Effect of Post-Harvest Handling Practices, Storage Technologies and Packaging Material on Post-Harvest Quality and Antioxidant Potential of Solanum Aethiopicum (Shum) Leafy Vegetable

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Abstract Several studies have supported the use of vegetables as foods as well as medicinal plants. However, most especially for the leafy types of vegetables, their high moisture content gives them a short shelf life. On average Solanum aethiopicum (Shum) has a shelf life of one day, making it unable to keep fresh for a long time. The objective of this study was to determine the effect of post-harvest handling practices and storage technology on the post-harvest quality and antioxidant activity in S. aethiopicum, as well as determine the packaging material that could be able to maintain a high post-harvest quality during storage. The post-harvest handling and storage technologies were tested under three experimental conditions. Experiment one involved placing 2.0 kg of the harvested S. aethiopicum with roots intact (RI) and others with roots cut-off (RC) in a charcoal cooler (-CC), 21.0±1.00 °C, 95.67±3.01 %rh; in ambient storage (-AC), 23.8±2.86 °C, 69.38±6.72 % rh; and in cold room (-CR), 7.17±1.30 °C, 95.80±3.19 %rh. Experiment two involved storing 2.0 kg of S. aethiopicum in charcoal cooler with no water treatment (TT-) and in ambient storage while immersing in portable water for 2 to 3 seconds during the day (TT+). Experiment three involved packing 1.0 kg of S. aethiopicum sample of both RC and RI state to assess the effectiveness of the packaging materials (0.1 cm meshed perforated polyethylene (RC0.1), 0.5 cm meshed perforated polyethylene (RC0.5) and a 60 µm perforated polyethylene (RC60µm) in maintaining quality of the vegetables. The edible parts of the vegetable were tested for moisture content, percentage weight loss, chlorophyll content, polyphenol content and total antioxidant activity (as measures of post-harvest quality and shelf life) after every 24 hours. The antioxidant activity was determined by screening for free radical scavenging properties using diphenyl picryl hydrazyl (DPPH), Ferric reducing antioxidant power (FRAP) and ascorbic acid as standard. The results revealed that Shelf life was found to increase (from one day to four days) when the vegetable was intermittently immersed in portable water for 2 to 3 seconds after every one hour during the day for vegetables in ambient storage both with roots intact (RI(TT+)-AC and with roots cut-off RC(TT+)-AC). The samples stored in cold room and charcoal cooler showed slow and comparable reduction (percent) of weight for both intact and roots cut. The chlorophyll content decreased in all storage conditions, with ambient conditions showing the most rapid decrease. The total polyphenol fluctuated within relatively small limits for both with intact and roots cut-off when stored in cold room and charcoal cooler (6.25±0.05 to 9.35±0.05 mgGAE/gfw; respectively) within the four days of storage. Storage in ambient conditions indicated an increase in total polyphenol content from 9.35±0.05 to 14.77±0.12 mgGAE/gfw for that with roots intact (RI-AC) and to 13.65±0.06 mgGAE/gfw for roots cut-off (RC-AC). The increase in total polyphenol content in the ambient storage led to increased total antioxidant activity compared to that stored in cold room and charcoal cooler that remained almost constant. The 60 µm perforated polyethylene and 0.1 cm meshed perforated polyethylene retained more moisture (84.55±0.18 % and 85.20±0.03 %; respectively) and showed minimal percentage of weight loss (9.69±0.25 %) with the highest chlorophyll content (8.06±0.02 mg/g dwb) on day four when stored in the charcoal cooler, making it the best tested packaging material.

Keywords: Solanum aethiopicum, charcoal cooler, shelf life, total polyphenol, total antioxidant activity, Ferric reducing antioxidant power (FRAP), diphenylpicrylhydrazyl (DPPH), perforated polyethylene
1. Introduction

The Solanaceae vegetable family has over 1000 species worldwide with at least 100 indigenous species in Africa [1]. Several studies have supported use of these vegetables as foods and medicinal plants [1], but despite their high nutrient content and benefits these come with, there has been limited research on local vegetable varieties in Uganda [2]. Solanum aethiopicum is the most popular of the Solanaceae leafy vegetables grown in east and central Uganda, and consumed in most of the restaurants in Kampala [3] However, vegetables are sensitive to environmental changes and especially changes to availability of water and temperature [4]. Additionally, post-harvest losses account for 50% of the total vegetable loss in the developing world as a result of inadequate infrastructure, poor technology and over production [5]. Any change in quality due to post-harvest handling in the market therefore leads to losses to the farmer.

Vegetables are also seasonal, with peak production occurring in May, September and October [6] The highest yields of S. aethiopicum Shum group are got under warm humid conditions. The yield is significantly affected by limited water supply during growth, this necessitates growing S. aethiopicum during rainy seasons or where there is reliable supply of water [7]. Drought seasons always give low yields as droughts significantly affect the crop physiology [7].

Post-harvest handling practices for S. aethiopicum have been documented to be rudimentary [8] which are likely to affect the quality of the vegetables. Some of the several factors that affect post-harvest quality of a vegetable includes; temperature in storage room, percentage relative humidity, light intensity, carbon dioxide concentration [9], cutting which brings about wounding that promotes humidity, light intensity, carbon dioxide concentration [9], cutting which brings about wounding that promotes post-harvest losses account for 50% of the total vegetable loss in the developing world as a result of inadequate infrastructure, poor technology and over production [5]. Any change in quality due to post-harvest handling in the market therefore leads to losses to the farmer. Post-harvest handling practices for S. aethiopicum have been documented to be rudimentary [8] which are likely to affect the quality of the vegetables. Some of the several factors that affect post-harvest quality of a vegetable includes; temperature in storage room, percentage relative humidity, light intensity, carbon dioxide concentration [9], cutting which brings about wounding that promotes phytochemical synthesis [10,11], increased light transmission and oxygen concentration which increase chlorophyll loss leading to deterioration [12,13,14]. On average, the shelf life of S. aethiopicum has been reported locally to be one day. Uganda is known to be a fruit basket where most of the food is consumed in its fresh form, this calls for development of post-harvest technologies that can prolong the shelf life of harvested leafy vegetables.

One of the most recent innovations has been on drying of S. aethiopicum as a processing technology [15]. This had its own limitations in terms of acceptability, where the dried vegetable product was rated lower than the fresh vegetables. Therefore, post-harvest handling must be done in the best way possible in order to increase shelf life and availability of the vegetable in its fresh form. If post-harvest handling is not well done, there is likely to be a loss in terms of the amount and quality of vegetable. Different vegetables require different methods of post-harvest handling that may generally depend on the maturity, water content of the leaves, texture and the shelf life at optimum temperature [16], and the post-harvest handling method adopted determines the ultimate quality of the vegetable in terms of texture, color, taste, nutrient and phytochemical content [17]. There is need to maintain temperature as low as possible. Currently mechanical refrigerators are used which are energy intensive, unaffordable to local farmers and require constant supplies of electricity, unavailable in rural parts of the country. Despite the losses in quality and quantity, there is insufficient recorded information available regarding the influence of post-harvest handling practices on the moisture content, weight loss, phytochemical content and antioxidant activity of S. aethiopicum vegetable in Uganda.

The objective of this study was to determine the post-harvest handling practices, storage technology and packaging material that can maintain a high post-harvest quality of S. aethiopicum leafy vegetable during storage for longer shelf life. Thus the effect of handling practices, packaging material and storage conditions on physical attributes (moisture content and weight loss) and the phytochemical attributes (total anti-oxidant capacity, total polyphenols and chlorophyll content) of S. aethiopicum was determined, by comparing two different storage conditions including use of the charcoal cooler which is affordable by local farmers and the electrical refrigerator.

2. Materials and Methods

2.1. Chemicals

All chemicals and reagents used were of analytical grade, purchased from sigma Germany. The filter papers were purchased from Whatman, UK.

2.2. Procurement of Vegetable Samples

The S. aethiopicum vegetables were procured from local farms of Kabbubu, Nangabo Sub County, Wakiso district in central Uganda at maturity and subjected to different post-harvest handling and storage methods. All the analyses were done in the research laboratory of the Food Technology and Nutrition Department, Makerere University, Uganda. The edible parts of the plant (leaves) were removed, washed under running water, crushed and used to extract and determine polyphenols total antioxidant activity and chlorophyll. The samples used for moisture content determination were not washed.

2.3. Experimental Design for Handling Practices and Storage Technologies

Three experimental designs were set up (Figure 1), two for studying handling practices and storage technologies, and the third for suitability of packaging material in extending the shelf life of S. aethiopicum. All experiments were done in 3 replicates.

In experiment one, two (2.0) kg of the vegetables were placed in three (3) perforated buckets and then; one was
kept in cold room -CR (7.17±1.30 °C, 95.80±3.19 %rh), one in the fabricated charcoal cooler -CC (21.0±1.00°C, 95.67±3.01 %rh), and the other kept at ambient conditions -AC (23.8±2.86 °C, 69.38±6.72 %rh). In experiment two, 2.0 kg of the vegetables were placed in two perforated buckets then one was stored in the fabricated charcoal cooler and the other at ambient conditions (23.8±2.86 °C, 69.38±6.72 %rh). For those stored at ambient conditions, their leaves were dipped in portable water (as a treatment – TT+) for 2-3 seconds after every one hour for twelve hours each day for five days. In experiment three, one (1.0) kg of the vegetable with roots cut-off (RC) was each packaged in three packaging materials; high gauge (60 µm) perforated polyethylene material, 0.5 cm pore size meshed polyethylene material and 0.1 cm pore size meshed (gauze) polyethylene material. One lot was placed in cold room RC-CR (60µ, 0.5, 0.1) (7.17±1.30 °C, 95.80±3.19 %rh); and the other in the fabricated charcoal cooler RC-CC (60µ, 0.5, 0.1). The vegetables were kept under the respective treatment conditions for five days, with analysis for moisture content, weight loss, chlorophyll content, total polyphenol content and total antioxidant activity carried out each consecutive day.

2.4. Preparation of Extracts for Analyses

Extraction was done following Wissam et al., [18], with slight modifications. The edible parts of fresh leaves were blended and 1.0 g of the paste dissolved in 50 ml of 80% methanol solution in a conical flask placed in a thermostatic water bath shaker at 45°C for 20 minutes. The liquid extract was separated from solids by centrifugation at 2000 rpm for 10 minutes and the supernatant stored at -20°C. This extraction was done in 3 replicates.
2.5. Determination of Polyphenol Content

The total phenolic content was determined following Folin-Ciocalteau [19]. To 0.5 ml of extract, 2.5 ml of 10% Folin-Ciocalteau’s reagent dissolved in water was added, followed by 2.5 ml of 7.5% NaHCO₃, incubated at room temperature in the dark for 45 minutes and the absorbance determined at 765 nm. The mean value of the replicate samples for the absorbance was then calculated. The standard solutions of gallic acid of concentrations 0.01, 0.02, 0.03, 0.04, 0.05 mg per ml were used to construct a standard calibration curve as described [20] and the concentration of phenolics were read in mg per g of fresh sample.

2.6. Determination of Antioxidant Activity

2.6.1 Free Radical Scavenging Activity by the DPPH Method

The free radical scavenging activity was assayed following [21], and kept at -20 °C and a 0.1 mM DPPH was prepared by diluting 10 ml of the stock solution with 90 ml of methanol. Ascorbic acid was used as the standard, prepared in concentrations of 25, 50, 75, 100 and 125 µg/ml. Equal volumes of 1.5 ml of the standard and the sample were added, kept in the dark for 30 minutes and absorbance measured at 517 nm using Genesys 10-UV spectrophotometer (Thermo Electron Corporation, Madison WI, USA). The percentage inhibition of both standard and samples was calculated using the formula;

\[
% \text{ inhibition} = \frac{AB - AA}{AB} \times 100
\]

Where; AB is absorbance of blank sample and AA is absorbance of sample.

A plot of % inhibition against ascorbic acid concentration was generated to show a calibration curve for the Ascorbic Acid equivalent (mg/g) of fresh sample.

2.6.2 Radical Scavenging Activity by FRAP Method

Ferric reducing antioxidant power assay was performed following Iqbal, Salim, and Lim [22], with slight modifications. To the extract (1 ml) was added 1 ml of FRAP reagent, that was prepared by mixing of 300 mM sodium acetate buffer (pH 3.6), 10 mM 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ) solution and 20 mM FeCl₃.6H₂O in a ratio of 10:1:1, and diluted with water to a total volume of 4 ml. Ascorbic acid, used as the standard was prepared in concentrations of 25, 50, 75, 100 and 125 µg/ml following [23]. The mixture was incubated in a water bath at 37 °C for 30 minutes and absorbance determined at 593 nm using Genesys 10-UV spectrophotometer (Thermo Electron Corporation, Madison WI, USA). The quantification of chlorophyll a and b was done using the equation by [24] and results expressed in mg/g dwb.

\[
\text{Cha} = 12.25A_{663.2} - 2.79A_{646.8} \quad (1)
\]

\[
\text{Chb} = 21.5A_{646.8} - 5.1A_{663.2} \quad (2)
\]

2.8. Determination of Moisture Content

The moisture content was determined as described in AOAC [26]. A thoroughly washed Petri-dish was placed in the oven to dry and then weighed. A sample of 3.0 g of blended S. aethiopicum leaves was placed in the weighed Petri dish, and then placed in an oven to dry at 60 °C for 16 hours. The dish and dry sample were transferred to a desiccator to cool to room temperature before being weighed again. Every sample was analysed in 3 replicates.

2.9. Weight Loss

The vegetable was weighed (2.0 kg) on the day of harvest and stored under three different treatment conditions (cold room, charcoal cooler and ambient conditions). After every 24 hours the vegetables were weighed for five days and the percentage weight loss determined using the formula;

\[
\frac{W_0 - W_t}{W_0} \times 100 \quad (3)
\]

Where Wo is the weight on day zero and Wt is the weight within an interval of 24 hours.

2.10. Control Experiment

All the different parameters including vitamin C, chlorophyll content, moisture content, weight loss, total antioxidant capacity and total polyphenol were determined on the day of harvest (day zero) and the results compared with the results of the subsequent days.

2.11. Determination of Temperature and Humidity

Calibrated data loggers were put in the air space of the charcoal cooler, cold room and stored at ambient conditions to record the temperature and humidity changes after every 30 minutes for five days. The average temperature (°C) and relative humidity (%) were determined each day.

2.12. Data Analysis

The data analyzed by using a two-way Analysis of Variance. The significant differences were obtained using the Tukey HSD test (p≤0.05). All data was analyzed using SPSS version 16.0 for windows (SPSS, Inc., Chicago, IL, USA).
3. Results

The percentage moisture content of RC-CR decreased from 83.91 ± 0.18 % to 79.69 ± 0.69 % on day zero to day four; while RC-CC decreased from 83.91 ± 0.18 % to 78.95 ± 0.23 % on day zero to day four and RC-AC decreased from 83.91 ± 0.18 % to 22.01 ± 0.08 % on day zero to day four. RI-CR remained almost constant that is from 83.88 ± 0.08 % to 83.20 ± 0.01 % on day zero to day four, RI-CC and RI-AC decreased from 83.88 ± 0.08 % to 80.58 ± 0.19 % and 83.88 ± 0.08 % to 40.25 ± 1.06 % on day zero to day four, respectively. The fastest decrease in percentage moisture content was observed in ambient storage. Both RC-CR, RI-CR, RC-CC and RI-CC showed slight decrease in percentage moisture content from day zero to day four. The data showed that there was variation between the moisture content of RC-CR, RC-CC, RC-AC, RI-CR, RI-CC and RI-AC for each day in the five days of storage, which was significant (P≤0.05). The percentage weight loss of RC-CR increased from 0.00 ± 0.00 % to 34.42 ± 1.63 % on day zero to day four and RI-CR increased from 0.00 ± 0.00 % to 23.24 ± 2.4 % on day zero to day four. For RC-CC, the percentage weight loss increased from 0.00 ± 0.00 to 24.44 ± 0.04 % on day zero to day four. The percentage weight loss of RC-AC increased from 0.00 ± 0.00 % on day zero to 50.30 ± 0.33 % on day four and for RI-AC it increased to 42.24 ± 0.43 % on day four. *S. aethiopicum* RC_AC showed the most rapid increase in the percentage weight loss. There was variation between the percentage weight loss RC-CR, RC-CC, RC-AC, RI-CR, RI-CC and RI-AC for each day in the five days of storage which was significant (P≤0.05).

Figure 2. Percentage moisture content and percentage weight loss in *S. aethiopicum*, stored in cold room, charcoal cooler and at ambient condition. RI is *S. aethiopicum* with roots intact, RC is *S. aethiopicum* with roots cut-off. RI-CR is roots intact in cold room

Figure 3. Changes in the chlorophyll content of *S. aethiopicum* stored in cold room, charcoal cooler and at ambient condition. RI is *S. aethiopicum* with roots intact, RC is *S. aethiopicum* with roots cut-off. RI-CR is roots intact in cold room, RI-CC is roots intact in charcoal cooler, RI-AC is roots intact in ambient storage, RC-CR is roots cut-off in cold room, RC-CC is roots cut-off charcoal cooler and RC-AC is roots cut-off in ambient storage
The chlorophyll content decreased with duration in varying storage conditions for both *S. aethiopicum* with roots intact (RI) and with roots cut-off (RC). The chlorophyll content of RC-AC and RI-AC decreased from 18.86 ± 0.01 mg/g dwb on day zero to 9.87 ± 0.03 mg/g/dwb and 11.75 ± 0.03 mg/g/dwb respectively. In charcoal cooler, the chlorophyll content decreased from 86.86 ± 0.01 mg/g to 10.29 ± 0.01 mg/g dwb for RC-CC and 18.86 ± 0.01 mg/g dwb to 12.98 ± 0.01 mg/g dwb for RI-CC. For RC-CR, the chlorophyll content decreased from 18.86 ± 0.01 mg/g dwb to 12.37 ± 0.03 mg/g dwb on day four and for RI-CR decreased from 18.86 ± 0.01 to 13.51 ± 0.02 mg/g dwb. The most rapid decrease in chlorophyll content was observed in RC-AC as shown in Figure 2 above. The results showed that there was a statistically significant difference in the chlorophyll content of *S. aethiopicum* kept in the three different storage conditions and between RI and RC in each day of storage (P ≤ 0.05).

The total polyphenols of RC-CR decreased slightly with duration of storage from 9.35 ± 0.05 to 6.38 ± 0.13 mg GAE/g fw and for RI-CR the total polyphenol content remained relatively constant (Figure 3). The total polyphenol of RC-CC and RI-CC was shown to remain relatively constant. RI-AC and RC-AC showed increase in total polyphenols. However, for RI-AC the total polyphenols remained relatively constant from day zero to day one (6.25 ± 0.05 and 6.30 ± 0.15 mg GAE/g fw) but a significant increase was observed from day two to day five. RI-AC showed the highest increase in total polyphenols on day three and five (15.93 ± 0.26 and 14.77 ± 0.12 mgGAE/g fw respectively) compared to that with RC-AC (13.83 ± 0.08 and 13.65 ± 0.06 mgGAE/g fw respectively) (Figure 6). The results showed that there was a statistically significant difference (P ≤ 0.05) in the total polyphenols of *S. aethiopicum* stored in the different storage conditions in each day of the storage duration.

Figure 4. Variation in the total polyphenol content of *S. aethiopicum* with storage duration stored when stored in cold room, charcoal cooler and at ambient conditions. RI is *S. aethiopicum* with roots intact, RC is *S. aethiopicum* with roots cut-off. RI-CR is root intact in cold room, RI-CC is roots intact in charcoal cooler, RI-AC is roots intact in ambient storage, RC-CR is roots cut-off in cold room, RC-CC is roots cut-off charcoal cooler and RC-AC is roots cut-off in ambient storage

Figure 5. Variation in the total antioxidant activity (TAA) of the stored *S. aethiopicum* in cold room, charcoal cooler and at ambient condition. RI-FRAP and RC-FRAP are results for FRAP method and RI-DPPH and RC-DPPH are results for DPPH method. RI-CR is roots cut-off in cold room, RC-CC is roots cut-off in charcoal cooler and RC-AC is roots cut-off in ambient storage
RC-CR showed a constant TAA with both FRAP and DPPH methods on day zero and day one. A decrease was observed starting on day two to day four; 3.42 ± 0.03 mgAAE/g fw on day two to 2.3 ± 0.06 mgAAE/g fw on day five when using FRAP method and 4.38±0.12 mgAAE/g fw on day one to 3.49 ± 0.02 mgAAE/g fw on day four when using DPPH method. However, the TAA of RC-CC was observed to remain relatively constant. RI-AC showed a decrease in the TAA for the first two days (2.98±0.03 mgAAE/g fw on day zero to 2.48±0.03 mgAAE/g fw on day one using FRAP method and 4.38±0.03 mgAAE/g fw on day zero to 3.76±0.05 mgAAE/g fw on day one when using DPPH method) and from day two to day four the TAA when determined using the two methods. RC-AC showed an increase in the TAA right on day one and the increase continued to day four when using both FRAP and DPPH method. The results showed that there was a statistically significant difference (P ≤ 0.05) in the TAA between the storage conditions and that determined for the different days from day zero to day four.

The total polyphenol content of RI(TT⁺)-AC increased from 4.45 ± 0.02 mg GAE/g fw on day zero to 6.56 ± 0.05 mgGAE/gfw on day four and that of RI(TT¯)-CC increased from 4.45 ± 0.02 mgGAE/g fw to 10.96 ± 0.12 mgGAE/g fw. RC(TT⁺)-AC showed an increase in the total polyphenols from 4.51 ± 0.12 mgGAE/g fw on day zero to 7.6±0.05 mgGAE/g fw and that of RC(TT¯)-CC increased from 4.51±0.12 mgGAE/g fw to 11.98±0.1 mgGAE/g fw (Figure 6). The results showed that there was a slight increase in the total polyphenol content of S. aethiopicum RC(TT⁺)-AC. S. aethiopicum RC(TT⁺)-AC and RC(TT¯)-CC showed a faster increase in the total polyphenol content with storage compared with RI(TT⁺)-AC and RI(TT¯)-CC. This data showed that there was a significant difference (P≤0.05) in the total polyphenol content of S. aethiopicum, RI(TT⁺)-AC showed a more rapid increase in the TAA than RI. The results showed that there was a significant difference (P≤0.05) between TAA of the S. aethiopicum, TT⁺ and TT¯.

The percentage moisture content of S. aethiopicum, RI(TT⁺)-AC increased from 78.91±0.01 % on day zero to 88.70±0.03 % on day four and for RC(TT⁻)-AC, the percentage moisture content increased from 77.28±0.63 % on day zero to 88.77±0.12 % on day four. Percentage moisture content of S. aethiopicum RI(TT⁺)-CC decreased from 78.91±0.01 % on day zero to 75.79±0.16 % and that of RC(TT⁻)-CC decreased from 77.28±0.63 % on day zero to 75.81±0.05 % on day four as shown in Figure 7. The difference in the percentage moisture content of S. aethiopicum, TT⁺ and TT¯ was significant at P≤0.05. The chlorophyll content of S. aethiopicum, RI(TT⁺)-AC remained almost constant, that is 10.95±0.04 mg/g dwb on day zero to 10.25±0.01 mg/g dwb on day four while that of RC(TT⁺)-AC decreased from 10.94±0.01 mg/g dwb on day zero to 10.25±0.18 mg/g dwb on day four (Figure 8). The results showed a decrease in the chlorophyll content of S. aethiopicum, RI(TT⁻)-CC and RC(TT⁻)-CC. For S. aethiopicum, RI(TT⁺)-CC the chlorophyll content decreased from 10.95±0.04 mg/g dwb on day zero to 7.65±0.02 mg/g dwb on day four as for RC(TT⁻)-CC as shown in Figure 9. The chlorophyll content first increased from 10.94±0.01 mg/g dwb on day zero to 12.27±0.16 mg/g dwb on day one then decreased to 6.00±0.03 mg/g dwb on day four. There was a significant difference in the chlorophyll content of S. aethiopicum TT⁺ and TT¯ at P≤0.05.

Figure 6. Variation in the total polyphenol content of S. aethiopicum stored at ambient condition and intermittently immersed in portable water for 2-3 seconds every after one hour during day (TT⁺). RI(TT⁺)-AC is with roots intact in ambient storage, RI(TT¯)-CC is with roots intact in charcoal cooler, RC(TT⁺)-AC is roots cut-off in ambient and RC(TT⁺)-CC is roots cut-off in charcoal cooler.
Figure 7. Effect of immersing S. aethiopicum in portable water intermittently for 2-3 seconds after every one hour (TT⁺) on antioxidant activity (TAA), under different storage conditions. RI-DPPH and RC-DPPH is TAA determined using DPPH method of S. aethiopicum with roots intact and roots cut-off respectively. RI(TT⁺)-AC and RC(TT⁺)-AC is S. aethiopicum in ambient storage with water treatment but with roots intact and roots cut-off respectively. RI(TT⁺)-CC and RC(TT⁺)-CC is S. aethiopicum in charcoal cooler without water treatment but with roots intact and roots cut-off respectively.

Figure 8. Effect of percentage moisture content, of immersing harvested S. aethiopicum in portable water intermittently but stored at ambient condition (TT⁺) and that stored in charcoal cooler but without water treatment (TT¯). RI is with roots intact and RC is with roots cut-off. RI(TT⁺)-AC and RC(TT⁺)-AC is that with roots intact and roots cut-off respectively in ambient storage with water treatment. RI(TT¯)-CC and RC(TT¯)-CC is that with roots intact and roots cut-off respectively in charcoal cooler without water treatment.

The percentage moisture content of S. aethiopicum RC-CR60 µm and RC-CC60µm decreased from 86.91±1.76 % on day of harvest to 85.66±0.06 % and 84.55±0.18 % respectively on day four. The RC-CC0.5 showed the lowest moisture content on day four of 80.55±0.07 % while RC-CR0.1 showed the lowest moisture content on day four of 82.99±0.03 % as shown in Table 1. The results showed that all the packaging material had high moisture retention within the shoots of S. aethiopicum. The RC-CR60µm polyethylene retained most of the water. The analysis showed that there was a significant difference in the moisture content of the packaged S. aethiopicum stored in cold room and charcoal cooler for each day among the three different packaging materials.

Table 1. Moisture content of packaged S. aethiopicum stored in cold room and charcoal cooler

<table>
<thead>
<tr>
<th>Duration (days)</th>
<th>Percentage moisture content of packaged and stored S. aethiopicum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RC-CR0.1</td>
</tr>
<tr>
<td>Zero</td>
<td>86.90±1.76</td>
</tr>
<tr>
<td>One</td>
<td>85.22±0.96</td>
</tr>
<tr>
<td>Two</td>
<td>84.88±0.04</td>
</tr>
<tr>
<td>Three</td>
<td>83.93±0.19</td>
</tr>
<tr>
<td>Four</td>
<td>82.99±0.03</td>
</tr>
</tbody>
</table>

Values are expressed as means ± standard deviation. *Values not sharing common superscript within the same row are significantly different (P ≤ 0.05) using Tukey HSD test. RC-CR is cold room and RC-CC is charcoal cooler. RC-CR0.1, RC-CR0.5 and RC-CR60µm is S. aethiopicum with roots cut-off and packaged in 0.1 cm meshed perforated polyethylene, 0.5 cm meshed perforated polyethylene, 60 µm perforated polyethylene respectively and kept in cold room. RC-CC0.1, RC-CC0.5 and RC-CC60µm is S. aethiopicum with roots cut-off and packaged in 0.1 cm meshed perforated polyethylene, 0.5 cm meshed perforated polyethylene, 60 µm perforated polyethylene respectively and kept in charcoal cooler.
The percentage weight loss of *S. aethiopicum* RC-CR0.1 and RC-CC0.1 increased from 0.00±0.00 % to 11.33±0.23% and 0.00±0.00 % to 12.57±0.27 % respectively. For the *S. aethiopicum* RC-CR0.5 and RC-CC0.5 the percentage weight loss increased from 0.00±0.00 % on day of harvest to 9.80±0.18 % and from 0.00±0.00 % to 12.49±0.05 % respectively as that of *S. aethiopicum* RC-CR60µm increased from 0.00±0.00 % to 8.83±0.72 % from 0.00±0.00 % to 9.69±0.25 % respectively. *S. aethiopicum* RC-CR0.1 and RC-CC0.1 showed the highest increase in percentage weight loss as RC-CR60µm perforated polyethylene showed the lowest percentage weight loss.

These results show that there was a faster increase in weight loss of packaged *S. aethiopicum* stored in charcoal cooler and also there was a significant difference (p≤0.05) in the percentage weight loss of the packaged *S. aethiopicum* stored in cold room and charcoal cooler for each day among the three different packaging materials.

![Figure 9. Effect on chlorophyll content, of immersing harvested *S. aethiopicum* in portable water intermittently but stored at ambient condition (TT⁺) and that stored in charcoal cooler but without water treatment (TT¯). RI; with roots intact and RC; with roots cut-off. RI(TT⁺) AC and RC(TT⁺) CC is *S. aethiopicum* in ambient storage with water treatment but with roots intact and roots cut-off respectively. RI(TT⁺) CC and RC(TT⁺) CC is *S. aethiopicum* in charcoal cooler without water treatment but with roots intact and roots cut-off respectively. Effect on chlorophyll content, of immersing harvested *S. aethiopicum* in portable water intermittently but stored at ambient condition (TT⁺) and that stored in charcoal cooler but without water treatment (TT¯). RI, with roots intact and RC, with roots cut-off. RI(TT⁺)-AC and RC(TT⁺)-AC is *S. aethiopicum* in ambient storage with water treatment but with roots intact and roots cut-off respectively. RI(TT⁺)-CC and RC(TT⁺)-CC is *S. aethiopicum* in charcoal cooler without water treatment but with roots intact and roots cut-off respectively.](image)

### Table 2. Percentage weight loss of packaged *S. aethiopicum* stored in cold room and charcoal cooler

<table>
<thead>
<tr>
<th>Duration (days)</th>
<th>Percentage Weight loss of packaged <em>S. aethiopicum</em> in different storage condition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RC-CR0.1</td>
</tr>
<tr>
<td>Zero</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>One</td>
<td>4.59 ± 0.25</td>
</tr>
<tr>
<td>Two</td>
<td>5.24 ± 0.63</td>
</tr>
<tr>
<td>Three</td>
<td>7.86 ± 0.28</td>
</tr>
<tr>
<td>Four</td>
<td>11.33 ± 0.23</td>
</tr>
</tbody>
</table>

Values are expressed as means ± standard deviation. Values within the same row and column are significantly different (P ≤ 0.05) using Tukey HSD test.

### Table 3. Chlorophyll content of packaged *S. aethiopicum* stored in cold room and charcoal cooler

<table>
<thead>
<tr>
<th>Duration (days)</th>
<th>Chlorophyll content (mg/g dwb) of packaged <em>S. aethiopicum</em> in different Storage conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RC-CR0.1</td>
</tr>
<tr>
<td>Zero</td>
<td>19.88±0.03</td>
</tr>
<tr>
<td>One</td>
<td>18.30±0.36</td>
</tr>
<tr>
<td>Two</td>
<td>17.58±0.23</td>
</tr>
<tr>
<td>Three</td>
<td>17.07±0.01</td>
</tr>
<tr>
<td>Four</td>
<td>14.67±0.02</td>
</tr>
</tbody>
</table>

Values are expressed as means ± standard deviation. Values within the same row and column are significantly different (P ≤ 0.05) using Tukey HSD test.
The chlorophyll content of *S. aethiopicum* RC-CR0.1 and RC-CR0.5 decreased from 19.88±0.03 mg/g dwb to 14.67±0.02 mg/g dwb and 14.70±0.09 mg/g dwb respectively as shown in Table 3.

The chlorophyll content of *S. aethiopicum* decreased from 19.88±0.03 mg/g dwb on day of harvest to 19.21±0.08 for RC-CR60µm and to 8.06±0.02 mg/g dwb for RC-CR0.1. *S. aethiopicum* RC-CC0.5 showed the most rapid decrease in chlorophyll content followed by *S. aethiopicum* RC-CR0.5 whereas RC-CR60µm lowest decrease in chlorophyll content. Packaged *S. aethiopicum* stored in charcoal cooler showed a more rapid decrease in chlorophyll compared to that stored in cold room for all the three packaging materials. There was a variation between the chlorophyll content of packaged *S. aethiopicum* in the three different packaging materials and within the storage condition which was significant at p≤0.05.

The polyphenol content of *S. aethiopicum* RC-CR0.1, RC-CR0.5 and RC-CR60µm increased from 3.30±0.21 mgQE/gfw on day zero to 5.48±0.03 mgQE/gfw, 5.52±0.13 mgQE/gfw and 5.55±0.09 mgQE/gfw on day four respectively. The polyphenol content of *S. aethiopicum* RC-CC0.1, RC-CC0.5 and RC-CC60µm increased from 3.30±0.21 mgQE/gfw on day zero to 5.89±0.01 mgQE/gfw, 4.13±0.03 mgQE/gfw and 4.55±0.05 mgQE/gfw on day four respectively.

The total polyphenol content increased with storage duration in both cold room and charcoal cooler for all the three packaging materials. The result analysis showed that there was a significant difference (P ≤ 0.05) in the total polyphenol content with in the three different packaging materials and storage conditions except on day four the RC-CR0.5 and RC-CR60µm also RC-CC0.5 and RC-CC60µm showed no significant difference (P≤0.05) as shown in Table 4.

The TAA of *S. aethiopicum* RC-CR0.1, RC-CR0.5 and RC-CR60µm increased from 1.32±0.18 mgAAE/gfw on day zero to 1.82±0.03 mgAAE/gfw, 1.97±0.15 mgAAE/gfw and 2.05±0.02 mgAAE/gfw on day four respectively and RC-CC0.5 and RC-CC60µm increased from 1.32±0.18 mgAAE/gfw on day zero to 2.47±0.02 mgAAE/gfw, 2.12±0.15 mgAAE/gfw and 2.11±0.02 mgAAE/gfw on day four respectively when using FRAP method. When using DPPH method, the TAA of *S. aethiopicum* packaged RC-CR0.1, RC-CR0.5 and RC-CR60µm increased from 2.02±0.02 mgAAE/gfw on day zero to 2.73±0.03 mgAAE/gfw, 2.78±0.03 mgAAE/gfw and 3.13±0.03 mgAAE/gfw on day four respectively while RC-CR0.1, RC-CR0.5 and RC-CR60µm increased from 2.02±0.02 mgAAE/gfw on day zero to 3.73±0.02 mgAAE/gfw, 3.20±0.05 mgAAE/gfw and 2.89±0.05 mgAAE/gfw on day four respectively. The TAA of the packaged *S. aethiopicum* increased with storage duration from day of harvest to day three and then slightly decreased, when determined using both FRAP and DPPH methods, for all the three different packaging materials for *S. aethiopicum* stored in cold room as shown in Figure 10.

The TAA of that stored in charcoal cooler generally increased with storage duration from day of harvest to day four when packaged in the three different packaging materials as shown in Figure 10. There was a variation between the TAA of packaged *S. aethiopicum* in the three different packaging materials and within the storage condition which was significant at p≤0.05.

**Figure 10.** Variation in the total antioxidant activity (TAA) of packaged *S. aethiopicum* and stored in cold room (CR) and charcoal cooler (CC). CR-DPPH and CC-DPPH is TAA of *S. aethiopicum* stored in CR and CC respectively when determined using DPPH method. CR-FRAP and CC-FRAP is TAA of *S. aethiopicum* stored in CR and CC respectively when determined using FRAP method. RC-CR0.1, RC-CR0.5 and RC-CR60µm is *S. aethiopicum* with roots cut-off and packaged in 0.1 cm meshed perforated polyethylene, 0.5 cm meshed perforated polyethylene, 60 µm perforated polyethylene respectively and kept in cold room. RC-CC0.1, RC-CC0.5 and RC-CC60µm is *S. aethiopicum* with roots cut-off and packaged in 0.1 cm meshed perforated polyethylene, 0.5 cm meshed perforated polyethylene, 60 µm perforated polyethylene respectively and kept in charcoal cooler.
4. Discussion

There are several factors that affect post-harvest quality of a vegetable, these include temperature in storage room, percentage relative humidity, light intensity, oxygen concentration, and carbon dioxide concentration [9]. The first indicator of deterioration of vegetables is moisture loss due to low relative humidity and high temperature, weight loss due to moisture loss and yellowing due to ethylene production [27]. The moisture loss is indicated by wilting, discoloration, and shriveling due to loss of turgidity and firmness [28]. The moisture content of the stored *S. aethiopicum* decreased with storage duration due to evaporation [29] resulting from fast air movement, low moisture content in the air and the thin waxy skin with numerous pores on the leaves. In the current study, the vegetables were subjected to different storage conditions with different temperatures and relative humidity and the findings indicated a difference in the moisture content in the vegetables stored under different conditions. Storage in the cold room and charcoal cooler showed higher retention of moisture compared to ambient storage which agrees with other studies [30,31]. This finding suggest that the lower the temperatures and the higher the relative humidity (experienced in both cold storage and charcoal cooler technology), the lower the rate of moisture loss and evaporation. Different vegetables respond to temperature variations after harvest and in storage differently [32]. Most of them however require a combination of low temperature ranging from 0°C to 10°C and high percentage relative humidity ranging from 85 % to 98 % [33]. This relative humidity can be achieved in either cold room or charcoal cooler. The low relative humidity below the optimum and high temperatures experienced in the ambient storage often lead to a higher rate of moisture loss. The rapid reduction in moisture content is seen to have caused a rapid increase in percentage weight loss (Figure 2). These agree with the findings reported elsewhere [34], confirming that any decrease in percentage moisture content leads in increase in percentage weight loss.

There was a decline in chlorophyll content with longer duration of storage of the leafy vegetable in all the three storage conditions. However, storage in ambient conditions revealed a faster decline most probably due to the high light intensity, leading to chlorophyll degradation and production of yellowish pigment as reported [35,36,37,38]. The light intensity in the charcoal cooler and cold room was relatively regulated because the vegetables were kept closed inside the structures and; particularly for the charcoal cooler, the inner walls were lined with black cloth which naturally regulates light. Such a mechanism can reduce the rate of chlorophyll degradation. Additionally, the decline in chlorophyll content was more rapid for the *S. aethiopicum* with roots cut-off. It is postulated that it could be due to the increased production of ethylene levels resulting from wounding [39,40]. The high temperatures in ambient storage are known to cause an increase in the activity of polyphenol oxidase whose optimum temperature is 30°C [41] and its activity lead to browning.

Polyphenols are important chemicals synthesized by plants for protection especially from harsh conditions like water stress, high temperatures and wounding [10,11,42,43,44]. This study established that ambient storage has harsh conditions for storage for example high temperature and low relative humidity which are known causes of high polyphenol content in stored *S. aethiopicum*. The levels are not comparable to those under cold room and charcoal cooler storage conditions, irrespective of whether or not they have roots. The high light intensity in the ambient storage has also been reported to lead to increased polyphenol content in stored vegetables [35,45]. In this current study, the polyphenol content of *S. aethiopicum* in ambient decreased after day three, which could be attributed to the increase in the polyphenol oxidase activity [41]. In this study, the total polyphenol content of *S. aethiopicum* stored in cold room and charcoal cooler remained almost constant most likely due to the low temperature and high relative humidity. This reduced the moisture loss and resulted in regulation of enzyme activity. The increase in total polyphenol of *S. aethiopicum* with roots cut-off stored in ambient storage was faster, probably because of cutting which resulted in wounding of the vegetable leading to increase in total polyphenol content [46]. This was controlled in cold room and charcoal cooler storage conditions. The increase in the total antioxidant activity can be attributed to the increase in the total polyphenol content with storage duration. The positive linear correlation between total antioxidants and polyphenol content has been shown in several studies [47,48,49,50,51,52,53].

When the vegetable in ambient storage was intermittently immersed in portable water, a practice being carried out in markets to prolong shelf life, the total polyphenol and chlorophyll content remained almost constant probably due to cooling that reduced the enzyme activity for the biosynthesis of polyphenols and ethylene. This also increased the moisture on the leaf surface, maintaining a high relative humidity around the leaves leading to reduced evaporation [54]. Packaging prolongs

### Table 4. Total polyphenol (T.P) content of packaged *S. aethiopicum* stored in cold room and charcoal cooler

<table>
<thead>
<tr>
<th>Duration (days)</th>
<th>T.P content (mgQE/gfw) of packaged <em>S. aethiopicum</em> stored in cold room and charcoal cooler</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RC-CR</td>
</tr>
<tr>
<td>Zero</td>
<td>3.50 ± 0.21a</td>
</tr>
<tr>
<td>One</td>
<td>3.20 ± 0.05b</td>
</tr>
<tr>
<td>Two</td>
<td>4.75 ± 0.01c</td>
</tr>
<tr>
<td>Three</td>
<td>6.10 ± 0.06a</td>
</tr>
<tr>
<td>Four</td>
<td>5.48 ± 0.03a</td>
</tr>
</tbody>
</table>

Values are expressed as means ± standard deviation. a,b,cValues not sharing common superscript within a row are significantly different (P ≤ 0.05) using Tukey HSD test. RC-CR is cold room and RC-CC is charcoal cooler. RC-CR0.1, RC-CR0.5 and RC-CR60µm is *S. aethiopicum* with roots cut-off and packaged in 0.1 cm meshed perforated polyethylene, 0.5 cm meshed perforated polyethylene, 60 µm perforated polyethylene respectively and kept in cold room. RC-CC0.1, RC-CC0.5 and RC-CC60µm is *S. aethiopicum* with roots cut-off and packaged in 0.1 cm meshed perforated polyethylene, 0.5 cm meshed perforated polyethylene, 60 µm perforated polyethylene respectively and kept in charcoal cooler.
shelf life [55,56,57,58] and different packaging materials give different shelf life and quality of stored vegetables [56,57,59]. The 60 µm perforated polyethylene showed higher moisture retention. This can be attributed to the increased relative humidity in the inside [60] as the polyethylene does not allow water penetration [58]. The perforation reduces water vapour condensation [54] hence maintaining a high relative humidity inside the material and reducing water loss from the vegetable [54]. The polyethylene material has been reported to be good materials for decreasing physicochemical changes and nutrient quality changes [14,61]. Packaging is reported to reduce chlorophyll loss from vegetables [12]. The loss in chlorophyll content was more in the 0.1 cm meshed perforated polyethylene and 0.5 cm meshed perforated polyethylene. This is probably due to the large pore size which increase light transmission and oxygen concentration within the packaging material. This increased light transmission and oxygen concentration increases chlorophyll loss leading to deterioration [12,13,14].

The total polyphenol content of S. aethiopicum increased in all the packaging material, probably due to the post-harvest biochemical reactions that set in with moisture loss and wounding, resulting from the cutting off of roots and the roughness of the packaging material, that promotes phytochemical synthesis [10,11]. This increase in the total polyphenol content also lead to the increase in the total antioxidant activity of S. aethiopicum in all packaging materials as shown in Figure 10. The best parckaging material studied for storage of S. aethiopicum was the 60 µm perforated polyethylene because of its ability to reduce moisture loss, reducing air and oxygen saturation inside the packaging hence probably increasing carbon dioxide concentration inside the packaging. This material is also smooth; this prevents wounding the leaves of the packaged vegetable. A proper packaging material should not be rough to avoid injuring the packaged vegetable contained inside as injuring a vegetable reduces the shelf life of the leafy vegetable [62].

5. Conclusion and Recommendations

Deterioration of harvested S. aethiopicum occurs more rapidly when the roots of the vegetable are cut-off. The percentage relative humidity is the most prominent factor that affected the moisture content and weight of stored S. aethiopicum. Cutting off of roots leads to increase in polyphenol biosynthesis and chlorophyll loss with storage duration as a result of increase in ethylene production of the vegetable leaves. The total antioxidant activity of S. aethiopicum increases with storage duration, however the quality of the vegetable in terms of moisture content, weight, nutritional quality has reduced by day four. Therefore, the consumer ought to consume this vegetable in the first four days if it is to be fed on when fresh. The freshness of the vegetable can be maintained by immersing the leaves intermittently in portable water for the first two to three days for vegetables in ambient storage however due to a lot of moisture, oxygen and microorganisms in the environment, rotting sets in and the quality of the vegetable reduces sharply. Therefore, the stored S. aethiopicum must be kept away from contact with water to prevent rotting. This makes the charcoal cooler a better affordable storage technology as it allows no contact of the vegetable with water yet it maintains a high relative humidity required. Packaging of S. aethiopicum prolongs self life and 60 µm perforated polyethylene packaging material is a preferred for S. aethiopicum.

Acknowledgements

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