SYNERGISTIC EFFECT OF SALTS AND CELLULOSE BASED COATING ON SHELF LIFE OF KINNOW (C*ITRUS RETICULATE*)

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ABSTRACT

Kinnow (*Citrus reticulate Blanco*) is achieving core consideration globally owing to its nutrition and high juice content. In Pakistan kinnow export is limited due to inadequate postharvest handling and lack of appropriate storage practices. Considering these issues, the present study was designed to evaluate the effect of hydroxy-propyl-methylcellulose (HPMC) coating in combination with $CaCl_2$ and $MgSO_4$ on shelf life extension of kinnow. Fruits were treated with different levels of $CaCl_2$ and$MgSO_4$followed by HPMC coating (3 and 5%) and stored at 10°C with 80% relative humidity for 6 weeks. Fruits were analyzed for various physico-chemical parameters on weekly basis. During this study lower fruit firmness ($0.24 Nm^{-2}$), loss in weight (0.64 %) and ethylene production ($0.039\ \mu L \cdot kg^{-1} \cdot hr^{-1}$) was observed in fruits treated with 1% $CaCl_2$ + 1% $MgSO_4$ + 5% HPMC (T_6) during storage of 42 days. Minimum chilling injury indexes0.22% and 0.61% were recorded in treatments T_4 and T_6, respectively. T_6showed higher values of titer able acidity (0.29%) and ascorbic acid contents (39.82mg/100g).Minimum TSS (9.62°Brix) were found in fruits of T_6. Overall, T_6 showed significantly better results for various parameters, as compared to all other treated and control fruits.

Key words: Citrus coating, Post-harvest, Hydroxy-propyl-methylcellulose, Physicochemical, shelf life.

INTRODUCTION

Among the tropical and subtropical countries, Pakistan is blessed with best agro climatic as well as food topographic conditions which provide a promising environment for successful cultivation of various fruits. Today Pakistan has great potential for citrus production which is grown in more than 52 countries of the world. Citrus species produced in Pakistan are well known due to their best quality and good sensory profile. Citrus occupies 29.55% area of total fruit cultivation in Pakistan. While land under kinnow cultivation accounts 60 % of total citrus acreage (GOP, 2003-2004).

Sargodha district in Punjab shows more potential in citrus production, processing, storage and exports. Citrus fruits are highly nutritious due to organic acids and carotenoids (Ansari and Feridoon, 2008). In Pakistan magnitudes of postharvest losses are 35-40% (PHDEB, 2006) due to lack of appropriate pre and post-harvest management, insufficient packing houses, inappropriate transport infrastructure, and limited cold chain facilities with traditionally slow marketing system. The quality of kinnow starts deteriorating right after harvesting if not properly managed.

In modern ages, coatings and edible films have received extraordinary attention for their use over synthetic coatings. Edible coatings retard dehydration, inhibit respiration, retain volatile compounds, improve texture and reduce micro flora. Coatings and edible films can be categorized in to three different types on the basis of their components *i.e.* composites, lipids and hydrocolloids. Different types of flavors, pigments, antioxidants, antimicrobial agents, firming agents and vitamins can be incorporated into edible coatings to improve and modify their functional properties. In the past few years, polysaccharides have been used as coating material for different fruits. These polymers proved to be having advantages due to their low cost, availability and biodegradability. Many derivatives of cellulose like carboxy-methylcellulose, methyl cellulose and hydoxy-propyl cellulose are produced commercially (Olivas and Barbosa-Canovas, 2005).

Cellulose based wax coating like (HPMC) is widely used on different species of the fruits, due to its non-toxic effects. Cellulose based compounds are water soluble and semi synthetic in nature which improve color and maintain fruit firmness. They are usually used as thickening agent, binders, film formers, water retention agents, protective colloids and emulsifiers. Cellulose coating with emulsions of salts and plant material showed prominent results to extend the shelf life of fruit (Adetunji *et al.*2012).

Different salts such as KCl, $CaCl_2$ and Na_2HCO_3 along with cellulose based coating are supportive to maintain firmness and reduce physiological weight loss. Decrease in weight loss of oranges was observed when treated with emulsion of HPMC, beeswax and shellac along with calcium chloride. In various experiments $MgSO_4$wasmostly used for tomato, cucumber and carrot but 1st time in this project it was used on citrus along with HPMC and $CaCl_2$ to extend the shelf life. The aim of the present study was to find out the effectof cellulose based coating assimilated with $MgSO_4$ and $CaCl_2$on kinnow to extend the shelf life and improve quality parameters.

MATERIALS AND METHODS

Raw material: Kinnow mandarin (*Citrus reticulate Blanco*) used in this study were harvested in mid-February from the orchard of University of Agriculture, Faisalabad. Mature fruits were harvested from four directions of the plant canopy to get homogeneous fruit samples. Kinnows were harvested at commercially mature stage when the upper part of the fruit skin showed glossy appearance with soft skin which is easy to peel off. Harvesting was performed by clipping the fruit stalk just above the point of attachment to the fruit. After that kinnow fruits were placed in plastic buckets (100fruits per bucket) and transported to the laboratory of National Institute of Food Science and Technology University of Agriculture, Faisalabad. Cellulose based coating HPMC (Sigma-Aldrich) and salts of calcium and magnesium (Fluka) were used with different concentrations.

Preparation of wax emulsion: Wax emulsion was prepared by dispersing HPMCin hot water at 80°C with glycerol (2 parts HPMC: 1 part glycerol) which is used as a plasticizer.

Pre treatment: Before wax coating kinnow fruits were sorted and graded to obtain uni form size for further post harvest processing. Then kinnow fruits were disinfected by immersion in diluted bleach (0.1% sodium hypochlorite) for 3-5 minutes followed by rinsing with tap water to remove dirt, spray residues and extraneous matter from the field. Thereafter,kinnows were placed on paper towels and allowed to dry in air at room temperature (25°C) under fan at moderate speed.

Treatment layout: Fruits were randomly divided into seven sets, each contained63 fruits. Each set was treated by different treatments including: uncoated/control fruits (T_0), fruits coated with 2% $CaCl_2$ + 3% HPMC (T_1), 2% $CaCl_2$ + 5% HPMC (T_2), 2% $MgSO_4$ + 3% HPMC (T_3), 2% $MgSO_4$ + 5% HPMC (T_4), 1% $CaCl_2$ +1% $MgSO_4$ + 3% HPMC (T_5) and 1% $CaCl_2$ +1% $MgSO_4$ + 5% HPMC (T_6). The fruits were dipped in above mentioned chemicals for 3 minutes at room temperature. Afterwards, they were dried at room temperature with natural air convection for 2–3 hours. After drying both, treated and untreated kinnow fruits were placed in small pre-cleaned perforated plastic baskets and stored at 10°C with 80% relative humidity in a chamber (Memmert ICH-260-C) equipped with automatic temperature and relative humidity control system.

Quality analysis: Physical properties like firmness and juice weight were determined by adopting the standard methods. Data concerning to fruit firmness was recorded with the help of digital penetrometer (53205Digital Fruit Firmness Tester, Agri. Measurement Instrument, Turoni, Italy). Juice weight was obtained by subtracting peel weight, rag weight and seed weight from total fruit weight. Physiological loss in weight was expressed as percentage decrease in fruit weight as described by Thakur *et al.* (2002). Incidence of chilling injury was determined as percentage of the number of decayed fruits from the initial number after an indicated period of storage. Rates of ethylene production were measured by the static system. A known quantity of fruits per treatment was weighed and sealed with ethylene meter (Model # drager PAC-III) in 3L container for 1 hour. After 1 hour the meter was removed from container and the reading (C_2H_4) was displayed in ppm on screen. The rate of ethylene production was calculated in $\mu L \cdot kg^{-1} \cdot h^{-1}$.

Biochemical quality characteristics of the treated fruits were estimated from the juice sample taken from each treatment. The quality attributes such as total soluble solids, titratable acidity and ascorbic acid were determined by using standard procedures of AOAC (2006). Total soluble solids (TSS) were examined by using a portable hand Rafractrometer (Model BS Eclipse 3-45) and the results were expressed as °Brix. Titratable acidity (TA) was measured by titration of juice sample with 0.1 N NaOH, and expressed as percentage of citric acid/100 ml of juice. Ascorbic acid was measured by 2, 6- dichlorophenol indophenol titration solution to end point, and expressed as ascorbic acid in mg per 100mL of juice.

Statistical analysis: The obtained data for each parameter was interpreted by applying Complete Randomized Design with factorial arrangements.Level of significance ($P>0.05$) was determined (ANOVA) as described by Steel *et al.* (1997).

RESULTS AND DISCUSSION

Physical analysis: Means regarding the effect of physical parameters (firmness and juice weight) showed significant differences at $P<0.05$ for treatment, storage and their interaction. Fruits treated with 1% $CaCl_2$ + 1% $MgSO_4$ + 5%HPMC (T_6) showed significantly high fruit firmness (1.2Nm^2) as compared to all other treatments. Lower fruit firmness was observed in untreated fruits (0.22Nm^2).Firmness decreased significantly with the passage of time. Before storage it was 1.2Nm^{2}and after 42 days it decreased up to 0.24Nm^{2}in case of T_6 as shown in Figure 1. According to results T_6 showed significantly higher juice weight (44.18%) as compared to T_0(38.56%). Maximum juice weight 42.76% was observed in initial day of storage but after 42 days it reduced to 40.14% under applied storage conditions as depicted in Figure 2.With previous findings firmness of kinnow was slightly better with HPMC compared to uncoated samples and that difference could be due to cultivar and storage conditions (Contreras-Oliva *et al.* 2012). Alteration in firmness and juice weight are less in those fruits which were treated with HPMC + Moringa as compared to untreated (Adetunji *et al.*2012). Generally, firmness of citrus decreases during the ripening processes. That is altered due to breakdown of starch molecules to form sugars in the presence of hydrolytic enzymes. Abdel-Aziz *et al.* (2000) indicated same results for weight loss of juice in controlled temperature and humidity conditions. A decline in juice weight was attributed to transpiration rate and dehydration during storage period.

Physiological analysis: Statistical analysis of physiological parameters showed that treatments, storage and their interaction significantly affected loss in weight, chilling injury and ethylene production. Minimum loss in weight was recorded (0.64%) in T_6, whereas maximum weight loss was observed in T_0 (2.46%). The weight loss among treated samples ranged from 1.12% to 3.25%. However, with the storage period, T_6 exhibited minimum loss in weight as compared to rest of the treatments. Lowest value of ethylene was observed in fruits of T_6 (0.039 $\mu L \cdot kg^{-1} \cdot h^{-1}$) as compared to untreated fruits which shows maximum ethylene production (0.096 $\mu L \cdot kg^{-1} \cdot h^{-1}$). Before storage kinnow mandarin produced 0.028 $\mu L \cdot kg^{-1} \cdot h^{-1}$ ethylene, whereas after 42 days it was 0.114 $\mu L \cdot kg^{-1} \cdot h^{-1}$as shown inFigure 3. Higher index of chilling injury (3.59%) was noted in fruits of T_0, whereas T_6 showed lower chilling injury (0.61%) as depicted in Figure 4. The fruits which were analyzed after 42 days storage showed higher index of chilling injury (3.63%) than the fruits being analyzed after 35, 28, 21, 14 and 7 days during storage interval with chilling injury indexes 2.88, 2.38, 2.08, 1.35 and 1.28%, respectively. Fruit weight is an important parameter for fruit quality. According to Silvia *et al.* (2009) Valencia oranges treated with HPMC-lipid emulsion along with sodium benzoate stored for 60 days at 5°C significantly reduced the weight loss, which indicated the effectiveness of these coatings as a moisture barrier as shown inFigure 5. At the end of this storage period, weight losses on all samples were around 3.9-4.4%. Weight loss in orange fruit during ripening is due to both transpiration and respiration. Transpiration is associated with water vapor pressure of the surrounding atmosphere and the fruit surface. Weight loss contributed by respiration is a result of carbon atoms, in the form of carbon dioxide molecules, leaving the fruits. Ethylene production also increased the ripening of fruits which ultimately decrease the shelf life of fruits. Generally, different types of wax materials especially cellulose based wax was helpful for the reduction in rate of respiration and ethylene production in kinnow during storage period because ethylene production in different wax coated fruits was found 90% lower than uncoated ones. Most physiological and biochemical changes involved during ripening of fruits are softening, change of colour from green to yellow, loss of acidity, conversion of starch to sugars, formation of cuticular waxes, synthesis of aromatic compounds, changes in flavor, color and texture of fruits(Kays and Paull, 2004).

Different factors like type of cultivar, storage conditions and postharvest treatments may affect the development of chilling injury in oranges and mandarins. Similarly, Perez *et al.* (2002) concluded that development of chilling injury in the Fortune mandarins took place when stored for specific duration (>60 days) at below 8°C temperature. According to some scientists chilling injury was slight in initial 30 days of experimentation while, severe chilling injury developed after 5 weeks of storage.

Biochemical analysis: During storage data related to biochemical parameters such as total soluble solids, titratable acidity and ascorbic acid contents showed significant results for storage and treatments as depicted in Table 1 & 2.T_6 fruits showed minimum TSS (9.62°Brix), whereas maximum TSS were observed in T_0(11.34°Brix) during entire duration. As the storage time increased TSS also increased, maximum TSS (11.80°Brix) were observed after 35 days of interval. High titratable acidity was observed in fruits of T_6(0.29%) as compared to all other treatments. Fruits of controlled treatment showed minimum acidity (0.22%). After 42 days of storage, acidity level decreased from 0.34 to 0.15%. Maximum vitamin C (39.82 mg/100g) was recorded in fruits of T_6as compared to controlled treatment which showed minimum (22.17mg/100g). Vitamin C decreased with the passage of time, in initial day maximum vitamin C was 40.27 (mg/100g) and after 42 days of storage, it was 37.84 (mg/100g).

Above mentioned results depicted that increase in TSS was due to the increase in rate of respiration and hydrolysis of starch in cold chamber. Thakur *et al.* (2002) also found similar results in kinnow mandarin during cold storage after 40 days. This effect was opposed by Babalar *et al.* (2007) who concluded a rapid increased in TSS in strawberry fruits. A correlation of inversely proportional was found between TSS and TA during storage. TA was higher in those fruits which are coated with emulsion of CMC (1.1%) along with soybean and sodium oleate stored for 27 days at 25°C and 75% relative humidity with storage time (Hasan, 2004). According to some other observations, decrease in titratable acidity may be due to decrease in oxygen transfer rate from the external medium to fruit, as the CMC amount in the emulsions increased. When oxygen transfer rate decreased, the glucose was subjected to anaerobic fermentation and produced alcohol, which decreased titratable acidity. Coated fruit significantly maintained vitamin C contents due to its binding layer which plays an important role to prevent the oxidation process in fruit. Vitamin C decreased with storage time when stored at 4°C, reported by Keditsu *et al.* (2003). The decrease in ascorbic acid level was related with a reduced capability of preventing oxidative damage. During storage period, higher level of vitamin C was observed in fruits coated with HPMC+ moringa extract than in control samples, similar to that was reported by Dang *et al.* (2010), who found that the content of vitamin C of coated fruits higher than that of the control. Moreover, Randhawa *et al.* 2014 analyzed various organic acids from Pakistani citrus species and observed the effect of refrigeration storage on their deterioration. They measured degradation percentage of different organic acids and depicted that vitamin C was highest (51.43+2.06 mg/100ml) in kinnow and decreased from 11 to 15 % during entire storage. Hence different factors responsible for oxidation of ascorbic acid include exposure to oxygen, metals, light, heat, and alkaline pH (Chitravathi *et al.* 2014).

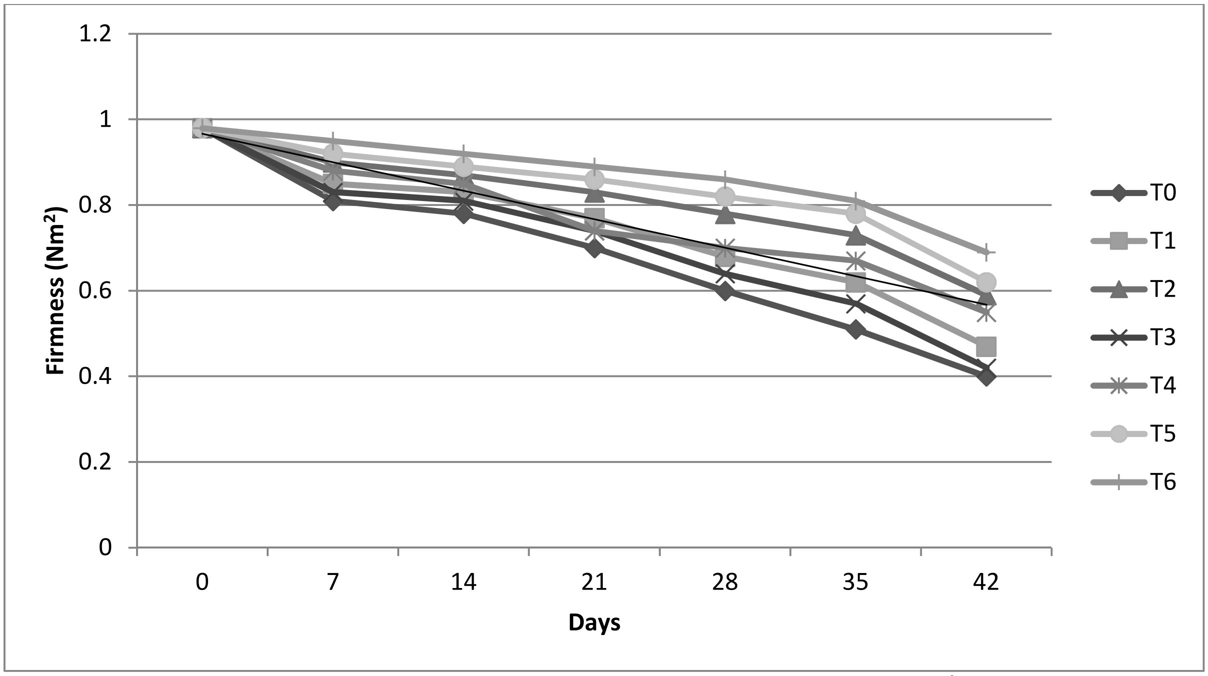

Figure 1. Effect of Ca and Mg salts along with cellulose based coating on Firmness (Nm^2) under controlled storage

Figure 2. Effect of Ca and Mg salts along with cellulose based coating on Juice weight (%) under controlled storage.

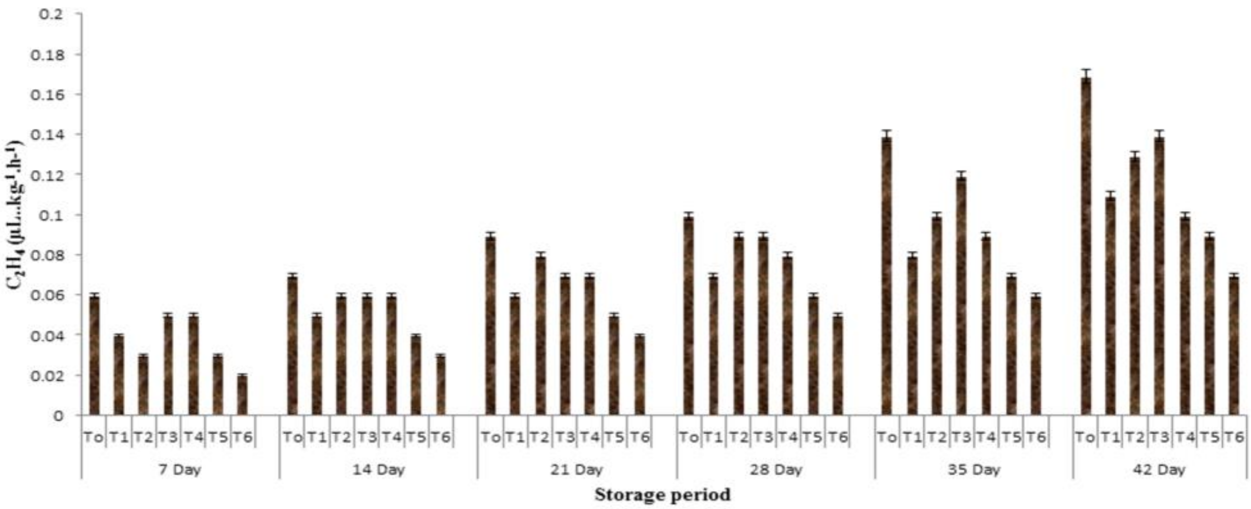

Figure 3. Effect of Ca and Mg salts along with cellulose based coating on Ethylenegas production (µL•kg-[1]•h-[1]) under controlled storage.

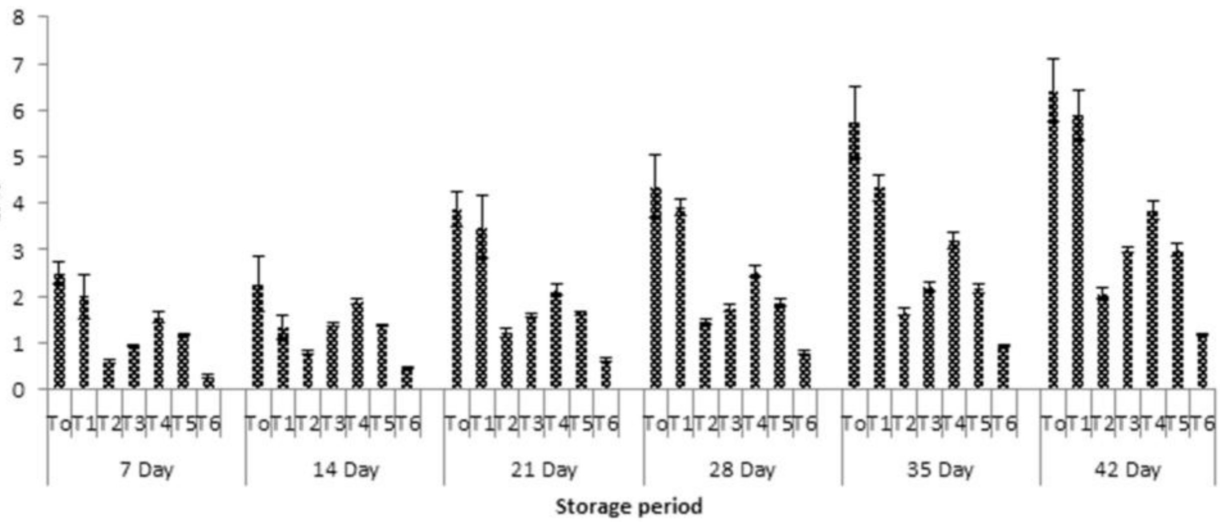

Figure4. Effect of Ca and Mg salts along with cellulose based coating on Chilling injury (%) under controlled storage.

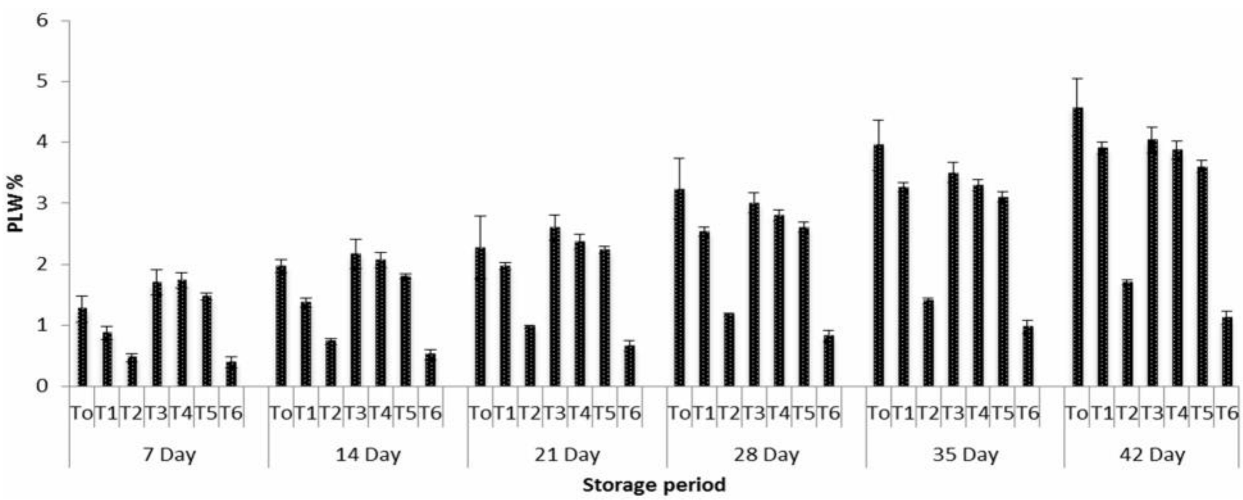

Figure 5. Effect of Ca and Mg salts along with cellulose based coating on %Loss of Weight (%) under controlled storage.

Table 1. Effect of Ca and Mg salts along with cellulose based coating on Biochemical parameters in different days of storage.

Storage (Days)	Total soluble solids (Brix)	Titratableacidity (%)	Vitamin C (mg/100g)
0	9.607^{G}	0.345^{A}	40.271^{A}
07	9.995^{F}	0.316^{B}	39.733^{B}
14	10.427^{E}	0.282^{C}	39.338^{BC}
21	10.871^{D}	0.252^{D}	38.995^{CD}
28	11.331^{C}	0.224^{E}	38.614^{DE}
35	11.809^{A}	0.195^{F}	38.195^{EF}
42	11.693^{B}	0.159^{G}	37.848^{F}

Table 2. Effect of Ca and Mg salts along with cellulose based coating on biochemical parameters in different treatments during storage.

Treatment	Total soluble solids (Brix)	Titratableacidity (%)	Vitamin C (mg/100g)
T_0	11.34^{A}	0.222^{E}	22.17^{E}
T_1	10.65^{E}	0.230^{E}	25.79^{CD}
T_2	11.18^{B}	0.285^{B}	26.67^{D}
T_3	10.99^{CD}	0.263^{C}	27.29^{B}
T_4	11.04^{C}	0.229^{E}	28.57^{BC}
T_5	10.91^{D}	0.250^{D}	27.10^{B-D}
T_6	9.62^{F}	0.295^{A}	39.829^{A}

Conclusion: This study revealed the comparative analysis on the effects of cellulose based coating with salts concentration under controlled storage condition on the quality and the shelf-life of *citrus reticulate.* Results depicted that the combination, where 5% cellulose coating along with 1% calcium and magnesium saltswere used, found best for maintaining the quality and extending the shelf-life of kinnow over other treatments or control. Hence, coated samples exhibited the least physiological loss in weight, juice % and less chilling injury with higher titratable acidity, ascorbic acid and lower total soluble solids. Moreover, fruit firmness was also maintained inhibiting ethylene production. Keeping in view the results, it can be concluded that the treatment T_6 was the most effective in maintaining the quality characteristics and extending the shelf-life of kinnow.

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