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Effect of spontaneous fermentation of cowpea leaves (Vigna unguiculata) on proximate composition, mineral content, chlorophyll content and beta-carotene content

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Abstract: The practice of fermentation has been used throughout the world for centuries as a means of preservation and or improvement of food safety and quality. Fermented foods gain unique sensory qualities and an increased shelf-life. The effects of fermentation of cowpea leaves (Vigna unguiculata) on the proximate composition, mineral content, chlorophyll and beta-carotene content of the leaves were investigated. The fermentation method was adopted from sauerkraut technology, as practiced in cabbage preservation. Supplementation of the substrate by addition of glucose at 1, 2 and 3% levels was also applied. The fermentation process was monitored by evaluation of the titratable acidity, pH and microbial population development. The treatment was compared to those of the blanched solar dried and fresh solar dried cowpea leaves at P< 0.05. Fermentation with the addition of the highest concentration of glucose (3%) gave the highest concentration of lactic acid of 0.6% and the lowest pH of 4.7. Fermented samples had the least crude protein and moisture content of 13.2 and 6.2%, respectively while crude fibre, ash and soluble carbohydrates of fermented samples increased. The fermented samples showed a significant ($p < 0.05$) reduction of iron, calcium, magnesium and zinc. At the end of 90 days storage study period the solar dried leaves retained the highest amount of chlorophyll and beta-carotene of 1590.7µg/g and 1.5µg/g, respectively. It was concluded that fermentation of cowpea leaves coupled with solar drying could be of potential for small scale producers as a method of enhancement of the keeping and nutritive quality. However, like other pulses, cowpeas contain several antinutritional factors, which may limit their consumption and affect the digestibility and bioavailability of nutrients.

Keywords: Cowpea leaves, fermentation, solar dried, nutritive quality, proximate composition

Introduction

Cowpea [*Vigna unguiculata (L.) Walp.]* is an important warm-season legume grown primarily in the semi-arid tropics that includes parts of Asia, Africa, Southern Europe, Southern United states and South America (Singh, 2003). In the tropics it is a dual-purpose legume, being used as leafy vegetables, grain, as fresh cut-and-carry forage, and for hay and silage, especially for eastern and southern Africa (Chweya and Eyzaguirre, 1999; Whitbread and Lawrence, 2006). The peas are a good source of protein and energy and are widely consumed all over the world, mainly in rural populations, and satisfy a considerable proportion of the protein requirements (Oiye *et al.,* 2009). Drought tolerance, short growing period and its multi-purpose use make cowpea a very attractive alternative for farmers who cultivate in marginal, drought-prone areas with low rainfall and less developed irrigation systems, where infrastructure, food security and diminishing malnutrition are major challenges (Keller, 2004; Weinberger and Msuya 2004). Despite its economic and social importance in developing parts of the world, cowpea has received relatively little attention from a research standpoint and it can, therefore, be considered as a neglected crop (Schippers, 2002; Singh *et al.,* 2003; Timko and Singh, 2008).

Pre-treatments, together with the drying method and the storage condition influence the quality of dehydrated foods. Among the pre-treatments used in dehydrated vegetables are the addition of chemical compounds, osmotic pre-treatments and blanching (Nieves *et al.,* 2001). Blanching is one of the most widely used pre-treatments and aims to inactivate enzymes, removal of air, hydrolyse and solubilise pectin. However, blanching has been known to cause an undesirable change in texture and loss of

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nutrients on account of leaching, especially minerals and the water-soluble vitamins and heat-induced losses (Pradeep and Susanta, 2001). Preservation of vegetables by lactic acid bacteria fermentation has been successfully employed in cow peas (Torres *et al*., 2005). The preservation mechanism is through lowering the pH in the vegetable by acidification through fermentation, leading to the inhibition of some microbial deteriorative changes.

ß-Carotene and chlorophyll has been found in most yellow, orange, dark green leafy vegetables and fruits such as kale, pumpkin, spinach, papaya, apricots, and peaches. Maintenance of the naturally colored pigments in stored foods has been a major challenge in food processing (Ihl *et al*., 1998). Duration and temperature of storage are two of the important factors responsible for the loss of pigments and color, and special care must be taken to produce food that retains its bright attractive color during freezing. Change in color during storage may therefore be used as a tool to evaluate the product quality.

The major constraints to the industrial use of cowpea by food companies in Africa include the lack of reliable statistics on production, the strong price fluctuations during the year, the low quality of the raw material in terms of physical defects, and the lack of primary processors. Processing as offered by fermentation would offer an opportunity for a more constant source of cowpeas with nutritive quality. While fermentation seems to enhance the nutritive value of legumes (Torres *et al.,* 2005), like other pulses, cowpeas contain several antinutritional factors, which limit their consumption and affect the digestibility and bioavailability of nutrients. Most of the published data on nutritive value of cowpeas is based on the seed (Singh *et al.,* 2003; Torres *et al.,* 2005; Oiye *et al.,* 2009). There is limited work on the cowpeas leaves especially on matters of nutrition and the effects of various preservation methods on nutrition quality. This is despite the knowledge that other green leafy vegetables have been recognized as a cheap and abundant potential sources of vitamins, minerals and protein (Aletor *et al.,* 2002). This is because of their ability to utilize a wide range of virtually unlimited and readily available primary materials (Klijn *et al.,* 1995).

Fermentation processes not only extend shelf-life in vegetable products as kimchi and sauerkraut, but impart desirable flavor attributes to the final product (Breidt et al., 2007). The nutritional content of a food may also be enhanced as a result of fermentation. Complex, indigestible polymers can often be catabolized through fermentation to digestible, useful carbohydrates. Liberation and synthesis of previously unavailable nutrients can also be a benefit of the fermentation process (Chavasit et al., 1991). Establishing the effect of fermentation and dehydration on composition of the cowpea leaves would enlighten consumers on their nutritive value and thus create a shift towards their consumption. Determining the fermentation conditions of cowpea leaves would also be a step forward in achieving an alternative enhancement of the keeping and nutritive quality for this vegetable. This would contribute to the development of primary processing for the food industry. This study investigated the fermentation of cowpea leaves coupled with solar drying to determine whether it has a potential for small-scale producers as a method of enhancement of the keeping and nutritive quality.

Materials and Methods

The cowpeas were planted in a field within an agricultural research station in Kenya, whose location lies at 0^{o} 22 S, 35^{o} 56 E and at 2267M above sea level. The field was used because of the available infrastructure and closeness to the university research laboratory. The daily temperatures range from 22°C to 28°C. The rain is erratic with an average of 600 to 900 mm annually. The soil is sandy and volcanic with an average depth of 0.6 m. Variety *Ken kunde1* from Kenya Seed Company was used in the study and no fertilization was done. The experimental design used was a completely randomized design replicated two times (36 days and 42 days) at $\alpha = 0.05$. Four locations were randomly selected in the field and the harvest was made at two different dates at each location, therefore a data of eight trials was used. The leaves were harvested fresh after 36 and 42 days of growth from germination. Only the tender young leaves were harvested during the study. The samples were packed loosely in two-ply polythene (HDPE) bags to minimise heat build-up. From each locality 500 grams each of leafy vegetables were washed and cut into thin slices and spread on medium density (0.926 – 0.940 g/cc) polythene bags after which they were analysed.

Determination of proximate composition

Proximate analyses of the cowpea leaves were determined according to the Association of Official Analytical Chemists (1984 and1995) methodology.

Crude protein (AOAC, 1995)

Micro-Kjeldahl method was used where 0.2 g of the dried sample was accurately weighed and

placed into micro-Kjeldahl digestion tubes. Into the sample, 10 ml of concentrated nitrogen free sulphuric acid was added to each tube and one selenium tablet added as a catalyst. The samples were digested in a digester (2012 digester Foss Tecator) at 445^{0}C for 3 hours. The digested samples were cooled to room temperature then distilled using Kjeldahl distillation unit (2200 kjeltec auto distillation Foss tecator). The distillate was collected in a 15 ml 0.1M HCl in which a mixed indicator of methyl red and methylene blue had been added. The excess HCl was titrated against 0.1M NaOH. The calculations were as follows:

$$\% \text{ crude protein} = (V_1 - V_2) \times (M \times 1.4 \times 6.25)/W.$$

Where V_2 is volume of HCl used in test portion, V_1 is volume of HCl used in blank test; M is molarity of acid and W is weight of test portion.

Moisture content

The oven drying method (Method 14.003, AOAC, 1984) was used where 2.0 g of samples were accurately weighed and transferred into aluminium dishes. The samples were dried in an oven at 105^{o}C for 6 hours and cooled in a desiccator for 10 minutes. Weights were taken at intervals of 1 hour until a constant weight was reached then calculations done to determine percentage for weight loss.

% Moisture content
= (weight of original sample – weight of dry sample) /(weight of original sample)

Crude fibre

Determination of crude protein was adopted from, AOAC, 1984 method 7.067 with modifications. In the determination of crude fibre, 2.0 g of the samples were accurately weighed and placed in a graduated beaker. To each sample, 100 ml of boiling water was added. Twenty five mililiters of 2.04 M H_2SO_4 was added and the contents topped up to 200 ml. The samples were boiled for 30 minutes on a hot plate. The samples were filtered by a filtering pump (Azlon, England) through a glass wool stack in filtering sticks. The samples were thoroughly washed with boiling water. This procedure was repeated with 1.78 M KOH. The samples were then washed with 70% ethanol and transferred to a crucible. The samples were dried at 105^{0}C for 3 hours in an oven after which they were cooled in a dessicator for 10 minutes and weighed. The samples were heated to ash in a muffle furnace (Gallenkamp size 2) for 3 hours, cooled to room temperature in a desiccator and weighed again.

% Crude fibre
= (weight of sample after oven drying - weight of sample after heating to ash) / original weight of the sample

Soluble carbohydrates

%Soluble carbohydrates
= 100 – (% moisture content + % crude protein + % ash content + % crude fibre)

Ash content

Gravimetric method was used where 2.0 g of sample was accurately weighed and placed into silica crucibles (Method 13.002, AOAC, 1995). The samples were ashed in a muffle furnace (Gallenkamp size 2) at 550^{0}C for 3 hours. The crucibles were cooled in a desiccator to room temperature and weighed. Ash content was calculated as a percentage of the dry sample. That is;

% Ash
= (100 – weight of original sample – weight of ash)/ (weight of original sample) x 100

Mineral content

Determination of calcium, iron, magnesium and zinc were done by using an Atomic absorption spectrophotometer (AAS) (AOAC, 1995). Two grams of dry sample were accurately weighed and digested in a digester (2012 digester Foss Tecator) using 10 ml of concentrated H_2SO_4 and 5 ml of concentrated HNO_3. The samples were cautiously heated at 250^{o}C until vigorous reaction subsided. Heating continued to 450^{o}C for 3hours. The digested samples were filtered through a Whatmans filter (No. 40) into a 100 ml flat-bottomed flask and contents topped up to the mark with distilled water. One millilter of 10% w/v of Lanthanum Chloride salt was added to each sample and run into an atomic absorption spectrophotometer (AAS) (CTA-2000 AAS Chemtech Analytical). Concentrations of minerals were obtained from a calibration curves made from standards of iron, zinc, calcium and magnesium.

Blanching

The harvested fresh leaves were weighed and washed to remove soil particles. They were then blanched in water at 95^{o}C for 90 seconds in wire baskets (Neives *et al*., 2001). The samples were cooled and then solar dried in drying trays and packed in paper bags. They were kept at room temperature for subsequent analysis.

Solar drying

Fresh cowpea leaves were dried in a solar drier and packed in paper bags. These acted as a positive control experiment with no treatment applied except for solar drying (Neives *et al*., 2001).

Fermentation

Fermentation of cowpea leaves was done according to Neives *et al*. (2001) with modifications. Harvested leaves were washed and divided into batches of 1 Kg each for the fermentation by addition of glucose at 1, 2 and 3%. Others were cut into pieces of 5mm and stuffed into fermentation bowls in batches of 1 Kg each. They were then fermented in 2.5% brine prepared using boiled water in plastic containers. Brine samples were drawn from the containers on days 1, 3, 7, 14 and 21 for the analysis of titratable acidity and pH done as follows.

Titratable acidity

For the determination of titratable acidity, 10 ml of brine sample from each fermenter was drawn and titrated against 0.1M NaOH. 1% phenolphthalein indicator was used to give the end point. The results were given as % lactic acid as follows.

% Lactic Acid = (ml of alkali x molarity of alkali x 9)/(volume of the sample in ml).

pH levels

The pH of the brine samples from each fermenter was measured by pH meter (Consort C 830 multiparameter analyzer, Belgium, calibrated with pH 4.00 and 7.00 buffer.

Microbial determination

For microbial determination, 1 ml of the brine sample from each fermenter was serially diluted to 10^{-3} for day 1 and to 10^{-6} on subsequent days. One milliter of diluents was pour plated. For day one, dilutions of 10^{-2} and 10^{-3} was pour plated while dilutions 10^{-5} and 10^{-6} were pour plated for the other days in deMan Rogosa Sharpe (MRS) agar for total lactic acid bacteria (LAB) determination and in potato dextrose agar (PDA) for yeast determination. MRS was prepared in the microbiology laboratory of Dairy and Food Science and Technology Department of Egerton University while PDA was bought from Liofilchem Company, Italy. Plates for MRS were incubated at 32^{o}C in an incubator for 24hours while those for PDA were kept at 24^{o}C and counting done after 48 hours.

Storage studies

Determination of total chlorophyll

For determination of total chlorophyll, spectroscopic characteristics of the aggregated chlorophyll extracted in plant crude extracts was done with some modification as described by Canjura *et al.* (1991). In brief, 2.0 g of the leaf sample was weighed and placed in a mortar. They were mixed with 15 ml of acetone and about 5.0 g of acid-washed sand. The mixture was ground with a pestle to extract the chlorophyll, and then the residues rewashed with 15 ml portions of acetone until the extract was colourless. The combined extract was made up to 40 ml with acetone and absorbance measured at 645 and 663nm in a UV spectrophotometer (Novaspec II Pharmacia Biotech). The two wavelengths are known to give the best spectroscopic characteristics of the aggregated chlorophyll a and b as extracted in plant crude extracts. Two 1 mg samples of chlorophyll a + b were obtained from sigma chemical company. These were used to prepare stock solutions in 80 % (v/v) acetone. The stock solutions were then used at six different concentrations to generate standard calibration curves. Total chlorophyll (chlorophyll a + chlorophyll b) concentration was calculated from absorbances at 645 nm and 663nm as follows:

Chlorophyll a (µg/g) = (12.7A663 – 2.69A645) x 40/2
Chlorophyll b (µg/g) = (22.9A645 – 4.68A663) x 40/2
Total chlorophyll (µg/g) = (20.2A645 + 8.02A663) x 40/2
The total chlorophyll content for the fresh leaves averaged 1800(µg/g) at day zero before the treatments.

Determination of beta-carotene

This was done using the spectrophotometric method as described by Laval-Martin (1985) with some modifications. In brief, two grams of the sample was weighed, finely chopped and placed in a mortar and 15 ml of acetone added. The mixture was ground with a pestle and mortar with acid-washed sand added to extract the chlorophyll, and then the residues rewashed with 15 ml portions of acetone until the extract was colourless. The extract was made up to 100 ml mark with acetone. Out of this, 25 ml were evaporated to dryness in a rotary vacuum evaporator (vacuum rotary evaporator type 349/2) and the residue washed in about 1 ml of petroleum ether. The solution was introduced into a chromatographic column prepared using silica gel 60 (0.063-0.2 mm/ 70-230 mesh ASTM). The column was prepared using silica gel 60 (0.063-0.2 mm/ 70-230 mesh ASTM) as stationary phase in a 1cm internal diameter chromatographic column. Five grams of

silica gel 60 were made into slurry using petroleum ether as the mobile phase and poured to make a 10cm packed column. Beta-carotene was eluted with petroleum ether and collected as a yellow pigment. It was eluted to a volume of 10 ml and read in a UV spectrophotometer (Novaspec II Pharmacia Biotech) at an absorbance of 440 nm. The concentrations were read on a standard curve prepared using known concentrations of pure beta-carotene obtained from sigma chemical company (ET Monks, Kenya). For the preparation of the standards, 0.001 g of pure beta-carotene were dissolved in 1000 ml pet-ether and standards prepared. This made 1000 ppm (1000µg/ml). These standards were; 0.5 µg/ml, 1.0 µg/ml, 1.5 µg/ml, 2.0 µg/ml, 2.5 µg/ml and 3.0 µg/ml whose absorbance was read at 440 nm. The beta carotene content in the leaves before any treatment averaged 2.3µg/ml.

Data analysis

All samples were analyzed in triplicates. Data obtained from each sample location was initially analyzed separately by running a single ANOVA and thereafter data were pooled to perform the combined analysis of the various sample locations in the field. Mean concentration of proximate composition for each treatment was compared according to the multiple means comparison technique of Fishers LSD test (a = 0.05), under the restriction of a significant treatment effect for a 3 way analysis of variance (Blanched solar dried, Fermented with the concentrations of the glucose substrates, fermented and solar dried). Graphical modelling of the concentration changes of ß-carotene and chlorophyll was conducted with the software package STATISTICA5.0/Graph.

Results

The changes in microbial population in the fermentation of cowpea leaves are summarized in Figures 1 and 2. For lactobacilli, the maximum population was reached in day three except for samples fermented with the addition of 3% glucose whose maximum population was reached on day seven (Figure 1). For yeasts (Figure 2) the maximum population was reached at day seven. A decrease in population for both lactobacilli and yeast was realized from day 14th to day 21st

The production of acid and decrease in pH occurred fastest in samples fermented with the addition of glucose. The 3% added glucose exhibited the highest lactic acid development and the lowest pH. The lowest pH reached in this study was 4.7 for 3% added glucose on day 7th. A maximum lactic acid of 0.6 % was attained at the same day. For the samples fermented with the addition of 1% glucose and the cut and fermented samples, the rise was gradual attaining a maximum lactic acid of 0.35% at day 14th as shown by figures 3 and 4 respectively.

Solar dried samples had significantly higher crude protein than fermented samples at $P < 0.05$ with the highest content exhibited by fresh solar dried samples. Samples fermented with the addition of 3, 2 and 1% glucose did not show any significant differences in crude protein content. Moisture content was significantly higher in samples fermented with the addition of 3% glucose as compared with other samples. Cut samples had the least moisture content. Fresh solar dried and samples fermented with the addition of 1 and 2% glucose were not significantly different. Crude fibre ash and soluble carbohydrates were relatively high in fermented samples as compared to the rest. Cut samples had significantly high soluble carbohydrates as compared to samples fermented with the addition of 1, 2 and 3% glucose, respectively.

At $P< 0.05$, iron content did not show any significant difference between fresh solar dried, blanched and samples fermented by addition of glucose. However, samples fermented with the addition of 2 and 3% glucose were significantly different from samples fermented with the addition of 1% glucose and blanched solar dried samples. Cut, fermented and solar dried samples had the least content of iron. Magnesium and zinc did not differ significantly among samples fermented with the addition of glucose but significantly differed from cut, fermented and solar dried samples. Blanching did not affect Magnesium and zinc as shown in Table 2.

The total chlorophyll content for the fresh leaves averaged 1800 (µg/g) at day zero before the treatments. From Figure 5, chlorophyll decrease ceases after week 10. Samples fermented with the addition of 2 and 3% glucose showed no significant difference. Samples fermented with the addition of 2% glucose and blanched solar dried samples did not differ significantly with samples fermented with the addition of 1% glucose at $P< 0.05$. From Figure 5, chlorophyll content stabilized after the tenth week.

The beta carotene content in the leaves before any treatment averaged 2.3 µg/ml. The cut, fermented and solar dried samples and samples fermented with the addition of 1% glucose had the least of beta-carotene during the storage period of 12 weeks as shown in Figure 6. It was also observed that the beta-carotene decrease ceases after week 10 as it occurred with

Table 1. The proximate composition (%) of cowpea leaves after different treatments.

Treatment	Crude protein	Moisture content	Crude fibre	Ash	Soluble carbohydrates
Fresh solar dried Blanched solar dried	35.97^{a}	11.56^{b}	12.76^{d}	13.08^{c}	26.64^{e}
Fermented with the addition of;	27.03^{b}	9.60^{c}	11.76^{e}	9.49^{d}	42.14^{b}
3% glucose	15.19^{e}	14.36^{a}	16.29^{c}	22.37^{a}	31.80^{d}
2% glucose	14.63^{cd}	12.18^{b}	18.93^{a}	22.84^{a}	31.58^{d}
1% glucose	14.04^{de}	11.45^{b}	17.61^{b}	22.61^{a}	34.30^{c}
Cut, fermented and solar dried	13.18^{e}	6.20^{d}	16.27^{c}	19.66^{b}	44.70^{a}

Means in same the column followed by the same letter are not significantly different ($P < 0.05$)
Mean separation done by Duncan's Multiple Range Test

Table 2. The mineral content (µg/g) of cowpea leaves under different treatments.

Treatment	Iron	Calcium	Magnesium	Zinc
Fresh solar dried Blanched solar dried	1.50^{ab}	16.37^{a}	0.67^{a}	0.08^{a}
Fermented with the addition of;	1.98^{a}	15.21^{ab}	0.64^{a}	0.07^{ab}
3% glucose	1.66^{ab}	13.42^{bc}	0.29^{c}	0.04^{c}
2% glucose	1.45^{b}	10.89^{c}	0.29^{c}	0.05^{bc}
1% glucose	1.98^{a}	12.86^{bc}	0.28^{c}	0.06^{abc}
Cut, fermented and solar dried	0.89^{c}	14.52^{ab}	0.38^{b}	0.08^{a}

Means in same column followed by the same letter are not significantly different ($P< 0.05$)
Mean separation done by Duncan's Multiple Range Test

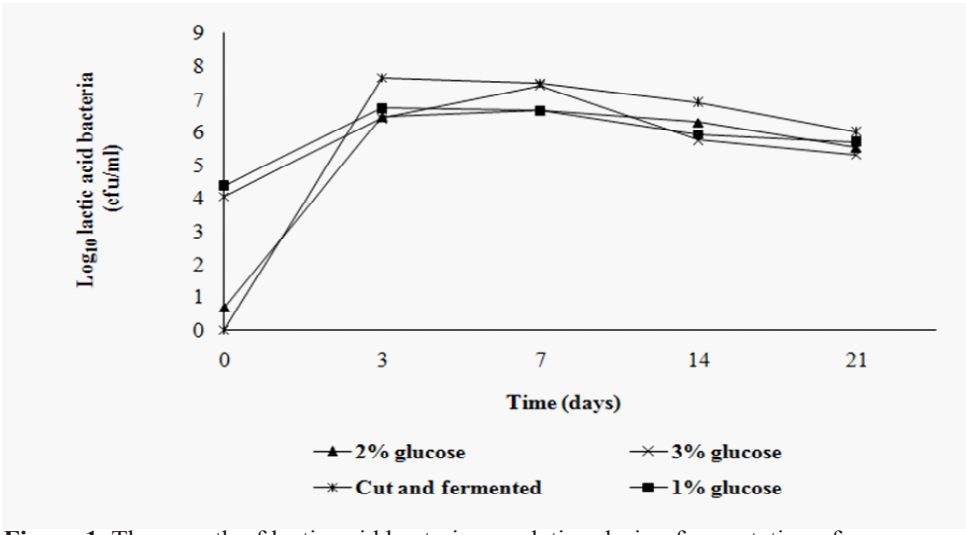

Figure 1. The growth of lactic acid bacteria population during fermentation of cowpea leaves

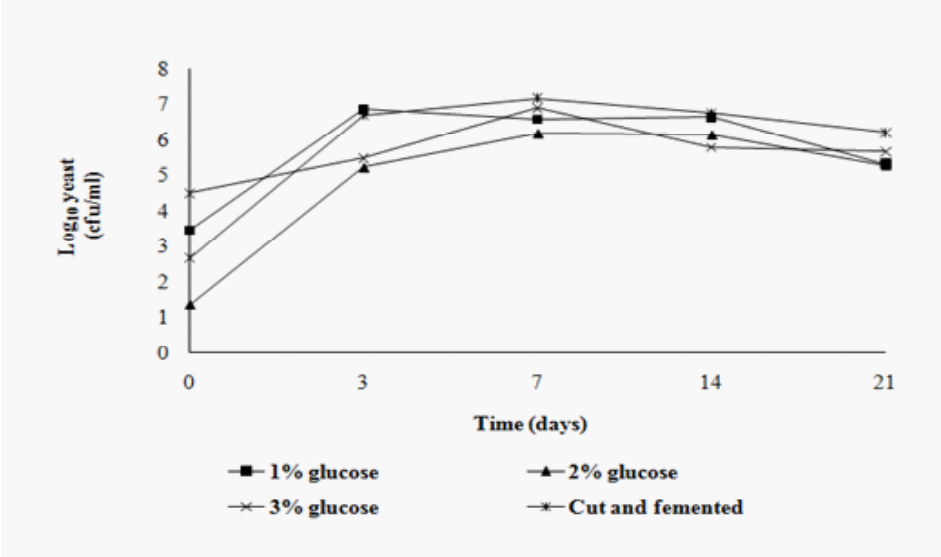

Figure 2. The growth of yeast population during fermentation of cowpea leaves

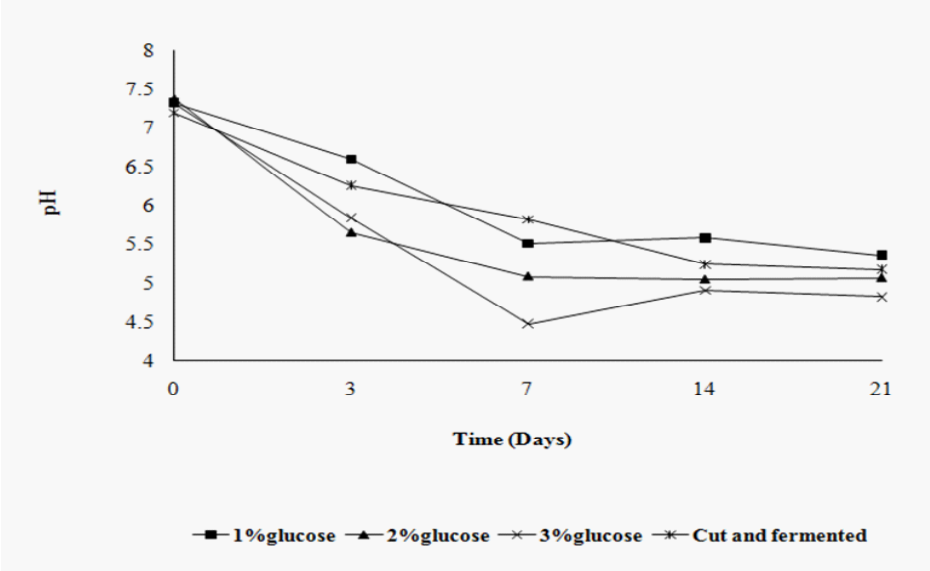

Figure 3. The pH profile during fermentation of cowpea leaves.

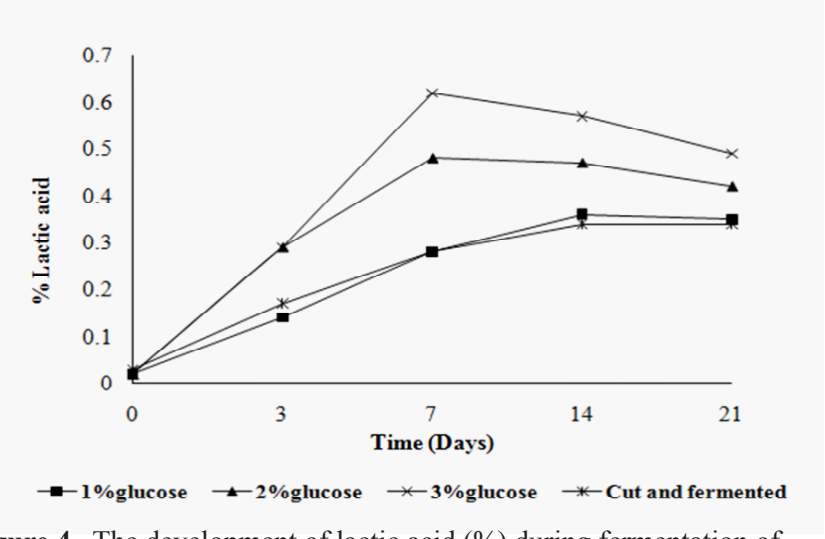

Figure 4. The development of lactic acid (%) during fermentation of cowpea leaves.

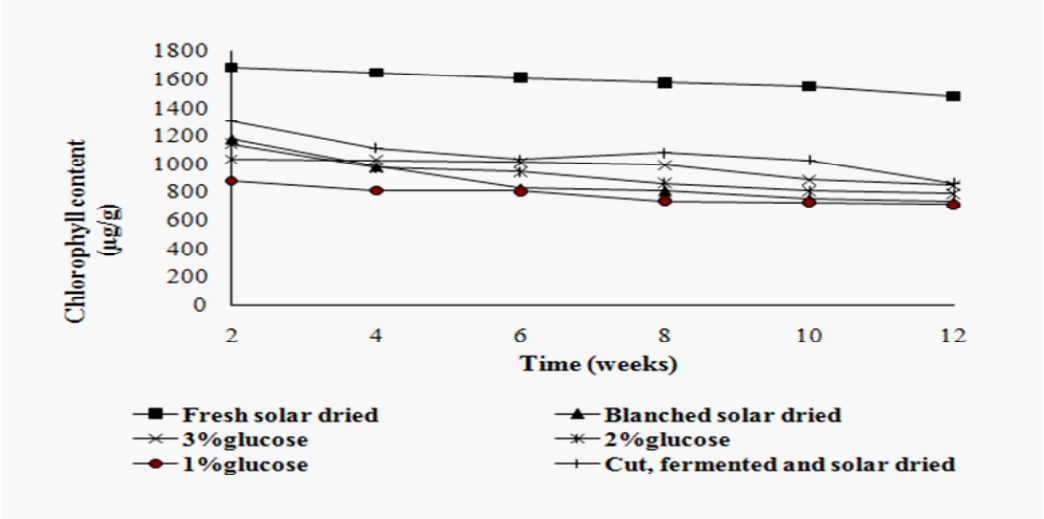

Figure 5. The changes in chlorophyll content during a 3- months storage period of dried cowpea leaves.

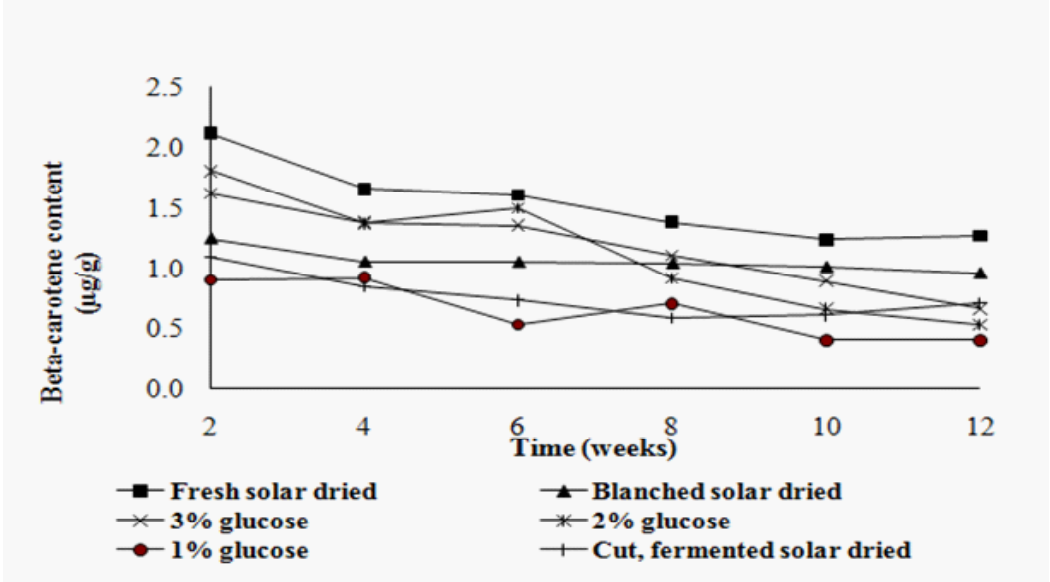

Figure 6. The changes in beta-carotene content during a 3-months storage period of dried cowpea leaves.

chlorophyll (Figure 5). Blanched solar dried samples exhibited the least changes during storage. It seemed that by extrapolation, in extended storage period at ambient temperatures, blanched samples would potentially retain a higher beta-carotene than other treatments.

Discussion

Proximate composition of any food is a reflection of its nutritive value. The higher the value of protein, soluble carbohydrates, crude fibre, ash and vitamins, the higher the nutritive value of that particular food. The higher moisture content of fermented leaves could be attributed to the high content of single cell protein due to high microbial count. Ash content increased significantly for fresh solar dried leaves for the fermented leaves. This could be attributed to addition of 2.5% sodium chloride at the start of fermentation. Green leafy vegetables are normally consindered to be a very good source of minerals and with fermentation, the mineral content tends to rise (Torres *et al.,* 2005). This is an implcation that fermented cow peas leaves are a superior source of minerals as compared to fresh leaves. Thus, fermentation should be encouraged as they stabilise and even icrement the minerals content as assessed by the total ash. The low protein content can be attributed to protein utilization by the fermentative microorganisms. The draining of the fermentation solution that goes with most of the dissolved nutrients is a major factor contributing to low nutrient content of fermented leaves.

Blanched solar dried samples exhibited the least changes during storage. This suggests that in extended storage period at ambient temperatures, blanched samples would potentially retain a higher beta-carotene than other treatments. In a related study, Mepba *et al*., (2007) observed that sun-drying at 30± 10C and a RH 80-85% for 10hr resulted in a mean moisture contents of 35.6% with insignificant (P> 0.05) crude fiber and total ash contents of treated vegetables. Similarly, Mandhyan *et al*., (1999) observed that sun-drying at 34-39^{0}C and RH 40-75% for 16hr caused significant reductions (60.5%) in moisture contents of spinach, cabbage, carrots and peas. Accordingly, reductions in moisture contents resulted in corresponding increases in dry matter contents due to concentration of soluble solids with relatively chemically stable products. Ajayi and Onayemi (1997) similarly observed that blanching had variable effects on dry matter composition with insignificant reductions in lipid, crude fiber, toal ash and carbohydrate contents but had no effect on the protein content of the vegetables.

Lactic acid fermentation involves production of lactic acid and carbon dioxide among other by-products of fermentation. This phenomenon is as a result of the multiplication of the lactic acid bacteria facilitated by availability of fermentable substrate. The high microbial count in the beginning of fermentation is attributed to presence of excess glucose. According to Sanchez *et al.* (2000) substrates (glucose, fructose and sucrose) are consumed fast within the first 1-4 days of fermentation hence the maximum population. In sauerkraut, the cabbages used have a sugar content of 1.2 to 2.2%. This is enough to complete fermentation and addition of more fermentable sugar is not required (Viander *et al.,* 2005). Other green vegetables with less fermentable sugars have 1 to 3% sugar added to complete fermentation (Viander *et al.,* 2005). The same study found that no substrates were detected in brine after 22 days of fermentation. Products found in the study by Sanchez *et al.* (2000) were lactic acid and acetic acid in the ratios of 3.0:5.6 respectively in both spontaneous and controlled fermentations. Ethanol was formed in large amounts in the case of spontaneous fermentation. Succinic and formic acids were formed in both fermentations, but the equilibrium concentrations were significantly different from each other. Succinic acid was higher in spontaneous fermentation. Some of the carbon was converted to CO_2. *Lactobacilli* spp. thrive well in salty conditions thus the witnessed fast growth. The higher acidity in samples added glucose could be attributed to higher content of glucose which acts as a substrate in fermentation.

The existence of yeasts in large numbers and low sugar content in cowpea leaves could be factors leading to low acidity as other by-products of yeast fermentation are produced hence the witnessed reduced pH and lactic acid after day 7. Low protein content can be attributed to protein utilization by the fermentative microorganisms. The draining of the fermentation solution that goes with most of the dissolved nutrients is major factor contributing to low nutrient content of fermented leaves. Fleming *et al*. (1995) studying fermentation of sauerkraut found that the substrates are either converted into organic acids, ethanol and CO_2 or converted to mannitol. Trail *et al*. (1996) observed that a high ratio of volatile to non-volatile acids has a positive effect on flavour of sauerkraut. This has been observed in cucumber (Chavasit *et al.,* 1991) and kimchi fermentation (Fleming *et al.,* 1995). Montana *et al.* (1993) obtained a carbon recovery of almost 100% in case of *L. Plantarum* in green olive fermentation. This was achieved through taking aseptic precaution in olive

preparation. The high content of ash in fermented leaves could be due to added salt as found out by Mutegi (2002). The addition of salt as a means has also been found to favour the growth conditions of desirable microorganisms by increasing ionic strength and the osmolarity of the medium thereby extracting water and additional nutrients from the tissues of the leaves to be fermented. Lactic acid bacteria tolerate high salt concentrations. The salt tolerance gives them an advantage over other less tolerant species and allows the lactic acid fermenters to begin metabolism, which produces acid that further inhibits the growth of non-desirable organisms (Fleming *et al.,* 1995).

Exposure of vegetable tissue cells by cutting is another factor responsible for loss of nutrients as the oozing of nutrients and other substrates takes place. As acidity increases, magnesium decreases an indication of replacement of magnesium ions by hydrogen ions in chlorophyll molecule (Nieves *et al.,* 2001). Other factors influencing the changes in mineral content are leaching and the draining of the brine solution that preceded drying and goes with it the minerals in the solution.

Fermentation of cowpea leaves coupled with solar drying could be of potential for small scale producers as a method of enhancement of the keeping and nutritive quality. However, like other pulses, cowpeas contain several antinutritional factors, which may limit their consumption and affect the digestibility and bioavailability of nutrients (Torres *et al.,* 2005; Oiye *et al*., 2009). The antinutritional problems linked with the presence of tannins or trypsin inhibitors can be easily avoided with appropriate dehulling and heat treatment. The flatulent sugars are not a limiting factor; cowpea has lower raffinose content than soybean (Torres *et al.,* 2005). Taking into consideration the good nutritional value and the positive image of cowpea for African consumers, it can be concluded that cowpea could be a good source of protein for industrial product manufacturing. Cowpea is rich in lysine and also as event from this study the iron content was not affected by the processing methods. Consequently it can be used to balance cereals. Its deficiency in sulphurous amino acids is addressed when it is combined with milk protein and/or cereals known for their high methionine and cystine content.

According to Nieves *et al.* (2001), chlorophyll is an important food quality in terms of sensory attributes. Blanching which is an important pre-processing heat–treatment of vegetables inevitably causes separation and losses of watersoluble nutrients as minerals, water soluble vitamins and sugars (Mepba *et al.,* 2007). However in the absence of blanching as it happens with fermentation, peroxidases, lipases and lipoxygenases break down the chlorophyll forming pheophytins thus the observed reduction in chlorophyll content and development of the brown colour. From this study it is also evident that blanching affects chlorophyll content. Some of it is lost with the blanching water.

The nutritional properties of ß-carotene have been studied extensively, and its role as an antioxidant has been widely reported (Palozza and Krinsky, 1992). The tissue maintenance function of vitamin A is apparently related to its antioxidant function, most or all of which can be taken over by ß-carotene. ß-carotene is the principal precursor of vitamin A, which is involved in vision, cell differentiation, synthesis of glycoproteins, mucus secretion from the epithelial cells, and overall growth and development of bones (Guerra–Vargas *et al*., 2001). Diets that are deficient in vitamin A have precipitated the death of children from measles, diarrhea, and other diseases because of impaired immunity (Guerra–Vargas *et al*., 2001). Thus the focus in recent years has been the methods which can effect preservation and retention of ß -carotene in different fruits and vegetables by several of cooking and different methods of food preservation on the ß –carotene. Blanching followed by freezing is an effective preservation technique for the retention of ß -carotene in fruits and vegetables. This study had similar findings as the blanching followed by fermentation on the cow peas had a stabilizing effect on the ß – carotene levels. This may be because of greater chemical extractability and loss of moisture and soluble solids which further concentrate the sample. Inactivation of certain oxidative enzymes takes place and it results in the breakdown of some structures leading to a higher bioavailability of ß-carotene (Guerra–Vargas *et al.,* 2001). Heat treatment such as blanching, cooking and steaming help to release bound carotenoids and render them to be easily extractable and hence the ß -carotene content stability. As the storage period increases, there is a decrease in the ß-carotene content of the cow peas. This loss of ß -carotene could be due to non-oxidative changes (cis - trans isomerization, epoxide formation or heat degradation of tissues) or oxidative changes on exposure to light and oxygen (Aruna *et al*., 1999).

Most studies have shown that lactic acid bacteria work best at temperatures of 18 to 22ºC. The *Leuconostoc* species, which initiate fermentation, have an optimum temperature range of 18 to 22ºC (mesophiles). Temperatures above 22ºC, favour the *Lactobacillus* species (Fleming *et al.,* 1995). Equilibrated brine cover solution containing around 5

to 6% sodium chloride, 0.1 to 0.4% calcium chloride, and 0.05 to 0.2% acetic acid provides desirable conditions for LAB, such as *Lactobacillus plantarum*, to predominate during vegetable fermentation (Breidt *et al.,* 2007). Since vegetables are mostly composed of water (Hutkins 2006), the added salt in the brine cover solution draws water and sugars from the cucumber fruit, which further aids to initiate the fermentation (Jay *et al.,* 2005). *L. plantarum* utilizes the available carbohydrates, such as glucose and fructose, reducing the pH by lactic acid production. Fermented vegetables can be stored in the tanks for a year or longer after the fermentation process.

Conclusions

The study established that fermentation and subsequent solar drying of cowpea leaves has a stabilising effect on the nutritional and quality attributes of the vegetables. Addition of a 3% glucose in the fermenting cow leaf matrix results in a rapid development of lactic acid indicative of increased microbial activity. It was concluded that fermentation of cowpea leaves coupled with solar drying could be of potential for small scale producers as a method of enhancement of the keeping and nutritive quality. However cowpeas contain several antinutritional factors, which may limit their consumption and affect the digestibility and bioavailability of nutrients.

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References

Aletor, O. R., Oshodi, A. A. and Ipinmoroti, K. 2002. Chemical composition of common leafy vegetables and functional properties of their leaf protein concentrates. Journal of Food Chemistry 78: 63–68.

Ajayi, J. K. and Onayemi, O. 1977. Effect of steam blanching and chemical treatments on the quality characteristics of some common leafy vegetables grown in Nigeria. Nigerian Food Journal 1: 68-71.

AOAC, 1995. Association of Official Analytical Chemists, (1995).Official Methods of Analysis. Association of Official Analytical Chemists. 16th Ed., Arlington, VA.

Aruna, K., Vimala, V., Dhanalakshmi, K. and Reddy, V. 1999. Physio-chemical changes during storage of Papaya fruit (Carica papaya L.). Journal of Food Science and Technology 36: 428- 433.

Breidt, F., McFeeters, R, F. and Díaz-Muñiz, I. 2007. Fermented vegetables. In: Doyle MP, Beuchat LR, editors. Food Microbiology: Fundamentals and Frontiers. 3rd ed. Washington D.C.: ASM Press. p 783-793.

Canjura, F. L., Schwartz, S. J. and Nunes, R. V. (1991). Degradation kinetics of chlorophylls and chlorophyllides. Journal of Food Science 56: 1639–1643.

Chavasit, V., Hudson, J. M., Torres, J. A. and Daeschel, M. A. 1991. Evaluation of fermentative bacteria in model low salt cucumber juice brine. Journal of Food Science 56: 462-465.

Chweya, J.A. and. Eyzaguirre, P.B. 1999. The Biodiversity of Traditional Leafy vegetables. IPGRI –International Plant Genetic Research Institute, Rome, Italy. 69 p.

Fleming, H. P., Kyung, K.H. and Breidt, F. 1995. Vegetable fermentations. In: Biotechnology. (Eds). Rehm, H. J., Reed, G., VCH, Weinheim, p. 629-661. Germany.

Guerra-Vargas, M., Jaramillo-Flores, E., Dorantes-Alvarez, L. and Hernandez-Sanchez, H. 2001. Carotenoid retention in canned pickled Jalapeno peppers and carrots as affected by Sodium Chloride, Acetic Acid and Pasteurization. Journal of Food Science 66: 620-625.

Hutkins, R. W. 2006. Microbiology and Technology of Fermented Foods. Ames, IA: Blackwell Publishing. 473 p.

Ihl, M., Monslaves. M. and Bifani, V. 1998. Chlorophyllase inactivation as a measure of blanching efficiency and color retention of artichokes (Cynara scolymus L). Lebensm Wiss Technology 31: 50 – 56.

Jay, M., Loessner, J. and Golden, A. 2005. Modern Food Microbiology. Heldman DR, editor. New York, NY: Springer. 790 p.

Keller, G. B. 2004. African Nightshade, eggplant, spiderflower – production and consumption aspects of traditional vegetables in Tanzania from the farmers point of view. Goettingen: Georg-August Universität, Germany, MSc Thesis.

Klijn, N., Weerkamp, A. H. and de Vos, W. M. 1995. Genetic marking of Lactococcus lactis shows its survival in the human gastrointestinal tract. Applied and Environmental Microbiology 61: 2771-2774.

Laval-Martin, D. 1985. Spectrophotometric method of controlled pheophytinization for the determination of both chlorophylls and pheophytins in plant extracts. Analytical Biochemistry 149: 121-129.

Mandhyan, B. L., Abroal, C. M. and Tijagi, H. R. 1988. Dehydration characteristics of winter vegetables. Journal of Food Science and Technology 25 (1): 20-22.

Montano, A., Sanchez, A.H. and Castro, A. 1993. Controlled fermentation of Spanish-type green olives. Journal of Food Science 58: 842-844.

Mepba, H. D., Eboh, L. and Banigo, D. 2007. Effects of processing treatments on the nutritive composition and consumer acceptance of some Nigerian edible leafy vegetables, African Journal of Food and Nutritional Development 7: 1- 18

Mutegi C. K. 2002. Preservation of kale by lactic fermentation and solar drying. Msc. Thesis, University of Nairobi, Nairobi.

Nieves, S., Gabriela, C. and Jose, B. 2001. The effect of blanching on the quality of dehydrated broccoli florets. European Research Technology 213: 474-479.

Oiye, S. O., Shiundu, K. M. and Oniang'o, R. K. 2009. The Contribution of African Leafy vegetables to vitamin A intake and the influence of income in rural Kenya. African Journal of Food and Nutritional Development 9: 1- 6.

Palozza, P. and Krinsky, N.1992. Communication. ß-carotene and ß-tocopherol are synergistic antioxidants. Archives of Biochemistry and Biophysics 297: 184- 187.

Pradeep, S. N. and Susanta, K. R. 2001. The effect of blanching on quality attributes of dehydrated carrots during long-term storage. European Research Technology 212: 445-448.

Sanchez, A.H., Montano, A., Castro, A. and Rejano, L. 2000. Comparative study on chemical changes in olive juice and brine during green olive fermentation. Journal of Agriculture and Food Chemistry 48: 5975-5980.

Schippers, R. 2002. African Indigenous Vegetables; An Overview of the Cultivated Species 2002 - on CD-ROM; Aylesford, UK: Natural Resources International Limited

Sidney, W. D. 1984. Official methods of analysis, Association of Official Analytical Chemists. 14th Ed. AOAC, Virginia, USA.

Singh, B. B., Chambliss O. L. and Sharma, B. 1997. Recent advances in cowpea breeding. Pp 30-49. In: Advances in cowpea research. (Eds) B. B. Singh, D.R. Mohan Raj, K.E Dashiell and Jackai, L.E.N. Co publication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural sciences (JIRCAS). IITA, Ibadan, Nigeria.

Singh, B.B., Ajeigbe, H.A., Tarawali, S.A., Fernandez-Rivera, S. and Abubakar, M. 2003. Improving the production and utilization of cowpea as food and fodder. Field Crops Research 84(1-2): 169-177.

Torres, A., Frias, J., Guerra, M. and Vidal-Valverde. C. 2005. Influence of fermentation on the nutritional value of two varieties of Vigna sinensis. European Food Research and Technology 220 (2): 31-36.

Timko, M. and Singh, B. 2008. Cowpea, a Multifunctional Legume In Genomics of Tropical Crop Plants. Editors Moore, P. and Ming, R. Publisher Springer New York. 227-258.

Trail, A. C., Fleming, H. P., Young, C. T. and Mcfeeters, R. F. 1996. Chemical and sensory characterization of commercial sauerkraut. Journal of Food Quality 19: 15-30.

Britta Viander , B., Maarit, M. and Palva, A. 2005. Impact of low salt concentration, salt quality on natural large-scale sauerkraut fermentation. Food Microbiology 20 (4): 391-395.

Wahua, T. 1999. Applied Statistics for Scientific Studies. Africa Link Press, Aba, Nigeria, 1999; 129-155.

Weinberger, K. and Msuya, J. 2004. Indigenous vegetables in Tanzania – significance and prospects. Technical Bulletin No. 31, Shanhua, Taiwan: The World Vegetable Centre.

Whitbread, A. and Lawrence, J. 2006. Cowpea fact sheet for Grain and Graze. Australian Government – Land & Water Australia. Online 21.11.2009 from: lwa.gov.au/files/ products/grain-and-graze/pn20434/pn20434.pdf.