The Pharma Innovation Journal 2021; 10(3): 746-753

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(3): 746-753

www.thepharmajournal.com
Received: 02-01-2021
Accepted: 06-02-2021

Srilatha V
S.V. Agricultural College, ANGRAU, Tirupati, Andhra Pradesh, India

Karthik Reddy P
S.V. Agricultural College, ANGRAU, Tirupati, Andhra Pradesh, India

Raveendra Reddy M
Regional Agricultural Research Station, ANGRAU, Tirupati, Andhra Pradesh, India

Anitha T
Regional Agricultural Research Station, ANGRAU, Tirupati, Andhra Pradesh, India

Mamatha NC
S.V. Agricultural College, ANGRAU, Tirupati, Andhra Pradesh, India

Lavanya Kumari P
S.V. Agricultural College, ANGRAU, Tirupati, Andhra Pradesh, India

Corresponding Author:
Srilatha V
S.V. Agricultural College, ANGRAU, Tirupati, Andhra Pradesh, India

Chemical interventions for extending shelf life of minimally processed bitter gourd (*Momordica charantia* L.)

Srilatha V, Karthik Reddy P, Raveendra Reddy M, Anitha T, Mamatha NC and Lavanya Kumari P

DOI: https://doi.org/10.22271/tpi.2021.v10.i3k.5874

Abstract
The minimal processing procedures like peeling, chopping and slicing would cause physiological effects which affect the quality of minimally processed bitter gourd products. The present investigation was carried out to study the impact of different chemicals on quality of minimally processed bitter gourd. The bitter gourd slices were treated by dipping in T_1-100 ppm sodium hypochlorite (NaoCl), T_2 - 2% citric acid, T_3 - 2% ascorbic acid, T_4 - 2% calcium chloride ($CaCl_2$), T_5 - 0.1% sodium benzoate, T_6 - 1% vinegar, T_7 - 100 ppm NaOCl+ 0.5% citric acid, T_8 - 100 ppm NaOCl+ 0.5% ascorbic acid, T_9 - 100 ppm NaOCl+ 2% $CaCl_2$, T_{10} - 100 ppm NaOCl + 0.1% sodium benzoate, T_{11} -100 ppm NaOCl+ 1% vinegar and T1$_2$ - distilled water as control to study their impact on quality during 21 days of refrigerated storage. Among the treatments, T_9 (100 ppm NaOCl + 2% $CaCl_2$) was found to be the best to treat minimally processed bitter gourd which record the least physiological loss in weight, better retention of chlorophyll, high carotenoid content, low peroxidise enzyme activity and delayed microbial contamination.

Keywords: Bitter gourd, minimally processed products, shelf life

Introduction
Bitter gourd (*Momordica charantia* L.), a member of cucurbitaceae family is variously known as bitter melon, bitter squash, balsam pear, barela, African cucumber and bitter cucumber. Bitter gourd 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. The antioxidant property is due to the presence of ascorbic acid and carotenoids and it is also used as hypoglycaemic agent for diabetic patients (Cefalu *et al.*, 2008) [9].
The demand for fresh and minimally processed fruits and vegetables has grown exponentially in the last few years due to change in consumer attitude and an increase in preference for ready to eat, fresh-cut, easy to use and pre-cut foods. The recent increase in fruit and vegetable production over the past decade has expanded the market for minimally processed products which enable the consumer to simply open and use them. Bitter gourd is one of the most popular vegetable among the pre-cut vegetables. A number of minimal processing procedures including peeling, chopping and slicing had physiological effects including increase in respiration rate, change in membrane permeability, water loss, ethylene production, loss of chlorophyll, microbial spoilage, enzymatic browning, tissue softening etc. in bitter gourd (Rico *et al.*, 2007) [28]. The shelf life of minimally processed fruits and vegetables can be enhanced by a variety of physical and chemical treatments as well as effective storage and packaging systems (Ugo De Corato, 2019) [39].
Among the chemical treatments, hydrogen peroxide, chlorine-based solutions, organic acids and preservatives are the widely used chemicals to prevent browning reactions, prevent ethylene production, reduce respiration rate and water loss and minimal postharvest infections (Lopez-Galvez *et al.*, 2013) [20]. In addition, chemical treatments can minimize the microbial growth and lessen the deterioration of texture in minimally processed produce (Bhaskaran *et al.*, 2013).
Sodium hypochlorite is a chlorine based solution and is the most widely used disinfecting agents for decontaminating fresh as well as minimally processed products as it is a powerful oxidizing agent (Guan and Fan, 2010) [14] and the recommended level is about 50 – 200 ppm (Ramos *et al.*, 2013) [10].

The addition of chemical disinfectant sodium hypochlorite is effective in reducing the microbial load and has been used to sanitize pre-cut fruits and vegetables (Sutsow, 1997, Soliva-Fortuny RC, Martin-Belloso, 2003) [33].
Organic acids have been widely used to slow down the enzymatic and non-enzymatic browning, microbial growth and to retain texture of fresh produce (Aguayo *et al.*, 2003) [1]. Citric acid, being a chelating agent could inactivate enzymes by binding to transition metals in the metal-enzyme complex (Jiang *et al*., 1999) [16], which have been used in various food processing systems. The use of reducing compounds such as ascorbic acid and its derivatives is very effective in controlling enzymatic browning (He Q, Luo Y 2007) [15].
Calcium chloride helps to delay ripening, reduces the susceptibility to chilling injuries, suppress senescence, less postharvest decay, reduced incidence of physiological disorders and improves the storage and marketable life of perishable products by maintaining their firmness and quality (El-Ramady *et al*., 2015) [11]. Calcium based treatments have also been used as preservative and firming compound in fruits and vegetable industry for extending shelf life of fruits and vegetables (Ugo De Corato, 2019) [39], since Ca^{+2} ion maintains firmness by cross linking with cell wall and middle lamella pectin (Rico *et al*., 2007) [28] which helps to maintain cell wall integrity. Manganaris *et al.* (2007) [25] suggested that calcium treatment can increase the tissue firmness and reduce the susceptibility to physiological disorders and lower the risk of salt-related injuries in peaches. Post-harvest application of $CaCl_2$ extend the storage life of pears up to 2 months, plums up to 4 weeks and apples up to 6 months at 0-2ºC with excellent colour and quality (El-Ramady *et al*., 2015) [11].
The large cut surfaces of minimally processed products and nutrient-rich tissues provide an ideal environment for microbial growth leading to spoilage of fresh cut produce. Sodium benzoate is a weak acid and widely used preservative having antimicrobial action as it disrupts the normal metabolism by accumulating the protons and anions inside the microbial cell (Lopez-Malo *et al*., 2007) [20]. As a result of raw material processing, the fresh cut produce shelf life is limited from few days to few weeks (Ugo De Corato, 2019) [39]. The combination of chemical reducing agent, an acidulant and a chelating agent would be highly effective to enhance shelf life and quality of fresh-cut products. However, internalization of bacteria and unreachable sites of fruits and vegetables are the major limitations of applying anti-microbial and anti-browning agents (Mahajan *et al*., 2014) [24]. Immersion in antioxidants such as ascorbic acid and citric acid has found promising in preventing enzymatic browning of apple rings (Lozano de- Ganzales *et al.*, 1993 [22]; Siroli *et al.*, 2014 [31]) on peeled potatoes (Sapers and Miller, 1995) [29] and on papaya (Sivaramakrishna *et al*., 2018) [32]. Wang *et al.*, (2007) [40] reported that, fresh cut bitter gourd stored at 2 ^{0}C had storage life of 4 days. Sheetal Devi *et al.*(2019) [30] found that fresh-cut bitter gourd washed with disinfectant had reported 28 days storage life at 5 ^{0}C. The challenge of the present research work was to provide promising strategies for enhancing the quality of fresh-cut bitter gourd.

Materials and Methods

The present investigation entitled as effects of chemical treatments on quality of minimally processed bitter gourd (*Momordica charantia L*.) was carried out on a commercially grown bitter gourd hybrid Varsha grown at horticultural garden, S.V. Agricultural College, Tirupati, Andhra Pradesh during rabi 2019-20. Fruits are marked after fruit set and harvested at 8 days after fruit set. The bitter guard fruits were cleaned removed the tail ends and processed by slicing into rings of one cm thickness using a stainless-steel knife. The treatments consist of T_1-100 ppm sodium hypochlorite (NaOCl), T_2 - 0.5% citric acid, T_3 - 0.5% ascorbic acid, T_4 - 2% calcium chloride (w/v), T_5–0.1% sodium benzoate (w/v), T_6 - 1% vinegar (v/v), T_7 - 100 ppm $NaOCl_2$ + 0.5% citric acid, T_8 - 100 ppm $NaOCl_2$ + 0.5% Ascorbic acid, T_9 - 100 ppm $NaOCl_2$ + 2% calcium chloride, T_{10}- 100 ppm $NaOCl_2$ + 0.1% sodium benzoate, T_{11} -100 ppm $NaOCl_2$ + 1% vinegar and T_{12} - distilled water as control. The treatments were imposed by dipping the bitter guard slices for 30 seconds in chemical solutions and stored at 5 ^{0}C in refrigerator. The treatments were replicated thrice with 100 g of sample size. Data were collected at 7, 14 and 21days of storage on loss in physiological weight, ascorbic acid content, total chlorophyll, total carotenoids, peroxidise enzyme activity and microbial count.

Physiological loss in weight (%)

The electronic weight balance, with an accuracy of 0.01 g, was used to measure the weight of the bitter guard slices before cutting. Weight loss was calculated as the difference between the initial weight and weight during the measurement period, and was defined as a percentage (% of initial weight).

Estimation of ascorbic acid content

The content of ascorbic acid in the bitter guard slices is measured using 2, 6-dichlorophenol-indophenol titration method (AOAC, 1980) [4]. Initially 5 g fresh pulp was macerated and diluted with 4% oxalic acid. After filtering through muslin cloth, the volume was adjusted to 25 ml with 4% oxalic acid. 5 ml of aliquot was titrated against indophenols dye solution (dye was prepared by dissolving 42 mg sodium bicarbonate into a small volume of distilled water and then 52 mg 2,6-dichloro phenol indophenol, and volume was made up to 200 ml with distilled water) until a light pink colour appears. The results are expressed as mg of ascorbic acid per 100 g of bitter gourd pulp.

Estimation of total carotenoid content

The extraction and calculation of total carotenoids was done as per the method described by de Carvalho *et al.* (2012) [10]. 15 g of bitter gourd sample was crushed to paste till colourless in a mortar and pestle by adding 3 g of celite 454 and 25 ml of acetone. 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 × 104) / (A1cm1% × weight of sample)

Peroxidase enzyme activity assessment

The peroxidase activity was determined spectrophotometrically at 25 °C with a UV-1601 PC UV-visible spectrometer (Shimadzu Corporation, Kioto, Japan) at 470 nm using guaiacol as substrate and H_2O_2 as hydrogen donor (Ong *et al.*, 2013) [27]. The substrate mixture contained

10 ml of guaiacol solution at 0.01 ml/ml, 10 ml of hydrogen peroxide solution at 3 mg/ml and 100 ml of 0.05 mol/l sodium phosphate buffer (pH 6.5). The reaction cuvette contained a total volume of 3 ml with 2.9 ml substrate mixture and 0.1 ml crude extract. One unit of activity is defined as a change in absorbance of 0.001/min. The blank sample contained only 3 ml of substrate mixture.

Microbial count

The total mesophilic microorganisms present in the minimally processed slices were counted at 7th, 14th and 21st day of its storage life. Plate count agar medium (0.5% peptone, 0.25% yeast extract, 0.1% glucose, 1.5% agar) is used to enumerate the aerobic mesophilic microorganisms present in the bitter gourd samples. A Quebec colony counter is used to count the number of viable counts and is expressed as colony forming units (CFU) per millilitre of sample.

The data were subjected to statistical analysis of repeated measures mixed ANNOVA as well as one way ANNOVA by Tukey's post hac test at 7, 14, 21 days of storage for multiple comparison among the treatments using SPSS (20 version). Data were presented as mean ± SD and significance was tested at $p \leq 0.05$.

Results and Discussions

The nutritional composition of fresh bitter gourd fruits were calculated before imposing the treatments and the proximate composition is 0.81 g/g of total chlorophyll, 26.71 µg/g of total carotenoids, 78.63 mg/g of ascorbic acid and 0.25 /min/g of peroxidise activity.

From the results of repeated measures mixed ANNOVA, significant impact of treatments, storage period and their interaction were observed (Table 1). At the end of 21 days storage period, among the treatments bitter guard slices treated with 100 ppm NaOCl + 2% $CaCl_2$ (T_9) recorded 9.24% loss in physiological weight, 0.65 mg/g total chlorophyll, 19.10 µg/g total carotenoids, 47.43 mg/g ascorbic acid, lowest peroxidise activity (1.06 /min/g) and less microbial contamination (6.82 cfu/g). Similarly data were statistically analysed at 7, 14 and 21 days of refrigerated storage to know the treatment effects at storage intervals and the results are as follows.

Table 1: Pooled analysis of variance for biochemical parameters of minimally processed bitter guard cv. Varsha for 21 days storage period

Treatments	Physiological loss in weight (%)	Total chlorophyll content	Total carotenoids	Peroxidase activity	Ascorbic acid	Microbial count
T1	15.18^{f}	0.31^{fg}	14.28^{c}	2.05^{e}	34.02^{cd}	7.78^{de}
T2	14.08	0.37^{ef}	15.48^{bc}	2.28^{e}	38.52^{bc}	8.60^{g}
T3	13.67^{e}	0.38^{de}	15.33^{bc}	2.29^{e}	38.41^{bc}	8.84^{g}
T4	12.19^{c}	0.43c^{de}	15.56^{bc}	1.51^{bc}	40.00^{bc}	8.16^{f}
T5	13.22^{de}	0.38^{de}	17.40^{ab}	1.99^{de}	38.03^{bc}	7.62^{cd}
T6	12.90^{d}	0.43c^{de}	15.37^{bc}	2.02^{de}	38.55^{bc}	8.08^{f}
T7	10.88^{b}	0.56^{b}	15.80^{bc}	1.43b^{c}	42.37^{ab}	7.99^{ef}
T8	11.66^{c}	0.44^{cd}	15.94^{bc}	1.62^{c}	41.38^{ab}	7.48^{bc}
T9	9.24^{a}	0.65^{a}	19.10^{a}	1.06^{a}	47.43^{a}	6.82^{a}
T10	10.29^{b}	0.61^{b}	17.52^{ab}	1.27^{ab}	39.06^{bc}	7.29^{b}
T11	11.78^{c}	0.47^{c}	15.20^{bc}	1.72^{cd}	41.29^{ab}	7.93^{ef}
T12	15.92 ^{g}	0.29^{g}	13.90^{c}	2.86^{f}	30.87^{d}	10.57^{h}
Total	12.58	0.44	15.91	1.84	39.16	8.10
S.V						
Weeks (*F* value)	9767.48**	1366.080**	1049.291**	933.612**	796.412**	25625.18**
Treatments (F value)	257.08**	89.160**	6.429**	69.423**	9.993**	418.341**
Weeks * Treatment (F value)	28.68**	10.077**	3.893**	15.445**	1.734*	29.385**

* Significant at 5% level

** Significant at 1% level

Same set of alphabets indicates insignificant difference (Tukey's)

T_1-100 ppm NaOCl

T_2 - 0.5% citric acid,

T_3 - 0.5% ascorbic acid,

T_4 - 2% calcium chloride

T_5 – 0.1% sodium benzoate

T_6 - 1% vinegar

T_7 - 100 ppm NaOCl + 0.5% citric acid,

T_8 - 100 ppm NaOCl + 0.5% ascorbic acid,

T_9 - 100 ppm NaOCl + 2% calcium chloride,

T_{10} - 100 ppm NaOCl + 0.1% sodium benzoate,

T_{11} -100 ppm NaOCl + 1% vinegar and

T_{12} - distilled water as control

Physiological Loss in Weight (%)

There was a progressive increase in the loss of weight during postharvest storage of pre-cut bitter gourd (Table 2). After 7 days of storage, bitter gourd slices have recorded least loss in physiological weight in all the treatments and gradually increased at 14 days and 21 days after storage except in T_9 (100 ppm $NaOCl_2$ + 2% calcium chloride). At the end of the 21-day storage, the lowest physiological loss in weight (9.24%) is recorded by bitter gourd slices treated with 100 ppm $NaOCl_2$ + 2% calcium chloride (T_9) while, the control treatment has recorded the highest loss in physiological weight (15.92%). The weight loss in fruits is largely associated with water loss through transpiration and respiration (Kays, 1997) [17]. The significant reduction in loss of physiological weight of bitter melon slices dipped in 2% $CaCl_2$ may be due to the ability of calcium in cross linking the cell wall with middle lamella pectin which helps to maintain cell wall integrity (El-Ramady *et al.*, 2015) [11]. These results are in accordance with the findings of Yadav and Swathi (2011) [41] who reported that the lowest physiological loss in weight was recorded by 2% $CaCl_2$ pre-treated bitter melon slices and in jabuticaba (Garcia *et al.*, 2019) [12].

Table 2: Effect of chemical pre-treatments on Physiological loss weight (%) of minimally processed bitter guard cv. Varsha

Treatments	7 days after storage	14 days after storage	21 days after storage	Pooled Mean
T_1	6.85 ± 0.24^{ef}	14.93 ± 0.18^{h}	23.75 ± 0.64^{f}	15.18^{g}
T_2	6.27 ± 0.11^{d}	14.14 ± 1.00^{g}	21.83 ± 0.43^{e}	14.08^{f}
T_3	6.48 ± 0.51^{de}	12.96 ± 0.07^{f}	21.57 ± 0.79^{e}	13.67^{f}
T_4	6.12 ± 0.12^{d}	11.97 ± 0.17^{e}	18.47 ± 0.46^{c}	12.19^{c}
T_5	6.04 ± 0.08^{d}	13.92 ± 0.14^{g}	19.71 ± 0.34^{d}	13.22^{de}
T_6	6.20 ± 0.17^{d}	12.90 ± 0.24^{f}	19.59 ± 0.54^{d}	12.90^{d}
T_7	5.51 ± 0.36^{c}	10.07 ± 0.08^{c}	17.07 ± 0.24^{b}	10.88^{b}
T_8	6.26 ± 0.08^{d}	10.68 ± 0.39^{cd}	18.04 ± 0.26^{c}	11.66^{c}
T_9	4.44 ± 0.29^{a}	8.42 ± 0.30^{a}	14.86 ± 0.41^{a}	9.24^{a}
T_{10}	4.99 ± 0.10^{b}	9.33 ± 0.30^{b}	16.53 ± 0.50^{b}	10.29^{b}
T_{11}	6.09 ± 0.08^{d}	10.75 ± 0.35^{d}	18.48 ± 0.45^{c}	11.78^{c}
T_{12}	7.19 ± 0.22^{f}	15.25 ± 0.24^{h}	25.32 ± 0.91^{g}	15.92^{h}
F value	29.106**	109.67**	102.37**	257.082**

* Significant at 5% level
** Significant at 1% level
Same set of alphabets indicates insignificant difference (Tukey's)

Total chlorophyll content

Significant difference in the retention of chlorophyll among the treatments was observed at 7, 14 and 21 days of storage (Table 3**).** The chlorophyll during the entire storage period was highest in the slices treated with 2% $CaCl_2$ in combination with 100 ppm NaOCl. The pigment contents reduced significantly with the storage time. At the end of 21-day of storage, the highest retention of chlorophyll (0.55 mg/g) was reported in T_9 (100 ppm $NaOCl_2$ + 2% Calcium chloride) and lowest (0.14 mg/g) was reported in T_{12} (Control). The colour retention of minimally processed vegetables not only affects the shelf life but also an important sensory factor considered by the consumers. Yellowing due to loss of chlorophyll is the most visible symptom of senescence in green vegetables which can be minimized by post harvest dip with calcium salts (Leon *et al*., 2010). Keutgen *et al.* (2012) [19, 18] also reported the post harvest retention of green colour in spinach leaves was correlated with calcium content.

Table 3: Effect of chemical pre-treatments on Total chlorophyll content (mg/g) of minimally processed bitter guard cv. Varsha

Treatments	7 days after storage	14 days after storage	21 days after storage	Pooled Mean
T_1	0.51 ± 0.03^{f}	0.25 ± 0.02^{f}	0.18 ± 0.01^{gh}	0.31^{fg}
T_2	0.56 ± 0.04^{ef}	0.36 ± 0.04^{ed}	0.20 ± 0.01^{fg}	0.37^{ef}
T_3	0.61 ± 0.03^{de}	0.33 ± 0.04^{e}	0.19 ± 0.03^{fg}	0.38^{de}
T_4	0.67 ± 0.03^{bc}	0.38 ± 0.03^{ed}	0.24 ± 0.02^{ef}	0.43^{cde}
T_5	0.59 ± 0.01^{de}	0.36 ± 0.07^{ed}	0.20 ± 0.01^{fg}	0.38^{de}
T_6	0.61 ± 0.021^{d}	0.41 ± 0.02^{d}	0.26 ± 0.03^{de}	0.43^{cde}
T_7	0.69 ± 0.06^{b}	0.55 ± 0.05^{b}	0.44 ± 0.06^{b}	0.56^{b}
T_8	0.62 ± 0.03^{cd}	0.32 ± 0.02^{e}	0.37 ± 0.02^{c}	0.44^{cd}
T_9	0.77 ± 0.02^{a}	0.63 ± 0.02^{a}	0.55 ± 0.03^{a}	0.65^{a}
T_{10}	0.72 ± 0.01^{ab}	0.60 ± 0.01^{ab}	0.51 ± 0.04^{a}	0.61^{b}
T_{11}	0.62 ± 0.07^{cd}	0.49 ± 0.04^{c}	0.30 ± 0.01^{d}	0.47^{c}
T_{12}	0.53 ± 0.03^{f}	0.22 ± 0.02^{f}	0.14 ± 0.01^{h}	0.29^{g}
F value	18.76**	47.58**	84.61**	89.160**

* Significant at 5% level
** Significant at 1% level
Same set of alphabets indicates insignificant difference (Tukey's)

Ascorbic acid content

A significant reduction in the content of ascorbic acid was observed throughout in all the treatments (Table 4). However, T_9 (100ppm NaOCl + 2% $CaCl_2$) and T_3 (2% ascorbic acid) treatments recorded highest ascorbic acid content 68.37 and 66.53 g/100g; 46.29 and 41.29 g/100g; 27.62 and 20.94 g/100g at 7, 14 and 21 days after storage, respectively. Ascorbic acid is the most sensitive vitamin, which degrades relatively faster by the exposure to heat, light, and oxygen. The loss of ascorbic acid content might be partly due to large surface area of the sliced bitter gourd. Vitamin C loss was also reported recently in stored fresh-cut cantaloupe (Beaulieu and Lea, 2007; Gil et al., 2006) [7, 13] in cabbage (Tirawat *et al*., 2013) [37] and in lettuce (Sheetal Devi *et al*., 2019) [30]. Dipping of bitter gourd slices in 2% ascorbic acid as pre-treatment might have fortified the ascorbic acid content. Higher retention of ascorbic acid in $CaCl_2$ treated bitter gourd slices might be due to the less loss of water from pre-cut products (Yadav and Swathi, 2011 [41]; El-Ramady *et al*., 2015) [11].

Table 4: Effect of chemical pre-treatments on ascorbic acid (mg/g) content of minimally processed bitter guard cv. Varsha

Treatments	7 days after storage	14 days after storage	21 days after storage	Pooled Mean
T_1	54.64 ± 3.15^{e}	34.01 ± 2.34^{ab}	13.39 ± 1.53^{gh}	34.02^{cd}
T_2	61.57 ± 2.71^{abcd}	38.78 ± 1.04^{ab}	15.20 ± 1.10^{fg}	38.52^{bc}
T_3	66.53 ± 3.14^{abcd}	41.48 ± 1.95^{ab}	20.94 ± 0.89^{fg}	42.98^{bc}
T_4	60.45 ± 0.78^{cde}	39.99 ± 0.63^{ab}	19.54 ± 0.51^{b}	39.99^{bc}

T_5	59.01 ± 1.02^{de}	38.03 ± 0.17^{ab}	17.05 ± 0.68^{cdef}	38.03^{bc}
T_6	60.96 ± 4.00^{bcde}	38.55 ± 1.94^{ab}	16.15 ± 0.29^{ef}	38.55^{bc}
T_7	67.50 ± 2.09^{ab}	41.29 ± 0.82^{ab}	18.12 ± 0.98^{cde}	42.32^{ab}
T_8	64.10 ± 3.99^{abcd}	41.38 ± 2.11^{ab}	18.66 ± 0.33^{bcd}	41.38^{ab}
T_9	68.37 ± 5.75^{a}	46.29 ± 2.14^{a}	27.62 ± 3.56^{a}	47.43^{a}
T_{10}	60.05 ± 5.77^{abc}	29.72 ± 2.26^{b}	15.10± 0.79^{b}	34.95^{bc}
T_{11}	61.72 ± 3.79^{abc}	38.41 ± 2.09^{ab}	16.53 ± 0.44^{def}	38.88^{ab}
T_{12}	45.01 ± 3.32^{f}	35.12 ± 1.05^{ab}	12.46 ± 1.14^{h}	30.87^{d}
F value	9.87**	1.26*	28.15**	9.99**

* Significant at 5% level
** Significant at 1% level
Same set of alphabets indicates insignificant difference (Tukey's)

Total carotenoids content

From the results it was observed that, irrespective of the treatment there was a significant progressive decline in the total carotenoids of bitter gourd slices (Table 5). Among the treatments, bitter gourd slices dipped in 100 ppm NaOCl + 2% $CaCl_2$ (T_9) has recorded the highest total carotenoids content at 7, 14 and 21 days of storage (24.20, 18.03 and 15.05 µg/100g, respectively).While the lowest total carotenoids content of 23.60, 12.10, 6.00 µg/100g at 7, 14 and 21 days of storage, respectively was observed in control and it was at par with T_1 (100 ppm NaOCl). The decrease in total carotenoids towards the later part of the storage period might be due to the degradation of the carotenoid pigments, which is further accelerated by decline in the content of natural ascorbic acid during raw material processing. Oxidative protection of carotenoids by ascorbic acid has previously had also been reported (Biacs and Daood, 2000) [8].Yen *et al.* (2008) found that, the ascorbic acid being a well known antioxidant can reduce the oxidation of cellular components including carotene. Yadav and Swati (2011) [41] in bitter gourd and Jinto and James (2011) [17] and Sivaramakrishna *et al.*, (2018) [32] in papaya reported the effectiveness of calcium chloride in retention of carotenoid content in minimally processed products. The reduction in carotenoid content of bitter gourd samples treated with NaOCl might be due the hypochlorous acid mediated oxidation (Martano *et al.*, 2011) [26]

Table 5: Effect of chemical pre-treatments on total carotenoid content (µg/g) of minimally processed bitter guard cv. Varsha

Treatments	7 days after storage	14 days after storage	21 days after storage	Pooled Mean
T_1	22.33 ± 0.84^{a}	12.13 ± 0.76^{d}	8.38 ± 0.65^{d}	14.28^{c}
T_2	23.23 ± 1.75^{a}	12.93 ± 1.42^{c}	10.28 ± 0.99^{c}	15.48^{bc}
T_3	22.47 ± 1.07^{a}	12.47 ± 1.07^{c}	11.04 ± 0.07^{c}	15.33^{bc}
T_4	22.57 ± 2.41^{a}	12.73 ± 1.78^{c}	11.39 ± 0.54^{c}	15.56^{bc}
T_5	24.43 ± 0.91^{a}	15.00 ± 2.08^{bc}	12.76 ± 0.55^{b}	17.40^{ab}
T_6	22.40 ± 1.61^{a}	12.37 ± 1.62^{c}	11.36 ± 0.56^{c}	15.37^{bc}
T_7	22.27 ± 2.51^{a}	14.33 ± 1.12^{cd}	10.81 ± 1.14^{c}	15.80^{bc}
T_8	23.67 ± 0.78^{a}	14.00 ± 0.98^{cd}	10.16 ± 1.04^{c}	15.94^{bc}
T_9	24.20 ± 2.00^{a}	18.03 ± 1.16^{a}	15.05 ± 0.15^{a}	19.10^{a}
T_{10}	22.27 ± 2.90^{a}	17.13 ± 2.06^{ab}	13.16 ± 1.02^{b}	17.52^{ab}
T_{11}	22.07 ± 1.79^{a}	13.50 ± 0.66^{cd}	10.03 ± 0.32^{c}	15.20^{bc}
T_{12}	23.60 ± 1.76^{a}	12.10 ± 0.20^{d}	6.00 ± 0.31^{e}	13.90^{c}
F value	0.58*	5.72**	30.14**	6.43**

* Significant at 5% level
** Significant at 1% level
Same set of alphabets indicates insignificant difference (Tukey's)

Peroxidase enzyme activity

There was a significant and steady increase in peroxidise (POD) activity with the string of storage period. Changes in POD activity of pre-cut bitter gourd slices were clearly distinguished by the different treatments (Table 6). Control (T_{12}) recorded the highest POD activity (1.37, 2.83 and 4.36 /min/g at 7, 14 and 21 days after storage, respectively) followed by T_9 treatment (100 ppm NaOCl + 2% $CaCl_2$) which recorded 0.53, 1.06 and 1.60 /min/g at 7, 14 and 21 days after storage, respectively. POD activity is an indicator of quality deterioration such as flavour loss and various biodegradation reactions. Calcium prevents the destruction of cell compartments. Effectiveness of calcium salts in reducing the POD activity was also reported in egg plant (Barbagallo *et al.*, 2012) [5] and in apple (Alandes *et al.*, 2006) [2].

Table 6: Effect of chemical pre-treatments on peroxidise activity (/min/g) of minimally processed bitter guard cv. Varsha

Treatments	7 days after storage	14 days after storage	21 days after storage	Pooled Mean
T_1	1.16 ± 0.11^{de}	1.92 ± 0.10^{def}	3.08 ± 0.10^{de}	2.05^{e}
T_2	1.33 ± 0.13^{e}	2.10 ± 0.19^{f}	3.41 ± 0.43^{ef}	2.28^{e}
T_3	1.29 ± 0.22^{e}	2.05 ± 0.23^{f}	3.54 ± 0.43^{f}	2.29^{e}
T_4	0.88 ± 0.10^{bc}	1.73 ± 0.12^{cd}	1.92 ± 0.11^{ab}	1.51^{bc}
T_5	1.35 ± 0.08^{e}	2.14 ± 0.07^{f}	2.49 ± 0.44^{c}	1.99^{de}
T_6	1.15 ± 0.07^{de}	1.92 ± 0.09^{def}	2.98 ± 0.10^{d}	2.02^{de}
T_7	0.81 ± 0.07^{b}	1.53 ± 0.13^{bc}	1.95 ± 0.19^{ab}	1.43b^{c}
T_8	1.03 ± 0.05^{cd}	1.79 ± 0.08^{de}	2.05 ± 0.19^{b}	1.62^{c}

T_9	0.53 ± 0.08^{a}	1.06 ± 0.10^{a}	1.60 ± 0.16^{a}	1.06^{a}
T_{10}	0.68 ± 0.04^{ab}	1.31 ± 0.18^{b}	1.83 ± 0.18^{ab}	1.27^{ab}
T_{11}	1.02 ± 0.06^{cd}	1.98 ± 0.04^{ef}	2.17 ± 0.08^{bc}	1.72^{cd}
T_{12}	1.37 ± 0.13^{e}	2.83 ± 0.15^{g}	4.36 ± 0.12^{g}	2.86^{f}
F value	17.08**	33.31**	42.93**	69.42**

* Significant at 5% level
** Significant at 1% level
Same set of alphabets indicates insignificant difference (Tukey's)

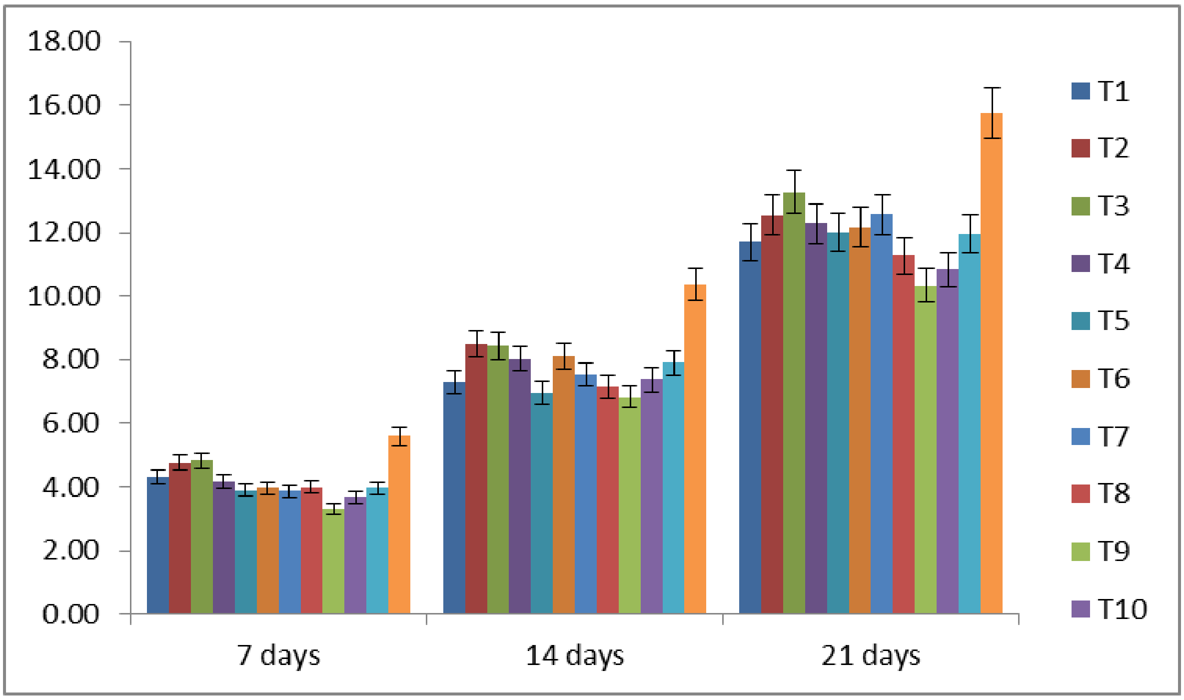

Fig 1: Effect of chemical pre-treatments on microbial count (cfu/g) content of minimally processed bitter guard cv. Varsha

T_1-100 ppm NaOCl	T_7 - 100 ppm NaOCl + 0.5% citric acid,
T_2 - 0.5% citric acid,	T_8 - 100 ppm NaOCl + 0.5% ascorbic acid,
T_3 - 0.5% ascorbic acid,	T_9 - 100 ppm NaOCl + 2% calcium chloride,
T_4 - 2% calcium chloride	T_{10} - 100 ppm NaOCl + 0.1% sodium benzoate,
T_5 – 0.1% sodium benzoate	T_{11} -100 ppm NaOCl + 1% vinegar and
T_6 - 1% vinegar	T_{12} - distilled water as control

Microbial count

The effect of different chemical treatments on total microbial count of pre-cut bitter gourd slices were evaluated for its shelf life study. Total mesophilic count was significantly differed among the treatments at 7, 14 and 21 days after refrigerated storage (fig.1). Slices treated with 100 ppm NaOCl + 2% $CaCl_2$ (T9) recorded less viable counts (3.30, 6.83 and 10.33 X10^6cfu/ml respectively) and is closely followed by T_{10} (100 ppm NaOCl + 0.1% sodium benzoate). Sodium benzoate has antimicrobial action due to the accumulation of protons and anions inside the microbial cell which disrupts the normal metabolism (Lopez-Malo *et al.*, 2007) [20]. Washing of different fresh-cut vegetables with acidified sodium hypochlorite effectively inhibited the microbial population by inhibition of microbial protein synthesis (Sun *et al.* 2012 [34]; Trinetta *et al.*, 2012) [38]. Calcium salts may reduce water activity, which can lead to delayed microbial growth (Luna-Guzman and Barret, 2000). Silveier *et al.* (2011) reported that calcium salt treatments can reduce microbial population effectively in fresh cut 'Galia' melon. The combination of the NaOCl with calcium chloride not only reduced the microbial counts and also helped to slow down the microbial growth which might be due to the combined effect of calcium which strengthens the cell wall and antimicrobial effect of NaOCl. Significant reduction in aerobic mesophilic counts with NaOCl in combination with $CaCl_2$ was also reported by Sunthon *et al.* (2016) [35] in fresh cut rose apple, Luo *et al.* (2011) [23] in fresh cut apple and Allendo *et al.* (2009) in fresh-cut cilantro.

This study revealed that the dipping the pre-cut bitter gourd slices in 100 ppm sodium hypochlorite + 2% $CaCl_2$ was relatively best in maintaining the quality parameters like physiological loss in weight, chlorophyll content, ascorbic acid content, total carotenoids and reduction in aerobic mesophilic microorganisms and delay in microbial growth during refrigerated storage. It has been possible to extend the shelf life of minimally processed bitter gourd samples upto 21 days under refrigerated storage by treating with biologically safe chemicals like NaOCl in combination with $CaCl_2$.

References

1. Aguayo E, Allende A, Artés F. Keeping Quality and Safety of Minimally Fresh Processed Melon. European Food Res. Tech 2003;216:494-499.
2. Alandes L, Hernando I, Quiles A, Perez-Munuera I, Lluch MA. Cell Wall Stability of Fresh-cut Fuji Apples Treated With Calcium Lactate. J. Food Sci 2006;71(9):615-620.

3. Allende A, Mc Evoy JL, Tao Y, Luo Y. Antimicrobial Effect of Acidified Sodium Chlorite, Sodium Chlorite, Sodium Hypochlorite, ond Citric Acid On *Escherichia coli* O157:H7 and Natural Microflora of Fresh-cut Cilantro. Food Control 2009;20:230-234
4. AOAC. Association of Official Analytical Chemists. Methods of analysis. 17th Ed. Association of Official Analytical Chemistry, Washington, USA 2000.
5. Barbagallo RN, Chisari M, Caputa G. Effects of Calcium Citrate and Ascorbate as Inhibitors of Browning and Softening in Minimally Processed 'Birgah' Egg Plants. Postharvest Biology and Technology 2012;73:107-114.
6. Baskaran SA, Upadhyay A, Kollanoor Johny A, Upadhyaya I, Mooyottu S. Efficacy of Plant Derived Antimicrobials as Antimicrobial Wash Treatments for Reducing Enterohemorrhagic *Escherichia Coli* O157: H7 on Apples. J. Food Sci 2013;78(9):1399-1404
7. Beaulieu JC, Lea JM. Quality changes in cantaloupe during growth, maturation, and in stored fresh-cuts prepared from fruit harvested at various maturities. J. Am. Soc. Hortic. Sci 2007;132(5):720-728.
8. Biacs PA, Daood HG. High-performance Liquid Chromatography and Photodiode-Array Detection of Carotenoids and Carotenoid Esters in Fruits and Vegetables. Journal of Plant Physiology 1994;143:520-25.
9. Cefalu WT, Ye J, Wang ZQ. Efficacy of Dietary Supplementation with Botanicals on Carbohydrate Metabolism in Humans. Endocrine, Metabolic and Immune Disorders-Drug Targets 2008;8:78-81.
10. de Carvalho LMJ, Gomes PB, de Oliveira Godoy RL, Pacheco S, Do Monte PHF, Ramos SRR. Total Carotenoid Content, A-Carotene And B-Carotene of Landrace Pumpkins (*Cucurbita moschata* Duch): A Preliminary Study. Food Research International 2012;47(2):337-340.
11. El-Ramady HR, Domokos-Szabolcsy É, Abdalla NA, Taha HS, Fári M. Post-harvest management of fruits and vegetables storage. In: Sustainable agriculture reviews, springer, Cham 2015,65-152p.
12. Garcia LGC, Silva EP, Silaneto CDM, Vilasboas EVB, Asqueri ER, Damiani C *et al*. Effect of the addition of calcium chloride and different storage temperatures on the post harvest of jabuticaba variety Pingi de Mel. Food Science and Technology 2019;39(1):26-269.
13. Gil MI, Aguayo E, Kader AA. Quality Changes and Nutrient Retention in Fresh-cut Versus Whole Fruits During Storage. Journal of Agriculture and Food Chemistry 2006;54(12):4284-4296.
14. Guan W, Fan X. Combination of sodium chlorite and calcium propionate reduces enzymatic browning and microbial population of fresh-cut “Granny Smith” apples. Journal of Food Science 2010;75:72-77.
15. He Q, Luo Y. Enzymatic browning and its control in fresh-cut produce. Stewart Postharvest Review 2007;3:1-7.
16. Jiang Y, Pen L, Li J. Use of citric acid for shelf life and quality maintenance of fresh-cut Chinese water chestnut. Journal of Food Engineering 1999;63:325-328.
17. Jinto and James. Influence of Pre-treatments on post harvest quality attributes and shelf life extension of papaya (*Carica papaya* L) fruits stored at low temperature. Kays, S. Post harvest physiology of perishable plant products. Georgia. Exon Press 2011.
18. Keutgen AJ, Poberezny J, Wszelczynska E. Non-invasive quality determination of spinach under simulated sale conditions and prediction of possible changes. Journal of Elementology 2012;17(2):69-278.
19. Leon AP, Frezza D, Logegaray VR, Harris M, Chiesa A. Calcium chloride dip and post harvest behaviour of butter head lettuce minimally processed. Acta Horticulture 2010;875:191-204.
20. Lopez-Galvez F, Ragaert P, Palermo LA, Eriksson M, Devlieghere F. Effect of new sanitizing formulations on quality of fresh-cut iceberg lettuce. Postharvest Biology and Technology 2013;85:102-108.
21. López-Malo J, Barreto-Valdivieso E, Palou and Martín FS. *Aspergillus Flavus* growth response to cinnamon extract and sodium benzoate mixtures. Food Control 2007;18(11):1358-1362
22. Lozano-de-Ganzales, Diane M, Barrett, Ronald E, Wrolst, Robert W Durst. Enzymatic browning inhibited in fresh and dried apple rings by pineapple juice. Journal of Food Science 1993;58:399-404.
23. Luo Y, Lu S, Zhou B, Feng H. Dual effectiveness of sodium chlorite for enzymatic browning inhibition and microbial inactivation on fresh-cut apples. LWT Food Science and Technology 2011;44:1621-1625.
24. Mahajan PV, Caleb OJ, Singh Z, Watkins CB, Geyer M. Postharvest treatments of fresh produce. Philosophical Transactions of Royal Society A 2014;372:201-303
25. Manganaris GA, Vasilakakis M, Diamantidis G, Mignani I. The effect of postharvest calcium application on tissue calcium concentration, quality attributes, incidence of flesh browning and cell wall physicochemical aspects of peach fruits. Food Chemistry 2007;100:1385-1392.
26. Martano G, Vogl C, Bojaxhi E, Bresgen N, Eckl P, Stutz H. Solid-Phase extraction and gc-ms analysis of potentially genotoxic cleavage products of β-carotene in primary cell cultures. Analytical and bioanalytical chemistry 2011;400(8):2415-242.
27. Ong G, Yap C, Maziah M, Tan S. Synergistic and antagonistic effects of zinc bioaccumulation with lead and antioxidant activities in *Centella Asiatica*. Sains Malaysiana 2013;42(11):1549-1555.
28. Rico D, Martín-Diana AB, Barat JM, Barry-Ryan C. Extending and measuring the quality of fresh-cut fruit and vegetables: A Review. Trends in Food Science and Technology 2007;8(7):373-86.
29. Sapers GM, Miller RL. Heated ascorbic/citric acid solution as browning inhibitor for pre- peeled potatoes. Journal of Food Science 1995;60:762-776.
30. Sheetal Devi, Deependra Kumar Mahato, Sarvesh Singh, Surendra Prasad Singh. Optimization of process variables for retention of functional properties of bitter melon (*Memordica charantia* L.) and its shelf life extension. Current Agriculture Research Journal 2019;7(1):74-89.
31. Siroli LF, Patrignani F, Serrazenetti DI, Tabanelli G, Manohari C, Tappi S. Efficacy of natural antimicrobials to prolong the shelf-life of minimally processed apples packaged in modified atmosphere. Food Control 2014;46:1-6.
32. Sivaramakrishna VNP, Dorajee Rao AVD, Giridar K, Latha P. Quality and shelf life evaluation of minimally processed papaya using chemical pre-treatments. International Journal of Pure and Applied Bioscience 2018;6(1):1276-1282.
33. Soliva-Fortuny RC, Martin-Belloso O. New advances in

extending the shelf life of fresh-cut fruits: A Review. Trends in Food Science and Technology 2003;14:341–353.
34. Sun SH, Kim SJ, Kwak SJ, Yoon KS. Efficacy of sodium hypochlorite and acidified sodium chlorite in preventing browning and microbial growth on fresh-cut produce. Preventive Nutrition and Food Science 2012;17(3):210-216
35. Sunthon Mola, Apiradee Uthairatanakij, Varit Srilaong, Sukunya Aiamla-or, Pongphen Jitareerat. Impacts of sodium chlorite combined with calcium chloride and calcium ascorbate on microbial population, browning and quality of fresh-cut rose apple. Agriculture and Natural Resources 2016;50(5):331-337.
36. Suslow TV. Post harvest chlorination – basic properties and key points for effective disinfection. Technical Report. Publication no. 8003 by the regents of the University of California, CA (USA) 1997.
37. Tirawat D, Kunimoto H, Noma S, Igura N, Shimoda M. Efficacy between the rapid hydrothermal pasteurization and sodium hypochorite treatments. Food and Nutrition Sciences 2013;4(6):636.
38. Trinetta V, Morgan M, Linton R. chlorine dioxide for microbial de contamination of food. A. Demirci, M.O. Ngadi (Eds.), Microbial Decontamination in the Food Industry: Novel Methods and Applications, Wood head Publishing Series in Food Science, Technology and Nutrition, Cambridge, UK 2012,533-562p.
39. Ugo De Corato. Improving the shelf-life and quality of fresh and minimally-processed fruits and vegetables for a modern food industry: a comprehensive critical Review from the traditional technologies into the most promising advancements, Critical Reviews in Food Science and Nutrition 2020;60(6):940-975
40. Wang L, Li Q, Cao J, Cai T, Jiang W. Keeping quality of fresh cut bitter gourd (*Momordica charantia*.) at low temperature of storage. J. Food Proc. Preserv 2007;31:571-582.
41. Yadav, Swati. Enhancement of shelf life of minimally processed bitter gourd (*Momordica charantia* L.). M.Sc. thesis submitted to CCSHAU 2011.