

Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com



E-ISSN: 2278-4136 **P-ISSN:** 2349-8234 JPP 2019; SP2: 567-571

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Passive modified atmospheric packaging of minimally processed bitter gourd

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Abstract

The consumer demands for fresh-like quality and convenience have given rise to minimally processing. Hence a study was taken up to increase the shelf life of fresh cut bitter gourd by passive modified atmospheric packaging. The fresh-cut was packaged using LDPE pouches with a thickness of 100 μ due to their less permeability to oxygen and stored at $30\pm2^{\circ}$ C and $8\pm2^{\circ}$ C temperature. The respiration rate was decreased to 30 % by storing at lower temperature. The changes in the quality parameters such as physiological loss in weight (4.2 %), colour value 'a' (-7.56), chlorophyll content (17.46 mg/100g), ascorbic acid (63.88 mg/100g) and titratable acidity (0.78 %) was observed at $8\pm2^{\circ}$ C. The shelf life of fresh-cut bitter gourd by passive modified atmospheric packaging was extended up to 12 days at $8\pm2^{\circ}$ C based on their acceptable limit of bacterial (4.56 × 10⁵ cfu/g) and fungal (3.28 × 10³) cfu/g growth.

Keywords: Passive modified atmospheric packaging, bitter gourd, minimal processing

1. Introduction

Bitter gourd (*Momordicacharantia*) is a member of *cucubitaceae* family and it is one of the most popular vegetables cultivated in China, Taiwan, Pakistan, India, and Philippines for their immature fruits and sometimes for the tender leafy shoots (Yamaguchi, 1983) ^[21]. The immature fruits, called bitter melon, bitter gourd or balsam pear are harvested at developmental stages up to seed hardening. Bitter gourd has important role as a source of carbohydrate, proteins, vitamins, minerals and other nutrients in human diet (Ali *et al.*, 2008) ^[3] which are necessary to maintain proper health. The beneficial health effects of bitter gourd have been attributed due to the presence of antioxidants that act as receptors of free radicals. Ascorbic acid and β carotene are the antioxidants present in bitter gourd at higher concentration. Apart from its antioxidant property, it also has many medicinal applications and is used as hypoglycemic agents for diabetic patients (Cefalu *et al.*, 2008) ^[5] and it is also beneficial against piles, blood and respiratory disorders and cholera.

Changes in lifestyle patterns has leads to increased demand for cut vegetables as the people do not have time to prepare vegetables at home as well as in hotels. Because of these factors consumption of minimally processed products has significantly increased (Allende *et al.*, 2006)^[1]. As a result, the market demands for 'fresh-cut' vegetables have increased rapidly (Day, 2001)^[7].

Though many vegetables are part of our dietary habit, the technology of ready to eat or ready to cook form of minimal processing is available only for few vegetables. Thus it is necessary to develop techniques to preserve bitter gourd. Keeping in view the above prospective, the present investigation on preservation of fresh-cut bitter gourd has been taken with the following objective to conduct studies for enhancing the shelf life of fresh-cut bitter gourd.

2. Materials and methods

1. Passive modified atmospheric packaging of fresh cut bitter gourd

The CO 1 variety of bitter gourd procured from the university orchard of Tamilnadu Agricultural University, Coimbatore. The thicknesses of the packaging materials (LDPE and PP) were measured using thickness meter. The oxygen and carbon dioxide transmission rates were found with the help of Manometric permeability tester (M/s. PBI Dansensor, USA).

The bitter gourd was prepared by slicing two distal parts off with sharp sterile stainless steel knives, removing the inner part and seed cavity and sliced along the longitudinal axis into 1cm thick cubes. Sodium hypochlorite (100ppm) was used for pre-treatment because it acts as a surface disinfectant which reduces the microbial growth in fresh-cut bitter gourd. Treated samples were shade dried for 15 min to remove the surface moisture and approximately 100g of slices were packaged in packaging material. The test samples were stored under ambient (30 ± 2 °C) and refrigerated condition (8 ± 2 °C).

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Institute of Agriculture, Tamil Nadu Agricultural University, Kumulur, Tiruchirappalli, Tamil Nadu, India The pre-treated bitter gourds of known quantity were kept in the container for the measurement of respiration rate. The containers were closed and the lid was made air tight by wrapping with plastic tape and stored at ambient and refrigerated conditions (temperature, humidity). The gas samples were drawn at an interval of one hour through septum using needle and gas concentration was found out using gas analyzer (Make: PBI Dansensor Model: Checkmate II). The respiration rate with respect to oxygen and carbon dioxide were calculated using the gas composition. 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).

$$Ro_{2} = \frac{y^{ti}o_{2} - y^{tf}o_{2} \times V}{100 \times M \times (t_{f} - t_{i})} \qquad (1)$$

$$Rco_{2} = \frac{y^{ti}co_{2} - y^{ti}co_{2} \times V}{100 \times M \times (t_{f} - t_{i})} \qquad (2)$$

Where Ro₂- Respiration rate in terms of O₂ evolved respectively, m³/kg/h, Rco₂-Respiration rate, in terms of CO₂ evolved respectively, m³/kg/h, V - Free volume inside the container, yo₂^{ti}- Volumetric concentration of O₂ at initial time (%), yco₂^t -Volumetric concentration of CO₂ at final time (%), M - Mass of the stored product kg, t_i and t_f- initial and final time respectively, h.

2. Physico-Chemical and Microbiological characteristics of Vegetables

2. 1. Physiological loss in weight (PLW)

Fresh-cut vegetables were weighed with the help of an electronic balance (Make: Avery; Model: OC-51) at regular intervals. The initial and final weights of the leaves were recorded and the percentage loss in weight was calculated. (Mathad, 2003)^[11].

Physiological loss in weight (%) =	Initial Weight – Final Weight	100 (2)
	Initial weight	× 100 (3)

2.2. Colour

Hunter lab colour flex meter (Make: Hunter Lab, Model: $45^{\circ}/0^{\circ}$) was used for the measurement of colour. It works on the principle of collecting the light and measures energy from the sample reflected across the entire visible spectrum. The meter uses filters and the mathematical models called Hunter model which rely on "standard observer curves" that define the amount of red, green, and blue (primary lights) required to match a series of colours across the visible spectrum. All the three standard CIE colour parameters 'L', 'a', 'b' was observed for daylight colour.

2.3. Chlorophyll

The total chlorophyll content was determined using a spectrophotometer assay with a (Ranganna, 1995)^[15]. The chlorophyll content in the fresh-cut bitter gourd at different atmosphere and at storage periods were determined by using 80 per cent acetone. The absorbance of the solution at 645 and 663nm against the solvent (80% acetone) blank was recorded. The amount of chlorophyll present in the extract mg chlorophyll per 100mg tissue using the following equation:

Chlorophyll $(mg/100g) = 20.2 (A645) + 8.02 (A663) \times V/100 \times W$ ------(4)

Where A = absorbance at specific wavelengths, (nm), V = final volume of the chlorophyll extract in 80% acetone and W = fresh weight of sample. (g)

2.4. Ascorbic acid content

Ascorbic acid content were determined by visual titration using 2,6-dichlorophenol- indophenol (Ranganna, 1995)^[15]. Ascorbic acid in mg per 100ml of vegetable was calculated.

Ascorbic acid $(mg/100g) = 0.5/V1 \times V2/5ml \times 50ml/$ wt of sample $\times 100$ ------(5)

2.5. Titrable acidity

Titrable Acidity was determined by titrating a known volume of vegetable juice with 0.1N NaOH to an end point of permanent pale pink colour using phenolphthalein as indicator. The NaOH required neutralizing the juice; the titrable acidity was calculated and expressed as per cent citric acid.

Titrable Acidity =
$$\frac{N \times V \times \text{Equivalent weight of acid} \times 100}{\text{Weight of the sample taken } \times 100}$$
 ------(6)

2.6. Microbial Analysis

The qualities of fresh-cut vegetables are based on the number and kind of microorganisms present, which was assessed by standard plate count method (Allen, 1953)^[2]. Commonly used media for the enumeration of bacteria and fungi are nutrient agar medium and Martin's Rose Bengal Agar medium.

2.7. Sensory Evaluation

Sensory evaluation of the vegetables was done as per the procedure outlined by Mangaraj *et al.* (2009)^[10] by the panel of semi-trained judges for appearance, color, flavor, texture, taste and overall acceptability using 9-point Hedonic scale varying from like extremely (rated as 9) to dislike extremely (rated as 1).

2.8. Statistical Analysis

Statistical analysis was carried out to study the effect of different parameters on all the dependent variables. Analysis of variance (ANOVA) was conducted with Factorial Completely Randomized block Design (FCRD) using the statistical software AGRES.

3. Results and discussion

1. Passive Modified atmospheric packaging of fresh-cut vegetables

The permeability of low density poly ethylene (LDPE) and poly propylene (PP) packaging materials of different thickness was assessed and is presented in the figure. 1. From the figure it was observed that, the permeability decreased with thickness of the packaging film for both O₂ and CO₂. The permeability of O₂ in the films leads to more respiration and deteriorate the quality of the product. Hence to maintain the quality of the product, the film LDPE-100 μ with low permeability to O₂ and CO₂ were selected for further study. Ati and Hotchkiss, (2002)^[4] reported that LDPE and PP films with a thickness of 25 to 100 μ are most commonly used for storage of minimally processed vegetables.

During respiration, the fresh-cut bitter gourd consumes O_2 and produces CO_2 a result of metabolic activity. Meyer *et al.* (1973) reported that the plant materials during respiration takes oxygen and break the organic reserves to simpler molecules of CO_2 and water with release of energy. The

respiration rate of pre-treated fresh-cut bitter gourd stored at ambient and at refrigerated condition was determined experimentally in a closed and permeable system.

The rate of respiration with respect to O_2 and CO_2 samples stored under ambient condition was ranging between 87 -41.72 ml/ kg h and 40.6 - 29 ml/kg h respectively Figure 1. The reduction in respiration rate may be due to the depletion of O_2 inside the container as time progresses. At refrigerated condition, the respiration rate with respect to both O_2 and CO_2 have come down by 30 per cent and it was decreasing as the storage period progressed. Smyth *et al.* (1998) ^[17] also reported a rapid decrease of respiration rate over time for cut iceberg lettuce at 5°C.

In ambient condition $(30\pm2^{\circ}C)$, it was more $(RO_2: 41.7 \text{ ml/kg})$ h and RCO₂: 29 ml/kg) and the samples got spoiled in 36 h itself. Igbal et al. (2009)^[8] also reported similar results for shredded carrot that refrigeration had a significant impact on respiration rate by decreasing the temperature from 20°C to 0°C. From the results it is concluded that the fresh-cut bitter gourd stored at refrigeration condition showed lower RO2 and RCO₂. The lower respiration rates of O₂ will increase the shelf life of the fresh-cut bitter gourd by restricting the metabolic activity at refrigerated condition. The statistical analysis on the respiration rate of O₂ and CO₂ at ambient and refrigeration condition under closed system showed that the treatments did not significantly influence the respiration rate. The storage period, temperature had a significant effect on RO₂ and RCO₂ $(p \le 0.01)$ and their interactions significantly influences the respiration rate.

2. Physico-chemical characteristics for MAP of fresh-cut bitter gourd.

The physico-chemical analysis of fresh-cut bitter gourd was carried out for physiological loss in weight, colour, chlorophyll, ascorbic acid, titratable acidity, bacterial and fungal growth.

The physiological loss in weight increased with storage period. The respiration and the transpiration of water from the product attributed to PLW of the samples (Wills *et al.*, 1989) ^[20]. The Ambient stored samples showed maximum loss of 5.2 per cent at 3 rd of storage. The minimum loss of 4.2 per cent was observed in LDPE films with 12 days of storage at $8\pm2^{\circ}$ C. Kudachikar *et al.* (2011) ^[9] reported that for robusta banana packaged in LDPE pouches showed lesser PLW due to low water vapor transmission rate. The treatments did not show significant difference on PLW at both ambient and refrigerated conditions. However the factors, the storage period and packaging material individually had a significant effect on the PLW (p≤ 0.01) and their interactions were also found to be significant.

The more negative value of 'a' indicates the green colour. The minimum change was observed in LDPE packaged bitter gourd at 12 days of storage. Sothornvit and Kiatchanapaibul (2009)^[18] reported that colour change of fresh-cut asparagus dependent on storage period. The treatments did not significantly influence the 'a' value at both ambient and refrigerated conditions. The factors, the storage period and packaging material individually had a significant effect on the 'a' value ($p \le 0.01$) and their interactions were also found to be significant.

The initial chlorophyll content of the fresh-cut bitter gourd was 19.56 mg/100g. At ambient condition, the control sample (T1) had maximum loss of chlorophyll content (12.21mg/100g) in 3 days of storage. The loss of chlorophyll content in LDPE pouches were 23.6 per cent under passive MAP. The chlorophyll content decreased with increase of storage period. Roura *et al.* (2000)^[16] reported that processing induced the decrease of chlorophyll content during storage in swiss chard leaves. The treatments did not show significant difference on the chlorophyll content. However the factors, the storage period, packaging material, packaging atmosphere individually had a significant effect on the chlorophyll content ($p \le 0.01$) and their interactions were also found to be significant.

The minimum loss of ascorbic acid was seen in T9 (63.52 mg/100g) in 12 days of storage compared to other treatment and the maximum loss was observed in control (52.34 mg/100g) after 3 days of storage. The ascorbic acid content of fresh-cut bitter gourd under MAP decreased with storage period. This result is in agreement with Wang *et al.* (2007)^[19] for minimally processed bitter gourd where there was a decrease in ascorbic acid content (58 %) over storage under refrigerated condition (10°C). The statistical analysis showed that the treatments are not significant. The storage period and packaging material individually had a significant effect on ascorbic acid (P≤0.01) and their interactions also had a significant effect.

The titrable acidity increased with storage period. The titrable acidity of fresh-cut bitter gourd in LDPE increased under passive MAP by 11.7 per cent at the end of 12 days of storage Piga *et al.* (2003)^[14] also reported increase of titrable acidity (29 %) during the storage of fresh-cut bitter gourd under refrigerated condition (10°C) for 7 days. From the statistical analysis it was observed that the treatments did not significantly influence the titrable acidity at both ambient and refrigerated conditions. However the factors, the storage period, packaging material individually had a significant impact on the titrable acidity (p ≤ 0.01) and their interactions were also found to be significant (p ≤ 0.01).

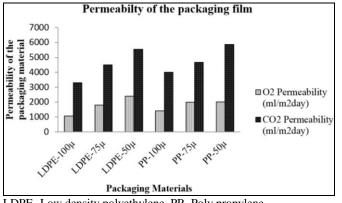
The initial load of bacteria and fungi seen in fresh-cut bitter gourd was 3.02×10^5 cfu/g and 2.56×10^3 cfu/g respectively. Nguyen and Carlin (1994) also reported that bacterial (10³-10⁶ cfu/g) and fungi counts (10^3 - $10^4cfu/g$) were seen in fresh-cut vegetables. The less bacterial $(4.56 \times 10^5 \text{cfu/g})$ and fungi count $(3.28 \times 10^3 \text{cfu/g})$ were found in LDPE packages at refrigerated condition at 12 days of storage. Corbo et al. (2004)^[6] reported that for cactus pear fruit, lower storage temperature significantly reduced the microbial growth. From the statistical analysis, the treatments did not show significant difference on the bacterial and fungal growth at both ambient and refrigerated conditions. The storage period, packaging material and packaging atmosphere individually had a significant effect on the bacterial and fungal growth ($p \le 0.01$). Their interactions were also significant with bacterial and fungal growth.

The shelf life of the product is mainly depending on the microbial and sensory quality. Based on the results obtained for the microbial analysis, the samples packed in LDPE film was found to be within the limits till 12 days of storage.

Table 1: Effect of Storage Period on Physio Chemical and Microbiological Parameters for Passive Map of Fresh Cut Bitter Gourd.

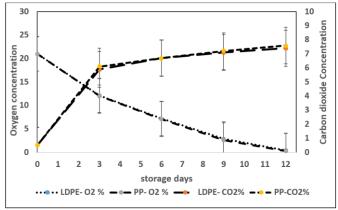
Parameters		Ambient Storage (30±2°C) Storage period (days)		Refrigerated storage (8±2°C) Storage period (days)			
	Initial						
		3	6	3	6	9	12
Physiological loss in weight (%)	0	5.2	*	1.1	2.4	3.6	4.2
Colour value a	-7.82	-6.88	*	-7.76	-7.7	-7.62	-7.56
Chlorophyll content (mg/100 g)	19.56	12.21	*	18.82	18.36	17.81	17.46
Ascorbic acid (mg/100 g)	77.17	52.34	*	70.04	68.7	66.26	63.88
Titrable Acidity %	0.88	0.76	*	0.85	0.83	0.8	0.78
Bacteria (10 ⁵ cfu/g)	3.02	8.12	*	3.59	3.86	4.2	4.56
Fungi (10 ³ cfu/g)	2.56	5.89	*	2.87	2.97	3.18	3.28

Samples got spoiled



LDPE- Low density polyethylene, PP- Poly propylene

Fig 1: Permeability of the Packaging Film



LDPE- Low density polyethylene packaged sample, PP- Poly propylene sample

O2%- Oxygen concentration, CO2%- Carbon dioxide concentration

Fig 2: Head Space Gas Analysis of fresh-cut bitter gourd in Passive MAP condition

4. Conclusion

The polythene films (LDPE) with a thickness of 100u were found to have lesser permeability to oxygen and carbon dioxide. The temperature was the most important factor influencing the respiratory activity and the incidence of weight loss in fresh-cut bitter gourd. Biological reactions increase with rise in temperature. The MAP studies showed that under refrigerated condition was having a minimum values in the physiological loss in weight (4.2 %), colour value 'a' (-7.56), chlorophyll content (17.46 mg/100g), ascorbic acid (63.88 mg/100g) and titrable acidity (0.78 %) after 12days of storage. The minimum growth of bacteria and fungi were found as 4.56×10^5 cfu/g (LDPE) and 3.28×10^3 cfu/g in 12 days and in sensory evaluation also maximum overall acceptability of 8 was recorded under refrigerated condition. Passive MAP technology in LDPE was more

suitable for fresh-cut bitter gourd.

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