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Effect of Composite Coatings of Sodium Alginate and Neem Leaf Extract on Retention of Antioxidant Properties of Kinnow Mandarin under Low Temperature Storage

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ABSTRACT

Kinnow mandarin fruit possesses high nutraceutical value, but it exhibits heavy loss in antioxidant quality during long-term storage. The effect of sodium alginate coating and combination of Sodium Alginate (SA) and Neem Leaf Extract (NLE) coatings was evaluated on cold stored Kinnow fruits. The results revealed that fruits applied with composite coating exhibited slower decline in the levels of phenols (7.89%), flavonoids (16.8%) and carotenoid content (6.64%) during the storage as compared to the control. Coated fruits also retained better antioxidant activity as compared to the control during storage period. In addition, SA +NLE coating reduced the spoilage, loss in weight, loss of juice content, acidity and vitamin C in the Kinnow fruits. Similarly, at the end of 75 days storage period, maximum TA, TSS, juice content, Ascorbic acid, carotenoid content, TPC, TFC and anti-oxidant activity was maintained in 2% SA + 20% NLE coating, hence being the most effective coating.

Keywords: Antioxidant properties, *Azadirachta indica*, Nutraceutical value, Postharvest management, Shelf life.

INTRODUCTION

Fruits of citrus are widely consumed around the world for their high nutritional content, bioactive components, pleasant flavor and widespread availability. These fruits, including mandarins, are prone to heavy postharvest losses (25–30%) due to a lack of proper post-harvest management procedures and poor storage conditions (Kahramanoglu *et al*., 2020). The harvest season for Kinnow mandarin (*Citrus nobilis* L. × *Citrus deliciosa* L.) runs from mid-January to mid-February under Northern India conditions. In spite of its nutritional benefits and commercial eminence, the fruit availability during off-season is very limited owing to its poor shelf life of 8-10 days (Mahawar *et al.,* 2020). The short harvest period of Kinnow causes a surplus in local markets, which is accompanied by post-harvest losses. Postharvest losses are typically caused by decay produced by wide range of pathogenic fungi, as well as lack of scientific procedures for management, both of which must be skillfully resolved (Petriacq *et al*., 2018). Shelf life of fruits can be enhanced by various ways such as the application of films and coatings, controlled atmospheric storage, modified atmospheric packaging, low temperature storage, or by the use of growth regulators, fungicides, waxes, oil coatings, chemicals, irradiation and packing materials (Nasrin *et al*., 2020). Use of chemicals have been discouraged as a means of reducing fruit losses around the world as new approaches for food safety arise in the postharvest supply chain. Plant extracts and natural substances are the best alternative to control pre-harvest and post-harvest diseases in fruits (Prasad *et al*., 2022).

Edible coatings derived from

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polysaccharides are starch, alginate and cellulose. Among these, alginate is a biopolymer with a coating function that is isolated from brown sea algae (Phaeophyceae) and has lately received a lot of interest for protecting fruit quality over long-term storage. Alginate coatings have been used to prolong the shelf life of a variety of fruits, including mango (Rastegar *et al*., 2019) and avocado (Iniguez-Moreno *et al*., 2021). Some reports suggest that efficacy of alginate coatings can be enhanced by enriching it with natural extracts. Alginate enriched with rhubarb extract was found to extend the shelf life of peaches (Li *et al*., 2019). The potency of coating of chitosan enriched with pomegranate peel extract was reported in sustaining the quality of pear fruit under low temperature storage (Megha *et al*., 2021).

Alginate-based films enriched with antifungal components can boost antifungal activity and lengthen the shelf life of the fruit. Neem (*Azadirachta indica*) extract has been used as a pesticide and fungicide in Asia for ages. The active ingredient in neem, Azadirachtin, is thought to have growth-regulating, insecticidal, and fungicidal activities. The application of neem coatings to enhance the storage life of grapefruit (Khan *et al*., 2021), Kinnow (Rashid *et al*., 2020), and other crops has been investigated. However, these studies do not provide information on combined effect of neem leaf extract with other compounds. Postharvest storage of kinnow fruit using composite coatings of sodium alginate and neem leaf extract was unexplored till date. Therefore, the study aimed to provide an alternate to synthetic waxes for enhancing the shelf life of Kinnow at low temperature in an eco-safe way.

MATERIALS AND METHODS

Plant Materials

Fruits of Kinnow mandarin were harvested during mid-January, 2021, at maturity (Total Soluble Solids 11.00±0.17° Brix, Acidity 0.83±0.02%). The harvested fruits were transferred to Post-Harvest Laboratory, Department of Fruit Science, Punjab Agricultural University, Ludhiana (situated at 30˚ 53’ 57” N Latitude and 75˚48’ 20” E Longitude) India, for storage studies. Fruits were sorted out to remove diseased and undesirable fruits, washed with chlorinated water and shade dried.

Coating Preparation and Treatments

Preparation of sodium alginate coating (2%) was done by dissolving sodium alginate powder (20 g) in 800 mL of distilled water, stirred on magnetic stirrer for 1 hour at 70°C to make a clear solution. After cooling the alginate solution, 1.0% glycerol was added as a plasticizer and the final volume was adjusted to 1 L with distilled water. For Neem Leaf Extract (NLE), fresh and mature neem (*Azadirachta indica*) leaves were collected, washed and dried under shade. Dried leaves were ground to powdery form using Willy Mill and 1 L of distilled water was used to soak neem leaf powder (200 g) for 6 hours. Afterwards, it was filtered with muslin cloth. The obtained filtrate was utilized as stock solution (100%) to prepare dilutions of coating solutions of varied strengths. Combined coating of 2% SA+10% NLE was prepared by mixing 20 g sodium alginate powder, 1.0% glycerol, 20 g calcium chloride, 100 mL NLE stock solution and making the volume to 1000 mL with distilled water in the procedural manner. Similarly, for preparing 2% SA+20% NLE and 2% SA+40% NLE coating, 200 mL and 400 mL of NLE stock solutions were used, respectively.

For storage trials, 800 fruits were made into five lots with 160 fruits in each lot, with four replications in each lot. There were ten fruits in each replication. Four lots were given different coatings of 2% SA, 2% SA+10% NLE, 2% SA+20% NLE, 2% SA+40% NLE and the fifth lot was kept untreated as control. Packing of fruits was done in ventilated 3-ply corrugated fiber board (CFB) boxes (5% perforation) and

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kept at 5-6°C and 90 to 95% RH for 75 days. The fruits were tested for physical and biochemical parameters at 30, 45, 60 and 75 days intervals.

Observations Recorded

Physiological Loss in Weight (PLW)

The fruit weight was recorded after each storage interval and percent PLW was calculated using the following formula:

$$\text{loss in fruit weight (\%)} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \text{ X } 100$$

Spoilage

The number of spoilt fruits and the total number of fruits were counted on each storage interval for calculating the percent of spoilt fruits as follows:

$$\text{Spoilage (\%)} = \frac{\text{number of spoilt fruits}}{\text{total number of fruits}} \text{ X } 100$$

Total Soluble Solids (TSS)

TSS was measured with the help of a digital refractometer (ATAGO, PAL-1, Japan) and quoted as 'degree brix'.

Titratable Acidity (TA)

To determine TA, titration of 2 mL juice was done against 0.1N NaOH with 2 to 3 drops of phenolphthalein until a light pink color developed and stated as a percentage of citric acid.

Juice Content

Fruit weight was noted and juice was extracted with juice extractor and weighed. Juice content was calculated as follows:

$$\text{Juice content (\%)} = \frac{\text{Juice weight (g)}}{\text{Fruit weight (g)}} \text{ X } 100$$

Sensory Rating

The fruits were evaluated by a panel of four judges for general assessment of taste, texture, flavor and appearance of the fruits with the help of 9-point Hedonic scale.

Ascorbic Acid Content (AA)

Homogenization of pulp (0.2 g) was done in 3 mL of Tri-chloroacetic acid (10%) and centrifuged at the speed of 10,000 rpm for 10 minutes. Folin-ciocalteu reagent (0.2 mL) was freshly prepared and added to the supernatant and incubated at 37°C for twenty min. Absorbance of the samples was measured against the blank at 760 nm (Jagota and Dani, 1982).

Carotenoid Content

Carotenoid content was measured by the method given by Barnes *et al*., 1992.

Preparation of methanolic extract from pulp:

Pulp of fruit was dried in oven at 65°C. 300 mg of dried pulp powder was mixed with 80% methanol (10 mL) and kept for 24 h after vigorous shaking. The samples were refluxed in the water bath at 80°C for 1 hours, then, filtered. The total volume of filtrate was made up to 15 mL by using 80% methanol. This extract was stored at 4°C temperature until further analysis. The methanolic extract was prepared according to the previous study (Widodo *et al.,* 2019) and it was used for the biochemical analysis of TPC, TFC and antioxidant activities.

Total phenolic content of the samples was calculated in terms of milligram of gallic acid equivalent (Swain and Hillis, 1959). The flavonoid content of samples was calculated in terms of a milligram of rutin equivalent (Balabaa *et al*., 1974).The antioxidant activity of the kinnow fruit extract was determined using Williams *et al.* (1995) method. Hydroxyl ion scavenging activity was calculated as inhibition percentage of antioxidant activity (Li *et al*., 2008).

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Statistical Analysis

The experiment was set up in a completely randomized design (factorial) with four replications. Using SAS 9.3 software, analysis of the results was done using two-way variance analysis (storage duration x treatments) with $P \leq 0.05$ significance level. The least significant square test was followed to determine the variations among the means, and the data was reported as mean ± SE.

RESULTS

Loss in Weight (%)

Loss in weight increased progressively along with the storage period (Table 1). Mean loss in weight was minimum (3.94%) in the fruits coated with 2% SA+20% NLE, followed by the fruits coated with 2% SA+10% NLE extract. Highest mean weight loss (6.05%) was recorded in uncoated fruits. At 75 days, control fruits showed a weight loss of 8.53%, whereas it was 6.30% in 2% SA+20% NLE treatment.

Spoilage (%)

All the treatments showed a wide variation in spoilage percentage under low temperature storage (Table 1). No or negligible spoilage was seen in all fruits up to 45 days of storage. After 45 days, the spoilage percentage started increasing progressively and it was maximum after 75 days of storage. Minimum mean spoilage percentage (1.96%) was noted in the fruits coated with 2% SA+20% NLE, whereas maximum mean spoilage (6.57%) was in the control fruits.

Total Soluble Solids (TSS)

TSS of the fruits was 10.50 °Brix at harvest, which increased initially along with increase in storage duration (Table 1). TSS increased gradually and reached a peak level of 12.75 °Brix in 2% SA+20% NLE treated fruits after storage for 60 daysand then declined to 12.08 °Brix after 75 days. However, in the control fruits, initial increase was rapid and it reached a peak value of 12.45 °Brix on 45th day and then decreased rapidly to 10.13 °Brix after 75 days of storage.

Titratable Acidity (TA)

Irrespective of treatments, TA content of fruits decreased as the storage time progressed (Table 1). The rate of decline in acidity was slower in coated fruits than in the control fruits. After the completion of storage period, 2% SA+20% NLE coated fruits had the highest titratable acidity (0.51%), which was considerably higher than the TA of the control fruits (0.38%), 2% SA (0.42%) and 2% SA+40% NLE coated fruits (0.44%).

Juice content (%)

Juice content was found to be maximum (49.80%) at the harvest stage of the fruits, and declined along with increase in storage interval (Table 1). Untreated fruits showed the greatest decline of approximately 25% in juice content from the beginning to the end of 75 days storage. However, treated fruits showed a lesser decrease in juice percentage; being lowest in 2% SA+20% NLE coated fruits, which retained 43.48% juice content on 75th day of storage.

Sensory Rating

Sensory quality was tested on the basis of appearance, taste, sweetness, flavor, texture and aroma. The best mean sensory rating (7.72) was given to the fruits coated with 2% SA+20% NLE. At 0 day, all fruits were given an average rating of 7.9, which increased upto 30 days. However, the control fruits showed significant decrease in

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Table 1. Effect of sodium alginate and neem leaf extract coatings on PLW, Spoilage, TSS, TA, juice content, Sensory rating, AA and total carotenoid content of Kinnow fruits during low temperature storage.

Parameter	Treatments	Storage time (days)				
		0	30	45	60	75
Physiological loss in weight (%)	2% SA	-	2.98±0.39^{bcd}	3.97 ± 0.34^{bcd}	5.46±0.47^{fgh}	7.87±0.47^{fgh}
	2% SA+10% NLE		2.93±0.31^{bcd}	3.52 ± 0.31^{cde}	4.95±0.43^{h}	6.58±0.43^{h}
	2% SA+20% NLE		1.51±0.31^{e}	3.51 ± 0.35^{cde}	4.42 ±0.38^{hi}	6.30±0.38^{hi}
	2% SA+40% NLE		2.82±0.37^{bc}	3.87 ± 0.34^{bcd}	5.53±0.48^{fgh}	7.36±0.48^{fgh}
	Control		4.02±0.35^{a}	4.43 ± 0.38^{a}	7.23±0.63^{defg}	8.53±0.63^{defg}
Spoilage (%)	2% SA	0.00±0.00^{k}	0.00±0.00^{k}	0.59±0.05^{fgh}	7.20± 0.61^{fgh}	11.35±0.96^{fgh}
	2% SA+10% NLE		0.00±0.00^{k}	0.00±0.00^{k}	4.86±0.41^{h}	8.83±0.58^{h}
	2% SA+20% NLE		0.00±0.00^{k}	0.00±0.00^{k}	4.60±0.39^{hi}	5.20±0.44^{hi}
	2% SA+40% NLE		0.00±0.00^{k}	0.26±0.02^{fgh}	6.35±0.54^{fgh}	9.11±0.77^{fgh}
	Control		0.00±0.00^{k}	0.83±0.07^{defg}	12.73±1.08^{defg}	19.28±0.78^{defg}
TSS (^{0}Brix)	2% SA	10.50±0.17^{ij}	11.35±0.19^{fgh}	12.00±0.19^{bcd}	12.23±0.22^{abcd}	11.23±0.20^{gh}
	2% SA+10% NLE		11.08±0.18^{h}	11.83±0.21^{cdef}	12.65±0.19^{a}	11.95±0.22^{bcde}
	2% SA+20% NLE		11.05±0.19^{hi}	11.78±0.19^{cdefg}	12.75±0.23^{a}	12.08±0.22^{bcd}
	2% SA+40% NLE		11.30±0.23^{fgh}	11.95±0.22^{bcde}	12.30±0.22^{abc}	11.40±0.21^{efgh}
	Control		11.73±0.19^{defg}	12.45±0.20^{ab}	11.25±0.21^{gh}	10.13±0.21^{j}
Titratable acidity (%)	2% SA	0.83±0.02^{a}	0.70 ± 0.02^{c}	0.58 ± 0.02^{d}	0.50 ± 0.02^{ef}	0.42± 0.01^{gh}
	2% SA+10% NLE		0.76 ± 0.02^{b}	0.67 ± 0.02^{c}	0.55 ± 0.02^{de}	0.47 ± 0.02^{fg}
	2% SA+20% NLE		0.77 ± 0.02^{b}	0.69 ± 0.02^{c}	0.60 ± 0.02^{d}	0.51 ± 0.02^{ef}
	2% SA+40% NLE		0.70 ± 0.02^{c}	0.67 ± 0.02^{c}	0.52 ± 0.02^{ef}	0.44 ± 0.01^{g}
	Control		0.57 ± 0.01^{d}	0.51 ± 0.01^{ef}	0.47 ± 0.01^{fg}	0.38 ± 0.01^{h}
Juice content (%)	2% SA	49.80±0.64^{a}	48.07 ± 0.62^{bc}	46.61±0.60^{bcd}	44.20±0.57^{fgh}	41.56±0.56^{jk}
	2% SA+10% NLE		48.09±0.62^{abc}	47.15±0.61^{bcd}	44.82±0.58^{efg}	42.79±0.55^{hij}
	2% SA+20% NLE		48.24±0.66^{ab}	48.09±0.62^{abc}	45.66±0.59^{def}	43.48±0.56^{ghi}
	2% SA+40% NLE		48.05±0.78^{bc}	47.03±0.61^{bcd}	44.09±0.57^{fgh}	41.76±0.54^{ijk}
	Control		46.37±0.60^{cde}	44.10±0.57^{fgh}	40.21±0.52^{k}	37.23±0.72^{l}
Sensory quality rating	2% SA	7.9±0.18^{a}	8.00 ± 0.14^{bc}	7.50 ± 0.22^{bcd}	7.30±0.23^{fgh}	6.00 ± 0.22^{jk}
	2% SA+10% NLE		8.40 ± 0.14^{abc}	8.00 ± 0.22^{bcd}	7.50±0.08^{efg}	6.30±0.18^{hij}
	2% SA+20% NLE		8.60 ± 0.37^{ab}	8.10 ± 0.23^{abc}	7.60±0.28^{def}	6.40±0.27^{ghi}
	2% SA+40% NLE		8.10 ± 0.24^{bc}	7.50 ± 0.32^{bcd}	7.30±0.22^{fgh}	6.10±0.26^{ijk}
	Control		8.00 ± 0.20^{cde}	7.50 ± 0.22^{fgh}	6.60±0.18^{k}	5.60±0.14^{l}

Table 1 continued…

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Continued of Table 1. Effect of sodium alginate and neem leaf extract coatings on PLW, Spoilage, TSS, TA, juice content, Sensory rating, AA and total carotenoid content of Kinnow fruits during low temperature storage.

Parameter	Treatments	Storage time (days)				
		0	30	45	60	75
Ascorbic acid (mg 100 g^{-1} FW)	2% SA	42.66±0.39^{a}	40.52 ± 0.37^{de}	38.49±0.35^{fgh}	36.83±0.34^{jk}	34.22±0.50^{n}
	2% SA+10% NLE		41.82±0.47^{abc}	40.52±0.37^{de}	38.02±0.35^{hi}	36.12±0.46^{kl}
	2% SA+20% NLE		41.94±0.36^{ab}	40.71±0.40^{cde}	38.33±0.41^{gh}	36.90±0.54^{ijk}
	2% SA+40% NLE		41.35±0.33^{bcd}	39.60±0.36^{ef}	37.72±0.34^{hij}	34.74±0.51^{mn}
	Control		39.25±0.36^{fg}	37.76±0.34^{hij}	35.51±0.32^{lm}	33.68±0.50^{n}
Total carotenoid content (µg g^{-1} FW)	2% SA	12.62±0.23^{bcde}	13.38±0.29^{ab}	12.35±0.27^{cde}	11.92±0.26^{efg}	10.98±0.24^{hi}
	2% SA+10% NLE		13.12±0.33^{abc}	12.92±0.28^{abc}	12.70±0.23^{bcd}	11.54±0.25^{fgh}
	2% SA+20% NLE		13.09±0.18^{abc}	13.00±0.29^{abc}	12.79±0.28^{bc}	12.36±0.27^{cde}
	2% SA+40% NLE		13.39±0.29^{ab}	12.66±0.28^{bcde}	12.35±0.27^{cde}	11.14±0.24^{hi}
	Control		13.68±0.30^{a}	12.00±0.26^{def}	11.19±0.25^{gh}	10.39±0.23^{i}

acceptability and decreased to a minimum of 5.6 at 75^{th} day of storage. On 75^{th} day, maximum rating (6.4) was given to fruits coated with 2% SA+20% NLE.

Ascorbic Acid (AA) Content

During the period of storage, Kinnows coated with the combinations of SA and NLE had higher ascorbic acid (AA) content than the controls (Table 1). Untreated fruits had the greatest drop in AA content (21.05%) from the beginning to the end of storage, whereas this decline was 13.50% and 15.33% in fruits coated with treatment of 2% SA+20% NLE and 2% SA+10% NLE coatings, respectively. Furthermore, after 75 storage days, fruits treated with combined 2% SA+20% NLE and 2% SA+10% NLE coatings preserved higher AsA (36.90 and 36.10 mg 100 g^{-1} FW, respectively) than those treated with 2% SA alone (34.22 mg 100 g^{-1} FW).

Carotenoid content

Fruits had an initial increase in total carotenoids content from harvest to 30 days of storage, with the largest increase in the control fruits (Table 1). Following that, the carotenoid concentration in all treatments declined up to 75 days of low temperature storage. In comparison to the control (10.39 µg g^{-1} FW), the fruits treated with 2% SA+20% NLE (12.36 µg g^{-1} FW) exhibited the maximum retention of total carotenoid content after the completion of storage period.

Total Phenolic Content (TPC)

For all the treatments, a constant decrease in TPC of fruits was witnessed as the period of storage progressed (Figure 1). When comparing untreated fruits to the SA combined with NLE or pure SA treated ones, the results showed that the later had higher (11.92 mg g^{-1} DW) TPC. During storage, the TPC content of the control fruits decreased rapidly compared to coated fruits. In addition, when compared to alone SA treatment, combined SA and NLE coatings (20 and 10%) retained higher TPC. At the conclusion of 75 days of storage, 2% SA+20% NLE coated fruits had the highest TPC (10.78 mg GAE g^{-1} DW) in comparison to other treated fruit lots.

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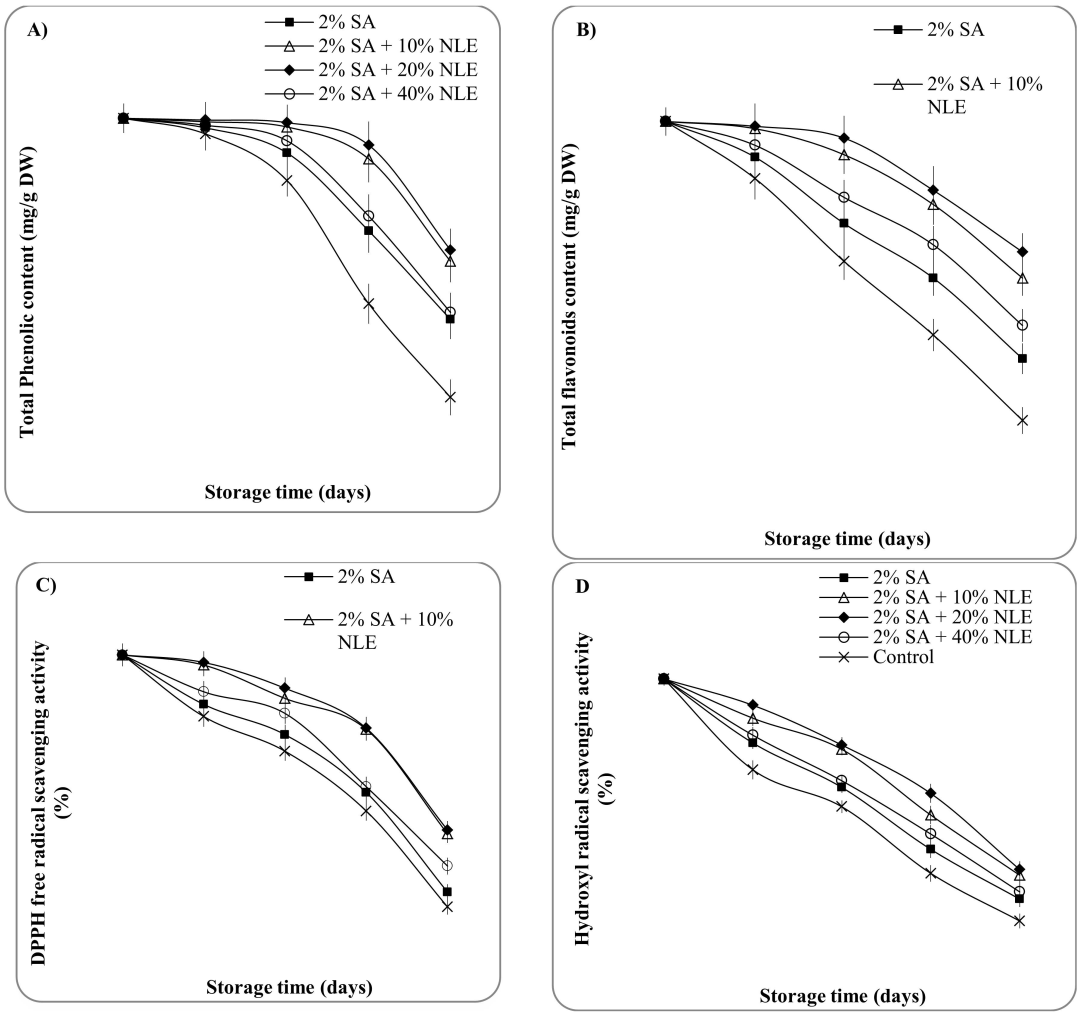

Figure 1. Effect of sodium alginate and neem leaf extract coatings on: (A) TPC, (B) TFC, (C) DPPH radical scavenging activity, and (D) Hydroxyl ion scavenging activity of Kinnow fruits during low temperature storage. The vertical bar represents the ± SE of mean for quadruplicate replications (P≤ 0.05).

Total Flavonoids Content (TFC)

Along with the progression of storage time, TFC levels were found to decrease progressively in all the fruits. Fruits coated with combination of coatings (SA+NLE) maintained more TFC than the control fruits (Figure 1). Kinnow fruits coated with 2% SA+20% NLE retained maximum TFC (2.49 mg RE g^{-1} DW) towards the end of the storage period, which was substantially more than TFC in fruits coated with SA alone (2.04 mg RE g^{-1} DW) and control fruits (1.78 mg RE g^{-1} DW). TFC loss was 23.35% lower in fruits coated with 2% SA + 20% NLE than in uncoated fruits at 75th day of cold storage.

Antioxidant activity

DPPH free radical scavenging activity varied from 16.82 to 28.23% during the 75 days storage period of Kinnow fruits (Figure

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1). DPPH radical scavenging activity was highest (28.23%) at the initiation of storage period, whereas it gradually declined to the minimum level towards the end of storage period. Irrespective of the storage interval, DPPH free radical scavenging activity was observed to be more in the coated fruits as compared to the control fruits. The DPPH inhibiting activity was lowest in control fruits (16.82%) and highest in fruits coated with 2% SA+20% NLE (20.30%).

The OH· scavenging activity ranged between 16.43 and 24.82% during the storage period (Figure 1). The OH· scavenging activity was highest (24.82%) at the initiation of the storage interval, which gradually declined to lowest value after 75 days of storage. At 75th day of storage, the uncoated fruits had the lowest OH· scavenging activity (16.43%). Kinnow fruits coated with 2% SA+20% NLE (18.21%) had 1.10 times higher OH· scavenging activity than the control at 75th day of storage, followed by 2% SA+10% NLE (18.01%), 2% SA+40% NLE (17.43%), and 2% SA treatment (17.19%).

DISCUSSION

Although kinnow fruit is rich in vitamins and antioxidants, it goes through metabolic alterations when stored. Substandard postharvest management practices result in quality degradation of fruits, reducing the consumer health benefits and endangering food security (Mahajan *et al*., 2022). Therefore, to obtain the nutritional benefits, it is critical to preserve the antioxidant capacity of fruits throughout storage. Edible coatings act as a barrier and hinder the water loss from the fruit surface to the atmosphere, and hence prevent dehydration. Coatings also reduce the uptake of oxygen by the fruit, thus slowing down the rate of respiration. These were the probable reasons for the lower loss of weight in coated fruits. The results were also in accordance with the studies conducted in other fruit crops like plums, peaches and strawberries, which showed that the coating of sodium alginate combined with some ingredients having antifungal activity can reduce the weight loss (Valero *et al*., 2013).

The fruits decayed due to various fungi rot, making the fruits inedible with bad odor and other undesirable biochemical changes. The results revealed that all the coated Kinnow fruits had a longer shelf life than the control fruits due to significantly lesser percentage of spoilage as compared to the control. Addition of 20% NLE to 2% SA coating could be a favorable approach in reducing the postharvest losses in Kinnow mandarin as neem extract acts as an antimicrobial agent. The sodium alginate-$CaCl_2$ edible coating also delayed the surface mold growth in strawberries for up to 15 days (Alharaty and Ramaswamy, 2020). In the beginning, the breakdown of insoluble polysaccharides into simple sugars increases total soluble solids. Following that, it gradually declined, probably due to a decrease in the carbohydrates and pectin levels, partial protein breakdown, and glycoside sub-unit breakdown during respiration (Belay *et al.,* 2019). The highest mean TSS (11.57%) was observed in fruits coated with 2% SA + 20% NLE, likely due to the decreased respiration rate, whereas the controls exhibited lowest TSS levels, potentially related to increased respiratory losses in these fruits due to the lack of a barrier to obstruct gas flow in the fruits. Similar TSS levels (11.17%) were acknowledged by Deshmukh *et al.* (2020) in Nagpur mandarin. During storage, the organic acids being consumed during the processes like respiration could be held accountable for the fall in acid content of Kinnow fruits (Shakir *et al*., 2022). Lesser decrease of acidity in coated fruits could be due to the property of sodium alginate in lowering the rate of respiration (Alharaty and Ramaswamy, 2020). It has been found that edible coating shows the effect in decreasing acidity losses in alginate and chitosan coated pear fruits (Adhikary*et al*., 2022). After 30 days, the acceptability of fruits started decreasing gradually due to

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degradative processes that took place in the fruits. Upon completion of 30 days of storage, the fruits became more acceptable, probably due to some desirable changes like optimum increase in TSS and decrease in acidity, which made an appropriate blend of taste. This decrease was slower in the coated fruits as the coatings form a barrier for gases and slowed the respiration rate and transpiration losses, thereby maintaining the quality of the fruits. On 75th day, maximum rating (6.4) was given to fruits coated with 2% SA+20% NLE treatment. Similarly the sodium alginate-$CaCl_2$ edible coating was known to preserve the sensory properties of the cut strawberry fruits (Alharaty and Ramaswamy, 2020).

Ongoing breakdown processes and moisture loss probably result in decrease in juice content during storage. Lower decline in juice content in coated fruits could be due to the protective shield effect of coating (Nath *et al*., 2013). AA is an important nutritional component in Kinnow that is frequently lost due to oxidation during storage. The effectiveness of NLE coatings enriched with SA in minimizing AA losses in fruits during storage could be attributed to the development of a defensive barrier against gas permeability on the fruit surface (Kumari *et al*., 2015). Citrus juice is the most complex natural source of carotenoids. Results revealed that the total carotenoid content increased upto 30 days of storage and then showed a decline, which may be due to conversion of chlorophyll to carotenoids in initial stages of storage and later degradation of carotenoids. The highest retention of carotenoids in 2% SA+20% NLE coating was presumably attributable to carotenoids' lower susceptibility to oxidation and geometric isomerization of their polyene chain. Similar results were also obtained by Baswal *et al*. (2020) earlier in Kinnow.

Phenolic compounds are principle secondary metabolites that function as strong antioxidants. TPC alterations throughout the postharvest period are influenced by genetics, temperature, and environmental factors (Rasouli *et al*., 2019). Oxidative degradation of phenolics due to cell structure breakdown induced by senescence leads to decline in total phenolic content during storage (Ali *et al*., 2019). Coatings create a semipermeable barrier over the fruit, altering the internal atmosphere by increasing CO_2 and/or decreasing O_2, and slowing the pace of nutritional degradation in mandarins. Similar lower decline of phenols in alginate-coated fruits were also demonstrated by Bose *et al*. (2019) in strawberry. Flavonoids are the most common type of polyphenol found in plants, and they offer significant protection against a variety of chronic diseases because of their antioxidant and anti-inflammatory activities (Zhang and Tsao, 2016). Slower decline of total flavonoids in coated fruits than control might be attributed to delay in senescence caused by coatings. Similar results were found by use of alginate coating incorporated spirulina coating by Rastegar and Atrash (2021) in mango.

Fruit antioxidant activity is strongly linked to polyphenols, which include flavonoids, therefore, variations in phenolic content may affect antioxidant activity (Haider *et al*., 2020). Furthermore, ascorbic acid, as an important member of the antioxidant family, may affect the antioxidant nature of fruit. The oxidation of phenolics, carotenoids and AA compounds in fruits often signifies a decrease in antioxidant activity during storage (Saleem *et al*., 2020). The decrease in these antioxidant components accompanied by the accelerated senescence with the progression of storage period reduced the DPPH radical scavenging activity (Ali *et al*., 2021). However, application of NLE and SA application, on the other hand, considerably lowered the decline of DPPH activity in coated fruits. Similarly, alginate coating preserved markedly higher DPPH activity in mango fruits (Rastegar *et al*., 2019). Hydroxyl radical scavenging activity showed a similar decreasing trend along with increase in storage period as seen in DPPH antioxidant activity, which was probably due to decrease in the amount of antioxidants. The fruit

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extracts of *Muntingiacalabura* had dose-dependent hydroxyl radical scavenging action (Preethi *et al*., 2010).

CONCLUSIONS

The effectiveness of natural compounds such as sodium alginate combined with neem leaf extract on fruit antioxidant activities was deliberated in this study. The use of combined coatings containing 2% SA and 20% NLE was found to be an effective technique for minimizing the loss in antioxidants. The current study found that sodium alginate combined with neem leaf extract was beneficial in retaining TSS, acidity, juice content, and antioxidant levels in Kinnow by effectively retaining compounds such ascorbic acid, phenolics, and carotenoids. The coating of 2% SA + 20% NLE developed an effective barrier to gas exchange and slowed the metabolic processes, delaying senescence and, so, maintaining flavonoid and antioxidant levels. Based on these findings, the use of treatment of 2% SA + 20% NLE is an effective and nature-friendly approach for preserving the bio-active components of Kinnow throughout storage. As a result, sodium alginate combined with neem leaf extract could be an effective method as a commercial substitute to replace synthetic material for increasing postharvest life of Kinnow fruits.

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تاثیر پوشش های ترکیبی آلژینات سدیم و عصاره برگ چریش (Neem) بر حفظ خواص آنتی اکسیدانی نارنگی کینو (Kinnow mandarin) در دمای پایین در انبار

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چکیده

میوه نارنگی کینو(Kinnow) ارزش غذایی بالایی دارد، اما در طول نگهداری طولانی مدت، کیفیت آنتی اکسیدانی زیادی را از دست می دهد. اثر پوشش آلژینات سدیم (sodium alginate) و ترکیب پوشش‌های آلژینات سدیم (SA) و عصاره برگ چریش (NLE) بر روی میوه‌های کینو در انبارسرد بررسی شد. نتایج نشان داد که در طول نگهداری، در مقایسه با شاهد، میوه های دارای پوشش ترکیبی کاهش آهسته تری در مقدار فنل (۷.۸۹%)، فلاونوئیدها (۱۶.۸%) و محتوای کاروتنوئید (۶.۶۴%) داشت. میوه‌های پوشش‌داده‌شده نیز در

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مقایسه با شاهد فعالیت آنتی‌اکسیدانی بهتری را در طول دوره نگهداری حفظ کردند. افزون بر این، پوشش SA + NLE باعث کاهش فساد، کاهش وزن، کاهش محتوای آب میوه، اسیدیته و ویتامین C در میوه‌های کینو شد. همانند این، در پایان دوره نگهداری ۷۵ روزه، حداکثر TA، TSS، محتوای آب میوه، اسید اسکوربیک، محتوای کاروتنوئید، TPC، TFC و فعالیت آنتی اکسیدانی در پوشش ۲٪ SA + 20٪ NLE حفظ شد، درنتیجه، این پوشش موثرترین پوشش است.

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