# Comparative Characterization of Trends and Patterns of Physical and Chemical Attributes of Optimal and Traditional Processed Cowpea Leaves 

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#### Abstract

Seasonality in the availability of cowpea leaves has often limited their utilization and thus the promotion of preservation techniques that convert the vegetables into storable and stable forms. The recommendations for the use of highly mechanized techniques in preservation are brought into question due to limited affordability among resource-constrained households that prefer less costly approaches. Therefore, this study used statistical techniques of principal component analysis to comparatively evaluate the trends of physicochemical quality of the two diverse approaches of processing cowpea leaves. The study evaluated dehydrated cowpea leaves of different processing techniques from farmer groups and optimally processed using modern techniques for nutritional composition, phytochemical compounds, and colour changes. Sun drying techniques that excluded blanching had the least content of beta-carotene and ascorbic acid, $2.65 \pm 0.95$ and $21.80 \pm 1.24 \mathrm{mg} / 100 \mathrm{~g}$ dry weight basis (dwb), respectively, accompanied by the most significant ( $p<0.001$ ) deterioration of colour $(7.74 \pm 3.49$ ) than techniques that included. Whereas the antinutrients declined, the difference did not significantly differ $(p>0.05)$ based on preservation techniques. With factor analysis determining optimal nutritional quality for cowpea leaves at 8 weeks after emergence, sun drying had the highest loss of beta-carotene and ascorbic acid, $66.7-80.1 \%$ and $53.7 \%-58.3 \%$, respectively ( $p<0.001$ ), whereas mineral leaching, reduction of antinutrients, and colour changes were more pronounced in dehydration techniques incorporating fermentation as pretreatment. For the traditional preservation techniques, increasing retention of minerals resulted in aggravated losses of betacarotene and ascorbic acid, whereas in the mechanized techniques, this was not the case. In concluding that the mechanized techniques have a better combination of attenuating losses of micronutrients, the study recommends that in promoting the utilization of traditional preservation techniques, low-cost processes like steam blanching can help improve the nutritional quality of the product.


## 1. Introduction

The vast utilization of cowpea leaves in sub-Saharan Africa (SSA) for food and nutrition security is due to their rich nutritional composition and their production in a variety of agroecological zones [1, 2]. Western and eastern Africa accounted for $85.1 \%$ and $7.8 \%$ of the 14.4 million hectares of global production area of the crop, respectively [3]. The crop has a dual purpose of utilization for its grains and vegetables,
which has made it popular among many communities in SSA [4]. Moreover, the vegetable is rich in phytochemicals with health-promoting properties that have aided the continued push for their utilization, including among urban communities [2,5]. Moreover, the crop has been exploited for nonfood uses for fodder [6, 7]. Its relative importance in the agricultural sector is due to its high productivity and stability, tolerance to environmental stress, economic viability, and low environmental impact coupled with its
capacity to promote environmental conservation [8]. Additionally, the crop has production flexibility to permit its production in mono and mixed cropping [9].

Cowpea leaves constitute one of the most consumed African leafy vegetables in Kenya [10]. The coastal areas of the country are among the regions with the highest production and consumption of the vegetable [7]. Thus, the vegetable forms a major component of their diet. However, seasonal availability of the crop often constrains its extended utilization among households. Reliance on fresh forms often exposes communities to shortages of such vegetables, especially in areas where there is much reliance on subsistent production [11]. Communities in the arid and semiarid lands (ASALs) of the country often incorporate traditional preservation techniques in order to enhance their utilization of the vegetable [11]. Traditional processing of the vegetables ranges from sun drying techniques to hurdle technology of blanching or cooking and drying and fermentation [12]. Over a quarter of the households in coastal areas were found to be reliant on traditional processed vegetables to overcome the shortage occasioned by seasonal availability [11]. The nutritional quality of processed products differs based on the technique utilized in processing. Whereas, Kirakou et al. [5] recommended blanching and fast-drying techniques, including solar drying for use in the processing of cowpea leaves due to their maximum nutrient retention; Owade et al. [11] established that sun drying, a more affordable technique, is the most utilized in the cowpea leaves value addition in the coastal and eastern arid and semiarid lands (ASALs) in Kenya. Therefore, it is not sufficient to be dismissive of these technologies as less efficient ways of availing vegetables for consumption despite the limited practice among communities.

This research study contributes to the promotion of the adoption of value-added techniques among producing households to enhance the all-season availability of the vegetable. The study sought to establish the trends and patterns in the retention and degradation of physicochemical attributes in value-added cowpea leaves subjected to either optimal or traditional processing techniques. The goal of this study was to use comparative statistical approaches to evaluate optimal and traditional processing techniques of cowpea leaves. This approach presents an objective way of mapping the differences and similarities in the physicochemical quality of vegetables subjected to different processing techniques. The study will shape nutrition information that is disseminated in nutrition interventions that promote value-added practices, especially in resourceconstrained settings in SSA.

## 2. Materials and Methods

2.1. Study Design. The study was undertaken in two phases. In the first phase, a survey was conducted among farmer groups processing cowpea leaves in Taita Taveta county, located between the latitudes $2^{\circ} 30^{\prime}$ and $4^{\circ} 30^{\prime}$ South and the longitudes $37^{\circ} 36^{\prime}$ and $39^{\circ} 14^{\prime}$ East and Kitui county that lies between the latitudes $0^{\circ} 10^{\prime}$ South and $3^{\circ} 0^{\prime}$ South and the latongitudes $37^{\circ} 50^{\prime}$ East and $39^{\circ} 0^{\prime}$ East. The study examined
the methods of processing cowpea leaves. Optimized processing techniques were selected from a review conducted by Owade et al. [7]. The selected dehydration techniques for cowpea leaves included solar drying, sun drying, and oven drying compared to local processing techniques that included sun and shade drying.

### 2.2. Phase I

2.2.1. Sample Collection and Preparation. A total of 30 samples of dehydrated cowpea leaves were obtained from 4 and 2 farmer groups in Taita Taveta and Kitui counties, respectively, who practised value-added practices for cowpea leaves. Samples were collected based on the processing technique: eight fresh, four shredded sun dried, two unshredded sun dried, two blanched sun dried, and two shadow dried from Taita Taveta county and four fresh, four shredded sun dried, and four unshredded sun dried from Kitui county. For this reason, the county of residence of the group was treated as a block rather than a factor. Samples were collected based on batches available during the weeklong study in the 2 areas. All samples were collected in May from Taita Taveta county and in October from Kitui county, 2020, when the leaves are most available, about 4 weeks after emergence, for at the time is when value addition of leaves are most practised. Collected samples, each weighing 2 kg , were placed in air-tight sterile polythene bags and placed in cooler boxes at -10C for transportation to the University of Nairobi Laboratories for analysis. Landraces were used. The 30 samples were subjected to compositing where $\sim 200 \mathrm{~g}$ obtained from each batch were mixed in a plastic tub based on similarity of the processing technique and similar farmer group to minimize effects of extraneous outliers due to individual variations in sample types. A total of 12 composites were obtained and evaluated for colour changes before being frozen awaiting nutritional analysis.

### 2.3. Phase II

2.3.1. Experimental Designs. This study utilized a combination of two experimental designs: the full factorial arrangement in the evaluation of the optimal maturity stage for harvesting of the cowpea leaves (Repert. Bot. 1:779.1843) and the completely randomized experimental study in the evaluation of the optimal processing. In the full factorial experiment, the experimental factors were the period of maturity and the variety of the cowpeas. On the other hand, in the completely randomized study, the experimental factor was the processing technique.
2.3.2. Evaluation of Optimal Stage of Maturity for Harvesting of Cowpea Leaves. (i) Experimental Arrangement. Two different varieties of cowpeas, Machakos 66 (M66, a dual purpose variety) and Kunde Mboga (predominantly for the leafy vegetables), were subjected to evaluation of their maturity indices and nutritional quality. The experiment was done in three different blocks to eliminate the effect of extraneous factors such as gradients of the soil and moisture.

The planting was done in two different planting seasons (April to August) and (September to August) in three different blocks at the University of Nairobi Field Station. The spacing of the plants was 60 cm by 30 cm as determined by Muniu [13]. The leaves of the different varieties were harvested at intervals of four weeks after emergence (WAE), transported to the laboratory, and stored at $-20^{\circ} \mathrm{C}$ awaiting analysis for nutrient and antinutrient contents.
(ii) Study Site. The study was done at the field station located at the College of Agriculture and Veterinary Sciences at the University of Nairobi, Nairobi County, Kenya. The field is located West of Nairobi County along the latitudes $1^{\circ}$ $15^{\prime} \mathrm{S}$, the longitudes $36^{\circ} 44^{\prime} \mathrm{E}$, and an altitude of 1820 m above sea level [14]. The area has an annual rainfall of 1060 mm , which has a bimodal distribution with long rains between March and May and short rains between October and December [15, 16]. The temperature ranges from 13.7 to $24^{\circ} \mathrm{C}$. The soils of the area are deep well-drained and dark reddish-brown to dark brown [14].
2.3.3. Evaluation of Optimal Dehydration of Cowpea Leaves. Optimal processing techniques of cowpea leaves have higher retention of the physicochemical quality when hurdle technology, a combination of pretreatment and dehydration, is used [7]. The study employed a completely randomized experimental design with the investigative factor being the dehydration technique. Kunde Mboga variety of cowpea leaves was harvested at optimal maturity, washed, cut, and divided into 2 batches. The first batch ( 15 kg ) was steam blanched for 2 min followed by immersion in ice-cold water and divided into 6 equal parts. Two parts each were dried using a forced air oven drier (at a temperature of $60^{\circ} \mathrm{C}$ for six hours), solar (at a maximum temperature of $70^{\circ} \mathrm{C}$ ), and the open sun on a raised platform. Drying was done till the leaves attained a moisture content below $15 \%$. The second batch was divided into 4 equal parts of 2.5 kg , and sugar and salt were added to each portion at $5 \%$ and $2 \%$, respectively, as established in our earlier study [17]. Fermented vegetables were dried in a forced air oven (at a temperature of $60^{\circ} \mathrm{C}$ for six hours) and solar dried (at a maximum temperature of $70^{\circ} \mathrm{C}$ ) till moisture content below $15 \%$ was attained. Dried cowpea leaves were evaluated for colour changes and then stored at $-20^{\circ} \mathrm{C}$ awaiting evaluation of nutrient and antinutrient composition.

### 2.4. Analysis of Physicochemical Attributes of Processed Cowpea Leaves

2.4.1. Determination of Proximate Composition. The proximate composition was determined as moisture, crude fat, crude ash, crude fibre, and crude protein contents in duplicates as per the methods $950.46,960.39,920.153,962.09$, and 955.05 of AOAC [18], respectively. The carbohydrate content was thereafter determined using the difference method as per the procedure described by Greenfield and Southgate [19]. The energy values of the traditionally preserved cowpea leaves were determined by multiplying the protein, carbohydrate, and fat contents ( $\mathrm{g} / 100 \mathrm{~g}$ ) by 17, 17,
and 37 , respectively, and separately adding the values for each sample.
2.4.2. Determination of Vitamin C Content. Ascorbic acid content was determined in duplicates as per method 967.21-1968 [20]. Standardization of the dichlorophenolindophenol (DCPIP) reagent was accomplished by titrating it three times with 2 mL of standard ascorbic acid solution ( $0.02 \%$ in $5 \%$ metaphosphoric acid). The titration was done until a rose-pink colour persisted for $>5 \mathrm{~s}$. A blank of $5 \%$ metaphosphoric acid was titrated three times. To a 10 g sample of the dehydrated cowpea leaves, 60 mL of $5 \%$ metaphosphoric acid was added and filtered using gravity through glasswool into a 100 mL volumetric flask. This was made to volume and 10 mL was placed into a 100 mL conical flask and titrated against DCPIP. The titre of the dye was determined as per equation (1). The amount of ascorbic acid in the dried vegetables was determined as per equation (2).

$$
\begin{equation*}
\operatorname{Titre}(F)=\frac{n}{b-a} \tag{1}
\end{equation*}
$$

where $n$ is the mg of ascorbic acid per ml of titrated standard solution, in this case, it is ( mg of ascorbic aci $\mathrm{d} \times 2$ ) $/ 50, a$ is the titre of the standard used, and $b$ is the titre of the blank.

$$
\begin{equation*}
\operatorname{Ascorbic} \operatorname{acid}\left(m g g^{-1}\right)=x-c \times \frac{f}{e} \times \frac{v}{y} \tag{2}
\end{equation*}
$$

where $x$ is the titre volume used for the sample, $c$ is the titre used for the blank, $f$ is the mg of ascorbic acid equivalent to 1 ml of DCPIP solution, $e$ is the assayed volume ( 2 ml ), $v$ is the volume of the initial assay solution $(10 \mathrm{ml})$, and $y$ is the volume of sample aliquot ( 10 ml ).

### 2.4.3. Determination of Beta-Carotene Content.

 Beta-carotene was determined calorimetrically using the spectrophotometry method adopted through modification of the methods described by Biswas et al. [21].Preparation of a standard curve: A stock solution of betacarotene ( $5 \%$ purity, Sigma Aldrich) was dissolved in acetone to make a concentration of $1 \mathrm{mg} / \mathrm{ml}$. The stock solution was used to make working solutions of $32,16,8,4,2,1$, $0.025,0.125,0.062,0.03$, and $0 \mu \mathrm{~g} / \mathrm{ml}$. A standard curve was generated on a UV-VIS spectrophotometer (Hitachi U-2900, Tokyo, Japan). The concentration was expressed in mg/ml. All standards were protected from the light by covering them with aluminium foil.

Sample preparation: Dried samples of the vegetables ( 1 g ) were mixed with 5 ml of chilled acetone and left at $4^{\circ} \mathrm{C}$ for 15 minutes with occasional shaking, vortexed at high speed for 10 minutes, and centrifuged at $1370 \times g$ for 10 minutes. The supernatant was collected in a tube and the extraction was repeated until a clear supernatant with no colouration was obtained. The supernatant was filled to a volume of 50 ml . The supernatant was passed through a Whatman paper no. 42 and the absorbance read at 450 nm . The concentration of beta-carotene was calculated as per the following equation.

$$
\begin{equation*}
b=\frac{C \times V}{M} \tag{3}
\end{equation*}
$$

where $b$ is the beta-carotene in $\mathrm{mg} / \mathrm{g}, C$ is the concentration determined as per the calibration curve, $V$ is the volume of the extract in ml , and $M$ is the weight of the sample used in extraction.
2.4.4. Determination of Mineral Content. The mineral (calcium, zinc, and iron) content was determined in duplicates using an atomic absorption spectrometer as per the AOAC [18] methods. A 2 g sample of cowpea leaves was ashed at $550^{\circ} \mathrm{C}$, followed by boiling in 10 ml of $20 \%$ hydrochloric acid in a beaker. The boiled solution was filtered into a 100 ml standard flask and then read using atomic absorption spectrometry (Buck Scientific 210 VGP, USA).
2.4.5. Determination of Oxalate Content. The oxalate content of the traditionally preserved cowpea leaves was determined in duplicate as per the procedures by AOAC [18] methods. About 1 g of preserved cowpea leaf samples were weighed into a 100 ml conical flask. To it, 75 ml of $3 \mathrm{~mol} / \mathrm{l}$ of $\mathrm{H}_{2} \mathrm{SO}_{4}$ was added and the solution was stirred using a magnetic stirrer. The solution was filtered through Whatman filter paper no. 1, and the filtrate was collected in a 250 ml conical flask. From this sample filtrate, 25 ml of it was titrated against hot $\left(80-90^{\circ} \mathrm{C}\right) 0.1 \mathrm{~N} \mathrm{KMnO}_{4}$ solution, with a persistent faint pink colour ( 30 seconds) indicating the endpoint. The oxalate content was calculated as 1 ml of 0.1 N of $\mathrm{KMnO}_{4}$ is equivalent to 0.006303 g of oxalate.
2.4.6. Determination of Nitrate Content. The nitrate content of the traditionally preserved cowpea leaf and cowpea vegetable samples was determined in duplicate by modification of procedures described by Gaya and Alimi [22]. Samples of the vegetable were ground using a mortar and pestle and to 10 g of the ground samples, 70 ml distilled water was added followed by 2.5 ml of $4 \% \mathrm{NaOH}$. The mixture was heated at $80^{\circ} \mathrm{C}$ for 25 minutes, with occasional shaking during heating. Thereafter, the resultant solution was filtered into a 100 ml volumetric flask through a fluted filter paper and filled to mark with distilled water to form a mixture 2 . About 4 ml of mixture 2 was pipetted into an icecold test tube followed by the addition of 1 ml of $1 \% \mathrm{Ag}_{2} \mathrm{SO}_{4}$, 7 ml of $98 \% \mathrm{H}_{2} \mathrm{SO}_{4}$, and 1 ml of $5 \%$ phenol solution to form mixture 3 that was left to stand in the dark for 20 minutes while occasionally shaking. Mixture 3 was transferred into a 50 ml separating funnel and toluene was added (mixture 4) and further shaken for 5-10 minutes to mix. The upper phase of mixture 4 (organic phase) was retained, while the aqueous phase was discarded. The organic phase was washed twice with 10 ml of distilled water, and each time, the aqueous phase was discarded. The organic phase was extracted further by the addition of 10 ml of $10 \% \mathrm{Na}_{2} \mathrm{CO}_{3}$ and shaken for a minute. The extract was collected in a test tube. The absorbance was read at 407 nm in a UV-VIS spectrophotometer (Hitachi U-2900, Tokyo, Japan). Standard curves
were generated by varying the concentrations of sulphuric acid, $\mathrm{Na}_{2} \mathrm{CO}_{3}$, and the phenol and reaction time of standard nitrogen nitrate solution. The quantity of nitrates was calculated as shown in equation (4):

$$
\begin{equation*}
\text { Nitrate }=\frac{C \times S}{W \times F} \tag{4}
\end{equation*}
$$

where $C$ is the concentration of the nitrates in the samples as per the calibration curve, $S$ is the volume of filtrate used to read the absorbance, $W$ is the weight of slurry used, and $F$ is the total volume of the filtrate.
2.4.7. Determination of Total Phenolic Compounds. The total phenolic content of the preserved samples of cowpea leaves was determined using the Folin-Ciocalteu procedure that was adopted through modification of the methods described by Abong et al. [23]. A 5 g sample of the vegetables was subjected to extraction by adding 5 ml of methanol followed by a twenty-four-hour extraction at $25^{\circ} \mathrm{C}$. The extract was centrifuged at $3226 \times g$ for 10 min , and the resulting supernatant was used to determine the total phenolic content. To an aliquot of 1 ml of methanolic extract in a 10 ml volumetric flask, 2.5 ml of tenfold dilution of Folin-Ciocalteu reagent ( $1: 10$ dilution with distilled water) was added, followed by 2 ml of $7.5 \%(\mathrm{w} / \mathrm{v})$ sodium carbonate solution. The mixture was topped to volume and incubated at $45^{\circ} \mathrm{C}$ for 15 minutes. The samples were read against a standard calibration curve of gallic acid monohydrate prepared by obtaining $0.25,0.5,1.0,1.5$, and $2.0 \mathrm{mg} / \mathrm{ml}$, followed by a similar treatment as the methanolic extracts. The calibration curve of the standard was in $\mathrm{mg} / \mathrm{ml}$ with an $R^{2}$ of 0.995 . Distilled water was used as the blank. The samples were read at 765 nm using a UV-VIS spectrophotometer (Hitachi U-2900, Tokyo, Japan), and the total phenolic content was expressed as mg per gallic acid equivalent (GAE) per gram as per equation (5):

$$
\begin{equation*}
P=\frac{C \times V}{M} \tag{5}
\end{equation*}
$$

where $P$ is the total phenolic content in $\mathrm{mg} / \mathrm{g}, C$ is the concentration determined as per the calibration curve, $V$ is the volume of the extract in ml , and $M$ is the weight of the sample used in extraction.
2.4.8. Determination of Flavonoid Contents. The flavonoid content of the samples was determined using the aluminium chloride colourimetric procedure by modifying the procedures described by Abong et al. [23]. A standard calibration curve was generated using a catechin solution. From a stock solution of $100 \mu \mathrm{~g} / \mathrm{ml}$ ( $\mathrm{w} / \mathrm{v}$ of methanol) of catechin, aliquots of $0.1,0.2,0.4,0.6,0.8$, and 1.0 ml were obtained and transferred into five 10 ml volumetric flasks containing 4 ml of water, followed by addition of sodium nitrite and left to rest for five minutes. After five minutes, 0.3 ml of $10 \%(\mathrm{w} / \mathrm{v})$ aluminium chloride was added and allowed to rest further for six minutes. To the rested mixture, 2 ml of 1 N sodium hydroxide was added and filled to volume. The standard curve was calibrated in $\mathrm{mg} / \mathrm{ml}$ with an $R^{2}$ of 0.995 . The
methanolic extract obtained as per the extraction procedures for determining total phenolics was subjected to treatment similar to catechin standards. The concentration of total flavonoids was determined in milligrams of catechin equivalents per gram (mg. CE. $\mathrm{g}^{-1}$ ) as per equation (6):

$$
\begin{equation*}
F=\frac{C \times V}{M}, \tag{6}
\end{equation*}
$$

where $F$ is the total flavonoid content in $\mathrm{mg} / \mathrm{g}, C$ is the concentration determined as per the calibration curve, $V$ is the volume of the extract in ml , and $M$ is the weight of the sample used in extraction.
2.4.9. Determination of Antioxidant Activity. The antioxidant activity of the leaves was determined using the 2, 2-diphenyl-1picrylhydrazyl (DPPH) procedure by modifying the methods described by Abong et al. [23]. Methanolic extract of preserved samples of cowpea leaves was prepared by mixing 0.25 g of the sample with 10 ml of $80 \%(\mathrm{v} / \mathrm{v})$ of methanol, with overnight extraction in a shaker. About 1 ml of the methanolic extract, standard Trolox solutions ( $0,5,10,25$, and $50 \mu \mathrm{~g} / \mathrm{ml}$ ), and blank were pipetted into boiling tubes and $0.002 \%$ of DPPH (prepared using absolute methanol) was added to each. The mixture was shaken briefly and read immediately upon the addition of DPPH at 515 nm in a UV-VIS spectrophotometer (Hitachi U-2900, Tokyo, Japan). A standard calibration curve of Trolox was used to calculate the antioxidant activity of the preserved cowpea leaves in $\mu \mathrm{M}$ Trolox equivalents (TE) per 100 g dry weight.
2.5. Determination of Colour Changes. The $\mathrm{L}^{*}, \mathrm{a}^{*}$, and $\mathrm{b}^{*}$ and chroma and hue angles of the dried cowpea leaves were determined as per the procedures described by the manufacturer (PCE Instruments, 2014). Using the CSCQ3 software, the hue, chroma, and $\Delta \mathrm{E}$ were calculated based on equations (7)-(10). The value of $L^{*}$ represented the lightness of the vegetable samples (more positive values have lighter colour intensity), the value $a^{*}$ represented the measure of redness (positive), greyness (zero), or greenness (negative), and the value $b^{*}$ represented the measure of yellowness (positive), greyness (zero), or blueness (negative).

$$
\begin{equation*}
\text { Hue angle }(\mathrm{Ho})=\arctan (b / a)(\text { for }+a \text { and }+b \text { values }), \tag{7}
\end{equation*}
$$

$$
\begin{align*}
\text { Hue angle }(\mathrm{Ho})= & \arctan (\mathrm{b} / \mathrm{a})+180(\text { for }-\mathrm{a} \text { and } \\
& +\mathrm{b} \text { values or for }-\mathrm{a} \text { and }-\mathrm{b} \text { values }), \tag{8}
\end{align*}
$$

Chroma angle $\left(C^{o}\right)=\sqrt{a^{2}+b^{2}}$,

$$
\Delta \mathrm{E}=\sqrt{\left(a_{1}^{*}+\mathrm{a}_{2}^{*}\right)^{2}+\left(b_{1}^{*}+\mathrm{b}_{2}^{*}\right)^{2}+\left(L_{1}^{*}+L_{2}^{*}\right)^{2}}
$$

2.6. Statistical Analysis. Statistical analysis of the data was done using the $R$ language for programming (ver. 4.0.3, [24]). A one-way analysis of varaince with blocking was used to test for mean differences induced by local processing techniques on
the physical and chemical qualities of cowpea leaves. For means that were significantly different, $p<0.05$, Tukey's honest significant difference (HSD) in the Agricolae package was used to separate them. One-way ANOVA, without blocking, was used to test differences in means of physical and chemical attributes of optimally preserved cowpea leaves and means were separated using Tukey's HSD. Principle component analysis was used to map patterns of nutrient retention in the samples. The data for optimization of the maturity stage of cowpea leaves were analyzed using two-way ANOVA. Akaike's Information Criterion of the AICmodav package was used to select the model that best explained the variation of the nutritional composition of cowpea leaves, and Tukey's HSD of the Agricolae package was used to separate means.

## 3. Results and Discussion

3.1. Physicochemical Qualities of Traditionally Processed Cowpea Leaves. There was a significant $(p<0.05)$ difference in the proximate composition of cowpea leaves based on the processing technique (Table 1). Whereas the crude fat content ( $4.3 \pm 0.3 \mathrm{~g} / 100 \mathrm{~g}$ dry weight) was high in blanched and sun-dried leaves than in fresh and other preserved samples, there was a decline in the crude ash content ( $p<0.05$ ). This low crude ash content is pronounced with significantly ( $p<0.05$ ) low mineral, iron, and calcium contents in the blanched and sun-dried leaves (Table 2). The leaching of minerals explained the declining sodium, iron, and zinc contents in the blanched dehydrated vegetables. The use of water rather than steam blanching aggravates the loss of the minerals in water [25]. On the positive end, the moisture levels reported in the traditionally preserved products were within the recommended limit by specific standards that permit up to $15 \%$ [26]. The moisture content established in this study was also within the range of documented studies by Owade et al. [7]. It is imperative to maintain moisture below $15 \%$ in order to prevent quality deterioration occasioned by microbial growth due to less optimal moisture content [27]. Hag et al. [28] established a critical limit of $\leq 14 \%$ for the growth of microorganisms in dehydrated African leafy vegetables.

The utilization of artisanal traditional processing for preservation resulted in a significant loss $(p<0.05)$ of micronutrients. Sun-dried leaves had the least amount of beta-carotene and ascorbic acid. This is caused by losses induced by photo-oxidation activity catalyzed by UV radiation [29]. UV-induced oxidation converts the beta-carotene from the provitamin A form to derivatives with less vitamin A activity. Additionally, exposure to factors such as heat that induces drying and oxygen also accelerates the oxidation of both beta-carotene and ascorbic acid [30]. Without blanching, the losses are aggravated due to increased oxidation of the two micronutrients with antioxidant activity [25, 29]. Whereas the antioxidant activities of the preserved samples significantly ( $p<0.001$ ) decreased with the application of traditional preservation techniques, the antinutrient content in the vegetables remained invariably high (Table 3). Moreover, degradation in colour also occurred with a significantly high deviation ( $p<0.001$ )

Table 1: Proximate composition of traditional preserved cowpea leaves (per $100 \mathrm{~g} / \mathrm{dmb}$ ).

| Processing <br> technique | Moisture $(\mathrm{g})$ | Crude protein $(\mathrm{g})$ | Crude fat $(\mathrm{g})$ | Crude fibre $(\mathrm{g})$ | Crude ash $(\mathrm{g})$ | Carbohydrates $(\mathrm{g})$ | Energy values <br> $(\mathrm{kcal})$ |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| S1 | $10.3 \pm 0.3^{\mathrm{b}}$ | $33.3 \pm 3.0^{\mathrm{a}}$ | $4.3 \pm 0.3^{\mathrm{a}}$ | $15.9 \pm 2.3^{\mathrm{a}}$ | $8.2 \pm 1.0^{\mathrm{b}}$ | $51.7 \pm 4.4^{\mathrm{a}}$ | $371.2 \pm 7.9^{\mathrm{a}}$ |
| S2 | $87.0 \pm 0.6^{\mathrm{a}}$ | $31.0 \pm 0.4^{\text {ab }}$ | $2.9 \pm 0.4^{\mathrm{b}}$ | $15.5 \pm 0.5^{\mathrm{a}}$ | $14.1 \pm 0.4^{\mathrm{a}}$ | $47.8 \pm 0.9^{\mathrm{b}}$ | $341.0 \pm 2.0^{\mathrm{c}}$ |
| S3 | $10.6 \pm 0.3^{\mathrm{b}}$ | $27.6 \pm 1.1^{\mathrm{b}}$ | $1.9 \pm 0.5^{\mathrm{c}}$ | $15.1 \pm 0.7^{\mathrm{a}}$ | $15.0 \pm 1.2^{\mathrm{a}}$ | $52.3 \pm 2.2^{\mathrm{a}}$ | $336.7 \pm 8.3^{\mathrm{b}}$ |
| S4 | $11.0 \pm 0.5^{\mathrm{b}}$ | $29.8 \pm 1.0^{\mathrm{c}}$ | $1.9 \pm 0.2^{\mathrm{c}}$ | $14.5 \pm 2.3^{\mathrm{a}}$ | $13.4 \pm 0.5^{\mathrm{a}}$ | $52.8 \pm 2.6^{\mathrm{a}}$ | $347.4 \pm 9.9^{\mathrm{b}}$ |
| \%CV | 109.1 | 15.3 | 104.6 | 15.5 | 19.3 | 8.0 | 5.1 |
| HSD | 29.6 | 1.78 | 3.02 | 14.6 | 4.3 | 1.55 | 6.49 |
| $P$ value | $<0.001$ | $<0.001$ | $<0.001$ | 0.050 | $<0.001$ | $<0.001$ | $<0.001$ |

The values are mean $\pm$ SD of duplicates. Values with different letters in the superscript along the column are statistically different. S1, blanched sun dried; S2, fresh leaves; S3, shadow dried; S4, unblanched sun dried. All the variables are in dry matter basis except for moisture content.

Table 2: Micronutrient composition of traditional preserved cowpea leaves ( $\mathrm{mg} / 100 \mathrm{~g}$ dry matter basis).

| Processing technique | Beta-carotene | Vitamin C | Zinc | Iron | Calcium | Sodium |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: |
| S1 | $4.13 \pm 1.96^{\mathrm{ab}}$ | $27.99 \pm 7.06^{\mathrm{b}}$ | $2.27 \pm 0.92^{\mathrm{a}}$ | $15.18 \pm 6.11^{\mathrm{b}}$ | $36.30 \pm 6.31^{\mathrm{b}}$ | $16.60 \pm 5.89^{\mathrm{b}}$ |
| S2 | $8.40 \pm 8.17^{\mathrm{a}}$ | $90.56 \pm 33.57^{\mathrm{a}}$ | $5.59 \pm 4.53^{\mathrm{a}}$ | $75.93 \pm 18.80^{\mathrm{a}}$ | $51.34 \pm 3.12^{\mathrm{a}}$ | $75.84 \pm 19.52^{\mathrm{a}}$ |
| S3 | $3.55 \pm 0.57^{\mathrm{a}}$ | $66.92 \pm 11.41^{\mathrm{a}}$ | $2.06 \pm 1.89^{\mathrm{a}}$ | $21.79 \pm 5.77^{\mathrm{b}}$ | $38.73 \pm 6.97^{\mathrm{b}}$ | $16.42 \pm 1.53^{\mathrm{b}}$ |
| S4 | $2.65 \pm 0.95^{\mathrm{a}}$ | $21.80 \pm 1.24^{\mathrm{b}}$ | $3.31 \pm 0.77^{\mathrm{a}}$ | $32.94 \pm 7.84^{\mathrm{b}}$ | $36.78 \pm 6.18^{\mathrm{b}}$ | $16.68 \pm 1.67^{\mathrm{b}}$ |
| \%CV | 78.8 | 30.2 | 58.2 | 71.7 | 15.6 | 61.2 |
| HSD | 4.46 | 55.6 | 3.29 | 34.2 | 10.1 | 30.2 |
| $P$ value | 0.032 | $<0.001$ | 0.294 | $<0.001$ | 0.003 | 0.034 |

The values are mean $\pm$ SD of duplicates. Values with different letters in the superscript along the column are statistically different. S1, blanched sun dried; S2, fresh leaves; S3, shadow dried; S4, unblanched sun dried.

Table 3: Antinutrient content and antioxidant activity of traditional preserved cowpea leaves ( $\mathrm{mg} / 100 \mathrm{~g}$ dry matter basis).

| Processing <br> technique | Nitrates $(\mathrm{mg})$ | Oxalates (mg) | Total phenolics (mg GAE) | Flavonoids (mg CE) | Antioxidant activity ( $\mu \mathrm{M} \mathrm{TE}$ ) |
| :--- | :---: | :---: | :---: | :---: | :---: |
| S1 | $509.02 \pm 138.55^{\mathrm{b}}$ | $151.90 \pm 25.73^{\mathrm{b}}$ | $20.75 \pm 2.64^{\mathrm{a}}$ | $4.45 \pm 2.17^{\mathrm{ab}}$ | $21.90 \pm 12.16^{\mathrm{b}}$ |
| S2 | $731.19 \pm 73.48^{\mathrm{a}}$ | $142.86 \pm 29.83^{\mathrm{b}}$ | $23.10 \pm 9.91^{\mathrm{a}}$ | $7.78 \pm 1.67^{\mathrm{a}}$ | $45.01 \pm 1.55^{\mathrm{a}}$ |
| S3 | $389.96 \pm 11.72^{\mathrm{c}}$ | $141.62 \pm 28.99^{\mathrm{b}}$ | $17.02 \pm 1.19^{\mathrm{a}}$ | $1.92 \pm 0.11^{\mathrm{b}}$ | $3.25 \pm 2.67^{\mathrm{b}}$ |
| S4 | $495.26 \pm 245.62^{\mathrm{bc}}$ | $191.85 \pm 21.63^{\mathrm{a}}$ | $25.69 \pm 2.73^{\mathrm{a}}$ | $6.39 \pm 2.69^{\mathrm{ab}}$ | $20.40 \pm 6.17^{\mathrm{b}}$ |
| \%CV | 20.2 | 15.3 | 20.5 | 46.8 | 44.9 |
| HSD | 177.7 | 35.8 | 21.9 | 5.73 | 23.0 |
| $P$ value | $<0.001$ | $<0.001$ | 0.092 | 0.016 | $<0.001$ |

The values are mean $\pm$ SD of duplicates. Values with different letters in the superscript along the column are statistically different. S1, blanched sun dried; S2, fresh leaves; S3, shadow dried; S4, unblanched sun dried.
occurring in preservation techniques that did not combine blanching (Table 4). The colour coordinates for $\mathrm{b}^{*}$, $\mathrm{a}^{*}$, and chroma and hue angles were significantly different from the fresh vegetables. It is recommended that in processing, such leaves are subjected to blanching as a pretreatment as it attenuates loss of beta-carotene and antioxidants and improves colour retention [25, 31]. Pretreatment like blanching is known to improve colour retention through attenuating continued oxidation of coloured pigments [25].

### 3.2. Physicochemical Qualities of Optimally Processed Cowpea Leaves

3.2.1. Optimization of the Stage of Maturity for Harvesting Cowpea Leaves. The promotion of cowpea leaves in the food security initiative hinges on their rich micronutrient and phytochemical composition [6]. Using the WSSplot, the optimal number of clusters was determined as three for the classification of the nutritional and antinutrient contents of
cowpea leaves harvested at different maturity stages. Cluster one had the optimal content of protein and micronutrients (Table 5). This had the advantage of establishing optimal trends of increasing nutrient content while minimizing the accumulation of antinutrients. Whereas seasonal variation had no difference in loading in the different clusters, the variety of cowpea leaves and the stage of maturity of leaves differed in loading amongst the three clusters. Kunde Mboga variety and cowpea leaves harvested at eight weeks after emergence (WAE) had the highest loading in cluster one (Tables 6 and 7). In choosing the most optimal period of harvesting, the cluster with the highest number of positive values for the normalized means of protein and micronutrient contents and the highest number of negative values for the antinutrient contents was selected and cluster two met this criterion.

Independent variable for Model_1 is the main effect of type of variety, season, and period of harvesting in weeks after emergence; Model_2 is main effect of type of variety; Model_3 is main effect of season; Model_4 is main effect of

Table 4: Colour changes of traditional preserved cowpea leaves.

| Processing technique | L | a | b | C | H | $\Delta \mathrm{E}$ |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: |
| S1 | $43.10 \pm 1.39^{\mathrm{ab}}$ | $-1.23 \pm 0.32^{\mathrm{c}}$ | $7.00 \pm 1.28^{\mathrm{ab}}$ | $7.11 \pm 1.26^{\mathrm{ab}}$ | $100.19 \pm 3.06^{\mathrm{a}}$ | $3.33 \pm 0.90^{\mathrm{b}}$ |
| S2 | $40.77 \pm 3.55^{\mathrm{b}}$ | $0.36 \pm 0.36^{\mathrm{a}}$ | $2.47 \pm 1.70^{\mathrm{b}}$ | $2.53 \pm 1.69^{\mathrm{b}}$ | $80.82 \pm 12.42^{\mathrm{b}}$ | NA |
| S3 | $45.11 \pm 3.52^{\mathrm{a}}$ | $-1.10 \pm 0.23^{\mathrm{c}}$ | $8.47 \pm 2.78^{\mathrm{ab}}$ | $8.54 \pm 2.78^{\mathrm{ab}}$ | $97.60 \pm 1.42^{\mathrm{a}}$ | $5.39 \pm 3.41^{\mathrm{ab}}$ |
| S4 | $47.82 \pm 3.52^{\mathrm{a}}$ | $-0.72 \pm 0.59^{\mathrm{b}}$ | $9.67 \pm 2.18^{\mathrm{a}}$ | $9.72 \pm 2.12^{\mathrm{a}}$ | $95.06 \pm 4.78^{\mathrm{a}}$ | $7.74 \pm 3.49^{\mathrm{a}}$ |
| \%CV | 6.06 | 60.5 | $22.1 \%$ | 21.6 | 9.49 | 66.1 |
| HSD | 4.2 | 0.37 | 6.7 | 6.8 | 10.1 | 2.4 |
| $P$ value | $<0.001$ | $<0.001$ | $<0.001$ | $<0.001$ | $<0.001$ | $<0.001$ |

The values are mean $\pm$ SD of duplicates. Values with different letters in the superscript along the column are statistically different. S1, blanched sun dried; S2, fresh leaves; S3, shadow dried; S4, unblanched sun dried.

Table 5: Normalized means of clustered nutrient and antinutrient composition of cowpea leaves harvested at different maturity stages.

| Chemical composition | Cluster 1 | Cluster 2 | Cluster 3 |
| :--- | :---: | :---: | :---: |
| Moisture content $(\mathrm{g})$ | 0.86 | 0.26 | -0.83 |
| Crude protein $(\mathrm{g})$ | 0.47 | 0.28 | -0.65 |
| Crude ash $(\mathrm{g})$ | 1.36 | -0.43 | -0.03 |
| Crude fat | -0.72 | 0.62 | -0.57 |
| Crude fibre | 2.02 | -0.46 | -0.32 |
| Carbohydrate $(\mathrm{g})$ | -1.33 | 0.49 | -0.08 |
| Beta-carotene $(\mathrm{mg})$ | 0.18 | 0.75 | -1.22 |
| Vitamin C $(\mathrm{mg})$ | 0.07 | -0.34 | 0.47 |
| Nitrates (mg) | -0.24 | -0.42 | 0.75 |
| Oxalates (mg) | -1.15 | 0.58 | -0.29 |
| Total phenolics (mg) | 1.43 | -0.24 | -0.35 |
| Flavonoids (mg) | 1.06 | -0.02 | -0.49 |
| Zinc (mg) | 1.96 | 0.62 | -0.05 |
| Iron (mg) | 1.30 | 0.10 | -0.49 |
| Sodium (mg) | 0.63 | 0.44 | -0.98 |
| Calcium (mg) | 2.08 | -0.44 | -0.39 |
| Total antioxidant $(\mu \mathrm{M} \mathrm{TE})$ | -0.57 | 0.85 | -1.00 |

TABLE 6: Loading of independent variables for optimization of stage of maturity of cowpea leaves in clusters.

| Independent variables |  | Clusters |  |  |
| :--- | :---: | :---: | :---: | :---: |
|  |  | 1 | 2 | 3 |
| Seasons | Season 1 | 50 | 50 | 50 |
|  | Season 2 | 50 | 50 | 50 |
|  | 4 | 50 | 33 | 0 |
| Variety | 8 | 0 | 67 | 0 |
|  | 12 | 50 | 0 | 100 |
|  | Machakos 66 | 100 | 33 | 0 |
|  | Kunde Mboga | 0 | 67 | 100 |

period of harvesting in weeks after emergence; and Model_5 is the interaction factors of type of variety, season, and period of harvesting in weeks after emergence.

Seasonal variation had no effect on the micronutrient or antinutrient content of cowpea leaves. The beta-carotene, sodium, and calcium contents were higher in the Kunde Mboga variety than in the dual-purpose variety of "Machakos 66" (Table 8). Similarly, the flavonoids and total phenolics had higher concentrations in the Kunde Mboga variety than in "Machakos 66" (Table 9). The Kunde Mboga is an improved variety that was developed for its vegetables rather than its grains, thus the better nutrition profile of the
vegetables than the M66, dual-purpose, and variety. With an increasing period of growth, cultivated cowpea leaves accumulate more antinutrients. This is not the trend for the micronutrients and protein content Muchoki [32]; Kirigia et al. [2]. Cowpea leaves like other African leafy vegetables present the property of high content of phytochemicals including antinutrients [33]. Beta-carotene was higher in cowpea leaves harvested at 8 WAE than at 4 or 12 WAE. Zinc and calcium contents increased with increasing WAE, whereas sodium content decreased. The interaction between variety and stage of harvest affected zinc and crude fibre contents (Table 10). At 4 WAE, cowpea leaves from Machakos 66 variety had higher crude fibre content than "Kunde Mboga." At 12 WAE, leaves from the latter had higher crude fibre content than the former (Figure 1). Zinc content in the leaves harvested from "Machakos 66" decreased over time of harvest. In the Kunde Mboga variety, it increased (Figure 2). Whereas it is desirable to have higher micronutrient content in the vegetable, the fibre content should not be high. More mature leaves tend to have higher fibre content Ohler et al. [34], thus being tougher for consumption as vegetables [35].
3.2.2. Nutrient Composition of Optimally Processed Cowpea Leaves. The hurdle concept (combination of two preservation techniques) offers the advantage of attenuating quality losses in the vegetables while improving the desirable product attributes such as sensory and textural properties [7, 29]. The focus is primarily on higher retention of micronutrients such as vitamins and minerals, with the loss of the latter first depicted by crude ash content. Fermented dehydrated vegetables had significantly ( $p<0.001$ ) high crude ash than blanched leaves (Table 10). In optimizing the fermentation process of cowpea leaves, Owade et al. [17] added salt (sodium chloride) at a proportion of $2 \%(\mathrm{w} / \mathrm{w})$, explaining the elevated crude ash level in the fermented dehydrated leaves. The fibre content in the fermented dehydrated leaves significantly ( $p<0.001$ ) declined, whereas the moisture content significantly ( $p<0.001$ ) increased. Soluble fibre is also broken down during lactic acid fermentation Nyman [36], so the fermented leaves have lower fibre content than the blanched. Oven drying techniques achieved the least amount of moisture of all the dehydration techniques ( $p<0.001$ ), which is desirable for prolonging the shelf-life of the dried product. Dried leaves with high

Table 7: AIC model selection criterion for independent factors affecting nutrient and antinutrient composition of cowpea leaves harvested at different maturity stages.

| Response variables | Delta values for models prediction (AIC weight \%) <br> Model_1 |  |  |  | Model_2 |
| :--- | :---: | :---: | :---: | :---: | :---: |

Independent variable for Model_1 is the main effect of type of variety, season and period of harvesting in weeks after emergence; Model_2 is main effect of type of variety; Model_3 is main effect of season; Model_4 is main effect of period of harvesting in weeks after emergence; and Model_5 is interaction factors of type of variety, season and period of harvesting in weeks after emergence.

Table 8: Main effect crop variety and stage of harvesting on the micronutrient content of cowpea leaves.

| Independent variable |  | Micronutrient (per 100 g dry matter basis) |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Beta-carotene (mg) | Ascorbic acid (mg) | Zinc (mg) | Iron (mg) | Sodium (mg) | Calcium (mg) |
| Crop variety | Kunde Mboga | $17.81 \pm 1.06{ }^{\mathrm{Aa}}$ | $64.02 \pm 57.63{ }^{\text {Aa }}$ | $7.04 \pm 3.83{ }^{\text {Aa }}$ | $26.79 \pm 10.68{ }^{\text {Aa }}$ | $58.74 \pm 10.59^{\text {Aa }}$ | $40.95 \pm 30.84{ }^{\text {Aa }}$ |
|  | $\begin{gathered} \text { Machakos } \\ 66 \end{gathered}$ | $14.78 \pm 1.49^{\text {Ab }}$ | $82.37 \pm 24.54{ }^{\text {Aa }}$ | $6.29 \pm 1.30^{\mathrm{Aa}}$ | $27.30 \pm 10.35^{\text {Aa }}$ | $12.21 \pm 3.59^{\text {Ab }}$ | $16.67 \pm 8.77^{\text {Ab }}$ |
| \%CV |  | 7.93 | 60.5 | 42.9 | 38.9 | 22.3 | 78.7 |
| HSD |  | 1.09 | 37.5 | 2.42 | 8.9 | 6.7 | 19.1 |
| $P$ value |  | <0.001 | 0.321 | 0.53 | 0.906 | <0.001 | 0.016 |
| Stage of harvesting (WEA) |  | $16.04 \pm 1.85{ }^{\text {Bb }}$ | $89.01 \pm 53.99^{\text {Ba }}$ | $6.06 \pm 1.89^{\text {Bab }}$ | $23.62 \pm 6.80^{\mathrm{Ba}}$ | $42.79 \pm 1.81{ }^{\text {Ba }}$ | $15.51 \pm 6.34^{\text {Bb }}$ |
|  | 8 | $17.84 \pm 1.36^{\mathrm{Ba}}$ | $55.42 \pm 8.51{ }^{\text {Ba }}$ | $5.20 \pm 1.01^{\text {Bb }}$ | $29.81 \pm 9.27^{\mathrm{Ba}}$ | $33.56 \pm 0.36^{\mathrm{Bb}}$ | $15.98 \pm 5.05^{\text {Bb }}$ |
|  | 12 | $15.00 \pm 1.76^{\mathrm{Bb}}$ | $75.15 \pm 5.55^{\mathrm{Ba}}$ | $8.74 \pm 3.72^{\mathrm{Ba}}$ | $27.71 \pm 13.89{ }^{\text {Ba }}$ | $30.07 \pm 2.97^{\text {Bb }}$ | $54.93 \pm 9.74^{\mathrm{Ba}}$ |
| \%CV |  | 7.93 | 60.5 | 42.9 | 38.9 | 22.3 | 78.7 |
| HSD |  | 1.56 | 56.6 | 3.4 | 13.4 | 7.4 | 19.6 |
| $P$ value |  | $<0.001$ | 0.544 | 0.058 | 0.450 | $<0.001$ | <0.001 |

The values are mean $\pm$ SD of duplicates. Values with similar uppercase letters followed by a different lowercase in the superscript along the column are statistically different.

Table 9: Main effect crop variety and stage of harvesting on the antinutrient and antioxidant contents of cowpea leaves.

| Independent variables |  | Antinutrient content and antioxidant activity per 100 g dry matter basis |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Nitrates (mg) | Oxalates (mg) | Total phenolics (mg GAE) | Flavonoids (mg CE) | Total antioxidant activity ( $\mu \mathrm{M}$ TE) |
| Crop variety | Kunde <br> Mboga | $278.71 \pm 5.78{ }^{\text {Aa }}$ | $1.74 \pm 0.65^{\text {Aa }}$ | $26.08 \pm 5.83{ }^{\text {Aa }}$ | $8.16 \pm 5.07^{\text {Aa }}$ | $26.40 \pm 8.94{ }^{\text {Aa }}$ |
|  | $\begin{gathered} \text { Machakos } \\ 66 \end{gathered}$ | $429.30 \pm 72.94{ }^{\text {Aa }}$ | $2.16 \pm 0.60^{\text {Aa }}$ | $15.66 \pm 1.57^{\text {Ab }}$ | $5.90 \pm 4.05^{\text {Ab }}$ | $21.19 \pm 5.49^{\text {Aa }}$ |
| \%CV |  | 59.8 | 32.6 | 17.8 | 30.6 | 53.6 |
| HSD |  | 179.8 | 0.54 | 3.2 | 1.8 | 10.8 |
| $P$ value |  | 0.096 | 0.115 | $<0.001$ | 0.02 | 0.329 |
| Stage of harvesting (WEA) | 4 | $621.79 \pm 134.42^{\text {Ba }}$ | $1.58 \pm 0.20^{\text {Aa }}$ | $4.04 \pm 2.08^{\text {Ac }}$ | $0.90 \pm 0.15^{\mathrm{Bb}}$ | $21.22 \pm 5.73^{\text {Ba }}$ |
|  | 8 | $206.24 \pm 35.22^{\text {Bb }}$ | $2.79 \pm 0.20^{\text {Aa }}$ | $23.15 \pm 8.58{ }^{\text {Ab }}$ | $9.73 \pm 1.77^{\mathrm{Ba}}$ | $33.94 \pm 8.90^{\mathrm{Ba}}$ |
|  | 12 | $234.00 \pm 59.71{ }^{\text {Bb }}$ | $1.49 \pm 0.30^{\text {Aa }}$ | $35.42 \pm 6.90^{\text {Aa }}$ | $10.47 \pm 1.58{ }^{\text {Ba }}$ | $16.21 \pm 2.88^{\mathrm{Ba}}$ |
| \%CV |  | 59.8 | 32.6 | 17.8 | 30.6 | 53.6 |
| HSD |  | 266.9 | 0.80 | 4.7 | 2.7 | 16.1 |
| $P$ value |  | 0.001 | 0.770 | <0.001 | <0.001 | 0.441 |

The values are mean $\pm$ SD of duplicates. Values with similar uppercase letters followed by a different lowercase in the superscript along the column are statistically different.

Table 10: Proximate composition of optimally dried cowpea leaves (per 100 g ).

| Processing <br> technique | Moisture $(\mathrm{g})$ | Crude protein <br> $(\mathrm{g})$ | Crude fat $(\mathrm{g})$ | Crude fibre $(\mathrm{g})$ | Crude ash (g) | Carbohydrates |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: |

*Significant at $p<0.05$. **Significant at $p<0.01$. The values are mean $\pm$ SD of duplicates. Values with different letters in the superscript along the column are statistically different. A, blanched oven dried; B, blanched sun dried; C, blanched solar dried; D, fermented oven dried; E, fermented sun dried; F, fermented solar dried; G, fresh leaves.


Figure 1: Effect of the interaction between crop variety and weeks after emergence (WAE) on crude fibre content of cowpea leaves. Error bars indicate the standard error of the means.


Figure 2: Effect of the interaction between crop variety and weeks after emergence (WAE) on the zinc content of the leaves. The error bars indicate standard error of the means.
moisture content encourage microbial spoilage of the product and thus will have a short shelf-life [28].

The effect of photo-oxidation in the reduction of the labile micronutrients explains the loss of $66.7-80.1 \%$ in beta-carotene and $53.7-58.3 \%$ in ascorbic acid content in the sun-dried vegetables (Table 11). Hurdle technology combining fermentation and sun drying had the least
retention of beta-carotene (19.8\%) and ascorbic acid (41.7\%). Combining dehydration techniques with fermentation resulted in a reduction in iron and zinc contents in the vegetables as compared to those combining dehydration with blanching. On the other hand, the sodium content of all the dehydrated leaves combined with fermentation was relatively high, more than even the fresh vegetables ( $p<0.001$ ). Incorporating salting in the dehydration processes for enhanced preservation resulted in reduced beta-carotene, ascorbic acid, zinc, and iron contents, whereas sodium, calcium, and moisture contents were relatively higher [37]. The fermentation period of 16 days enhances the leaching of micronutrients from the processed leaves into water [17].

Incorporating fermentation in the processing of dehydrated cowpea leaves significantly ( $p<0.001$ ) reduced the antinutrient contents of the leaves (Table 12). The nitrates, followed by the oxalates, had the highest decline when fermentation techniques were included in the processing. It is desirable that the two compounds are low in foods due to their negative effects on the bioavailability of micronutrients [38]. It is also desirable that the techniques retain the physical attributes, such as the colour of the products. The use of a combination of two preservation techniques in the processing of cowpea leaves seeks to minimize quality loss while improving sensory and textural properties [7, 29]. Whereas all dehydration techniques induced deterioration of the colour of the preserved samples, sun dried samples processed through hurdle technology had the highest deviation ( $p<0.001$ ), see Table 13. Exposure to UV radiation during sun drying destroys the colour pigments, including the chlorophyll and the carotenoids, thus the high deviation in colour [39].

### 3.3. Comparative Characterization of Retention of Physico-

 chemical Quality of Optimally and Traditional Processed Cowpea Leaves. Essentially, dehydrated vegetables should have a closer similarity in quality to fresh vegetables when cooked to enhance consumer acceptability of these preserved forms. In finding the blanched solar-dried leaves as the most acceptable in the evaluation of the impact ofTable 11: Micronutrient composition of optimally processed cowpea leaves ( $\mathrm{mg} / 100 \mathrm{~g}$ dry matter basis).

| Processing technique | Beta-carotene | Ascorbic acid | Zinc | Iron | Calcium | Sodium |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: |
| A | $22.65 \pm 0.06^{\mathrm{a}}$ | $136.00 \pm 1.88^{\mathrm{b}}$ | $8.90 \pm 2.42^{\mathrm{b}}$ | $17.07 \pm 3.31^{\mathrm{ab}}$ | $14.12 \pm 4.00^{\mathrm{b}}$ | $19.95 \pm 4.00^{\mathrm{c}}$ |
| B | $7.34 \pm 0.03^{\mathrm{f}}$ | $76.83 \pm 0.60^{\mathrm{c}}$ | $6.54 \pm 0.28^{\mathrm{bc}}$ | $25.99 \pm 8.44^{\mathrm{a}}$ | $19.84 \pm 2.06^{\mathrm{b}}$ | $15.81 \pm 2.06^{\mathrm{c}}$ |
| C | $16.80 \pm 0.05^{\mathrm{c}}$ | $51.06 \pm 0.19^{\mathrm{e}}$ | $13.91 \pm 0.86^{\mathrm{a}}$ | $23.03 \pm 10.58^{\text {ab }}$ | $19.75 \pm 2.19^{\mathrm{b}}$ | $15.85 \pm 2.19^{\mathrm{c}}$ |
| D | $13.41 \pm 0.00^{\mathrm{e}}$ | $38.82 \pm 0.06^{\mathrm{f}}$ | $2.20 \pm 0.63^{\text {de }}$ | $8.17 \pm 4.09^{\mathrm{b}}$ | $19.08 \pm 1.55^{\mathrm{b}}$ | $102.12 \pm 1.55^{\mathrm{a}}$ |
| E | $4.38 \pm 0.11^{\mathrm{g}}$ | $69.27 \pm 0.87^{\mathrm{d}}$ | $1.35 \pm 0.37^{\mathrm{e}}$ | $14.15 \pm 3.50^{\mathrm{ab}}$ | $17.15 \pm 3.50^{\mathrm{b}}$ | $87.51 \pm 3.50^{\mathrm{a}}$ |
| F | $15.21 \pm 0.07^{\mathrm{d}}$ | $75.46 \pm 0.85^{\mathrm{c}}$ | $5.26 \pm 1.12^{\text {cd }}$ | $9.07 \pm 2.69^{\mathrm{b}}$ | $16.38 \pm 2.14^{\mathrm{b}}$ | $94.52 \pm 5.14^{\mathrm{a}}$ |
| G | $22.06 \pm 0.04^{\mathrm{b}}$ | $165.97 \pm 0.11^{\mathrm{a}}$ | $13.51 \pm 1.34^{\mathrm{a}}$ | $22.65 \pm 0.52^{\mathrm{ab}}$ | $46.29 \pm 4.72^{\mathrm{a}}$ | $27.90 \pm 4.72^{\mathrm{c}}$ |
| \%CV | 41.1 | 69.7 | 64.2 | 84.4 | 30.3 | 55.5 |
| HSD | 0.1666 | 2.46 | 3.37 | 16.0 | 9.91 | 20.2 |
| $P$ value | $<0.001$ | $<0.001$ | $<0.001$ | 0.009 | $<0.001$ | $<0.001$ |

The values are mean $\pm$ SD of duplicates. Values with different letters in the superscript are statistically different. A, blanched oven dried; B, blanched sun dried; C, blanched solar dried; D, fermented oven dried; E, fermented sun dried; F, fermented solar dried; G, fresh leaves.

Table 12: Antinutrient content of optimally processed cowpea leaves ( 100 g dry matter basis).

| Processing technique | Nitrates $(\mathrm{mg})$ | Oxalates $(\mathrm{mg})$ | Total phenolics $(\mathrm{mg}$ GAE) | Flavonoids $(\mathrm{mg}$ CE) | Antioxidant activity $(\mu \mathrm{M} \mathrm{TE})$ |
| :--- | :---: | :---: | :---: | :---: | :---: |
| A | $232.89 \pm 31.40^{\mathrm{c}}$ | $274.85 \pm 8.78^{\mathrm{a}}$ | $43.36 \pm 6.30^{\mathrm{ab}}$ | $9.78 \pm 0.25^{\mathrm{ab}}$ | $37.18 \pm 3.12^{\mathrm{a}}$ |
| B | $303.31 \pm 22.63^{\mathrm{b}}$ | $106.44 \pm 1.0 \mathrm{c}^{\mathrm{d}}$ | $44.96 \pm 2.20^{\mathrm{ab}}$ | $9.38 \pm 0.08^{\mathrm{ab}^{\mathrm{b}}}$ | $36.09 \pm 1.98^{\mathrm{a}}$ |
| C | $218.40 \pm 3.48^{\mathrm{c}}$ | $215.73 \pm 3.82^{\mathrm{b}}$ | $44.69 \pm 2.72^{\mathrm{ab}}$ | $10.31 \pm 0.32^{\mathrm{a}}$ | $36.07 \pm 0.94^{\mathrm{a}}$ |
| D | $180.54 \pm 38.74^{\mathrm{c}}$ | $128.04 \pm 7.76^{\mathrm{cd}}$ | $36.13 \pm 0.08^{\mathrm{bc}}$ | $8.27 \pm 0.83^{\mathrm{b}}$ | $38.08 \pm 0.63^{\mathrm{a}}$ |
| E | $181.60 \pm 4.83^{\mathrm{c}}$ | $116.55 \pm 15.37^{\mathrm{cd}}$ | $28.93 \pm 5.06^{\mathrm{c}}$ | $9.83 \pm 0.5 \mathrm{a}^{\mathrm{b}}$ | $44.10 \pm 8.31^{\mathrm{a}}$ |
| F | $181.60 \pm 4.83^{\mathrm{c}}$ | $137.12 \pm 4.88^{\mathrm{c}}$ | $31.71 \pm 0.12^{\mathrm{c}}$ | $9.00 \pm 1.00^{\mathrm{ab}}$ | $34.66 \pm 4.36^{\mathrm{a}}$ |
| G | $760.00 \pm 34.29^{\mathrm{a}}$ | $217.92 \pm 6.60^{\mathrm{b}}$ | $48.42 \pm 5.35^{\mathrm{a}}$ | $9.97 \pm 0.01^{\mathrm{ab}}$ | $38.86 \pm 0.91^{\mathrm{a}}$ |
| \%CV | 36.4 | 72.5 | 80.4 | 5.9 | 26.3 |
| HSD | 68.5 | 22.5 | 10.9 | 1.6 | 22.4 |
| $P$ value | $<0.001$ | $<0.001$ | $<0.001$ | 0.010 | 0.840 |

The values are mean $\pm$ SD of duplicates. Values with different letters in the superscript along a column are statistically different. A, blanched oven dried; B, blanched sun dried; C, blanched solar dried; D, fermented oven dried; E, fermented sun dried; F, fermented solar dried; G, fresh leaves.

Table 13: Colour changes of optimally processed cowpea leaves.

| Processing technique | L | a | b | C | H | $\Delta E$ |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: |
| A | $39.28 \pm 3.21^{\mathrm{ab}}$ | $-2.54 \pm 0.02^{\mathrm{c}}$ | $7.12 \pm 0.03^{\mathrm{a}}$ | $7.56 \pm 0.03^{\mathrm{ab}}$ | $109.60 \pm 0.09^{\mathrm{a}}$ | $16.62 \pm 1.95^{\mathrm{b}}$ |
| B | $37.77 \pm 3.46^{\mathrm{b}}$ | $-0.11 \pm 0.14^{\mathrm{bc}}$ | $1.93 \pm 0.70^{\mathrm{a}}$ | $1.94 \pm 0.71^{\mathrm{b}}$ | $92.53 \pm 2.73^{\mathrm{cd}}$ | $22.05 \pm 1.49^{\mathrm{a}}$ |
| C | $38.35 \pm 0.03^{\mathrm{b}}$ | $-0.25 \pm 0.40^{\mathrm{bc}}$ | $3.30 \pm 1.37^{\mathrm{a}}$ | $3.33 \pm 1.34^{\mathrm{b}}$ | $96.33 \pm 7.63^{\mathrm{bc}}$ | $20.48 \pm 0.92^{\mathrm{a}}$ |
| D | $50.13 \pm 6.17^{\mathrm{a}}$ | $0.80 \pm 0.35^{\mathrm{ab}}$ | $6.16 \pm 0.34^{\mathrm{a}}$ | $6.22 \pm 0.29^{\mathrm{ab}}$ | $82.51 \pm 3.58^{\mathrm{d}}$ | $15.35 \pm 0.27^{\mathrm{b}}$ |
| E | $37.77 \pm 3.46^{\mathrm{b}}$ | $-0.11 \pm 0.14^{\mathrm{bc}}$ | $1.93 \pm 0.70^{\mathrm{a}}$ | $1.94 \pm 0.71^{\mathrm{b}}$ | $92.53 \pm 2.73^{\mathrm{cd}}$ | $22.05 \pm 1.49^{\mathrm{a}}$ |
| F | $50.13 \pm 6.17^{\mathrm{a}}$ | $0.80 \pm 0.35^{\mathrm{ab}}$ | $6.16 \pm 0.34^{\mathrm{a}}$ | $6.22 \pm 0.2 \mathrm{ab}^{\mathrm{ab}}$ | $82.51 \pm 3.58^{\mathrm{d}}$ | $15.35 \pm 0.27^{\mathrm{b}}$ |
| G | $26.94 \pm 2.38^{\mathrm{b}}$ | $3.84 \pm 3.06^{\mathrm{a}}$ | $-10.22 \pm 5.64^{\mathrm{b}}$ | $11.06 \pm 6.04^{\mathrm{a}}$ | $105.70 \pm 0.02^{\mathrm{ab}}$ | NA |
| \%CV | 40.1 | 42.3 | 95.4 | 43.4 | 73.4 | 66.8 |
| HSD | 11.35 | 3.30 | 6.2 | 6.1 | 10.47 | 3.40 |
| $P$ value | $<0.001$ | $<0.001$ | $<0.001$ | 0.003 | $<0.001$ | $<0.001$ |

The values are mean $\pm$ SD of duplicates. Values with different letters in the superscript along a column are statistically different. A, blanched oven dried; B, blanched sun dried; C, blanched solar dried; D, fermented oven dried; E, fermented sun dried; F, fermented solar dried; G, fresh leaves.
preservation techniques on sensory attributes, deterioration of textural properties and colour was minimized by Natabirwa et al. [40]. Artisanal techniques that employ the use of sun drying techniques excluding blanching as a pretreatment result in alteration both in textural and colour properties [41]. The correlation maps generated through principal component analysis for the nutrient composition of locally processed cowpea leaves showed that with limited retention of beta-carotene content, the antioxidant activity and crude protein content also deteriorate (Figure 3). Additionally, the utilization of techniques that improved the retention of the minerals (sodium, calcium, zinc, and iron) aggravated the losses of antioxidant activity and beta-
carotene. However, blanching has been found to attenuate deterioration of antioxidants and colour, so their inclusion improves quality amelioration. The optimally processed cowpea leaves that incorporated blanching as a pretreatment had higher retention of crude protein and beta-carotene (Figure 4). The loss of the minerals was not aggravated by the use of processing techniques that improved the retention of beta-carotene. Even with blanching, the use of hot water as in the case of traditional processing rather than steam as in the optimal techniques has the disadvantage of aggravating the leaching of minerals [5]. Limited leaching of minerals coupled with attenuation of labile nutrients such as betacarotene improves nutrient retention.


Figure 3: Correlation of plots showing clustering of the physical and chemical attributes of locally processed cowpea leaves.

Variables located in the same quadrant are positively correlated; those located in opposed quadrants are negatively correlated. The distance of a variable from the origin measures the quality of representation in the 2 principal components (Dim $1=$ first principal component (micronutrients and physical attributes) and Dim $2=($ macronutrients and antioxidant activity). Variables closer to the margin of the circle are represented by the 2 principal components. $\mathrm{L}^{*}, \mathrm{a}^{*}$, and $\mathrm{b}^{*}$ are the coordinates for the colour space and PCA represents the principal component analysis.

Variables located in the same quadrant are positively correlated; those located in opposed quadrants are negatively correlated. The distance of a variable from the origin measures the quality of representation in the 2 principal components (Dim $1=$ first principal component (micronutrients and antinutrients) and Dim $2=$ second principal component (macronutrients)), with variables closer to the margin of the circle being represented by the 2 principal components. $\mathrm{L}^{*}, \mathrm{a}^{*}$, and $\mathrm{b}^{*}$ are the coordinates for the colour space and PCA represents the principal component analysis.


Figure 4: Correlation of plots showing clustering of the physicochemical attributes of optimally processed cowpea leaves.

## 4. Conclusion

This study concludes that the mix of techniques utilized in the traditional preservation of cowpea leaves lacks balance in the trends of retention of essential nutrients in the products. The incorporation of mechanized techniques introduces a balance and attenuates losses of these essential micronutrients. Even so, this should not be the reason for dismissing the traditional processing techniques as a means of improving vegetable availability among households for the leaves still had significant amounts of beta-carotene, zinc, and iron, some of the micronutrients whose deficiencies are prevalent in Africa. This study would thus recommend that initiatives promoting the utilization of similar traditional techniques of preservation evaluated in this study should coopt for some of the low-cost pretreatments such as steam blanching in order to improve the nutritional quality of the products.

## Data Availability

The data used and/or analyzed during the current study are available from the corresponding author upon request.

## Consent

This manuscript was presented as a part of a thesis at the Faculty of Agriculture, University of Nairobi Kenya. The source is cited as Owade [42].

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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