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Influence of Postharvest Applications of Some Edible Coating on Storage Life and Quality Attributes of Navel Orange Fruit During Cold Storage

Aml R.M. Youssef, Enas A.M. Ali, Hala E. Emam

Horticultural Crops Technology Dept., National Research Centre, 33 Bohouth St., Dokki, Giza, Egypt.

Abstract: The main benefits of edible active coatings are to maintain the quality and extend shelf life of fresh fruits and prevent microbial spoilage. This experiment was conducted to evaluate the influence of some edible coating such as chitosan concentrations (0.1, 0.5 and 1%), mango leaves extract and mango leaves extract with 2% gelatin) on Navel orange fruit to extending storage life and maintain fruit quality during two successive seasons of 2012 and 2013. Fruit quality characteristics i.e. fruit firmness, respiration rate, total acidity, soluble solid content, ascorbic acid content, juice weight, juice volume, cellulase and pectinase enzyme activities were assessed periodically after storage at 5^{o}C and 85-90% RH. Results indicated that chitosan coating at 1% and mango leaves extract with gelatin at 2% could be more effective in keeping fruit firm with the least respiration rate, titratable acidity and the highest value for soluble solid content, ascorbic acid, weight of juice and volume during storage period at 5^{o}C than all other coating treatment compared with untreated fruit (control). Navel orange fruits showed a great increase in cellulase activity compared with gradual decrease in Pectinase activity after cold storage period. The results of this study showed that chitosan coatings and mango leaves extract with gelatin at 2% have an excellent potential to be used on Navel orange fruit to maintain quality and extending shelf life.

Key words: orange fruit, edible coating, chitosan, mango leaves extract, gelatin, quality.

Introduction

Citrus is considered the most common popular fruit in the world and it takes second or third position after grapevines and apples[1]. Navel orange (*Citrus sinensis*, L. Osbeck)) is the most popular fruit crops among other citrus species in Egypt. Consumers usually judge the quality of fresh fruits on the basis of appearance and freshness at the time of purchase[2]. There are many successful postharvest techniques (such as controlled atmosphere, modified atmosphere packaging, plastic film packaging, etc.) which have become standard practice, however edible coatings / films are of great interest and continue to be extensively studied for their potential ability to maintain the quality of fresh fruits and vegetables[3]. Edible coatings as wax, oils, natural products and others have been used as an effective technology to keep the quality of postharvest fruits and vegetables[4]. Films and edible coatings are defined as a thin application of material that forms a protective barrier around an edible commodity and can be consumed along with the coated product[5]**.** Moreover, Edible coatings are used to create a modified atmosphere and to reduce weight loss during transport and storage[6]. Chitosan is a modified natural carbohydrate polymer derived from chitin, which has been found in a wide range of natural sources such as crustaceans, fungi, insects and some algae reported by[7]. Chitosan coating offers a defensive barrier against bacterial contamination and loss of moisture from the surface of food products, thus extending their shelf life. With limited increase in the concentration of chitosan coating, the beneficial effect of chitosan on postharvest

life fruits and quality of the food is enhanced. The present review delineates the preparation, properties and potential application of chitosan coatings for enhancing the postharvest life and quality of different types of fruits[8]. Chitosan coatings containing bergamot oil produced the most effective antimicrobial activity, and showed the greatest inhibition of the respiration rates in terms of both O_2 consumption and CO_2 generation[9].

Mango (*Mangifera indica* L.) leaves extract can be used as natural preservatives in food applications as natural antioxidant additive replacing toxic synthetic antioxidants such as butylhydroxytoluene (BHT)[10] and as anti-aging compound in cosmetic products[11]. Also, leaves are a rich source of phenolic compounds with strong antioxidant power, particularly mangiferin, a special xanthone commonly called as "super-antioxidant" because of their potent antioxidant capacity, and other phenolic compounds like quercetin, widely studied by its pharmacological properties[12]. On the other side, Gelatin is an important functional biopolymer widely used in foods to improve elasticity, consistency, and stability[13]. In addition, gelatin can be made from many different sources of collagen. Cattle bones, hides, pigskins, and fish are the principle commercial sources. As such, it may come from either agricultural or non-agricultural sources. There are no plant sources of gelatin, and there is no chemical relationship between gelatin and other materials referred to as vegetables gelatin, such as seaweed extracts[14].The characteristic features of gelatin are high content of the amino acids glycine, proline and hydroxyproline. Gelatin also has a mixture of single and double unfolded chains of hydrophilic character[15].

The objective of this study was to investigate the effectiveness of some postharvest coating treatments as chitosan with concentrations at (0.1, 0.5 and 1%), mango leaves extract and mango leaves extract with gelatin at 2%) on maintaining quality criteria, pectinase and cellulase activity and storage period extension of Navel orange fruit.

Material and Methods

Fruit.

Mature fruit were harvested from 20 years old trees of Navel Orange (*Citrus sinensis* L. osbeck) budded on Sour Orange (*C. aurantium* L.) rootstock in a private orchard at El- Menoufia Governorate during two successive seasons of 2012 and 2013. The maturity stage of Navel orange fruit is assessed from the color, fruit firmness, juice content, ssc, and acidity of the juice. Mature orange fruit harvested at full color stage and SSC (11%), TA (0.90%), firmness (13 Ib/inch^2), juice ratio (35%) and ascorbic acid (VC. 48 mg/100ml juice).Undamaged fruits, free from visual blemishes, uniform in shape, weight, color and firmness were harvested, graded, packed and transported immediately to the postharvest laboratory of Agricultural Development System (ADS) project in Cairo University.

Treatments.

On arrival, fruits were washed, air dried and were Coated by different concentration of chitosan solution (0.1, 0.5 and 1%), Mango leaves extract (*Mangifera Indica*., were collected from a private orchard free from insect infestation, infection or damage and carefully washed with tap water and left to dry in the dark at room temperature. The air-dried leaves were converted to powder and the air-dried powdered leaves (100 g) were macerated for 24 hr. with distilled water (1 L), and then filtered to obtain the water extract and Mango leaves extract added to 2 % gelatin or control fruit which treated with sterile distilled water then air-dried. Fruit packed in carton boxes and stored at 5^{o}C for 60 days in control temperature rooms (Bally sectional prefab. walls, Ins. USA) with relative humidity at (85-90%).Three replicates for each treatment and sampling time (15 days) were used and each replicate consists of 5 fruits. Gases, enzyme measurements and fruit quality were assessed as follow:

Fruit quality assessments.

Fruit firmness: Fruit firmness was determined using Ametek pressure tester. Firmness of 5 fruits from each replicate was measured at two opposite points on the equator of each fruit. Results were calculated as Ib/inch^2 [16].

Respiration rate: fruits of each sampling date were weighed and placed in 3-liter jars at 20°C. The jars were sealed for 24 hr. with a cap and a rubber septum. O_2 and CO_2 samples of the headspace were removed from a septum with a syringe and injected into Servomex Inst. (Model 1450C, Food Pack Gas Analyzer) to measure oxygen and carbon dioxide production. Respiration rate was calculated as ml CO_2/kg/hr.[17, 18].

Juice weight and volume were evaluated**.** Total acidity (expressed as citric acid) was determined by titrating 5 ml juice with 0.1N sodium hydroxide using phenolphthalein as an indicator, SSC content was measured to using a T/C hand refractometer Instrone, Brix-readings 0-30 ranges (Model 10430, Bausch and Lomb Co. Calif., USA). Ascorbic acid content was determined using 2 and 6 dichlorophenol indophenols' titration methods as described by[16].

Pectinase and Cellulase Activities: 0.5 ml of supernatant enzyme extraction were used and mixed in acetate and citrate buffer and incubated at 45 and 50°C for l0 min for pectinase and cellulase respectively. The reaction was stopped with 3 ml of 3, 5-dinitrosalicylic acid reagent, the color was obtained after heating for 10 min. and measured at wavelength of 570 nm and expressed as one unit of pectinase activity liberates 1 Mmol D-galactouronic acid in milliliter per min. While, Cellulase color was measured at wavelength of 5 % nm with shimadzu UV-VIS spectrometer model UV-240 and expressed as one unit of cellulose activity liberates1M mol glucose. Cellulose activity liberates 1M mol glucose in milliliter per min.[19, 20].

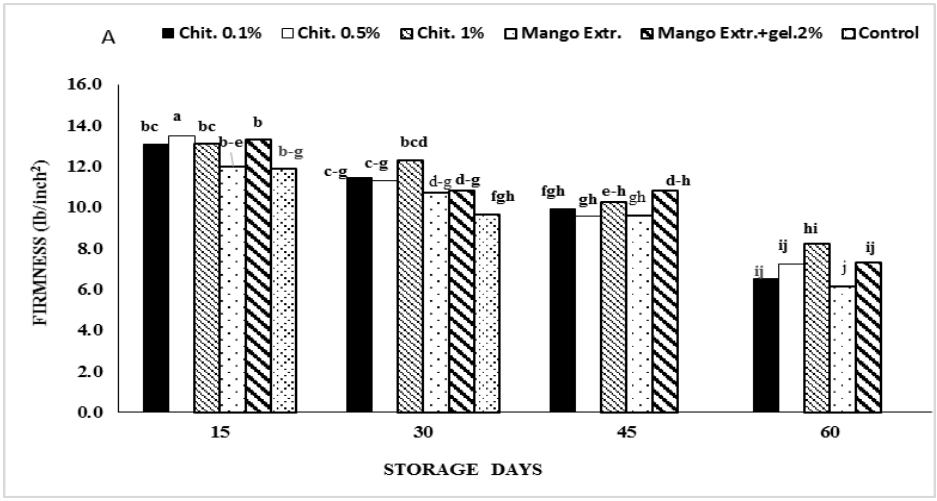
Statistical analysis: All data were subjected to statistical analysis according to the procedures reported by [21]. Treatments means were compared by Duncan‟s multiple range tests at the 5% level of probability in the two seasons of study.

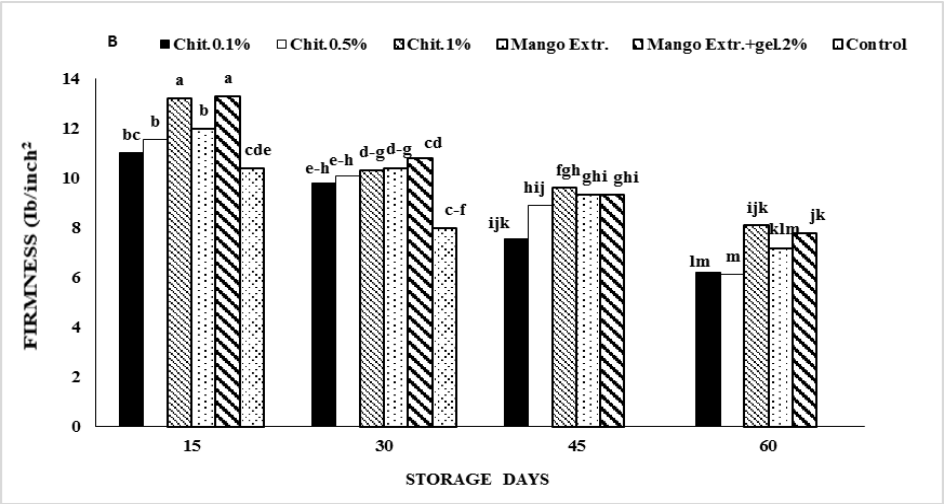
Results and Discussion

Fruit firmness: Fruit firmness is a major attribute that dictates the postharvest life and quality of fruit. chitosan coatings significantly reduced the loss in firmness of fruits during storage. Changes in fruit firmness of Navel Orange fruit varied after coating treatments by some natural compounds such as chitosan concentrations (0.1, 0.5 and 1%), mango leaves extract and mango leaves extract with 2% gelatin then storage at 5°C and RH, 85-90% compared with untreated fruit (control) during two successive seasons were illustrated in (Figs. 1 and 2).

The data cleared that, the firmness of coated fruit decreased gradually and significantly, towards the end of cold storage period recorded the less value after 60 days of storage at 5°C during two successive seasons. All the treatments gave rise to fruit with higher firmness values than untreated fruit (control), which recorded the less value (9.67 and 8.00 Ib/inch^2) at 30 days of storage period. Significant differences were noticed among all coating treatments. The highest values of fruit firmness recorded by fruit coated with 1.0 % chitosan (8.23 and 8.1 Ib/inch^2) and mango leaves extract with gelatin at 2 % (7.32 and 7.79 Ib/inch^2) followed by chitosan at 0.5 % (7.25 and 6.12 Ib/inch^2), chitosan at 0.1 % (6.52 and 7.15 Ib/inch^2). Meanwhile, orange fruits coated with mango leaves extract had the less firm at both season (6.14 and 6.21 Ib/inch^2).

The retention of firmness with chitosan coating is in agreement with the results of [22] who reported that papayas fruit (Solo cv.) coated with 1.5% chitosan were firmer than the control during 14 days storage at ambient temperature. The beneficial effect of the elevated chitosan concentration on firmness has also been reported for Murcott‟ tangor[23], papaya by[24] and for guava[25]. Also, coated Keitt mangoes with gelatin at 2% showed a significant delay in the changes of firmness compared to uncoated ones[26]. Meanwhile, the antioxidant treatment of Le Conte pear fruits gave the same effect in reducing the rate of fruit softening compared with untreated fruits, which recorded the lowest significant rate of fruit firmness showed by [27]. The obtained results could be explained by[28] which showed that antioxidants application improving postharvest quality in Yali pears may be due to a reduction membrane lipid peroxidation by enhancing the capacity of cells to scavenge reactive oxygen species.

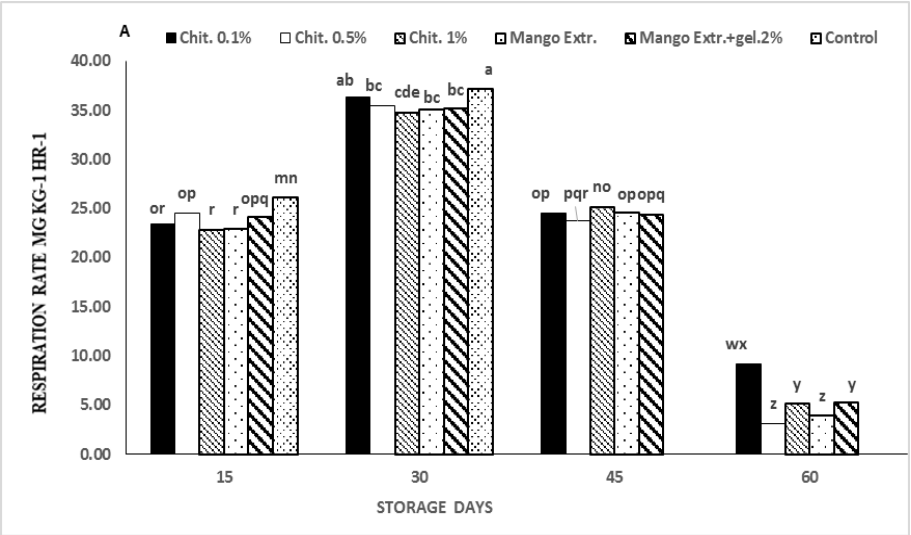


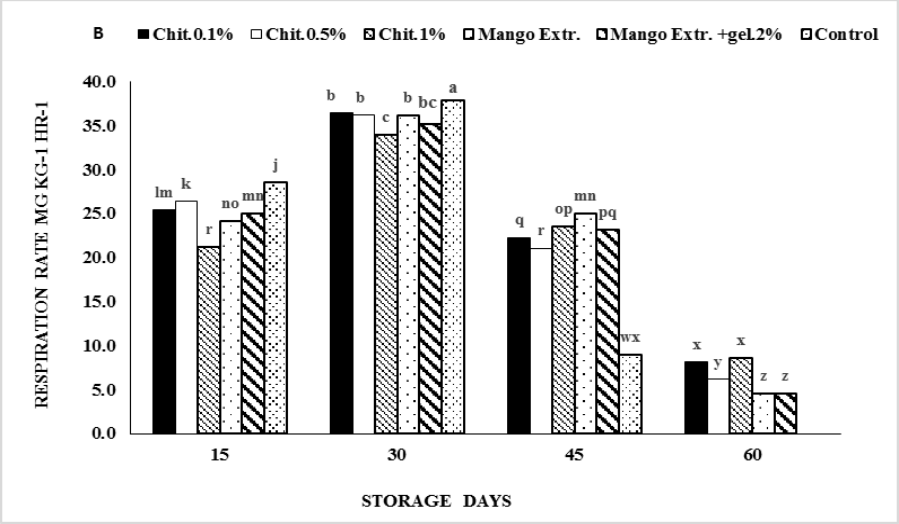


Figs. (1 and 2). Effect of some postharvest coating treatments of chitosan at 0.1, 0.5 and 1% , mango leaves extract and mango leaves extract with gelatin at 2% on firmness (Ib/inch^2).of Navel Orange fruit stored at 5º C and 85-90 % RH during 1st (A) and 2^{ed} (B)seasons.

Respiration rate: Postharvest coating treatments of Navel Orange fruit with chitosan at 0.1, 0.5 and 1%, mango leaves extract and mango leaves extract with gelatin at 2% throughout storage period at 5^0C during two successive seasons were elucidated in Figs. (3 and 4). Respiration rate (CO_2 production) of coated fruit resulted a noticeable significant increase reaching its peak value up to 30 days storage and then a decrease was observed at the end of storage period. On the other side, the fruit treated with chitosan at 1% appeared the least value of respiration rate followed by mango leaves extract with gelatin at 2% then mango leaves extract, chitosan at 0.5 and 0.1%, while untreated fruit recorded the maximum value of respiration rate.

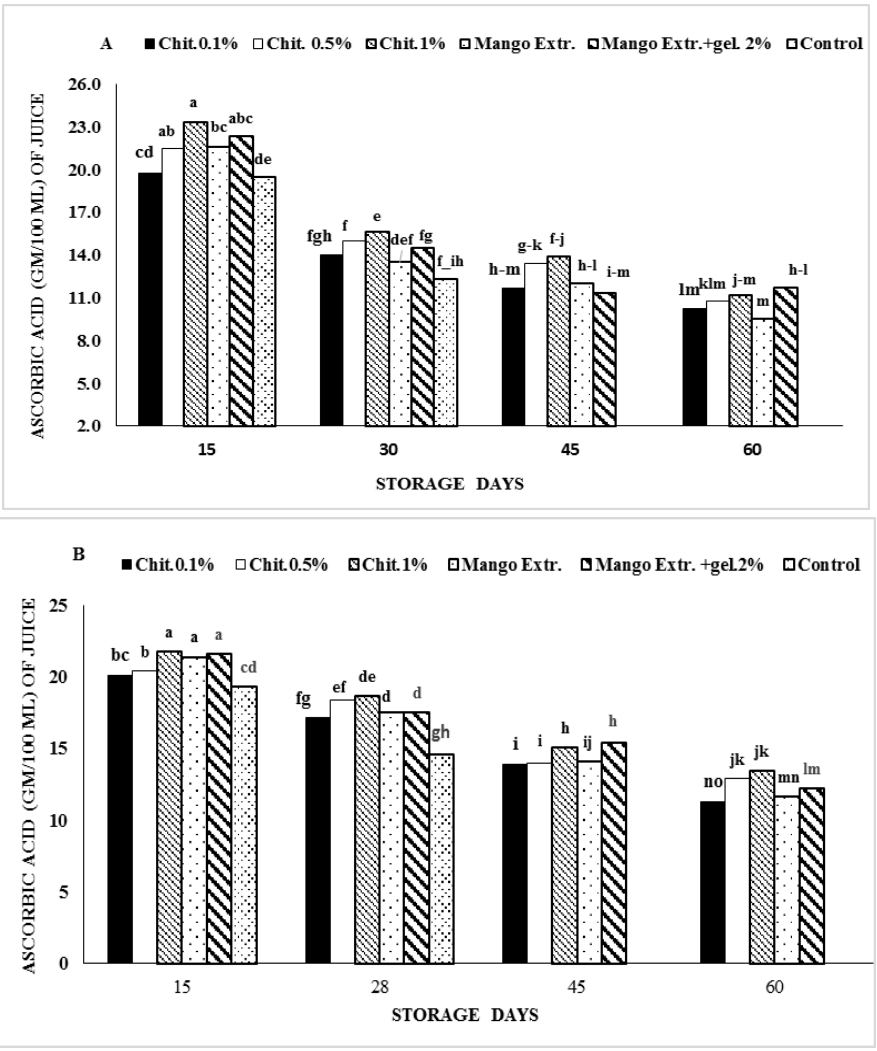
The rate of respiration reduces in grapes fruit coated with chitosan may be the reason of delayed senescence and a reduced susceptibility to decay[29]. The efficacy of chitosan in reducing production of internal CO_2 is reported on pears[30]**.** Also, the edible coatings of chitosan extend the shelf life of the fruit and vegetables by minimizing the rate of respiration and reducing the water loss found by [8]. On the other side,[28] found that ability of chitosan coatings with antioxidants to prolong storage quality of Chinese pear fruit because antioxidants decreased respiration rate.





Figs. (3 and 4). Effect of some postharvest coating treatments of chitosan at 0.1, 0.5 and1% , mango leaves extract and mango leaves extract with gelatin at 2% on respiration rate (mg kg^{-1} hr^{-1})) of Navel Orange fruit stored at 5°C and 85-90 % RH during 1*st* (A) and 2*ed* (B) seasons.

Ascorbic acid content: (Figs. 5 and 6) demonstrated the effect of some postharvest coating treatments such as chitosan at 0.1, 0.5 and1%, mango leaves extract and mango leaves extract with 2% gelatin at) on the fruit quality of Navel Orange cv. throughout storage period (60 days) at 5°C during the two successive seasons compared with untreated fruit. Ascorbic acid content decreased after 15 days of storage recorded the lowest value after 60 day‟s storage for all treatments with a little significant differences. In this case, Navel Orange fruit treated with chitosan at 1.0% had the maximum value of ascorbic acid (23.3 and 21.8) followed by mango leaves extract with 2% gelatin (22.32 and 21.6). Then mango leaves extract recorded (21.60 and 21.40), followed by chitosan at 0.5% (21.50 and 20.4) and 0.1 % (19.8 and 20.14).While untreated fruit recorded the minimum value of ascorbic acid content (19.5 and 19.35). These results agree with the findings of [31]who decided that, ascorbic acid of Kagzi lime fruits decreased when storage period increased. Also, [32]found that ascorbic acid decreased with increasing period of storage in kinnow fruits due to the action of some kinds of enzymes called ascorbic acid oxidase. The coated Keitt mangoes with gelatin at 2% showed a significant delay in the changes of ascorbic acid content compared to uncoated ones found by[26]. Meanwhile, Antioxidants treatments caused a general increase in fruit ascorbic acid content for “Alphonse” and “Badami” mango cultivars [33].



Figs. (5 and 6). Effect of some postharvest coating treatments of chitosan at 0.1, 0.5 and 1% , mango leaves extract and mango leaves extract with gelatin at 2% on ascorbic acid (mg/100 ml of juice) of Navel Orange fruit stored at 5ºC and 85-90 % RH during 1*st* (A) and 2*ed* (B) seasons.

Soluble solid content (SSC): This study have indicated that fruit of Navel orange fruit coated with different concentration of chitosan at 0.1, 0.5 and 1%, mango leaves extract and mango leaves extract with gelatin at 2% increased soluble solid content gradually and significantly to a peak after 30 days of cold storage at 5°C and RH, 85-90% thereafter, decreased gradually till the end of storage period compared to untreated fruit during the two successive seasons Tables (1 and 2). Fruit coated with chitosan at 1.0 % showed the highest value of SSC (15.9 and 14.8%) followed by mango leaves extract with gelatin at 2% (14.87 and 13.9%) during storage period. Meanwhile, coated fruit of chitosan at 0.1% recorded the lowest value of SSC (14.07 and 11.57%) compared with untreated fruit (13.07 and 10.8%) during both seasons respectively. The effect of chitosan coating on soluble solid content was probably due to the slowing down of respiration and metabolic activity, hence retarding the ripening process, modifying the internal atmosphere by reducing O_2 and/or elevating CO_2 and suppressing ethylene evolution reported by[34]. Moreover, the higher levels of total soluble solids in the fruit coated with chitosan may be due to protective O_2 barrier reduction of oxygen supply on the fruit surface, which inhibited respiration[35]. Coated Keitt mangoes with gelatin at 2% showed a significant delay in the changes of soluble solid content compared to uncoated ones found by[26]**.** In addition**,** treatment with antioxidants of Le Conte pear fruit showed more effective statistically in increasing SSC (%) throughout the progress of the storage periods reported by[27]. The increase in total soluble solids may be due to their influence in increasing photosynthetic pigment, which reflected on photosynthesis process and led to increase in carbohydrate content reported by[36].

Titratable acidity: Fruit acidity is important consumer variable as the balance of SSC and TA relates to overall taste and consumer acceptability. According to tables (1 and 2) it is clear that Navel orange fruits had gradual and significant decrease in titratable acidity during storage at 5^0C in the 1st and 2nd seasons after some edible coating treatments. coated fruit with chitosan at 1 % showed the minimum acid value after 60 days of storage (0.087 and 0.090%) followed by those coated of mango leaves extract with gelatin at 2% (0.11 and 0.10%), mango leaves extract (0.12 and 0.11%), chitosan 0.5 % (0.12 and 0.11%) and chitosan 0.1 % (0.13 and 0.13%) at the two successive seasons respectively. Control fruits kept only at 5^{o}C recorded the highest acid value (2.1 and 3.15) when compared with treated fruits. In this respect**,** titratable acidity is directly related to the concentration of organic acids present in the fruits observed by[37]. In addition, the results are similar with findings of [38] reported that the effect of chitosan on longan fruit decreased the titratable acidity during storage period. The higher levels of titratable acidity in coated fruit of gelatin at 2%, 4%, 8% and control may be due to protective O_2 barrier or reduction of O_2 supply to the fruit surface which inhibited respiration rate[39]. The percent total acidity (%)of pear fruits showed a slight reduction up to 45 days of cold storage and a gradual statistically decrease as storage period advanced for antioxidants treated and untreated fruit[27]**.** Moreover, The pear coating with antioxidants and stored helped maintain titratable acidity compared with control found by[28]. The reduction in juice acidity by antioxidants treatments could be attributed to its influence on increasing the tissue respiration and increasing ripening-associated activities[40].

Juice weight and volume: The juice weight and volume of Navel orange fruit treated by some edible coating throughout storage period at 5^{o}C during two successive seasons was elucidated in Tables (1 and 2). Fruits treated with chitosan at 0.1, 0.5 and 1%, mango leaves extract and mango leaves extract with gelatin at 2% results a noticeable significant increase in juice weight and volume reaching the maximum value up to 30 days of cold storage and then an decrease was observed at the end of storage period. Generally, results indicated that fruits treated with chitosan at 1% recorded the highest juice weight and volume) followed by mango leaves extract with gelatin at 2 %, mango leaves extract, chitosan at 0.5% chitosan at 0.1%. Coating treatments gave effective results by maintaining higher juice weight and volume recovery over control fruit, which gave the lowest value of juice weight and volume after 30 day's storage. The same trend was noticed in both seasons. These results were agree with those obtained by[41, 42, 43]. Also, these results are confirmed by [44] who reported that chitosan coatings have been barrier to moisture loss and therefore retarding dehydration of papaya fruit.

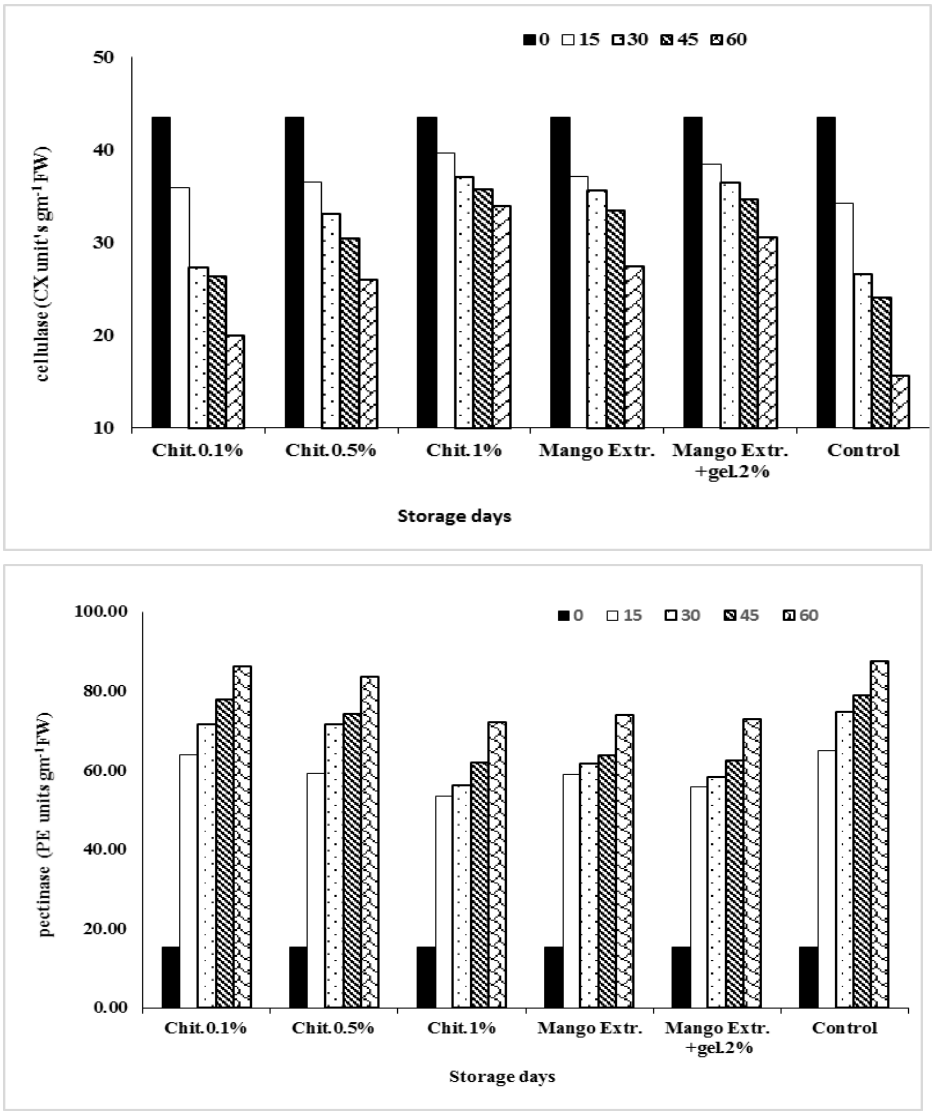
Table (1). Effect of some postharvest coating treatments of chitosan, mango leaves extract and mango leaves extract with gelatin at 2% on soluble solid concentration(%), total acidity (%),juice weight (gm) and juice volume(cm^3) of Navel Orange, fruit stored at 5º C and 85-90 % RH during first seasons.

Characters / Treatments	Storage in days	Chitosan 0.1%	Chitosan 0.5%	Chitosan 1%	Mango Extract.	Mango Extract. +gelatin.2%	Control
(SSC %)	15	13.23 b-f	12.5 d-g	14.4 abc	13.4 b-f	13.5 b-f	12.32 e-h
	30	14.07 a-e	14.27abcd	15.9 a	14.5 abc	14.87 ab	13.07 b-f
	45	10.63 h-k	11.13 g-j	12.85 c-g	12.43 d-h	12.7 c-g	_
	60	8.23 l	8.79 kl	10.13 ijk	9.56 jkl	9.93 jkl	_
Acidity (%)	15	0.84 b	0.92 b	0.84 b	0.9 b	1.01 b	2.21 a
	30	0.75 b	0.72 bc	0.62 cd	0.68 c	0.63 cd	2.1 a
	45	0.22 e	0.23 cd	0.19 d	0.18 d	0.18 d	_
	60	0.13 d	0.12 d	0.087 f	0.12 d	0.11 d	_
Juice weight (gm.)	15	77.5 i	77.7 i	112.33 c	83 gh	85.79 g	68.9 k
	30	95.96 e	102.25 d	137.14 a	109.5 c	115.33 b	90.2 f
	45	55.21 n	80.86 h	93.54 e	83.41 gh	83.41 gh	_
	60	48.4 o	48.4 o	72.9 g	51.9 n	71.91 j	_
Juice volume (cm3)	15	80.33 def	80.23 def	110 ab	83 def	85.67 cd	67.83 fgh
	30	93.54 bcd	95.48 bcd	115.3 a	102.3 abc	114 a	85.67cde
	45	52.67 hij	55.5 g-j	85.67 cde	80.86 def	83.41 def	_
	60	45.17 jk	48.4 ijk	71.91 efg	55.65 g-l	70.67 efg	_

Table (2). Effect of some postharvest coating treatments of chitosan, mango leaves extract and mango leaves extract with gelatin at 2% on soluble solid concentration (%), total acidity (%), juice weight (gm) and juice volume (cm^3) of Navel Orange fruit stored at 5° C and 85-90 % RH during second seasons.

Characters / Treatments	Storage in days	Chitosan 0.1%	Chitosan 0.5%	Chitosan 1%	Mango Extract	Mango Extract. +gelatin.2%	Control
(SSC %)	15	11.61 b-g	12.57 a-e	12.9 a-d	11.53b-g	12.4 a-e	11.03 c-h
	30	11.57 b-g	13.5 abc	14.8 a	12 b-f	13.9 ab	10.8 d-h
	45	10.33 e-i	10.33 e-i	11.5 b-h	10.7d-i	10.94 d-h	_
	60	8.27 ijk	9.00 hij	10.13a-i	9.3 g-j	9.66 f-j	_
Acidity (%)	15	1.05 b	0.93 b	0.89 b	0.92 b	0.9 b	1.94 b
	30	0.75 b	0.74 b	0.59 b	0.63b	0.61 b	3.15 a
	45	0.29 b	0.23 b	0.18 b	0.18 b	0.16 b	_
	60	0.13 b	0.11 b	0.09 b	0.11 b	0.1 b	_
Juice weight (gm.)	15	95.96 g	97.3 fg	114.33 c	98.4 fg	98.4 fg	89.2 i
	30	109.5 d	110.5 d	137.14 a	111 d	118 b	102.25 e
	45	81.81 k	90.86 hi	99.95 ef	92 h	97.12 g	_
	60	63.25 n	63.73 n	77.95 l	71.91 m	75.42 l	_
Juice volume (cm3)	15	86 g	89.33 f	113 b	96.65 fg	97.63 e	84.67 gh
	30	101.33 d	101.15 d	122 a	108.41 c	112.5 b	97.3 e
	45	75.21 i	85.67g	95.67 fe	86.83 fg	89.33 f	_
	60	53.33 m	58.4 l	75.2 i	67.5 k	70.66 j	_

Cellulase and Pectinase enzyme activities: The results illustrated in (Figs. 7 and 8) recorded that there were a significant increase in cellulase activity compared with gradual decrease in Pectinase activity of edible-coated Novel fruits gradually towards the end of cold storage period (60 days). Untreated fruit (control) showed a great increase in cellulase activity while coated fruit with 1% chitosan recorded the maximum value compared with the initial value at harvest. Pectinase activity decreased gradually after storage period at 5°C recorded its least value after eight weeks compared to its activity at harvest. These results confirmed with [45]whose reported that coating treatments affected changes in the cell-wall constituents by actually inhibiting the hydrolytic enzymes including pectinase and cellulase of pears during storage thereby reduction the rate of metabolic processes in fruit during ripening and storage.



Figs. (7 and 8). Effect of some postharvest coating treatments of chitosan at 0.1, 0.5 and1%, mango leaves extract and mango leaves extract with gelatin at 2% on fruit cellulase and pectinase activities (units /gm. FW.) of Navel orange fruit stored at 5ºC and 85- 90% RH during means of two seasons.

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