EFFECT OF PRE-TREATMENTS ON BIOCHEMICAL AND MICROBIAL PARAMETERS OF GUAVA FRUIT DURING STORAGE

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ABSTRACT

Freshly harvested and fully matured guava fruits (Lucknow-49) were hydro-cooled at 2 ± 1°C for 10 min and pretreated with different treatments viz., calcium chloride (2 %), hydrogen peroxide (1 %), benomyl (0.1 %), neem oil (2 %), lemon grass oil (0.2 %), cinnamon oil (4 %) and ozone (150 mg/h). Fruits were packed in 50 µm LDPE bags and stored at 10 ± 1°C. Control fruits without any pre-treatment were stored at low as well as room temperature. The biochemical, sensory and microbial parameters of the guava fruit were recorded at 5 days interval during storage. Minimum TSS (14.1 °Brix) and total sugar (8.94 %) were observed in ozone treatment, while maximum titratable acidity (1.13 %) and ascorbic acid (236 mg/100 g) was observed in ozone treatment on 30 days of storage. Maximum sensory score was found in ozone and in cinnamon oil treatments. Microbial parameters viz., total plate count, E.coli, salmonella and yeast and mould in the fruit were found absent for ozone, cinnamon oil, neem oil and hydrogen peroxide treatments. Maximum changes in biochemical, sensory and microbial parameters were found in control at room temperature followed by control at low temperature. Shelf life of guava fruit could be increased up to 30 days with minimum changes in biochemical, sensory and microbial parameters. when the fruits pretreated with ozone (150 mg/h) followed by packaging in 50 µm LDPE bags at 10 ± 1°C storage temperature.

KEY WORDS: Guava, pre-treatments, ozone, packaging, storage

INTRODUCTION

India is the largest producer of the guava fruit (Psidium guajava L.) in the world. Guava being climacteric fruit, it has very short shelf life and marketing of fresh fruits to distant places is very difficult. The short harvesting season, limited domestic demand and improper storage facility creates glut in the market and consequently loss to the fruit growers. Due to its short shelf-life, as much as 18-20 per cent of fruits perish, as post-harvest losses during different post-harvest operations (Anonymous, 2014). Pre-storage treatments viz., pre-cooling, certain chemicals, plant extracts (essential oils), inhibitors, ozonation or combinations, prior to packaging and storage play an important role to control insect pests, yeast and mould on the food surface; prevent bacterial and fungal rots; destroy pesticides and chemical residues, which
ultimately leads to improve the shelf life of fruits.

Chemicals have been widely used to reduce the incidence of post-harvest disease. Although effective, many of these materials have been removed from the market in recent years because of economic, environmental or health concerns. These problems associated with the use of chemicals have stimulated the produce industry to identify alternative treatments equivalent to chlorine in antimicrobial effectiveness. The plant extracts are generally assumed to be more acceptable and less hazardous than synthetic compounds for preventing fungal decay in organic fruits after harvest which is a novel preservation approaches. Ozonation is a noble technology that can be used to sanitize produce. A naturally occurring molecule, ozone is a powerful disinfectant. The potential utility of ozone in the produce industry depends on the fact that as an oxidizing agent, it is 1.5 times stronger than chlorine and is effective over a much wider spectrum of microorganisms than chlorine and other disinfectants.

At present, no specific pre-treatment technique is followed for guava fruit to control insect pests, bacterial and fungal rots on the food surface as well as destruction of pesticides and chemical residues prior to packaging and storage. However, good pre-treatment before storage reduces post-harvest losses, preserves quality and prolongs the shelf life of fresh guava fruit to fulfill the consumers and market demand. International markets reject fruits and vegetables containing unauthorized pesticides, with pesticide residues exceeding permissible limits and with inadequate labeling and packaging. Obviously, post-harvest management determines food quality and safety, competitiveness in the market and the profits earned by producers.

**MATERIALS AND METHODS**

**Raw material**

Freshly harvested and fully matured guava fruit cv. Lucknow-49 at colour breaker stage were procured and brought to the laboratory in plastic crates to avoid any physical damage. The fruits were graded on the basis of weight (120-150 g) to maintain homogeneity and damaged fruits were sorted out. The fruits were thoroughly washed with clean water to remove dust and dirt particles. Then, the fruits were hydro-cooled (2 ± 1 °C) using ice water for 10 minutes to remove field heat. The pre-cooled fruits were pre-treated with different treatments viz., calcium chloride (2 %), hydrogen peroxide (1%), benomyl (0.1%), neem oil (2%), lemon grass oil (0.2%), cinnamon oil (4%) and ozone (150 mg/h). The fruits were kept in different solutions for 10 minutes, while ozone treated fruits were kept for 8 minutes in ozone purifier. There were two control treatments. Control fruits were not treated with pre-treatments. Control fruits packed in LDPE bags were stored at 10 ± 1 °C storage temperature. The control fruits without packaging were stored at room temperature (22 ± 7 °C) with RH range of 35-60 per cent. The details of treatments are as under:

**Details of treatments**

**A.Independent parameters :**
1. Calcium chloride (CaCl₂, 2%) for 10 minutes
2. Hydrogen peroxide (H₂O₂, 1%) for 10 minutes
3. Neem oil (2%) for 10 minutes
4. Lemongrass oil (0.2%) for 10 minutes
5. Cinnamon oil (4%) for 10 minutes
6. Benomyl (0.1%) for 10 minutes
7. Ozone (150 mg/h) for 8 minutes
8. Control at 10 ± 1 °C storage temperature (with packaging)
9. Control at room temperature storage (22 ± 7 °C) (without packaging)

**B. Treatments: 09**

**C. No. of replications : 04**

**Storage study**

After application of pre-treatments, the fruits were dried under shade to remove surface moisture. Two fruits
together were packed in a 150 x 225 mm size of 50 µm thick LDPE bag with 80-100 mm headspace. The samples were stored at 10 ± 1 °C with 80-85 per cent relative humidity (RH) in cold chamber. There were two control treatments. Control fruits were not treated with pre-treatments. Control fruits packed in LDPE bags were stored at 10 ± 1 °C storage temperature. The control fruits without packaging were stored at room temperature (22 ± 7 °C) with RH range of 35-60 per cent.

**Biochemical parameters**

Total soluble solids (TSS) was measured by hand refractometer (range 0-90 %) and corrected at 20°C. Total sugar was determined by phenol sulphuric acid method as reported by Sadasivam and Manikam (1996), while ascorbic acid and titratable acidity was estimated as reported by Ranganna (2000).

**Sensory analysis**

Sensory characteristic in terms of overall acceptability of guava fruit was evaluated on the basis of appearance, pulp colour and taste after ripening of the fruits at room temperature (30 ± 2°C) for two days by covering gunny bag. Sensory characteristics of ripe fruits were evaluated by a panel of semi trained 10 judges using 9 point hedonic scale (Amerine et al., 1965).

**Microbial parameters**

Total plate counts was measured using N-agar method, E.coli was measured using EMB-agar method, Salmonella using SS agar method and yeast and mould was measured using PDA-agar method as suggested by Downes and Ito (2001).

**Statistical analysis**

The observations taken for various parameters of guava fruits at 5 days interval during storage were subjected to analysis of variance technique considering Completely Randomized Design with four replications as suggested by Panse and Sukhatme (1985). All the treatments of the experiment were compared at 5 per cent level of significance.

**RESULTS AND DISCUSSION**

**Biochemical parameters**

**Total soluble solids (**°Brix**)**

TSS of the fruit was enhanced with increased in storage period, which might be due to the increased in concentration of organic solutes as a consequence of water loss in the fruit. It is evident from the Figure 1 that TSS was observed minimum in ozone (14.1 °Brix) and maximum TSS was found in control at room temperature (16.5 °Brix) followed by control at low temperature (16.2 °Brix) at the end of storage period. The increased in TSS during storage was also reported by Wijewardane and Guleria (2009) in apple.

**Total sugar (%)**

The total sugar of the fruit was increased with increased in storage period. The increase in total sugar with storage period might be due to the release of sugars by the hydrolysis of polysaccharides. From the Figure 2, it was cleared that total sugars was observed minimum in ozone (8.94 %) and maximum in control at room temperature (13.28 %) followed by control at low temperature (12.11 %) at the end of storage period. The increased in total sugars with storage period was also reported by Eman et al. (2013) in mango.

**Titratable acidity (%)**

Titratable acidity of the fruit was decreased with increased in storage period. The decrease in titratable acidity might be due to the conversion of acids into sugars and also use of organic acids as respiratory substrate during storage. From the Figure 3, it was observed that titratable acidity was observed maximum in ozone (1.13 %) and minimum titratable acidity was recorded in control at low temperature (0.76 %) at the end of storage period. Similar results for titratable acidity were also reported by Eman et al. (2013) in mango.

**Ascorbic acid (mg/100g)**

Ascorbic acid of the fruit declined with advancement of storage period. The decrease in ascorbic acid might be due to the process of oxidation of ascorbic acid.
From the Figure 4, it was cleared that ascorbic acid was found maximum in ozone (236 mg/100 g) and minimum ascorbic acid was recorded in control at room temperature (162 mg/100 g) followed by control at low temperature (165 mg/100 g) at the end of the storage period. These results for ascorbic acid are in agreement with the results reported by Monaco et al. (2014) in mango.

**Sensory evaluation**

From the Table 1, it was cleared that maximum sensory score of guava fruit (7.6) was observed in ozone and cinnamon oil treatments, while minimum score of overall acceptability was recorded in control at room temperature (4.5) followed by control at low temperature (5.3) at the end of the storage period. These results are in conformity with the results reported by Antala et al. (2014) in guava.

**Microbial parameters**

**Total plate counts**

Total plate counts were found absent after pre-treatments of guava fruit at initial stage. The mean value of total plate count of control fruits at initial stage after hydro-cooling was 2×10² cfu/g. From the Table 2, it was cleared that total plate counts of the fruit increased with increased in storage period in benomyl, calcium chloride, lemon grass oil and control. However, it was found absent in the fruits treated with ozone, cinnamon oil, neem oil and hydrogen peroxide throughout storage period. Minimum total plate counts were recorded in benomyl (8×10⁵ cfu/g) and maximum total plate counts were recorded in control at room temperature (14×10⁴ cfu/g) followed by control at low temperature (11×10⁴ cfu/g) at the end of storage period. These results for total plate counts are in agreement with Bialka and Demirci (2007) in raspberry and strawberry.

**E. coli**

The effect of different pre-treatments on *E. coli* of guava fruit during storage is presented in Table 3. *E. coli* was not observed after and before pre-treatments at an initial stage including control. *E. coli* was found present in lemon grass oil (1×10² cfu/g) and control at low temperature (3×10² cfu/g) on 30 days of storage. It was also found in control at room temperature (2×10⁴ cfu/g) on 10 days of storage. Similar findings for *E. coli* were also reported by Achen and Yousef (2001) in apple.

**Yeast and mould**

Yeast and mould in guava fruits were found absent after pre-treatments at initial stage. From the Table 4, it was apparent that yeast and mould was found absent in the fruits for ozone, cinnamon oil, neem oil and hydrogen peroxide treatments during the entire storage period. Yeast and mould in calcium chloride, benomyl and lemon grass oil was observed 8×10² cfu/g, 11×10² cfu/g and 2×10⁴ cfu/g, respectively at the end of storage period. Maximum yeast and mould was recorded in control at room temperature (16×10⁴ cfu/g) followed by control at low temperature (3×10⁴ cfu/g) at the end of storage period. These results for yeast and mould are in conformity with the results reported by Aday et al. (2014) in strawberry and Abd-El-Latif (2016) in apple.

**CONCLUSION**

It can be concluded that ozone treatment (150 mg/h) followed by packaging in 50 µm LDPE bags at low temperature storage retained shelf life of fresh guava fruit up to 30 days with minimum change in biochemical, sensory and microbial parameters of the guava fruit.

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### Table 1: Effect of different pre-treatments on overall acceptability of guava fruit during storage

| Treatments        | Storage Period (Days) |      |      |      |      |      |
|-------------------|-----------------------|------|------|------|------|------|
|                   |                       | 5    | 10   | 15   | 20   | 25   | 30   |
| Calcium chloride  | 8.8                   | 8.1  | 7.7  | 7.4  | 7.2  | 6.8  |
| Hydrogen peroxide | 8.0                   | 7.5  | 7.1  | 6.9  | 6.7  | 6.0  |
| Neem oil          | 8.5                   | 8.1  | 7.7  | 7.5  | 7.0  | 6.7  |
| Lemon grass oil   | 7.7                   | 7.5  | 6.9  | 6.5  | 6.1  | 5.7  |
| Cinnamon oil      | 8.8                   | 8.4  | 8.2  | 7.9  | 7.8  | 7.6  |
| Benomyl           | 8.1                   | 7.8  | 7.4  | 7.2  | 7.0  | 6.4  |
| Ozone             | 8.8                   | 8.5  | 8.2  | 8.0  | 8.0  | 7.6  |
| Control-low temp. | 7.5                   | 7.0  | 6.5  | 6.2  | 6.0  | 5.3  |
| Control-room temp.| 6.5                   | 4.5  | -    | -    | -    | -    |
| S.Em.±            | 0.17                  | 0.27 | 0.22 | 0.29 | 0.22 | 0.31 |
| C.D. at 5 %       | 0.50                  | 0.77 | 0.63 | 0.86 | 0.65 | 0.91 |
| C.V. %            | 4.25                  | 7.1  | 5.77 | 8.19 | 6.38 | 9.61 |

### Table 2: Effect of different pre-treatments on total plate counts (cfu/g) of guava fruits during storage

| Treatments        | Storage Period (Days) |      |      |      |      |      |
|-------------------|-----------------------|------|------|------|------|------|
|                   |                       | 0    | 5    | 10   | 15   | 20   | 25   | 30   |
| Calcium chloride  | Ab                    | Ab   | Ab   | Ab   | Ab   | 1×10⁴ | 3×10⁴ |
| Hydrogen peroxide | Ab                    | Ab   | Ab   | Ab   | Ab   | Ab   | Ab   |
| Neem oil          | Ab                    | Ab   | Ab   | Ab   | Ab   | Ab   | Ab   |
| Lemon grass oil   | Ab                    | Ab   | Ab   | 1×10⁴ | 3×10² | 1×10⁴ | 5×10⁴ |
| Cinnamon oil      | Ab                    | Ab   | Ab   | Ab   | Ab   | Ab   | Ab   |
| Benomyl           | Ab                    | Ab   | Ab   | Ab   | Ab   | 5×10⁵ | 8×10⁵ |
| Ozone             | Ab                    | Ab   | Ab   | Ab   | Ab   | Ab   | Ab   |
| Control-low temp. | 2×10⁴                 | 10×10⁴ | 2×10⁵ | 3×10⁴ | 5×10⁵ | 8×10⁴ | 11×10⁵ |
| Control-room temp.| 2×10⁴                 | 2×10⁴ | 14×10⁴ |      |      |      |      |

### Table 3: Effect of different pre-treatments on E.coli (cfu/g) of guava fruits during storage

| Treatments        | Storage Period (Days) |      |      |      |      |      |
|-------------------|-----------------------|------|------|------|------|------|
|                   |                       | 0    | 5    | 10   | 15   | 20   | 25   |
| Calcium chloride  | Ab                    | Ab   | Ab   | Ab   | Ab   | Ab   | Ab   |
| Hydrogen peroxide | Ab                    | Ab   | Ab   | Ab   | Ab   | Ab   | Ab   |
| Neem oil          | Ab                    | Ab   | Ab   | Ab   | Ab   | Ab   | Ab   |
| Lemon grass oil   | Ab                    | Ab   | Ab   | Ab   | Ab   | Ab   | 1×10⁴ |
| Cinnamon oil      | Ab                    | Ab   | Ab   | Ab   | Ab   | Ab   | Ab   |
| Benomyl           | Ab                    | Ab   | Ab   | Ab   | Ab   | Ab   | Ab   |
| Ozone             | Ab                    | Ab   | Ab   | Ab   | Ab   | Ab   | Ab   |
| Control-low temp. | Ab                    | Ab   | Ab   | Ab   | Ab   | Ab   | 3×10⁴ |
| Control-room temp.| Ab                    | Ab   | 2×10⁴ |      |      |      |      |

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Table 4: Effect of different pre-treatments on yeast and mould (cfu/g) of guava fruits during storage

| Treatments         | Storage Period (Days) |
|--------------------|-----------------------|
|                    | 0        | 5        | 10       | 15       | 20       | 25       | 30       |
| Calcium chloride   | Ab       | Ab       | Ab       | Ab       | 3x10^2   | 8x10^2   |          |
| Hydrogen peroxide  | Ab       | Ab       | Ab       | Ab       | Ab       | Ab       |          |
| Neem oil           | Ab       | Ab       | Ab       | Ab       | Ab       | Ab       |          |
| Lemon grass oil    | Ab       | Ab       | Ab       | 1x10^4   | 8x10^2   | 2x10^4   |          |
| Cinnamon oil       | Ab       | Ab       | Ab       | Ab       | Ab       | Ab       |          |
| Benomyl            | Ab       | Ab       | Ab       | Ab       | 7x10^2   | 11x10^2  |          |
| Ozone              | Ab       | Ab       | Ab       | Ab       | Ab       | Ab       |          |
| Control-low temp.  | 2x10^2   | 3x10^2   | 7x10^2   | 9x10^2   | 13x10^2  | 1x10^4   | 3x10^4   |
| Control-room temp. | 2x10^2   | 4x10^4   | 16x10^4  | -        | -        | -        | -        |

Fig. 1: Effect of different pre-treatments on TSS of guava fruit
Fig. 2: Effect of different pre-treatments on total sugars of guava fruit

Fig. 3: Effect of different pre-treatments on titratable acidity of guava fruit
Fig. 4: Effect of different pre-treatments on ascorbic acid of guava fruit

\[ y = -8.8295x + 297.9 \]
\[ R^2 = 0.9987 \]