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Development and evaluation of Modified Atmosphere (MA) packages employing lamination technique for Royal Delicious apple

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Abstract

Modified atmosphere (MA) packages were prepared for Royal Delicious apple with an aim to achieve the optimum storage atmosphere within the packages in minimum possible time. Apples were harvested at their commercial maturity and respirtion rate of the fruits was measured at temperatures between 0 – 25°C. On the basis of preliminary investigations, air composition with 3% O_2 and 3% CO_2 was found appropriate for MA packaging of apple. The MA package was finalized with a size of 24 cm x 19 cm for 1 kg fill-weight of apples. As the gas transmission rates of individual polymeric films could not match the gas transmission requirements of MAP, judicious combinations of the films were made to bring gas transmission characteristics close to the required values. The equilibrium concentrations of O_2 and CO_2 were established within 36-72 h under different storage temperatures. The mathematical model, developed applying enzymatic kinetics based respiration equation coupled with the Arrhenius model, effectively predicted the equilibrium time and the levels of O_2 and CO_2 at equilibrium. MA packages were evaluated through assessment of different quality parameters of apple during storage and were found to increase its shelf life by 125-210% that of unpacked fruits at different storage temperatures.

Key words: Apple, Respiration rate, Gas transmission modelling, MA packaging, Quality

Introduction

The Delicious group of cultivars predominates the apple market and the Royal Delicious apples account for 50-60% of the total apple production in India. Post-harvest constraints like limited shelf life, susceptibility to many diseases and pest, faster fruit ripening at warmer temperatures etc. limit apples storage period (Kaul and Gupta, 1987; Kaushal and Sharma, 1995). It is important to prolong the storage period and improve its keeping quality during transport and marketing. Application of improved packaging systems is one such possibility.

Modified Atmosphere Packaging (MAP) is simple, economical, and effective way of extending shelf life of fresh commodities (Sandhya, 2010; Nicolais et al., 2011b). The modification of the storage atmosphere within the package is achieved by the interplay of two processes, the respiration rate of the packaged products and the gas permeation rates through the packaging materials (Smith et al., 1987; Fonseca et al., 2002; Mahajan et al., 2007; Meetoo, 2011). It is generally desirable to achieve an atmosphere low in O_2 and high in CO_2 to regulate the metabolism of the packaged produce, and the activity of decay-causing organisms to increase the storage life (Mangaraj et al., 2009a).

Rocha et al. (2004) stored apple for 6.5 months in MAP with good marketable quality using 100 µm polypropylene at 4°C and 85% R.H. The incidence of bitter pit that developed during controlled and modified atmospheres storage of 'Cox's Orange pippin apple for 5 weeks at 2°C was reduced from 50% to less than 5% (Hewett, 1984). Guan et al. (2004) packed 'Fuji apples' in optimal MAP that had good quality after 7 months storage at 0°C and 10°C with reduction of scald rate. Rocculi et al. (2006) reported that active modified atmosphere decreased the rate of oxygen consumption compared with passive MA, in particular at the beginning of storage.

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The objective of MAP design is to achieve the equilibrium concentrations of O_2 and CO_2 within the package as quickly as possible on account of the interactions of the produce with the package and the external atmosphere. These concentrations need to lie within the desired levels required for extending the storage life of the commodity (Oliveira et al., 2010; Costa et al., 2011). The desired levels of gas concentrations could be achieved by matching the film permeation rates for O_2 and CO_2 with the respiration rate of the packaged produce. As different products vary in their respiration rates and as MA-packages are exposed to a dynamic environment, each package needs to be optimized for specific productss (Tano et al., 2007; Montanez et al., 2010). A MAP system, which is not properly designed, may be ineffective or even may shorten the storage life of a commodity. If the desired atmosphere is not established rapidly the package would have no benefit, and the product might experience serious damages and its storage life might be shortened (Mahajan et al., 2007; Mangaraj and Goswami, 2009b). Therefore, it is important to carry out a systematic design of modified atmosphere packaging for different commodities by considering all factors affecting respiration and permeation. The present study was under taken to design MAP system for Royal Delicious apple and to evaluate its quality attributes under various storage temperatures.

Materials and Methods

Measurement of respiration rate of apple

Royal Delicious apples were harvested from an orchard at Shimla, Himachal Pradesh at their commercial maturity i.e. 125-130 days after full bloom. The respiration rate data for the fruits were experimentally determined for different temperatures using the closed system method (Mangaraj and Goswami, 2008). A mathematical model applying Michaelis-Menten type equation coupled with the Arrhenius model was developed for predicting the respiration rate of apple (Lee et al., 1991, 1996; Mangaraj and Goswami, 2008, 2009c; 2011a,b).

Measurement of GTR of selected films

With the objective of meeting MAP requirements for apple, BOPP and PVC films were selected considering gas permeability, water vapor transmission rate, sealing reliability, clarity, strength, and durability (Kader et al., 1989; Del Nobile et al., 2009). The gas transmission rates (GTR) of the films were determined employing equal pressure method (Mangaraj et al., 2009). Arrhenius-equation was fitted to the experimental data to depict the relationship of GTR with temperature (Exama et al., 1993).

Target level of MA package air composition for apple

Preliminary investigation with various designs of optimum combinations of O_2 and CO_2 for MA packaging of apple was carried out. Also, the MA packages were evaluated for sub-optimal air compositions with a view to provide a factor of safety against the development of deletarious levels of O_2 and CO_2 in the packages at any stage, throughout the distribution chain. On the basis of preliminary investigations and the sub-optimal package air composition, the MA packages for apple were designed with target air composition of 3% O_2 and 3% CO_2.

Modeling of gaseous exchange in MAP system

Once the fruit is sealed in the package, the O_2 and CO_2 concentration gradients develop due to respiration and the polymeric film serves as the regulator of O_2 inflow and CO_2 outflow. Considering that there is no gas stratification within the packages and that the total pressure is constant, the basic mass balance relationships describing the O_2 and CO_2 concentration changes in a package are:

Rate of O_2 inflow – Rate of O_2 consumed by product = Rate of O_2 accumulation in package space, and;
Rate of CO_2 generated by the fruits – Rate of CO_2 outflow = Rate of accumulation CO_2 in package space

Preparation of film laminates for MA Packaging of apple

The mass balance relationships and the results of preliminary investigation were used for the optimization of package parameters. The MA packaging system was restricted to medium size apples with each package of 24 cm x 19 cm weighing 1 kg. The GTR requirement of the MA package was calculated using respiration data (Mangaraj et al., 2012a,b). The GTR of the selected films were compared with the gas transmission requirement (GTR_{req}) of MAP for apple. Neither BOPP nor PVC film alone could meet the GTR_{req} of package satisfactorily. Thus, the two films were laminated (Figure 1) to bring the GTR of the laminates close to the required values (Ahvenainen, 2003; Mangaraj et al., 2011). The areas of the two individual films were optimized and two types of film laminates i.e. LFR-1 (BOPP-30μ + PVC-50μ) and LFR-2 (BOPP-45μ + PVC-35μ) were developed employing lamination technique (Prasad, 1995; Mangaraj et al., 2011).

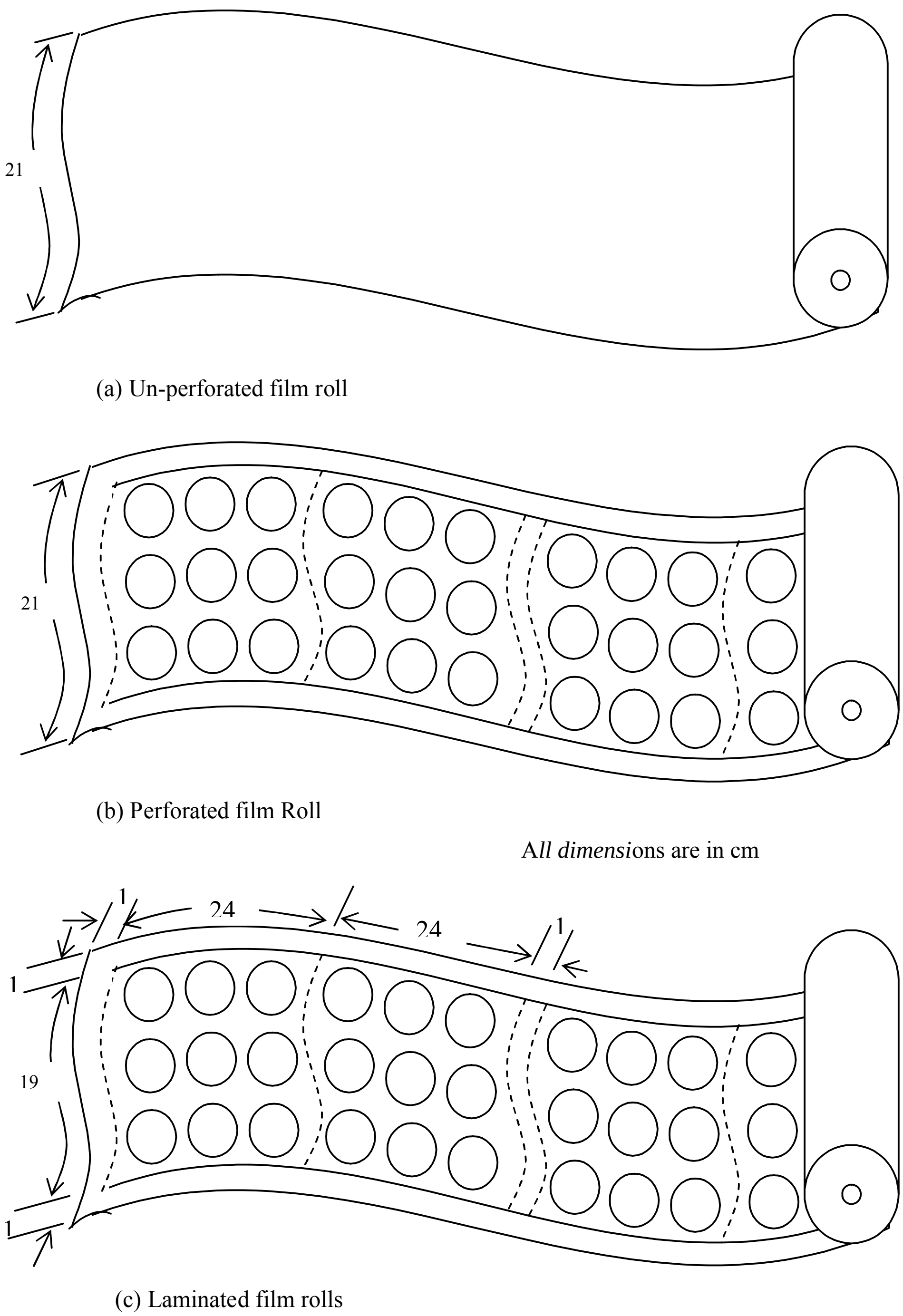

Figure 1. Laminated film rolls for MA packaging of fruits.

Optimization of the area of films for OTRreq of MAP

The areas of the laminated films and un-perforated film were optimized to match the oxygen transmission rate requirement of MAP by employing the following equations (Mangaraj et al., 2011; Mangaraj et al., 2012a).

$$OTR^{req} = OTR^{la}\ a_1 + OTR^{upf}\ a_2 \qquad (1)$$

$$a_1 = \frac{A_1}{A}\ ;\ a_2 = \frac{A_2}{A} \qquad (2)$$

$$a_1 + a_2 = 1 \qquad (3)$$

Where, OTR^{req} is the oxygen transmission requirements of film laminate for MAP; OTR^{la} is the oxygen transmission rate of the combined film; OTR^{upf} is the oxygen transmission rates of un-perforated single film; a_1 is the fractional area of laminated portion; a_2 is the effective fractional area of un-perforated film; A_1 is the area of laminated portion of the film laminate; and A_2 is the effective area of un-perforated film of the laminate.

Calculation of OTRla

The oxygen transmission rate of the combined film was measured experimentally. However, the values of OTR^{la} were also calculated by employing the following equation.

$$\frac{1}{OTR^{la}} = \frac{x_1}{\left(x\ OTR^1\right)} + \frac{x_2}{\left(x\ OTR^2\right)} \qquad (4)$$

Where, OTR^1 and OTR^2 are the oxygen transmission rates of individual films having thickness of x_1 and x_2, respectively and x is the thickness of the film laminate.

Determination of a1 and a2

The values of a_1 and a_2 were determined by incorporating the values of OTR^{req}, OTR^{la} and OTR^{upf} in equations (1) and solving this with equation (3).

Calculation of A1 and A2

The area of laminated portion (A_1) and effective area of un-perforated film (A_2) was calculated using equations (2) as shown in equations (5) and (6).

$$A_1 = a_1\ A \qquad (5)$$

$$A_2 = a_2\ A \qquad (6)$$

Calculation of size and nos. of circular disc for removal from film

For the development of film laminates, an area equal to A_2 was removed in the form of circular disc from the perforated film. The sizes and numbers of discs required to be removed from film was calculated by employing the following equation (7).

$$A_2 = \pi/4\left(d_1^2\ N_1 + d_2^2\ N_2 + ... + d_n^2\ N_n\right) \qquad (7)$$

Where, d_1, d_2, $d_3 \ldots d_n$ are the diameter of the circular disc to be removed, and N_1, N_2, $N_3 \ldots N_n$ are the numbers of discs to be removed from the film.

Calculation of OTR of film laminate (OTRfl)

The oxygen transmission rate of the film laminates was calculated by putting the values of a_1, a_2, OTR^{la} and OTR^{upf} in equation (1) and given in equation (8).

$$OTR^{fl} = OTR^{la}\ a_1 + OTR^{upf}\ a_2 \qquad (8)$$

If, $OTR^{fl} = OTR^{req}$ then, the MA package design can be considered to be successful.

Calculation of CTR of film laminate (CTRfl)

Similarly, the CTR value of the film laminate (CTR^{fl}) was calculated by putting the values of a_1, a_2, CTR^{la} and CTR^{upf} as given in equation (9).

$$CTR^{fl} = CTR^{la}\ a_1 + CTR^{upf}\ a_2 \qquad (9)$$

Where, CTR^{la} is the carbon dioxide transmission rates of the combined films and CTR^{upf} is the CTR of un-perforated film.

MA packages for apple

Using different coded film laminates i.e. LFR-1 and LFR-2, two types of MA packages (PCG-LFR1, PCG-LFR2) of size 24 cm x 19 cm were prepared. Six apples were inserted in each package and the packages were heat-sealed. Silicon rubber septums were glued to the packages to facilitate gas sampling. The MA packages were labeled and kept for subsequent storage study at 10, 15, 20 and 25°C temperature. The representative samples were taken from the fruit lot and the physico-chemical attributes were determined employing standard techniques and procedures.

Performance evaluation of MA packages

The performance of various packages was evaluated for their ability to establish equilibrium at target levels, and ability to extend the shelf life of the packaged fruit while maintaining quality. The experimental variation of in-pack O_2 and CO_2 concentrations (Y_{O2}^{in} and Z_{CO2}^{in}) from the time of sealing the fruits in the package to the time of establishing equilibrium were recorded at 6 h interval. The samples of package air were analyzed for the variation of O_2 and CO_2 concentration in the package with time using GC. The equilibrium concentration of O_2 (Y_{O2}^{eq}), CO_2 (Z_{CO2}^{eq}) and equilibrium time (t_{eq}) were subsequently determined. The different quality attributes such as PLW, volume reduction, firmness, TSS, titratable acidity, color (l*, a*, b*, hue angle, chroma, total color difference) of MA packaged as well as unpacked apples, with storage time were determined by standard methods (Ranganna, 1995). Various sensory attributes such as color, texture, taste and mouth feel of the fruit samples were evaluated using Fuzzy logic models (Das, 2005).

Statistical methodology

Three-factor analysis of variance was carried out to find the direct two-factor and three-factor interaction effects of temperature, storage system and storage period on the quality parameters of apple (Das and Giri, 1986). Response surface methodology (RSM) was employed to evaluate the effects of temperature, storage periods and their interaction on the quality parameters of MA packed and unpacked fruits (Khuri and Cornel, 1987; Mangaraj and Singh, 2011).

Results and Discussion

Respiration rate at transient state of apple

The effect of changes in O_2 and CO_2 concentrations on respiration rates at various levels of storage temperature under simulated transient state of MAP were analyzed. The decrease in O_2 concentration from 20.5% to 4.2% reduced R_{O2} and R_{CO2} from 9.76 to 2.3 and 11.5 to 2.19 cc/kg-h, respectively, for apple at 2.0% CO_2 concentration level and 10ºC storage temperature. For similar reduction in the O_2 concentration at 5% CO_2 level, R_{O2} and R_{CO2} were found to have reduced from 7.24 to 1.84 and 9.85 to 1.78 cc/kg-h, respectively. At 3% O_2 and 3% CO_2, the respective values of R_{O2} and R_{CO2} were found to be 1.93 and 2.11 cc/kg-h, and those under normal air were 9.43 and 11.76 cc/kg-h, respectively. The results indicated 78 to 82% reduction in respiration rate. Similarly at 15^{o}C, the percent reduction of R_{O2} and R_{CO2} from normal air to modified atmosphere of 3% O_2 and 3% CO_2 was found to be 80-85% (Figure 2). The percentage reduction in R_{O2} and R_{CO2} were found to be higher at higher levels of temperature.

Similarly increase in CO_2 reduces respiration rates at all the level of O_2. The effect of variation in O2 on respiration rates was found to be much higher than that of the variation in CO_2. The effect of CO_2 level on R_{O2} and R_{CO2} was found more pronounced at higher level of O_2 concentration. These results are in close agreement with those obtained by Talasila et al. (1992). Overall, at all the reference temperatures, R_{O2} decreased by decreasing the O_2 and increasing the CO_2 levels and this effect was more significant for low O_2 concentration and higher temperatures. Similar trends were observed for CO_2 production rates (R_{CO2}). Therefore, low O_2 and high CO_2 atmospheres imposed a decreasing trend in the respiration rate of apple, which should increase its shelf life.

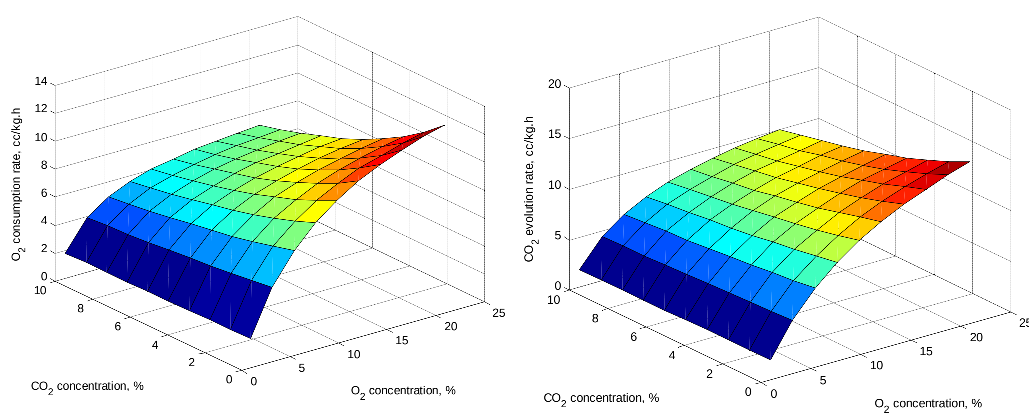

Figure 2. Respiration profile of apple under simulated transient state of MAP at 15ºC.

Gas Transmission Rates of Selected Polymeric Films

The O_2 transmission rate (OTR) and CO_2 transmission rates (CTR) of the selected films as well as the combined film laminates were determined at 10, 15, 20 and 25ºC and are given in Table 1. The gas transmission rates have been expressed for the total film thickness and not for unit film thickness. The GTR of the films increased with the increase in temperature. However, the magnitude of the increase varied with the film type and thickness. Among the selected films, the GTR and the TR of PVC film were found to be significantly higher as compared to BOPP film.

Equilibrium concentrations of O_2 and CO_2 in MA packages

The predicted as well as experimental values of Y_{O2}^{eq}, Z_{CO2}^{eq} and t_{eq} for various MA packages at different storage temperatures have been presented in Table 2.

Table 1. GTR of Selected Polymeric films and laminates at various temperatures.

Polymeric films/ Laminates	Film code	Thickness (μ)	Gas transmission rates (Cm^3 (m^2.h. ΔC)$^{-1}$)							
			10ºC, 90% RH		15ºC, 80% RH		20ºC, 75% RH		25ºC, 70% RH	
			OTR	CTR	OTR	CTR	OTR	CTR	OTR	CTR
BOPP-I	PFR-1	30	43.15	190.72	61.72	278.13	88.59	408.62	125.86	596.57
BOPP-II	PFR-2	45	25.76	87.19	38.54	171.42	57.93	263.57	79.13	368.72
PVC-I	PFR-3	25	650.91	3968.81	943.37	5830.16	1320.84	8263.27	1894.30	11992.64
PVC-II	PFR-4	35	417.59	2527.64	614.38	3784.81	846.17	5264.08	1289.38	8148.89
PVC-III	PFR-5	50	290.26	1712.58	431.29	2609.83	585.52	3579.78	896.45	5566.95
BOPP-I + PVC-III	LFR-1	80	92.22	428.967	132.89	629.79	188.53	915.48	271.98	1349.77
BOPP-II + PVC-II	LFR-2	80	43.69	150.95	65.33	294.37	97.78	451.01	134.26	633.21

Table 2. Predicted and Experimental Equilibrium Concentration of O2 and CO2 for PCG-LFR-1 Package at Different Storage Temperatures.

Temperature (ºC)	$Y_{O2}^{eq\text{-}pre}$ (%)	$Y_{O2}^{eq\text{-}exp}$ (%)	$Z_{CO2}^{eq\text{-}pre}$ (%)	$Z_{CO2}^{eq\text{-}exp}$ (%)	$t^{eq\text{-}pre}$ (h)	$t^{eq\text{-}exp}$ (h)
Package fill weight (W_p): 1.0 kg, V_{fp} : 890 ml						
10	3.11	3.17	3.53	3.74	60.00	66.00
15	3.14	3.22	3.51	3.68	54.00	58.00
20	3.31	3.24	3.59	3.74	46.00	50.00
25	3.30	3.17	3.90	4.17	40.00	42.00
Package fill weight (W_p): 0.9 kg, V_{fp}: 1024 ml						
10	3.21	3.25	3.73	3.86	72.00	80.00
15	3.19	3.23	3.66	3.79	66.00	74.00
20	3.28	3.19	3.62	3.73	62.00	60.00
25	3.23	3.16	3.90	4.11	56.00	54.00
Package fill weight (W_p): 0.95 kg, V_{fp}: 967 ml						
10	3.12	3.10	3.57	3.74	68.00	74.00
15	3.14	3.17	3.54	3.55	62.00	64.00
20	3.26	3.31	3.55	3.68	56.00	52.00
25	3.27	3.20	3.89	3.82	48.00	44.00
Package fill weight (W_p): 1.05 kg, V_{fp}: 818 ml						
10	3.01	3.06	3.37	3.49	58.00	62.00
15	3.08	3.11	3.40	3.52	50.00	52.00
20	3.23	3.16	3.44	3.48	44.00	46.00
25	3.20	3.12	3.71	3.65	38.00	34.00
Package fill weigh (W_p): 1.10 kg, V_{fp}: 762 ml						
10	3.00	2.94	3.34	3.45	52.00	58.00
15	3.05	3.10	3.35	3.50	46.00	50.00
20	3.20	3.13	3.39	3.34	40.00	36.00
25	3.15	3.17	3.60	3.52	36.00	34.00

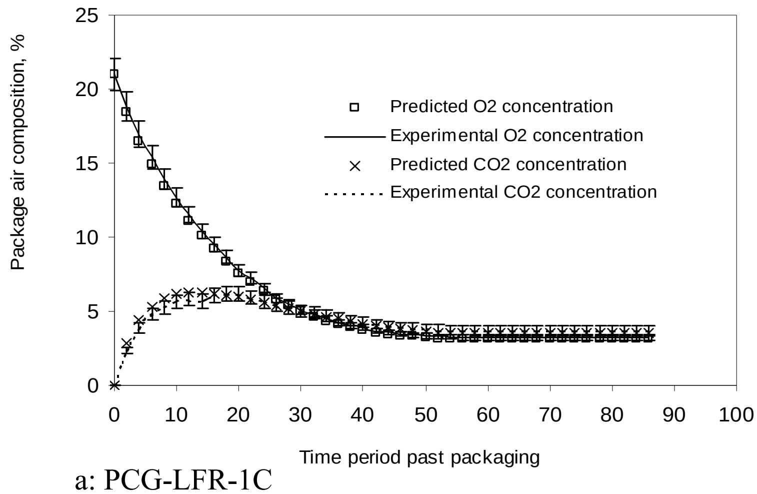

Figure 3. Experimental and predicted variation in package air composition with time for pcg-lfr-1c at 15ºC storage temperatures.

The experimental as well as predicted variations of O_2 and CO_2 levels in MA packages PCG-LFR-1C, at 15ºC storage temperatures have been shown in Figure 3. Most of the packages have established equilibrium at such levels of O_2 and CO_2, which were fairly close to the target levels. The predicted and experimental values of Y_{O2}^{eq}, Z_{CO2}^{eq} were found to be higher than the target levels for all the MA packages. There was good agreement between predicted as well as experimental values of Y_{O2}^{eq} and Z_{CO2}^{eq}. The experimental values of Y_{O2}^{eq} varied between 3.10 – 3.31% whereas those of Z_{CO2}^{eq} varied between 3.34 – 4.17% for all types of MA packages for apple, at all the reference temperature levels. During steady state period, the experimental values of O_2 and CO_2 were found to be nearly constant for an extended period of storage. By and large, all types of MA packages have accommodated varying fill weight between 0.9–1.1 kg and have established dynamic equilibrium state without causing any unfavorable deviation from the target levels of O_2 and CO_2 at all the reference storage temperatures.

Equilibrium time

For various packages, the predicted values of equilibrium time were found to have varied between 36–72 h; whereas those of experimental values varied between 34–80 h; for all types of MA packages for apple, respectively at all the reference storage temperatures as given in Table 2. The experimental values somewhat deviated from the predicted ones.

The development of quasi-equilibrium conditions and the variations in the free volume in the package (V_{fp}), because of the varying fill weight, were probably the cause of such deviations in equilibrium time (t_{eq}) values. In fact, small variations in V_{fp} are always possible in a flexible package. Hence, it is unrealistic to expect a constant value of V_{fp} in the flexible packaging system.

It has been seen that the variation in O_2 affects both R_{O2} and R_{CO2} significantly. With the variation in R_{O2} and R_{CO2}, the O_2 consumption as well as the CO_2 evolution of the package varies which in turn affects O_2 and CO_2 level in the internal atmosphere of the package. Thus, as O_2 decreases, R_{CO2} reduces which in turn reduces CO_2 in the internal atmosphere of the package. Reduction in CO_2 level tends to retrieve R_{CO2} slightly. However, the amount of reduction in CO_2 due to decrease in O_2 is greater than the amount retrieved. As such, with the decrease in O_2 level, CO_2 level also decreases, though by small amounts. Thus, though the equilibrium condition for CO_2 level appears to be approaching earlier than that of O_2 level but in true sense, CO_2 level becomes stable only when O_2 level attains equilibrium as shown in Figure 3. It implies that the equilibrium for both, O_2 and CO_2 is attained simultaneously in MA packaging. Also, in view of the fact that O_2 level has more pronounced effect on respiration rates than CO_2 level, the equilibrium time (t_{eq}) for O_2 level assume greater importance. The single equilibrium time (t_{eq}) approach, advocated in this study, is in agreement with the study of Rocculi et al. (2006), Torrieri et al. (2007), Rai and Paul (2007), González-Buesa et al. (2009), Tariq et al. (2009) and Mangaraj et al. (2011, 2012a,b).

Validation of the MAP model

The mathematical model using enzymatic kinetics based respiration equation coupled with the Arrhenius model was developed and solved numerically using MATLAB programme. The model was used to determine (i) the variation of O_2 and CO_2 with time within the MA packages; and (ii) the time to reach the equilibrium concentration within the MA package as well as the level of O_2 and CO_2 concentration at equilibrium state. The equilibrium O_2 and CO_2 concentration obtained from the model was verified against the experimental data for MA packaging of apple. The mean relative deviation moduli (E) between the equilibrium concentration of O_2 and CO_2 as predicted by the developed model and that obtained through experiment were found to be in the range of 5.92-8.60% and 7.14-9.35%, respectively. This indicates the developed model is in good agreement with the experimental data. The equilibrium time for both, O_2 and CO_2 has been observed to be attained simultaneously in MA packaging.

Quality assessment of MA packaged and unpackaged fruits

The variations in physico-chemical quality attributes of Royal Delicious apples under MA packaged and un-packaged condition with different storage temperature were evaluated and compared. The data of different quality attributes of apple at 15ºC for different storage periods is presented in Table 3. Variations of some important parameters with storage temperature are shown in Figure 4 to 6.

Table 3. Variations in Quality Attributes of MA Packed and Unpacked Apple at 15°C.

Quality parameters	Packaging system	Storage periods, days 4	17	31	45	59	73	87
PLW (%)	PCG-LFR-1	0.00	0.60	1.23	1.40	2.00	2.60	3.52
	PCG-LFR-2	0.00	0.68	1.31	1.72	2.40	3.10	3.87
	CS	0.00	2.62	4.37	9.38	14.39	20.34	28.56
Vr (%)	PCG-LFR-1	0.00	0.17	0.25	0.31	0.42	0.55	0.78
	PCG-LFR-2	0.00	0.18	0.24	0.38	0.45	0.68	0.91
	CS	0.00	1.71	3.72	4.82	5.72	6.34	7.40
Firmness (g)	PCG-LFR-1	7625.00	7560.00	7516.00	7240.00	6850.00	6510.00	6142.00
	PCG-LFR-2	7625.00	7540.00	7423.00	7180.00	6770.00	6420.00	6073.00
	CS	7625.00	7130.00	6730.00	6125.00	5740.00	4700.00	4238.00
Puncture strength (g)	PCG-LFR-1	397.00	390.00	383.00	361.00	328.00	281.00	247.00
	PCG-LFR-2	397.00	389.00	380.00	355.00	322.00	282.00	243.00
	CS	397.00	338.00	300.00	262.00	232.00	192.00	140.00
TSS (°Brix)	PCG-LFR-1	12.26	12.57	13.00	13.72	14.31	14.85	16.23
	PCG-LFR-2	12.26	12.60	13.12	13.68	14.45	15.00	16.45
	CS	12.26	13.14	14.40	15.52	15.92	16.68	14.52
TA (% malic acid)	PCG-LFR-1	0.25	0.242	0.232	0.221	0.21	0.196	0.182
	PCG-LFR-2	0.25	0.239	0.229	0.219	0.206	0.192	0.178
	CS	0.25	0.235	0.21	0.172	0.136	0.121	0.108
Ascorbic acid (mg/100ml of juice)	PCG-LFR-1	7.87	7.52	7.10	6.73	6.14	5.53	4.63
	PCG-LFR-2	7.87	7.54	7.00	6.82	5.92	5.38	4.55
	CS	7.87	6.82	5.12	4.27	3.24	2.52	1.69
Starch-Iodine Index	PCG-LFR-1	4.50	4.65	4.72	4.81	5.16	5.37	5.87
	PCG-LFR-2	4.50	4.69	4.83	4.75	5.22	5.32	5.79
	CS	4.50	4.92	5.65	6.19	6.82	7.52	8.29
L*	PCG-LFR-1	40.43	39.74	39.14	38.29	37.45	36.54	35.46
	PCG-LFR-2	40.43	39.60	38.90	38.14	37.23	36.32	35.39
	CS	40.43	39.36	38.24	37.00	35.26	33.75	32.12
a*	PCG-LFR-1	42.18	42.58	43.73	44.36	45.10	45.97	47.30
	PCG-LFR-2	42.18	42.73	43.90	44.50	45.22	46.11	47.42
	CS	42.18	43.96	45.61	47.38	47.92	47.56	46.85
b*	PCG-LFR-1	18.73	18.12	17.85	17.52	16.50	14.74	13.56
	PCG-LFR-2	18.73	17.99	17.63	17.35	16.33	14.72	13.48
	CS	18.73	17.46	15.84	13.78	12.54	12.40	12.26

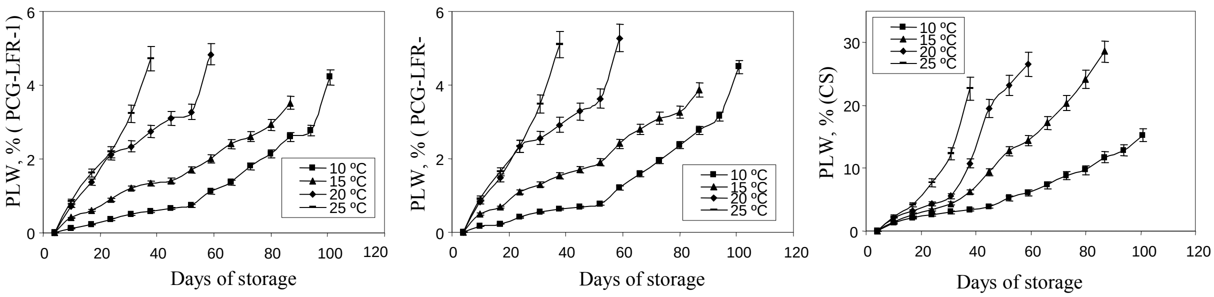

Figure 4. Variation in PLW (%) of MA packaged and control sample at various storage temperature.

During storage, an increase in PLW (%) was observed in all conditions. However, the fruits stored under MAP and at low temperature recorded slower changes. This might be due to the effect of high humidity atmosphere inside the package that reduced the rate of water loss from the fruits and also the films acting as a barrier to moisture loss from fruit surface to the environment. The activities of ripening enzymes (pectin esterase, polygalacturonase and cellulase) were reduced due to modified environment. Also the biochemical metabolic activity involved in different physiological processes was reduced and the carbohydrate utilized in physiological process was also low. The % PLW in unpackaged apples was 3.65-7.11 times higher than that of MA packaged apples at different storage temperatures (Figure 4). At elevated temperature, the reserve carbohydrate utilization increased, and hence the physiological loss in weight was more and so also the reduction in volume. The % reduction in volume of PCG-LFR-1 packed apple was found to be 10 - 14.6 times lower than that of the control at the given temperature ranges. In MAP, apart from the reduction in respiration and heat of respiration, the evaporation of water from the fruit surface is drastically reduced (Arvanitoyannis and Bosnea, 2004).

There was gradual decline in fruit firmness and puncture strength of apple in MA packed as well as control during storage. However, the application of MAP proved more effective in retention of higher fruit firmness of apple than normal (control) storage (Figure 5). The retention of relatively higher fruit firmness under MA condition at lower temperature could be attributed to slower metabolic activities leading to lower substrate utilization, minimum ripening changes, regulation of ethylene biosynthesis and cellular disintegration (Gorny and Kader, 1996; Hardenburg, 1971; Kader, 1986; Hind and Abu-Bakr, 2003; Goswami and Mangaraj, 2011; Mangaraj et al., 2012a). The loss of firmness in fruits is also attributed to the change in pectic substances (Mohsenin, 1980; Mangaraj et al., 2005). The increase of soluble pectins was retarded by lowering storage temperature and increasing CO_2 level (Awad and Jager, 2003). Also CO_2 level was reported to have pronounced effect on hydrolytic changes. In MAP, low O_2 and high CO_2 levels retard cellular disintegration of cellular walls, membrane permeability, senescence as well as hydrolytic changes which in turn help retaining flesh firmness for longer periods (Soliva-Fortuny, 2001; Nicolais et al., 2011a).

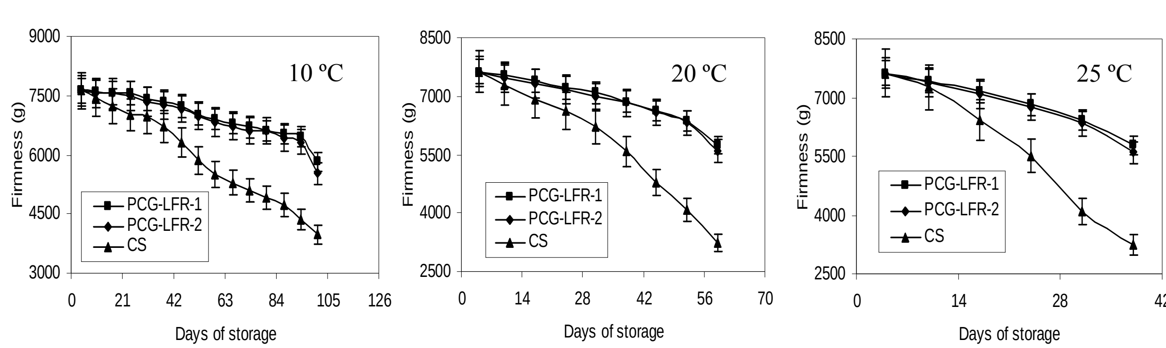

Figure 5. Variation in firmness (g) of MA packaged and unpackaged apple during storage.

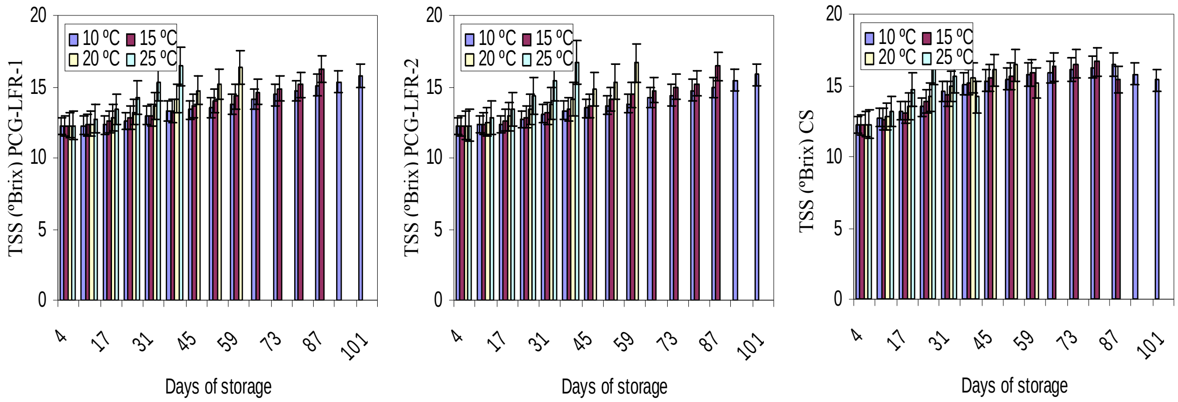

Figure 6. Variation in TSS (ºbrix) of MA packaged and control sample at different storage temperature.

The TSS content of apple was found to have increased with storage period. In case of MA packaged apples, the increase in TSS content was slow and gradual (Figure 6). The increase in TSS during storage is attributed to the numerous catabolic processes taking place in the fruit, preparing it for ripening and senescence. With time, starch gets hydrolyzed into mono and disaccharides (hydrolysis of insoluble polysaccharides in to simple sugar), which in turn may lead to an increase in TSS and sugars. On complete hydrolysis of starch, no further increase in TSS occured and subsequently TSS declined since they are the primary substrate for respiration (Podesedek et al., 2000; Johnston et al., 2001). These findings are further supported by the observation of Lelievre et al. (1997). Titratable acidity (TA) was found to have decreased with storage period. During storage, increased respiration is responsible for the declining of malic acid content, which is the principle metabolic substance together with sugar in apple. It was observed that the rate of decline in TA of MA packaged apple at low temperature was lower as compared to that of control. Similar experimental variations in TA values with storage period have also been reported by Lelievre et al. (1997) and Rocha et al. (2004).

The reduction in ascorbic acids in unpackaged apples was 2 - 2.5 times higher than that of MA packaged apples at different storage temperatures. It is reported that high oxygen level could be responsible for acceleration of ascorbic acid loss through increased oxidation by ascorbate oxidase during storage (Singh and Pal, 2008). The slow decrease of ascorbic acid content also influenced the shelf life of fruit. Ascorbic acid can scavenge radicals by inhibiting initiation and breaking chain propagation or suppressing formation of free radicals by binding to the metal ions, reducing hydrogen peroxide, and quenching superoxide and singlet oxygen. The extent of ascorbic acid loss decreases as oxygen level decreased, and carbon dioxide level increased (Lee and Kader, 2000). MA packaging was found effective in preventing the ascorbic acid losses as evident from the findings.

For most apple cultivars, rather than having a constant level, there is an increase in ethylene during the ripening process (Johnston et al., 2001). This increase in ethylene concentration may allow the different ripening characters to be co-ordinated so that starch conversion always occurs before excessive fruit softening or the production of attractant volatiles. The rate of increase in starch Iodine Index value of apple was slow and steady at lower temperature and under MAP but it was faster in the control fruits at higher temperatures. This indicated that the atmospheric modification retarded the amount of starch formation in the apple flesh (Table 3). The scores between 4 and 6 using 1 to 9 scales for starch-Iodine index were considered as matured but not ripened.

The values of L* and b* decreased, while that of a* increased during storage (Table 3). The royal delicious apple was harvested when 75 - 80% of the yellow skin was covered with red strip. The ripening of apple is governed by the radiation, enzyme activity and pigmentation. During storage, the surface area as well as the intensity of redness increased which is indicated by the increase in a* value (Mangaraj et al., 2006). When the whole surface area was covered with red strips then the apple achieved maximum redness and the apple was considered to have ripened completely. It was observed from the control stored fruits that after achieving total redness, the a* value decreased very slightly and remained constant throughout the storage periods, meaning thereby that the color did not change significantly after ripening. However, other physiological changes in the apple took place as indicated by the change in TSS, acidity, firmness (Table 3). Similar observation but reverse trend was observed for yellowness values. As the surface area for redness increased, the yellowness decreased. After certain period the b* value remained constant and decreased very slightly in control sample. It was observed that the fruits stored under MA packaging maintained better colour than unpacked apple. The retention of relatively higher colour values under MA condition may be due to the lower metabolic activities leading to retarded ethylene biosynthesis, lowering ripening changes and delaying senescence. As storage progressed, the redness increased, however, there was no shifting of colour spectrum during the entire period of study.

The ΔE of apple increased during storage. However, in control sample, it was high at the initial stage then decreased and remained constant (Figure 7). This was due to the facts that after complete redness, the overall colour did not change significantly. The ΔE value of MA packaged apple was lower than the air-stored. This indicates that the rate of total color change of MA packaged was lower than that of the control fruits. The effect of temperature on ΔE with different packaging system shows that rate of change of color is faster and higher at higher temperature during storage (Figure 7). The hue angle, which represents the pure / exact colour of the fruit, gradually decreased with storage time in MA packaged as well as unpackaged apple. The hue angle of fruits stored in air was decreased from 23.94 to 14º during storage at different temperatures reflecting the increase in redness. However, there was no shift of colour from one spectrum to other as the apple was harvested when the color was 75 - 80% red. During storage, only redness increased as is evident from the observations (Table 3). The hue angle of fruits under MAP was lower than that of the control for the same storage periods indicating the preservation of color pigment under modified atmosphere. The increase in chroma value during the storage period, reveals that the brightness increases till the development of full colour (Figure 7). However, in control sample the chroma values decreased after attainment of complete ripening. The color of the MA packaged fruits was comparable with the color at harvesting maturity (Mangaraj and Goswami, 2009d). The MA packaged apple was slightly lighter than that of the control during shelf life extended storage period as reflected in its higher L*, lower a*, higher hue angle, lower chroma and ΔE values. The increase in chroma value of apple indicates the increased in brightness or intensity of red colour during extended storage.

Altogether, Chlorophyllase is considered as the important enzyme in the pathway of chlorophyll degradation of apple. The MA packaging of fruits delayed the increases in the activities of cell wall hydrolytic enzymes and chlorophyllase activity that affects the fruit colour.

The results of the quality attributes of apple (cv. *Royal Delicious*) under MA packaging were in agreement with those found by Rocha et al. (2004), which stated apples (cv. Bravo de Esmolfe) packed in MA showed reduced weight loss, preserved better colour and firmness than fruits stored in air. Prasad (1995) reported that MA packed golden delicious apples retained orchard freshness for longer period of time with increased shelf life. The incidence of bitter pit that developed during storage of New Zealand 'Cox's Orange Pippin' apples were progressively reduced from 50% to less than 5% in MA Packaging (Hewett, 1984). Geeson et al. (1994) found that application of polymeric film lining systems for MA box packaging provided effective, reproducible atmospheres of 7-10% CO_2 and 5-7% O_2, which was beneficial in shelf-life extension of Bramley's Seedling and Cox's Orange Pippin apples, and hence was introduced into the UK fruit market. Guan et al. (2004) reported that Fuji apples in optimal MA package had good quality retention during storage. Dipping treatment and MA affected the respiratory activity of the packed product and preserved better quality of Golden Delicious minimally processed apples (Rocculi et al., 2006). Mondial Gala' apples stored under controlled atmospheres maintained quality parameters better, in addition to the highest titratable acidity and firmness values than the air stored fruits (Echeverria et al., 2008).

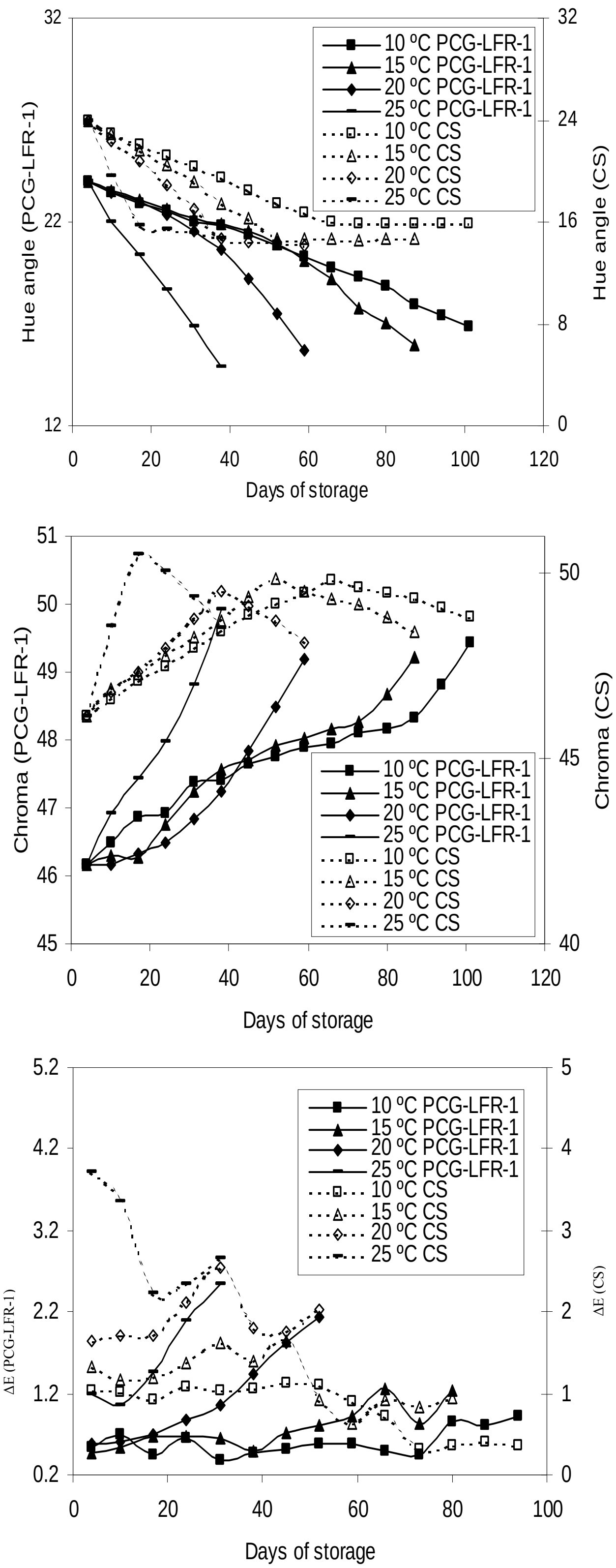

Figure 7. Colour values of MA packaged (PCG-LFR-1) and control stored apple at various temperatures.

ANOVA of quality parameters during storage

Three-factor ANOVA revealed the direct effect as well as two and three factor interaction effects of temperature, storage system and storage periods on quality parameters of apple at 1% level of significance. The results of the three factors ANOVA for some of the quality attributes of apple are given in Table 4.

Second order response surface regression was fitted to the experimental data on different quality parameters of apple. The regression coefficients of all the quality parameters obtained from response surface regression analysis are presented in Tables 5 to 7, which revealed the linear, quadratic and interaction regression coefficients of temperature, days of storage and their interaction are significant at 1% level of significance for all the variables except PLW and Reduction in Volume, for which significance is at 5% level of significance.

Table 4. ANOVA for Quality Parameters of MA Packed and unpacked Apple.

Sources of variation	DF	MSS	F-Value
PLW (%)			
Model	73	32.957783	322.81**
Replication	2	0.0621005	0.61
Days	5	120.3673185	1178.96**
Temp	3	108.4747963	1062.48**
Storage system	2	235.9413671	2310.97**
Days*Temp	15	20.8267859	203.99**
Days*Storage system	10	35.5647721	348.35**
Temp*Storage system	6	23.1515060	226.76**
Days*Temp*Storage system	30	6.6563895	65.20**
Error	142	0.102096	---
Firmness			
Model	73	1795609.2	6878.31**
Replication	2	49.85	0.19
Days	5	7777706.97	29793.5**
Temp	3	10331251.09	39575.2**
Storage system	2	9333356.51	35752.6**
Days*Temp	15	1545338.50	5919.62**
Days*Storage system	10	999983.55	3830.57**
Temp*Storage system	6	772586.87	2959.49**
Days*Temp*Storage system	30	157164.66	602.04**
Error	142	261.1	---
TSS			
Model	73	3.7890336	83.71**
Replication	2	0.0406000	0.01
Days	5	28.5697233	631.21**
Temp	3	17.7102179	391.29**
Storage system	2	11.5424014	255.02**
Days*Temp	15	1.3858149	30.62**
Days*Storage system	10	1.0272697	22.70**
Temp*Storage system	6	0.5719211	12.64**
Days*Temp*Storage system	30	0.7680910	16.97**
Error	142	0.0452615	---

Storage system: MAP and Control ; ** = Significant at 1%

Table 5. Parameters of Response Surface Analysis for PCG-LFR-1 of Apple.

Variables	b0	b1	b2	b11	b22	b12	R2
PLW	1.8326	-0.2468	-0.0486	0.0077	0.0002	0.0050	0.8942
Red.vol	1.7605	-0.2259	-0.0360	0.0061	-0.0002	0.0039	0.7414
Firmness	5923.72	227.8709	19.4673	-6.4944	-0.1266	-2.0641	0.9184
Puncture	246.07	20.7073	2.0234	-0.6187	-0.0129	-0.2047	0.9305
TSS	15.4818	-0.4395	-0.0318	0.0124	0.0002	0.0045	0.9552
Acidity	0.1875	0.0078	0.0007	-0.0002	-0.0001	-0.0001	0.9457
Ascacid	4.9529	0.3866	0.0316	-0.0108	-0.0002	-0.0039	0.9447
Starch II	6.0544	-0.1702	-0.0225	0.0044	0.0001	0.0015	0.8950
L*	43.7376	-0.4416	-0.0119	0.0120	0.0001	0.0042	0.9482
a*	35.1994	0.8604	0.0375	-0.0229	-0.0001	-0.0062	0.9119
b*	26.5583	-0.9739	-0.0947	0.0269	0.0005	0.0079	0.9335
Hue ang	36.8806	-1.6077	-0.1349	0.0440	0.0007	0.0127	0.9274
Chroma	44.0075	0.2666	-0.0150	-0.0067	0.0002	-0.0016	0.9044
ΔE	5.9119	-0.6822	-0.0760	0.0198	0.0003	0.0050	0.8499

Table 6. Parameters of Response Surface Analysis for PCG-LFR-2 of Apple.

Variables	b0	b1	b2	b11	b22	b12	R2
PLW	1.4150	-0.1623	-0.0575	0.0050	0.0003	0.0054	0.8700
Red.vol	1.8966	-0.2436	-0.0394	0.0067	-0.0001	0.0042	0.7567
Firmness	5680.05	254.5104	21.9174	-7.2367	-0.1477	-2.2184	0.9139
Puncture	235.13	22.5502	1.5645	-0.6793	-0.0086	-0.2000	0.9170
TSS	15.6269	-0.4520	-0.0336	0.0127	0.0001	0.0048	0.9521
Acidity	0.1813	0.0085	0.0008	-0.0002	-0.0001	-0.0002	0.9363
Ascacid	4.6152	0.4189	0.0345	-0.0117	-0.0002	-0.0041	0.9372
Starch II	5.8273	-0.1690	-0.0064	0.0049	0.0003	0.0009	0.7844
L*	43.7548	-0.4378	-0.0059	0.0119	-0.0001	0.0043	0.9355
a*	35.3345	0.8264	0.0405	-0.0218	-0.0002	-0.0067	0.9223
b*	27.2901	-1.0551	-0.0775	0.0290	0.0004	0.0078	0.8638
Hue ang	36.7602	-1.5751	-0.1331	0.0430	0.0007	0.0132	0.9252
Chroma	44.2698	0.2328	-0.0228	-0.0057	0.0002	-0.0015	0.8981
ΔE	5.4603	-0.6375	-0.0687	0.0191	0.0003	0.0047	0.8149

Table 7. Parameters of Response Surface Analysis for Control Stored Apple.

Variables	b0	b1	b2	b11	b22	b12	R2
PLW	11.4190	-1.0852	-0.4963	0.0175	0.0020	0.0427	0.9636
Red.vol	1.2372	-0.1095	-0.0987	-0.0005	-0.0002	0.0145	0.8704
Firmness	3736.36	527.9729	21.4651	-15.0395	-0.0801	-4.53420	0.9241
Puncture	196.35	27.4302	-0.9443	-0.7923	0.0083	-0.22830	0.9505
TSS	12.1702	-0.1180	0.1266	0.0050	-0.0008	-0.00020	0.8911
Acidity	0.2336	0.0033	-0.0003	-0.0001	-0.0001	-0.00010	0.9825
Ascacid	2.7140	0.6962	-0.0206	-0.0195	0.0002	-0.00600	0.9673
Starch II	6.8510	-0.2803	-0.0179	0.0069	0.0001	0.00370	0.9745
L*	51.2674	-1.4384	-0.0778	0.0402	0.0004	0.01240	0.8835
a*	31.6256	1.3970	0.0831	-0.0379	-0.0002	-0.01510	0.9425
b*	32.5417	-1.7606	-1.7606	0.0481	0.0010	0.01870	0.9549
Hue ang	46.2025	-2.8679	-0.2410	0.0779	0.0013	0.03110	0.9538
Chroma	47.2560	-0.0301	-0.1050	-0.0001	0.0008	0.00080	0.6689
ΔE	6.8790	-0.8480	-0.0634	0.0275	0.0001	0.00660	0.8801

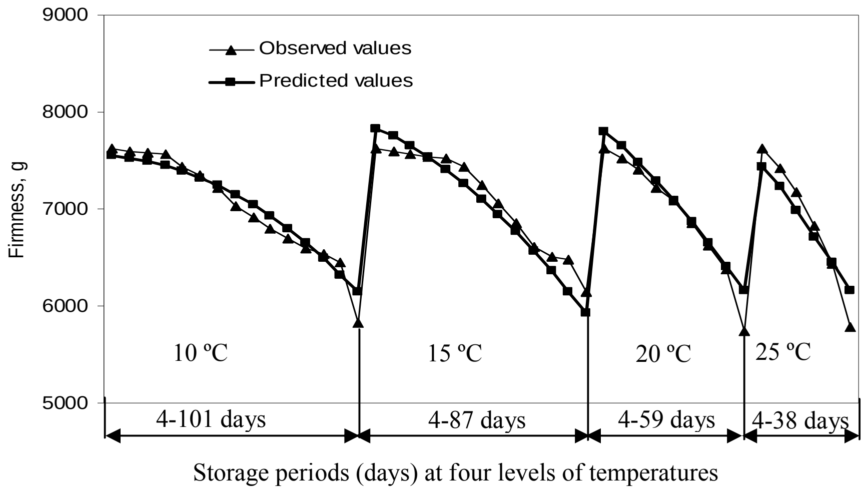

Figure 8. Experimentally observed and predicted values of firmness during different storage periods at various temperatures for apple.

From the regression coefficients (Table 5, 6, and 7) it is inferred that there are lower rates of changes in all the quality attributes of apple in MA packages as compared to the control samples during storage. It also indicates that the effect of temperature is more pronounced than the storage periods for the changes in quality parameters during storage.

The developed regression models were verified for all the quality parameters of fruits. The comparison of experimental and predicted quality parameters yielded the mean relative deviation modulus (E) value of less than 5, which indicated that the developed regression models have good agreements for predicting the quality attributes of MA packaged and unpackaged fruits during storage. The comparison of experimentally determined firmness of apple with predicted values under PCG-LFR-1 package is shown in Figure 8.

Conclusions

The laminated MA packages, developed in this study, were found to have met the GTR requirements of the MAP precisely. Most of the packages achieved equilibrium of O_2 and CO_2 levels, which were fairly close to the target levels. The experimental values of equilibrium levels of O_2 and CO_2 varied between 3.10 – 3.31% and 3.34 – 4.17%, respectively, for various MA packages of apple at different temperatures. Apples packed in MA packages exhibited reduced weight loss, reduced amount of starch formation, maintained good colour and preserved firmness as compared to the fruits stored in normal atmosphere. The shelf-life of fruits under MAP was extended up to 94, 80, 52 and 31 days at 10, 15, 20 and 25ºC storage temperatures, respectively. The MA packaging system increased the shelf life of apple by 125-210% as compared to the unpacked fruits at various storage temperatures with a quality comparable with the freshly harvested commodities. The mean relative deviation moduli between the equilibrium concentrations of O_2 and CO_2 predicted by the model and obtained through experiment were found to be 5.92-8.6% respectively, indicating good agreement with the experimental data. Three- factor ANOVA revealed that the direct as well as two and three factor interaction effects of temperature, storage system and storage periods on quality parameters of apple were significant at 1% level of significance.

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