$ were considered statistically significant for all the analysis. A Principal Component Analysis (PCA) was performed to determine the correlation patterns and variation between mineral and anti-nutrient content in ALVs.

3 Results and discussion

Compositional data for this study are given on a dry basis (db) except where otherwise stated.

3.1 Mineral contents of traditionally processed African leafy vegetables

Compared to their cooked counterparts, the mineral content of fermented ALVs was significantly ($p \leq 0.05$) lower for iron in wild jute and spider plant; for magnesium in spider plant, Ethiopian kale and amaranth; and for calcium in amaranth (Table 1). In contrast, compared to the cooked ALVs, the mineral content of fermented ALVs was significantly ($p \leq 0.05$) higher for iron in amaranth and Ethiopian kale; for zinc in cowpea leaves, wild jute and Ethiopian kale; for magnesium in wild jute and cowpea; and for calcium in cowpea, spider plant and wild jute. In this study,

Table 1. Mineral contents of traditionally processed African green leafy vegetable.

Vegetable		Fe	Zn	Mg	Ca	P	Al
		mg/100 g, db					
CP	COO	29.7 ± 0.6^{b*}	2.9 ± 0.1^{a*}	301 ± 5^{a*}	1840 ± 33^{a*}	340 ± 6^{b*}	32.4 ± 0.6^{c*}
	BLA	18.1 ± 0.1^{a}	3.0 ±0.3^{a}	268 ± 7^{a}	1850 ± 30^{a}	337 ± 4^{b}	13.5 ± 0.2^{a}
	FER	28.5 ± 0.5^{b}	3.4 ± 0.1^{b}	343 ± 4^{b}	2220 ± 33^{b}	303 ± 4^{a}	25.6 ± 0.6^{b}
SP	COO	61.7 ± 1.2^{c*}	12.0 ± 0.2^{b*}	437 ± 8^{b*}	2456 ± 43^{a*}	614 ± 11^{a}	75.8 ± 1.4^{c*}
	BLA	40.0 ± 0.5^{a}	11.8 ± 0.1^{b}	350 ± 1^{a}	2381 ± 29^{a}	617 ± 4^{a}	45.3 ± 0.1^{a}
	FER	52.8 ± 1.0^{b}	11.2 ± 0.1^{a}	377 ± 5^{a}	2660 ± 51^{b}	601 ± 3^{a}	56.0 ± 1.1^{b}
WJ	COO	29.5 ± 0.4^{c*}	3.7 ± 0.0^{a}	573 ± 1^{b}	1422 ± 10^{a*}	648 ± 7^{b*}	30.8 ± 0.3^{c*}
	BLA	10.4 ± 0.4^{a}	3.5 ± 0.1^{a}	528 ± 12^{a}	2452 ± 93^{b}	454 ± 10^{a}	6.4 ± 0.0^{a}
	FER	16.4 ± 0.3^{b}	4.5 ± 0.0^{b}	640 ± 7^{c}	2782 ± 29^{c}	570 ± 6^{c}	14.2 ± 0.3^{b}
A	COO	30.0 ± 0.6^{b*}	6.4 ± 0.0^{b*}	1365 ± 12^{b*}	1995 ± 21^{b*}	477 ± 3^{a*}	39.0 ± 0.4^{c*}
	BLA	26.4 ± 0.0^{a}	6.8 ± 0.1^{c}	1556 ± 7^{c}	1985 ± 11^{b}	570 ± 14^{b}	26.2 ± 0.3^{a}
	FER	33.2 ± 0.7^{c}	6.0 ± 0.1^{a}	1254 ± 16^{a}	1730 ± 28^{a}	476 ± 6^{a}	34.1 ± 0.1^{b}
EK	COO	13.4 ± 0.3^{a}	4.1 ± 0.1^{a}	561 ± 11^{c}	2890 ± 54^{b}	565 ± 11^{b}	9.7 ± 0.2^{a}
	BLA	20.0 ± 1.0^{b}	5.1 ± 0.1^{b}	421 ± 13^{a}	2216 ± 83^{a}	521 ± 2^{a}	42.3 ± 1.4^{c}
	FER	24.5 ± 0.2^{c}	5.2 ± 0.1^{b}	521 ± 10^{b}	2928 ± 34^{b}	555 ± 14^{b}	21.2 ± 0.3^{b}

Values are expressed as mean ± SD (n = 2). Mean values of each vegetable in the same column with different superscripts are significantly different at $p \leq 0.05$, CP= Cowpea, SP= Spider plant, WJ = Wild jute, A= Amaranth, EK = Ethiopian kale, FER=Fermented, BLA = Blanched, COO = Cooked, Values of cooked vegetable with a * are significantly different to the cooked Ethiopian kale at $p \leq 0.05$, db = dry basis

the cooking water was not discarded, minerals leached out and remained in cooked ALVs at analysis. However, the sugar-salt solution used for fermentation was drained out, which could have contributed to mineral losses through leaching. Also, the addition of salt encourages the growth of lactic acid bacteria (LAB) which increases the osmolarity and ionic strength of the fermentation medium, causing water and minerals to be extracted from vegetable tissues (Swain et al., 2014). The localisation of the mineral in the vegetable leaf matrix determines how the mineral reacts to processing (Raes et al., 2014). Minerals in the ALVs could have been located in different plant organelles attributing to variations in the influence of fermentation.

Compared to their cooked counterparts the mineral content of blanched ALVs was significantly ($p \leq 0.05$) lower for iron in cowpea, spider plant, wild jute and amaranth; for magnesium in spider plant and Ethiopian kale; for calcium in Ethiopian kale; and for phosphorous in amaranth and Ethiopian kale (Table 1). In contrast, compared to cooked counterparts, mineral contents of blanched ALVs was significantly ($p \leq 0.05$) higher for iron in Ethiopian kale; for zinc in Ethiopian kale and amaranth; for calcium in wild jute; and phosphorous in amaranth.

There are no studies available which compared mineral contents in blanched and cooked ALVs, however, previous studies which compared raw and blanched ALVs reported lower levels of iron and zinc in blanched ALVs (Acho et al., 2015; Mepba et al., 2007). ALVs were blanched for five minutes in the current investigation, which has been reported to be the effective maximum blanching time to minimise mineral losses through leaching (Acho et al., 2015), however, some losses were still apparent. The degree of heating, rate of leaching, duration of cooking, and surface area exposed to water determines the degree in which minerals are lost through processing (Lešková et al., 2006).

Compare to cooked counterparts, aluminium content of all blanched and fermented ALVs were significantly ($p \leq 0.05$) lower, except for Ethiopian kale with higher contents (Table 1). Spider plant had the highest aluminium contents. High content of aluminium in plant-based foods could be an indication of soil contamination (Salvo et al., 2018) and result in artificially high levels of essential minerals in food, especially iron. Previously, it was reported that normal cleaning before processing ALVs did not remove all impurities (van Jaarsveld et al., 2014). Additional soil impurities which were not removed by normal cleaning could have been removed through leaching out into the blanching and fermentation mediums. Spider plant's leaves generally grow nearer to the ground surfaces which makes it susceptible to soil contamination. High levels of aluminium content found in blanched and fermented Ethiopian kale could be attributed to cross-contamination with aluminium foil as the vegetable stuck to aluminium foil which lined the drying trays

3.2 Antinutrient contents of traditionally processed African green leafy vegetables

Compared to cooked counterparts, condensed tannins were significantly ($p \leq 0.05$) lower in all fermented ALVs except Ethiopian kale (Table 2). Waluchio (2016) also found a decrease of 86% of tannin contents after spontaneous fermentation of cassava leaves. During spontaneous fermentation, microbial activity results in tannic acid degradation into smaller compounds, and also the the activation of tannin acyl hydrolase, which hydrolyses the ester and depside bonds of tannic acid to form hydrolysable tannins (Macedo et al., 2011), free tannins leaches out into the fermentable medium which was discarded attributing to low levels.

Compared to cooked counterparts, condensed tannins were significantly ($p \leq 0.05$) lower in blanched spider plant and wild jute while higher contents were found in blanched cowpea. Lower levels of condensed tannins in some ALVs could possibly be due to leaching out into the blanching medium. High temperatures break down hydrogen bonds that exists between tannin complexes

Table 2. Antinutrients content of traditionally processed African green leafy vegetables.

Vegetable	Condensed Tannins	Total Phenolics		Total oxalates	Soluble oxalates	Insoluble oxalates	Phytates
		mg CE /100 g, db			mg/100 g, db		
CP	COO	137 ± 14^{b}	2022 ± 83^{a*}	3349 ± 224^{b*}	2967 ± 181^{b*}	382 ± 68^{a}	2263 ± 189^{a}
	BLA	174 ± 17^{c}	2083 ± 155^{a}	2226 ±177^{a}	2039 ± 58^{a}	187 ± 229^{a}	2131 ± 297^{a}
	FER	58 ± 5^{a}	2612 ± 55 ^{b}	2111 ± 51^{a}	2031 ± 49^{a}	80 ± 36^{a}	2025 ± 263^{a}
SP	COO	172 ± 7^{b*}	2040 ± 25^{a*}	2502 ± 55^{c}	2400 ± 64^{c}	102 ± 107^{a}	2437 ± 272^{b}
	BLA	140 ± 8 ^{a}	1951 ± 92^{a}	1862 ± 212^{b}	804 ± 24^{a}	1058 ± 226^{b}	1848 ± 272^{a}
	FER	129 ± 9^{a}	3022 ± 293^{b}	1272 ± 36^{a}	1234 ± 20^{b}	37 ± 55^{a}	1831 ± 246^{a}
WJ	COO	220 ± 22^{b*}	2491 ± 222^{a*}	3835 ± 403^{c*}	2470± 119^{b}	1361 ± 409^{b*}	2140 ± 408^{a}
	BLA	116 ± 12^{a}	2106 ± 74^{a}	1754 ±178^{a}	812 ± 21^{a}	943 ± 199^{b}	2296 ± 386^{a}
	FER	132 ± 13^{a}	2204 ± 176^{a}	2629 ± 227^{b}	2433 ± 43^{b}	196 ± 206^{a}	2194 ± 418^{a}
AM	COO	144 ± 16^{b}	1655 ± 95^{a*}	4680 ± 242^{c*}	4113 ± 197^{c}	567 ± 45^{a}	4408 ± 213^{b}
	BLA	132 ± 10^{b}	1857 ± 181^{a}	2126 ±87^{a}	1640 ± 25^{a}	485 ± 97^{a}	3866 ± 538^{ab}
	FER	78 ± 8 ^{a}	1637 ± 110^{a}	3408 ± 43^{b}	3219 ± 112^{b}	189 ± 78^{a}	3429 ± 532^{a}
EK	COO	147 ± 9^{a}	1103 ± 86^{a}	2627 ± 92^{c}	2334 ± 109^{c}	293 ± 72^{b}	1977 ± 90^{a}
	BLA	135 ± 14^{a}	1020 ± 95^{a}	1633 ± 24^{b}	408 ± 10^{a}	1226 ± 32^{c}	1751 ± 126^{a}
	FER	223 ± 18^{b}	1168 ± 103^{a}	843 ± 24^{a}	806 ± 17^{b}	37 ± 25^{a}	1831 ± 246^{a}

Values are expressed as mean ± SD (n = 4 for condensed tannins and phenolics, n = 6 for oxalates and phytates), Mean values of each vegetable in the same column with different superscripts are significantly different at $p \leq 0.05$, CP= Cowpea, SP= Spider plant, WJ = Wild jute, A= Amaranth, EK = Ethiopian kale, FER = Fermented, BLA = Blanched, COO = Cooked, Insoluble oxalates = (Total oxalates – soluble oxalates), (%) = (soluble oxalates / Total oxalates) x 100, Values of cooked vegetable with a * are significantly different to the cooked Ethiopian kale at $p \leq 0.05$, db = dry basis, CE = catechin equivalents.

and, hence, increase the extraction of tannins during analysis (Peng & Jiang, 2004), which may be a possible reason for the observed high levels of condensed tannins in blanched cowpea.

TPC was found to be significantly higher ($p \leq 0.05$) in fermented cowpea and spider plant than in their cooked counterparts. During fermentation, condensed tannins such as proanthocyanin are hydrolysed to phenols through the activation of polyphenol oxidase (Nkhata et al., 2018, Emmambux & Taylor, 2003). The transformation of tannins to phenols that occurs during fermentation increases phenolic contents (Taylor & Duodu, 2015).

Compared to cooked counterparts, significantly lower ($p \leq 0.05$) total oxalates and soluble contents were found in all fermented and blanched ALVS, except fermented wild jute with no significant changes, while blanched spider plant and Ethiopian kale had higher insoluble oxalates content (Table 2). During fermentation LAB degrade oxalates, thereby reducing the levels (Okoye & Ene, 2018, Goldfarb et al., 2007). Wild jute contain pectin (Ahmed et al., 2003), which probably influenced its processing to some extent as less medium was discarded before drying as it turned jelly-like possibly accounting for the inconsequential changes found in its soluble oxalate contents. During blanching, soluble oxalates are lost through leaching out into the blanching medium (Raes et al., 2014). Insoluble oxalates are only effectively reduced by prolonged thermal processing, (Savage & Klunklin, 2018)

Compared to cooked counterparts, blanched spider plant, fermented amaranth and spider plant had significantly ($p \leq 0.05$) lower phytate contents (Table 2). Similarly, lower levels of phytates were reported in a study in which spider plant was fermented for 48 hours (Akello, 2014). In contrast to the current study, significantly higher phytate levels were found in blanched *Moringa oleifera* leaves than in the cooked sample (Sallau et al., 2012). ALVs were blanched at the maximum blanching time of five minutes which served to minimise mineral losses and were further dried at 60 °C for 24 hours, which to some degree degraded some phytates in spider plant. During fermentation, endogenous phytase is activated resulting in degradation of phytate (Coulibaly et al., 2011), and LAB produces lactic acid, which decreases the pH to the optimal range for phytase activity increasing phytate degradation (Gupta et al., 2015). The extent of reduction of phytates likely was due to the amount of lactic acid produced in each ALV.

3.3 Relationship between minerals and antinutrients in traditionally processed African green leafy vegetables

The principal component analysis (PCA) (Figure 2) shows that total oxalates, soluble salts, and magnesium were positively correlated, indicating that oxalates in these ALVs probably predominantly existed in the form of magnesium oxalate. Magnesium oxalate has been associated with more solubility than calcium oxalate (Laranjinha et al., 2019). Insoluble calcium oxalates pose a health risk as it can accumulate in the renal glomeruli and forms kidney stones (Massey, 2007). Also, the strong association found between iron, zinc, and aluminium may suggest that a portion of iron and zinc from these ALVs may be from soil/dust contamination.

3.4 Bioaccessibility of iron and zinc from processed African leafy green vegetables

Compared to cooked counterparts, percentage iron bioaccessibility and the amount of bioaccessible iron (Table 3) in all the fermented and blanched ALVs showed no significant ($p > 0.05$) differences except for Ethiopian kale and blanched amaranth with lower contents. Blanched amaranth had lower

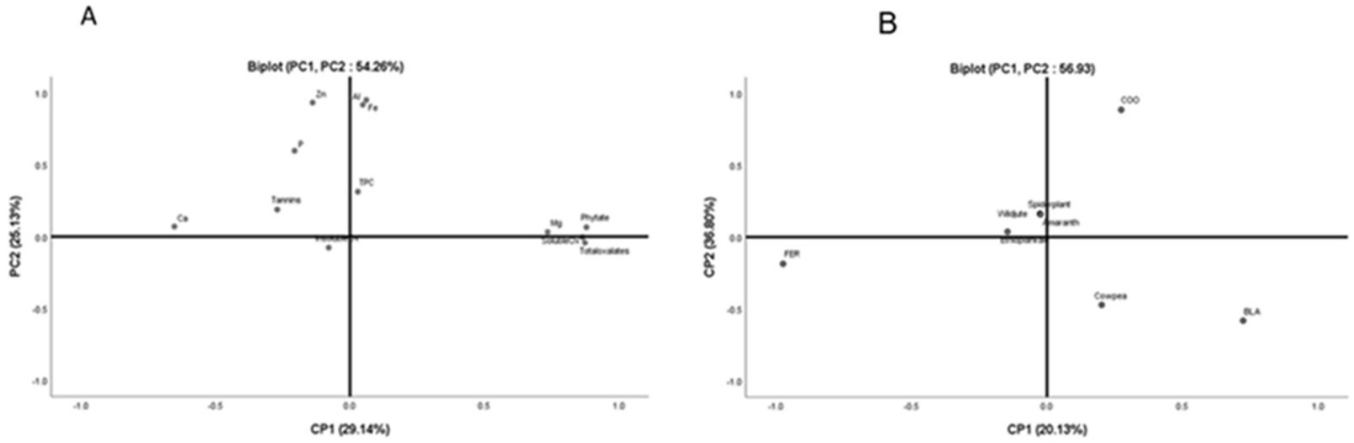

Figure 2. Principal component analysis of minerals and antinutrients of traditionally processed African leafy vegetables. (A) PCA loadings; phytate, total oxalate, soluble oxalate, insoluble oxalate total phenolic content (TPC), tannins, iron, zinc, phosphorus, magnesium, calcium, aluminium; (B) Green leafy vegetable species; spider plant, cowpea, wild jute, amaranth, Ethiopian kale and processing methods (COO = cooked, BLA = Blanched, FER = Fermented).

Table 3. The bioaccessibilities of iron and zinc of traditionally processed African green leafy vegetables.

Vegetable [#]		Bioaccessibility (%)		Amount bioaccessible (mg/100 g, db)	
		Fe	Zn	Fe	Zn
CP	COO	3.9 ± 0.5[a]	35.7 ± 4.4[a]	1.15 ± 0.14[a]	1.04 ± 0.13[a]
	BLA	2.1 ± 0.9[a]	24.2 ± 10.8[a]	0.38 ± 0.17[a]	0.73 ± 0.32[a]
	FER	3.7 ± 1.3[a]	30.8 ± 5.4[a]	1.06 ± 0.86[a]	1.02 ± 0.66[a]
SP	COO	2.1 ± 0.7[a]	15.7 ± 5.2[a]	1.30 ± 0.42[a]	1.88 ± 0.62[a]
	BLA	1.8 ± 0.3[a]	18.2 ± 5.3[a]	0.71 ± 0.12[a]	2.14 ± 0.62[a]
	FER	1.5 ± 0.3[a]	17.0 ± 3.0[a]	0.80 ± 0.16[a]	1.90 ± 0.33[a]
WJ	COO	1.6 ± 0.6[a]	22.5 ± 10.0[a]	0.47 ± 0.19[a*]	0.83 ± 0.37[a]
	BLA	4.4 ± 0.6[a]	25.1 ± 7.9[a]	0.46 ± 0.06[a]	0.89 ± 0.28[a]
	FER	2.3 ± 0.2[a]	12.2 ± 1.3[a]	0.37 ± 0.03[a]	0.55 ± 0.05[a]
A	COO	10.7 ± 2.3[a]	8.4 ± 4.6[a]	3.20 ± 0.68[b*]	0.54 ± 0.30[a]
	BLA	7.8 ± 0.4[a]	18.0 ± 16.3[b]	2.06 ± 0.64[a]	1.23 ± 1.10[a]
	FER	11.5 ± 4.7[a]	16.6 ± 10.1[b]	3.81 ± 1.26[b]	1.00 ± 0.61[a]
EK	COO	15.3 ± 3.6 [b]	40.9 ± 8.6 [a]	2.05 ± 0.38[b]	1.67 ± 0.35[a]
	BLA	2.5 ± 1.9[a]	16.6 ± 13.5[a]	0.51 ± 0.37[a]	0.85 ± 0.68[a]
	FER	2.6 ± 1.1[a]	22.3 ± 7.0[a]	0.63 ± 0.26[a]	1.16 ± 0.36[a]

Values are expressed as mean ± SD (n = 4), Mean values of each vegetable in the same column with different superscripts are significantly different at $p \leq 0.05$, CP= Cowpea, SP= Spider plant, WJ = Wild jute, A= Amaranth, EK = Ethiopian kale, FER = Fermented, BLA = Blanched, COO = Cooked, Values of cooked vegetable with a * are significantly different to the cooked Ethiopian kale at $p \leq 0.05$, [#] mineral availabilities expressed in two ways; mineral content in dialysate as a % of the total mineral in a vegetable, as well as the amount of minerals in the dialysate, db = dry basis.

iron contents (Table 1), which possibly influenced the amount of bioaccessible iron between the samples. Ethiopian kale has been found to contain high levels of β-carotene (Odongo et al., 2017), which enhances iron bioaccessibility (Gautam et al., 2010; Kruger et al., 2018). Drying result in losses of β-carotene and ascorbic acid in ALVs (Mutuli & Mbuge, 2018), which probably affected iron bioaccessibility in fermented and blanched samples. All processed ALVs had high levels of antinutrients (Table 2) which possibly influenced iron bioaccessibility. Phytate binds to iron making it unavailable for absorption (Gibson et al., 2010). Phytate/iron molar ratios of all processed ALVs ranged between 2.7 to 18.7 (Table 3) and were above the critical level of less than 1, which estimates iron bioavailability that is strongly impaired (Hurrell, 2004). ALVs had high levels of condensed tannins (58-220 mg CE/100 g) found in this study (Table 2) which had some inhibitory effects on iron bioaccessibility. Condensed tannins forms insoluble complexes with iron making it unavailable for absorption, and also binds with protein forming tannin-protein complexes inhibiting digestive enzyme activation that are crucial in breaking down food matrixes to release minerals in the lumen for absorption (Shahidi & Ambigaipalan, 2015). TPC form insoluble complexes with Fe^{3+} reducing the bioaccessibility of non-haem iron (Perron & Brumaghim, 2009), negatively influencing iron bioaccessibility. The strong positive association found between iron and aluminium contents (Figure 2) shows that some of the iron could be from extrinsic contamination

which has a questionable bioavailability. There is a controversy on the bioavailability of contaminant iron, human studies should be conducted to ascertain its bioavailability (Teklu, 2017).

Concerning zinc bioaccessibility, there were no significant ($p > 0.05$) differences found in percentage zinc bioaccessibility between cooked and fermented same type of ALVs, while higher amount of bioaccessible zinc was observed in blanched amaranth (Table 3), attributing to 50% lower total oxalate contents found in the blanched sample (Table 2). Oxalates binds to zinc forming an insoluble salt which are less absorbable and impairs zinc absorption (Lonnerdal, 2013). Phytate also has an inhibitory effect on zinc bioaccessibility (Gibson et al., 2010), forming insoluble complexes with zinc making the mineral unabsorbable. Phytate/zinc molar ratios of ALVs exceeded the critical level of 15 (four times higher) (Table 4), which predicts that zinc absorption from these ALVs is seriously impaired (Food Agriculture Organization, 2005). Calcium has the affinity to form complexes with phytate and zinc that are insoluble (Gibson et al., 2010). Phytate-calcium/zinc molar ratios for which zinc bioavailability is seriously impaired, are higher than the critical limit of 200 (Bindra et al., 1986). The phytate-calcium/zinc molar ratios in all processed ALVs were very high (921 to 3930) (Table 4), four to nineteen times higher than the critical limit of 200 which shows that the combined inhibitory effect of phytate and calcium on zinc was more pronounced.

3.5 The potential contribution of ALVs in this study towards absolute requirements for zinc and iron in women

For women aged 19 to 50 years a standard portion of 300 g (as consumed) of amaranth has the potential to contribute over 40% towards iron AR irrespective of processing (Figure 3 A) while spider plant could make the largest contribution of all the ALVs. However, *in vitro* dialysability assays does not entirely simulate all factors involved in human digestion because bioavailability has a physiological endpoint (Etcheverry et al., 2012), therefore these are not actual values but estimates.

Table 4. Phytate-mineral ratios of processed green leafy vegetables.

Molar ratios		CP	SP	WJ	A	EK
PA: Zn[1]	COO	78	20	48	69	47
	BLA	69	16	58	56	34
	FER	60	15	48	57	35
PA: Fe[2]	COO	7	3	6	13	13
	BLA	10	4	19	12	7
	FER	6	3	11	9	6
PA x Ca/ Zn[3]	COO	3585	1232	2038	3406	3419
	BLA	3187	921	3930	2789	1876
	FER	3316	982	3352	2443	2547

CP= Cowpea, SP= Spider plant, WJ = Wild jute, A= Amaranth, EK = Ethiopian kale, FER = Fermented, BLA = Blanched, COO = Cooked. [1](mg phytate/MW of phytate: mgZn/MW of Zn); [2](mg phyate/MW of phytate: mgFe/MW of Fe); [3](mg phytate/MW of phytate x mg Ca/MW of Ca / mg Zn/MW of Zn).

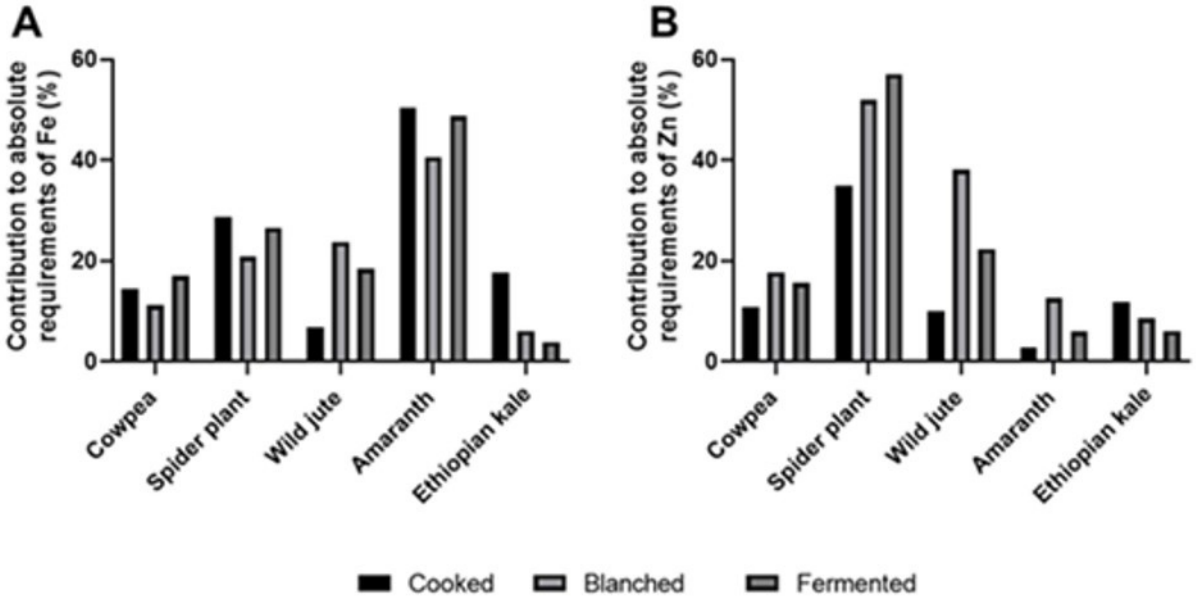

Figure 3. Contribution (%) of traditionally processed African leafy vegetables towards (A) absolute iron and (B) absolute zinc requirements of women aged 19 to 50 years.

3.6 Limitations and strengths of the study

These findings provide valuable information on predicting the direction of bioavailability of iron and zinc from processed ALVs to a wide audience in sub-Saharan Africa. ALVs are reconstituted and cooked for consumption when out of season, therefore the results of this study simulated the situation only after processing but not after preservation. ALVs were processed in duplicates, hence the low number of samples used could be a weakness of this study.

4 Conclusions

ALVs in this study were found to have relatively high levels of essential minerals, however, high antinutrient contents found possibly had an effect on their iron and zinc bioaccessibility. Therefore, to enhance iron and zinc bioaccessibility it is recommended that ALVs should be consumed concurrently with enhancers of iron and zinc. Further research is needed on the bioavailability of iron and zinc in humans. The current *in vitro* study can only predict the direction of iron and zinc bioavailability because *in vitro* models cannot simulate all in vivo conditions.

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