American Journal of Food Technology

ISSN 1557-4571

Academic
Journals Inc.

www.academicjournals.com

American Journal of Food Technology 1 (2): 129-138, 2006
ISSN 1557-4571

Passive Modified Atmosphere Packaging of Banana (Cv. Cavendish) Using Silicone Membrane

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Abstract: Silicone diffusion was used for the generation of passive modified atmosphere at low temperature to over come the deficiencies associated with LDPE based diffusion. The optimization of MAP variables (fill weight and silicone membrane diffusion area at a constant fill volume and storage temperature) was carried out using response surface methodology in terms of responses (head-space O_2, CO_2 and storage life). The derived quadratic equations were found to support experimental data obtained under the experimental conditions. The target predicted storage life and head-space gas composition could find concurrence with experimental data in support with the optimized variables derived. The optimized variables could be used for the scale up of silicone based passive MAP of banana in rigid containers, suitable for bulk storage, transportation as well as for retail marketing.

Key words: Banana, cavendish, modified atmosphere packaging, silicone membrane

Introduction

Banana is a major tropical fruit and cultivated extensively in India and also in the South-Asian tropics. India has an annual banana production of 16.81 million tonnes out of a total fruit production of 44.5 million tonnes (Indian Horticulture Database, 2002). However, this production is not being utilized for long distance transportation and exports due to lack of appropriate postharvest handling techniques. Passive modified atmosphere of banana in polyethylene wrappers/pouches has shown significant promise over the years. However, anaerobiosis due to excessive CO_2 accumulation (Abdel-Rahman *et al*., 1995) followed by off-flavour development (Kliebber *et al*., 2002) had been the major problem in addition to fogging of films. Use of ethylene scrubber was found to delay de-greening and softening of the fruit (Krishnamurthy and Kusalappa, 1985; Chamara *et al*., 2000).

Over the years the application of silicone membrane as a diffusion surface in MAP applications has gained increased popularity. Marcellin and Leteinturier (1966) used silicone membrane as an effective diffusion surface in an active modified atmosphere system where the initial modification of the gases was done by active technological control and subsequently appropriate atmosphere was maintained by the use of silicone membrane as a diffusion surface. However, the active MAP systems involving continuous use of mechanical devices for gas circulation and ventilation were found extremely cost intensive. Later on, silicone diffusion was reported for controlled atmosphere storage of leeks and celery (Gariepy *et al*., 1985; 1994). In fact, the Marcellin type of active modified atmosphere need to be considered as a controlled atmosphere mode of storage as the system needs initial setting of atmosphere and also continuous monitoring of the system. Reports exist on the use of silicone diffusion in support of passive modified atmosphere storage by means of silicone windows placed on laboratory scale containers for the storage of pre-cooled green asparagus (Gariepy *et al*., 1991). Sudheer *et al*. (1999) reported effective use of silicone membrane system for the passive modified atmosphere storage of strawberry in laboratory scale containers.

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Silicone membranes are made up of fine nylon fabric uniformly coated with silicone rubber with high permeability towards O_2, CO_2 and ethylene with O_2 and CO_2 permeation rate ratio at approximately 1:3 (Prabhanjan *et al*., 1995). A number of silicone membranes are being evaluated for their efficacy in MA applications. However, the reports on the use of silicone for banana storage under passive MAP are scanty and therefore, the present study aims at optimization of silicone membrane based passive MA in PET containers using set experimental variables. The optimization involves variables like fill weight for PET containers with specific volume in addition to the area of silicone based diffusion surface. The experimental variables have been coined keeping in view the retail marketing of banana fruits without escalating the cost of storage. The storage temperature has also been kept in the range practiced for banana storage without causing chilling injury.

Materials and Methods

Fruits

Fully mature banana (Cv. Cavendish) at 3/4 full stage in the form of fully developed fronds were procured from the local market of Mysore, India and brought to Defence Food Research Laboratory, Mysore, India for conducting the experiment. The fronds were cut into hands each having 10-12 fruits. Grading was carried out by selecting the fruits from middle portion of each hand and the fruit mass was in the range of 90-100 g each. Fruits with visible infections and mechanical injuries were also sorted out. The selected hands were subjected to surface sanitation by washing the fruits in chlorinated water (0.01%) followed by a wash in thiabendazole solution (0.02%). The surface moisture of the fruits was removed by placing the fruits in a stream of dehumidified air. The cut ends of the hands were sealed with white sterilized petroleum jelly containing 0.2% potassium sorbate.

Packaging

Modified atmosphere packaging of graded and sanitized fruits was carried out in cylindrical PET jars of 4 L capacity with lids. Each jar was provided with two rectangular silicone windows with specific diffusion area on opposite sides of the cylindrical walls. The experimental design consisted of 14 jars covering specific ranges of fill weight and silicone diffusion area. The experimental block was replicated 6 times and the mean values pertaining to the physico-chemical evaluation of the fruits have been reported. The silicone membrane used in the study was procured from trade in Canada and was evaluated for O_2, CO_2 and C_2H_4 permeation rates using automatic manometric gas permeability tester (Model-L100-5000, LYSSY AG, Switzerland) besides water vapour transmission rate.

Experimental Design

Response surface methodology was used for designing the experiment (Khuri and Cornell, 1987). A Central Composite Rotatable Design (CCRD) was used, taking 2 variables at 5 levels each with 6 centre points. The data pertaining to the coded and uncoded parameter values of the design have been presented in Table 1 and 2 under which 6 replications at the centre point and 2 at the other points were carried out. The range of parameter values was based on earlier studies on banana (Chauhan *et al*., 2004) and strawberry (Sudheer *et al*., 1999). The fruits were kept in PET jars and stored in a BOD incubator set at 13±1°C and 85-90% RH. Three response variable i.e., O_2, CO_2 at equilibrium and storage life were considered and optimized on the basis of maximum extension in storage life.

Head-space O_2 and CO_2

Head-space O_2 and CO_2 concentrations were analysed at a time interval of 12 h using an O_2/CO_2 head-space analyzer (PBI Dansensor, Checkmate 1900, Prg. Version 1.7, Ringsted, Denmark), Teflon septa and auto-injector. The instrumental analysis was based on solid state battery type zirconia sensor for O_2 and a self contained non-dispersive infrared sensor for CO_2. A minimum headspace gas volume of 5 mL was maintained for all the analysis.

Physico-chemical Evaluation of Fruits

Physico-chemical analyses of the fruits were carried out initially and at the end of storage life (after ripening). Respiration and ethylene synthesis rates were monitored using gas chromatographic method described by Hakim *et al.* (2004) and Gailard and Grey (1969), respectively. Ascorbic acid and titratable acidity were estimated by standard methods (AOAC, 1990), pH and soluble solids were measured by using pH meter (Century, Model CP 931, Century Instruments Private Limited, Chandigarh, India) and hand refractometer (Erma, Tokyo, Japan), respectively. Firmness of banana was measured at the geometric centre of the fruit, using texture analyzer (TAHDI, Stable Micro System, London, UK) equipped with 2 mm diameter cylindrical rod. The colour values in terms of L, a, b were recorded with a tristimulus colorimeter (Model 2810; Datalab India Pvt. Ltd., Silvasa, Gujrat, Inida) using D-65 illuminating conditions and 2° observer.

Sensory Evaluation and Storage Life

Storage life of the modified atmosphere stored banana fruits was determined on the basis of sensory evaluation carried out using 9-point hedonic scale when the fruits were at their maximum sensory acceptability after exposure to ethrel (0.01%) induced ripening at 30°C for a period of two days.

Optimization

The data obtained for equilibrated O_2, CO_2 and storage life under different experimental conditions were analyzed using multiple regression techniques. Response surface model was developed to represent the data and to determine the optimal conditions for various parameters, to obtain maximum storage life.

Colour Plotting

One of techniques used to visualize the response surface was to plot contours of the response surface equation. In a contour plot, lines or curves of constant response values are drawn on a graph or plane whose coordinate axes represent the levels of independent variables and the response is visualized perpendicular to the plane of paper. Series of contour plots of equal response were generated which provided useful information to interpret the contribution of independent response variables.

Results and Discussion

Modified atmosphere storage of banana has received significant attention over the years. Acedo and Bantista (1993) gave comprehensive account on the role of ethylene during banana ripening and indicated that the physiologically active concentrations of ethylene could lead to ethylene mediated symptoms besides causing profound influence on the metabolism of the fruit during storage. A number of reports also exist for the chemical regulation of ripening in case of bananas (Krishnamurthy and Kushalappa, 1985) as well as mangoes (Krishnamurthy and Joshi, 1989). Despite the information available on the chemical and hormonal regulation of banana ripening along with extensive reports on the modified atmosphere storage (Satyam *et al.*, 1992) attempts are desired at scaled up field level storage of the produce due to the limitations of LDPE mentioned earlier. Silicone membranes as a diffusion surface hold promise due to the low barrier properties and ideal permeation rates for O_2, CO_2 and ethylene which were found to be 49,500 mL/day/m^2/atm for oxygen, 24,8000 mL/day/m^2/atm for carbon dioxide and 11,2000 mL/day/m^2/atm for ethylene; the selected silicone membrane could permeate O_2 and CO_2 in the ratio of approximately 1:5. The physico-chemical characteristics of banana fruits used for the experiment have been given in Table 1.

Table 1: Physico-chemical characteristics of banana (n = 6)

Parameters	Raw*	Ripened*
Firmness (kg)	1.437±0.143	0.367±0.041
Ascorbic acid (mg/100 g)	14.28±0.33	11.12±0.26
pH	4.35±0.04	4.42±0.02
Pulp : Peel	1.38±0.03	1.54±0.04
Brix : Acid	25.91±0.08	136.12±1.06
Respiration rate (mg CO_2/kg/h)	28.65±2.44	96.28±3.85
Ethylene synthesis rate (μL/kg/h)	6.34±1.20	17.62±2.11
L-value	48.22±1.28	62.78±2.64
a-value	-8.964±0.316	8.378±0.250
b-value	21.843±0.167	25.747±0.154

*Mean±SD

Table 2: Coded and uncoded parameter levels

	Coded variables				
Parameters	+1.414	+1	0	-1	-1.414
Fill weight, g (X_1)	1155.33	1000.00	625.00	250.00	94.67
Silicone diffusion area, cm^2 (X_2)	11.66	10.00	6.00	2.00	0.34

Table 3: Effect of fill weight and silicone diffusion area on equilibrated head-space O_2, CO_2 concentrations and storage life (n = 6)

Coded variables		Uncoded variables				
X_1	X_2	Fill weight (g)	Diffusion area (cm^2)	O_2 (%)	CO_2 (%)	Storage life (Days)
-1	-1	250.00	2..00	14.1	1.8	18
0	0	625.00	6.00	6.0	9.0	22
0	0	625.00	6.00	6.0	9.0	22
0	0	625.00	6.00	6.0	9.1	22
0	-1.414	625.00	0.34	3.1	8.2	21
-1	1	250.00	10.00	16.8	2.3	15
1	1	1000.00	10.00	4.9	6.3	26
0	0	625.00	6.00	6.1	9.0	22
0	1.414	625.00	11.66	10.5	5.6	18
0	0	625.00	6.00	6.1	9.0	22
0	0	625.00	6.00	6.0	9.0	22
-1.414	0	94.67	6.00	17.9	1.1	14
1	-1	1000.00	2.00	2.2	12.1	23
1.414	0	1155.33	6.00	4.1	6.2	28

Optimization of Modified Atmosphere Storage

Optimization of storage variables is an important aspect of modified atmosphere storage. The optimization involved variables such as silicone membrane diffusion area and fill weight at constant fill volume and the responses studied were O_2 and CO_2 head-space concentrations and storage life at each permeation. The different levels of variables (i.e., fill weight and silicone diffusion area) and the equilibriated O_2, CO_2 and storage life at specific temperatures obtained under variable experimental conditions at a constant fill volume have been given in Table 2 and 3.

Equilibriated O_2

Head-space O_2 plays an important role in restricting the metabolic rate during fruit ripening and at the same time, the O_2 levels within the modified atmosphere containers need to be maintained above a threshold level to avoid anaerobiosis. As such, the O_2 levels within the PET jars with silicone windows ranged from 2.2 to 17.9% depending on the diffusion area and fill weight. The minimum and maximum values were obtained at specific experimental conditions of 1000 g fill weight, 2 cm^2 diffusion area in case of the minimum values, while the maximum values were recorded at 94.67 g fill weight and

Table 4: Analysis of variance of response variables

Source	df	Sum of squares		
		O_2	CO_2	Storage life
Model	5	318.02****	132.02****	182.75****
Total individual effect				
Fill weight (g)	1	234.54****	57.85****	160.20****
Diffusion area (cm^2)	1	31.46***	10.07**	2.25*
Combined effect of both variables				
Linear	2	266.00****	67.92**	162.42****
Quadratic	2	52.02****	54.18***	11.30**
Interactive	1	2.36*	9.92	9.00*
R^2 (%)		98.08	95.06	97.76

****Significant ($p \leq 0.0001$), ***Significant ($p \leq 0.001$), **Significant ($p \leq 0.005$), *Significant ($p \leq 0.05$)

Table 5: Coefficients of regression for dependent variables

Term	O_2, (%)	CO_2, (%)	Storage life, (Days)
Constant	6.03****	9.02****	22.00****
X_1	-5.41****	2.69****	4.47****
X_2	1.98****	-1.12***	-0.53
X_1^2	2.63****	-2.60****	-0.44
X_2^2	0.53	-0.97**	-1.19***
$X_1 X_2$	0.26	-1.57***	1.50**

****Significant ($p \leq 0.0001$), ***Significant ($p \leq 0.005$), **Significant ($p \leq 0.05$)

6 cm^2 diffusion area (Table 3). The data on the equilibriated O_2 level were analyzed employing the multiple regression technique to develop the response surface model (Table 4). Linear and quadratic models with and without interactions were tested for their adequacies to describe the response surface using t-test and R^2 values as test criteria (Khuri and Cornell, 1987). A quadratic model was found adequate to describe the variation in responses. The response function so developed was:

$$Y = 6.03 - 5.41X_1 + 1.98X_2 + 2.63X_1^2 + 0.53X_2^2 + 0.26X_1X_2 \qquad (1)$$

The sign and magnitude of coefficients indicated the effect of variables as the responses. Negative sign of the coefficients described decrease in response when the level of a variable increased, while positive sign indicated an increase (Table 5). Significant interactions suggested that the level of the one of the interaction variable could be increased while that of the other had decreased at constant value of the response. The F value and probabilities highlighted the dependent variables as most significant ($p \leq 0.001$), however the interaction term was found to be non-significant. The coefficient of determination of the regression model for equilibriated O_2 as a function of fill weight and silicone diffusion was more than 98.08% indicating the validity of the regression model (Fig. 1).

Equilibriated CO_2

CO_2 as an output of aerobic respiration attained equilibrium after specific time depending on variables i.e., fill weight, diffusion surface, features involving permeability as well as surface area and storage temperature. Fill volume also played an influencing role. As in the case of O_2, CO_2 equilibriation also has a crucial role in the optimization of any MAP system. As such, the period

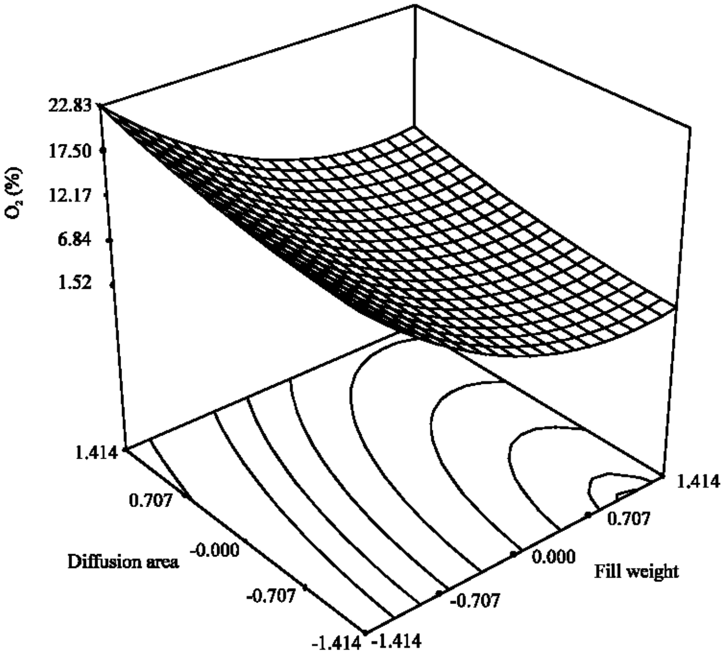

Fig. 1: Head-space O_2 concentration as a function of fill weight and silicone diffusion area

required for CO_2 equilibriation with silicone diffusion under various experimental variables was found to be in the range of 2-3 days under a storage temperature of 13±1°C. The coefficients of regression model and t-ratios have been shown in Table 4. The R^2 value for the regression model as a function of fill weight and diffusion area was more than 95.06% indicating the validity of regression model. The response function obtained was as follows:

$$Y = 9.02 + 2.69\ X_1 - 1.12\ X_2 - 2.60\ X_1^2 - 0.97X_2^2 - 1.57\ X_1X_2 \qquad (2)$$

Both the variables i.e., fill weight and diffusion area showed significant effect. The effect of fill weight was found to be more pronounced on CO_2 equilibriation than diffusion area as in the case of head-space O_2 profiles (Table 5). The regression coefficients showed a positive relationship between fill weight and equilibrium CO_2 concentration while other terms had a negative correlation (Fig. 2).

The modified atmosphere storage of fresh fruits has been extensively reported based on mathematical models and the predictions for O_2 and CO_2 profiles and found to have a higher extent of validity in several fruits i.e., blue berries (Song *et al.*, 2002) and tomato (Charles *et al.*, 2003). However, in the case of banana the literature is scarce on the mathematical modeling of MAP while there are no reports in the case of silicone based diffusion. Optimization of MAP variables using response surface methodology for different varieties using silicone membrane diffusion based passive MAP has certain intrinsic advantages i.e., the process drawn from the output results in variable optimization rather than mere testing of experimental results with a hypothesis for validation. Therefore, the optimized variables could be used as authentic means for field applications.

Storage Life

Storage life of fresh fruits with specific reference to highly perishable ones such as banana is as such a function of different variables under any modified atmosphere storage system. The storage life

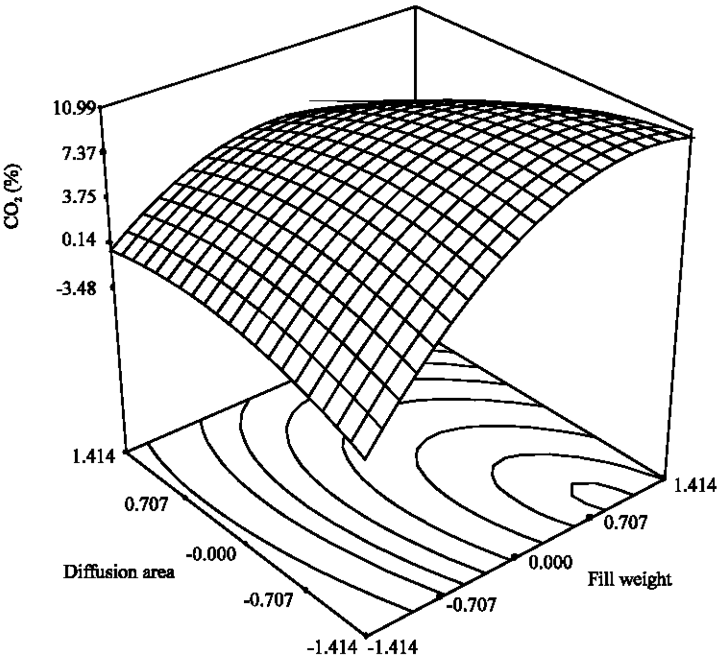

Fig. 2: Head-space CO_2 concentration as a function of fill weight and silicone diffusion area

is a unified expression of physico-chemical and sensory characteristics of a ripening fruit and derivation of the same considering different and specific variables described earlier can highlight the overall performance of the passive MAP using silicone membrane diffusion. The response function developed on the basis of t-test and R^2 values is as follows:

$$Y = 22.00 + 4.47 X_1 - 0.53 X_2 - 0.44 X_1^2 - 1.19 X_2^2 + 1.50 X_1X_2 \quad (3)$$

The overall storage life obtained during the passive MAP was found to be 14-28 days at 13±1°C. The t-ratio and probability of significance of coefficient of variables and interaction terms are given in Table 4 and 5. The R^2 value for the regression model as a function of fill weight and diffusion area was more than 97.76% indicating the validity of the regression model. The data as such showed effects of fill weight and effect of diffusion area on the overall shelf life of banana. Fill weight contour plot (Fig. 3) showed that up to a certain threshold fill weight, the selected fill volume could progressively increase the storage life beyond which due to an excessive fill weight a negative trend in terms of fall in storage life was observed, apparently due to setting of anaerobiosis and excessive condensation of moisture. In case of diffusion area beyond the threshold area, a fall in the storage life of the stored fruits was observed. It is imperative that the optimal O_2 and CO_2 gas compositions are attained within the head-space under a certain level of diffusion area and fill weight under standard fill volume and temperature conditions. Too low a diffusion area has also been found to be problematic causing anaerobiosis and discoloration of the fruit.

Based on the above optimization, the following variable conditions were obtained for a target storage life of beyond 28 days with head-space O_2 and CO_2 concentrations at equilibrium in the range of 3-6% under a fill volume of 4 L at a storage temperature of 13±1°C.

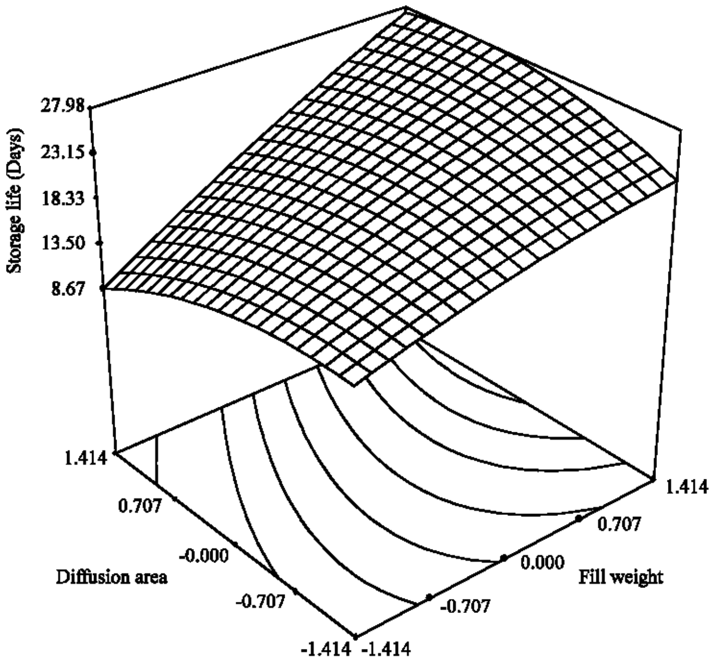

Fig. 3: Storage life as function of fill weight and silicone diffusion area

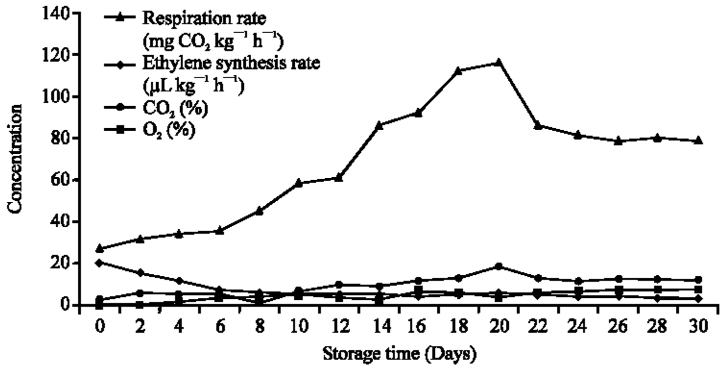

Fig. 4: Changes in head-space gaseous composition and respiration rate during storage of banana in optimized conditions at 13±1°C

Fill weight : 990 g
Silicone diffusion area : 6.14 cm^2
Equilibriated O_2 : 4.39%
Equilibriated CO_2 : 5.99%
Storage life : 29.98 days
Composite desirability : 85.5%

The predicted value of storage life under optimized conditions was about 30 days. The observations were experimentally verified and supported by actual laboratory data and storage life was found to be 28-29 days under MAP at 13±1°C.

Respiratory Activity and Ethylene Generations Under Optimized MAP Conditions

The results of respiratory activity and ethylene synthesis (Fig. 4) showed a typical climacteric pattern highlighting normal physiology of ripening of fruits without symptoms of excessive low O_2 stress or high CO_2 induced peel spotting, a common problem encountered in conventional passive MAP using low density polyethylene (Abdel-Rahman *et al*., 1995). The equilibriated O_2 and CO_2 levels were found to be in the range of 4-6% and as such the sensory quality was found to be in the range of 7.5 to 8.0 in terms of overall sensory scores.

Silicone diffusion can be used not only for providing diffusion windows for PET jars but also for suitably designed transport containers and bulk means of storage. The technique is inexpensive offering high potential for field applications to obtain high quality banana fruits using passive MAP techniques.

Conclusions

The optimization of silicone diffusion based modified atmosphere storage system in PET jars could lead to the derivation in responses i.e., head-space O_2, CO_2 and storage life using different variables i.e., fill weight and silicone diffusion area at constant fill volume and storage temperature. The derived quadratic equations were found to support the experimental data obtained under laboratory conditions. The validity observed was found to be significant ($p \leq 0.0001$) suggesting the efficacy of the derivation of responses using response surface methodology. The target predicted storage life and head-space gas composition concurred the experimental data supporting the overall dependability on the variable patterns derived. Since, the conventional LDPE based passive MAP for banana continues to show undesirable tendencies i.e., peel spotting, fogging of film and anaerobiosis, passive MAP with silicone diffusion can be used for field applications.

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