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Determination of changes on physical, chemical and organoleptic properties of selected leafy vegetables stored under different temperatures and relative humidity

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ABSTRACT

Fresh commodities which are harvested from the field tend to deteriorate if the proper cooling methods are not practiced. When the harvested commodities are exposed to an environment where temperature is lower than the commodity and the relative humidity is higher (≈95%), it reduces the rate of respiration and transpiration. This study has proved that the cold and humidified condition (temperature is lower by 3^0C than in-house condition and relative humidity; ≈95%) is effective for storing leafy vegetables which were tested for organoleptic properties, weight loss, colour loss and chlorophyll degradation (by spectrophotometric method) against in-house condition. But the effectiveness of storing leafy vegetables in cold and humidified condition was lower than refrigerated condition, with regard to some characteristics such as chlorophyll and colour degradation as the chlorophyll-a,b degradation, visible green colour loss and increment of yellowness were moderate compared to in-house and refrigerator conditions. But when it comes to characteristics such as freshness and weight loss, the cold and humidified condition was most effective than in-house or refrigeration condition as cold and humidified condition samples had lower cumulative loss throughout the testing period. The cold and humidified condition samples had the highest preference for appearance and overall acceptability for Sessile joyweed and higher preference for Water morning glory.

KEYWORDS: *cold and humidified condition, refrigerator, weight loss, chlorophyll-a,b, leafy vegetable*

1. INTRODUCTION

Fruits and vegetables consist of water, nutrients, vitamins, minerals, fiber and plant chemicals. Due to the water and nutrient content, these are highly susceptible for spoilage (1). If proper cooling methods are not practiced after harvesting these commodities lose its weight and the quality before reaches to the customer (1). Even after harvesting metabolic reactions such as respiration and transpiration take place in post-harvest commodities (fruits and vegetables), cause to deterioration and reduce the post-harvest life (1). Transpiration includes, moisture migration through the skin of the produce, evaporation of moisture from surface and convective mass transport of moisture to the surrounding (2). Evaporation which occurs at the produce surface is an endothermic process and it will cool the surface and reduce the transpiration by lowering the vapour pressure at the surface (2). The respiration which occurs within the commodity tends to increase its temperature and hence the transpiration cause to the raising vapour pressure at the surface of the commodity (2). Therefore, commodities need to be stored at low temperature, high humidity environment in order to minimize the loss due to transpiration (2).

Temperature is the critical factor to maintain the post-harvest quality of commodities during the storage period (1). When a produce is harvested its temperature is close to the ambient air temperature and can be reached to $>40^0$C if they are held in direct sunlight (1). At higher temperature, rate of respiration is extremely high. Due to that, the shelf life of the commodity can be gone down remarkably. Hence the field heat removal is very important, of which the commodities are exposing to an environment where the temperature is lower than the commodity (1). There are several methods which are used to store post-harvest commodities at lower temperature (1). They are, underground storage, in-ground storage, ice-cooled stores, ice-refrigeration, mechanical refrigeration, cool stores, modified atmosphere storage and controlled atmosphere (CA) storage (1).

1.1 Statement of the Problem

Generally, the ambient (in-house) condition is high in temperature and low in relative humidity and their fluctuations can be seen. Hence, the leafy vegetables stored under in-house condition tend to deteriorate rapidly. And most of refrigeration systems are operated at low relative humidity levels which have rapid drying effect. Though the fresh produce can be cooled and stored in refrigerators under low temperatures, commodities will be withered and shriveled by losing water due to the lower level of relative humidity inside the refrigerator. Though the respiration of commodities is lower in refrigerators due to low temperature, the transpiration is high as the relative humidity inside is lower. Under this circumstance, it is important to analyze the impact of temperature and relative humidity level to identify the proper condition for storing leafy vegetables to prolong the post-harvest life.

1.2 Purpose of the Study

The study specifically seeks to:

I. Determine the changes on physical, chemical and organoleptic properties in selected leafy vegetables, stored at cold and humidified condition against same vegetables kept at in-house and refrigerator condition.
II. Ascertain the impact of temperature and relative humidity on the storage life of selected leafy vegetables.

1.3 Objectives of the Study

I. To evaluate variation of sensory properties in selected leafy vegetables regarding to in-house, cold and humidified and, refrigerator condition.
II. To evaluate variation of weight and colour in selected leafy vegetables with regard to in-house, cold and humidified and refrigerator condition.

III. To evaluate the variation of chlorophyll-a, and chlorophyll-b concentration of leafy vegetables regarding to in-house, cold and humidified and, refrigerator condition.

2. METHODOLOGY

2.1 Collection of plant samples for the study

The two leafy vegetables (*Alternanthera sessilis* and *Ipomoea aquatica*) were selected for the experimental purpose. The required fresh leafy vegetables were harvested from a grower in Oruwala, western province in Sri Lanka. Fresh looking, healthy, not damaged, not bruised nor withered green colour leaves at right maturity level were selected.

After sorting the collected leafy vegetables, bunches of them were made by tying them. Bunches of leafy vegetables were placed in three conditions namely refrigerated, in-house and, cold and humidified condition along with manually perforated clear ziplock food storage bags. All treatments were replicated thrice and subjected to determine variation of sensory properties, weight, colour and chlorophyll-a,b concentration against period of storage.

2.2 Instrument for Data Collection

The weight measurements were taken using analytical balance (OHAUS PA 214, max 210 g, d= 0.0001g) and chromameter (LOVIBOND; RM 200,S/N:2010004647;2016-09) was used to take colour measurements in leafy vegetables. A vortex mixer (VELP SCIENTIFICA: ZX3, S/N: 132965, Hz: 50, W: 45) and centrifuge (HERMLE Z 306, S/N: 76170003, Max.drehzahl: 14000 1/min) were used in analysis of chlorophyll concentration. The absorbance for chlorophyll solutions was read using UV-VS spectrophotometer (SHIMADZU:UV MINI – 1240,A10934703413CD) and quartz glass (SHIMADZU high precision cell, 10mm light path).

2.3 Analysis of Organoleptic Properties

Triplicate samples from each treatment from each commodity were subjected to the sensory study which was carried out initially (Before beginning of the study) and time to time during the period of study with a view to find out best storage method for the studied two leafy vegetables. The sensory evaluation was carried out with a thirty members of semi-trained (discriminative) sensory panel for two sensory attributes, such as appearance and overall acceptability using a five point hedonic scale.

2.4 Analysis of Physical Properties

The total weight of each leafy vegetable sample was measured initially and after time to time during the study period with a view to determine weight variation of each sample against period of storage. In addition to that, inedible parts of each sample were also segregated and weighed along with the total weight measurement during the testing period. The data pertaining to the edible weight and cumulative weight loss were analyzed and graphically presented using the Excel 2013.

The colour of each leafy vegetable sample was measured initially (before beginning of the study) and time to time during the study period with a view to determine the color variation (L*,a* and b* values) of each sample. Colour measurements were taken ten times per sample.

2.5 Analysis of Chemical Properties

The chlorophyll-a, and chlorophyll-b concentration were determined using spectrophotometric method. The 80% of acetone was prepared by diluting (Analytical reagent: Min.Assay=99.5%, M.W=58.08, Weight per ml at 20^0C=0.791g) with distilled water. The leafy vegetable samples were taken and ground with mortar and

pestle to a fine paste. Then 0.5g of the paste was weighed accurately to the centrifuge tube (50ml) containing 10ml of 80% acetone and homogeneously mixed using Vortex mixer at 10Hz for 1min. The homogeneously mixed sample was centrifuged at 3000rpm for 10min. The supernatant was separated and 0.3ml of it was mixed with 2.7ml of 80% acetone. Then the absorbance was read at 663nm and 646nm using UV-VS spectrophotometer and quartz glass for the determination of Chlorophyll-a, and Chlorophyll-b content respectively (3). It was make sure to cover all the glassware and test tubes which contained chlorophyll solutions with aluminum foils and cotton lids covered with aluminum foil. The concentration of these pigments was calculated according to the equations given in Table 1.

Table 1: Equations to determine concentrations of chlorophyll-a and chlorophyll-b in µgml^{-1} in leafy vegetables

Solvent	**Pigment**	**Equation**
80% acetone	Chlorophyll-a (C_a)	$C_a = 12.25A_{663.2} - 2.79\ A_{646.8}$
	Chlorophyll-b (C_b)	$C_b = 21.5\ A_{646.8} - 5.1\ A_{663.2}$

Credits: Adapted from (4)

2.6 Method of Data Analysis

The results of the organoleptic properties were analyzed statically using IBM SPSS statistics 21. Friedman and Wilcoxon signed rank test were performed at 95% confidence level to identify the significance difference among three different conditions under tested attributes against period of study. The average edible weight % and colour measurements were statistically analyzed using Minitab 17. The linearity for the variation of edible weight % and colour with respect to time and treatment was determined using R square values (R-sq %).

3. RESULTS AND DISCUSSION

The Sessile joyweed (*Alternanthera sessilis*) and Water morning glory (*Ipomoea aquatica*) are leafy vegetables which are popular in Sri Lanka as "Mukunuwenna" & Kankun", and were tested for 89hours and 93 hours respectively.

Sessile joyweed (*Alternanthera sessilis*):

The Relative humidity (RH) in in-house, refrigerator and cold and humidified condition varied between 74-86%, 60-100% and 91-97% respectively during the testing period. The refrigerator humidity can vary widely. If it is closed for a longer period, then it can become rather dry (may be under 10%). But when the door of the refrigerator is opened moisture from the room condition might enter and condense. That moisture will raise the humidity inside the refrigerator. Hence a refrigerator which is being opened frequently will have high humidity and which has been closed for several days will be very dry. As the temperature inside the refrigerator is low (0^0C-4^0C) it may get saturated quickly with a small amount of water vapor and give 100% reading as RH.

After 89 hours, the Sessile joyweed samples placed in in-house condition had a withered look and almost all the leaves were either yellow or yellow-green in color. The samples placed in the refrigerator condition had a higher degree of wither than the samples placed in the in-house condition. However, color degradation of leaves in the refrigerator samples was lower than that of under in-house and, cold and humidified condition. But the samples placed in the cold and humidified condition were not much withered compared to refrigerator or the in-house condition samples. It was kept a fresh looking for long period compared to other two samples.

But the yellowness could be seen up to certain extent but lower than the in-house condition samples. The leaves of samples kept under in-house condition got more yellowness within a short time span and after 89 hours all the leaves were either fully or partially yellow. But the colour changing of leaves in the samples placed in the cold and humidified condition was lower than the in-house samples.

The results of three storage methods pertaining to appearance and overall acceptability after 40, 56 and 73hours storage periods were significantly difference ($p<0.05$) to each other according to the Wilcoxon Signed Rank Test. After 89hours, samples kept under refrigerator and cold and humidified conditions were not significantly different in appearance ($p=0.637$, $p>0.05$) and overall acceptability ($p=0.564$, $p>0.05$). According to the results, during 40 to 89 hours period, vegetables kept under cold and humidified condition had a higher preference than the refrigerator sample.

According to sensory evaluation, most of panelists, after 56 hrs of storage preferred to have vegetables kept under cold and humidified condition rather than keeping under refrigerator; because cold and humidified condition prevailed higher relative humidity. To maintain highest possible harvest quality, it is essential to give proper ventilation and maintaining proper RH during storage (5).

According to the results (Figure 1) throughout the testing period the edible weight was higher in the samples kept under cold and humidified condition than that of refrigerator or in-house samples.

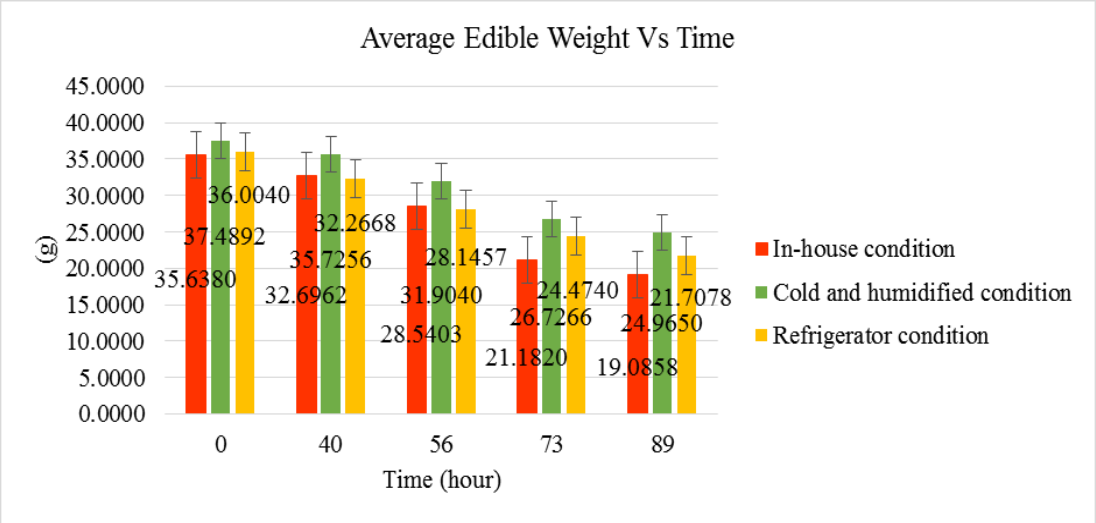

Figure 1: The variation of average edible weight of Sessile joyweed samples with time regarding to in-house, cold and humidified and refrigerator condition

According to the results (Figure 2) the average cumulative weight loss was lower throughout the testing period of the samples kept under cold and humidified condition than that of refrigerator or in-house condition. By subtracting the edible weight % by 100, the cumulative weight loss % can be calculated.

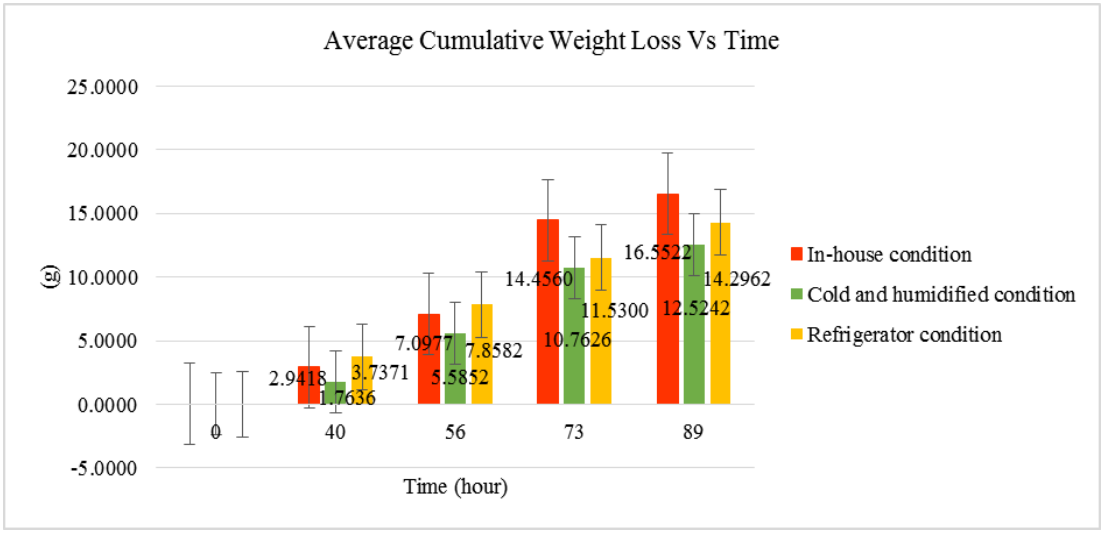

Figure 2: The variation of average cumulative weight loss of Sessile joyweed samples kept under in-house, cold and humidified and refrigerator condition

According to the statistical analysis the outcome indicates, edible weight % as Response, time as continuous variable, and D_H & D_R as categorical predictors. In here when the D_H & D_R categorical predictors are 0 0 the treatment was named as "In-house", when it is 1 0 the treatment was named as "Cold and humidified", when it is 0 1 the treatment was named as "Refrigerator". The Scatter plot (Figure 3) and R-sq % proved that the relationship between edible weight %, time and treatment was not linear, but most suitable one was cubic.

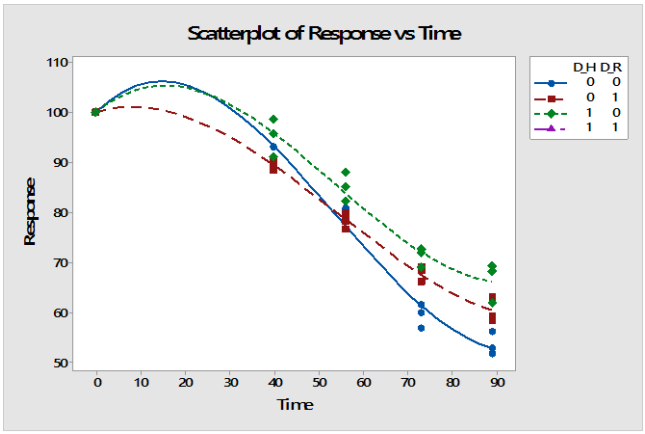

Figure 3: Scatter plot of change in edible weight % of Sessile joyweed samples with time and treatment

This may be can happen, because these are biological matters and their weight variation can be varied due to the variation in metabolic activities or respiration in the samples. The water content in horticultural crops (water activity (aw) of fresh fruits and vegetables is 0.97 - 1.00 (6)) is high and after harvesting they tend to desiccate due to loss of moisture. The heat which is generated by respiration is dissipated by transpiration in most of leafy vegetables such as lettuce (7). However, after harvesting commodities the transpiration is reduced dramatically (8). Rapid lowering the temperature of produce and maintaining produce at a constant low temperature cause to minimize the enzymatic process and other reaction processes that cause losses (9). The temperature and RH of the cold and humidified condition were kept as lower by 3^0C than in-house condition and 95% respectively. Due to that the cumulative weight loss of commodities in cold and humidified condition was lower than the in-house or refrigerator condition. It has been given the recommended storage guidance for (Florida-grown) green, leafy vegetable as temperature and RH equal 0^0C and 95-100% respectively for appropriate storage life of 10-14days (5).

Using L*, a*, b* values, the colour changes in fruits and vegetables can be evaluated. When the value L is positive, the colour of the product is light and if a* value changes from negative to positive the colour is changed from green to red. When the b* value changes from negative to positive the colour is changed from blue to yellow. When the produce deteriorates, the chlorophyll content in the leafy vegetables decrease. Hence the visible green colour is reduced while yellow colour is increased with time.

The, a* value at initial, after 56hours and after 89hours of Sessile joyweed samples in in-house, cold and humidified and refrigerator condition were "-9.1, -3.8 & 2.0", "-9.2, -9.8 & -7.3" and "-9.2,-8.8 & -8.7" respectively. Since a- represents green colour and a+ represents red color, if the, a* value is high in negative value means it is high in green colour. When consider the colour values after 56hours green colour was more in cold and humidified condition samples and lesser in in-house condition samples. When consider the overall variation in –a values, lower decrement was there in refrigerator samples and higher decrement was there in in-house condition samples. Even after 89 hours the green colour was lesser in in-house condition samples than the others. The b* value at initial, after 56hours and after 89hours in, in-house, cold and humidified and refrigerator condition were "17.1, 43.6 & 48.4", "16.6, 31.6 & 38.2" and "16.0, 17.8 & 21.6" respectively. Since b- represents blue colour and b+ represents yellow color, if the, b* value is high (in positive value) means it is high in yellow colour. When consider the overall variation in b values, lower increment was recorded in refrigerator samples and higher increment was with in-house condition samples. It was also proven that after 89hours the yellowness was higher in in-house condition samples and lower in refrigerator samples, but the yellowness in cold and humidified condition samples was in between those two samples.
When the green colour intensity is lost, the physical parameters (-a, -a/b values in colour measurements) are also increased (10). L* indicates the lightness. The L* value at initial of samples in, in-house, cold and humidified and refrigerator condition were 36.0, 36.2 and 36.4 respectively and after 89hours it was 64.4, 53.2 and 40.3 respectively due to the incensement of yellowness.

According to the statistical analysis the outcome indicates, a* or b* value as Response, time as continuous variable, and D_H & D_R as categorical predictors. In here when the D_H & D_R categorical predictors are 0 0 the treatment was named as "In-house", when it is 1 0 the treatment was named as "Cold and humidified", when it is 0 1 the treatment was named as "Refrigerator". According to the Scatter plot (Figure 4) and R-sq %, it was proved that the relationship between a*, time and treatment was not linear, the most suitable one was cubic.

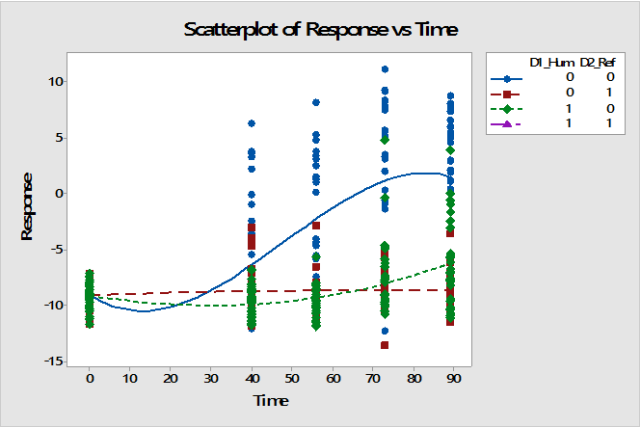

Figure 4: Scatter plot of change in Chromometer "a*" value of Sessile joyweed samples (regarding to each treatment) with time

The graphical representation (Figure 5) and R-sq % proved that the relationship between b*, time and treatment was not linear, but most suitable one was cubic. As these commodities are biological matters their colour variation can be varied due to the variation in metabolic activities.

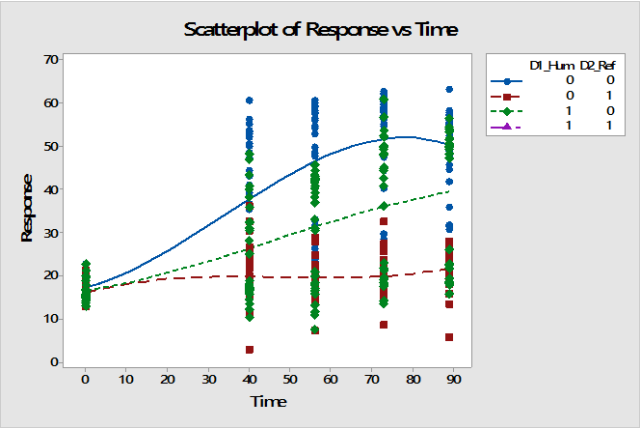

Figure 5: Scatter plot of change in Chromameter "b*" value of Sessile joyweed samples (regarding to each treatment) with time

The absorbance of chlorophyll-a, and chlorophyll-b in Sessile joyweed samples are given in Table 2.

Table 2: The variation of absorbance of chlorophyll-a and chlorophyll-b in Sessile joyweed samples (regarding to each treatment) with time

Time (hour)	In-house		Cold and humidified		Refrigerator	
	A663nm Ch-a	A646nm Ch-b	A663nm Ch-a	A646nm Ch-b	A663nm Ch-a	A646nm Ch-b
0	0.525	0.991	0.527	0.992	0.526	0.991
40	0.130	0.693	0.163	0.752	0.348	0.784
56	0.062	0.640	0.128	0.662	0.364	0.775
73	0.030	0.594	0.049	0.595	0.349	0.767
89	0.025	0.593	0.045	0.594	0.349	0.769

Table 3: The variation of concentration of chlorophyll-a and chlorophyll-b in Sessile joyweed samples (regarding to each treatment) with time

Time (hour)	Concentration (µg/ml)					
	In-house		Cold and humidified		Refrigerator	
	Ch-a	Ch-b	Ch-a	Ch-b	Ch-a	Ch-b
0	3.67 ± 0.56	18.62 ± 4.98	3.69 ± 0.57	18.65 ± 4.97	3.68 ± 0.56	18.63 ± 4.95
40	-0.35 ± 0.14	14.25 ± 1.33	-0.10 ± 0.21	15.34 ± 0.56	2.16 ± 0.62	15.08 ± 0.29
56	-1.03 ± 0.21	13.44 ± 0.03	-0.28 ± 0.61	13.58 ± 0.55	2.30 ± 0.43	14.81 ± 0.30
73	-1.28 ± 0.023	12.61 ± 0.30	-1.06 ± 0.04	12.55 ± 0.21	2.13 ± 0.56	14.72 ± 0.37
89	-1.39 ± 0.025	12.62 ± 0.02	-1.17 ± 0.05	12.54 ± 0.01	2.13 ± 0.09	14.74 ± 0.48

In in-house Sessile joyweed samples, the initial chlorophyll-a content (Table 3) was 3.67µg/ml. but after 40hours it was given as -0.35µg/ml (due to lower concentrations) and 40hours onwards the negative chlorophyll value was gradually increased. The same situation was occurred with cold and humidified condition sample too, however, the initial chlorophyll-a value was 3.69µg/ml and it was -0.10µg/ml after 40hours. Thus, negative value for cold and humidified condition sample was lower than that of in-house condition sample. It shows the chlorophyll-a content in the cold and humidified condition was higher than the in-house condition sample, even after 89hours, it was (-1.17µg/ml) for cold and humidified samples and (-1.39 µg/ml) for in-house condition samples. Reason for this consequence was degradation of chlorophyll-a after 40hours. The negative value for chlorophyll-a might be either due to the low chlorophyll-a concentration in measured sample or due to interference in spectrophotometric measurements, because in polar solvents, chlorophyll-b is more soluble than chlorophyll a due to its carbonyl group. It can be suspected that the negative chlorophyll values were given from the turning point (After 40hours) onwards due to the chlorophyll degradation. Hence it can be suspected the point after 40hours as the chlorophyll degradation point. But in refrigerator samples initial chlorophyll-a content was 3.68µg/ml and after 40hours it was 2.16µg/ml. Compared to the In-house and the cold and humidified condition samples, the chlorophyll-a concentration after 40hours in refrigerator sample was high. It might be due to the light in the refrigerator itself and it caused the variation in chlorophyll-a concentration. Four 40W (540 lumens) incandescent lights are traditionally used for refrigerators. However, the inside of the cold and humidified conditioned chamber was completely dark and the in-house condition samples were also placed in a closed shaded area to prevent harms from pests like rats.

The initial chlorophyll-b concentration in in-house condition samples was 18.62µg/ml and after 40hours it was decreased up to 14.25µg/ml and it was decreased further. The initial chlorophyll-b concentration in Cold and humidified condition samples was 18.65µg/ml and after 40hours it was 15.34µg/ml and it was decreased further. For Refrigerator condition samples initially, it was 18.63µg/ml and after 40hours it was 15.08µg/ml and it was also later decreased further. The Cold and humidified condition samples had higher chlorophyll-b concentration until 40hours. But after 89 hours refrigerator samples had higher chlorophyll-b concentration which was 14.74 µg/ml. However, in fruits and vegetables, chlorophyll-a, and chlorophyll-b occur approximately in 3:1 ratio (10). The initial chlorophyll a/b ratio in Sessile joyweeded for this study was 1:5.

According to research finding both the synthesis and the degradation (photo-oxidation) of chlorophylls are under irradiation, but however at high irradiance the rate of synthesis is overtaken by the rate of degradation (11). High Chlorophyll-b levels allow light interception in wider wavelength bands and as a result of that a larger amount of energy transaction to reaction centers is expected (11). Hence, plants in low light conditions produce a greater ratio of chlorophyll-b to chlorophyll-a molecules, increasing photosynthetic yield.

Chlorophylls degrade occur easily with heat, dilute acids, light and oxygen. In a previous study it has been elicited that the chlorophyll-a degraded 12 to 18 times faster than chlorophyll-b in green peas depending on temperature and also revealed that chlorophyll-a is more susceptible to thermal treatments (10). It has also been elicited that the activation energy of chlorophyll-b was less than chlorophyll-a (10, 12). The higher activation energy indicates that a small change in temperature is sufficient to degrade chlorophyll-a more rapidly (10). Hence as shown in results (Table 3) it can be possible to have chlorophyll-a, in low concentrations than the chlorophyll-b.

Water morning glory (*Ipomoea aquatica*):

The Relative humidity in in-house, refrigerator and cold and humidified condition varied between 75-80%, 50-100% and 88-95% respectively during the testing period of samples for Water morning glory. The appearance after 93hours clearly indicated the leaves of the samples placed under in-house condition were either almost yellow or yellow-green in colour and also had some kind of withered looking. The Refrigerator

condition samples had withered a lot even than the in-house condition samples. Even though the refrigerated samples had withered a lot, the leaves were green in color. Color degradation of leaves was also there but up to a lower level comparing to the in-house and cold and humidified condition samples. But after the testing period, the samples placed in the cold and humidified condition were less withered than the samples kept under refrigerator and in-house condition and also kept fresh looking for long period compared to other two samples.

According to the results of three storage methods pertaining to appearance and overall acceptability until 93hours, storage periods were significantly different ($p<0.05$) to each other according to the Wilcoxon Signed Rank Test. After 20hours, samples kept under refrigerator and in-house conditions were not significantly different in both appearance ($p=0.593>0.05$) and overall acceptability ($p=0.593>0.05$). During 42 to 93 hours period, vegetables kept under all three (cold and humidified, in-house and refrigerator) conditions were significantly different in both appearance and overall acceptability. According to sensory evaluation, most of panelists, until 75hours of storage preferred to have vegetables kept under cold and humidified condition rather than keeping under refrigerator or in-house condition. Though from 20hours to 42hours the preference for refrigerator samples were lower than the cold and humidified condition samples, after 93hours it has increased than the cold and humidified condition samples. That might be due to the yellowness appeared later with the samples in cold and humidified condition.
According to the results (Figure 6) the average cumulative weight loss was lower after 93hours in the samples kept under cold and humidified condition than that of refrigerator or in-house conditions.

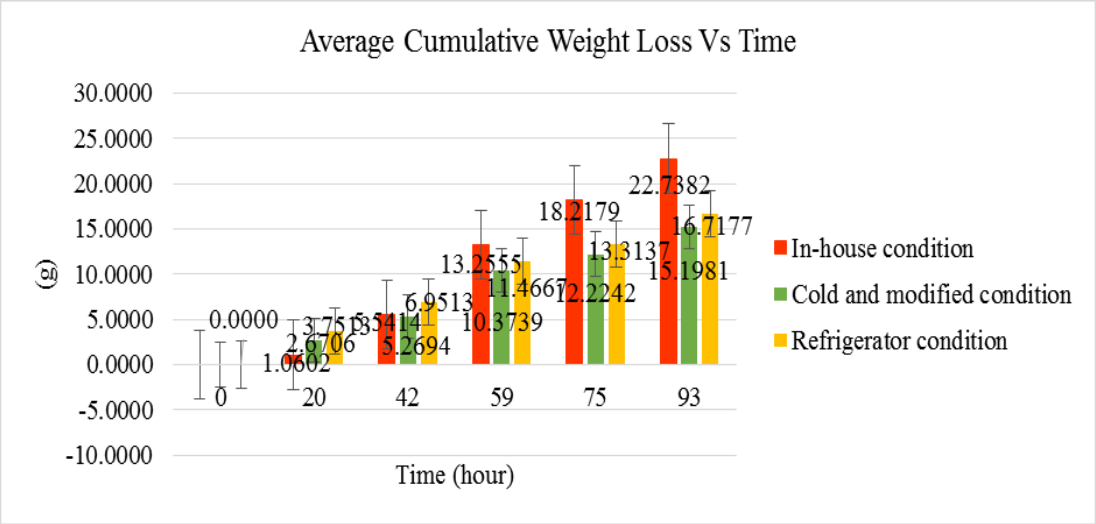

Figure 6: The variation of average cumulative weight loss of Water morning glory samples kept under in-house, cold and humidified and Refrigerator condition with time

With some estimates it has been suggested that the post-harvest loss of 30-50% is with the horticulture produce (fruits and vegetables) which is highly perishable. It has been given the recommended storage for (Florida grown) greens/leafy as temperature & RH equal 0 ^{0}C and 95-100% respectively for approximated 10-14days of storage life (5).

The outcome of statistical analysis indicates, edible weight % as Response, time as continuous variable, and D_H & D_R as categorical predictors as same as previous. According to the Scatter plot (Figure 7) and R-sq %, it was proved that the relationship between edible weight %, time and treatment was not linear, but most suitable one was cubic. This may be can happen due to the metabolic reactions occur in commodities even after harvesting.

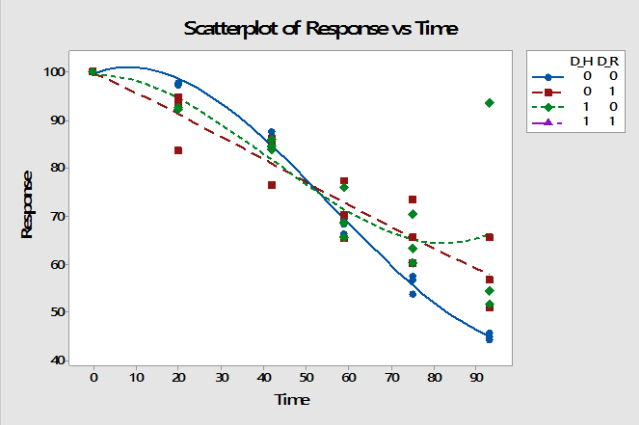

Figure 7: Scatter plot of change in edible weight % of Water morning glory samples with the time and treatment

The a* value at initial, after 59hours and after 93hours of Water morning glory samples in in-house, cold and humidified and refrigerator condition were "-6.7, -4.5 & 0.9", "-7.2, -6.6 & -6.25" and "-6.4, -8.6 & -8.9" respectively. When consider the colour values after 93hours green colour was lesser in in-house condition samples, more in refrigerator samples and moderate in cold and humidified samples. The b* value at initial, after 59hours and after 93hours of samples in in-house, cold and humidified and, refrigerator conditions were "12.0, 42.9 & 51.85", "13.0, 39.2 & 31.2" and "10.4, 19.6 & 24.26" respectively. As b+ represents yellow color the yellow colour after 93 hours, was more in in-house condition samples, lesser in refrigerator samples and moderate in cold and humidified condition samples. The L* value at initial and after 93hours of samples in in-house, cold and humidified and, refrigerator conditions were "22.5 & 59.2", "21.9 & 42.9" and "25.2 & 38.5" respectively. A previous study which was tested broccoli juice as the sample has been suggested that the change in L* and b* colour values may be due to pheophytin-pyropheophytin conversion or upon heating to degradation of other compounds present in the sample (13).

The outcome of statistical analysis indicates, a* or b* value as Response, time as continuous variable, and D_H & D_R as categorical predictors as same as previous. According to the graphical representation (Figure 8) and the R-sq %, it was proved that the relationship between a*, time and treatment was not linear, the most suitable one was cubic.

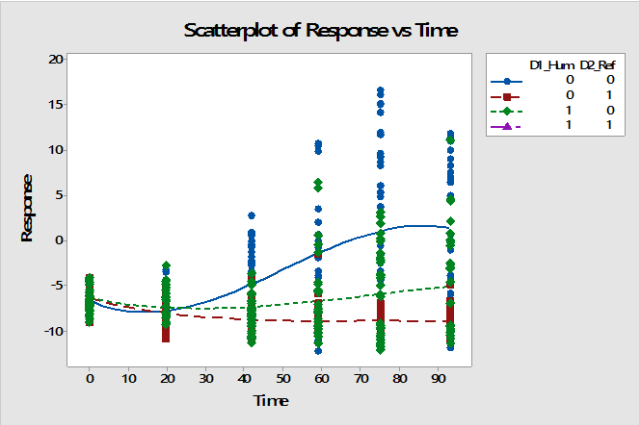

Figure 8: Scatter plot of change in Chromameter "a*" value of Water morning glory samples (regarding to each treatment) with time

The scatter plot (Figure 9) and R-sq % proved that the relationship between b*, time and treatment was not linear, but most suitable one was cubic.

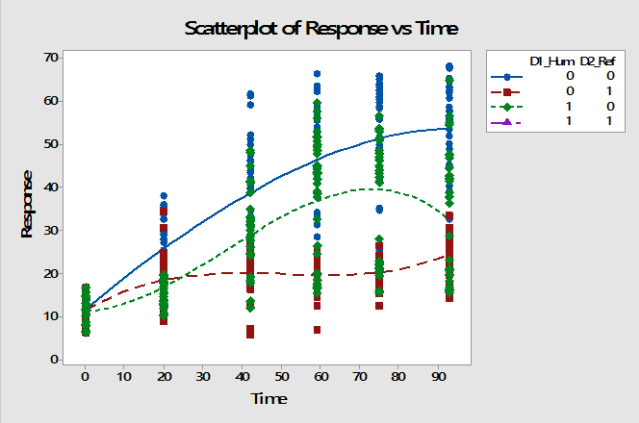

Figure 9: Scatter plot of change in Chromometer "b*" value of Water morning glory samples (regarding to each treatment) with time

The absorbance of chlorophyll-a, and chlorophyll-b in Water morning glory samples are given in Table 4.

Table 4: The variation of absorbance of chlorophyll-a and chlorophyll-b in Water morning glory samples (regarding to each treatment) with time

Time (hour)	**In-house**		**Cold and humidified**		**Refrigerator**	
	A663nm	**A646nm**	**A663nm**	**A646nm**	**A663nm**	**A646nm**
	Ch-a	**Ch-b**	**Ch-a**	**Ch-b**	**Ch-a**	**Ch-b**
0	0.287	0.704	0.285	0.702	0.286	0.702
42	0.049	0.622	0.135	0.649	0.184	0.659
59	0.014	0.606	0.061	0.619	0.090	0.633
75	0.013	0.606	0.045	0.617	0.069	0.627
93	0.011	0.609	0.033	0.616	0.065	0.614

Table 5: The variation of concentration of chlorophyll-a and chlorophyll-b in Water morning glory samples (regarding to each treatment) with time

Time (hour)	**Concentration (µg/ml)**					
	In-house		**Cold and humidified**		**Refrigerator**	
	Ch-a	**Ch-b**	**Ch-a**	**Ch-b**	**Ch-a**	**Ch-b**
0	1.55 ± 0.96	13.67 ± 0.28	1.54 ± 0.95	13.64 ± 0.25	1.54 ± 0.94	13.64 ± 0.21
42	-1.14 ± 0.09	13.12 ± 0.11	-0.16 ± 0.38	13.26 ± 0.27	0.42 ± 0.23	13.23 ± 0.18
59	-1.51 ± 0.01	12.96 ± 0.02	-0.98 ± 0.27	13.00 ± 0.07	-0.67 ± 0.15	13.16 ± 0.17
75	-1.53 ± 0.02	12.96 ± 0.05	-1.17 ± 0.04	13.03 ± 0.15	-0.90 ± 0.18	13.13 ± 0.11
93	-1.57 ± 0.01	13.05 ± 0.01	-1.31 ± 0.08	13.08 ± 0.01	-0.91 ± 0.05	12.88 ± 0.10

The chlorophyll-a concentration (Table 5) after 59hours and 93hours of Water morning glory samples in in-house, cold and humidified and, refrigerator condition were "-1.51µg/ml & -1.57µg/ml","-0.98µg/ml & -1.31µg/ml" and " -0.67µg/ml & -0.91µg/ml" respectively. The initial chlorophyll-a concentration in Water morning glory was positive in value. But after 42hours the chlorophyll-a concentration of samples kept under in-house and cold and humidified condition was negative in value and from that point onwards the negative value was gradually increased. But the chlorophyll-a concentration for Refrigerator samples was positive (0.42µg/ml) until 42 hours and after 59hours it was negative in value and then the negative value was gradually increased. Hence the chlorophyll-a after 93hours was lesser in in-house samples, more in Refrigerator samples and moderate in cold and humidified samples. It can be suspected that the point at which started to give negative value as the chlorophyll degradation point of Water morning glory. The chlorophyll-b concentration after 59hours of samples in in-house, cold and humidified and refrigerator condition were "12.96, 13.0 & 13.16" respectively. From initial to 93hours the remained chlorophyll-b concentration was higher than the chlorophyll-a concentration. Hence the chlorophyll a/b ratio would be low, but chlorophyll b/a ratio would be high. The initial chlorophyll a/b ratio in Water morning glory was 0.11 and b/a ratio was 8.8. As a function of temperature, chlorophyll-a, degrades faster than chlorophyll-b. And according to previous study the ratio of chlorophyll-a to chlorophyll-b decreases with increasing temperature and colour of peas which is green, approaches to yellow gradually (10). It has been elicited that the heat-induced loss of green colour is a consequence of both degradation of chlorophyll-a, and chlorophyll-b (14). As chlorophyll-a, has an intense blue-green colour, where chlorophyll-b has a yellow-green colour, hence it had been subjected that the loss of green colour is mainly due to pheophytinization of chlorophyll-a (15).

4. CONCLUSIONS

According to the sensory evaluation with regard to the appearance and overall acceptability the cold and humidified condition samples had the highest preference throughout the testing period for Sessile joyweed and higher preference for Water morning glory. There was no linear relationship either between edible weight %, period of storage and type of treatment (in-house, cold and humidified and refrigerator condition) or between colour (a* or b*), period of storage and type of treatment for both Sessile joyweed and Water morning glory commodities.

This study has proved that the cold and humidified condition (temperature is lower by 3^0C than in-house condition, RH≈95%) is effective for storing leafy vegetables namely Water morning glory and Sessile joyweed than in in-house condition. But effectiveness of it is lower than that of refrigerated condition, with regard to chlorophyll and colour degradation. But when it comes to characteristics such as freshness and weight loss, cold and humidified condition is more effective than in-house or refrigeration conditions.

5. RECOMMENDATIONS

Based on the findings of the study following recommendations were made:

- The change in physical and chemical properties with regard to low temperature (≈4^0C) and high relative humidity (≈95%) can be monitored on some other high respiratory leafy vegetables.
- The impact of light intensity for the degradation of chlorophyll-a, and Chlorophyll-b in leafy vegetables after harvesting can be tested with a light source. The variation of chlorophyll content of leafy vegetables can be measured regarding to the change of the intensity of the light source.

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