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Impact of Gas Composition, Temperature and Pre-Treatments on Mint Leaves Quality under Modified Atmosphere Packaging

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A B S T R A C T

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Respiration rate, sensory attributes, change in color, physiological loss in weight, chlorophyll and beta - carotene content and microbial loads were determined during storage of mint leaves at 10 and 27°C to find an optimal gas composition to extend the shelf life. The Low Density Poly Ethylene (LDPE) bags with a thickness of 152 µ which recorded the lowest permeability to oxygen (1067 ml/m^2/day) was selected and used for packaging mint leaves. The harvested mint leaves were cleaned and subjected to pre chilling and pre-cooling treatment and packaged in low density polyethylene (LDPE) bags with a product volume ratios Viz., 1:18, 1:11, and 1:8 to assess the respiration rate under ambient and refrigerated condition using the permeable system. Optimization of gas composition for MAP was done by calculating the respiration rate using Michaelis–Menten equation. Based on the respiration rate, a gas composition of 5% O_2, 5% CO_2 and 90% N_2 was found to be the best in the product volume ratio of 1:8 which recorded the lowest respiration rate, and a slight changes in the physico-chemical parameters, was recorded during the storage period of 30 days. The keeping quality of leaves stored under ambient conditions had a shelf life of 4 days when compared to 20 days under refrigerated condition. The MA packaged mint leaves kept under refrigerated condition had more shelf life than at ambient condition.

Introduction

Green leafy vegetables supply adequate amounts of vitamins, minerals, less fat, high dietary fibre, rich folic acid, vitamin C, potassium, magnesium and calcium. They are rich in beta-carotene, iron and good sources of zinc, manganese cobalt, copper and many other minerals. They are highly perishable due to loss of water, high senescence and loss of chlorophyll which leads to accumulation of CO_2 followed by yellowing and decay when stored at high temperature products [Paull, (1992); Yamauchi and Watada (1991) and Aharoni *et al.*, (1989)]. Leafy vegetables will respire even after they are cut and packaged. The physiological requirements must be met or they will rapidly deteriorate. During respiration, green leafy vegetables are constantly consuming oxygen and producing carbon dioxide, heat and water. These rates can be slower down by holding the fruit at low temperature. Leafy vegetables will modify the package environment and their physiology will be modified. The changes in gas composition of the package environment are referred to as MAP and this technology is central to maintaining the quality of shelf life of fresh produce. The atmosphere that exists inside a MAP is a function of the film and the product. Hence it is essential to know the respiratory requirements of the products and the permeability properties of the film. The appropriate atmosphere, and proper temperature, to be maintained for a give commodity to realise the optimum quality and postharvest life.

Permeability is typically slow compared to the normal movements of the gases in air, so that the films acts as a partial barrier to gas movement. When the permeation of gas is slow, close to zero, the film is called as barrier film. Flexible films vary in their rates of gas transmission, commonly known as oxygen transmission rate (OTR) or carbon dioxide transmission rate CO_2 TR). Films with intermediate gas diffusion rates are more applicable for packaging respiring commodities.

Quality characteristics of culinary herbs include fresh appearance, colour, aroma and flavour and lack of defects like decay and yellowing (Cantwell and Reid, 1993). Fresh mint leaves have very short shelf life under the ambient conditions. The production of high quality mint leaves possess unique challenges to food processors due to high water activity, respiration, senescence, and loss of chlorophyll, undesirable physiological changes, contamination and growth of micro flora. Hence there is a need to mitigate the above damages by proper handling and post - harvest processing techniques.

The respiration of fresh herbs can be reduced by many preservation techniques like low temperature, canning, dehydration, freeze-drying, controlled atmosphere, hypobaric and modified atmosphere. Dehydration also controls the activity of microorganisms by the removal of water under controlled conditions of temperature, pressure and relative humidity (Sandhya, 2010).

Rajesh (2001) stated that horticultural commodities are different from other food products as they are living organisms. The high respiration rate and other metabolic process associated with ripening of these products continue throughout the marketing cycle. Modified atmosphere packaging technique could be used effectively to inhibit both biological and chemical degradation (Jayas and Jeyamkondan, 2002).

Modified atmosphere packaging can be defined as ‘the enclosure of food products in a film in which the gaseous environment has been changed or modified to slow respiration rates, reduce microbiological growth, and retarded enzymatic spoilage with the intent of extending shelf life’. MAP is becoming an increasingly popular methods of shelf life extension of food products when an extended shelf life at refrigerated temperature is required.

MAP utilizes polymeric films with selective permeability for O_2, CO_2 and water vapour to create a modified atmosphere around the packaged product due to respiration of the product and the selective permeability of the packaging material (Guevara *et. al*., 2003). The atmosphere within the packaging changes over storage time due to factors such as product respiration and biochemical changes, as well as the slow diffusion of the gases through the packaging film. There are many factors to take into account with this technique, such film permeability (O_2, CO_2, water vapour) or temperature, that make it essential to fix the optimal conditions for each vegetable product (Fonseca, Oliveira, and Brecht, 2002). Application of reduced levels of O_2 and increased levels of CO_2 in the atmosphere surrounding fresh produce has several positive effects on respiration rate, ethylene production and sensitivity, texture losses, improves chlorophyll and other pigment retention, delays ripening and senescence reduces the rate of microbial growth and spoilage (Aguilera and Olivera, 2009).

This study has been carried out to evaluate the effect of MAP storage and temperature conditions on shelf life of mint leaves. The objective was the study of two MAP conditions: the first one combined with refrigeration at 7±1°C, is recommended for commercialisation and the second was carried out in order to compare these results with normal atmosphere conditions.

The evaluation was done by measuring the variation of physiological, physical, chemical and microbiological characteristics over the storage periods.

Materials and Methods

The mint leaves, which has a commercial utility as culinary herbs were taken for the study. After harvest, the mint leaves were trimmed cleaned and shade dried to remove the surface moisture (Fig. 1).

Pre treatments

The cleaned mint leaves are bundled @ 50, 75 and 100 g and are subjected to pretreatments like pre-chilling and pre-cooling to reduce the field heat.

Pre-chilling

Pre chilling treatment was given by soaking the bundles in chilled water for 10 minutes so that translocation of chilled water to the aerial parts of the leaves may occur without causing chilling injury, and to reduce the field heat. The chilling temperature and duration was optimized by conducting respiration studies. The temperature was optimized as 5˚C and the duration was 10 minutes for best results.

Pre-cooling

Pre cooling involves the removal of field heat from freshly harvested produce in order to slow down metabolism and reduce deterioration prior to transport or storage (Janick, 1986). Baird and Gafney (1976) pointed out that pre cooling is likely the most important of all the operations used in the maintenance of desirable, fresh and salable produce. To study and compare the effect cold water treatment, pre cooling was done at 7±2˚C for 10 minutes before the leaf bundles are subjected to modified atmospheric packaging. Prolonged exposure leads to chilling injury and short duration exposure does not have significant effect of processing. So 7±2˚C and 10 minutes duration was found to be the best one.

Permeability of packaging materials

Three packaging film of LDPE and PP bags of varying thickness 152 µ, 200 µ and 400 µ were tested for the permeability of gases, oxygen and Carbon dioxide using a permeability tester (M/s. PBI Dansensor, Lyssy Line of Permeability testers).

Gas analysis

The O_2 and CO_2 concentrations were measured with a MAP analyser (Make: PBI Dansensor Model: Checkmate II).

Every one hour the gas samples were drawn from the container through silicon rubber septum (fixed on the packaging material) using needle of the MAP analyser. With the recorded gas composition the respiration rate of oxygen and carbon dioxide were calculated.

Measurement of respiration rate

Respiration is a metabolic process, which consists of oxidative breakdown of organic matter present in the cells such as starch, sugars, acids, fats, proteins into simpler molecules such as carbon dioxide and water along with concurrent production of energy and other molecules which can be used by the cell for synthetic reactions (Wills *et.al.*, 1989). The extent of respiration can be measured by determining the amount of substrate loss, oxygen consumed, carbon dioxide liberated, heat produced and energy evolved (Pantastico *et. al.*, 1975).

Respiration study was conducted in three steps under ambient and refrigerated conditions.

Closed system without gas flushing,
Permeable system without gas flushing and
Permeable system with gas flushing

In the closed system PET (Polyethylene Teri phthalate) containers with 1.780 liters capacity were used. The top and bottom diameters, height of the container were 12.5, 11.5 and 17 cm, respectively. A single hole of one cm diameter was made on the top of the lid. A silicon septum was fitted into the hole using brass fittings to draw gas samples for analysis.

The respiration rate can be calculated by the change in oxygen concentration with time when the commodity was stored in a closed container as given below (Cameron *et.al.*, 1989).

$$Ro_2 = \frac{y^{ti}o_2 - y^{tf}o_2 \times V}{100 \times M \times (t_f - t_i)} \quad \text{--- (1)}$$

$$Ro_2 = \frac{y^{tf}co_2 - y^{ti}co_2 \times V}{100 \times M \times (t_f - t_i)} \quad \text{--- (2)}$$

Where,

Ro_2 and Rco_2	-	respiration rate, in terms of O_2 and CO_2 evolved respectively, m^3/kg/h
V	-	free volume inside the container
yo_2^{ti} and yo_2^{tf}	-	volumetric concentration of O_2 at initial and final time respectively, %
yco_2^{ti} and yco_2^{tf}	-	volumetric concentration of CO_2 at initial and final time respectively,%
M	-	mass of the stored product, kg
t_i and t_f	-	initial and final time respectively, h

The respiration rate was calculated by the change in oxygen concentration with time when the commodity was stored in a polymeric film (LDPE) was given below (Lakakul *et al.*, 1999).

$$Ro_2 = \frac{Po_2 \times A}{100 \times L \times M} \times (y^e o_2 - yo_2) \quad \text{--- (3)}$$

$$Rco_2 = \frac{Pco_2 \times A}{100 \times L \times M} \times (yco_2 - y^e co_2) \quad \text{---(4)}$$

Where,

A	-	package surface area, m^2
L	-	package thickness, m
M	-	mass of stored product, kg
Po_2 and Pco_2	-	film permeability coefficient for O_2 and CO_2 respectively, m^2 s^{-1}
$y^e o_2$ and yo_2	-	volumetric concentrations of O_2 outside and inside the package, respectively, %

Storage study

Storage study was conducted based on the optimized product volume ratio 1:8. LDPE film of thickness 152 μ was selected which has low permeability to oxygen. The gas composition of 5 per cent O_2, 5 per cent CO_2 and 90 per cent N_2 was chosen as best composition for storing mint leaves under MAP. All the parameters were analysed based on the statistical analysis using AGRES. The leaves were stored until they get spoiled. During the storage period, physiological, physical, bio-chemical and microbiological studies (Atmosphere composition, Colour, Physiological loss in weight (PLW), Chlorophyll, Beta-carotene and Microbial Analysis) were carried out to compare the results with the fresh leaves. Based on the results, the shelf life of mint leaves under modified atmosphere packaging was determined.

Statistical analysis

Statistical analysis was carried out to study the effect of different parameters (Pretreatments, storage conditions and product to free volume ratio) on all the dependent variables. Analysis of variance (ANOVA) was conducted with Factorial Completely Randomized block Design (FCRD) using the statistical software AGRES.

Results and Discussion

The experimental results of respiration rate of mint leaves, change in oxygen and carbon dioxide concentration in the pretreatments and storage temperatures are discussed. The quality aspects of the green leaves such as physiological, physical, chemical, microbiological and shelf life on the final quality of the modified atmosphere packaged mint leaves are also discussed based on the results obtained from the experiments.

Physicochemical and microbial analysis for fresh mint leaves

The physicochemical parameters such as moisture content, colour value, chlorophyll content and β-carotene for the fresh mint leaves were analyzed and presented in table 4 and the results of microbial analysis in table 5. From the above results it is clear that the fungal population was lower than the bacterial population. This may be due to the quality of water used for irrigating the crop.

Selection of packaging material

Packaging material is optimized based on the permeability. The permeability of Low Density Poly Ethylene (LDPE) and Poly Propylene (PP) packaging materials of different thickness were assessed and selected for the study based on their permeability rate. The permeability of the packaging materials is given in figure 2.

From the figure the maximum and minimum permeability to oxygen was observed for LDPE - 3 (2392 ml/m^2/day) and LDPE -1 (1067 ml/m^2/day). LDPE-1 was selected as the packaging material, since it has less permeability to oxygen (1067 ml/m^2/day) which was desirable for the study. More permeability to oxygen results in more availability of oxygen in the head space which increases respiration rate and results in decay of the product.

Determination of respiration rate under closed system

During respiration, O_2 is consumed and CO_2 is produced as the result of metabolic activity. Meyer *et al.*, (1973) reported that during respiration oxygen is taken in by plants and break the organic reserves to simpler molecules of CO_2 and water with release of energy.

The respiration study using closed system without gas flushing for mint leaves revealed that under ambient condition the respiration rate was more than the refrigerated condition. The result clearly shows that the temperature is the most important external factor influencing the respiration. Biological reactions generally increase by two or three-fold for every 10˚C rise in temperature within the range of temperatures normally encountered in the distribution and marketing chain (Zagory and Kader, 1988).

Table 1 states that the minimum RRO_2 was 0.1498 m^3/kg h under ambient condition and it was 0.0079 m^3/kg h, due to lesser oxygen utility for respiration under refrigeration condition. This is due to less metabolic activity). Control with the product to free volume ratio of 1:8 was found to be effective in reducing the respiration rate under both the conditions. The $RRCO_2$ under ambient and refrigerated conditions ranged from 0.1008 to 0.1072 m^3/kg h for the pre-cooled and control samples with the product to free volume ratio 1:8 after12 hours and between 0 - 0.1106 m^3/kg h. Smyth *et al.,* (1998) has also reported a rapid decrease of respiration rate over time for cut iceberg lettuce at 5˚C.

Determination of respiration rate under permeable system without gas flushing

From table 2 it was clear that the respiration rate RRO_2 of mint kept under two different temperatures vary greatly. Under ambient condition the maximum value for respiration rate attained after 3 hours, for the product volume ratio of 1:8 was 0.2570 m^3/kg h and the lowest value measured was 0.0514 m^3/kg h. For samples kept under refrigerated condition the lowest RRO_2 was 0.0218 m^3/kg h. Higher the temperature higher the respiration rate (Iqbal *et al.,* 2004).

The $RRCO_2$ of mint under refrigerated condition recorded the lowest value (0.0656 m^3/kg h). Respiration rate decreased with a decrease in O_2 concentration and temperature, and increased with a decrease in CO_2 concentration (Fonseca *et al.,* 2002).

Respiration rate using permeable system with gas flushing

It was observed from table 3 that respiration rate (RRO_2) under ambient condition was found to be minimum after 9 hours for the gas composition (O_2-5%, CO_2-5% and N_2-90%). The lowest value of respiration rate was 0.108 m^3/kg h and was due to less oxygen availability in the head space provided. The lowest respiration rate under refrigerated condition was 0.104 m^3/kgh. The $RRCO_2$ recorded a minimum value (0.201 m^3/kg h) for the above said gas composition under low temperature storage. The results are same as the results obtained by Fonseca *et al.,* (2002).

Optimization of gas composition

The respiration rate decreased with the decrease in temperature due to less reaction rate at lower temperatures (Zhang *et al.,* 2003). Kader *et al.,* (1992) reported that 3–5 per cent of O_2 and 4–5 per cent of CO_2 are more suitable for maintaining the quality and extending shelf life of fresh-cut produces at refrigerated condition. The gas composition was optimized based on the respiration rate under ambient and refrigerated conditions. The lowest respiration rate of O_2 obtained in the refrigerated system for mint leaves was 0.104 m^3/ kg h for the gas composition containing 5 per cent O_2, 5 per cent CO_2 and 90 per cent N_2 as mentioned in table 3 From the results, it was concluded that in gas flushed permeable system, the respiration rate of O_2 and CO_2 under refrigeration condition showed lesser respiration rate compared to ambient condition. The respiration rate decreased with the decrease of temperature (Li and Zhang, 2008).

Table.1 Effect of Product volume ratio, pretreatments and duration on respiration rate of mint under ambient and refrigerated condition for closed system

Product volume ratio	Treatment	Duration,h	Ambient condition		Refrigerated condition	
			Ro_2	Rco_2	Ro_2	Rco_2
1:17	control	3	0.3248	0.1848	0.0336	0.0504
		6	0.2352	0.1400	0.0336	0.0336
		9	0.1960	0.1232	0.0672	0.0168
		12	0.1820	0.1190	0.0336	0.0504
	Pre chilling	3	0.3024	0.1736	0.0336	0.0336
		6	0.2240	0.1400	0.0336	0.0504
		9	0.1978	0.1232	0.0672	0.0336
		12	0.1652	0.1078	0.0504	0.0672
	Pre cooling	3	0.3248	0.1848	0.0504	0.0336
		6	0.2352	0.1428	0.0336	0.0336
		9	0.1978	0.1288	0.0504	0.0168
		12	0.1680	0.1148	0.0336	0.0504
1:11	control	3	0.2071	0.1380	0.0327	0.0218
		6	0.1908	0.1298	0.0327	0.0218
		9	0.1647	0.1114	0.1526	0.0436
		12	0.1498	0.1008	0.0218	0.0436
	Pre chilling	3	0.2289	0.1453	0.0436	0.0218
		6	0.2197	0.1435	0.0218	0.0327
		9	0.1889	0.1162	0.1526	0.0436
		12	0.1644	0.1153	0.0436	0.0327
	Pre cooling	3	0.2107	0.1344	0.0654	0.0218
		6	0.1962	0.1308	0.0218	0.0218
		9	0.1683	0.1114	0.0763	0.0545
		12	0.1507	0.1072	0.0218	0.0218
1:8	control	3	0.2449	0.1501	0.0316	0.0474
		6	0.2119	0.1422	0.0158	0
		9	0.1825	0.1211	0.0237	0.0237
		12	0.2225	0.1725	0.0079	0.0237
	Pre chilling	3	0.2449	0.1580	0.0474	0.1027
		6	0.2133	0.1501	0.0158	0.0158
		9	0.1843	0.1299	0.0079	0.0237
		12	0.2185	0.1665	0.0079	0
	Pre cooling	3	0.2370	0.1553	0.0395	0.1106
		6	0.2067	0.1462	0.0158	0.0079
		9	0.1808	0.1281	0.0237	0.0158
		12	0.2172	0.1685	0.0079	0

Table.2 Effect of Product volume ratio, pretreatments and duration on respiration rate of mint under ambient and refrigerated condition for permeable system

Product volume ratio	Treatment	Duration,h	Ambient condition		Refrigerated condition	
			Ro_2	Rco_2	Ro_2	Rco_2
1:17	control	3	0.1103	0.3007	0.0568	0.1730
		6	0.0941	0.2496	0.0405	0.1362
		9	0.0827	0.2208	0.0373	0.1428
		12	0.0697	0.1851	0.0466	0.1532
	Pre chilling	3	0.1557	0.3858	0.0519	0.1305
		6	0.1241	0.2886	0.0349	0.0972
		9	0.1081	0.2539	0.0346	0.1026
		12	0.0941	0.2241	0.0332	0.1124
	Pre cooling	3	0.1265	0.3362	0.0795	0.1943
		6	0.1070	0.2745	0.0616	0.1681
		9	0.0930	0.2444	0.0546	0.1522
		12	0.0807	0.2117	0.0474	0.1461
1:11	control	3	0.09504	0.2622	0.0454	0.1297
		6	0.0772	0.2092	0.0319	0.0862
		9	0.0598	0.1663	0.0310	0.0953
		12	0.0524	0.1437	0.0311	0.0999
	Pre chilling	3	0.1350	0.3569	0.0421	0.1060
		6	0.1064	0.2708	0.0292	0.0743
		9	0.0918	0.2310	0.0274	0.0795
		12	0.0791	0.2005	0.0265	0.0703
	Pre cooling	3	0.1069	0.2954	0.0518	0.1155
		6	0.0907	0.2447	0.0432	0.1075
		9	0.0821	0.2215	0.0360	0.0953
		12	0.0689	0.1827	0.0324	0.0963
1:8	control	3	0.0842	0.2357	0.0454	0.1328
		6	0.0717	0.1960	0.0304	0.0948
		9	0.0605	0.1638	0.0273	0.0857
		12	0.0514	0.1415	0.0267	0.0944
	Pre chilling	3	0.1029	0.28187	0.0316	0.0866
		6	0.0786	0.2226	0.0271	0.0664
		9	0.0705	0.1874	0.0243	0.0656
		12	0.0610	0.1645	0.0219	0.0669
	Pre cooling	3	0.0948	0.2570	0.0429	0.1115
		6	0.0818	0.2208	0.0304	0.0770
		9	0.0753	0.1993	0.0251	0.0691
		12	0.0640	0.1699	0.0233	0.0678

Table.3 Effect of Product volume ratio, pretreatments and duration on respiration rate of mint under ambient and refrigerated condition for permeable system with gas flushing

Gas composition	Duration-h	Ambient condition		Refrigerated condition	
		RO_2	RCO_2	RO_2	RCO_2
O2- 3%, CO2-5% and N2-92%	3	0.3029	0.4878	0.3029	0.4878
	6	0.1620	0.2829	0.1612	0.2936
	9	0.1118	0.2147	0.1118	0.2265
O2- 4%, CO2-5% and N2-91%	3	0.3029	0.4878	0.2916	0.4594
	6	0.1620	0.2829	0.1539	0.2794
	9	0.1118	0.2147	0.1107	0.2194
O2- 5%, CO2-5% and N2-90%	3	0.2770	0.4807	0.2803	0.4452
	6	0.1515	0.2936	0.1474	0.2581
	9	0.1080	0.2194	0.1042	0.2005

Table.4 Physicochemical qualities of fresh mint leaves

Physicochemical qualities of fresh mint leaves					
Moisture content (%, wb)	**Colour value**			**Chlorophyll content (mg/g)**	**β-carotene (mg /g)**
	L	**a**	**b**		
86.7	42.24	-12.75	22.34	1.40	48.30

Table.5 Microbial analysis of fresh mint leaves

Microbial analysis of fresh mint leaves		
Replication	**Bacterial population x 10^5 cfu/mg**	**Fungi population x 10^3cfu/mg**
I	**15**	**2**
II	**5**	**1**
III	**9**	**2**

Table.6 Effect of storage period on colour value for mint leaves

Storage days	Colour value					
	Ambient condition			Refrigerated condition		
	L	a	b	L	a	b
0	40.24	-12.75	22.34	40.24	-12.75	22.34
4	31.56	-4.78	15.98	40.15	-9.43	22.11
8	-	-	-	39.72	-10.1	23.1
12	-	-	-	40.18	-8.9	21.86
16	-	-	-	37.28	-8.71	22.45
20	-	-	-	36.44	-7.95	21.62

Table.7 Effect of storage period on gas composition of mint leaves

Storage days	Ambient condition					Refrigerated condition				
	Gas composition %			RRO_2	$RRCO_2$	Gas composition %			RRO_2	$RRCO_2$
	O_2	CO_2	N_2			O_2	CO_2	N_2		
0	4.8	5.3	89.9	0.0972	0.0639	4.7	5.2	90.1	0.01458	0.0426
4	0	9.4	90.6	0.0025	0.0098	1.4	6	92	0.0018	0.0022
8	-	-	-	-	-	0.4	8.4	91.2	0.0012	0.0037
12	-	-	-	-	-	2	6.7	92.1	0.0008	0.0013
16	-	-	-	-	-	0.9	8.1	91.2	0.0005	0.0017
20	-	-	-	-	-	0.1	9.6	91	0.0005	0.0002

Table.8 Microbial load on the 20^{th} day of storage

Name of the green leaf	Bacterial population x 10^6 cfu/mg	Fungal population x 10^4cfu/mg
Mint leave	I 13 II 15 III 14	3.0 4.0 4.0

Methods of estimation of dependent variables

S.No.	Dependent variables	Method adopted
Physiological and physical properties		
1.	Physiological loss in weight	$\text{PLW}(\%) = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$
2.	Colour value	Anonymous, Colour flex meter (Hunter lab)
Biochemical properties and microbial load		
3.	Beta carotene	Ranganna (1979)
4.	Chlorophyll content	Ranganna (1979)
5.	Microbial load	Standard plate count method (Allen,1953)

Fig.1 Flow chart for modified atmosphere packaging of mint leaves

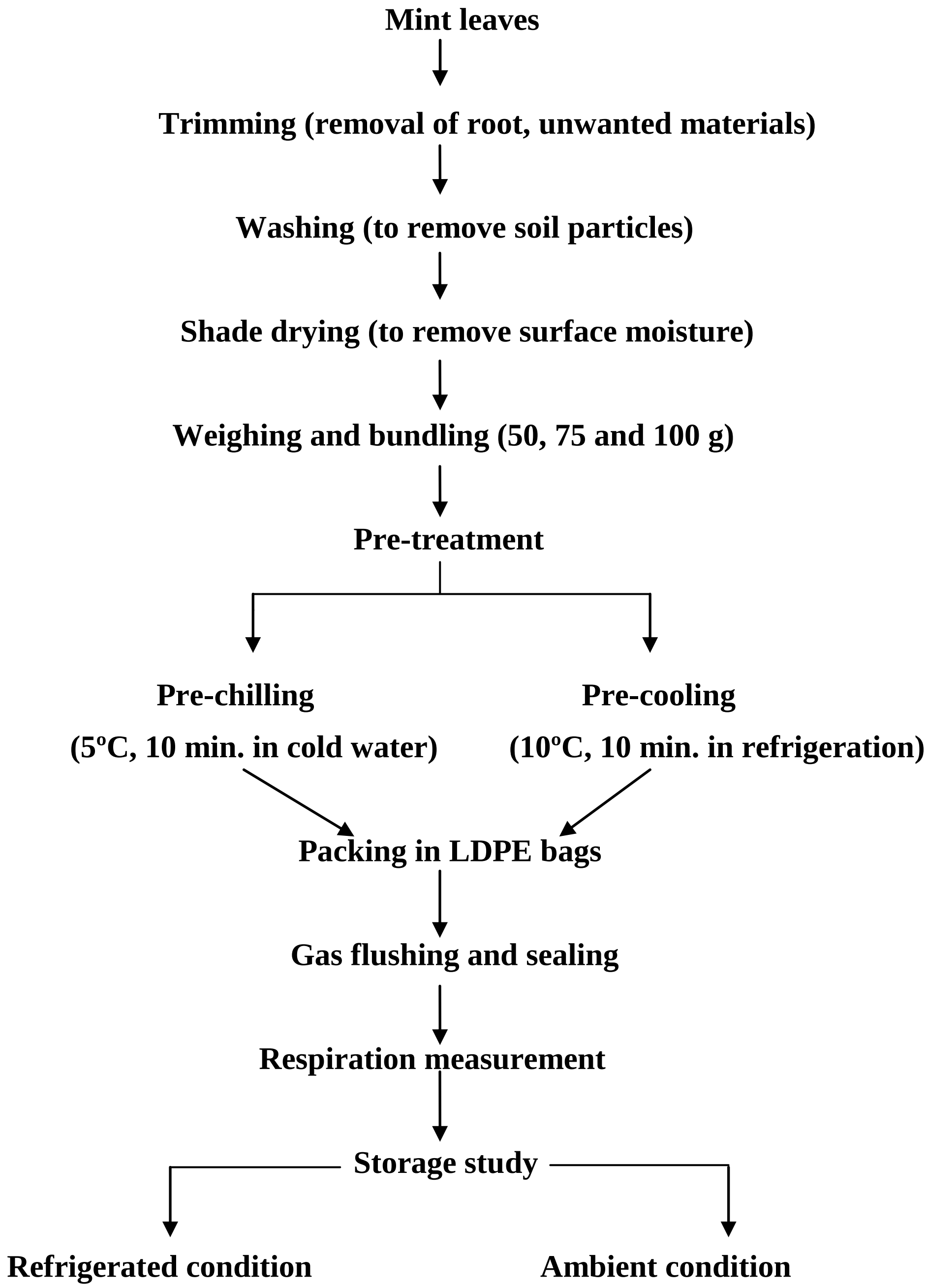


Fig.2 Permeability of the packaging materials

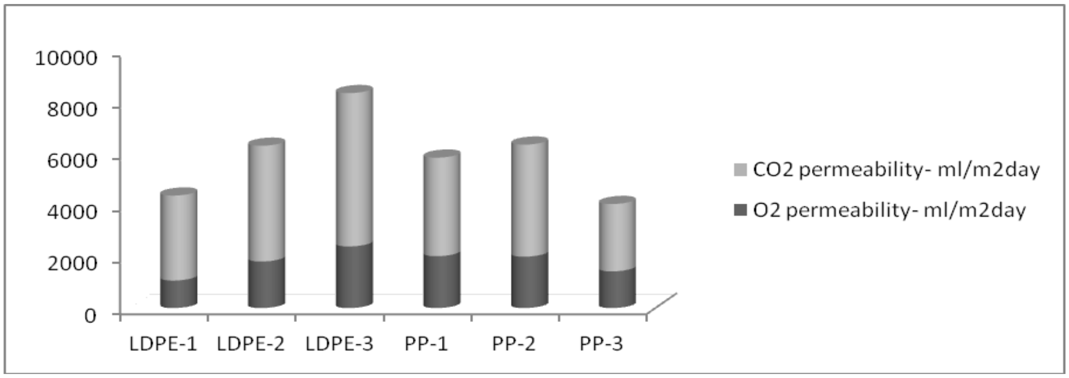


Fig.3 Physiological loss in weight of mint leaves during storage

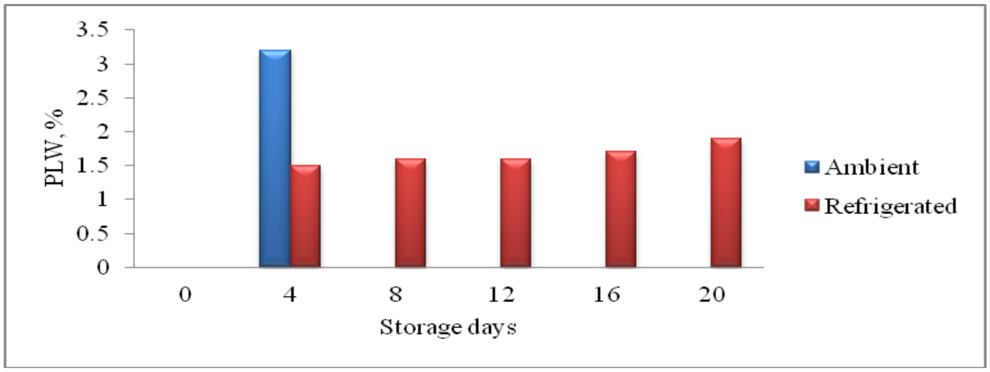


Fig.4 Change in respiration rate of O_2 during storage of mint leaves

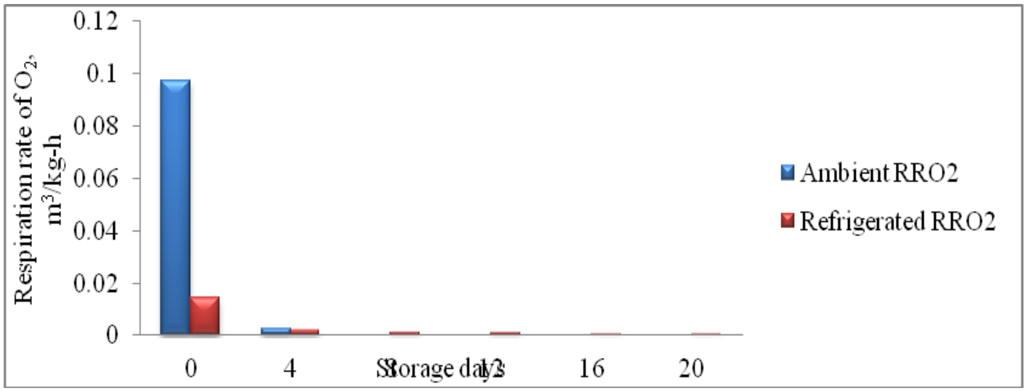


Fig.5 Change in respiration rate of CO_2 during storage of mint leaves

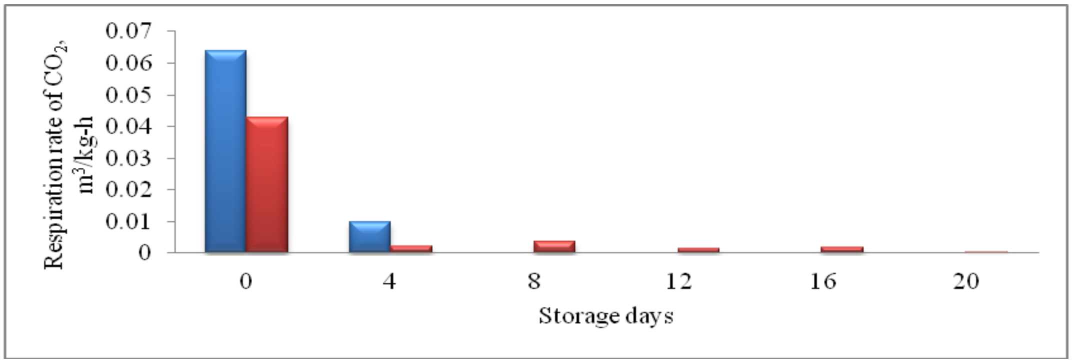


Fig.6 Change in chlorophyll content during storage of mint leaves

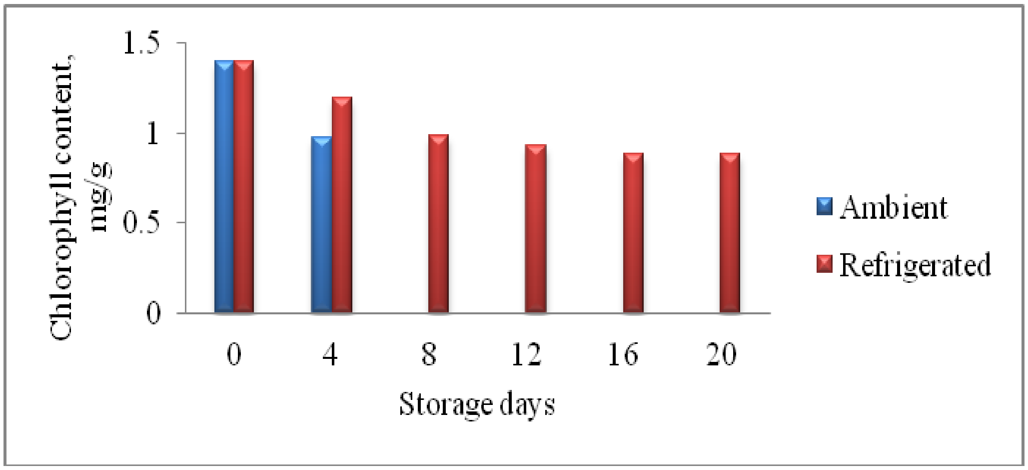
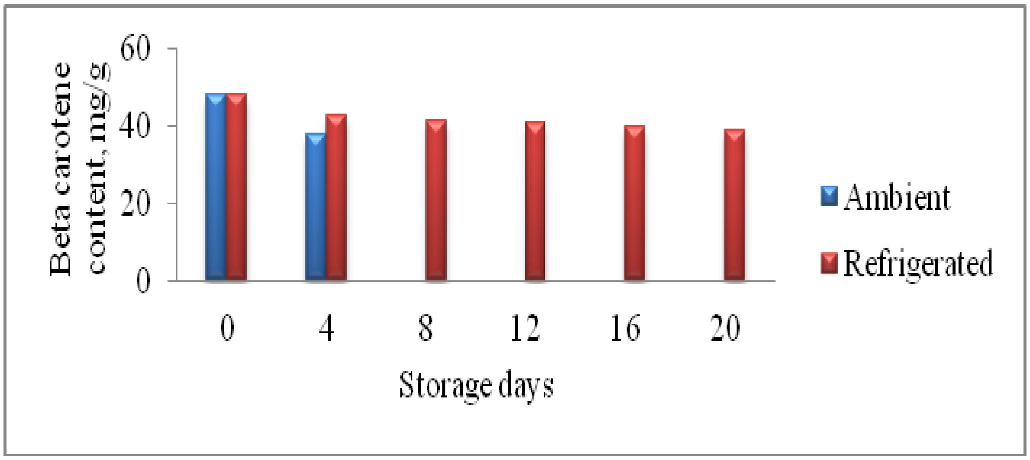


Fig.7 Change in β - carotene content during storage of mint leaves



The PLW was less, the colour value, chlorophyll and beta carotene contents were retained much in the case of 5 per cent O_2, 5 per cent CO_2 and 90 per cent N_2 gas composition. Hence, the composition was chosen for storage study along with pre cooling treatment and 1:8 as product to free volume ratio for mint leaves. The storage study was conducted under ambient condition and in refrigerated condition.

Effect of storage period on the physiological loss in weight of mint leaves (PLW)

It was inferred from figure 3. The weight loss was gradual from the initial day of storage to final day. The weight loss of 1.9 per cent was observed during the 20th day of storage for the sample kept under refrigerated condition. PLW was more under ambient condition than the refrigerated condition, and was reported as 3.2 per cent.

This was due higher temperature in the ambient atmosphere, which leads to high respiration than the refrigerated condition. The PLW may be mainly due to water loss as the product of respiration (Wills *et al.,* 1989) and also due to transpiration water loss (Moleyar and Narasimhan, 1994) during the storage period.

Effect of storage period on the colour value of mint leaves

The colour of mint leaves indicates the freshness of the leaves during the storage period. The 'L' value represents the brightness of the leaves and from the table 6, it was evident that the 'L' value of the mint leaves decreased from 40.24 to 36.44 on 20th day of storage. There was no much variation in the 'L' value of the leaves during storage. The 'a' value of the mint leaves under ambient condition changed to a large extent because the leaves have started decaying on the 4th day itself. The refrigerated stored leaves have shown a constant decrease in the green colour. At the 20th day of storage the 'a' value was – 7.95. The 'b' value changed to a lesser extent during the storage period. The 'b' at the beginning of storage was about 22.34 and at the 20th day was 21.62 respectively. Higher temperature inside the package degrades the colour of the mint leaves due to the bleaching or surface burning. Similar results were also reported by Kader *et al.,* (2002).

Effect of storage period on the gas composition of mint leaves

Temperature had significant impact on the respiration rate. At ambient condition the samples got spoiled after 4 days, whereas in refrigeration, the samples were in good condition up to 20 days, since O_2 consumption and CO_2 production were less due to low metabolic activity. Fonseca *et al.,* (2002) reported that at lower temperature the oxygen consumption and carbon dioxide generation were less. As per the general statement in the case of respiration the oxygen concentration decreases and carbon dioxide concentration increases.

The gas composition inside the gas flushed mint leaves also follows the same trend. From table 7 it was clear that under ambient condition (30ºC) decrease in O_2 concentration results an increase in CO_2 level. Under refrigerated storage condition the O_2 concentration was decreasing up to 8th day increasing on 12th day and was about 2 per cent, later on declines to 0.1 per cent on 20th day. Regarding CO_2 concentration it decreased to 6.7 per cent and increased to 9.6 per cent on 20th day of storage. The fluctuation in gas concentration during storage was mainly due to permeability of the packaging material to O_2 and CO_2 (Figs. 4 and 5).

Effect of gas composition on the respiration rate on mint leaves

The respiration rate was found to be high under ambient condition than refrigerated storage. It is evident from table 7 that RRO_2 value at 30ºC was 0.097 m^3/kg h and reduced to 0.0018 m^3/kg h on the 4th day. The leaves have started decaying after this period under ambient condition. In the case of refrigerated condition the initial value of RRO_2 was 0.0146 m^3/kg h and reduced to 0.0005 m^3/kg h. This was due to lower metabolic activity under this temperature.

The respiration rate of carbon dioxide followed the same trend as RRO_2. From table 7 it is clear that the $RRCO_2$ was 0.0639 in the initial period and reduced to 0.002 m^3/kg h at the fourth day of storage. Similarly in the refrigerated condition it was reduced to 0.0002 m^3/kg h on 20th day from 0.026 m^3/kg h in the beginning.

Temperature is the most important external factor influencing the respiratory activity of fruit and vegetables (Zagory and Kader, 1988). Watada *et al., (*1996) stated that low O_2 and elevated CO_2 atmospheres together with low storage temperature reduce the product respiration rate. Fonseca *et al.*, (2002) also reported the increase of shelf life of was found in shredded galega kale when stored under gas flushed samples.

Effect of gas composition on the chlorophyll and β - carotene content of mint leaves

From figure 6 it is clear that the chlorophyll content of the sample stored under ambient condition reduced to 0.971 mg/g against its initial values. Under refrigerated condition it varied between 1.4 mg/g – 0.883 mg/g. The chlorophyll content decreased with increase of storage period. Roura *et al.*, (2000) reported that processing induced the decrease of chlorophyll content during storage in swiss chard leaves.

In the fresh mint leaves the β - carotene content was 48.3 mg/g. The figure 7 stated that there was a sudden decrease in the value to about 37.81 mg/g under ambient condition. In the refrigerated condition the value on the 20th day was 38.67 mg/g.

Effect of gas composition on the microbial population of mint leaves

Maintaining the quality of the food product during storage was mainly due to inhibition of growth of spoilage microorganisms and in most cases the condition chosen were that those reduce the microbial growth. Microbial food spoilage was characterized by undesirable sensory changes to the odour, colour, flavour and sometimes texture of the food, making it inedible or unsaleable. Generally low O_2 and high CO_2 with low temperature condition was selected for safe food products in MAP.

The low level of oxygen may inhibit the surface growth of pathogenic anaerobic bacteria particularly *Clostridium botulinum* but it would not prevent anaerobic condition present on the body of the product (Hotchkiss, 1988). It is noted that the bacterial and fungal population during the storage period are within the limits (Table 8).

In the closed system the observed minimum respiration rate under the refrigerated condition was between 0–0.0079 m^3/kgh and for permeable system without gas flushing minimum respiration rate was 0.02187 m^3/kgh. In the permeable system with gas flushing minimum respiration rate observed was 0.1042 m^3/kgh, for the gas composition 5 per cent O_2, 5 per cent CO_2 and 90 per cent N_2.

The physiological loss in weight observed was less under refrigerated condition than in ambient condition for coriander and mint leaves. The colour value, chlorophyll and beta carotene content of mint leaves were not altered significantly up to 20 days. The microbial population for was within the permissible level after 20 days. Storage at refrigerated condition with modified atmosphere packaging was effective in extending the shelf life of mint leaves to 20 days compared to four days under the ambient condition.

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