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Modified Atmospheric Packaging Extends the Postharvest Shelf Life of *Mukunuwenna (Alternanthera sessilis* L.)

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ABSTRACT

Sessile Joyweed (Alternanthera sessilis) locally known as Mukunuwenna is the most widely cultivated and consumed leafy vegetable in Sri Lanka. However, it loses its shelf life within 3 to 4 days after harvesting under ambient conditions, which is a big issue in the market and at the household level. Modified atmospheric (MA) packaging has given promising results for many perishables to increase their postharvest shelf life which has not been tested for Mukunuwenna. Hence, the present experiment was carried out to evaluate the effect of modified atmospheric packaging on keeping quality of *Mukunuwenna*. The experiment was conducted using perforated and nonperforated polyethylene with the gauge of 150 and 300. Mukunuwenna bundles with 100 g were used per each treatment. Irrespective of the gauge, perforated polyethylene (PPE) packages reduced water loss remarkably. However, PPE was not effective in delaying yellowing and leaf shedding. Sealed non-perforated polyethylene (SNPPE) packages reduced water loss and leaf yellowing remarkably irrespective of the thickness. SNPPE delayed chlorophyll degradation significantly (P<0.05) compared to that of PPE and control samples. Total soluble solid (TSS) content increased continuously in control samples whereas it was constant between 5.5% and 7% in other treatments. Leaf wilting and withering were the major problematic causes to lose the keeping quality of control treatment whereas leaf yellowing and decaying were the identified causes in PPE and SNPPE treatments under ambient conditions (34.2±1.8 °C). Visual quality rating (VQR) test results indicated the postharvest shelf life of control samples, PPE in both thicknesses and SNPPE in both thicknesses as 3, 4 and 6 days respectively. SNPPE with 150 and 300 gauges extend postharvest shelf life of *Alternanthera sessilis* (*Mukunuwenna*) by 100%.

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Leafy vegetables have been playing an important role in the Sri Lankan diets since ancient era. Sri Lankans consume leafy vegetables not only as a food source but also as an indigenous medicine for curative and therapeutic purposes because of their nutritive and medicinal values. At present, there is a high demand on leafy vegetables all around the world because of their inherent characters. In Sri Lankan meals, leafy vegetables are an important component with rice and other curries. Of all the leafy vegetables, sessile joyweed (Alternanthera sessilis) locally known as Mukunuwenna/ ponnanganni is the most commonly consumed leafy vegetable in Sri Lanka (Gunasekera et al., 1990). That is mainly because of its taste and comparatively low cost; and large quantities are often consumed in a single meal (Balasuriya and Dharmaratne, 2007). Mukunuwenna contains phytochemicals such as antioxidants, phenols, ascorbic acid (Senarathne et al., 2016) and beta carotene (Chandrika et al., 2006) which possess various health benefits to consumers.

Even though Mukunuwenna contains various types of nutrients, it has a very short postharvest shelf life. At ambient conditions, it is as short as about 3-4 days due to leaf yellowing and wilting (Rajapakse and Beneragama, 2016). Wilting takes place mainly due to water loss from the commodity. Leaf yellowing is due to senescence-related degradation of chlorophylls. In ethylene sensitive perishables, the senescence is accelerated and governed mostly by ethylene. High respiration rate also accelerates the senescence of leafy vegetables by depleting food reserves within the commodity. Reducing water loss and delaying leaf yellowing/ senescence may help in extending the postharvest life Mukunuwenna. of Transpiration should be controlled to reduce the water loss from agricultural commodities. Vapor pressure deficit between the produce and the surrounding atmosphere should be reduced prevent or to minimize to transpirational water loss which helps preventing or delaying the leaf wilting. Ethylene biosynthesis and ethylene action inhibitors can effectively be used to delay leaf

senescence and yellowing in ethylene sensitive agricultural commodities. Modified atmospheric packaging (MAP) can be used to address these issues to increase the postharvest life of *Mukunuwenna* by delaying leaf yellowing and wilting.

MAP is 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 (Hintlian and Hotchkiss, 1986; Coles et al., 2003; Soltani et al., 2015). It modifies the composition of the internal atmosphere of a package which reduces the level of O_2 and increase the level of CO_2 or other inert gases. This will lead to reduce the respiration, prevent ethylene production due to low concentrations of O_2 and the competitive inhibition of ethylene action by higher concentrations of CO₂. Apart from that, modified atmospheric packaging creates a barrier which restricts movement of water through the film being used. This phenomenon helps to increase the relative humidity while reducing vapor pressure deficit, consequently reducing the transpiration. Passive modified atmospheric packaging is commonly practiced which modifies the atmospheric condition as a result of respiration of the product and gas transmission rates of the packaging film. It is a low-cost method compared to active modified atmospheric packaging and can easily be adopted by the people to extend the postharvest life of Mukunuwenna.

Therefore, the principle hypothesis of this study was to determine whether the modified atmospheric packaging has significant effects on extending the postharvest shelf life of Mukunuwenna. Specifically, the study intended to i) identify the effect of perforated and non-perforated plastic films as modified atmospheric packaging of Mukunuwenna, ii) compare the effectiveness of 150 and 300 gauge low density polyethylene (LDPE) in atmospheric packaging modified of Mukunuwenna, iii) compare the quality of modified atmospheric packed and non-packed Mukunuwenna in order to select the most suitable packing method and material for Mukunuwenna.

MATERIALS AND METHODS

Experimental location

Experiment was conducted in a laboratory at the National Institute of Post-Harvest Management, Anuradhapura. *Mukunuwenna* hybrid 'Thissa' was harvested from a selected commercial cultivation in Bangadeniya, Sri Lanka.

Sample preparation and experimental treatments

Cut ends of the harvested samples were dipped in water in buckets just after harvesting and transported to a shady place in the farm. The samples were then washed with water and made into bundles. The bundles were placed in water buckets again, stacked in plastic crates and transported to the laboratory of National Institute of Post-Harvest Management. The samples were cleaned to remove leaves with any defects including damaged, diseased and ripened leaves. Then, 2 inches from the base of the stems were removed to remove most matured leaves and bundles of 100 g Mukunuwenna stems were prepared for each treatment. Following four treatments were used as modified atmospheric packages, along with the control:

- 1. Sealed non perforated polyethylene bags of gauge 150 T1
- Perforated (4 holes with 1 mm diameter on each side) polyethylene bags of gauge 150 – T2
- Perforated (4 holes with 1 mm diameter on each side) polyethylene bags of gauge 300 – T3
- 4. Sealed non perforated polyethylene bags of gauge 300 T4
- 5. Control (without a package-Spraying water 4 times per day) CR

Measurements

Readings were taken daily with 3 replicates until the samples became unmarketable. Chlorophyll content (total chlorophyll content, *Ch a* and *Ch b*), leaf colour values (L*, a* and b*), weight loss, total soluble solid content and visual quality ratings were taken as readings.

Chlorophyll content

Leaf samples of 2.5 g from top, bottom and middle parts of the *Mukunuwenna* bundles were taken to quantify the chlorophyll content in each replicate from the treatments. Total weight of 2.5 g samples was ground using a mortar and a pestle and extracted using 80% acetone until the leaf samples became colourless. Spectrophotometer was used to quantify (Optical density (OD) at 660 and 642.5 nm) the chlorophyll content using the following equation (Ranganna, 1986).

- 1. Total chlorophyll content = (7.12 x OD at 660 nm) + 16.8 x OD at 642.5 nm)
- Chlorophyll a = (9.93 x OD at 660 nm) (0.777 x OD at 642.5 nm)
- Chlorophyll b = (17.6 x OD at 642.5 nm) (2.81 x OD at 660 nm)

Total soluble solids (TSS) content

Plant samples from top, bottom and middle parts of the *Mukunuwenna* bundles were taken and ground using mortar and pestle. The juice was extracted using a muslin cloth. TSS of extracted juices were measured by a temperature compensated digital refractometer (3810, Atago PAL-1) and expressed as a percentage.

Leaf colour values

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

Evaluation of weight loss and visual quality rating

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. Visual quality rating was developed for MA packed samples based on leaf colour, leaf shedding, leaf wilting and leaf decaying. Visual quality of *Mukunuwenna* was rated using 1–5 scale, i.e.5 = excellent (green and fresh), 4 = good (yellowing and/or wilting started), 3 = fair (10% leaf yellowing and/or wilting, no leaf shedding and decaying), 2 = poor (25% leaf

yellowing and/or wilting, leaf shedding and decaying started, unmarketable, but usable) and 1 = unusable (50% leaf yellowing and/or wilting, leaf shedding and decaying continues).

Experimental design and analysis

The experiment was conducted as a complete randomized design. Parametric data were analyzed using analysis of variance and means were compared using Duncan multiple range and LSD tests with SPSS statistical software 16.0. Visual quality rating was analyzed using Kruskal-Wallis test.

RESULTS AND DISCUSSION

Chlorophyll content

Chlorophyll content (total chlorophyll, chlorophyll a and b) was significantly different (P<0.05) among the treatments. Highest chlorophyll (total chlorophyll, chlorophyll a and b) content was observed in T4 throughout the storage period whereas the lowest

chlorophyll content was observed in control sample at the 4th day of the storage period while T3 showed its lowest total chlorophyll content at the 5th day of storage period (Figures 1, 2A and 2B). Significant (P<0.05) reduction in chlorophyll content was observed in the control sample at the 4th day of storage period where it became unmarketable. A significant (P<0.05) reduction of chlorophyll content in T2 and T3 samples were observed by the 5th day of storage period.

Colour values

Leaf colour change of *Mukunuwenna* is considered as a major visual index of marketability. The L*, a* and b* values indicate the colour directions and they were significantly different at the end of the storage period (P<0.05) among the treatments. There were variations in the colour values during the storage period and increment of L*, a* and b* values were observed from day one to the day that they lost their marketability (Figure 3).

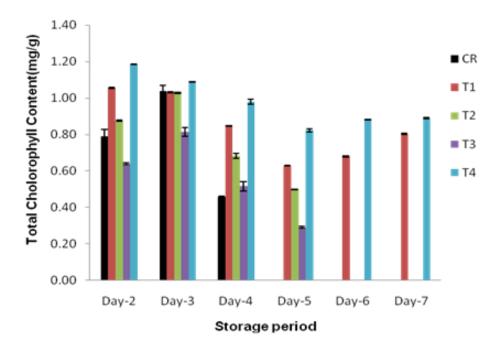


Figure 1: Total Chlorophyll content of Mukunuwenna at different treatments during the storage period.

Note:* All values are expressed in mg/g of fresh weight. CR- Control, T1- Sealed non perforated polyethylene bags of gauge 150, T2- Sealed perforated polyethylene bags of gauge 150, T3- Sealed perforated polyethylene bags of gauge 300, T4- Sealed non perforated polyethylene bags of gauge 300. Vertical bars show standard error of mean

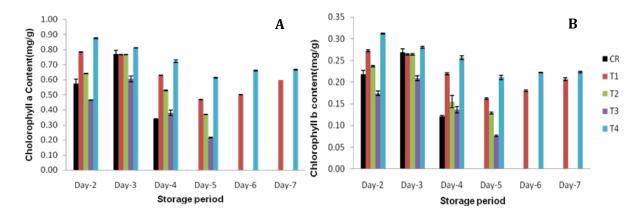


Figure 2: A) Chlorophyll *a* and B) Chlorophyll *b* content of Mukunuwenna at different treatments during the storage period.

Note:* All values are expressed in mg/g of fresh weight. Vertical bars show standard error of mean

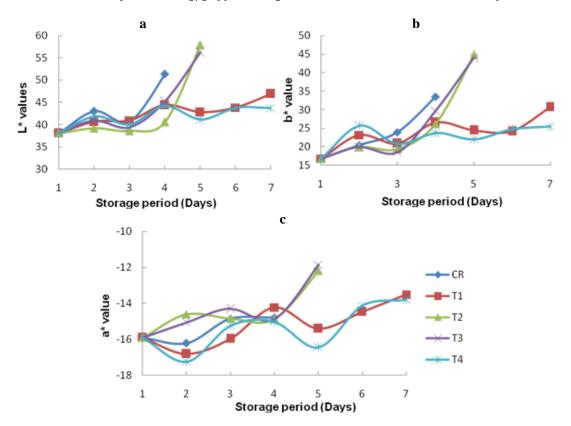


Figure 3: Changes in leaf color (L*, a* and b*) of Mukunuwenna under MA packing and control treatments over time.

Note: CR- Control, T1- Sealed non perforated polyethylene bags of gauge 150, T2- Sealed perforated polyethylene bags of gauge 150, T3- Sealed perforated polyethylene bags of gauge 300, T4- Sealed non perforated polyethylene bags of gauge 300.

Total soluble solid content

Total soluble solid (Brix degree) content of the treatment samples showed a significant difference (P<0.05) compared to the control

sample after the 2^{nd} day. Brix value of control sample showed a continuous increment during the storage period whereas other treatments did not, which remained between 5.7 to 7 % of brix value (figure 4).

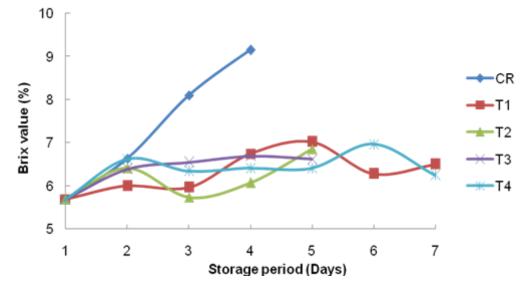


Figure 4: Changes in the brix value during the storage period with treatments.

Note: CR- Control, T1- Sealed non perforated polyethylene bags of gauge 150, T2- Sealed perforated polyethylene bags of gauge 150, T3- Sealed perforated polyethylene bags of gauge 300, T4- Sealed non perforated polyethylene bags of gauge 300.

Physiological weight loss

The physiological weight loss was greater in the control sample and it was significantly (P<0.05) different compared to other treatments. Weight loss percentage of control sample was 44.65 ± 6.28 % at the 4th day of storage period which was not in marketable condition whereas other treatments showed less than 3% of weight loss even at the unmarketable stage (Table 1).

Visual quality rating

Visual quality rating was conducted to evaluate the marketable postharvest life of *Mukunuwenna* after applying the treatments (Table 2). Control sample had 3 days of marketable life whereas T2 and T3 had 4 days and the highest postharvest shelf life was observed in the T1 and T4 samples achieving 6 days of marketable period after harvesting (Table 2).

Treatment	Time (Days of storage)								
Treatment	2	3	4	5	6	7			
CR	7.86±2.54	27.57±7.22	44.65±6.28	-	-	-			
T1	0.36±0.03	0.71±.02	$1.03 \pm .05$	1.44±.02	1.80±.04	2.13±.07			
T2	0.47 ± 0.08	0.79±.09	1.12±.09	1.54±.14	-	-			
Т3	0.51±0.09	0.99±.11	1.41±.21	2.01±.32	-	-			
T4	0.42±0.05	0.77±.09	1.10±.12	1.54±.22	1.93±.13	2.27±.14			

Table 1: Physiological weight loss (Percentage) of Mukunuwenna at different treatmentsduring the storage period

*All values are in percentage of weight loss. CR- control, T1- Sealed non perforated polyethylene bags of gauge 150, T2- Sealed perforated polyethylene bags of gauge 300, T4- Sealed non perforated polyethylene bags of gauge 300. Each value represents mean ± S.D. of three replicates.

	Time (Days of storage)								
Treatment	1(Initial)	2	3	4	5	6	7		
CR	5	4	3	1	-	-	-		
T1	5	5	5	4	4	3	2		
T2	5	5	4	3	1	-	-		
Т3	5	5	4	3	1	-	-		
T4	5	5	5	4	4	3	2		

Table 2: Visual Quality Rating of Mukunuwenna at Different Treatments during the Storage Period

1-5 scale, i.e.5 = excellent (green and fresh), 4 = good (yellowing and/or wilting started), 3 = fair (10% leaf yellowing and/or wilting, no leaf shedding and decaying), 2 = poor (25% leaf yellowing and/or wilting, leaf shedding and decaying started, unmarketable, but usable) and 1 = unusable (50% leaf yellowing and/or wilting, leaf shedding and decaying continues). CR- control, T1- Sealed non perforated polyethylene bags of gauge 150, T2- Sealed perforated polyethylene bags of gauge 150, T3- Sealed perforated polyethylene bags of gauge 300.

Moreover, above tested results revealed the path to interpret the properties which affect the shelf life of Mukunuwenna with MA packaging. Many foods (specially fruits and vegetables) are spoilt rapidly in air due to moisture loss or uptake, reaction with oxygen and the growth of aerobic microorganisms (Soltani et al., 2015) leading to a shorter shelf life. Modified atmospheric (MA) packaging has been identified as an effective method to increase the shelf life of perishables (Kirtil and Oztop, 2016) which has been used to modify the O_2 and CO_2 levels within the package headspace (Mangaraj et al., 2009) that helps to control the respiration rate. Moreover, MA packing maintains a higher relative humidity within the package where it minimizes the water loss from the commodity while keeping the freshness. Mukunuwenna is considered as a highly perishable leafy vegetable in Sri Lanka which has a higher respiration rate, leading to a shorter shelf life. In this experiment, we found that MA packed Mukunuwenna showed an extended postharvest shelf life.

Generally, green leafy vegetables including *Mukunuwenna* possess higher respiration rate while undergoing many physiological changes after harvest, shortening the postharvest shelf life. Leaf colour change from green to yellow is a major visible change due to degradation of chlorophyll which unmasks the carotenoid pigments underneath. Another visible index is the wilting of the plant samples due to water loss. Colour change from green to yellow was observed in T2 and T3 (Figure 3b, c) whereas

wilting due to physiological water loss was observed in control samples (Table 1 and 2) as major causes of ending the postharvest shelf life by making sessile joy weed unusable. T1 and T4 did not show considerable water loss, colour change and chlorophyll degradation compared to initial samples (Figures 1, 2, 3, 4 and Table 1) until these samples became unmarketable due to decaying and shedding of leaves after six days in storage (Table 2). However, variation of chlorophyll content that was observed during storage period may be due to the initiation of new leaves and shoots. In addition, the light environment was not taken into consideration during the storage period, hence it may be another reason affecting the variation of chlorophyll content (Ferrante et al., 2004).

Leaf colour values of L*, a* and b* gave a quantitative and scientific description about the colour changes during storage period. The L* value describes the changes of lightness and darkness of the *Mukunuwenna* samples. L* value was increased during the storage period irrespective of the treatments where control sample, T2 and T3 showed a rapid increment of lightness after 3rd day of storage whereas T1 and T4 did not show any rapid increment of L* value though these two treatments show higher L* value than initial sample at the end of the storage period (Figure 3 a).

The b* value indicates the blueness or yellowness of the samples. Positive values indicate the yellowness whereas negative values indicate the blueness. The control sample showed a continuous increment of b* value (Figure 3b) whereas T2 and T3 showed a rapid increment of b* value (yellow colour) and rapid reduction of chlorophyll content (Figure 3b and Figure 1) after 3rd day of storage period due to degradation of chlorophylls which may be due to production of ethylene.

MA packing deals with reduction of O_2 and increment of CO₂ as mentioned earlier, which create a stress condition on the Mukunuwenna samples. These samples were kept under ambient conditions with the mean temperature of 34.2±1.8 °C which causes a temperature stress on the samples. Due to pin holes in T2 and T3 samples, O₂ goes inside and high temperature, ethylene due to biosynthesis may have started. Hall and Smith (1995) observed that stresses lead to initiate the production of ethylene in such conditions. Ethylene production was continued without interruption with these conditions. Chlorophyllase is the enzyme responsible for the degradation of chlorophyll (Ladaniya, 2008) while ethylene induces the production of chlorophyllase (Trebitsh et al., 1993). Therefore, perforated MA packing must have led to degrade the cholorophyll at a higher rate once ethylene production started. T1 and T4 samples did not show a rapid increment of b* value or reduction of chlorophyll content during the storage period. That might be due to inhibition of ethylene production under lack of oxygen and competitive inhibition of ethylene action by CO₂. Golden *et al.* (2014) have described the effect of CO_2 against ethylene biosynthesis and the ethylene action.

The a* value describes the redness or greenness of the samples. Negative values indicate the green colour while positive values indicate the red colour. The control sample, T2 and T3 showed reduction of green value (Figure 3c) during the storage period which may be due to degradation of chlorophylls whereas T1 and T4 did not show regular pattern of reduction or increment during the storage period (Figure 3c). These results may have a link with the production of ethylene which directly affects the degradation of chlorophyll.

Physiological weight loss of the samples is one of the major causes of quality deterioration in fresh leafy vegetables mainly due to transpiration after harvesting. The transpirational water loss affects the loss of freshness as evidenced by wilting, shriveling, loss of firmness, crispness, and and succulence, which all are components of freshness (Ambuko et al., 2017). Physiological weight loss of control treatment was much higher than other 4 treatments where 7.9 %, 27.6% and 44.7 % in 2^{nd} , 3^{rd} and 4^{th} day of storage respectively. It was below 3% in other treatments even at the unusable stage (Table 1 and 2). Previous studies have shown that the leafy vegetables become unusable when they lose 3% of their weight (Ben-Yehoshua and Rodov, 2002; Ambuko et al., 2017). Similarly, in Mukunuwenna samples with MA packing irrespective of perforation, weight loss is less than 3% even at unusable stage. Furthermore, MA packing prevents water loss hence it prevent the products from wilting even though it becomes unusable due to chlorophyll degradation and decaying of leaves.

Total soluble solid (TSS) content gives an idea about the sugar content of the samples tested. Control samples showed a continuous increment of TSS during the storage period whereas other treatments did not show a considerable increment. The increase in TSS in control samples may be due to loss of water as evidenced by Wijesinghe and Sarananda (2002) for stored pineapples.

Visual quality rating gave а better understanding about the external appearance of the *Mukunuwenna* samples. Leaf yellowing, leaf wilting, leaf shedding and leaf decaying are the major visual quality characters used by the consumers to decide the quality. These quality attributes were used to rate the quality of the stored *Mukunuwenna* samples to evaluate the storable life. Visual quality rating revealed that, the control samples with spraying water 4 times a day lasts only for 3 days while the perforated MA packing in both gauges of 150 and 300 lasts marketable for 4 days (Table 2) irrespective of the thickness of LDPE plastic films. Samples stored using non perforated LDPE showed the highest postharvest shelf life of 7 days (Table 2) irrespective of the thickness of plastic films.

Aharoni *et al.* (1988) have reported similar results for 22 fresh herbs including watercress, parsley, dill and coriander in Israel. Contrasting results were observed by Taduri *et al.* (2017) for mango where, perforated film packages with 5% perforation as the best treatment for shelf life extension. Even though sealed non perforated film packages increased the postharvest shelf life in the present study, it caused a slight off odor which completely disappeared upon aeration, as was observed by Aharoni *et al.* (1988).

CONCLUSIONS

Experiment results showed that the postharvest shelf life of *Mukunuwenna* is 3 days at ambient conditions $(34\pm1.75 \text{ °C}; \text{ RH} 57\pm5\%)$ whereas modified atmospheric packaging with LDPE polyethylene gauge 150 (38 micron) and 300 (75 micron) with perforation extend the postharvest shelf life for 4 days which was a 33% increment. Moreover, sealed, non-perforated packaging increases the postharvest shelf life up to 7 days which is a 100% increment while maintaining the freshness.

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