Optimization of Process Variables for Retention of Functional Properties of Bitter Melon (*Momordica Charantia L.*) and its Shelf - Life Extension

SHEETAL DEVI,[1] DIPENDRA KUMAR MAHATO,[2]* SARVESH SINGH[2] and SURENDRA PRASAD SINGH[2]

[1]SAB Miller India Ltd., Sonipat, Haryana 131001, India
[2]Centre of Food Science and Technology, Banaras Hindu University (BHU), Varanasi- 221005, Uttar Pradesh, India

Abstract

This study was carried out to investigate the effect of sodium hypochlorite (NaClO), hydrogen peroxide (H_2O_2) and soaking time on vitamin C, carotenoid, antioxidant activity and total phenolic content for optimizing the pre - treatment for minimal processing of bitter melon slices and to evaluate its shelf life at different storage conditions. Fresh - cut bitter melon slices were given post-cutting treatment of wash solution containing NaClO (25 - 500ppm) and H_2O_2 (1 - 5%) solution for different time intervals (10 - 120 minutes). The treated samples were analyzed for Vitamin C content, carotenoid, DPPH inhibition activity, ABTS inhibition activity and total phenolic content (TPC) using Central Composite Rotatable Design (CCRD). The optimum condition for soaking achieved by CCRD was NaClO (25 ppm), H_2O_2 (1 %) and soaking time (56.32 min). The minimally processed bitter melon at optimized wash water treatment was packed in LDPE pouch of 0.1 mm thickness and were evaluated for shelf-life under modified atmospheric packaging, MAP 1 (5 % O_2, 25 % CO_2 & 70 % N_2) and MAP 2 (10 % O_2, 20 % CO_2 & 70 % N_2) stored at temperature of 5, 20 and 35 °C, respectively. Based on microbiological, colour and textural analysis, MAP 1 was found to be better compared to MAP 2 and the shelf - life of fresh - cut bitter melon slices was prolonged up to twenty-eight days of storage.

Introduction

Bitter melon (*Momordica charantia L.*), a member of Cucurbitaceae family is variously known as bitter gourd, bitter squash, balsam pear, karalla, African cucumber and bitter cucumber. It has twice the calcium and potassium content with respect to spinach and banana, respectively. It is rich in minerals like iron, zinc, phosphorus, sodium, and magnesium as well as a fair source of vitamin C that contributes about 55% of total vitamins.[1] Minimally processed vegetables, also referred to as ready-to-use, fresh-cut or pre-cut produce, are raw fruits and vegetables that undergo through various stages like washing, peeling, slicing, and chopping before actually being packaged for further processing and consumption. The market value of minimally processed fruits and vegetables are limited by their short shelf life. This is accountable to the tissue damage during processing stages and the microbial growth during storage. The washing of fresh-cut products with chlorinated water (50-200 mgL^{-1} of active chlorine) reduces the levels of various pathogenic microorganisms. For this purpose, sodium hypochlorite (NaClO) is most widely used as a sanitizer in the fresh-cut industry.[2] In addition to this, hydrogen peroxide (H_2O_2), which is highly oxidative in nature due to the release of O_2 in aqueous solutions, has antimicrobial activity both against gram-positive and gram-negative bacteria.[3] H_2O_2 in the range of 1-5% is used as a sanitizer for food contact surfaces as well as for packaging materials in aseptic filling operations. The antimicrobial efficiency of 4-5% H_2O_2 is comparable to 100-200 ppm of chlorine treatment.[4]

The major concern regarding minimally processed fruits and vegetables are on their microbiological quality, safety, processing and packaging issues.[5] As minimally processed vegetables require storage before cooking, thus, may possess a potential food safety issue.[6] Minimally processed vegetables are usually contaminated with food-borne pathogens such as *Escherichia coli* O157: H7 and *Listeria monocytogenes.* According to Rodgers, Cash,[7] the treatment of whole lettuce, strawberries, and cantaloupe with 100 ppm chlorine for 5 minutes reduced *L. monocytogenes* and *E. coli* O157:H7 to non-detectable levels. Both these pathogens have been reported in a variety of vegetables like leafy vegetables, bitter melon, cabbage, celery, cucumber, radish, tomato and bean sprouts.[8] However, the use of modified atmosphere packaging (MAP) have not been reported with any outbreaks. The MAP makes use of different levels of O_2 and CO_2 for packaging purpose, where 3-8% CO_2 and 2-5% O_2 is recommended for fruits and vegetables for MAP storage.[9]

Sodium hypochlorite (100-10,000 ppm) and H_2O_2 (5%) have been used for sanitization of "chile de árbol" pepper (*Capsicum frutescens L.*) fruit with the exposure time varying from 30-120 minutes.[10] de São José and Vanetti[11] used 5% of H_2O_2 for the decontamination of watercress, parsley and strawberries. Similarly, Meireles, Fulgêncio[12] minimally processed the vegetable plants using NaClO solution. Till date, there are no reports available for the combined effects of NaClO and H_2O_2 with other hurdles like freezing and MAP for the processing and preservation of bitter melon. Therefore, the novelty of this study lies on the use of combined effect of NaClO and H_2O_2 as a sanitizer for decontaminating the wash water during minimal processing of bitter melon in the range of (25-500 ppm) and (1-5%) respectively for the exposure time of (10-120) minutes. Therefore, this study was conducted to evaluate the effects of NaClO and H_2O_2 with soaking time assisted by different MAP gas composition and storage temperatures for the retention of functional properties of bitter melon and its shelf - life evaluation based on microbial growth and physico - chemical properties. Bitter melon, being a good source of vitamin C, carotenoid, antioxidant activity and total phenolic content, these nutritional parameters were selected as responses. The above techniques are also free from any environmental hazards and are effective in maintaining various quality parameters of bitter melon as compared to other preservation technologies.

Materials and Methods
Materials

Fresh bitter melon was procured from the local market of Varanasi, India. All chemical reagents used in this work were of analytical grade and purchased from Merck (Merck, Darmstadt, Germany) and Sigma (Sigma-Aldrich, Gillingham - Dorset, England). The LDPE pouch of 0.1 mm thickness was purchased from Sigma - Aldrich for MAP packaging of minimally processed bitter melon.

Sample Preparation for Minimal Processing

Mature bitter melon of uniform size and dark green colour were selected for the study. They were first sorted and washed in running water. The tail ends of cleaned bitter melon were removed followed by slicing into rings of one cm thickness using a stainless-steel knife. Bitter melon slices were soaked in NaClO (25 - 500 ppm) and H_2O_2 (1 - 5 %) solution for different time intervals (10-120 minutes) for optimizing the pre-treatment. The sanitizers and soaking time were selected as per the earlier research focused on the synergistic effect of NaClO and H_2O_2 on microbial decontamination.[13]

Experimental Design

Response Surface Methodology (RSM) was used to optimize the process variables for the functional properties of bitter melon. The effects of three independent variables: A (Sodium hypochlorite), B (Hydrogen peroxide) and C (Soaking time) at levels 25-500 ppm, 1-5% and 10-120 minutes respectively on five response variables: Vitamin C content, carotenoid, DPPH inhibition activity, ABTS inhibition activity and Total phenolic content (TPC) were evaluated using Central Composite Rotatable Design (CCRD). All the experiments were carried out in triplicate. The effects of the independent variables (A(X1), B(X2) and C(X3)) on the dependent variable Y was evaluated using the Eq. below:

$$Y_n = \alpha_0 + \alpha_1 X_1 + \alpha_2 X_2 + \alpha_3 X_3 + \alpha_{11} X_1^2 + \alpha_{22} X_2^2 + \alpha_{33} X_3^2 + \alpha_{12} X_1 X_2 + \alpha_{13} X_1 X_3 + \alpha_{23} X_2 X_3$$

Where, Y_n represents the response variable, α_0 is a constant; α_1, α_2 and α_3 are the coefficients of the linear effects; α_{12}, α_{13} and α_{23} are the coefficients of the interaction between the factors; and α_{11}, α_{22} and α_{33} are the coefficients of the quadratic effect.

The data were analyzed for analysis of variance (ANOVA) using Design-Expert 9.0.2.0 (State Ease, Inc.). The fitness of the polynomial equation was estimated using the coefficient of determination (R^2) and at a level of 5% significance.

Proximate Analysis

The official methods AOAC[14] was used to determine moisture, proteins, fibre and ash content. The moisture content of fresh - cut bitter melon slices and the minimally processed at optimum condition were determined using a rapid moisture analyzer (MB 45, Switzerland). Protein content was estimated using Automatic Kjeldahl method (Kjeldah 2006 Digester and Kjeltec® 2300 Analyzer unit, Hoganas, Sweden).

Estimation of Vitamin C Content

The vitamin C was analyzed by the titration method. Ten grams of sample was taken, blended with 3% metaphosphoric acid (HPO_3) and made up to 100 mL with HPO_3. It was then filtered and centrifuged. Ten milliliters of samples were taken for the titration and titrated against the standard dye solution (2,6 - dicholorophenol endophenol).[15] Prior to this, the dye was standardized using 5 ml of standard ascorbic acid and 5 ml of HPO_3 and then titrated with dye solution to obtain a dye factor as:

Dye factor = 0.5 / titre

Then the dye factor is used in the actual calculation of Vitamin C as:

mg of ascorbic acid per 100g = titre × dye factor × volume made up ×100 / aliquot of extract taken for estimatiom × volume used

Estimation of Carotenoid Content

The extraction and calculation of total carotenoid was done as per the method described by de Carvalho, Gomes.[16] To about 15 g of sample, 3 g of celite 454 (Tedia, Ohio, USA) and 25 ml of acetone was added and crushed to paste in a mortar & pestle till colourless. The extract obtained was then transferred to a separating funnel containing 40 ml of petroleum ether. The acetone was removed by the addition of water and discarding the aqueous phase. The extract was then subjected to anhydrous sodium sulphate to remove the remaining moisture present. Finally, the volume was made up with petroleum ether and absorbance was read at 450 nm by UV-1800 spectrophotometer (Shimadzu, Japan). The total carotenoid is given by:

Carotenoid content (μg / g) = (Absorbance × volume used × 10^4) / ($A_{1cm}^{1\%}$ × weight of sample)

Where,

$A_{1cm}^{1\%}$ = 2592, is the extinction coefficient of β - carotene in petroleum ether.

Extract for Antioxidant Inhibition Analysis

Ten grams of minimally processed bitter melon slices were crushed to paste in mortar and pestle followed by the addition of 100 ml of ethanol (70%). The mixture was homogenized properly and filtered using Whatman no 42. The sample extract thus obtained was used for DPPH, ABTS and TPC analyses.

Antioxidant Inhibition Activity Analysis
DPPH (2, 2 Diphenyl-1-Picrylhydrazyl) Method

The test was performed as per the method by Apak, Gorinstein.[17] To 0.2 mL of sample extract, 1 mL of DPPH solution (80 µg / mL ethanol) was added. The blank was taken as 0.2 mL distilled water with 1 mL DPPH solution. Both the sample and blank were stored in a dark at room temperature for 30 minutes. After this, the samples were centrifuged at 3000 rpm for 15 minutes and the absorbance was measured at 515 nm using UV-1800 spectrophotometer (Shimadzu, Japan).

$$DPPH \% = (A_{blank} - A_{sample}) / A_{blank} \times 100$$

Where, A is the absorbance of samples and blank

ABTS (2, 2'- Azino - Bis (Ethyl Benzthiazoline - 6 - Sulfonic Acid) Method

The test was carried out as per the ABTS method described by Shalaby and Shanab.[18] The stock solution of ABTS was prepared by mixing 7mM ABTS with 2.45 mM potassium persulfate (1:1, v/v) and kept for 4 - 16 hrs for the reaction to complete and give stable absorbance. $ABTS^{•+}$ solution was then diluted with ethanol to an absorbance of 0.700 ± 0.05 at 734 nm for the measurements. Then, 0.1 mL of sample extract was mixed to 0.9 mL of ABTS•+ solution, mixed properly and absorbance was taken after 15 minutes at 734 nm. The antioxidant is measured by a decrease in absorbance as:

$$ABTS \% = (A_{blank} - A_{sample}) / A_{blank} \times 100$$

Where, A is the absorbance of samples and blank $(ABTS^{•+})$

Total Phenolic Content (TPC) Analysis

The analysis for TPC was performed using Folin-Ciocalteau reagent as per the method described by Ainsworth and Gillespie[19] with slight modification. Approximately 0.5 mL of sample extract was taken to which 3 mL of distilled water was added followed by 2 mL of Folin - Ciocalteau reagent. Then after 3 min, 2.0 mL of sodium carbonate 7.5 % (w / v) was added and the reaction mixtures were kept for 45 min in an incubator shaker in dark condition. After the appearance of blue colour, the absorbance was measured at 765 nm using UV - 1800 spectrophotometer (Shimadzu, Japan). Gallic acid was used to plot a standard calibration curve. The total phenolic content of the sample was determined by plotting the spectrophotometer readings of the samples against the standard calibration curve and the amount estimated was expressed in terms of gallic acid equivalents (GAE / g).

MAP Packaging of Minimally Processed Bitter Melon at Optimized Pre - Treatment

The predicted values obtained by the desirability function method with the highest desirability for optimization was verified by experimental values. The minimally processed bitter melon slices at optimized pre - treatment were weighed in 50 g and packaged in low-density polyethylene (LDPE) pouches of 0.1 mm thickness, one set with MAP 1 (5% O_2, 25% CO_2 & 70% N_2) and the other set with MAP 2 (10% O_2, 20% CO_2 & 70% N_2) using modified atmospheric packaging machine (Induac Saurabh Engineers, INDIA). The MAP 1 and 2 gaseous levels were selected based on recommended (around 5% O_2 and 10% CO_2) gas composition for fruits and vegetables storage.[9] The sample in LDPE pouch at the normal atmospheric gas condition was taken as control. Each set of MAPs packaged bitter melon samples were stored at temperature of 5 °C, 20 °C and 35 °C in triplicate for a period of one month and microbial growth, colour and textural changes were monitored at an interval of 7 days.

Microbiological Analysis

Total plate count, total fungal count and coliform count were done according to method AOAC.[20] The serial dilution was done in a test tube with 9 mL of 0.9% NaCl solution. One gram of sample was added to the first test tube and then serially diluted up to 10^{-5} dilution factor. 1 mL of each dilution was pour plated for plate count agar (PCA), Potato dextrose agar (PDA) and Violet Red Bile Agar (VRBA). The plates were incubated at 37 °C for 48 hours in an inverted position. The plate counts were expressed as colony - forming units (CFU)/g.

Physical Properties of Stored Bitter Melon Samples
Colour and Textural Analysis

The color was determined by using a colorimeter (Konica Minolta, Japan). The color parameters were L* (L* = 0, black and L* = 100, white), a* (-a* = greenness and + a* = redness), and b* (-b* = blueness and + b* = yellowness). The colorimeter was calibrated with a white calibration tile.[21]

The texture analysis (hardness) was performed using a texture analyzer (Model CT3, Brookfield Engineering Laboratories, USA) with slight modification in methods used by Park, Kim.[22] 5 g of sample was put on a circular platform. It was compressed to 60% with a rod - type probe (TA 10, 25.4 hmm diameters) at a speed of 0.5 mm/s in order to carry out the TPA analysis. Hardness was determined and recorded in triplicates.

Statistical Analysis

The physicochemical analysis was performed in triplicates and the results were reported as a mean ± standard deviation. The data were then analyzed for analysis of variance (ANOVA) at $P < 0.05$. All the statistical analyses were performed using Design-Expert 9.0.2.0 software.

Results and Discussion

The effects of sodium hypochlorite (NaClO), hydrogen peroxide (H_2O_2) and soaking time on the functional properties of minimally processed bitter melon have been evaluated. The individual as well as the synergistic effects of NaClO and H_2O_2 with respect to soaking time were used to optimize the level of sanitizers for pre-treatment. The optimum condition obtained was further carried for minimal processing of bitter melon followed by MAP packaging and shelf life evaluation.

Effect of Pre - Treatment on Proximate Composition

Proximate composition of bitter melon slices before and after minimal processing using NaClO and H_2O_2 are shown in Table 1. These data are in accordance with the findings of Donya, Hettiarachchy[23] and Bangash, Arif[24] for bitter melon. The minimal processing by the use of selected combined sanitizers does not significantly impact on the proximate composition of bitter melon. Similarly, Sapers, Miller[25] when treated the mushroom with H_2O_2, they observed no significant changes in the proximate analysis. This supports our findings regarding the effect of minimal processing on the proximate composition of bitter melon which remains almost unaffected.

Table 1: Nutritional evaluation of bitter melon before and after pre-treatment at optimum condition

S.No.	Parameters	Quantity (%)	
		Before pre-treatment	**After pre-treatment**
1.	Moisture Content	90.20 ± 1.10a	91.20 ± 1.10a
2.	Protein Content	2.27 ± 0.51b	2.47 ± 0.25b
3.	Dietary fibre	2.75±0.42c	2.80±0.35c
4.	Total Ash	1.46 ± 0.25d	1.32 ± 0.17d

*(a-d) same letters denote non-significant difference at p ≤ 0.05

Effect of Pre-Treatment on Vitamin C Content

From the response plot (Figure 1), it is observed that the variables NaClO, H_2O_2 and soaking time adversely affected the vitamin C content of the minimally processed bitter melon. Vitamin C acts as a reducing agent and thus has an inhibitory effect on the H_2O_2 as reported by Carnelio, Khan.[26] Weston, Ito[27] and D'Aquino, Suming[28] have also reported antagonist effects of NaClO on vitamin C. The vitamin C content was slightly reduced after washing the fresh-cut lettuce with NaClO solution as compared to the unwashed samples.[29] The decrease in vitamin C content was similar to findings by Tirawat, Kunimoto[30] showing the significant decrease in vitamin C content in cabbage. The interaction effect between NaClO & H_2O_2 and between H_2O_2 & soaking time had a negative effect while the interaction between NaClO & soaking time had a positive effect. The regression equation obtained for the vitamin C content is given below:

Vit.C = +76.40 - 1.36 × A - 0.68 × B - 1.07 × C - 0.11 × AB + 0.034 × AC - 0.12 × BC + 0.24 × A^2 - 50.037 × B^2 - 0.44 × C^2

Effect of Pre - Treatment on Carotenoid Content

The effect of variables NaClO, H_2O_2 and soaking time on carotenoid content is shown in Figure 2. The interaction between NaClO & soaking time and between H_2O_2 & soaking time had a negative effect. The possible reason for the decrease in carotenoid is due to its oxidation. In an experiment conducted by Sommerburg, Langhans,[31] it was found that the carotenoid cleaved into products
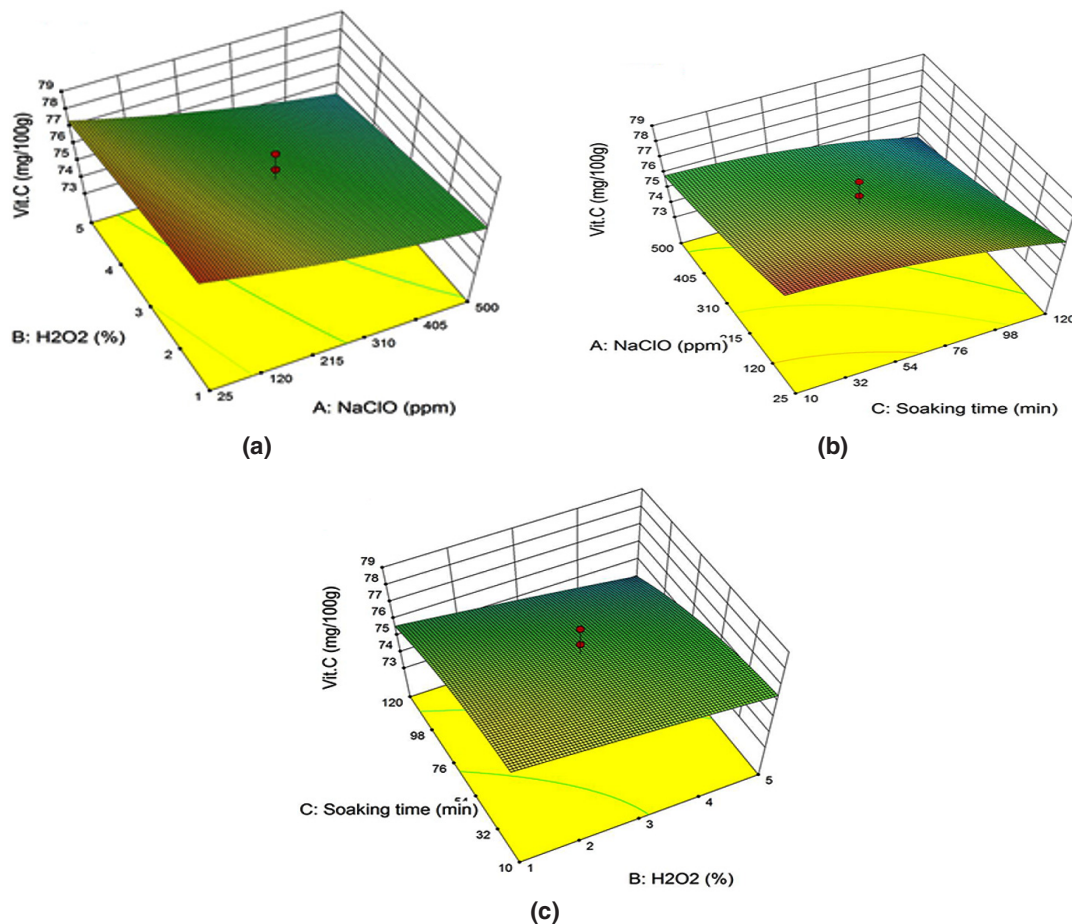

(a)

(b)

(c)

Fig. 1: Response surface plots of vitamin C as influenced by levels of (a) NaClO and H$_2$O$_2$ (b) NaClO and soaking time (c) H$_2$O$_2$ and soaking time for bitter melon slices
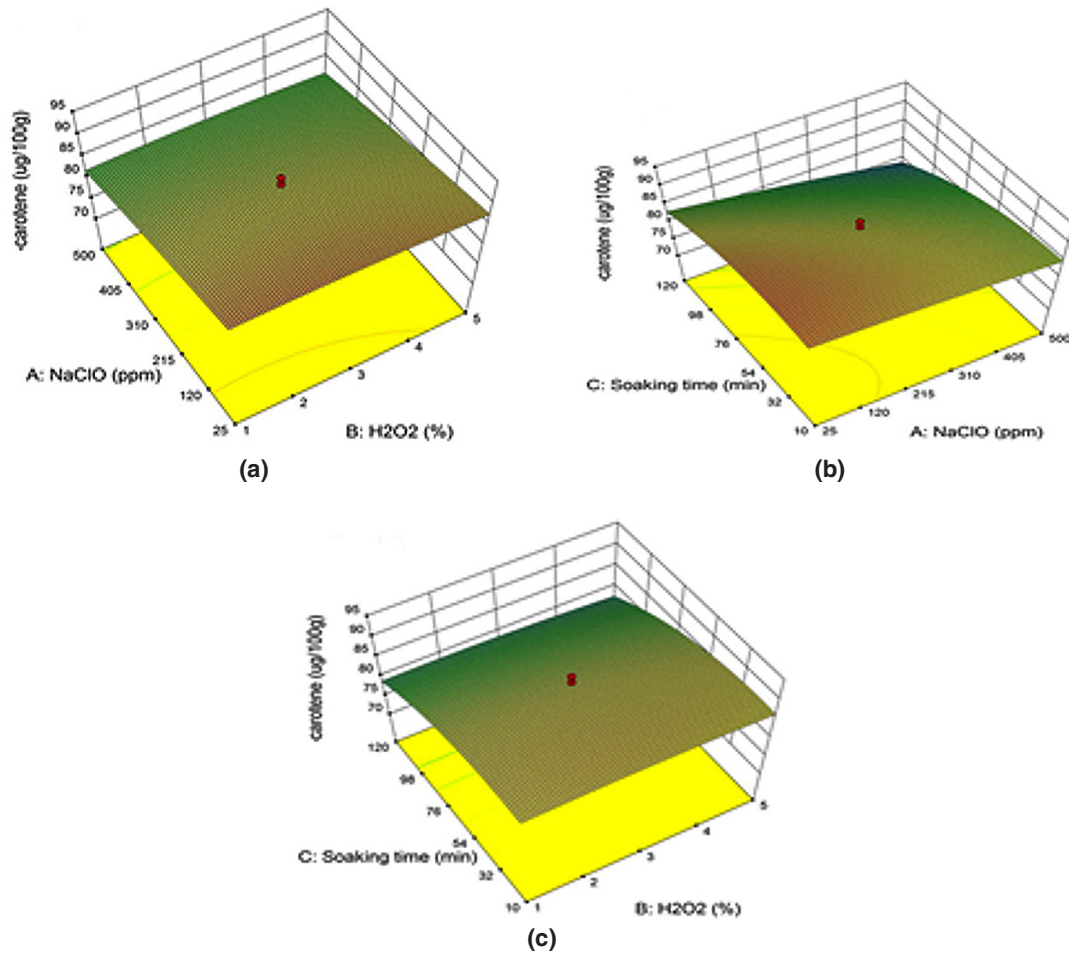
Fig. 2: Response surface plots of carotenoid as influenced by levels of (a) NaClO and H₂O₂ (b) NaClO and soaking time (c) H₂O₂ and soaking time for bitter melon slices

after oxidation mediated by hypochlorous acid in presence of sodium hypochlorite (HOCl/-OCl). HOCl/-OCl interacts with more lipophilic constituents i.e. carotenoids and is able to destroy it.[32] Similarly, the oxidative stress on carotenoid is induced by H₂O₂ which is the reason behind the decrease in carotenoid content. This is in accordance with the findings of Martano.[33] Müller[34] also observed a significant decrease in both α and β carotene levels in carrot by increasing the concentration of NaClO from 20 ppm to 200 ppm. Similarly, Martín-Diana, Rico[35] reported that when fresh-cut carrots were treated with 150 ppm of NaClO (pH 9.3) for 5 min, a carotenoid loss of 30% was obtained. However, a positive interaction effect between NaClO & H₂O₂

was observed. The regression equation for the response carotenoid is reported as:

Carotenoid = +87.80464 - 5.70171E - 003 × A + 0.49744 × B + 0.080589 × C + 5.78947E - 004 × AB - 1.02775E - 004 × AC - 2.18182E - 003 × BC - 5.99585E - 003 × A² - 0.12300 × B² - 9.48059E - 004 × C²

Effect of Pre-Treatment on DPPH Inhibition Activity

The selected variable conditions had a positive effect (Figure 3) on the DPPH inhibition activity of the minimally processed bitter melon. This is supported by the findings of Ribeiro, Valentão[36] and
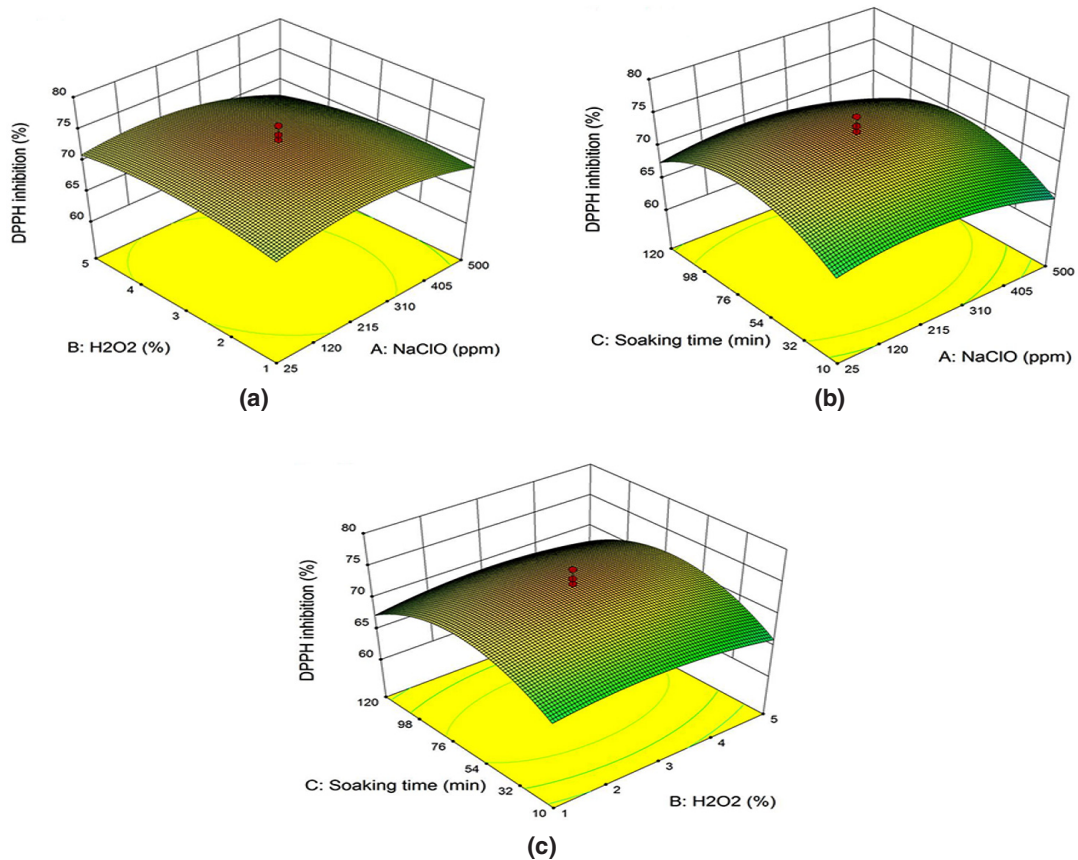
(a)

(b)

(c)

**Fig. 3: Response surface plots of DPPH inhibition activity as influenced by levels of
(a) NaClO and H$_2$O$_2$ (b) NaClO and soaking time (c) H$_2$O$_2$ and soaking time for bitter melon slices**

Mendoza-Sánchez, Guevara-González.[37] The H$_2$O$_2$ reacts with superoxide in the presence of certain transition metal ions give rise to a highly-reactive oxidizing species, i.e. the hydroxyl radical (•OH). This is responsible for influencing the DPPH activity positively. The interaction effect between NaClO & H$_2$O$_2$ had a negative effect while the interaction of the two with soaking time separately had positive effects. The equation below describes the regression for the DPPH inhibition activity.

DPPH Inhibition Activity = 61.43945 + 0.020863 × A + 2.19398 × B + 0.20286 × C - 1.36579E003 × AB + 7.75120E - 006 × AC + 3.44318E - 003 × BC - 4.20255E - 005 × A^2 - 0.36547 × B^2 - 1.60411E - 003 × C^2

Effect of Pre-Treatment on ABTS Inhibition Activity

The selected variable conditions had a positive effect (Figure 4) on the ABTS inhibition activity of the minimally processed bitter melon similar to that on DPPH inhibition activity. This is because antioxidants react with H$_2$O$_2$ and/or with derivate oxidizing species that inhibit the ABTS$^{•+}$ radical formation which finally leads to overestimation of antioxidant capacity.[38] The interaction effect between NaClO & H$_2$O$_2$ showed negative effect while the interaction between NaClO & soaking time and between H$_2$O$_2$ & soaking time had positive effects. The regression equation for the ABTS inhibition activity is:

ABTS Inhibition Activity = +66.50981+ 8.22336E - 003 × A + 3.17341 × B + 0.14174 × C - 2.12632E

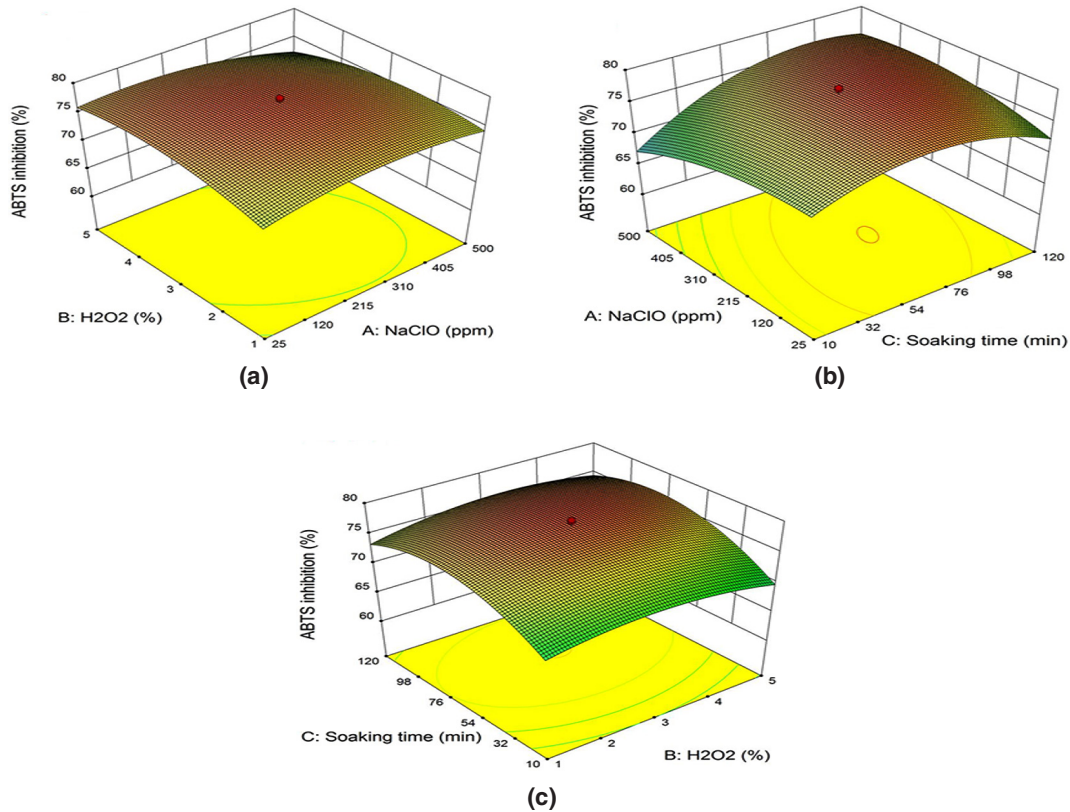**Fig. 4: Response surface plots of ABTS inhibition activity as influenced by levels of (a) NaClO and H$_2$O$_2$ (b) NaClO and soaking time (c) H$_2$O$_2$ and soaking time for bitter melon slices**

-003 × AB + 1.77608E - 004 × AC + 4.25000E - 003 × BC - 3.11494E - 005 × A^2 - 0.47240 × B^2 - 1.27917E - 003 × C^2

Effect of Pre-Treatment on Total Phenolic Content
All the selected variables *viz.*, NaClO, H$_2$O$_2$ and soaking time had a positive effect (Figure 5) individually on the total phenolic content. This is supported by the findings of Vandekinderen, Van Camp[39] who reported an increase in total phenolic content in minimally processed leek when treated with NaClO. The phenolic compounds leach into wash water because of their hydro-solubility. This is prevented by the wash water containing NaClO and H$_2$O$_2$ which could be the reason behind the increase in total phenolic content. The interaction effect between NaClO and H$_2$O$_2$ had a negative effect while the interaction of these two variables with soaking

time had positive effects. The regression equation for the Total Phenolic Content is as follows:

Total Phenolic Content = + 5.17290 + 8.62333E - 004 × A + 0.26719 × B + 0.016247 × C - 7.89474E - 005 × AB + 1.43541E - 005 × AC + 3.40909E - 004 × BC - 3.52113E - 006 × A^2 - 0.036395 × B^2 - 41628E - 004 × C^2

Validation of the Predicted Model
The suitability of the model for the responses was determined at the optimum conditions of NaClO (25 ppm), H$_2$O$_2$ (1 %) and soaking time (56.32 min). The predicted values for vitamin C content, Carotenoid, DPPH inhibition activity, ABTS inhibition activity and total phenolic content and the experimental data obtained for these responses are shown in Table 2. The experimental values for the responses were
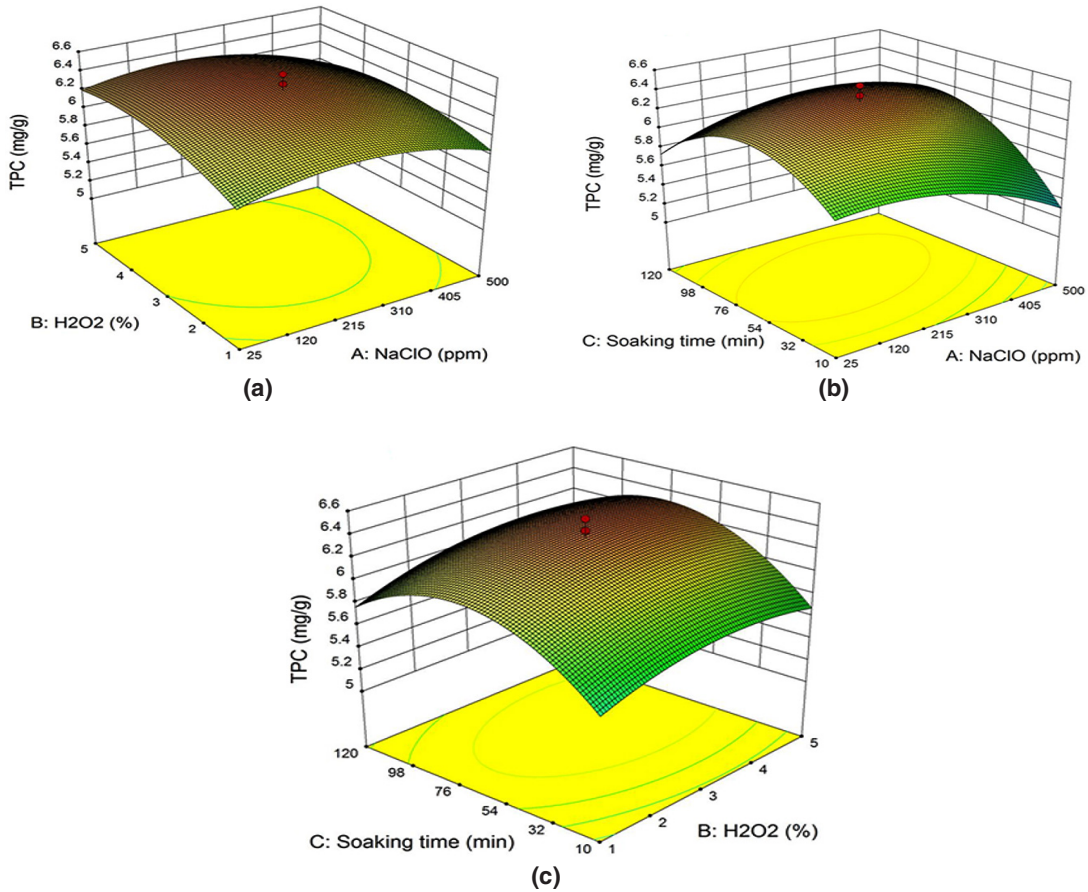
Fig. 5: Response surface of total phenolic content as influenced by levels of (a) NaClO and H₂O₂ (b) NaClO and soaking time (c) H₂O₂ and soaking time for bitter melon slices

Microbial Growth in MAP of Minimally Processed Bitter Melon

The combined effects of sanitizers (NaClO & H_2O_2), MAP and temperature (5 °C, 20 °C & 35 °C) on total plate count, total fungal count and coliform count were evaluated (Table 3) in bitter melon for its shelf-life study. The sample in LDPE pouch at atmospheric condition was taken as control. The control sample was spoiled after 4 days at 5 °C and 1 day at 20 °C & 35 °C, therefore, no analysis was done with control after spoilage. There was no coliform growth reported during 28 days of storage, however, bacterial and fungal growth were higher in MAP 2 compared to MAP 1 at higher temperature. An earlier study with the test solutions of NaClO & H_2O_2 in combination with modified atmosphere technology were effective in inhibiting the growth of the microorganisms. It

was reported by Pereira, Kothe[40] that no coliforms growth detected at 45 °C in the minimally processed radicchio (Cichorium intybus L.) samples sanitized with NaClO solution (150 ppm) and H_2O_2 solution (4 %). The combined effects of NaClO and H_2O_2 on the elimination of A. hydrophila adhered to polythene under static and dynamic conditions have also been evaluated.[13] This supports our findings where no coliforms were detected till the 28th day of storage at either temperature. The total plate count and total fungal count significantly increased with the increase in temperature of storage as well as with the concentration of oxygen from 5 to 10% and carbon dioxide from 20 to 25% in MAP 2 compared to MAP 1. It was also observed that the MAP 1 stored at 5 °C showed lesser fungal and total count as compared to that MAP 2 stored at higher temperatures. These

Table 2: Validation of the experimental and predicted values for the optimization of process variables

Optimum value for design variable			Vitamin C content (mg /100g of dry matter)		Carotenoid (μg/100 g of dry matter)		DPPH inhibition activity (%)		ABTS inhibition activity (%)		Total phenolic content (mg/g)		Desirability
Sodium hypochlorite (ppm, A)	Hydrogen peroxide (%,B)	Soaking time (min,C)	Predicted value	Experimental value	Predicted value	Experimental value	Predicted value	Experimental value	Predicted value	Experimental value	Predicted value	Experimental value	
25	1	56.32	78.68	75.36	89.31	86.66	70.27	68.52	73.75	71.25	5.92	5.75	0.802

Table 3: Microbial counts (total plate count, total fungal count and coliform count) (cfug-1) of minimally processed and MAP packaged bitter melon slices during storage at three different temperatures

Storage (days)	MAP	Total Plate Count (PCA) (cfug⁻¹)			Total Fungal Count (PDA) (cfug⁻¹)			Coliform count (VRBA) (cfug⁻¹)		
	Temp. →	5°C	20°C	35°C	5°C	20°C	35°C	5°C	20°C	35°C
0	MAP 1	0	0	0	0	0	0	0	0	0
	MAP 2	0	0	0	0	0	0	0	0	0
	Control	0	0	0	0	0	0	0	0	0
7	MAP 1	100 ±8[a]	230±10[a]	300±11[a]	20±4[a]	32±3[a]	40±2[a]	0	0	0
	MAP 2	105±6[a]	250±8[b]	340±12[b]	22±2[a]	35±5[a]	50±5[b]	0	0	0
	Control	*	*	*	*	*	*	*	*	*
14	MAP 1	700±11[b]	890±12[c]	1020±15[c]	40±5[b]	50±7[b]	65±8[c]	0	0	0
	MAP 2	970±8[c]	1560±15[d]	2830±17[d]	48±7[b]	72±10[c]	85±11[d]	0	0	0
	Control	*	*	*	*	*	*	*	*	*
21	MAP 1	3165±14[d]	3986±24[e]	4590±19[e]	130±10[c]	155±16[d]	230±18[e]	0	0	0
	MAP 2	4690±15[e]	5970±20[f]	7864±22[f]	250±16[d]	360±12[e]	580±16[f]	0	0	0
	Control	*	*	*	*	*	*	*	*	*
28	MAP 1	6872±25[f]	8452±24[g]	10542±30[g]	1010±25[e]	1260±15[f]	1530±20[g]	0	0	0
	MAP 2	10132±50[g]	11562±42[h]	13542±52[h]	1476±32[f]	1883±23[g]	2235±25[h]	0	0	0
	Control	*	*	*	*	*	*	*	*	*

MAP 1 (O₂-5%, CO₂-25% & N₂-70%); MAP 2 (O₂-10%, CO₂-20% & N₂-70%), (a-h) same letters denote non-significant difference at p ≤ 0.05
*indicates the sample got spoiled

Table 4: Colour and textural changes in minimally processed and MAP packaged bitter melon slices during storage at three different temperatures

Storage (days)	MAP	L*			Colour a*			b*			Texture (hardness, N)		
Temp. →		5°C	20°C	35°C	5°C	20°C	35°C	5°C	20°C	35°C	5°C	20°C	35°C
0	MAP 1	79 ±0.58a	79 ±0.58a	79 ±0.58a	-2.41 ±0.14a	-2.41 ±0.14a	-2.41 ±0.14a	30.14 ±0.21a	30.14 ±0.21a	30.14 ±0.21a	35.68 ±1.08a	35.68 ±1.08a	35.68 ±1.08a
	MAP 2	79 ±0.58a	79 ±0.58a	79 ±0.58a	-2.41 ±0.14a	-2.41 ±0.14a	-2.41 ±0.14a	30.14 ±0.21a	30.14 ±0.21a	30.14 ±0.21a	35.68 ±1.08a	35.68 ±1.08a	35.68 ±1.08a
	Control	79 ±0.58a	79 ±0.58a	79 ±0.58a	-2.41 ±0.14a	-2.41 ±0.14a	-2.41 ±0.14a	30.14 ±0.21a	30.14 ±0.21a	30.14 ±0.21a	35.68 ±1.08a	35.68 ±1.08a	35.68 ±1.08a
7	MAP 1	78.97 ±0.11b	78.45 ±0.21b	76.55 ±0.19b	-2.27 ±0.07b	-2.19 ±0.05b	-1.84 ±0.15b	34.29 ±0.44b	35.5 ±0.28b	36.77 ±0.21b	33.28 ±0.46b	32.01 ±0.05b	31.78 ±0.11b
	MAP 2	78.45 ±0.19c	76.06 ±0.71c	74.82 ±0.13c	-2.13 ±0.09b	-2.07 ±0.05b	-1.28 ±0.05b	34.84 ±0.06c	35.94 ±0.06c	37.16 ±0.05c	32.29 ±0.19c	32.06 ±0.23b	31.02 ±0.30c
	Control	*	*	*	*	*	*	*	*	*	*	*	*
14	MAP 1	74.45 ±0.11d	70.67 ±0.09d	68.71 ±0.20d	-1.26 ±0.07c	-1.12 ±0.09d	-0.61 ±0.06d	37.55 ±0.11d	40.25 ±0.03d	42.24 ±0.15d	31.16 ±0.81d	28.86 ±0.17c	27.78 ±0.14d
	MAP 2	74.09 ±0.13e	70.16 ±0.07e	68.08 ±0.55e	-1.29 ±0.07c	-1.05 ±0.05d	-0.48 ±0.02e	37.92 ±0.03e	40.92 ±0.04e	43 ±0.11e	30.69 ±0.34e	28.26 ±0.18d	26.81 ±0.12e
	Control	*	*	*	*	*	*	*	*	*	*	*	*
21	MAP 1	65.63 ±0.17f	60.45 ±0.19f	58.86 ±0.02f	1.56 ±0.02d	1.91 ±0.06e	2.49 ±0.05e	42.59 ±0.04f	46.7 ±0.05f	50.49 ±0.05f	28.73 ±0.35f	24.29 ±0.35e	22.21 ±0.43f
	MAP 2	64.2 ±0.07g	60.46 ±0.02g	58.19 ±0.06g	1.92 ±0.04e	2.26 ±0.06f	2.74 ±0.06f	43.57 ±0.09g	47.44 ±0.12g	50.9 ±0.09g	28.23 ±0.57f	23.96 ±0.33f	21.24 ±0.34g
	Control	*	*	*	*	*	*	*	*	*	*	*	*
28	MAP 1	58.57 ±0.09h	55.75 ±0.03h	52.66 ±0.02h	2.47 ±0.02f	2.95 ±0.07g	3.2 ±0.07h	45.78 ±0.01h	50.37 ±0.12h	55.76 ±0.11h	25.38 ±0.48g	20.42 ±0.27g	18.4 ±0.45h
	MAP 2	57.66 ±0.10i	54.44 ±0.21i	52.49 ±0.05i	2.94 ±0.05g	2.98 ±0.03g	3.58 ±0.04i	46.29 ±0.06i	50.67 ±0.12i	56.41 ±0.07i	22.44 ±0.30h	15.48 ±0.26h	11.93 ±0.67i
	Control	*	*	*	*	*	*	*	*	*	*	*	*

MAP 1 (O_2-5%, CO_2-25% & N_2-70%); MAP 2 (O_2-10%, CO_2-20% & N_2-70%), (a-i) same letters denote non-significant difference at $p \leq 0.05$
*indicates the sample got spoiled

found to be significant with the predicted values at the 5 % level of significance.

Microbial Growth in MAP of Minimally Processed Bitter Melon

The combined effects of sanitizers (NaClO & H_2O_2), MAP and temperature (5 °C, 20 °C & 35 °C) on total plate count, total fungal count and coliform count were evaluated (Table 3) in bitter melon for its shelf-life study. The sample in LDPE pouch at atmospheric condition was taken as control. The control sample was spoiled after 4 days at 5 °C and 1 day at 20 °C & 35 °C, therefore, no analysis was done with control after spoilage. There was no coliform growth reported during 28 days of storage, however, bacterial and fungal growth were higher in MAP 2 compared to MAP 1 at higher temperature. An earlier study with the test solutions of NaClO & H_2O_2 in combination with modified atmosphere technology were effective in inhibiting the growth of the microorganisms. It was reported by Pereira, Kothe[40] that no coliforms growth detected at 45 °C in the minimally processed radicchio (Cichorium intybus L.) samples sanitized with NaClO solution (150 ppm) and H_2O_2 solution (4 %). The combined effects of NaClO and H_2O_2 on the elimination of A. hydrophila adhered to polythene under static and dynamic conditions have also been evaluated.[13] This supports our findings where no coliforms were detected till the 28th day of storage at either temperature. The total plate count and total fungal count significantly increased with the increase in temperature of storage as well as with the concentration of oxygen from 5 to 10% and carbon dioxide from 20 to 25% in MAP 2 compared to MAP 1. It was also observed that the MAP 1 stored at 5 °C showed lesser fungal and total count as compared to that MAP 2 stored at higher temperatures. These observations are supported by the earlier findings on bitter melon.[41-43]

Colour and Textural Changes in MAP of Minimally Processed Bitter Melon

The colour and textural changes were observed over the storage period in MAP of minimally processed bitter melon (Table 4). The lightness of the product in MAP 2 decreased more compared to Map 1 with the increasing number of days as well as with the increase in storage temperature. Further, the values for greenness shifted towards redness more rapidly in MAP 2 and the changes were more significant at higher temperature. Similar trend was observed for yellowness of the samples over storage. Furthermore, the texture (hardness) of the sample was seen to decrease faster at higher temperature in MAP 2 as in against MAP 1. These findings are supported by the earlier research on fresh-cut bitter melon packaged in active MAP in LDPE and their physico-chemical changes over the storage period.[44]

Conclusion
This study revealed that the wash water solution of sodium hypochlorite (25 ppm), hydrogen peroxide (1%) and soaking time (56.32 min) was relatively optimum in maintaining the functional properties: vitamin C, carotenoid content, antioxidant activity (DPPH & ABTS) and total phenolic content without significantly affecting the proximate composition of the bitter melon. The use of modified atmosphere packaging (MAP) with the gas composition of MAP 1 (5% O_2, 25% CO_2 & 70% N_2) combined with cooling at 5 °C was sufficient to prolong the shelf-life of minimally processed bitter melon for up to 28 days of storage. The low oxygen concentration along with low temperature was effective in reducing the growth of the microorganisms. It has been possible to extend the shelf life of minimally processed bitter melon by 28 days in combination of hurdles and MAP. The result is of significant interest due to low cost and easy availability of the commodity and chemicals which can link the agricultural sector to a more prosperous and sustainable industrial application.

Conflict of Interest
The authors declare no conflict of interest.

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