



## RESEARCH PAPER

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## Influence of drying and cooking methods on the nutritional value of four native mucilaginous vegetables consumed in Benin

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### Abstract

Native vegetables are rich in macronutrients and bioactive micronutrients, including antioxidants. Post-harvest treatments and different ways of cooking vegetables affect their nutritional and functional qualities. The impact of drying and cooking methods on the nutritional value and antioxidant properties of *Adansonia digitata* L., *Bombax brevisuspe* Sprague, *Ceiba pentandra* (L.) Gaertn leaves and *Grewia venusta* Fresen flowers was investigated. The protein, lipid, fibre, mineral and polyphenol contents of vegetables were analysed using reference methods as per AOAC (Association of Official Analytical Chemists). The results show that shade-dried during harmattan increases the protein (27.63%) and lipid (77.66%) contents of *A. digitata*, the protein (19.25%) and fibre (30.55%) contents of *B. brevisuspe*, the fibre (24.59%) contents of *G. venusta* and decreases the lipid (-2.90%) contents of *C. pentandra*. Cooking results in higher losses of iron (-16.26% to -71.96%), calcium (-52.09% to -78.13%) and vitamin C (-44.78% to -75.77%). Cooking also affects the antioxidant activity of the four vegetables, resulting in a decrease in polyphenol levels (-74.48%) of *A. digitata*, (-87.56%) of *B. brevisuspe*, (-80.03%) of *C. pentandra* and (-94.26%) of *G. venusta*. To ensure that the quality and quantity of nutrients in these vegetables are preserved, it is important to review traditional drying and cooking methods.

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## Introduction

Some woody species are used for cooking by their leaves, seeds and flowers. Generally, young tender leaves are harvested, cooked as a vegetable for relish and served mainly with starchy food (maize meal) (Mampholo *et al.*, 2015). Therefore, the consumption of TLVs can alleviate micronutrient deficiencies, which is especially important amongst women and children living in rural households (Mampholo *et al.*, 2015). It has been shown that baobab leaves are a pool of essential and protective compounds and minerals (Ogbaga *et al.*, 2017). The staple diets in sub-Sahara Africa, which consist largely of cereals, roots and tubers, are low in calcium and at the same time high in phytic acid, oxalic acid and tannic acids that can hinder the bioavailability of calcium. Baobab leaves are widely used, cooked as spinach, and frequently dried often powdered and used for sauces over porridges, thick gruels of grains or boiled rice (Abioye *et al.*, 2014). Nutritional iron deficiency arises when the iron concentration of the diet and/or its absorption from the intestine are suboptimal. Baobab leaves and fruit pulp have the potential of rectifying the problems (Habte and Krawinkel, 2017). In general, all types of baobab leaves were very rich in Ca, with higher levels in old than in young leaves of all types (Chadare *et al.*, 2014). Cooking brings many benefits for both the sanitary quality and the improvement of taste of food. Green vegetables are most prominent to seasonal fluctuations, growing well in the rainy season and usually scarce and costly during the dry season rice (Abioye *et al.*, 2014; Ogbaga *et al.*, 2017). Colour is an important quality attribute of food, which influences consumers' choice and preferences (Pathare *et al.*, 2013). Traditional mucilaginous vegetables are staple food for many people in Africa particularly in the Central region of the continent (Rahul *et al.*, 2015). In center and south Benin, the leaves and flowers usually dried are used for a soup known as slimy. Despite the importance of traditional vegetables in the diet, understanding of postharvest processing of traditional vegetables is limited. In order to improve the nutritional contents of mucilaginous vegetables-derived foods and

enhance the consumption level, it is important to understand the phytochemical and proximate contents of the vegetables shade-dried or cooked. The objectives of this study are to determine the nutritional content of traditional mucilaginous vegetables and to assess effects of processing method on chemical composition of *Adansonia digitata*, *Bombax brevicuspe*, *Ceiba pentandra* leaves and *Grewia venusta* flowers.

## Materials and methods

### Materials

The leaves of *A. digitata* and *B. brevicuspe* were collected in the village of Ouèdèmè, those of *C. pentandra* were harvested in Djallouma and the flower buds of *G. venusta* in Aglamidjodji in the department of Collines in central Benin during the dry season. These plants were previously authenticated by National Herbarium (University of Abomey-calavi, Benin). The fresh leaves and the flower buds of those species collected were washed under tap water. The fresh leaves were subsequently separated into 3 portions. To obtain shade dried samples, the leaf tissues (first portion matured) collected were spread in shade where there was no exposure to sunlight 36 hours during. The shade dried matured leaves were ground in a ceramic mortar and pestle into a powdered form. The ground and sieved powder was then transferred into air tight plastic containers and brought to laboratory and labelled accordingly. One portion was retained raw and others were cooked at 100°C during 15mn. A sample of 5g of each plant material portion was soaked in 100ml of distilled water for 24hrs. The solution was filtered using approximately 11cm diameter whatman filter paper. The extract was subsequently collected after 24 hrs and immediately used for phytochemical analyses (Ogbaga *et al.*, 2017).

### Methods

In this study, the leaves of *A. digitata*, *B. brevicuspe*, *C. pentandra* and the flowers of *G. venusta* that were shade-dried or cooked were analysed and compared to fresh vegetables.

*Physico-chemical analyses of dried vegetable powders*  
*Determination of mineral and vitamin C contents of*  
*raw vegetable powders*

Minerals such as calcium, iron and magnesium were determined according to the spectrophotometric method (AOAC, 1990).

*Dosage of vitamin C*

Ascorbic acid or vitamin C was extracted in the presence of a solution of metaphosphoric acid/acetic acid and measured by 2,6-dichlorophenol indophenol calibrated by the standard vitamin C of known concentration (Pongracz *et al.*, 1971). An amount (10mL) of acid (metaphosphoric acid / acetic acid) was added to 1g of powder of the extract and the mixture was stirred for 30 minutes and filtered on whatman paper N°1. 3mL of the filtrate was titrated with 2,6-dichlorophenol indophenol until a persistent champagne rose coloration appeared. The volume of 2,6-dichlorophenol indophenol (Ve) added was noted. A solution of metaphosphoric acid-acetic acid was also titrated with 2,6-dichlorophenol indophenol. The volume added (Vo) was noted. A 1mg/mL vitamin C solution prepared just before the assay was also titrated with 2,6-dichlorophenol indophenol under the same conditions. The volume paid (Vs) was noted. Each assay was performed in triplicate.

*Determination of lipid, protein and dietary fibre*  
*content of raw vegetable powders*

The samples were analysed for dry matter, crude lipid, crude protein and crude fibre. Dry matter was determined according to standard methods (AOAC, 2000). Crude lipid was extracted with petroleum ether by Soxhlet method (AOAC, 1990). Crude protein content was calculated by converting the nitrogen content ( $N \times 6.25$ ), determined by the Kjeldahl method (AOAC, 2000). Fibre determination is done according Osborne and Voogt (1978) method.

*Determination of secondary metabolite (total*  
*polyphenols) contents of raw vegetable extracts*

*Sample extraction*

The leaves of *A. digitata*, *B. brevisuspe*, *C. pentandra* and the flower of *G. venusta* were crushed. Water was used as an extraction solvent. The extracts were concentrated in a vacuum.

*Total polyphenols*

The polyphenol dosage method chosen uses the Folin-Ciocalteu reagent. The Folin-Ciocalteu reagent is phosphotungstic acid ( $H_3PW_{12}O_{40}$ ) and phosphomolybdic acid ( $H_3PMO_{12}O_{40}$ ) mixture. In basic solution, polyphenols reduce the yellow-coloured Folin-Ciocalteu reagent to blue-coloured tungsten and molybdenum oxide. The intensity of this blue color indicates the content of total polyphenol in the mixture. The latter shows a maximum absorption at 760nm, the intensity of which is proportional to the amount of polyphenols in the sample (Khadhri *et al.*, 2013). To quantify the total polyphenols in our samples, we used the method of Singleton *et al.* (1999) with some modifications using 96-well microplates with a transparent bottom. To 20 $\mu$ L of each sample contained in a well, we added 100 $\mu$ L of Folin-Ciocalteu reagent and then shake it for 30 seconds before incubating for 5 minutes. An addition of 80 $\mu$ L of  $Na_2CO_3$  to 75 mg.mL<sup>-1</sup> was made in each well to end the reaction. The absorbance readings of the wells were taken at 760nm. The polyphenols calibration was made from gallic acid (0.2mg/mL) with variable concentration from 0.04 to 0.16 mg.mL<sup>-1</sup>.

*Statistical analysis*

Calculations of nutrient and secondary metabolite content from vegetables were performed using Excel software to determine if there are differences between the sample types. Where significance was indicated, a one-way ANOVA with a Tukey post-hoc test was conducted. Different letters represent significant differences at  $P < 0.05$ .

**Results and discussion**

*Proximate composition of raw and dried mucilaginous*  
*vegetables consumed in Benin rural areas*

The analysis was carried out on fresh vegetables as control as well as the shade dried vegetables. The proximate composition of the shade dried mucilaginous vegetables examined in this study is presented in Table 1. The protein content after shade dried ranged from  $10.67 \pm 0.97g/100g$  (*C. pentandra*) to  $23.66 \pm 0.45g/100g$  (*B. brevisuspe*). Elegbede (1998) observed that increase in protein content of

dried leafy vegetables compared to fresh ones may occur as a result of loss of moisture which in turn has an influence on dry matter. These differences might be due to leaf maturity stage, environmental factors and harvesting method. These results are also in agreement with the studies done by Ogbaga *et al.* (2017) which showed that dried leaves retained good amounts of protein. Thus, dried vegetables could be good and affordable sources of protein for poor community of developing countries in order to fight against protein deficiency (Oulaï *et al.*, 2016). The presence of the highest number of glycosides specifically cardiac-glycosides in shade-dried samples relative to other samples suggest that the former have higher cholesterol lowering properties (Ogbaga *et al.*, 2017). Furthermore, Adak *et al.* (2017) showed that one effect frequently observed when drying foods is shrinkage, which considerably affects their structure and texture.

**Table 1.** The protein, lipid and fibre content of fresh (raw) and shade dried mucilaginous vegetables.

n=3	(g/100g)	Protein	Lipid	Fibre
<i>Adansonia</i>	Fresh	17.19±0.77 <sup>a</sup>	1.03±0.34 <sup>a</sup>	9.42 ± 1.00 <sup>a</sup>
<i>digitata</i>	Shade dried	21.94±0.23 <sup>b</sup>	1.83±0.17 <sup>b</sup>	10.7±0.89 <sup>a</sup>
<i>Bombax</i>	Fresh	19.84±1.18 <sup>a</sup>	1.71±0.33 <sup>a</sup>	11.29 ± 1.17 <sup>a</sup>
<i>brevicuspe</i>	Shade dried	23.66±0.45 <sup>b</sup>	1.98±0.13 <sup>a</sup>	14.74±0.49 <sup>b</sup>
<i>Ceiba</i>	Fresh	9.26±1.98 <sup>a</sup>	2.75±0.43 <sup>a</sup>	24.95 ± 0.60 <sup>a</sup>
<i>pentandra</i>	Shade dried	10.67±0.97 <sup>a</sup>	2.67±0.24 <sup>a</sup>	28.63±0.62 <sup>b</sup>
<i>Grewia</i>	Fresh	13.01±1.54 <sup>a</sup>	0.12±0.04 <sup>a</sup>	13.01± 1.74 <sup>a</sup>
<i>venusta</i>	Shade dried	11.80±0.47 <sup>a</sup>	0.68±0.28 <sup>b</sup>	16.21±0.66 <sup>b</sup>

\*Values are mean± standard deviation (3 replicates)

\*Values with same superscript between fresh and dried are not significantly different at p >0.05

In addition, it would be interesting to determine the amino acid profile of these leafy vegetables in order to evaluate some essential amino acids contents (Oulaï *et al.*, 2016). The lipid content was observed to range between 0.68±0.28 (*G. venusta*) and 2.67±0.24g/100g (*C. pentandra*). These values were closed to 2.46 of shade drying for baobab leaves (Abioye *et al.*, 2014) but leafy vegetables could not be considered as a rich source of lipid, which is in agreement with the fact that they are considered as “heart friendly food” (Oulaï *et al.*, 2016). The lipid increase in vegetables due to drying is most probably an apparent increase caused by loss of other vegetable components. Dietary fiber may be classified into three major groups (cellulose, non-cellulose and lignin)

according to their structure and properties. All the samples had increased crude fibre content with values ranging from 10.7±0.89g/100g (*A. digitata*) to 28.63±0.62 g/100g (*C. pentandra*). The shade dried samples had the highest values compared to the control samples (9.42±1.00-24.95±0.60g/100g). These values are however higher than those reported by Abioye *et al.* (2014) who reported a value of 8.13g/100g for baobab leaves. The results obtained in this study showed that shadow drying had concentration effect on the nutrients contents of the studied leafy vegetables, which would be related to leaf maturity stage of the dried vegetables. This increase in these levels would also be related to drying conditions; 36 hours under the shade during the harmattan. According to Abioye *et al.* (2014) shade drying represented a better method of ensuring high retention of nutrients while keeping aesthetic parameters that make for good acceptability. Dried vegetables can provide easy way of utilization as might be processed into powder, which is easy to use and/or mix in soup or porridge to enhanced micronutrients utilization to needy women at reproductive age and children below age of five years (Chege *et al.*, 2014; James and Matemu, 2016). According to Ogbaga *et al.* (2017), the variations might be due to stage of maturity of the sample.

*Proximate composition of raw and cooked mucilaginous vegetables consumed in Benin rural areas*

The analysis was carried out on fresh vegetables as control as well as the cooked vegetables. The proximate composition of cooked mucilaginous vegetables examined in this study is presented in Table 2. The high protein content in these vegetables can eliminate deficiencies amongst children, pregnant women and poor people living in rural areas which indicates that the TLVs can be used for building and repairing of body tissue, regulation of body process and formation of enzyme and hormones (Misra and Misra, 2014). Lipids help with brain function, joint mobilization and even energy production, they also help the body to absorb fat-soluble vitamins such as vitamins A and E (Adeniyi *et al.*, 2012). Fibre intake has a number of health benefits, including

maintenance of healthy laxation and the reduced risk of cardiovascular disease (Ehilé *et al.*, 2017). After 15mn, the residual contents of protein, lipid and fibre were: protein (10.21-16.20g/100g), lipid (1.04-3.74g/100g), fibre (11.00-31.00g/100g). The reduced protein contents of cooked leafy vegetables could be attributed to the severity of thermal process during cooking to protein degradation (Oulaï *et al.*, 2014). Protein digestibility is a major index of protein quality because a certain amount of amino-acids may be present in a food and it may not necessarily be available to the organism for nourishment. The denaturation of the protein and reduction in amino acid availability by cross-linking, racemization, degradation and formation of complexes with sugar may result in loss of digestibility. Therefore, when attempting to estimate protein quality, one of the first factors that must be evaluated is its digestibility. The relatively low level of protein in cooked leafy vegetables necessitates supplementation with animal proteins or proteins from legumes to have an impact on protein-energy malnutrition (Nangula *et al.*, 2010). However, 15 min cooked leaves of *A. digitata* and *B. brevicuspe* could be considered as non-negligible sources of proteins in view to their contents (16.20±0.60 and 13.00±0.09g/100g) which are higher than the minimal value (12%) recommended for protein foods (Ali, 2009).

**Table 2.** The protein, lipid and fibre content of raw and cooked (15mn) mucilaginous vegetables.

n=3	(g/100g)	Protein	Lipid	Fibre
<i>Adansonia digitata</i>	Raw	17.19±0.77 <sup>a</sup>	1.03±0.34 <sup>a</sup>	9.42 ± 1.00 <sup>a</sup>
	Cooked	16.20±0.60 <sup>a</sup>	1.92±0.22 <sup>b</sup>	12.06±0.48 <sup>b</sup>
<i>Bombax brevicuspe</i>	Raw	19.84±1.18 <sup>a</sup>	1.71±0.33 <sup>a</sup>	11.29 ± 1.17 <sup>a</sup>
	Cooked	13.00±0.09 <sup>b</sup>	1.04±0.13 <sup>b</sup>	11.00±0.69 <sup>a</sup>
<i>Ceiba pentandra</i>	Raw	9.26±1.98 <sup>a</sup>	2.75±0.43 <sup>a</sup>	24.95 ± 0.60 <sup>a</sup>
	Cooked	11.60±0.57 <sup>a</sup>	3.74±0.19 <sup>b</sup>	31.00±0.57 <sup>b</sup>
<i>Grewia venusta</i>	Raw	13.01±1.54 <sup>a</sup>	0.12±0.04 <sup>a</sup>	13.01± 1.74 <sup>a</sup>
	Cooked	10.21±0.75 <sup>a</sup>	1.23±0.09 <sup>b</sup>	24.67±0.58 <sup>b</sup>

\*Values are mean± standard deviation (3 replicates)

\*Values with same superscript between raw and cooked are not significantly different at p >0.05

As concern lipids, the low values contents at 15 min (1.04-3.74g/100g) in the studied cooked leafy vegetables corroborate the findings of many authors which showed that leafy vegetables are poor sources of fat (Ejoh *et al.*, 1996; Oulaï *et al.*, 2014).

Cooking of all selected leafy vegetables resulted in an increase (24.24-89.62%) in their crude fibres content at 15 min of cooking except *B. brevicuspe* (-02.56%). This finding is in agreement with some reports that cooking caused increase in soluble fibres content, with corresponding decrease in insoluble fibres content (Lintas and Cappelloni, 1998; Oulaï *et al.*, 2014). The increased temperature during cooking leads to breakage of weak bonds between polysaccharides and the cleavage of glycosidic linkages, which may result in solubilization of the dietary fibres (Svanberg *et al.*, 1997; Oulaï *et al.*, 2014).

Iron is required for blood formation and is said to be an important element in the diet of pregnant women, nursing mothers, infants convulsing patients and elderly to prevent anaemia and other related diseases (Alinor and Oze, 2011). Since iron deficiency (anaemia) is common in African region where baobabs grow, the leaves represent an important source of iron (Ogbaga *et al.*, 2017). Calcium is the most abundant mineral in humans existing as hydroxyapatite and very important to humans for its role in blood clotting, muscle contraction, neurological function, bone and teeth formation (Senga Kitumbe *et al.*, 2013). Magnesium is a cofactor in over 300 enzymatic reactions that regulate various biochemical reactions in the body including protein synthesis, muscle function, blood glucose, blood pressure and heart rate regulation (Rude *et al.*, 2009). Table 3 shows iron, calcium and magnesium composition of the cooked vegetables. The registered losses at 15mn were as follow: iron (16.26-71.96%), calcium (52.09-78.13%), magnesium (29.43-88.11%). There was a difference between raw and cooked mucilaginous vegetables. However, Chadare *et al.* (2014) reported that iron is not easily lost during cooking (5mn); this might be related to stable chemical binding to compounds that probably inhibit iron release such as phytates, calcium, and polyphenols. It can be estimated that the heat treatment is rather short and as such would not affect to a high extent the mineral content of the vegetables. According to Chadare *et al.* (2014) the higher Ca solubility in the cooked leaves may have resulted from the degradation of fibrous components.



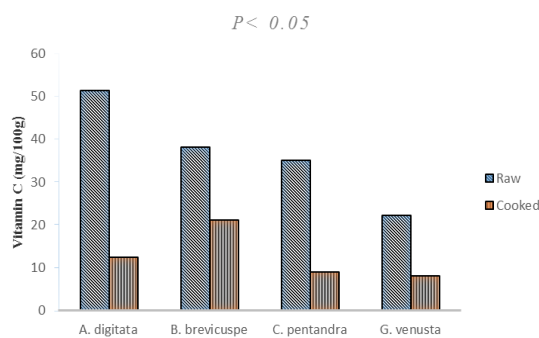
**Table 3.** The content of iron, calcium and magnesium in fresh (raw) and cooked (15mn) mucilaginous vegetables.

n=3	(mg/100g)	Iron	Calcium	Magnesium
<i>Adansonia digitata</i>	Raw	25.11±0.52 <sup>a</sup>	357.09±38.71 <sup>a</sup>	278.00±36.93 <sup>a</sup>
	Cooked	7.04±0.33 <sup>b</sup>	168.43±6.85 <sup>b</sup>	196.18±8.89 <sup>b</sup>
<i>Bombax brevicuspe</i>	Raw	6.70±0.43 <sup>a</sup>	107.34±3.36 <sup>a</sup>	518.05±46.01 <sup>a</sup>
	Cooked	3.00±0.20 <sup>b</sup>	51.42±3.02 <sup>b</sup>	61.56±1.61 <sup>b</sup>
<i>Ceiba pentandra</i>	Raw	18.03±0.57 <sup>a</sup>	475.56±7.56 <sup>a</sup>	667.55±46.96 <sup>a</sup>
	Cooked	6.01±0.22 <sup>b</sup>	104.00±4.17 <sup>b</sup>	165.11±3.46 <sup>b</sup>
<i>Grewia venusta</i>	Raw	20.54±1.49 <sup>a</sup>	228.70±26.82 <sup>a</sup>	478.23±18.62 <sup>a</sup>
	Cooked	17.20±0.46 <sup>b</sup>	88.21±1.15 <sup>b</sup>	76.18±0.22 <sup>b</sup>

\*Values are mean± standard deviation (3 replicates)

\*Values with same superscript between raw and cooked are not significantly different at p > 0.05

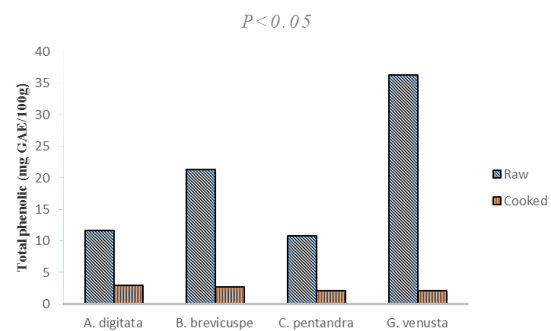
The effect of cooking time on vitamin C and polyphenols contents is depicted in fig. 1 and 2, respectively. Vitamin C is a powerful antioxidant essential for healthy formation of bone and teeth (Misra and Misra, 2014), it plays a huge role in maintaining an healthy lifestyle, and preventing diseases (Adjatin *et al.*, 2013). Losses of vitamin C contents at 15 min of cooking were (44.78-75.77%). These values support the results obtained for other studies which indicate losses up to 50-75% in cooked vegetables (Singh and Harshal, 2016). Prabhu and Barrett (2009) reported that leaves of the vegetables may absorb a large amount of cooking water and this can lead to dilution and further reduction in the level of vitamins in the cooked products. However, Hossain *et al.* (2017) reported that there was a significant increase of vitamin C content in boiled Indian spinach leaf samples and the percent gain due to boiling were 14.81.



**Fig. 1.** Effect of cooking on vitamin C content of mucilaginous vegetables consumed in Benin.

According to Rickman *et al.* (2007) the apparent increase may be attributed to loss of soluble solids: the rate of diffusion of ascorbic acid out of the cell

may be slower than that of other solids such as sugars. Another possible reason for the increment of vitamin C is that mucilaginous texture (non-starch polysaccharide) might slow down vitamin C degradation during cooking practices (Hossain *et al.*, 2017). It is important noting that ascorbic acid is a water-soluble antioxidant that promotes absorption of soluble iron by chelating or by maintaining the iron in the reduced form (Yamaguchi *et al.*, 2001).



**Fig. 2.** Effect of cooking on polyphenols content of mucilaginous vegetables consumed in Benin.

Vitamin C, a well-known potent dietary water soluble antioxidant, can react with peroxy radicals, and help control lipid peroxidation of cellular membranes (Hossain *et al.*, 2017). With regard to the drastic decrease of vitamin C during cooking, consumption of cooked leafy vegetables may be supplemented with other sources of vitamin C such as tropical fruits to cover the daily need for humans (40mg/day) as recommended by food and agriculture organization (Fao, 2004). Polyphenols have been linked to the reduced risk of developing chronic diseases such as, Alzheimer’s disease, cancer, inflammatory bowel syndrome and cardiovascular diseases in the human body (Saxena *et al.*, 2012).

Losses of polyphenols contents at 15 min of cooking were (74.48 – 94.26%). The decrease of the polyphenolic compounds content could be due to leaching or heat lability of specific flavonoids (Oulāi *et al.*, 2014). According to Moyo *et al.* (2017) the decrease of total phenolic content (TPC) may perhaps be due to the increase in temperature that accelerates reactions leading to oxidation processes and thereby prompting the available phenolics to be oxidized to

form compounds that do not react with the Folin-Ciocalteu reagent that is used in TPC analysis. In addition, phenolics available could have formed complexes with other nonphenolic compounds such as proteins and mineral ions (Moyo *et al.*, 2017). The negative impact of cooking on polyphenols content ( $p < 0.05$ ) may affect nutritional and functional properties of vegetables previously underlined. However, boiling and blanching could have induced hydrolysis of the plant cell wall, thereby releasing the dietary fiber-bound phenols with high antioxidant activity or encouraging the formation of compounds with higher antioxidant activity (Moyo *et al.*, 2017).

### Conclusion

Shade drying was found most suitable among the drying methods because it was able to retain most of the nutrients with significant difference in proximate contents from other drying methods. On the overall, shade drying represented a better method of ensuring high retention of nutrients while keeping aesthetic parameters that make for good acceptability. Therefore shadow drying should be encouraged as a way of ensuring all year round supply of vegetables and micronutrients to all risk groups. In order to reduce anti nutritional factor contents, traditional leafy vegetables are usually blanched and cooked for soups preparation. The cooking method not only affects the nutritional composition of the food, but also the level of available bioactive compound. Although the processing by heat increases food digestibility because it breakdown the food to complex proteins and carbohydrates, however vitamins, minerals and some other beneficial nutrients are lost. This food based approach may serve as a means of reducing “hidden hunger” in developing countries accompaniment for starchy foods, and value-added products. Future research on kinetic parameters describing vitamins decomposition during cooking should be carried out.

### Conflict of interests

The authors did not declare any conflict of interest

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