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Effect of Modified Atmospheric Packaging on Extending Shelf Life of Curry Leaves (*Murraya koenigii* L.)

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Abstract: Curry leaves tree (*Murraya koenigii* L.) is a popular leafy spice and a leafy vegetable used in Sri Lanka and many other countries, except its usage for medicinal purposes due to phytochemicals present in the plant. Even though it is a very useful and commonly used herb, the freshness and the post-harvest shelf life under ambient condition is around for two days which is a huge limitation for marketing. Modified atmospheric packaging (MAP) has used as an effective method to extend the shelf life of fresh commodities including leafy vegetables. Hence, this experiment was planned to evaluate the effectiveness of MAP on post-harvest shelf life of Curry leaves. Experimental treatments were perforated and non-perforated low density polyethylene bags with gauge 150 (38 micron) and 300 (75 micron) where control samples without package. Thirty gram bundles of fresh curry leaves were used per each treatment. Leaf color values (L*, a* and b*), leaf defoliation percentage, physiological weight loss, chlorophyll content, total carotene content and visual observations were used as measurements. Control samples existed only for 2 days where completely dried out at 3rd day of storage. Irrespective of the thickness, perforated polyethylene packages extended the shelf life for 4 days. Sealed polyethylene of thickness 75 micron showed one day of storage life where it produce unpleasant odor at 2nd day of storage. Sealed polyethylene thickness of 38 micron extended the shelf life up to 6 days which was 200% increment of storage period. At 6% defoliation level, samples were considered as unmarketable and end of storage life.

Keywords*:* Leaf color, Leafy vegetable, MA packaging, Post-harvest, Spice

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Introduction

Curry leaves (*Murraya koenigii*) has been used as a leafy spice, leafy vegetable and a medicinal plant since ancient era and it is an important component in every meal preparations in Sri Lanka where, it considered as an important and specific plant for its characteristic aroma and flavor. Curry leaves contain mahanine, koenine, koenigine, koenidine, girinimbiol, girinimibine, koenimbine, O-methyl murrayamine A, Omethyl mahanine, isomahanine, bismahanine, bispyrayafoline as phytochemicals (Saini and Reddy, 2015; Gahlawat *et al*., 2014). Rajendran *et al.,* (2014) found that, linalool, elemol, geranile acetate, myrcene, allo-ocimene, α-terpinene, (E)-β-ocimene as main compounds in curry leaf oilextraction. In addition, it is equipped with antibacterial, antifungal, antiprotozoal, anticancer, hepatoprotective, anti-thrombic activities, hypoglycemic and hypolipidemic properties due to the phytochemicals presence in the plant (Rajendran *et al.,* 2014; Ganesan *et al.,* 2013; Kamleshiya *et al.,* 2012; Iyer and Devi, 2008). Curry leaves exhibited the highest total phenolic content with 137.39 ±1.35 EGA (mg)/extracts (g), and showed the best total reducing capacity among 14 tested edible plants in Sri Lanka (Galketiya *et al*., 2017).As well as, it is used as antiemetic, antidiarrheal, dysentery, febrifuge, blood purifier, tonic, stomachic, flavoring agent in curries and chutneys (Saini and Reddy, 2015).

Even though curry leaves possess high medicinal and therapeutic values, the post-harvest shelf life in fresh form is very low. Being a perishable, it cannot be retained fresh for more than a day under ambient condition (Ambrose *et al*., 2015). It undergoes leaf drying, defoliation/abscission, wilting and color change after harvesting under ambient condition. Loss of greenness or color change is due to the degradation of chlorophylls (Yamauchi and Watada, 1991) and fresh curry leaves rapidly lose their moisture and get wilted (Ambrose *et al*., 2015). Respiration, transpiration and ethylene production may be the physiological processes affecting the postharvest shelf life of fresh curry leaves other than mechanical injuries which are physical damages. In order to keep in fresh form, it is required to control the respiration, transpiration, ethylene production and mechanical damages to overcome leaf drying, defoliation/abscission, wilting and color change into an unacceptable state.

In order to reduce the water loss or to control the transpiration, it is necessary to reduce the vapor pressure deficit between the commodity and the surrounding environment. Respiration could be controlled by lowering the oxygen level, increasing CO_2 or inert gas concentration around the produce. Mechanical damages can be minimized by adopting proper harvesting and post-harvest handling practices. Ethylene biosynthesis and ethylene action inhibitors can effectively be used to delay leaf senescence, yellowing

and abscission in ethylene sensitive agricultural commodities (Iqbal *et al.,* 2017).

By modifying the gas concentration around the food, shelf life can be extended by reducing respiration and transpiration where, kirtil and Oztop (2016) identified modified atmospheric (MA) packaging as an effective method to increase the shelf life of perishables. Modified atmospheric packaging is known to modify the gas composition inside the package (Mangaraj *et al*., 2009) where fresh commodities modifies it with their respiration leading reduction of oxygen concentration and increase the carbon dioxide concentration while MA packaging can be defined as the packaging of a perishable product in an atmosphere which has been modified so that its composition is other than that of air (Soltani *et al*., 2015; Coles *et al*., 2003; Hintalin and Hotchkiss, 1986). MA packaging directly affect to reduce the respiration while affecting ethylene biosynthesis because of low amount of oxygen. In addition, higher level of carbon dioxide acts as a competitive inhibitor for ethylene action. The film being used as packaging material, acts as a moisture barrier which prevents transpiration by increasing the relative humidity inside the package leading reduction of vapor pressure deficit. Therefore, there is a possibility to use modified atmospheric packaging to extend the shelf life of Curry leaves in fresh form. Hence an experiment was conducted to find out the possibility of extending post-harvest shelf life of curry leaves using modified atmosphere created using different thickness at ambient storage.

Materials and Methods

Experimental Location

Experiment was conducted in a laboratory at the National Institute of Post-Harvest Management, Anuradhapura. Locally available Curry leaves were harvested at partially matured stage from Anuradhapura District.

Sample Preparation and Experimental Treatments

Harvested samples were put into water buckets just after harvesting and transpor ted to a shady place. Then the samples were transported to the laboratory of National Institute of Post-Harvest Manage ment. After transporting, samples were washed and allowed to drain the excess water and cleaned to remove leaves with any defects including damaged, diseased and over matured leaves. After that, partially matured compound leaves (with leaflets) were used to make bundles of 30 g for each treatment. Four treatments were used as modified atmospheric packages along with the control sample. The treatments were as follows,

1. Sealed polyethylene bags of gauge 150 – T1
2. Perforated (4 holes with 1 mm diameter on each side) polyethylene bags of gauge 150 – T2

3. Sealed polyethylene bags of gauge 300 – T3
4. Perforated (4 holes with 1 mm diameter on each side) polyethylene bags of gauge 300 – T4
5. Control (without a package) –T5/CR

Measurements

Readings were recorded daily with 3 replicates until the samples became unmarketable. Chlorophyll content (total chlorophyll content, *Ch a* and *Ch b*), total carotene content, leaf color values (L*, a* and b*), weight loss and leaf defoliation/ abscission percentage were taken as readings.

Chlorophyll Content and Total Carotene Content

Leaf samples of 2.5 g from top, bottom and middle parts of the Curry leaves bundles were taken to quantify the chlorophyll and carotene content in each replicate from the treatments. Total weight of 2.5 g samples were chopped using mortar and pestle and juice was extracted using 100%acetone as a solvent until the leaf samples became colorless and spectrophotometer was used to quantify (Optical density (OD) at 662, 645 and 470 nm) the chlorophyll content and total carotene content using below equations (Şükran *et al*.,1998).

1. Chlorophyll a = (11.75 x OD at 662 nm) – (2.350 x OD at 645 nm)
2. Chlorophyll b = (18.61 x OD at 645 nm) – (3.960 x OD at 662 nm)
3. Total carotene = (1000 x OD at 470 nm) – (2.270 x Chlorophyll a – 81.4 x Chlorophyll b)/227
4. Chlorophyll a+b were calculated

Leaf Colour Values

Leaf color was measured using Hunter lab color difference meter (CR 400, Konica Minolta) and the L*, a* and b* values were recorded (McGuire, 1992). Color values from top, bottom and middle parts of each bundle were taken and then mean value was calculated for each replicate.

Defoliation/Abscission Percentage

Abscission or the separation of leaflets from the compound leaves were measured as weight. Then the defoliated weight was expressed as percentage in each replicate with below equation.

Defoliation percentage

$$= \frac{Defoliated\ weight}{Sample\ weight} \times 100$$

Evaluation of Physiological Weight Loss

Physiological weight loss was recorded by subtracting final weights from the initial weights of stored samples and expressed as a percentage of weight loss with reference to the initial weight. Below equation was used.

Physiological Weight Loss

$$= \frac{Initial\ weight - Final\ weight}{Initial\ weight} \times 100$$

Evaluation for Odour Generation

Five-point hedonic scale was used to check odor generation with 5 was given to like very much and 1 for dislike very much.

Experimental Design and Analysis

The experiment was conducted as a complete randomized design using 3 replicates per treatment. Data were analyzed using analysis of variance and means were compared using Duncan's multiple range and LSD tests with SPSS statistical software 20.0.

Results and Discussion

Chlorophyll Content

Total Chlorophyll content (chlorophyll a+ chlorophyll b) was significantly different among treatments ($P<0.05$) (Figure 1). T3 samples showed gradual reduction of chlorophyll content and the lowest was observed at 4th day of storage whereas T1, T2 and T4 samples didn't show significant reduction even at unmarketable stage (Figure 1). In contrast, *Mukunuwenna* (*Alternanthera sessilis* L.) showed chlorophyll degradation and color change where it was considered as a main sign to determine the marketability (Kumara and Beneragama, 2020). Degradation of chlorophyll in the T3 may be due to anaerobic respiration because of high carbon dioxide concentration results from the higher barrier properties.

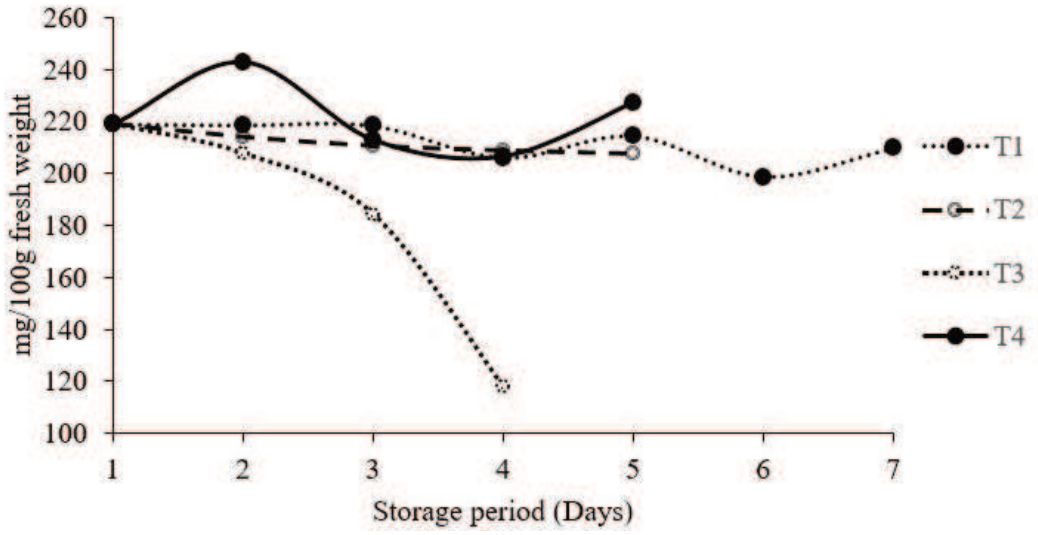

Figure 1: Changes in chlorophyll (a+b) content during storage period. T1- Sealed polyethylene bags of gauge 150, T2- Perforatedpolyethylene bags of gauge 150, T3- Sealed polyethylene bags of gauge300, T4- Perforated polyethylene bags of gauge 300.

Total Carotene Content

Total carotene content was not significantly different (P>0.05) among the treatments during storage period up to marketable stage (Figure 2).However continuous significant (P<0.05) reduction of total carotene content was observed in T3samples within tested period. According to the results, gauge 300 non-perforated LDPE affected to degrade the total carotene content whereas other treatments didn't significantly degrade (P>0.05) at the end of the marketable period. Reason for the degradation of carotene content may be the high barrier properties of LDPE 300-gauge plastic film which blocks the oxygen to move in to the package hence anaerobic respiration takes place and destroy the pigments.

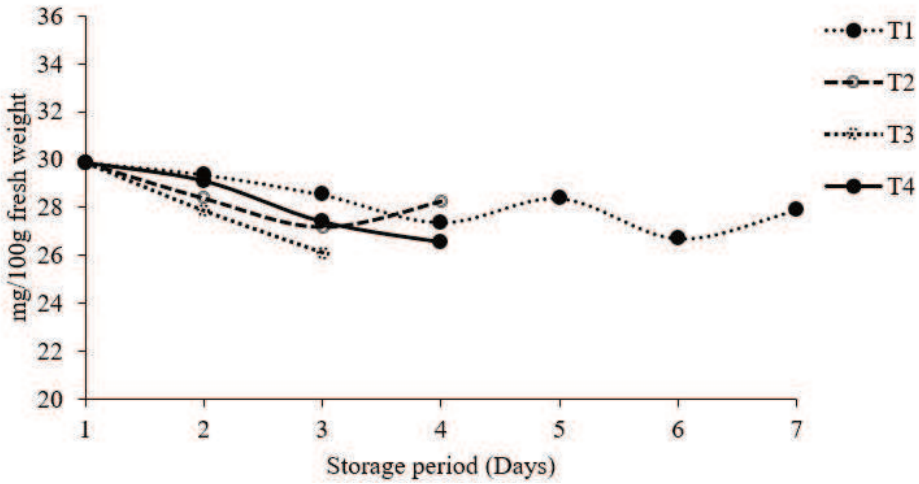

Figure 2: Changes in total carotene content during storage period. T1- Sealed polyethylene bags of gauge 150, T2- Perforated polyethylene bags of gauge 150, T3- Sealed polyethylene bags of gauge 300, T4- Perforated polyethylene bags of gauge 300.

Leaf Colour Values

Leaf color changes were measured as L*, a* and b* values. Color values were significantly different in control samples compared with other treatments (P<0.05). Change of leaf color was not much observable during the storage period except for control and T3samples. Rapid increment of L* and b* values were observed for control samples after 2nd day whereas reduction was observed in a* value (Figure 3). T3 samples showed reddish brown color patches on 3rd day of storage hence increment of b* value whereas other treatments didn't. Ambrose et al. (2015) observed browning effects in both 38 and 75 micron non-perforated LDPE in their experiment for curry leaves. However, in present study, 75 micron LDPE non perforated treatment showed brown patches, whereas 38 micron LDPE non-perforated treatment didn't show browning effect. McGuire (2000) also indicated that the curry leaves didn't show visible color changes stored under 1ºC for 15 days in sealed bags

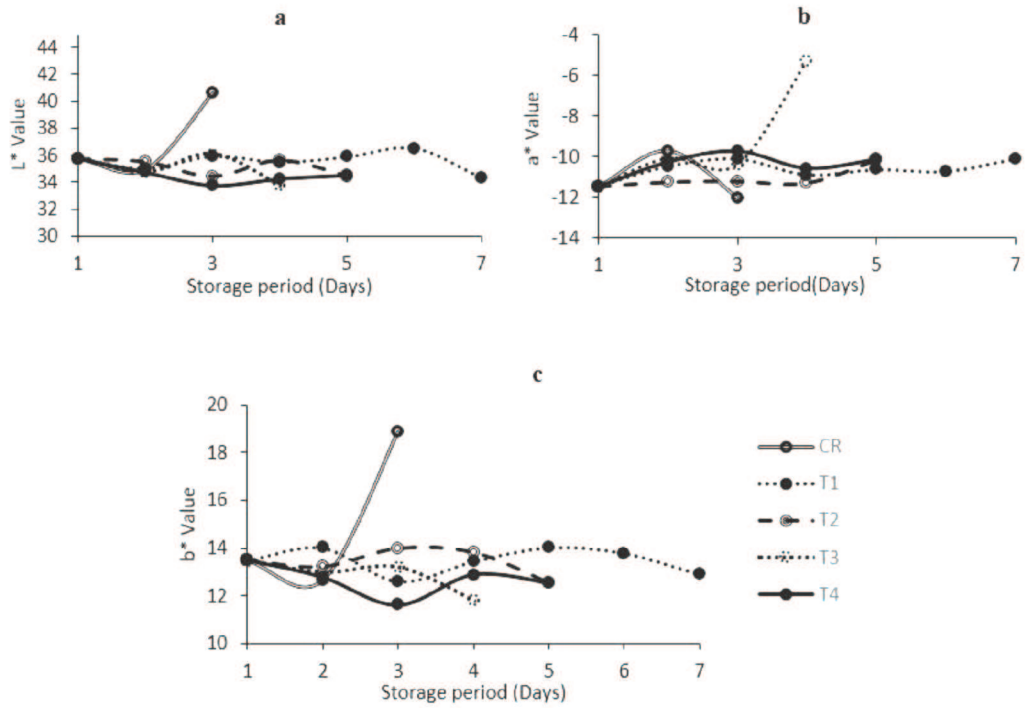

Figure 3: Changes in leaf color (L*, a* and b*) during storage period.T1- Sealed polyethylene bags of gauge 150, T2- Perforated polyethylene bags of gauge 150, T3- Sealed polyethylene bags of gauge 300, T4- Perforated polyethylene bags of gauge 300, CR /T5- Control.

Physiological Weight Loss

Physiological weight loss was the highest in control sample and it was significantly different ($P<0.05$) compared with other treatments. Lowest physiological weight loss was observed in T1 samples (Table 1) and it was lower than 3% even at 7th day of storage. Similar results were obtained for *Mukunuwenna* (*Alternanthera sessilis* L.) by Kumara and Beneragama (2020) under similar conditions in similar packaging material. Results of the present study revealed that the physiological weight loss could be reduced by using suitable packing materials with appropriate vent areas. One reason for the reduction of physiological weight loss may be the high relative humidity inside the package which is a result of high moisture barrier properties of LDPE packaging material where directly reduce the transpirational water loss by reducing vapor pressure deficit between the surrounding atmosphere and the leaves. Weight loss from leafy vegetables leads to reduce the marketability and usability where, Ben-Yehoshua and Rodov (2002); Ambuko *et al.,* (2017) indicated that the leafy vegetable loss their marketability when they lose 3% of their weight. Positively, MA packaging could be an effective method to reduce the weight loss of leafy vegetables.

Table 1: Physiological weight loss (Percentage) of Curry leaves at different treatments during the storage period

Treatment	Time (Days of storage)					
	2	3	4	5	6	7
T1	0.73±0.05	1.03±0.08	1.57±0.09	2.08±0.06	2.43±.0.08	2.99±.0.06
T2	1.52±0.39	2.26±.0.23	3.48±0.48	4.15±0.52	-	-
T3	0.88±0.17	1.96±0.23	-	-	-	-
T4	0.85±0.32	1.64±0.20	2.29±0.24	2.82±0.29	-	-
T5/CR	12.88±5.21	46.97±3.87	-	-	-	-

All values are in percentage of weight loss. T1- Sealed polyethylene bags of gauge 150, T2- Perforated polyethylene bags of gauge 150, T3- Sealed polyethylene bags of gauge 300, T4- Perforated polyethylene bags of gauge 300, T5/CR- Control. Each value represents mean ± S.D. of three replicates.

Defoliation Percentage

Curry leaves were considered as unmarketable at 6% defoliation stage. Control samples and T3 samples didn't show leaf defoliation even at unmarketable stage. T2 and T4 samples subjected to more than 6% defoliation at 5th day of storage where T1 samples at 7th day of storage (Table 2).

Table 2: Defoliation percentage of Curry leaves at different treatments during the storage period

Treatment	Time (Days of storage)				
	3	4	5	6	7
T1	2.65±1.45	2.78±0.93	4.81±0.25	4.96±0.25	8.88±.4.54
T2	0	2.67±.0.76	8.43±3.86	-	-
T3	0	-	-	-	-
T4	0.70±0.10	3.65±1.50	23.69±14.70	-	-
T5/CR	0	-	-	-	-

All values are in percentage of defoliated weight.T1- Sealed polyethylene bags of gauge 150, T2- Perforated polyethylene bags of gauge 150, T3- Sealed polyethylene bags of gauge 300, T4- Perforated polyethylene bags of gauge 300, T5/CR- Control. Each value represents mean ± S.D. of three replicates.

Moreover, when considering the results of odor generation and visual color changes, T3 samples produced bad odor (results not shown) at 2nd day of storage and formed brown color patches at 3rd day of storage. Control samples were completely dried out at 3rd day of storage. Other treatments didn't produce bad odor (results not shown), brown color patches or they didn't wilt or dry. As mentioned earlier, control and T3 samples didn't defoliate. However Storage life was decided for control samples by physiological weight loss and leaf drying. Control samples completely dried off at 3rd day of storage and physiological weight loss was nearly 50% (46.97±3.87) where it was considered as unmarketable. Even at 2nd day of storage, control samples started to drying and physiological weight loss was 23% higher than the allowable loss at marketability where it was considered as 3% (Ben-Yehoshua and Rodov, 2002; Ambuko *et al.,* 2017). Storage life for T3 samples decided by the odor generation and browning. Storage life of T3 samples were considered as only one day because of unpleasant odor generation and development of brown patches. Storage life of T1,T2 and T4 samples were decided by defoliation percentage. According to the results, T1 extended the shelf life of Curry leaves for 6 days, T2 and T4 for 4 days. Shelf life was not extended by T3 and it was less than one day compared with control samples where it was 2 days in control. Ambrose *et al*., (2015) also found that perforated film packaging extended the shelf life of curry leaves for 4 days in India. However they decided it by color scoring. Defoliation (falling of leaflets from leaves)was a major issue under MA packaging conditions in present study where it was considered as main parameter to decide storage life except for T3 and control samples as stated earlier.

Conclusions

Results of the present study showed that the post-harvest shelf life of Curry leaves was 2 days under ambient conditions (31.64±1.84 ºC; 53.21±9.40 RH). Modified atmospheric packaging with sealed LDPE gauge 300 (75 micron) exhibited only one day of post-harvest life. Perforated LDPE of gauge 300 (75 micron) and 150 (38 micron) showed 4 days of post-harvest shelf life which was 100% increment where sealed LDPE gauge 150 showed post-harvest shelf life of 6 days which was 200% increment. Finally it can be concluded that sealed low density polyethylene of gauge 150 (38 micron) could be used as an effective method to maintain the keeping quality of fresh curry leaves under ambient conditions (31.64±1.84 ºC; 53.21±9.40 RH).

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