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

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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 T1 -100 ppm sodium hypochlorite (NaOCl), T2 - 2% citric acid, T3 - 2% ascorbic acid, T4 - 2% calcium chloride (CaCl2), T5 - 0.1% sodium benzoate, T6 - 1% vinegar, T7 - 100 ppm NaOCl+ 0.5% citric acid, T8 - 100 ppm NaOCl+ 0.5% ascorbic acid, T9 - 100 ppm NaOCl+ 2% CaCl2, T10 - 100 ppm NaOCl + 0.1% sodium benzoate, T11 -100 ppm NaOCl+ 1% vinegar and T12 - distilled water as control to study their impact on quality during 21 days of refrigerated storage. Among the treatments, T5 (100 ppm NaOCl + 2% CaCl2) 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++ ion maintains firmness by cross linking with cell wall and middle lamella pectin (Rico et al., 2007) [24] 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 CaCl₂ extend the storage life of pears up to 2 months, plums up to 4 weeks and apples up to 6 months at 0-2°C 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 antimicrobial 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 [32]); 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) [16] reported that, fresh cut bitter gourd stored at 2°C had storage life of 4 days. Skeethal Devi et al.(2019) [10] found that fresh-cut bitter gourd washed with disinfectant had reported 28 days storage life at 5°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 T₁-100 ppm sodium hypochlorite (NaOCl), T₂ - 0.5% citric acid, T₃ - 0.5% ascorbic acid, T₄ - 2% calcium chloride (w/v), T₅ -0.1% sodium benzoate (w/v), T₆ - 1% vinegar (v/v), T₇ - 100 ppm NaOCl; + 0.5% citric acid, T₈ - 100 ppm NaOCl; + 0.5% Ascorbic acid, T₉ - 100 ppm NaOCl; + 2% calcium chloride, T₁₀ - 100 ppm NaOCl; + 0.1% sodium benzoate, T₁₁ -100 ppm NaOCl; + 1% vinegar and T₁₂ - distilled water as control. The treatments were imposed by dipping the bitter guard slices for 30 seconds in chemical solutions and stored at 5°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 with 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 guard 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 aqeous 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) / ([A/cm1% × 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 H₂O₂ 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 ANOVA as well as one way ANOVA by Tukey’s post hoc test at 7, 14, 21 days of storage for multiple comparison among the treatments using SPSS (20 version).

**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 ANOVA, 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% CaCl2 (T9) 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<sup>a</sup>               | 0.31<sup>b</sup>          | 14.28<sup>c</sup> | 2.05<sup>b</sup>   | 34.02<sup>cd</sup> | 7.87<sup>bc</sup> |
| T2         | 14.08<sup>a</sup>               | 0.37<sup>a</sup>          | 15.48<sup>bc</sup>| 2.28<sup>c</sup>   | 38.52<sup>bc</sup> | 8.60<sup>b</sup>  |
| T3         | 13.67<sup>a</sup>               | 0.38<sup>bc</sup>         | 15.33<sup>bc</sup>| 2.29<sup>c</sup>   | 38.41<sup>bc</sup> | 8.84<sup>b</sup>  |
| T4         | 12.19<sup>a</sup>               | 0.43<sup>bc</sup>         | 15.56<sup>c</sup> | 1.51<sup>ab</sup>  | 40.00<sup>b</sup>  | 8.16<sup>b</sup>  |
| T5         | 13.22<sup>a</sup>               | 0.38<sup>bc</sup>         | 17.40<sup>bc</sup>| 1.99<sup>bc</sup>  | 38.03<sup>bc</sup> | 7.62<sup>cd</sup> |
| T6         | 12.90<sup>a</sup>               | 0.43<sup>bc</sup>         | 15.37<sup>bc</sup>| 2.02<sup>bc</sup>  | 38.55<sup>bc</sup> | 8.08<sup>bc</sup> |
| T7         | 10.88<sup>b</sup>               | 0.56<sup>b</sup>          | 15.80<sup>bc</sup>| 1.43<sup>b</sup>   | 42.37<sup>bc</sup> | 7.99<sup>bc</sup> |
| T8         | 11.66<sup>a</sup>               | 0.44<sup>bc</sup>         | 15.94<sup>c</sup> | 1.62<sup>c</sup>   | 41.38<sup>bc</sup> | 7.48<sup>bc</sup> |
| T9         | 9.24<sup>a</sup>                | 0.65<sup>a</sup>          | 19.10<sup>bc</sup>| 1.06<sup>c</sup>   | 47.43<sup>c</sup>  | 6.82<sup>a</sup>  |
| T10        | 10.29<sup>b</sup>               | 0.61<sup>a</sup>          | 17.52<sup>bc</sup>| 1.27<sup>bc</sup>  | 39.06<sup>bc</sup> | 7.29<sup>b</sup>  |
| T11        | 11.78<sup>c</sup>               | 0.47<sup>c</sup>          | 15.20<sup>c</sup> | 1.72<sup>cd</sup>  | 41.29<sup>bc</sup> | 7.93<sup>cd</sup> |
| T12        | 15.92<sup>f</sup>               | 0.29<sup>f</sup>          | 13.90<sup>f</sup> | 2.86<sup>f</sup>   | 30.87<sup>cd</sup> | 10.57<sup>bc</sup>|
| 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)

T1 - 100 ppm NaOCl
T2 - 0.5% citric acid,
T3 - 0.5% ascorbic acid,
T4 - 2% calcium chloride
T5 - 0.1% sodium benzoate
T6 - 1% vitamin B12

T7 - 100 ppm NaOCl + 0.5% citric acid,
T8 - 100 ppm NaOCl + 0.5% ascorbic acid,
T9 - 100 ppm NaOCl + 2% calcium chloride,
T10 - 100 ppm NaOCl + 0.1% sodium benzoate,
T11 - 100 ppm NaOCl + 1% vitamin B12

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 T9 (100 ppm NaOCl + 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% calcium chloride (T9) 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) [117]. The significant reduction in loss of physiological weight of bitter melon slices dipped in 2% CaCl2 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) [113]. 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% CaCl2 pre-treated bitter melon slices and in jaboticaba (Garcia et al., 2019) [12].

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Ascorbic acid content
A significant reduction in the content of ascorbic acid was observed throughout in all the treatments (Table 4). However, T_3 (100ppm NaOCl + 2% CaCl_2) and T_4 (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) [19]. 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].

| Treatments | 7 days after storage | 14 days after storage | 21 days after storage | Pooled Mean |
|------------|----------------------|-----------------------|----------------------|-------------|
| T_1        | 54.64 ± 3.15        | 43.01 ± 2.34           | 13.39 ± 1.53         | 34.02       |
| T_2        | 61.57 ± 2.71         | 38.78 ± 1.04           | 15.20 ± 1.10         | 38.52       |
| T_3        | 66.53 ± 3.14         | 41.48 ± 1.95           | 20.94 ± 0.89         | 42.98       |
| T_4        | 60.45 ± 0.78         | 39.99 ± 0.63           | 19.54 ± 0.51         | 39.99       |

* 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% 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.

| Treatments | 7 days after storage | 14 days after storage | 21 days after storage | Pooled Mean |
|------------|----------------------|-----------------------|----------------------|-------------|
| T_1        | 0.51 ± 0.03         | 0.25 ± 0.02           | 0.18 ± 0.01          | 0.31        |
| T_2        | 0.56 ± 0.04         | 0.36 ± 0.04           | 0.20 ± 0.01          | 0.37        |
| T_3        | 0.61 ± 0.03         | 0.33 ± 0.04           | 0.19 ± 0.03          | 0.38        |
| T_4        | 0.67 ± 0.03         | 0.38 ± 0.03           | 0.24 ± 0.02          | 0.43        |
| T_5        | 0.59 ± 0.01         | 0.36 ± 0.07           | 0.20 ± 0.01          | 0.38        |
| T_6        | 0.61 ± 0.02         | 0.41 ± 0.02           | 0.26 ± 0.03          | 0.43        |
| T_7        | 0.69 ± 0.06         | 0.55 ± 0.05           | 0.44 ± 0.06          | 0.56        |
| T_8        | 0.62 ± 0.03         | 0.32 ± 0.02           | 0.37 ± 0.02          | 0.44        |
| T_9        | 0.77 ± 0.02         | 0.63 ± 0.02           | 0.55 ± 0.03          | 0.65        |
| T_10       | 0.72 ± 0.01         | 0.60 ± 0.01           | 0.51 ± 0.04          | 0.61        |
| T_11       | 0.62 ± 0.07         | 0.49 ± 0.04           | 0.30 ± 0.01          | 0.47        |
| T_12       | 0.53 ± 0.03         | 0.22 ± 0.02           | 0.14 ± 0.01          | 0.29        |
| 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)
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% CaCl₂ (T₃) has recorded the highest total carotenoids content at 7, 14 and 21 days of storage (24.20, 18.03 and 12.46 μg/g at 7, 14 and 21 days after storage, respectively). 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] 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 ascorbic acid during raw material processing. Oxidative protection of carotenoids by ascorbic acid has previously has also been reported (Biacs and Daood, 2000) [8] and Yadav and Swati (2011) [41] in bitter gourd and Jinto and James (2011) [17] and Sivaramakrishna et al., (2018) [12] 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 |
|------------|----------------------|----------------------|----------------------|-------------|
| T₁        | 22.33 ± 0.84ₐ         | 12.13 ± 0.76ₐ        | 8.38 ± 0.65ₐ         | 14.28ₐ      |
| T₂        | 23.23 ± 1.7ₕ         | 12.93 ± 1.4₂         | 10.28 ± 0.9ₐ         | 15.48ₕ      |
| T₃        | 22.47 ± 1.0ₗ         | 12.47 ± 1.ₐₗ         | 11.04 ± 0.0ₗ         | 15.33ₜ      |
| T₄        | 22.57 ± 2.ₗₗ         | 12.7ₗₗ ± 1.ₗₗ       | 11.39 ± 0.ₗₗ         | 15.ₗₗₗ      |
| T₅        | 24.4ₗₗ ± 0.ₗₗ       | 15.₀ₗₗ ± 2.₀ₗₗ      | 12.7ₗₗ ± 0.ₗₗ         | 17.ₗₗₗ      |
| T₆        | 22.4₀ ± 1.ₗₗ       | 12.ₗₗ ± 1.₂ₗₗ       | 11.ₗₗ ± 0.ₗₗ         | 15.ₗₗₗ      |
| T₇        | 22.2ₗₗ ± 2.ₗₗ      | 14.ₗₗ ± 1.₂ₗₗ      | 10.ₗₗ ± 1.₂ₗₗ       | 1ₗₗₗₗ      |
| T₈        | 23.ₗₗ ± 0.ₗₗₗ      | 1ₗₗ ± 0.ₗₗₗ       | 10.ₗₗ ± 0.ₗₗₗ      | 1ₗₗₗₗ      |
| T₉        | 24.₂ₗₗ ± 2.₀ₗₗ     | 1ₗₗ ± 0.ₗₗₗ      | 1₀ₗₗ ± 2.ₗₗₗ      | 1ₗₗₗₗ      |
| T₁₀       | 22.₀ₗₗ ± 2.ₗₗₗ     | 1ₗₗ ± 0.ₗₗₗ      | 1₀ₗₗ ± 2.ₗₗₗ      | 1ₗₗₗₗ      |
| T₁₁       | 23.ₗₗ ± 0.ₗₗₗ    | 1ₗₗ ± 0.ₗₗₗ    | 1₀ₗₗ ± 2.ₗₗₗ    | 1ₗₗₗₗ      |
| T₁₂       | 2ₗₗ ± 0.ₗₗₗ   | 1₀ₗₗ ± 2.ₗₗₗ   | 6.₀ₗₗ ± 0.ₗₗₗ   | 1ₗₗₗₗ      |
| F value   | 0.ₘₗₗ ± 0.ₘₗₗ | 5.ₘₗₗ ± 0.ₘₗₗ | 3.₀ₘₗₗ ± 0.ₘₗₗ | 3.ₘₗₗₗ      |

* 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 (T₁₂) 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 T₃ treatment (100 ppm NaOCl + 2% CaCl₂) 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) [12].

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 |
|------------|----------------------|----------------------|----------------------|-------------|
| T₁        | 1.₁ₖ ± 0.₁₁ₖ        | 1.₂ₖ ± 0.₁³ₖ        | 3.₀ₖ ± 0.₁₀ₖ        | 2.₀₅ₖ      |
| T₂        | 1.₃ₖ ± 0.₁₃ₖ        | 2.₁ₖ ± 0.₁₉ₖ        | 3.₄₁ ± 0.₄₃ₖ        | 2.₉ₗₖ      |
| T₃        | 1.₂ₗₖ ± 0.₂₂ₗₖ      | 2.₀ₗₖ ± 0.₂₇ₗₖ      | 3.₃ₗₖ ± 0.₄₃ₗₖ      | 2.₂ₗₖ      |
| T₄        | 0.₃ₗₖ ± 0.₁₉₃ₗₖ    | 1.ₗₖ ± 0.₁₂ₗₖ    | 1.ₗₖ ± 0.₁₁₇ₖ    | 1.₅₁ₗₖ      |
| T₅        | 1.ₓₗₖ ± 0.₀₈ₘₗₖ   | 2.₁ₗₖ ± 0.₀₇ₘₗₖ   | 2.₄ₗₖ ± 0.₄₄ₘₗₖ   | ₁.₉ₗₘₖ      |
| T₆        | 1.₁ₗₖ ± 0.₀₇ₘₗₖ   | 1.ₗₖ ± 0.₀₉ₘₗₖ   | 2.₉ₘₖ ± 0.₁₀₉ₘₖ   | 2.₀₂ₗₘₖ      |
| T₇        | 0.ₖₗₖ ± 0.₀₇ₖₗₖ | 1.ₗₖ ± 0.₁₃ₖₗₖ | 1.₉ₖ ± 0.₁₉ₖₗₖ | 1.₄₃ₖₗₖ    |
| T₈        | 1.₀ₓ ± 0.₀₅₉ₘₗₖ | 1.ₗₖ ± 0.₀₈₉ₘₗₖ | 2.₀₅ ± 0.₁₉₉ₘₗₖ | 1.₆₂₉ₘₖ |
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| Treatment | Microbial Count (cfu/ml) | Treatment | Microbial Count (cfu/ml) | Treatment | Microbial Count (cfu/ml) | Treatment | Microbial Count (cfu/ml) | F value |
|-----------|--------------------------|-----------|--------------------------|-----------|--------------------------|-----------|--------------------------|---------|
| T1        | 0.53 ± 0.08a             | T9        | 1.06 ± 0.10a             | T7        | 1.60 ± 0.16a             |          |                          | 17.08** |
| T10       | 0.68 ± 0.04ab            | T8        | 1.06 ± 0.10a             | T10       | 1.06a                    |          |                          | 33.31** |
| T11       | 1.02 ± 0.06cd            | T11       | 1.98 ± 0.04df            | T11       | 1.27ab                   |          |                          | 42.93** |
| T12       | 1.37 ± 0.13e             | T12       | 2.83 ± 0.15f             | T12       | 2.86f                    |          |                          | 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/ml) content of minimally processed bitter guard cv. Varsha

T1 - 100 ppm NaOCl
T2 - 0.5% citric acid
T3 - 0.5% ascorbic acid
T4 - 2% calcium chloride
T5 - 0.1% sodium benzoate
T6 - 1% vinegar
T7 - 100 ppm NaOCl + 0.5% citric acid
T8 - 100 ppm NaOCl + 0.5% ascorbic acid
T9 - 100 ppm NaOCl + 2% calcium chloride
T10 - 100 ppm NaOCl + 0.1% sodium benzoate
T11 - 100 ppm NaOCl + 1% vinegar and 1% vinegar
treated with 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% CaCl₂ (T9) recorded less viable counts (3.30, 6.83 and 10.33 X10⁶ cfu/ml respectively) and is closely followed by T₁₀ (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) [30]. 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 ‘Galía’ 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 CaCl₂ was also reported by Sunthon et al. (2016) [35] in fresh cut rose apple, Luo et al. (2011) [33] 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% CaCl₂ 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 up to 21 days under refrigerated storage by treating with biologically safe chemicals like NaOCl in combination with CaCl₂.

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