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EFFECT OF COOLBOT^{TM} COLD STORAGE AND MODIFIED ATMOSPHERE PACKAGING ON THE SHELF LIFE AND POSTHARVEST QUALITY OF COLLARDS

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ABSTRACT

Collard (*Brassica oleracea var.acephala* L.) is a nutritious leafy vegetable that is widely cultivated and consumed in Kenya. However, collard is highly perishable with a shelf life of one to two days at ambient conditions, which limits its consumption. Exploring storage options and packaging methods that can extend the shelf life of collards, can avert quantity and quality losses of the vegetable. This will ensure sustained income to producers and continued vegetable supply to consumers. The study aimed to evaluate the effectiveness of CoolBot^{TM} Technology and Modified Atmosphere Packaging (MAP) as options to preserve quality and extend the shelf life of collards. Two experiments were conducted at Kabete and Juja sub-counties using freshly harvested collards from the University of Nairobi Kabete farm. In each experiment, homogenous batch of freshly harvested collards were first divided into four batches. The vegetable batches were then subjected to two storage options (CoolBot^{TM} cold room; 5±2 °C, 75±20% RH, and ambient conditions; 25±10 °C, 50±15% RH), which were further split into two packaging treatments (packaged using Xtend® MAP, and unpackaged). The experimental layout was a 2 by 2 factorial arranged in a completely randomized design with three replications. Measurements were performed daily to determine cumulative weight loss, yellowing, wilting and color changes. Biochemical assay was also performed to determine the changes in vitamin C and beta-carotene content. Results show that CoolBot^{TM} cold storage extended the shelf life of collards by 6 days without MAP and 13 days with MAP, in comparison to collards stored at ambient conditions. The slow rate of deterioration under CoolBot^{TM} storage (with or without MAP) was evidenced by delayed yellowing, wilting, and reduced weight loss compared to ambient conditions, in the two experimental sites. The loss in vitamin C content was significantly higher ($p \leq 0.05$) in collards under ambient conditions than those under CoolBot^{TM} storage. In ambient conditions, unpackaged collards lost 77.7% of their vitamin C content while the packaged collards lost 57.7% by end of shelf life (day four). In CoolBot^{TM} storage, packaged collards lost 18.4% whereas the unpackaged lost 26.3% of their initial vitamin C content. In CoolBot^{TM} storage, packaged collards lost 26.2% while the unpackaged lost 44.1% of their initial Vitamin C by day seven. These results demonstrate a synergistic effect of CoolBot^{TM} cold storage and MAP in preserving quality and extending the shelf life of collards.

Key words: CoolBot^{TM} Technology, Xtend, Collards, Postharvest Quality, Shelf life

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INTRODUCTION

Collard (*Brassica oleracea var.acephala.L*) is the most popular leafy vegetable in Kenya and its production value accounts for 18.4% of total value of exotic vegetables [1]. Collard is a major source of antioxidants ranked fourth vegetable in its antioxidant capacity after sweet potato, mustard, and kale greens and has great ability in binding with bile acids and being excreted from the body, thus lowering blood cholesterol levels [2]. Like other leafy vegetables, collards are highly perishable since they contain high moisture content (>90%). The water loss through transpiration results in reduced marketable weight and loss of aesthetic attributes, which affects marketability of the vegetables. Temperature greatly influences the physiological and metabolic activities within harvested leafy vegetables [3]. For every 10°C rise in temperature, the rate of deteriorative processes such as respiration, ethylene evolution, water loss and microbial growth is said to increase by 2-3 folds (referred as Q10) [4] thus shortening the shelf life of perishables like vegetables. The high perishability of leafy vegetables coupled with poor temperature management contributes to high postharvest losses. Maintaining low temperatures accompanied with increased relative humidity within the storage chamber can minimize moisture loss from the stored produce [5]. Cold storage temperatures slow down the rate of respiration, transpiration, and biochemical changes, all which are responsible for deterioration of leafy vegetables [6].

Coolbot technology uses an electronic gadget "CoolBot^{TM}" which is fixed on a normal air conditioner and overrides its thermostat enabling it to cool the storage room to the desired temperatures depending on the optimum temperature range for the stored produce [7]. A CoolBot^{TM} cold room has three components: an insulated room, electronic CoolBot^{TM} gadget, and a compatible air conditioner. This technology has been utilized to extend the shelf life of mango (*Mangifera indica*) where the shelf life was extended by 18 days and 16 days compared to ambient conditions [8-9], and Onions (*Allium cepa* L.) where loss reduction of 25% and additional value of $1.5 was realized [10]. Previous studies recommend more research on CoolBot^{TM} technology in combination with other technologies such as waxing, MAP and on different perishable commodities for wider adoption of the technology [9]. CoolBot^{TM} cold rooms are environmentally friendly because they utilize electricity more efficiently and are relatively affordable compared to conventional cold rooms [7]. Adoption of CoolBot^{TM} technology can enable smallholder farmers to extend the shelf life and ensure an extended marketing period for their produce. It can also enhance farmers' incomes and ensure constant availability of fresh vegetables to consumers.

Use of modified atmosphere packaging (MAP) provides a humid environment around the produce and causes a change in gas composition within the package resulting in reduced metabolic processes [11]. Modified atmosphere-packaging causes a reduction in O_2 levels and a rise in CO_2 within the film, as the stored produce utilize O_2 and release CO_2 during respiration. The altered gas composition on stored products reduces respiration rate, causes retardation of fungal infections on the commodities, and inhibits the ethylene effects in causing deterioration [12]. Modified atmosphere packaging promote slow rate of the change in color of stored produce which could be attributed to delay in biosynthesis of color pigments like anthocyanin and carotenoids due to

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reduced activities of metabolic processes involved in their biosynthesis [13]. In addition, MAP creates a humid environment around the produce thus reducing water loss that can cause shriveling of fruits and wilting of leafy vegetables [14]. Combination of cold storage and MAP has been shown to create a synergistic or additive effect that is superior to either technology applied independently [9-15]. The objective of this study was to evaluate the effect of CoolBot™ storage and modified atmosphere packaging (Xtend®) on the shelf life and postharvest quality of collards.

MATERIALS AND METHODS

Mature collard leaves were harvested at eight weeks from transplanting, from the university of Nairobi, Kabete farm during cool morning hours (<08:00 hrs). The harvested collard leaves were transported in plastic crates to Kabete and Juja experimental sites. At the experimental sites, the vegetables were sorted for uniformity based on size and freedom from damage. At each experimental site, a homogenous batch of freshly harvested vegetables was divided into four batches which were subjected to four different treatments. The treatments included collards packaged using Xtend® MAP or not packaged and either stored in the CoolBot™ cold room (5±2°C, 75±20% RH) or stored at ambient room conditions (25±10°C, 50±15%RH). The CoolBot™ gadget was sourced from Store-it-Cold LLC, USA. The experimental layout was a 2 x 2 factorial arranged in a completely randomized design with three replications.

Data collection

Room temperature and relative humidity

Room temperature and percent relative humidity inside the CoolBot™ cold room and ambient room were monitored hourly using HUATO data loggers (Model HE17x, Huato Electric Co., Ltd., Shenzhen, China). The data was retrieved at the end of the experiment by downloading the recorded data using HUATO app [8].

Percentage cumulative weight loss

Three batches of four leaves each were marked 1 to 3 in each treatment and used to measure cumulative weight loss using a digital weighing balance (Model Libror AEG-220, Shimadzu Corp., Kyoto, Japan). Data were collected daily where the initial weight of a batch of collard was recorded as (W1) and the new weight of the same batch was recorded as (W2) on each sampling day. The percent cumulative weight loss was calculated as follows:

$$Cumulative\ weight\ loss\ (100\%) = \frac{W1-W2}{W1}\ x\ 100$$

Color change

Three batches of collard containing three leaves each were marked in each treatment and used to determine the hue angle using a Minolta color meter (Model CR-200, Osaka, Japan). The color meter was calibrated using a white paper.

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Wilting

A sample of three batches of collards were randomly selected daily from each treatment and wilting determined as described by Namweha *et al.* [16] then expressed as a percentage. A scale ranging from 1-5 was used: 1 = 0–19%; 2 = 20–39%; 3 = 40–59%; 4 = 60–79%; 5 = > 80%.

The end of marketable shelf life of stored collards was considered for vegetables with a score of two and above.

Yellowing

A sample of three batches of collard were randomly selected daily from each treatment and their yellow color assessed visually using a non-linear color rating scale described by Singh *et al.,*[17] with slight modification. A scale ranging from 1-5 was used:

1 = 0–19%; 2 = 20–39%; 3 = 40–59%; 4 = 60–79%; 5 = > 80%

The end of marketable shelf life of stored collards was considered for vegetables with a score of two and above.

Vitamin C content

The ascorbic acid content in the samples was determined by High Performance Liquid Chromatography (HPLC) method following the procedures described by Vikram *et al.* [18]. In short, approximately 3 g of fresh sample was weighed and extracted with 0.8% metaphosphoric acid. This was made to 20 ml of extract and then centrifuged at 10,000 rpm. The supernatant was filtered and diluted with 10 mL of 0.8% metaphosphoric acid. This was passed through 0.45 µ filter and 20 µL injected into the HPLC machine. Various concentrations of ascorbic acid standards were also made to make a calibration curve. High Performance Liquid Chromatography analysis was done using Shimadzu 20 A series equipped with UV-VIS detector (20 AD) with a C-18 ODS column (250 mm x 4.6 mm x 5 ul). The mobile phase was 0.8% metaphosphoric acid, at 0.9 mL/min flow rate and wavelength of 266 nm.

Overall Shelf Life

The shelf life was determined by counting the number of days the collards stayed in storage until a pre-determined end stage. This was based on parameters such as yellowing and wilting, which are considered the principal causes of postharvest losses and poor quality in leafy vegetables [19].

Statistical Analysis

Data of the two experimental sites were subjected to contrast analysis, and finding no experimental differences, they were combined and analyzed together. Analysis of variance (ANOVA) was carried out to show the effects of storage options, MAP, and their interactions as fixed effects on traits measured. Differences were considered significant when the p-values were <0.05. Statistical analyses were performed using R version 4.0.2 software.

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RESULTS AND DISCUSSION

Temperature and relative humidity changes in the CoolBot^{TM} and ambient room conditions

During the storage period ambient room temperatures fluctuated between 16.3°C-27.6°C in Juja as compared to14.5°C-20.1°C in Kabete. High temperatures were observed between 0900hrs and 1500hrs. The CoolBot^{TM} cold rooms maintained the preset temperatures of 5±2°C throughout the experiment period. CoolBot^{TM} technology resulted in cool temperatures throughout the storage periods in the two experimental sites, which slowed the deterioration rate of collards. Other than the low temperatures, CoolBot^{TM} cold storage also resulted in high percent relative humidity relative to the ambient conditions. The low temperatures and high humidity decreased the vapor pressure difference which in turn reduced water loss from the stored produce [20], hence reduced the deterioration rate of the collards. Previous authors also found temperature to be a major factor that determines the postharvest quality of green leafy vegetables [21-22].

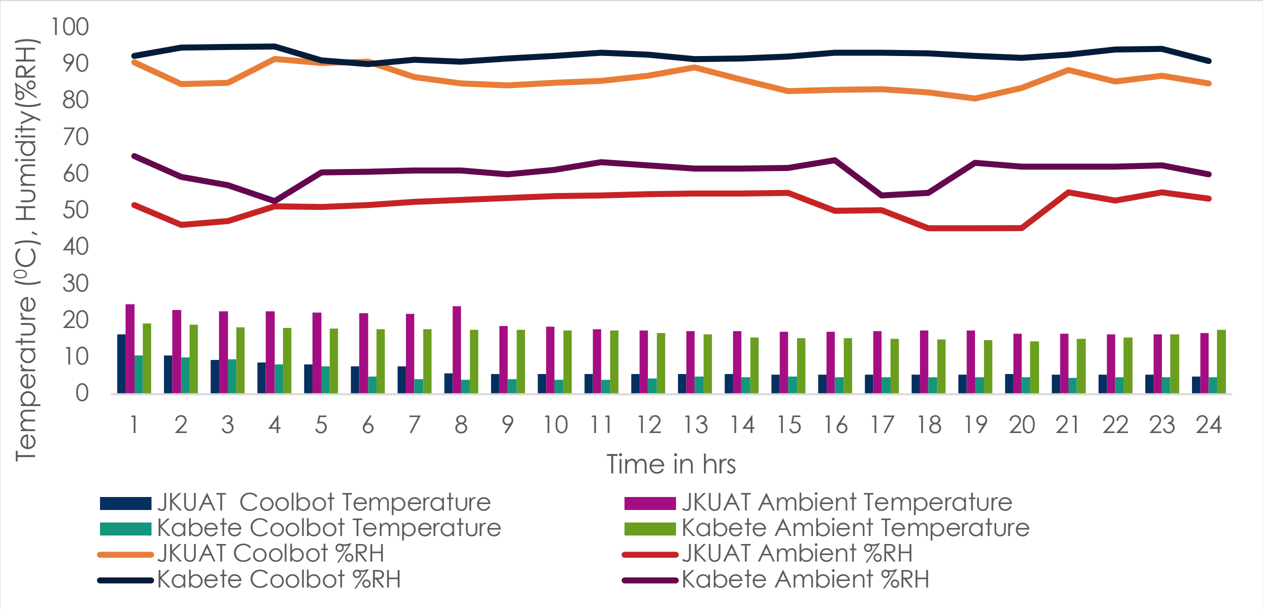

Figure 1: Changes in temperature and relative humidity in the CoolBot^{TM} and ambient room conditions in Kabete and Juja during the first 24hrs of storage

Percentage Cumulative Weight Loss

A gradual increase in weight loss was observed in all collards during storage irrespective of the storage option and/or packaging. However, collards stored under CoolBot^{TM} cold rooms retained a higher percent of their initial weight when compared to those stored in ambient conditions (Figure 2). In ambient storage conditions, unpackaged collards lost 23.5% while packaged collards only lost 3.4% of their initial weight after three days of storage. In converse, collards stored in CoolBot^{TM} and left unpackaged lost 9.1% of their initial weight on the same day while the packaged collards only lost 1.8%. The changes in weight can be attributed to the water lost through transpiration and substrate breakdown during respiration [19]. The results of the present study are in accordance with the findings by Ambuko *et al.* [5] in Amaranth who stated that the rate of PWL was influenced by temperature and relative humidity which affects the vapor pressure difference between the products and the environment and can be controlled by reducing temperature and increasing relative humidity hence the lower losses recorded under CoolBot^{TM} storage. Water loss is influenced by temperature and relative humidity whereby high temperatures and low humidity results in increased vapor pressure deficit and more water is lost from the produce to the surroundings [23]. This explains the higher percent weight loss recorded by ambient stored collards compared to cold storage. Past studies indicate that water loss is dependent on relative humidity, temperature, air movement and atmospheric pressure [24], and the rate of physiological weight loss is dependent on respiration and transpiration and is accelerated with high temperatures [25]. Rapid weight loss at higher storage temperatures were also reported in brussels sprouts (*Brassica oleracea var gemmifera*) [26], spinach (*Spinacia oleracea)* and fenugreek (*Trigonella foenum-graecum)* [27].

Use of Xtend® bags was significant ($p \leq 0.05$) in reducing weight loss both under CoolBot^{TM} storage and ambient conditions, when compared to the unpackaged collards. Combination of CoolBot^{TM} cold storage and modified atmosphere packaging (Xtend® bags) significantly ($p \leq 0.05$) reduced weight loss whereby the collards only lost 5.5% of their initial weight at the end of marketable shelf life (14 days). Further reduction in weight loss observed in MAP packaged collards can be explained by the fact that, Xtend® MAP provides a humid microenvironment within the packages reducing water loss through transpiration and causes a reduction in respiration rate due to reduced O_2 levels within the package [12]. The present findings corroborate Kumar and Singh [28], who reported negligible weight loss at the end of storage in MAP packaged broccoli (*Brassica oleracea var. italica)* compared to control samples.

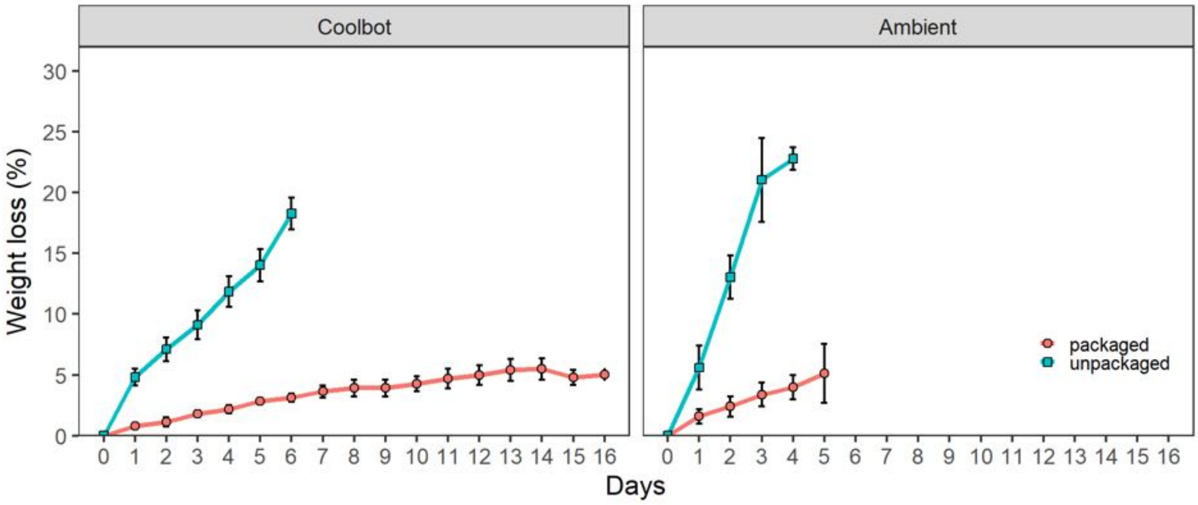

Figure 2: Cumulative weight loss of collards stored under CoolBot^{TM} cold storage and ambient storage conditions, and either packaged in Xtend bags or left unpackaged. Data from two separate experiments in Kabete and Juja were combined and shown as means ± SE; n = 4 replicate batches

Color change

The Hue angle of the stored collards reduced during storage, but the changes were more rapid for the collards stored under ambient conditions. A significant ($p \leq 0.05$) difference on color change was observed between the collards stored under CoolBot^{TM} storage and ambient conditions. Hue angle of collards stored under ambient conditions dropped after four days of storage, from 126 to 66 for the packaged, and from 120 to 60 for the unpackaged collards. However, collards under CoolBot^{TM} storage recorded 112.3 and 109.6 (green color) on the same day for the packaged and unpackaged respectively (Figure 3). Use of modified atmosphere packaging (Xtend bags) to complement CoolBot^{TM} storage further delayed the undesirable color changes in collards.

Color is one of the important factors that determine the marketability and consumer acceptance of leafy vegetables, and color change is the first visible symptom of senescence in many horticultural crops and may compromise their economic value. Therefore, slowing down the rate of color change is critical in extending the shelf life of vegetables. In the current study, the slower rate of color change in CoolBot^{TM} stored collards can be attributed to the low temperatures leading to reduced metabolic activities. Artes *et al*. [13] attributes color change to delay in biosynthesis of anthocyanin and carotenoids resulting from reduced metabolic processes due to low temperatures. In the current study, a gradual change in color from green to yellow was observed in all the stored collards. However, the changes were slower for the collards stored in cold storage as compared to ambient conditions. This can be attributed to the low temperatures in the cold rooms which slowed the rate of chlorophyll degradation hence delaying cascade mechanisms that trigger senescence and formation of the yellow color. In contrast, collards under ambient conditions showed rapid color changes due to higher temperatures which speeded up chlorophyll degradation [29]. Manolopoulou *et al.* [22] reported a stronger reduction in hue angle as the storage temperature increased in lettuce and broccoli.

The positive effects of MAP on color could be due to decreased metabolic processes responsible for both chlorophyll degradation and carotenoids synthesis or other processes that facilitate unmasking of preexisting color pigments [30]. Similar results of higher chlorophyll retention at low temperatures and packaging were reported by Negi and Roy [31].

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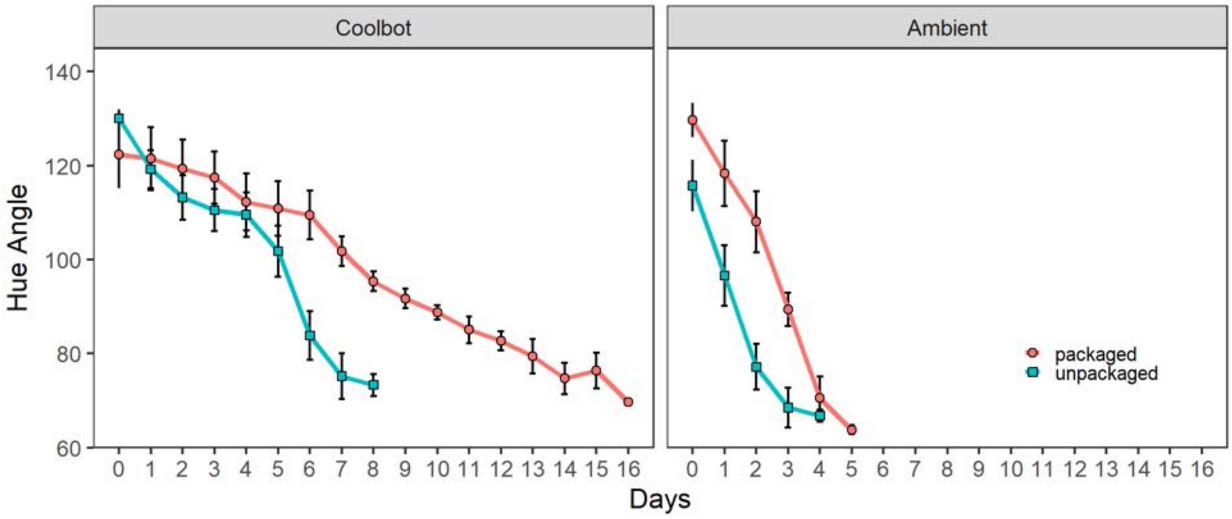

Figure 3: Changes in Hue angle of collards stored under CoolBot^{TM} cold storage and ambient storage conditions, and either packaged in Xtend bags or left unpackaged. Data from two separate experiments in Kabete and Juja were combined and shown as means ± SE; n = 4 replicate batches

Yellowing

Yellowing in collards was significantly ($p \leq 0.05$) delayed under CoolBot^{TM} cold storage when compared to ambient conditions. Yellowing was 12.5% high in the first day of storage for collards under ambient room conditions. In converse, there were no yellowing signs in the first day of storage for collards under CoolBot^{TM} cold storage, with yellowing signs appearing in the fifth day of storage (Figure 4). A 54.2% yellowing was recorded for collards stored at ambient room condition at the end of marketable shelf life (three days), while collards stored in CoolBot^{TM} showed 25% yellowing at day seven (end of shelf life). This concur with previous studies in amaranth and broccoli where yellowing was shown to occur more rapidly as storage temperature increased [5-32]. The process of senescence and yellowing in plants is related to the action of the plant's defense system and the action of antioxidants and enzymes such as superoxide dismutase, catalase, and peroxidase [33]. High temperatures and low relative humidity slow down the activities of superoxide dismutase, catalase, and peroxidase, eventually lowering the oxygen radical production that leads to the yellowing [34]. The low rate of yellowing for the cold stored collards can be attributed to the low temperatures and high humidity as reported in spinach [35]. Use of Xtend® bags in combination with cold storage delayed the yellowing of the stored collards more than in the ambient conditions. This can be attributed to the low temperatures achieved in the CoolBot^{TM} hence decreased rate of chlorophyll degradation [29] and the fact that MAP alters gas composition within the package which reduces the rate of metabolic activities responsible for both chlorophyll degradation and carotenoid synthesis or other processes involved in unmasking of preexisting color pigments [30]. Chlorophyll degradation has been shown to be associated with leaf yellowing in harvested leafy vegetables [36]. Findings of present study are in accordance with results reported by Garande *et al.* [27].

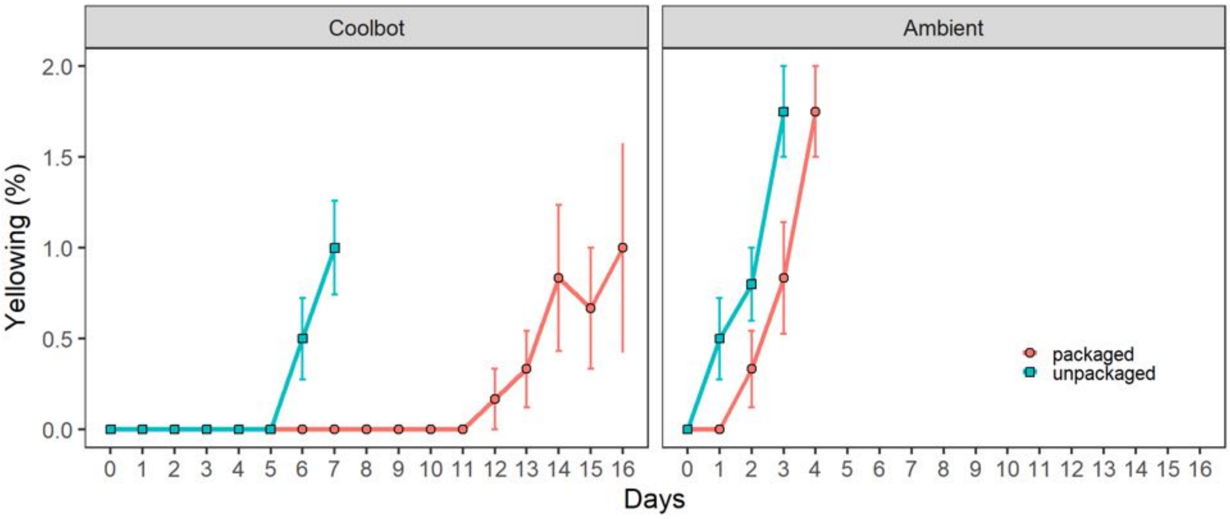

Figure 4: Yellowing changes in collards stored under CoolBot^{TM} cold storage and ambient storage conditions, and either packaged in Xtend bags or left unpackaged. Data from two separate experiments in Kabete and Juja were combined and shown as means ± SE; n = 4 replicate batches

Wilting

Wilting for collards under CoolBot^{TM} cold storage was 25% for the unpackaged, while the packaged collards showed no signs of wilting, at the end of marketable shelf life (7 days) (Figure 5). Use of modified atmosphere packaging (Xtend bags) significantly ($p \leq 0.01$) reduced wilting of collards stored under both CoolBot^{TM} and ambient conditions. Furthermore, collards packaged in Xtend bags showed no signs of wilting during the storage period. Use of MAP in cold storage greatly reduced wilting of the stored collards. Wilting in vegetables is caused by water loss which leads to reduced cell turgidity [37]. Vegetables contain >90% water, hence very susceptible to water loss leading to wilting. For most leafy vegetables, weight loss of up to 3% significantly affects their aesthetic value and marketability [5]. Temperature and relative humidity affect the vapor pressure difference (VPD) between the produce and its environment. In this regard, the lower the VPD, the lower the water loss from the stored produce [37]. The relatively lower temperatures and high relative humidity in the CoolBot^{TM} cold rooms contributed to lower VPD and subsequently reduced the physiological weight loss and wilting in collards. Similar results have been reported in leafy amaranth stored in evaporative coolers [5]. The high temperature and low relative humidity recorded in ambient room conditions resulted in increased rate of respiration and transpiration, which in turn increased moisture losses resulting in loss of turgidity whose direct impact is wilting [38]. Modified Atmosphere packages creates a humid environment around the produce thus reducing water loss that can cause shriveling of fruits and wilting of leafy vegetables [14].

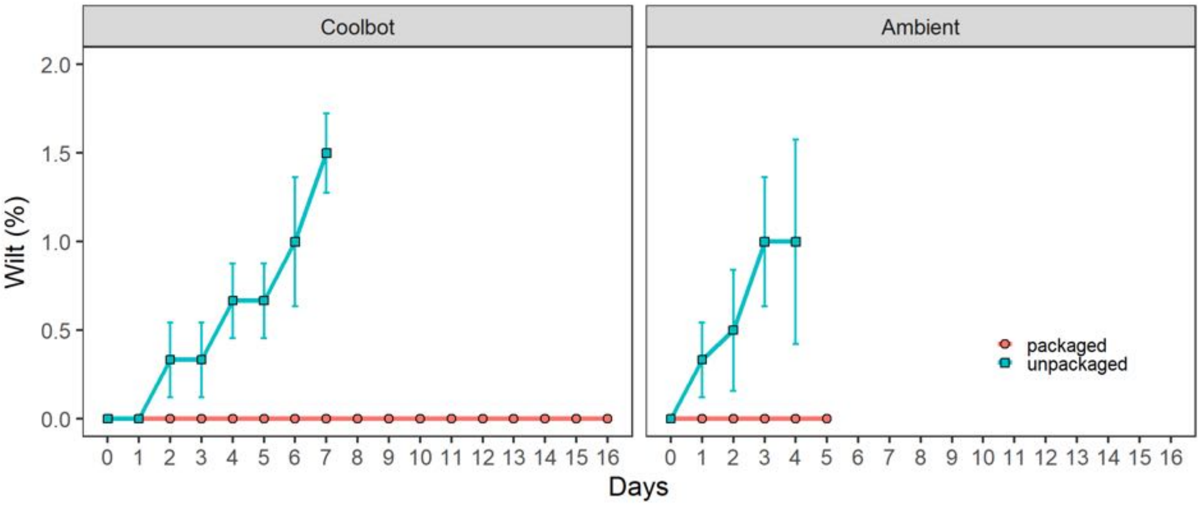

Figure 5: Wilting changes in collards stored under CoolBot^{TM} cold storage and ambient storage conditions, and either packaged in Xtend bags or left unpackaged. Data from two separate experiments in Kabete and Juja were combined and shown as means ± SE; n = 4 replicate batches

Vitamin C content

The Vitamin C content decreased gradually during the storage period for all the stored collards irrespective of storage option or packaging. However, significant ($p \leq 0.05$) losses in vitamin C content were observed under ambient storage conditions compared to CoolBot^{TM} cold storage (Figure 6). Collards stored at ambient conditions and left unpackaged lost 77.7% while the packaged collards lost 57.7% of their initial vitamin C by day 4. In converse, collards stored under CoolBot^{TM} cold storage and left unpackaged lost 26.3% while the packaged collards lost 18.4% by day 4. Importantly, collards under CoolBot^{TM} cold storage and packaged in Xtend bags retained higher vitamin C content (65.7mg/100g) at the end of shelf life (14 days).

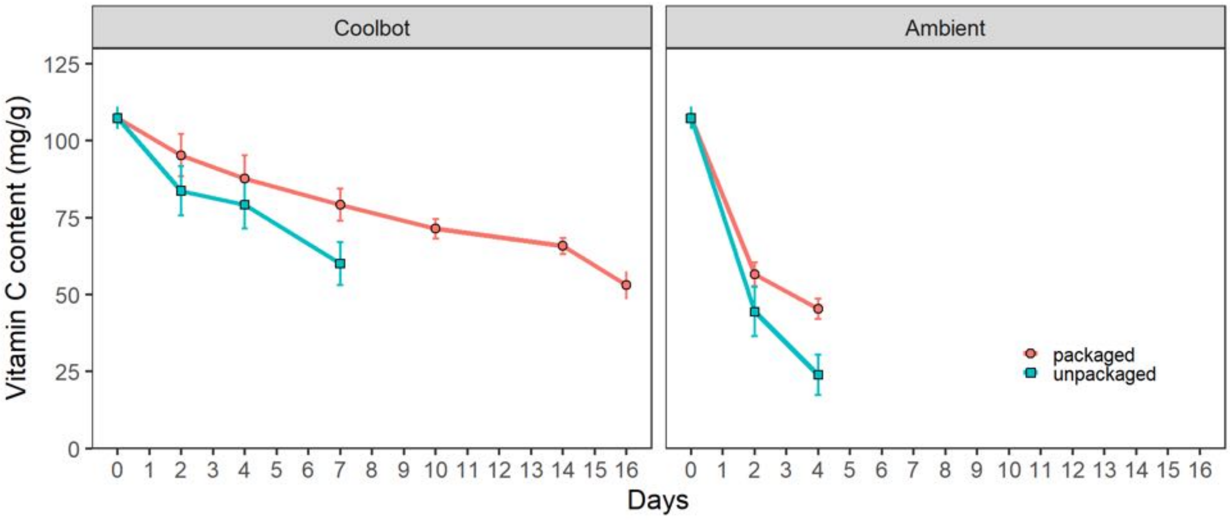

Figure 6: Vitamin C content of collards stored under CoolBot^{TM} cold storage and ambient storage conditions, and either packaged in Xtend bags or left unpackaged. Data from two separate experiments in Kabete and Juja were combined and shown as means ± SE; n = 4 replicate batches

Loss in vitamin C has been shown to correlate positively with increased storage temperature in broccoli [32] and higher retention of vitamin C content was observed for vegetables in refrigerated storage compared to room temperatures [27], which agrees with the present study. Conditions that favor wilting were shown to result in increased vitamin C loss in vegetables such as spinach, cabbage, snap beans and collards [39]. Kader *et al.* [19] attributes the loss in ascorbic acid content to both temperature and water loss, and also stated that ascorbic acid is easily oxidized and losses are enhanced by higher temperatures. This explains the higher losses recorded under ambient conditions (higher temperatures, low relative humidity) in the present study. The rate of degradation of vitamin C depends on temperature which is a critical factor involved in its destruction. Therefore, temperature management after harvest is the most important factor in maintaining vitamin C content of fruits and vegetables. Generally, fruits and vegetables show a gradual decrease in Ascorbic acid content as the storage temperature or duration increases. In the current study, use of modified atmosphere packaging (Xtend® bags) further reduced loss of vitamin C and this can be linked to reduced enzymatic oxidation due to altered gas composition in the package and reduced water loss from the stored collards. Previous studies have shown that loss of Vitamin C correlated positively with that of water loss through transpiration [14], hence the higher losses recorded for the unpackaged collards in ambient temperatures. Higher retention of Vitamin C for MAP packaged broccoli has been reported [28] and Vitamin C content was shown to be better preserved in MAP stored spinach when compared to normal air [40]. The stability of ascorbic acid is generally enforced by maintaining low temperature during storage [27].

Overall shelf life

CoolBot^{TM} cold storage extended the shelf life of collards by up to 6 days for the unpackaged and 13 days for the packaged collards compared to ambient conditions. Use of modified atmosphere packaging had greater effect when used under CoolBot^{TM} storage as it further extended the shelf life by 9 days. This can be explained by the low temperatures coupled with high humidity thus reducing the rate of deteriorative processes. The high relative humidity and low temperature recorded under CoolBot^{TM} storage technology in the present study offered favorable conditions to reduce metabolic processes taking place inside the fresh collards which reduced the deteriorative processes hence the increase in shelf life of collards observed. CoolBot^{TM} technology has successfully been utilized to preserve quality and extend shelf life of mango [8-9] and vegetables (eggplants, chilli and okra) [7].

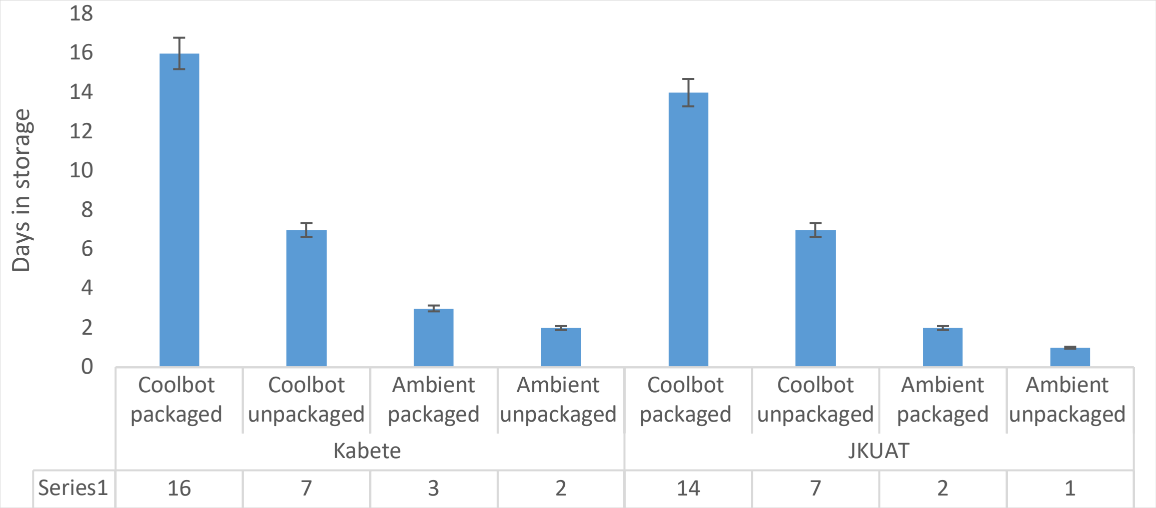

Figure 7: Shelf life of collards stored under CoolBot$^{\text{TM}}$ cold storage and ambient storage conditions, and either packaged in Xtend® bags or left unpackaged. Data from two separate experiments in Kabete and Juja. Top bars represent SE of means ($p \leq 0.05$)

Figure 8: Collards stored at (A) Ambient without MAP Day 4 **(B)** Ambient+MAP Day 4 **(C)** CoolBot^{TM} without MAP Day 4 **(D)** CoolBot^{TM} + MAP Day 4 **(E)** CoolBot^{TM} without MAP Day 8 **(F)** CoolBot^{TM} + MAP Day 8 **(G)** CoolBot^{TM} +MAP Day 10 **(H)** CoolBot^{TM} +MAP Day 14

CONCLUSION

Generally, all deteriorative processes such as weight loss, undesirable color changes, wilting and loss of vitamin C were delayed under CoolBot^{TM} storage and use of Xtend MAP led to further delay and reduction of these processes. Combination of CoolBot^{TM} technology and MAP had greater effect than when the technologies were used individually hence, found to be more effective for retaining the postharvest quality. CoolBot^{TM} technology was effective in preserving postharvest quality and extending shelf life of fresh collards for up to 16 days compared to 1-2 days shelf life under ambient conditions. CoolBot^{TM} storage is an effective technology that can benefit an organized group of small-scale farmers, aggregators and other actors along the vegetable value chains. Further studies on its effectiveness to preserve quality of different perishable commodities are recommended.

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Competing Interest Statement

The authors declare no conflict of interest.

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