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**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  $2 \pm 1$  °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  $10 \pm 1$  °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  $10 \pm 1$  °C storage temperature.

**Keywords:** Guava, Pre-Treatments, Chemical, Essential Oil, 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% of fruits perish as post-harvest losses during different post-harvest operations (Anon., 2014). Pre-storage treatments viz., pre-cooling, certain chemicals, plant extracts (essential oils), inhibitors, ozonation or combination 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 ( $2 \pm 1$  °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 for ozone treated the fruits were kept for 8 minutes in ozone purifier.

### 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  $10 \pm 1$  °C with 80-85 % 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  $10 \pm 1$  °C storage temperature. The control fruits without packaging were stored at room temperature ( $22 \pm 7$  °C) with Rh range of 35-60 %.

### Details of treatments

A. Independent parameters :

1. Calcium chloride ( $\text{CaCl}_2$ , 2%) for 10 minutes
2. Hydrogen peroxide ( $\text{H}_2\text{O}_2$ , 1%) for 10 minutes
3. Neem oil (2%) for 10 minutes
4. Lemon grass 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  $10 \pm 1$  °C storage temperature (with packaging)

9. Control at room temperature storage ( $22 \pm 7$  °C) (without packaging)

B. Treatments : 09

C. No. of replications : 04

### 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, while ascorbic acid and titratable acidity was estimated as reported by Ranganna.

### 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 ( $30 \pm 2$  °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. All the treatments of the experiment were compared at 5 per cent level of significance. The analysis of variance (ANOVA), standard error of mean (SEM), critical difference (CD), coefficient of variance (CV) and mean values were tabulated and the level of significance was reported as suggested by Panse and Sukhatme (1985).

## Results and discussion

### Biochemical parameters

#### Total soluble solids (°Brix)

TSS of the fruit enhanced with increase in storage period. The increase in TSS with storage period might be due to the increase 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 increase in TSS during storage was also reported by Wijewardane and Guleria (2009) in apple.

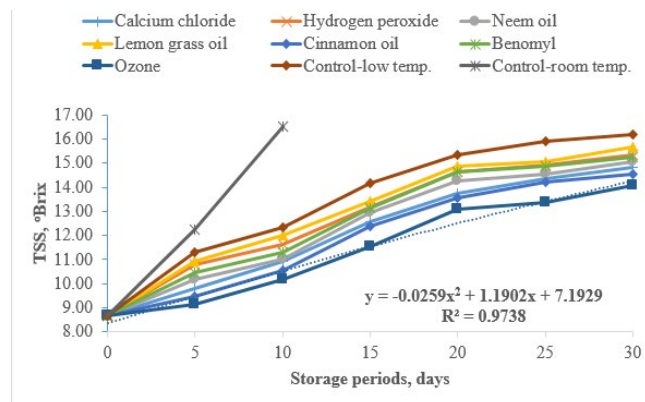


Figure 1 Effect of different pre-treatments on TSS of guava fruit

**Total sugar (%)**

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

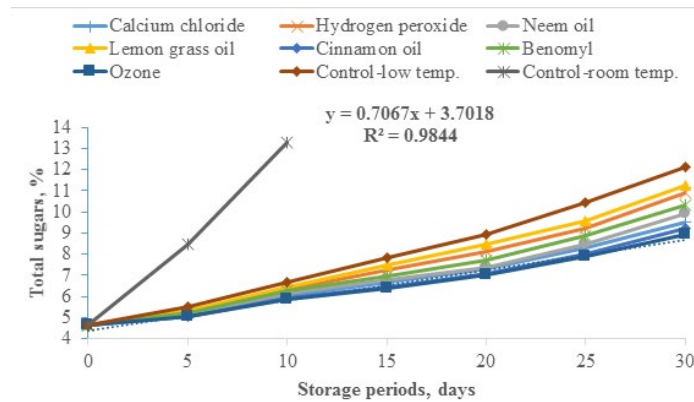


Figure 2 Effect of different pre-treatments on total sugars of guava fruit

**Titrateable acidity (%)**

Titrateable acidity of the fruit decreased with increase in storage period. The decrease in titrateable 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 can be observed that titrateable acidity was observed maximum in ozone (1.13 %) and minimum titrateable acidity was recorded in control at low temperature (0.76 %) at the end of storage period. Similar results for titrateable acidity were also reported by Eman et al. (2013) in mango.

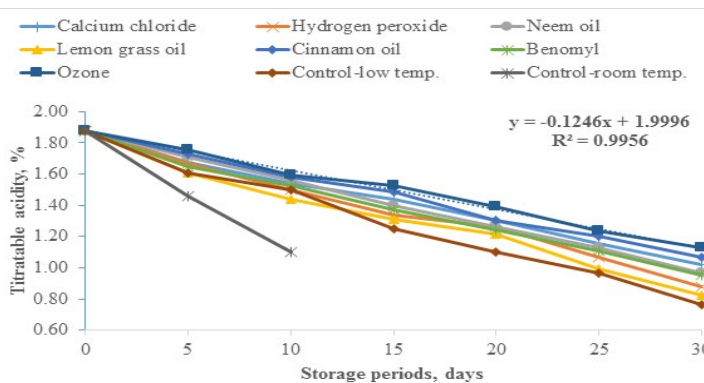


Figure 3 Effect of different pre-treatments on titrateable acidity of guava fruit

### 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 is clear 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.

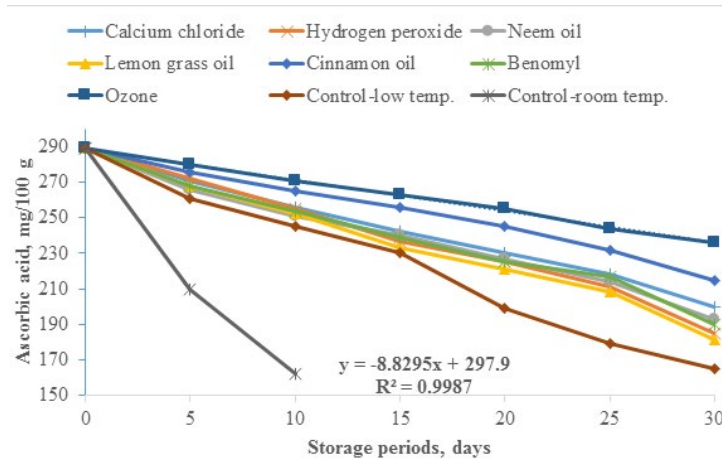


Figure 4 Effect of different pre-treatments on ascorbic acid of guava fruit

### Sensory evaluation

From the Table 1, it is clear 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.

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

## Microbial parameters

### Total plate counts

Total plate counts was 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  $2 \times 10^2$  cfu/g. From the table 2, it is clear that total plate counts of the fruit increased with increase 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 was recorded in benomyl ( $8 \times 10^2$  cfu/g) and maximum total plate counts was recorded in control at room temperature ( $14 \times 10^4$  cfu/g) followed by control at low temperature ( $11 \times 10^4$  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.

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	$11 \times 10^2$	$3 \times 10^4$
Hydrogen peroxide	Ab	Ab	Ab	Ab	Ab	Ab	Ab
Neem oil	Ab	Ab	Ab	Ab	Ab	Ab	Ab
Lemon grass oil	Ab	Ab	Ab	$1 \times 10^2$	$3 \times 10^2$	$1 \times 10^4$	$5 \times 10^4$
Cinnamon oil	Ab	Ab	Ab	Ab	Ab	Ab	Ab
Benomyl	Ab	Ab	Ab	Ab	Ab	$5 \times 10^2$	$8 \times 10^2$
Ozone	Ab	Ab	Ab	Ab	Ab	Ab	Ab
Control-low temp.	$2 \times 10^2$	$10 \times 10^2$	$2 \times 10^4$	$3 \times 10^4$	$5 \times 10^4$	$8 \times 10^4$	$11 \times 10^4$
Control-room temp.	$2 \times 10^2$	$2 \times 10^4$	$14 \times 10^4$	-	-	-	-

### 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 ( $1 \times 10^2$  cfu/g) and control at low temperature ( $3 \times 10^2$  cfu/g) on 30 days of storage. It was also found in control at room temperature ( $2 \times 10^4$  cfu/g) on 10 days of storage. Similar findings for E.coli were also reported by Achen and Yousef (2001) in apple.

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	30
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	$1 \times 10^2$
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	$3 \times 10^2$
Control-room temp.	Ab	Ab	$2 \times 10^4$	-	-	-	-



### Yeast and Mould

Yeast and mould in guava fruits were found absent after pre-treatments at initial stage. From the Table 4, it is apparent that yeast and mould was found absent in the fruits for ozone, cinnamon oil, neem oil and hydrogen peroxide treatments during entire storage period. Yeast and mould in calcium chloride, benomyl and lemon grass oil was observed  $8 \times 10^2$  cfu/g,  $11 \times 10^2$  cfu/g and  $2 \times 10^4$  cfu/g, respectively at the end of storage period. Maximum yeast and mould was recorded in control at room temperature ( $16 \times 10^4$  cfu/g) followed by control at low temperature ( $3 \times 10^4$  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.

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	Ab	$3 \times 10^2$	$8 \times 10^2$
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	$1 \times 10^2$	$8 \times 10^2$	$2 \times 10^4$
Cinnamon oil	Ab	Ab	Ab	Ab	Ab	Ab	Ab
Benomyl	Ab	Ab	Ab	Ab	Ab	$7 \times 10^2$	$11 \times 10^2$
Ozone	Ab	Ab	Ab	Ab	Ab	Ab	Ab
Control-low temp.	$2 \times 10^2$	$3 \times 10^2$	$7 \times 10^2$	$9 \times 10^2$	$13 \times 10^2$	$1 \times 10^4$	$3 \times 10^4$
Control-room temp.	$2 \times 10^2$	$4 \times 10^4$	$16 \times 10^4$	-	-	-	-

### Conclusions

It may be concluded that ozone treatment (150 mg/h) followed by packaging in 50  $\mu$ m 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 fruit.

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