EFFECT OF BEE WAX COATINGS ON PHYSIOLOGICAL CHANGES IN FRUITS OF SWEET ORANGE CV. “BLOOD RED”

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ABSTRACT

An experiment was conducted to evaluate the influence of bee wax coating on physiological changes in fruits of sweet orange cv. “Blood Red” at Horticulture Department, University of Arid Agriculture, Rawalpindi during 2004-05. Bee wax at the rate of 1.3 and 5% along with constant level of 0.5% benlate was used for proposed study. Results indicated that all waxing treatments maintained weight loss, firmness, pH, TSS, titratable acidity, TSS/acid ratio, reducing sugars, non-reducing sugars, total sugars and ascorbic acid. In this study, bee wax used at the rate of 5% along with 0.5% benlate gave better results for citrus storage than all other treatments and is recommended for waxing of Blood Red oranges kept at room temperature during the months of January, February and March in Rawalpindi conditions.

Key Words: Bee wax, Physiological changes, Sweet orange, Blood Red

INTRODUCTION

Citrus is the most important fruit crop being grown in Pakistan on an area of 0.19904 million hectares, producing 2.2945 million tons annually. Sargodha, Faisalabad, Sheikhupura and Multan districts of the Punjab province are the main citrus growing areas of Pakistan which constitutes major part of the export. Pakistan exported 96540 tones of citrus fruits, worth Rs. 185.75 millions during 2007 – 08. Kinnow account for 80% of the total, while oranges constitute 8% (GoP, 2009).

Blood Red oranges having attractive shade on skin and deep red to purplish-pigmented pulp with special delightful aroma that makes the commodity in great demand in country and as a potential candidate for export. Their demand increases from February onwards with increase in prices. Sweet oranges have great demand in Gulf, Europe and East Asia had to increase the export volume; it is necessary for the fruits to remain fresh and in good quality during the transit. But under ordinary conditions, sweet oranges cannot be stored for long duration due to various physiological and pathological disorders (Malik, 1994); as a result farmers have to sell their produce in bulk causing a glut condition in the market. In this way, farmers cannot get good prices and lose share in the profit.

Various types of skin coating materials (Bee wax, paraffin wax, carnauba wax, CaCl₂, shellac and CMC etc.) have been used to restrict moisture loss from the surface of Kinnow. Feutrell’s early, Pineapple and Valencia late cultivars in Pakistan (Ahmad et al., 1997; Farooqi et al., 1981). Post harvest treatments are used to reduce the loss of moisture from surface of fruits through evaporation, transpiration and respiration, metabolic activities within the fruits especially respiration and the effect of decay-causing microorganism (Malik., 1994). Post harvest handling operations should be conducted according to the requirement of the commodity. Pre-cooling or washing, drying, grading, proper packing, transportation and storage are all crucial in maintaining taste, flavour and edibility of fruits (Meena and Yadav, 2001). Keeping in view the high quality of Blood Red oranges produced in this area, the studies were designed to standardize the wax coating formulations for enhancement of shelf life with low deterioration in quality.

Citrus fruits have a natural waxy layer on the outer surface that is partially removed during washing. An extra discontinuous layer of wax applied artificially with sufficient thickness and consistency to prevent anaerobic respiration within the fruit provides the necessary protection against decay organisms. Tiny injuries and scratches on the surface of fruits can be sealed by wax application. Another obvious advantage of waxing is the enhancement of the gloss of fruits and vegetables. Appearance is therefore improved, making the produce more acceptable to the consumers (Pantastico, 1997).

Keeping in view the importance to increase the post harvest life of sweet oranges the present studies was carried out to evaluate the effect of bee wax on physiological characteristics of sweet oranges cv. “Blood Red”.

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MATERIALS AND METHODS

The experiment was conducted at the Postgraduate Laboratory of Horticulture Department, University of Arid Agriculture Rawalpindi during 2004 – 05. Blood Red oranges were harvested at proper physiological stage of maturity (when the fruits attained a specific color according to the variety and area) from Haripur in January 2004 and January 2005 (Javed et al., 1987). The fruits were brought to Horticulture Department Laboratory, UAAR in crates. These fruits were sorted out to remove diseased or bruised ones. The fruits were washed using tap water to remove dirt and spray residues and dried under fan. The clean dried fruits were then divided into 4 main lots each containing 90 fruits. Each treatment was replicated three times.

The treatments were as follows:

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₀</td>
<td>Control</td>
</tr>
<tr>
<td>T₁</td>
<td>Fruits treated with 1% Bee wax + 0.5% Benlate</td>
</tr>
<tr>
<td>T₂</td>
<td>Fruits treated with 3% Bee wax + 0.5% Benlate</td>
</tr>
<tr>
<td>T₃</td>
<td>Fruits treated with 5% Bee wax + 0.5% Benlate</td>
</tr>
</tbody>
</table>

All lots of fruits were packed according to the experimental layout and stored at room temperature in the existing laboratory during 2004 – 05 (temperature vary from 12-19°C).

Data Collection

Data on randomly selected fruits in each treatment per replication were recorded at 7 days interval during the experiment on the following parameters.

Weight loss (%)

For the determination of weight loss during storage, 3 fruits were marked at the start of experiment from each treatment and kept separate for periodic weighing be electric balance to calculate weight loss during storage. The percent weight loss was calculated as under:

\[
\text{Percent weight loss} = \left( \frac{\text{Weight of fresh fruit (g)} - \text{weight after interval (g)}}{\text{Weight of fresh fruits (g)}} \right) \times 100
\]

Firmness (Kg/cm²)

Firmness of fruits was measured by using penetrometer. (Wagner Model FT 327 having 28lbs capacity). For this needle of instrument was inserted into the peel of fruit and reading was noted on meter in kilograms per square centimeter (Kg/cm²). (This instrument was not available in laboratory during 2004)

pH of fruit juice

pH of fruit juice was measured by using digital pH meter (model: Knick 646) according to Association of Official Analytic Chemist (AOAC) method No. 981.12-b (1990)

Total Soluble Solids (Brix)

Total soluble solids were determined according to AOAC (1990) using hand refractometer at room temperature. The extracted juice from each lot was shaken well and the representative samples were placed on dry refractometer prism and readings were taken directly.

Titratable Acidity of juice (g/100 ml of juice)

Titratable acidity of freshly extracted juice from each lot was determined by the standard method of AOAC, 1990.

Total Soluble Solids/Acid ratio

Total Soluble Solids (TSS)/acid ratio was calculated for all the samples by using following formula:

\[
\text{TSS/Acid ratio} = \left( \frac{\text{TSS}}{\text{Total Acid contents}} \right)
\]
Reducing Sugars (%)
Reducing sugars of juice was estimated as described by Hortwitz (1960).

Total Sugars (%)
Total sugars of juice were determined using the methods described by Hortwitz (1960).

Non-reducing Sugars (%)
The non-reducing sugars were determined by using the following formula (Hortwitz, 1960).

Non-reducing sugars (%) = Total Sugars (%) – [Reducing sugars (%) X 0.95]

Ascorbic Acid (mg/100ml of juice)
Ascorbic acid was determined by the indophenol’s Titration method used by Rusk, (1963).

RESULTS AND DISCUSSION

Weight Loss (%)
During 2004, maximum weight loss (28.24%) was recorded with control and it was non-significantly different from 1% bee wax that showed 23.11% value of weight loss. Minimum weight loss (17.80%) was recorded with 5% bee wax and it was non significantly different from 3% bee wax that showed 19.17 values of weight loss. During 2005 maximum weight loss (17.76 %) was recorded with control treatment. 5% bee wax showed minimum weight loss (10.41%). It was at par with 3% bee wax that showed 12.44% value Table I.

Table 1 Effect of bee wax coating treatments on the weight loss (%), firmness (Kg/cm²), pH, TSS and titratable acidity (g/100ml of juice) of sweet orange cv. “Blood Red” during 2004 and 2005

<table>
<thead>
<tr>
<th>Bee Wax (%)</th>
<th>Weight Loss (%)</th>
<th>Firmness (Kg/cm²)</th>
<th>pH</th>
<th>Total Soluble Solids (Brix)</th>
<th>Titratable Acidity (g/100ml of juice)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>28.24 A</td>
<td>17.76 A</td>
<td>3.68 A</td>
<td>3.53 A</td>
<td>12.57 A</td>
</tr>
<tr>
<td>1%</td>
<td>23.11 A</td>
<td>13.86 B</td>
<td>5.13 A</td>
<td>4.39 C</td>
<td>11.64 C</td>
</tr>
<tr>
<td>3%</td>
<td>19.17 B</td>
<td>12.44 B</td>
<td>4.69 BC</td>
<td>3.51 BC</td>
<td>11.79 BC</td>
</tr>
<tr>
<td>5%</td>
<td>17.8 B</td>
<td>10.41C</td>
<td>4.32 C</td>
<td>3.35 B</td>
<td>11.09 D</td>
</tr>
<tr>
<td>6%</td>
<td>17.76 A</td>
<td>12.02 ABC</td>
<td>4.39 C</td>
<td>3.35 B</td>
<td>11.09 D</td>
</tr>
<tr>
<td>9%</td>
<td>17.96 A</td>
<td>12.2 AB</td>
<td>4.43 C</td>
<td>3.53 A</td>
<td>12.35 A</td>
</tr>
</tbody>
</table>

Note: All means sharing similar letters are statistically non-significant at 0.05 probability level

Maximum weight loss in control is due to the high rate of transpiration and respiration. Minimum weight loss in treatment 5% bee wax might be due to wax coating, which acts as barrier between inner and outer environment of fruit. Hence retained weight in fruits through out the storage period. Also wax coating decreased the rate of respiration (Grierson, 1987). These results are in line with findings of Yuniarti and Suhardi (1992) who reported that lower weight loss was observed in case of treated mangoes as compare to control. Similar views were expressed by Lim-Byung et al., (1998) who showed that wax coating decreased the rate of respiration and transpiration which intern resulted in reduced weight loss, shrivel and increased shelf life (Thai et al., 2001 and Hagenmaier and Baker, 1994). In 2005 the weight loss is higher than 2004. This was due to the high temperature in year 2005 than in 2004. During 2004, the data of storage intervals showed that all storage intervals differed significantly with each other Table III. Maximum weight loss (41.88%) was obtained on 56th day. In this case rate of transpiration increased due to ripening, while minimum weight loss (0.00%) on 1st day while during 2005, the data of storage intervals showed that all storage intervals differed significantly with each other Table III. Maximum weight loss (28.07%) was obtained on 56th day, while, minimum weight loss (0.00%) on 1st day.

Weight loss means the amount of water lost from fruits or vegetables and it is related to the shelf life of produce. These results further affirmed the findings of Attia (1995), who reported that with the passage of time weight loss increased. The effect of bee wax on weight loss of fruit is significant; however there was a slightly less loss of weight in fruits treated with the paraffin wax, which might be due to the uniform covering of the paraffin wax emulsion with even thickness.

Firmness (Kg/cm²)
Application of 5% bee wax in 2005 retained maximum firmness (5.13 Kg/cm²) throughout the storage period. It was at par with 3% bee wax which showed 4.69 Kg/cm² values of firmness, respectively. While control
have lowest value in terms of firmness (4.43 Kg/cm²) and proved to be poorer. It was at par with 3% bee wax and 1% bee wax that showed 4.69 and 4.60 Kg/cm² values of firmness Table I.

5% bee wax gives shine to the fruits, as it is surface finisher by reducing the evaporation and also avoid the wrinkling in fruits, also, it reduces the movement of water molecules from cell structure as the ratio of unbound water with the bound water in the cells maintains the firmness of fruits (Greg and Santi, 1987). Krishnamurthy (1989) also found better result relating firmness, texture and taste in mangoes treated with Tal-prolong (sugar based chemical used for coatings).

The data in Table III (during 2005) depicts that 1st day shows maximum scores (5.56 Kg/cm²). It was at par with 7th, 14th and 21st days that showed 5.34, 5.24 and 5.15 Kg/cm² values of firmness, respectively, while 56th day shows minimum scores (3.88 Kg/cm²) in terms of firmness. It was at par with 42nd and 49th days that showed 4.21 and 4.01 Kg/cm² values of firmness, respectively (Table III).

Maximum score in the 1st day might be the increased activity of pectic enzymes. As softening of orange fruit is closely related to the increased activity of pectic enzymes especially pectinesterase and polygalacturonase (Salunkhe and Desai, 1984). Furthermore, due to wax coatings, there was reduction in cell wall loosening and respiration rate which intern increased the cell integrity. All these factors might have increased the firmness in fruit of sweet orange. While minimum score in firmness might be due to the increased storage period pectic enzymes must be lost or degraded which intern reduce the firmness of fruit. These results are correlated with the findings of Ladaniya and Sonker (1997) who reported maximum retention of natural freshness and firmness was recorded when fruits were waxed and stored for up to 21 days of storage in case of Nagpur mandarin. Further these findings acquiesce with Alam and Paul (2001) who reported that the firmness of kinnow fruits decreased with storage period.

The effect of bee wax on fruit firmness is non significant, but small variation might be due to uniform and even covering of paraffin wax which might reduce the respiration and transpiration.

**pH of Fruit Juice**

Table I (in Year 2004) showed that maximum pH (3.68) was recorded with control. Minimum value of pH (3.39) was obtained from treatment 5% bee wax. It was at par with 3% bee wax that showed 3.51 value of pH. However in Year 2005 showed that maximum pH (3.53) was recorded for control and it was non-significantly differred from 1% and 3% bee wax that showed 3.42 and 3.43 value in terms of pH respectively. Minimum value (3.35) was obtained for 5% bee wax and it was non-significantly differred from 1% and 3% bee wax showed 3.42 and 3.43 values in terms of pH, respectively.

Increase in pH in control might be due to high rate of metabolic activities hence acidity decreased but pH increased and also due to the high TSS contents. Decrease the pH in 5% bee wax might be due to the hydrolysis of protein and fermentation of carbohydrates. pH might be decreased due to the oxidation of certain food components like aldehyde and ketones forming corresponding acids. The results are in line with findings of Manzano and Diaz (2001) which was harvested ‘Valencia’ orange pH content was analyzed during five storage periods (initial, 15, 30, 45 and 60 days). pH content was between 3.84 and 4.17.

The data pertaining to storage intervals (in Year 2004) showed that 56th day had maximum value (3.99). 1st day had minimum value (3.39) in terms of pH that was non-significantly differred from 28th, 7th, 14th and 21st days showed 3.4, 3.41, 3.43 and 3.45 values, respectively. The data showed that storage intervals (in Year 2005) showed that 56th day had maximum value (3.61) that was non-significantly differred from 49th day showed 3.6 value of pH. 1st day had minimum value (3.19) in terms of pH that was non-significantly differred from 7th and 14th days showed 3.29 and 3.33 values, respectively, Table III.

The change in pH during storage period might be due to number of reasons; firstly, the alteration of biochemical condition of fruit due to treatments secondly, due to lower rate of respiration and metabolic activity, pH increase but at a slower rate particularly at the end of storage period, as there might be the saturation of atmosphere inside the pack with water vapors. These results are similar with findings of Biasi and Zanette (2000) who revealed that gibberellic acid and wax solution had slight increased pH with storage.

The effect of bee wax on pH of fruit is almost similar, but there was a slight pH suppressing behavior of paraffin wax treated fruits.
Total Soluble Solids (Brix)

The data in year 2004 indicate that treatment control retained maximum TSS (12.57 brix) throughout the storage period though statistically it was at par with 1% bee wax, which showed 12.11 brix in terms of TSS, respectively. While, 5% bee wax have lowest value (11.09 brix) in terms of TSS. It was at par with 3% bee wax that showed 11.79 brix. Similar trend was observed in 2005 control having maximum TSS (12.28 brix) throughout the storage period though statistically it was at par with 1% bee wax which showed 12.20 brix, while, 5% bee wax have lowest value (11.64 brix) in terms of TSS. It was at par with 3% bee wax that showed 12.02 brix in terms of TSS Table I.

Increased TSS in control by accumulating different solutes in vacuoles of cells and fruits goes ripen and starch in hydrolyzed into sugars. Possible reason in reduction of TSS in 5% bee wax was due to the fact that fact that these retard the hydrolysis of starch into sugars and also the conversion of polysaccharides in to disaccharides and monosaccharides by changing the biochemical activities. The results accede to the waxed with Semperfresh (1%), at ambient temperature. Moreover the results correlate with findings of Manzano and Diaz (2001) that analyzed TSS during five storage periods (initial, 15, 30, 45 and 60 days) and reported that TSS content ranged between 9.11 to 10.24%.

The data presented in Table 3 (in Year 2004) depicts that 56th days storage shows maximum scores (12.77 brix) of TSS, but was at par with 49th and 42nd days that showed 12.17 and 12.07 brix, respectively. Day 1st shows minimum scores (11.29 brix) in terms of TSS. It was at par with 7th, 14th and 21st days that showed 11.36, 11.56, and 11.65 brix in terms of TSS, respectively. In Year 2005 depicts that 56th day shows maximum scores (12.90 brix) in terms of TSS. It was at par with 49th, 42nd, and 35th days that showed 12.76, 12.49 and 12.31 brix in terms of TSS, respectively. 1st day shows minimum scores (11.30 brix). It was at par with 7th and 14th days that showed 11.41 and 11.50 brix, respectively Table III.

The decrease in TSS in 1st day may be due to more utilization of sugars than conversion of complex carbohydrates into simple sugars by the fruit to fulfill energy demand. The changes in TSS are directly correlated with hydrolytic changes in the starch concentration during the post harvest period. These changes result in the conversion of starch to sugar, which in an important index of ripening process (Kays, 1997). Many different solutes are accumulated in vacuoles of cells as the fruit ripens. Fruit contain much starch that hydrolysis to sugars as fruit ripens. At the same time proteopectin in the cell wall hydrolyze to soluble pectins thus the total soluble contents of such fruits gradually increases after harvest (Ryogo, 1988). These results are correlated with the findings of Ladaniya and Sonker (1997) who reported maximum retention of TSS was recorded when fruits were waxed and stored for up to 21 days of storage of Nagpur manfrin.

The data indicated that effect of bee wax on TSS is non significant, but the level of TSS remained slightly lesser in the paraffin coated fruits than of bee wax coated fruits. It might be due to uniform coating of paraffin wax, which might reduce the respiration and transpiration and therefore reducing the metabolic activities i.e. conversion of starch to sugar.

Titratable Acidity of Juice (g/100 ml of Juice)

The data of wax coating treatments (during 2004) exhibited that all treatments differed significantly with each other. Maximum acidity (0.85 g/100 ml of juice) was observed in treatment control. Least value (0.65 g/100 ml of juice) was recorded 5% bee wax. The data of wax coating treatments (during 2005) exhibited that all treatments differed significantly with each other. Maximum acidity (0.87 g/100 ml of juice) was observed in treatment control. Least value (0.68 g/100 ml of juice) was recorded 5% bee wax Table I.

Increase in acidity in control treatment might be due to the formation of carbonic acid (acidosis) or it might be due to the fermentation of sugars resulting in production of acids while, decrease in acidity in 5% bee wax might be due to the fact that as fruit ripens, it diminishes its malic and citric acid contents and favored the formation of sugars (Martinez et al., 1997). The results are in line with the findings of Lim-Buying et al., (1998) who observed that the effect of prowax F coating on apples during room (15-20 °C) storage and found that titratable acidity decreased faster in control fruits than in wax coated fruits.

The data of storage intervals during 2004 showed that all treatments differed significantly with each other. 1st day had higher value (0.91 g/100 ml of juice) of titratable acidity whereas 56th day had least value (0.61 g/100 ml of juice) for acidity. The data of storage intervals during 2005 showed that all treatments differed significantly with
each other. 1st day had higher value (0.91 g/100 ml of juice) of titratable acidity whereas 56th day had least value (0.64 g/100 ml of juice) Table III.

Table III  Effect of storage interval (days) on the weight loss (%), firmness (Kg/cm²), pH, TSS and titratable acidity (g/100ml of juice) of sweet orange cv. “Blood Red” during 2004 and 2005

<table>
<thead>
<tr>
<th>Storage Interval (Days)</th>
<th>Weight Loss (%)</th>
<th>Firmness (Kg/cm²)</th>
<th>pH</th>
<th>Total Soluble Solids (Brix)</th>
<th>Titratable Acidity (g/100ml of juice)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0 I</td>
<td>0 I</td>
<td>5.56 A</td>
<td>3.39 D</td>
<td>3.19 E</td>
</tr>
<tr>
<td>7</td>
<td>4.97 H</td>
<td>3.54 H</td>
<td>5.34 A</td>
<td>3.41 CD</td>
<td>3.29 DE</td>
</tr>
<tr>
<td>14</td>
<td>10.44 G</td>
<td>6.82 G</td>
<td>5.24 A</td>
<td>3.43 CD</td>
<td>3.33 CDE</td>
</tr>
<tr>
<td>21</td>
<td>15.57 F</td>
<td>10.15 F</td>
<td>5.15 A</td>
<td>3.45 CD</td>
<td>3.37 BC</td>
</tr>
<tr>
<td>28</td>
<td>21.18 E</td>
<td>12.89 E</td>
<td>4.45 B</td>
<td>3.4 D</td>
<td>3.47 BC</td>
</tr>
<tr>
<td>35</td>
<td>26.69 D</td>
<td>16.22 D</td>
<td>4.39 B</td>
<td>3.49 BC</td>
<td>3.47 BC</td>
</tr>
<tr>
<td>42</td>
<td>32.06 C</td>
<td>20.22 C</td>
<td>4.21 BC</td>
<td>3.6 BC</td>
<td>3.52 B</td>
</tr>
<tr>
<td>49</td>
<td>36.92 B</td>
<td>23.97 B</td>
<td>4.01 C</td>
<td>3.56 B</td>
<td>3.6 AB</td>
</tr>
<tr>
<td>56</td>
<td>41.88 A</td>
<td>28.07 A</td>
<td>3.88 C</td>
<td>3.99 A</td>
<td>3.61 A</td>
</tr>
</tbody>
</table>

Note: All means sharing similar letters are statistically non-significant at 0.05 probability level

The decrease in acidity on 56th day was probably due to the consumption of citric acid by the microorganisms as a source of carbon as this acidity is higher in coated fruits; it is possible that the O2 accumulated internally in the fruits tissue caused acidosis after dissolving and forming carbonic acid (Carrillo et al., 1995). Titratable acidity is directly related to the concentration of organic acids present in the fruits. Organic acids exist as free acids (citric acid), anions (citrate) or are combined as salt (sodium citrate) (Kays, 1997). Increase in acidity on 1st day might be due to the fermentation of sugars resulting in production of acids. Acidity is often used as an indication of maturity; acid decreases on ripening of fruit (Bhattacharya, 2004). It has also been reported that during the in the ripening of fruits, malic acid disappear first, followed by citric acid (which result in reduction in amount of titratable acidity), suggesting the catabolism of citrate via malate (Mattoo et al., 1975; Salunkhe and Desai, 1984). Disappearance of malic and citric acid during ripening process may be the main factor responsible for the reduction in titratable acidity during the storage. The microorganisms may use citric acid as the carbon source, hence resulting in reduction in titratable acidity and the low acidity contents at the end of storage period as described by Badshah et al., (1997) and Batu and Thompson (1998). Also the results are similar with findings of Jiang and Li (2001) that reported the effect of chitosan coating on longan fruit and found the titratable acidity decreased during storage.

The effect of bee wax on intractable acidity is statistically similar, but small variation might be due to uniform coating of paraffin wax, which might reduce the respiration and transpiration and therefore reducing the metabolic activities i.e. conversion of acids.

TSS/Acid Ratio

TSS/Acid ration during 2004 as depicted in Table II indicates that treatment control retained maximum TSS/acid ratio (18.78) throughout that storage period. While, 5% bee wax have lowest value (14.27). It was at par with 3% bee wax that showed 15.13 value of TSS/acid ratio. Table II (in Year 2005) indicates that control retained maximum TSS/acid ratio (17.81) throughout that storage period. It was at par with 1% bee wax that showed 16.55. While, 5% bee wax have lowest value (13.66). The higher change in TSS/acid ratio in control treatment is directly correlated with hydrolytic changes in the starch concentration (conversion of starch to sugars). While slow increase in 5% bee wax probably due to the consumption of citric acid by the microorganisms during the post harvest period, which is and important index of ripening process (Carrillo et al., 1995; Kays, 1997). Javed et al., (1987) found that TSS/acid ratio of waxed blood red oranges were lower than those of un-waxed fruits.

The data presented in Table IV (in Year 2004) depicts that 56th days shows maximum scores (19.31) in terms of TSS/acid ratio while 1st day shows minimum scores (12.49) (see Table IV). Table IV (in Year 2005) depicts that 56th day shows maximum scores (19.53) while 1st day shows minimum scores (10.63). It was at par with 7th day that showed 12.93 values of TSS/acid ratio. With the passage of time degradation of ascorbic acid lead to more TSS as structural formula of ascorbic acid is similar to glucose there fore decrease in ascorbic acid is correlated with increase in TSS/acid ratio. Also, this might be because of higher sugars than acids. Manzano and Diaz (2003) reported that ‘Valencia’ oranges fruits were harvested, sorted, graded and treated with a wax coating (Primafresh and Cerabar “Dr”) and found that TSS/acid ratio was increased with the passage of time.
Reducing Sugars (%)

During 2004 maximum reducing sugar contents (4.78%) was observed in treatment control where as least value (3.85%) was recorded in 5% bee wax after subsequent storage period. However during 2005 maximum reducing sugar contents (4.38%) was observed in control after subsequent storage period whereas minimum reducing sugar contents (3.62%) was recorded in 5% bee wax Table II.

Table II Effect of bee wax coating treatments on the TSS/Acid ratio, reducing sugars (%), non-reducing sugars (%), total sugars (%), ascorbic acid (mg/100ml of juice) of sweet orange cv. *Blood Red* during 2004 and 2005

<table>
<thead>
<tr>
<th>Bee Wax (%)</th>
<th>TSS/Acid Ratio</th>
<th>Reducing Sugars (%)</th>
<th>Non-reducing Sugars (%)</th>
<th>Total Sugars (%)</th>
<th>Ascorbic Acid (mg/100ml of Juice)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>18.78 A</td>
<td>17.81 A</td>
<td>4.78 A</td>
<td>4.38 A</td>
<td>5.56 A</td>
</tr>
<tr>
<td>3%</td>
<td>15.13 CD</td>
<td>15.88 CD</td>
<td>3.88 C</td>
<td>3.87 C</td>
<td>5.09 BC</td>
</tr>
<tr>
<td>5%</td>
<td>14.27 E</td>
<td>13.66 D</td>
<td>3.85 D</td>
<td>3.62 D</td>
<td>4.91 C</td>
</tr>
</tbody>
</table>

Note: All means sharing similar letters are statistically non-significant at 0.05 probability level

Higher reducing sugars in control treatment might be the effect of enzymes present in the fruit, especially related with pectinases enzyme by decreasing activity, it might affect the other enzymes, Sucrose synthase in responsible for the starch accumulation and many affect the starch to sugar conversion (Anon, 2004). The slow rate of increase in sugar in treatment 5% bee wax might be due to use of waxes which affect the activity of mitochondria and some enzymes as described by Wills and Rigney (1979). The results are in line with the findings of Gal et al., (1990) who observed the effect of Fruitex (3% total solids) (wax emulsion) on blood red oranges during room storage and found that reducing sugars increased slowly in wax coated fruits than control during storage.

During 2004 all the storage intervals displayed a significant difference in the reducing sugar contents. Data also revealed that 56th day showed the best value (5.04%) of reducing sugars and 1st day showed the least value (3.33%) of reducing sugars. While during 2005 all the storage intervals displayed a significant difference in the reducing sugar contents. Data also revealed that 56th day showed the best value (4.68%) and 1st day showed the least value (3.33%) of reducing sugars (Table IV). It has been reported that the starch, which is built up in green fruit, is progressively replaced by reducing sugars as development continues. Early in ripening sugar reach their maximum concentration and about the same time as the peak in organic acids (Hobson and Harman, 1986). Moreover, Syamal (1991) reported that the total soluble solids and reducing super percentage increases during ripening. During the normal ripening, the reducing super contents tend to increase through the stages of maturity (Salunkhe and Desai, 1984). Also the results are similar with findings of Ahmad et al., (1986) who reported the effect of seal-britex-65 wax on blood red oranges and found that reducing storage increased during storage.

Non-reducing Sugars (%)

The non-reducing sugars during 2004 showed maximum content (5.56%) in treatment control. Least value (4.91%) was recorded in 5% bee wax. It was non-significantly different form 3% bee wax that showed 5.09% value of non-reducing sugars after subsequent storage period. Whereas Year 2005 showed maximum non-reducing sugar content (5.34%) was observed in control where as least value (5.04%) was recorded in 5% bee wax after subsequent storage period Table II. Application of 5% bee wax delayed the respiration and ripening, so the ripening and changes associated with ripening tent to slow down the pattern of accumulation of sucrose was more visible. Also waxes affect the enzymes present in the fruit, especially related with pectinases enzyme by decreasing its activity; it might affect the other enzymes like acid invertase and sucrose synthase responsible for the sucrose metabolism and starch accumulation (Wang et al., 1993). The data of storage intervals during 2004 on non-reducing sugar contents was exhibited in (Table IV). All the storage intervals displayed a significant difference in the non-reducing sugar contents. Data also revealed that 56th day showed the best value (5.66%) of reducing sugars and 1st day showed the least value of non-reducing sugars (4.62%). However in year 2005 all the storage intervals displayed a significant difference in the non-reducing sugar contents. Data also revealed that 56th showed the best value (6.70%) of reducing sugars and 1st day showed the least value of non-reducing sugars (4.53%).
oxidative deterioration as well as mild oxidation of ascorbic acid results in the formation of dehydroascorbic acid. Control treatment might be due to increased respiration causing loss of ascorbic acid. Ascorbic acid is susceptible to decrease in oxygen level, which might lead to lesser loss of ascorbic acid from fruits. Maximum ascorbic acid content was recorded in wax coating degradation of fruits in comparison with control treatments.

Sucrose synthase and may affect the starch accumulation and may affect the starch to sugar conversion. The slow rate of increase in sugar in 5% bee wax may be due to use of wax which affects the activity of mitochondria and some enzymes as described by Wills and Rigney (1979). As waxes affect the enzymes present in the fruit, especially related with pectinases enzyme by decreasing its activity. Sucrose synthase is responsible for the starch accumulation and may affect the starch to sugar conversion (Anon, 2004). The results are in line with findings of Ahmad et al., (1986) who reported the effect of seal-britex-65 wax on blood red oranges and found that non-reducing increased during storage due to enzyme activity. The effect of both waxes on non-reducing sugar is statistically similar.

Total Sugars (%)
The data regarding total sugars presented in (Table II) (during 2004) showed significant difference between all the storage intervals. Maximum total sugar content (10.10%) was observed in control where as least value (8.76%) of total sugars was recorded in 5% bee wax after subsequent storage period. During 2005, maximum total sugar content (9.52%) was observed in control where as least value (8.90%) of total sugars was recorded in 5% bee wax after subsequent storage period. The slow rate of increase in sugar in 5% bee wax might be due to use of wax which affects the activity of mitochondria and some enzymes as described by Wills and Rigney (1979). As waxes affect the enzymes present in the fruit, especially related with pectinases enzyme by decreasing its activity. Sucrose synthase is responsible for the starch accumulation and may affect the starch to sugar conversion (Anon, 2004). The results are in line with findings of Ahmad et al., (1986) who reported the effect of seal-britex-65 wax on blood red oranges and found that total sugars increased in highly in control fruits as compared to control during storage.

During 2004 all the storage intervals displayed a significant difference in the total sugar contents. Duncan Multiple Range test also revealed that 56th day showed the maximum value of total sugars (10.83%) and 1st day showed the least value (7.8%) of total sugars. The data of storage intervals during 2005 on total sugar contents was exhibited in (Table IV). All the storage intervals displayed a significant difference in the total sugar contents. Data revealed that 56th day showed the best value of total sugars (11.35%) and 1st day showed the least value (7.86%) of total sugars. With the passage of time respiration, transpiration and other metabolic processes enhanced. Due to this starchy are converted into sugars and reducing sugar quantity increased. The results are in line with findings of Gul et al., (1990) who observed that the effect of Fruitex (3% total solids) (wax emulsion) on blood red oranges during room storage and found that non-reducing sugars increased during storage. Also the results are similar with findings of Ahmad et al., (1986) who reported the effect of seal-britex-65 wax on blood red oranges and found that non-reducing increased during storage due to enzyme activity. The effect of both waxes on non-reducing sugar is statistically similar.

Ascorbic Acid (mg/100ml of juice)
The data of treatments during 2004 showed that significant significant difference among all treatments. In this case treatment 5% bee wax had minimum value (43.64 mg/100 ml) of ascorbic acid. Maximum value (50.36 mg/100 ml) was recorded for control. While during 2005 showed that 5% bee wax had minimum value (44.33 mg/100 ml) of ascorbic acid. Maximum value (50.68 mg/100 ml) was recorded for control Table II. Minimum ascorbic acid in 5% bee wax treatment might be due to the ascorbic acid not susceptible to oxidative in fruits. Also due to the wax coating degradation of carbon dioxide into ethylene and water (Wills et al., 1981) resulting in a decrease in oxygen level, which might lead to lesser loss of ascorbic acid from fruits. Maximum ascorbic acid in control treatment might be due to increased respiration causing loss of ascorbic acid. Ascorbic acid is susceptible to oxidative deterioration as well as mild oxidation of ascorbic acid results in the formation of dehydroascorbic acid.

Note: All means sharing similar letters are statistically non-significant at 0.05 probability level
The presence of oxygen accelerates oxidation process in fruits (Ahmad, 1982). The results are in line with findings of Kumar et al., (2000): they found that ascorbic acid decreased with increasing period of storage in fruits of kinnon. But the decreased in ascorbic acid was less in coated fruits as compare to control.

The mean of storage interval (during 2004) showed that all treatments differed significantly with each other. 1st day had highest value (58.62 mg/100 ml of juice) of ascorbic acid where as 56th days had lowest value (39.60 mg/100ml of juice) of ascorbic acid. The remaining values lie in between these minimum and maximum values. However during 2005 showed that 1st day had highest value (58.91 mg/100 ml of juice) of ascorbic acid where as 56th days had lowest value (38.27 mg/100ml of juice) of ascorbic acid. The remaining values lie in between these minimum and maximum values Table IV. According to Bhattacharya (2004) that the Vit. C content of fresh fruit was maximum just before ripening and then decreases due to the action of enzymes called ascorbic acid oxidase. Usually much of the ascorbic acid is transferred to juice and oxidized. The results congregate with the finding of Verma and Dashora (2000) who narrated that when storage period proceeded, TSS increased while ascorbic acid and acidity of Kagzi lime fruits decreased.

**CONCLUSION AND RECOMMENDATIONS**

It was concluded that 5% bee wax in combination with 0.5% benlate proved to be best wax that were very effective in improving the overall quality and extending the shelf life of fruits. Our conclusion about bee wax concentration should be further tested by conducting systematic research studies for increasing the shelf life of citrus.

**REFERENCES**


