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PROLONGING STORAGE AND SHELF LIFE OF "ANNA" APPLE FRUITS BY USING CHITOSAN AND SOME NATURAL ANTIOXCIDANTS

Neven M.N. Nagy*

Hort. Dept., Fac. Agric., Damanhour Univ., Egypt

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ABSTRACT: This study was carried out during two successive seasons of 2016 and 2017 in a private orchard located in Abo Hommos district, Beheira Governorate, Egypt to evaluate the effect of some postharvest treatments included: 2% (*W/V*) chitosan, 200 ppm citric acid, 200 ppm ascorbic acid, 2 mmol/l salicylic acid, chitosan plus citric acid, chitosan plus ascorbic acid and finally chitosan plus salicylic acid, all at the above- mentioned concentrations on decay, firmness as well as other quality parameters of "Anna" apple fruits during cold storage at 2±1^{o}C with 90- 95% RH for three months and after post- storage shelf life at ambient temperature for five days. The results revealed that fruit decay, fresh weight loss, electrolyte leakage, total sugars, total soluble solids (TSS) as well as anthocyanin and carotene contents were increased, while firmness, vitamin C and acidity were decreased with the progress of cold storage and post- storage shelf life periods. Furthermore, the results indicated that all applied treatments reduced the incidence of fruit decay and fruit fresh weight loss. Furthermore, postharvest treatments retarded the loss of fruit firmness, lessened electrolyte leakage of fruits and were more effective on keeping the physical and chemical quality properties relative to the control treatment. Finally, this study emphasized that the addition of chitosan to antioxidant acids was more effective than the sole applications of each component on mitigating the incidence of decay, retarding the loss of firmness, reducing weight loss and electrolyte leakage beside keeping other physical and chemical characteristics, thus prolonging the storage and post- storage shelf life of "Anna" apples, especially the combination included chitosan and salicylic acid. Therefore, this strategy is easy to apply, new, cheap and safe that can ensure increases of "Anna" apple fruits marketability and the growers income.

Key words: Apple, coating, chitosan, antioxidants, decay, electrolyte leakage, pigments, firmness, fruit quality, storage, shelf life.

INTRODUCTION

Apple (*Malus domestica* L.) is considered one of the most important fruit consumed in the world. Meanwhile, consumers demanded apple fruits and the concept has been increasing by the public awareness of health and nutrition, confirmed the requested superior fruit quality with prolonged shelf life. "Anna" apple has been categorized as an excellent commercial cultivar under Egyptian conditions (low chilling requirement).

There are many problems facing apple producers and consumers such as firmness which deemed one of the most important features of apple quality. Consumers desire a crispy apple flesh so keeping fruit firmness from orchard to consumers has been one of the main purposes to maintain fruit quality. In addition, blue mold decay caused by *Penicillum expansum* is another issue standing up to apple growers and consumers since it causes a major postharvest diseases (**Simonaitiene *et al.*, 2014**).

Chitosan had the effectiveness on controlling blue mold decay of apples and it can be a promising alternative in controlling postharvest diseases (**Li *et al.*, 2015**). Moreover, many literature referred to that chitosan had anti-

* **Corresponding author: Tel. :** +201005059761
E-mail address: Neven_Nagy 75@ yahoo.com

browning virtue and can reduce decay, maintain tissue hardness and prolong shelf life periods for many horticulture commodities (**Liu *et al*., 2014; Li *et al*., 2015**). Chitosan also was able to reduce the changes of total anthocyanin degradation and to prevent color deterioration during cold storage (**Varasteh *et al*., 2012**). In the same trend, **Jiang *et al.* (2018)** reported that a novel chitosan formulation namely Kadozan resulted in reducing fruit respiration rate, retarding the increase of cell membrane permeability, keeping higher content of anthocyanin, maintaining quality and prolonging shelf life of harvested litchi fruits.

Citric and ascorbic acids are two natural and organic antioxidants having auxinic action, moreover, citric and ascorbic acids provided control on most fungi such gray mold (*Botrytic cinera*) and white mold (*Sclertinia sclerotiorum)* in various crops (**Elad, 1992**). Such antioxidants were successful in decreasing decay, weight loss and fruit softening during cold storage and marketing period of Le Conte pear fruits (**Hafez *et al*., 2010**). In addition, **Abd El-Wahab (2015)** emphasized that antioxidants like salicylic, ascorbic and citric acids were more effective on extending postharvest life as well as citric acid has been used to prevent browning, maintain the quality and prolong fruit shelf life (**Queiroz *et al*., 2011**).

Moreover, salicylic acid (SA) is a simple phenolic component, an endogenous hormone, natural and safe signaling molecule applied for maintenance of postharvest quality (**Asghari and Aghdam, 2010**). Positive effect of SA was found on controlling of fungal diseases (**Xu and Tian, 2008; Zhang *et al*., 2008; Mandal *et al*., 2009; Pila *et al*., 2010**), anthracnose disease (**Zainuri *et al*., 2001**) and the incidence of postharvest diseases (**Khademi *et al*., 2012**). Moreover, SA can prolong the postharvest shelf life of harvested fruits by delaying the development of disease incidence (**Terry and Joyce, 2004**) and by inducing disease resistance of fruits (**Yao and Tian, 2005; Aghdam *et al*., 2011**). Salicylic acid was very effective on delaying fruit ripening (**Mohammadi and Aminifrad, 2013**) and fruit senescence thus, increasing storability by decreasing weight loss, fungal decay and maintaining firmness (**Khademi and Ershadi, 2013**) besides improving health promoting properties of fruits (**Lu *et al*., 2011; Dokhanieh *et al*., 2013**). Chilling injury and electrolyte leakage as well as vitamin C loss were decreased by applying SA (**Sayyari *et al*., 2009**). Similarly, **Shafiee *et al.* (2010)** noticed that SA was capable to reduce weight loss beside retain fruit firmness and hue angle. Therefore, SA prolongs storage life and shelf life (**Awad, 2013; Khademi and Ershadi, 2013; El-Shazly *et al*., 2013; Ali *et al*., 2013**).

There are insufficient studies about the influence of using chitosan and antioxidants on apples. The combination of chitosan and citric acid applied to control browning and to improve storage life (**Ducamp-Collin *et al*., 2008; Liu *et al*., 2014**) was able to reduce the undesirable changes of fruits and to protect fruit tissue from injury (**Zhang *et al*., 2017**).

Therefore, this study aims to assess the effect of chitosan, antioxidants especially citric, ascorbic and salicylic acids in addition to their combinations on keeping quality of "Anna" apple fruits during cold storage period and their post- storage shelf life trying to achieve the proper application to prolong its storage and shelf life as well as their marketability, especially fruits of this cultivar appear on May and end their season on June or July. Consequently, extending storage duration for extra months is desirable to raise the income of apple growers by increasing fruit price in this time.

MATERIALS AND METHODS

This study was carried out during the two successive seasons 2016 and 2017 by using 5 years old "Anna" apple trees, grown in a private orchard located in Abo Hommos district, Beheira Governorate, Egypt. The trees were budded on Malus apple rootstock, spaced at 4×4 meters, in a sandy soil, under drip irrigation and received the common horticulture practices applied in the orchard. A number of 2160 apple fruits were hand-harvested on the last week of May and were sound, free from obvious pathogens, uniform in shape, size plus peel color, as possible. The collected apple fruits were carefully transported to the laboratory immediately, washed under tap water, dipped in sodium hypochloride (0.05%) for 3 min. for surface sterilization, rinsed with distilled water, air dried and then divided into eight groups (treatments). Each treatment was consisted of three replicates (each one included 30 fruits) meaning 90 fruits per treatment for each cold storage date. Fruits were immersed for 3 min. in one of the following solutions: Control (water), 2% (*W/V*) chitosan (in 1% acetic acid), 200 ppm citric acid, 200 ppm ascorbic acid, 2 mmol/l salicylic acid, chitosan plus citric acid, chitosan plus ascorbic acid and finally chitosan plus salicylic acid, all at the used concentrations. Tween- 20 was added to all solutions at 0.05%, as a surfactant agent. The treated- fruits were air dried, apple fruits of each replicate were placed in clean plastic containers and then stored in a commercial cold chamber at 2±1^{o}C and 90- 95% RH for three months. Samplings were periodically taken every month for assessments. In addition, another 45 apple fruits were collected to determine the initial physical and chemical quality parameters before storage (initial or zero time).

Quality Assessment of Fruits

Periodical physical and chemical evaluations were made on 15 apple fruits per replicate of all applied treatments during cold storage and similar number of fruits was left on shelf for 5 days at ambient temperature after each storage period (post- storage shelf life).

Determination of Physical and Chemical Characteristics

Decay (%)

The number of decayed fruits (due to fungi, bacteria or any visible pathogenic microorganisms) was recorded at each sampling date and was calculated per each sample as a percentage of the total fruit number at the beginning of cold storage.

Weight loss (%)

Apple fruits were individually labeled and weighed before starting cold storage (initial weight) and at each sampling date of cold storage (one month interval), the difference of weight loss was assessed relative to the initial weight and weight loss percentage was calculated using the equation of **Ghoname (1992)**.

Fruit firmness (Newton)

Firmness was measured as (Ib/in^2) on the two opposite sides at the tropic of the fruit, after removing the peel, using Effigi pressure tester (mod. Ft 327). The values of readings were converted to Newton unit (Ib/in^2 ×4.448), since this unit is the required one for scientific writing (**Verma and Jashi, 2000**).

Electrolyte leakage (%)

The percentage of electrolyte leakage was calculated according to the procedure of **Ahrenes and Ingram (1988)**.

Freshly prepared juice of apple fruits per each sample was extracted by using electric fruit juicer to assess the following measurements:

Total sugars (%)

Total sugars was extracted and evaluated by using the phenol-sulphuric acid colorimetric method according to **Egan *et al.* (1987)**.

Total soluble solids (TSS) content

The TSS as Brix was measured at 20^{o}C by using a hand refractometer.

Total acidity (%)

It was determined by titration with 0.1 N sodium hydroxide in the presence of phenolphthalein indicator (**AOAC, 2000**).

Vitamin C content

It was determined and expressed as mg L-ascorbic acid/100 ml fruit fresh juice using the method described by **AOAC (2000)**.

Anthocyanin content of peel (mg/100 g fresh weight)

The amount of anthocyanin content was assessed by using the technique of **Fuleki and Francis (1968)**.

Carotene content of peel (mg/100 g fresh weight)

Carotene was extracted, assessed and calculated the amount of total carotene of apple fruit peel according to the procedure of **Wintermans and Mots (1965)**.

Statistical Analysis

The results of this study were statistically analyzed using split plot in completely randomized design as described by **Steel and Torrie (1980)**. The treatments were allocated in the main plots, while the periods were assigned in the sub plots. Analysis of variance was achieved and comparisons were done by using the new least significant difference (NLSD) at 0.05 level which was calculated by using SAS statistical software (**SAS, 1996**).

RESULTS

The Effect of the Postharvest-Applied Treatments, Time Factor and their Interactions on Physical Characteristics of "Anna" Apple Fruits During Cold Storage and After Post- Storage Shelf Life Periods in the Two Seasons of 2016 and 2017

With regard to the effect of postharvest treatments on decay percentage in "Anna" apple fruit, the results in Table 1 indicate that all applied treatments significantly reduced decay percentage relative to the control which achieved the highest fruit decay in both seasons. Moreover, the combined addition of chitosan and salicylic acid gave an advantage in reducing decay percentage as compared with the individual application of such acid in both seasons. The sole treatments of applied acids had similar effect on fruit decay in both seasons. In the first season, it could be observed that the differences among chitosan and all applied treatments except control were not enough to be significant. Citric, ascorbic and salicylic acids were identical in their effect on fruit decay in both seasons.

Apple fruits were also affected by postharvest treatments, regardless the effect of post-storage shelf life periods. The results in Table 1 obviously show that chitosan alone or the combinations of chitosan and antioxidants gave the least fruit decay in both seasons. On the other hand, control treatment achieved the highest values of fruit decay in a consistent manner in the two seasons.

Concerning the effect of cold storage periods on fruit decay, the results in Table 1 reveal that fruit decay started to appear after the second month of cold storage and tended to increase to achieve the highest percentage after the third month of cold storage. This trend of results was consistent in both seasons. Similar results were obtained concerning the effect of shelf life after cold storage periods on fruit decay.

The interaction between postharvest treatments and cold storage periods on fruit decay was presented in Table 1. The results showed that control treatment resulted in an increase in fruit decay between the second and third months in both seasons as compared with other treatments except with salicylic acid in the first season. On the contrary, chitosan plus antioxidants were able to control fruit decay after the second and third months of cold storage and resulted in the least fruit decay comparing with the sole application of ascorbic and salicylic acids. The highest percentages of fruit decay at the end of cold storage were obtained with control treatment in both seasons.

Decay percentage of "Anna" fruits was influenced by the interaction between treatments and post-storage shelf life periods. The results listed in Table 1 indicate that the highest fruit decay on shelf was found by the control treatment after the third period of post- storage shelf life, while the least decay, in general, was observed by applying chitosan alone or mixed with antioxidants. Chitosan plus ascorbic acid or salicylic acid treatments were more effective on alleviation the incidence of fruit decay after the last period of post-storage shelf life in both seasons. Moreover, chitosan and the sole application of antioxidant acids were identical in their effects on fruit decay. Control treatments resulted in the highest fruit decay percentage when compared with other applied treatments in the two seasons at the third post- storage shelf period.

The results shown in Table 2 indicate that all applied treatments, regardless of the cold storage periods, were more effective on reducing apple fruit weight loss percentages as compared with the control which resulted in the highest percentages of weight loss in both seasons. Moreover, the addition of chitosan to all applied antioxidants, namely citric, ascorbic and salicylic acids caused a great reduction of fresh weight loss comparing with the individual treatments of each component. Furthermore, using salicylic acid alone or combined with chitosan achieved the lowest fresh weight loss percentages in a consistent manner during the two seasons. All applied antioxidant treatments resulted in lower weight loss than the sole application of chitosan in both seasons.

Table 1. Effect of the postharvest-applied treatments, time factor and their interactions on fruit decay percentage of "Anna" apple fruits during cold storage and after post-storage shelf life periods in the two seasons of 2016 and 2017

Treatment (T)	Decay (%)								
	Cold storage period (P) (month)					Post- storage shelf life period (P) (5 days)			
	0	1	2	3	Mean	1	2	3	Mean
					2016 season				
Control	0.00^{e*}	0.00^{e}	11.11^{b}	22.22^{a}	8.33^{a}	0.00^{f}	15.55^{b}	24.66^{a}	13.40^{a}
Chitosan	0.00^{e}	0.00^{e}	0.00^{e}	6.66^{bcd}	1.66^{bc}	0.00^{f}	2.22^{ef}	11.11^{bcd}	4.44^{c}
Citric acid	0.00^{e}	0.00^{e}	4.44^{cde}	8.88^{bc}	3.33^{bc}	0.00^{f}	6.66^{cdef}	15.55^{b}	7.40^{b}
Ascorbic acid	0.00^{e}	0.00^{e}	4.44^{cde}	8.88^{bc}	3.33^{bc}	0.00^{f}	8.88^{bcde}	13.33^{bc}	7.40^{b}
Salicylic acid	0.00^{e}	0.00^{e}	6.66^{bcd}	11.11^{b}	4.44^{b}	0.00^{f}	8.88^{bcde}	13.33^{bc}	7.40^{b}
Chitosan plus citric acid	0.00^{e}	0.00^{e}	0.00^{e}	4.44^{cde}	1.11^{c}	0.00^{f}	0.00^{f}	8.88^{bcde}	2.96^{cd}
Chitosan plus ascorbic acid	0.00^{e}	0.00^{e}	0.00^{e}	2.22^{de}	0.56^{c}	0.00^{f}	0.00^{f}	6.66^{cdef}	2.22^{d}
Chitosan plus salicylic acid	0.00^{e}	0.00^{e}	0.00^{e}	2.22^{de}	0.56^{c}	0.00^{f}	0.00^{f}	4.44^{def}	1.48^{d}
Mean	0.00^{c}	0.00^{c}	3.33^{b}	8.33^{a}	-	0.00^{c}	5.27^{b}	12.24^{a}	-
					2017 season				
Control	0.00^{g}	0.00^{g}	17.78^{b}	26.66^{a}	11.11^{a}	0.00^{i}	22.22^{b}	33.33^{a}	18.52^{a}
Chitosan	0.00^{g}	0.00^{g}	2.22^{fg}	8.88^{de}	2.78^{cd}	0.00^{i}	6.66^{fghi}	15.55^{bcde}	7.41^{bcd}
Citric acid	0.00^{g}	0.00^{g}	8.88^{de}	17.78^{b}	6.67^{b}	0.00^{i}	11.11^{defg}	20.00^{bc}	10.37^{b}
Ascorbic acid	0.00^{g}	0.00^{g}	6.66^{def}	11.11^{cd}	4.44^{bc}	0.00^{i}	8.89^{efgh}	15.55^{bcde}	8.15^{bc}
Salicylic acid	0.00^{g}	0.00^{g}	11.11^{cd}	15.55^{bc}	6.67^{b}	0.00^{i}	13.33^{cdef}	17.78^{bcd}	10.37^{b}
Chitosan plus citric acid	0.00^{g}	0.00^{g}	4.44^{efg}	4.44^{efg}	2.22^{cd}	0.00^{i}	6.66^{fghi}	11.11^{defg}	5.92^{bcd}
Chitosan plus ascorbic acid	0.00^{g}	0.00^{g}	0.00^{g}	4.44^{efg}	1.11^{d}	0.00^{i}	2.22^{hi}	6.66^{fghi}	2.96^{d}
Chitosan plus salicylic acid	0.00^{g}	0.00^{g}	0.00^{g}	2.22^{fg}	0.56^{d}	0.00^{i}	4.44^{ghi}	6.66^{fghi}	3.70^{cd}
Mean	0.00^{c}	0.00^{c}	6.39^{b}	11.39^{a}	-	0.00^{c}	9.44^{b}	15.83^{a}	-

- The results in the table were subjected to angular transformation and * means values having the same letter (s) were not significantly different according to NLSD at 0.05 level.

Table 2. Effect of the postharvest-applied treatments, time factor and their interactions on weight loss percentage of "Anna" apple fruits during cold storage and after post-storage shelf life periods in the two seasons of 2016 and 2017

Treatment (T)	Fresh weight loss (%)								
	Cold storage period (P) (month)					Post-storage shelf life period (P) (5 days)			
	0	1	2	3	Mean	1	2	3	Mean
	2016 season								
Control	0.00	2.85	5.95	10.13	4.73	3.96	7.44	21.00	10.80
Chitosan	0.00	2.73	3.97	8.57	3.82	3.25	4.93	14.25	7.48
Citric acid	0.00	2.36	3.63	6.30	3.07	3.00	4.61	11.39	6.33
Ascorbic acid	0.00	2.11	3.52	6.21	2.96	2.91	4.36	11.25	6.17
Salicylic acid	0.00	2.00	3.20	6.19	2.85	2.73	3.27	11.00	5.67
Chitosan plus citric acid	0.00	1.62	2.73	5.55	2.47	2.31	3.15	8.47	4.64
Chitosan plus ascorbic acid	0.00	1.50	2.66	5.32	2.37	2.09	3.01	8.26	4.45
Chitosan plus salicylic acid	0.00	1.38	2.45	5.16	2.25	2.00	2.99	8.09	4.36
Mean	0.00	2.07	3.51	6.68	-	2.78	4.22	11.71	-
NLSD	T= 0.1132		P= 0.0712	T*P= 0.2021		T= 0.0914	P= 0.0689	T*P= 0.1911	
	2017 season								
Control	0.00	4.01	6.98	12.31	5.83	4.23	9.59	24.17	12.66
Chitosan	0.00	3.77	4.53	10.00	4.58	4.00	6.66	17.78	9.48
Citric acid	0.00	3.60	4.71	8.21	4.13	4.00	6.31	14.66	8.32
Ascorbic acid	0.00	3.00	4.37	8.08	3.86	3.91	6.13	14.3٠	8.11
Salicylic acid	0.00	3.00	4.23	8.01	3.81	3.73	6.02	14.11	7.95
Chitosan plus citric acid	0.00	1.98	2.60	5.76	2.59	2.22	4.27	8.92	5.14
Chitosan plus ascorbic acid	0.00	1.72	2.31	5.45	2.37	2.19	4.11	8.6٠	4.97
Chitosan plus salicylic acid	0.00	1.66	2.22	5.19	2.27	2.07	4.00	8.10	4.72
Mean	0.00	2.84	3.99	7.88	-	3.29	5.88	13.83	-
NLSD	T= 0.0468		P= 0.0516	T*P= 0.1462		T= 0.0979	P= 0.0710	T*P= 0.2021	

Assessing the effect of applied treatments on weight loss, regardless of the post- storage shelf life, proved that all used treatments resulted in lower percentages of weight loss as compared with control which gave apple fruits with the highest weight loss in both seasons. Moreover, using chitosan plus antioxidants such as citric, ascorbic and salicylic acids were more helpful on reducing fruit weight loss than using chitosan and antioxidant compounds individually. Chitosan plus salicylic acid caused the lowest percentages of weight loss in a consistent manner during the two seasons.

With regard to the effect of cold storage periods at 2±1°C on fresh weight loss, regardless of the treatments, the results in Table 2 show that fruit weight loss gradually increased as the cold storage period advanced to reach to the highest value at the third month of cold storage in the two successive seasons.

Furthermore, post-storage shelf life periods also caused an obvious effect on fruit weight loss. Since, the percentage of weight loss in "Anna" apples tended to increase significantly with the progress of post-storage shelf life periods.

Table 2 also displayed the effect of interaction between various applied treatments and cold storage periods on the percentage of weight loss in "Anna" apples stored at 2±1°C for three months during 2016 and 2017 seasons. The results revealed that after the first month of cold storage, weight loss percentages were significantly lower as compared with the second and third months of cold storage in all treatments. At the end of storage, control treatment resulted in the highest percentage of weight loss comparing with other treatments. Chitosan plus either ascorbic acid or salicylic acid treatments achieved the lowest percentages of weight loss after the third month of cold storage. Moreover, at this time, the addition of chitosan to the antioxidant compounds reduced fruit weight loss in comparison with the individual treatment of chitosan or antioxidants.

The effect of interaction between various-used treatments and post-storage shelf life periods was presented in Table 2. Control treatment caused a significant increase in weight loss after all post- storage shelf life periods. On the other hand, apples of other applied treatments had the lowest percentages of weight loss starting from the first period of post-storage shelf life till the last one. The most effective reduction was noticed at the last period of post-storage shelf life in apples treated with chitosan plus salicylic acid. The trend of results was similar in both seasons.

From the results mentioned in Table 3, it could be concluded that the highest significant value of pulp firmness of "Anna" apples was found with each of chitosan plus ascorbic or salicylic acids which were significantly similar during the two seasons. Moreover, the individual application of any of citric, ascorbic and salicylic acids was more effective in retarding the loss of firmness than chitosan treatment. Control fruits had significantly the lowest pulp firmness as compared with other ones in a consistent manner during the two seasons. The results presented in Table 3 also show that there was a similar effect of ascorbic acid to that of citric acid treatment in both seasons.

The behavior of various- applied treatments, regardless the post-storage shelf life periods, was nearly similar to that effect of the same treatments, regardless the cold storage periods. Significant differences were found between chitosan plus ascorbic acid and chitosan plus citric acid treatments in the first season only. The application with chitosan plus salicylic acid gave the lowest significant loss in pulp firmness in both seasons. Moreover, the individual applications of ascorbic and salicylic acids were significantly different in both seasons.

The influence of both cold storage and post-storage shelf life periods on "Anna" apples tissue firmness was reported in Table 3. The results revealed that tissue firmness was considerably reduced with the advancement of either cold storage or post- storage shelf life durations in both seasons.

With regard to "Anna" apple tissue firmness as affected by the interaction between treatments and cold storage periods, the results was tabulated in Table 3. The results revealed that the loss of firmness significantly appeared after the first month of cold storage in all treatments as compared with initial firmness. After the first month of cold storage, it could be also noticed

Table 3. Effect of the postharvest-applied treatments, time factor and their interactions on firmness of "Anna" apple fruits during cold storage and after post-storage shelf life periods in the two seasons of 2016 and 2017

Treatment (T)	Firmness (Newton)								
	Cold storage period (P) (month)					Post- storage shelf life period (P) (5 days)			
	0	1	2	3	Mean	1	2	3	Mean
					2016 season				
Control	60.04	57.13	51.00	31.15	49.83	55.27	42.14	25.03	40.81
Chitosan	60.04	57.45	54.03	32.60	51.03	55.16	44.05	29.99	43.06
Citric acid	60.04	57.13	54.71	39.40	52.82	55.00	45.28	36.44	45.57
Ascorbic acid	60.04	58.85	54.82	38.85	53.14	56.00	45.33	35.97	45.76
Salicylic acid	60.04	58.93	55.66	41.61	54.06	56.00	50.07	38.60	48.22
Chitosan plus citric acid	60.04	58.99	55.84	49.65	56.13	57.39	52.00	46.46	51.95
Chitosan plus ascorbic acid	60.04	59.10	56.00	53.54	57.17	57.22	54.14	50.18	53.84
Chitosan plus salicylic acid	60.04	59.87	56.00	54.08	57.50	57.00	55.00	53.65	55.21
Mean	60.04	58.43	54.76	42.61	-	56.13	48.50	39.54	-
NLSD	T= 0.9323		P= 0.4048	T*P= 1.1401		T= 0.6788	P= 0.5962	T*P= 1.6812	
					2017 season				
Control	62.15	58.08	47.12	32.47	49.95	54.68	41.31	28.44	41.47
Chitosan	62.15	58.29	52.10	36.92	52.36	55.91	50.93	33.85	46.89
Citric acid	62.15	58.47	55.73	40.27	54.15	57.05	52.76	37.20	49.00
Ascorbic acid	62.15	58.91	55.89	42.31	54.81	56.93	52.82	39.16	49.63
Salicylic acid	62.15	59.13	56.00	44.14	55.35	57.72	54.19	40.87	50.93
Chitosan plus citric acid	62.15	59.76	56.88	52.91	57.92	57.83	54.54	49.46	53.94
Chitosan plus ascorbic acid	62.15	59.83	56.91	54.97	58.46	57.91	55.00	51.35	54.75
Chitosan plus salicylic acid	62.15	59.98	57.12	55.55	58.70	57.91	56.00	55.16	56.35
Mean	62.15	59.05	54.72	44.94	-	56.99	52.19	41.93	-
NLSD	T= 0.6922		P= 0.7113	T*P= 2.0111		T= 0.8625	P= 0.7489	T*P= 2.1181	

that the addition of chitosan to the three- used antioxidants did not cause a significant change in such firmness relative to salicylic acid treatment. At the end of cold storage, chitosan plus citric, ascorbic or salicylic acid treatments achieved the lowest significant loss in firmness in the two seasons. Moreover, firmness values of apples treated with antioxidants and chitosan individually had higher firmness than untreated ones at the end of storage in both seasons.

Assessing the interaction between treatments and post- storage shelf life periods was reported in Table 3. The results declared that after the first period of post- storage shelf life, chitosan treatment had the same influence on such firmness comparing with the control. Moreover, firmness of chitosan- treated fruits was similar to those treated with the individual applications of antioxidants in both seasons. The control treatment resulted in a significant lower firmness than the combinations of chitosan plus antioxidants which were similar in their firmness. At the last period of post-storage shelf life, it could be observed that apples treated with chitosan plus salicylic acid had significantly the highest firmness in comparison with other treatments. The least value of fruit firmness was obtained with control treatment. The addition of chitosan to antioxidants was more effective on reducing the loss of firmness than the individual treatments of each component. These results were true in both seasons.

The Effect of the Postharvest-Applied Treatments, Time Factor and Their Interactions on Chemical Characteristics of "Anna" Apple Fruits During Cold Storage and After Post-Storage Shelf Life Periods in the Two Seasons of 2016 and 2017

Effect of chitosan, applied antioxidants and their combinations, regardless of cold storage periods, on the percentage of electrolyte leakage was listed in Table 4. The results indicated that untreated apples had the highest electrolyte leakage percentages while chitosan plus salicylic acid treated ones had the lowest percentages in both seasons. Moreover, there was a great advantage when chitosan was mixed with antioxidants in reducing electrolyte leakage percentages of "Anna" apples in comparison with the sole applications of each compound. Citric and ascorbic acids, regardless of cold storage periods, caused a similar effect on electrolyte leakage percentage of apples in a consistent manner during the two seasons.

The effect of applied treatments, regardless of post-storage shelf life periods, took nearly the same pattern obtained above. On the other side, citric and ascorbic acids were similar in their effect on electrolyte leakage percentage, only in the first season.

Concerning the influence of both cold storage and post-storage shelf life periods on the percentage of electrolyte leakage of "Anna" apple fruits, the results shown in Table 4 revealed that the electrolyte leakage percentage tended to significantly increase as the cold storage or post- storage shelf life durations progressed during the two seasons.

Table 4 also referred to the effect of interaction between various applied treatments during cold storage or shelf life periods in the two successive seasons. The results confirmed that the percentage of electrolyte leakage started to increase after the first month and continued to increase with the progress of cold storage period in all applied treatments to reach to the highest percentages in untreated fruits comparing with other treated ones in both seasons. The treatment of chitosan plus salicylic acid achieved the lowest electrolyte leakage in "Anna" apples at the end of storage in both seasons.

Similar results were obtained with the interaction between chitosan, antioxidants plus their combinations and post- storage shelf life periods during the two seasons. After the first period of post- storage shelf life, chitosan plus each of antioxidants caused lower electrolyte leakage as compared with chitosan and control treatments. In addition, citric acid was similar to chitosan in its effect on electrolyte leakage in the two seasons. Moreover, the results listed in Table 4 refer to the similar effect on electrolyte leakage with chitosan plus either ascorbic or salicylic acid treatments at the end of post-storage shelf life period in both seasons.

The results shown in Table 5 reveal the effect of various treatments on the percentage of total sugars in "Anna" apples, regardless the effect of cold storage periods. Untreated fruits had the highest percentage of total sugars as

Table 4. Effect of the postharvest-applied treatments, time factor and their interactions on electrolyte leakage percentage of "Anna" apple fruits during cold storage and after post- storage shelf life periods in the two seasons of 2016 and 2017

Treatment (T)	Electrolyte leakage (%)								
	Cold storage period (P) (month)					Post- storage shelf life period (P) (5 days)			
	0	1	2	3	Mean	1	2	3	Mean
	2016 season								
Control	7.25	17.37	22.72	24.18	17.88	19.76	23.00	31.19	24.65
Chitosan	7.25	9.14	12.95	19.59	12.23	10.97	15.63	21.25	15.95
Citric acid	7.25	8.63	9.39	18.85	11.03	9.11	12.89	21.08	14.36
Ascorbic acid	7.25	8.59	9.39	18.57	10.95	9.00	12.73	20.92	14.22
Salicylic acid	7.25	8.44	9.11	17.96	10.69	8.70	12.51	19.29	13.50
Chitosan plus citric acid	7.25	8.41	9.00	15.22	9.97	8.61	11.30	16.27	12.06
Chitosan plus ascorbic acid	7.25	8.11	9.00	12.96	9.33	8.53	11.11	13.83	11.16
Chitosan plus salicylic acid	7.25	8.02	8.77	12.04	9.02	8.42	10.40	13.74	10.85
Mean	7.25	9.59	11.29	17.42	-	10.38	13.69	19.69	-
NLSD	T= 0.2933		P= 0.2223		T*P= 0.6333	T= 0.2949	P= 0.2376	T*P= 0.6723	
	2017 season								
Control	9.11	22.12	29.37	31.96	23.14	25.45	30.15	30.73	28.77
Chitosan	9.11	11.71	16.33	28.37	16.38	13.92	23.64	30.78	22.78
Citric acid	9.11	11.54	14.18	28.01	15.71	13.74	22.12	30.24	22.03
Ascorbic acid	9.11	11.29	16.20	25.60	15.55	13.11	21.60	27.56	20.75
Salicylic acid	9.11	11.00	16.06	23.95	15.03	13.00	19.33	25.73	19.35
Chitosan plus citric acid	9.11	10.10	13.06	23.69	13.99	12.06	19.07	25.34	18.82
Chitosan plus ascorbic acid	9.11	10.08	13.00	22.73	13.73	12.00	16.12	24.26	17.46
Chitosan plus salicylic acid	9.11	11.00	13.00	22.13	13.81	12.00	16.03	23.58	17.20
Mean	9.11	12.36	16.40	25.81	-	14.41	21.00	27.27	-
NLSD	T= 0.2182		P= 0.2391		T*P= 0.6763	T= 0.5773	P= 0.2491	T*P= 0.7041	

Table 5. Effect of the postharvest- applied treatments, time factor and their interactions on total sugars percentage of "Anna" apple fruits during cold storage and after post-storage shelf life periods in the two seasons of 2016 and 2017

Treatment (T)	Total sugars (%)								
	Cold storage period (P) (month)					Post- storage shelf life period (P) (5 days)			
	0	1	2	3	Mean	1	2	3	Mean
	2016 season								
Control	10.16	10.63	11.15	13.16	11.28	10.92	11.75	13.98	12.21
Chitosan	10.16	10.47	11.00	11.95	10.89	10.91	11.36	12.85	11.70
Citric acid	10.16	10.33	10.89	11.68	10.77	10.56	11.05	12.40	11.33
Ascorbic acid	10.16	10.30	10.73	11.53	10.68	10.50	11.00	12.05	11.18
Salicylic acid	10.16	10.30	10.60	11.40	10.62	10.50	11.00	12.00	11.16
Chitosan plus citric acizd	10.16	10.20	10.69	11.31	10.59	10.49	11.83	11.91	11.41
Chitosan plus ascorbic acid	10.16	10.20	10.65	11.00	10.50	10.41	10.72	11.55	10.89
Chitosan plus salicylic acid	10.16	10.20	10.51	10.62	10.37	10.47	10.72	11.40	10.86
Mean	10.16	10.33	10.77	11.58	-	10.59	11.17	12.26	-
NLSD	T= 0.2361	P= 0.1922		T*P= 0.5401		T= 0.3413	P= 0.1751	T*P= 0.4952	
	2017 season								
Control	10.53	11.27	12.45	13.47	11.93	11.92	12.93	13.91	12.92
Chitosan	10.53	11.05	12.08	12.98	11.66	11.63	12.60	13.36	12.53
Citric acid	10.53	10.98	11.96	12.73	11.55	11.37	12.22	13.13	12.24
Ascorbic acid	10.53	10.83	11.73	12.41	11.37	11.25	12.08	12.90	12.07
Salicylic acid	10.53	10.80	11.52	12.12	11.24	11.11	11.94	12.64	11.89
Chitosan plus citric acid	10.53	10.69	11.00	12.00	11.06	10.93	11.71	12.57	11.73
Chitosan plus ascorbic acid	10.53	10.64	11.00	11.90	11.02	10.90	11.63	12.31	11.61
Chitosan plus salicylic acid	10.53	10.60	11.00	11.72	10.96	10.87	11.45	12.00	11.44
Mean	10.53	10.86	11.59	12.42	-	11.25	12.07	12.85	-
NLSD	T= 0.2354	P= 0.1972	T*P= 0.5572			T= 0.2325	P= 0.2000	T*P= 0.5652	

compared with other treated ones. On the other hand, chitosan combined with all kinds of antioxidant treatments had lower total sugars percentage than the sole applications of each component and this was significant in the second season. Moreover, the effect of chitosan treatment on total sugars was similar to that obtained with citric or ascorbic acid treatment. There was no significant difference between ascorbic acid and salicylic acid treatments in their effect on total sugars of apples. Furthermore, using chitosan plus antioxidant acid treatments significantly had the same effect as salicylic acid on total sugars. The obtained results were in a consistent manner in both seasons.

Similar trend was found concerning the effect of postharvest-applied treatments, regardless of post-storage shelf life periods with small differences. Chitosan plus either ascorbic acid or salicylic acid treatments had the same effect on total sugars percentage and resulted in the least total sugars percentage relative to control and chitosan treatments.

The influence of cold storage and post-storage shelf life durations on total sugars percentage of “Anna” apples was reported in Table 5. The results indicated that after the first month of cold storage, total sugars percentage did not change relative to the initial time of storage and then tended to significant increase as cold storage period advanced to achieve the highest value after the third month of cold storage in the two seasons.

The same trend was found with the effect of post-storage shelf life periods since the greatest increase in total sugars was noticed after the third period of post-storage shelf life in both seasons. The increase of such total sugars percentage started after the first period of post-storage shelf life.

Changes in total sugars content of “Anna” apples as affected by the interaction between treatments and cold storage period were presented in Table 5. The results declared that after the first month of cold storage in both seasons, all treatments gave fruits with similar total sugars to those at the initial except control in the second season. By the end of cold storage, control and chitosan treatments caused great increases in total sugars in the two seasons. Chitosan plus salicylic acid treatment resulted in significant decrease in total sugars of apples as compared with control, chitosan, citric and ascorbic acids after the third month, in both seasons.

Total sugars percentage also was influenced by the interaction between treatments and post-storage shelf life periods. The results reported that after the first period of post- storage shelf life, total sugars percentages did not significantly change in most applied treatments, in both seasons. It could be found that total sugars values in the control, sole applications of antioxidants and the combination included chitosan plus citric acid significantly increased as compared with that obtained after the first period of post- storage shelf life. At the end of post- storage shelf life duration, untreated apples had significantly higher total sugars content relative to those of other treatments except chitosan in the second season, while the least content was found in fruits treated with chitosan plus either ascorbic acid or salicylic acid.

The effect of chitosan, antioxidants and their combinations, regardless of both cold storage and post-storage shelf life durations, on the percentage of total soluble solids (TSS) was presented in Table 6. From these results, it could be noticed that control treatment resulted in the highest percentage of TSS comparing with other treatments either during cold storage or after post- storage shelf life periods. Salicylic acid and applied combinations included chitosan with either citric acid or ascorbic acid were similar in their effect on TSS percentage and caused low TSS in both seasons during cold storage. The sole treatment of chitosan achieved a great increase in TSS and that was similar to control, only in the first season but was comparable to citric acid treatment in the second season. Chitosan plus salicylic acid treatment led to the lowest TSS percentages in both seasons.

Identical effect was observed when discussing the effect of treatments, regardless of post-storage shelf life periods, on TSS where control and chitosan treatments caused higher TSS percentages than other applied treatments in the first season and higher than salicylic acid and chitosan plus antioxidants in the second season.

Table 6. Effect of the postharvest- applied treatments, time factor and their interactions on total soluble solids (TSS) percentage of "Anna" apple fruits during cold storage and after post- storage shelf life periods in the two seasons of 2016 and 2017

Treatment (T)	TSS (Brix)								
	Cold storage period (P) (month)					Post-storage shelf life period (P) (5 days)			
	0	1	2	3	Mean	1	2	3	Mean
	2016 season								
Control	13.00	13.97	15.00	16.14	14.53	14.59	15.63	17.51	15.91
Chitosan	13.00	13.80	14.71	15.98	14.37	14.00	15.27	16.70	15.32
Citric acid	13.00	13.61	14.43	15.48	14.13	13.93	14.86	16.00	14.93
Ascorbic acid	13.00	13.57	14.13	15.13	13.96	13.90	14.75	16.00	14.88
Salicylic acid	13.00	13.52	14.00	15.00	13.88	13.72	14.54	15.87	14.71
Chitosan plus citric acid	13.00	13.38	14.00	14.91	13.82	13.86	14.48	15.53	14.62
Chitosan plus ascorbic acid	13.00	13.38	13.95	14.97	13.82	13.80	14.39	15.67	14.62
Chitosan plus salicylic acid	13.00	13.38	14.00	14.80	13.79	13.71	14.33	15.67	14.57
Mean	13.00	13.58	14.28	15.30	-	13.94	14.78	16.12	-
NLSD	T= 0.1882		P= 0.1244		T*P= 0.3512	T= 0.2506	P= 0.1174	T*P= 0.3321	
	2017 season								
Control	13.44	14.64	15.67	17.38	15.28	15.07	16.99	18.80	16.95
Chitosan	13.44	14.37	15.39	17.00	15.05	14.81	15.87	17.84	16.17
Citric acid	13.44	14.14	15.17	16.67	14.86	14.90	15.77	17.37	16.01
Ascorbic acid	13.44	14.00	15.00	16.38	14.71	14.67	15.68	17.17	15.84
Salicylic acid	13.44	14.00	14.90	16.17	14.63	14.40	15.48	16.94	15.61
Chitosan plus citric acid	13.44	14.00	14.80	16.06	14.58	14.50	15.38	16.52	15.47
Chitosan plus ascorbic acid	13.44	13.98	14.80	15.54	14.44	14.50	15.17	16.20	15.29
Chitosan plus salicylic acid	13.44	13.81	14.60	15.60	14.36	14.33	15.17	16.00	15.17
Mean	13.44	14.12	15.04	16.35	-	14.65	15.68	17.10	-
NLSD	T= 0.2239		P= 0.1688		T*P= 0.4771	T= 0.2833	P= 0.2167	T*P= 0.6131	

Moreover, the chitosan and antioxidant combinations resulted in the least TSS relative to the sole applications of chitosan, citric acid and ascorbic acid in the first season.

From the results tabulated in Table 6, it could be revealed that the percentage of TSS tended to increase with the progress of either cold storage or post-storage shelf life periods in the two seasons.

Concerning the interaction effect between postharvest- applied treatments and cold storage periods, it could be generally noticed that the percentages of total soluble solids in "Anna" apples were not different with all applied treatments after the first month of cold storage. Furthermore, chitosan was similar to the control in TSS percentage in the second and the third months of cold storage. By the end of cold storage, salicylic acid, ascorbic acid and the formulations consisted of chitosan and antioxidants achieved the lowest percentages of TSS as compared with the control in both seasons.

After the first period of post- storage shelf life, it could be found that all treatments were similar in their effect on TSS percentage with the exception of control treatment which caused a significant increase in TSS, only in the first season. After the second period of post- storage shelf life, untreated "Anna" apples had higher percentages of TSS as compared with those of other treatments in the two successive seasons. After the third post- storage period of shelf life, TSS percentages were higher for untreated apples followed by chitosan treated ones as compared with those of other treatments. Moreover, the addition of chitosan to antioxidants, namely citric, ascorbic and salicylic acids resulted in less TSS when compared with other treatments in both seasons.

Acidity content was also affected with various applied treatments where the least acidity was found by control treatment relative to other treatments, regardless cold storage and post- storage shelf life periods in both seasons (Table 7). On the contrary, using chitosan added to antioxidants resulted in a similar effect on acidity and achieved the highest acidity in the two seasons during cold storage or after post-storage shelf life, except chitosan plus citric acid treatment which was significantly different after post-storage shelf life in the first season. Moreover, the individual application of chitosan led to significant higher acidity than control either during cold storage or after post-storage shelf life in a consistent manner in both seasons. The effect of chitosan was also similar to that of citric acid during cold storage in both seasons and after post-storage shelf life in the second one. Ascorbic acid and salicylic acid treatments were comparable in their influence on acidity content during cold storage and after post-storage shelf life. In a similar way, salicylic acid and chitosan plus citric acid had the same effect in such acidity in both seasons.

With regard to the influence of cold storage and post-storage shelf life durations on acidity content of apples, the results listed in Table 7 show that acidity tended to decrease gradually and significantly with the advancement of cold storage and post-storage shelf life periods to reach to the least value after the last periods of cold storage and post- storage shelf life. This trend was consistent in the two successive seasons.

Changes in acidity of "Anna" apples due to the interaction between treatments and cold storage periods were shown in Table 7. The results clearly indicate that in both seasons, there were generally significant decreases in acidity after the first month of cold storage relative to the initial acidity in all treatments. Control and chitosan treatments caused lower acidity than chitosan plus antioxidants which led to the lowest percentages of acidity. Furthermore, the differences among control and all treatments at this time were not big enough to be significant in both seasons. After the second month of cold storage, untreated apples had the least acidity comparing with other treated ones. At this time, it could be also noticed that chitosan treatment gave fruits with acidity similar to that found in apples treated ones with the sole application of citric acid. By the end of cold storage, the least acidity was obtained with control treatment while the highest acidity was reported in chitosan added to salicylic acid in the two seasons. Chitosan-treated apples had a similar acidity to those treated with citric acid.

Table 7. Effect of the postharvest- applied treatments, time factor and their interactions on total acidity percentage of "Anna" apple fruits during cold storage and after post-storage shelf life periods in the two seasons of 2016 and 2017

Treatment (T)	Total acidity (%)								
	Cold storage period (P) (month)					Post-storage shelf life period (P) (5 days)			
	0	1	2	3	Mean	1	2	3	Mean
					2016 season				
Control	0.413	0.390	0.341	0.300	0.361	0.368	0.320	0.281	0.323
Chitosan	0.413	0.396	0.363	0.331	0.376	0.379	0.347	0.316	0.347
Citric acid	0.413	0.398	0.368	0.340	0.380	0.382	0.352	0.327	0.353
Ascorbic acid	0.413	0.400	0.375	0.355	0.385	0.389	0.367	0.340	0.365
Salicylic acid	0.413	0.400	0.379	0.360	0.388	0.391	0.370	0.349	0.370
Chitosan plus citric acid	0.413	0.406	0.383	0.369	0.392	0.393	0.377	0.357	0.375
Chitosan plus ascorbic acid	0.413	0.403	0.391	0.377	0.396	0.399	0.381	0.367	0.382
Chitosan plus salicylic acid	0.413	0.407	0.395	0.380	0.398	0.400	0.388	0.370	0.386
Mean	0.413	0.400	0.374	0.352	-	0.387	0.362	0.338	-
NLSD	T= 0.0075	P= 0.0056		T*P= 0.0158		T= 0.0061	P= 0.0045	T*P= 0.0126	
					2017 season				
Control	0.436	0.412	0.360	0.321	0.382	0.385	0.339	0.302	0.342
Chitosan	0.436	0.418	0.382	0.347	0.395	0.400	0.364	0.330	0.364
Citric acid	0.436	0.420	0.387	0.353	0.399	0.404	0.370	0.336	0.370
Ascorbic acid	0.436	0.425	0.393	0.362	0.404	0.409	0.378	0.346	0.378
Salicylic acid	0.436	0.427	0.397	0.369	0.407	0.411	0.381	0.354	0.382
Chitosan plus citric acid	0.436	0.430	0.400	0.373	0.409	0.418	0.388	0.360	0.388
Chitosan plus ascorbic acid	0.436	0.428	0.405	0.381	0.412	0.417	0.393	0.369	0.393
Chitosan plus salicylic acid	0.436	0.431	0.409	0.389	0.416	0.420	0.400	0.377	0.399
Mean	0.436	0.423	0.391	0.361	-	0.408	0.376	0.347	-
NLSD	T= 0.0068	P= 0.0045	T*P= 0.0127		T= 0.0096		P= 0.0061	T*P= 0.0173	

In Table 7, it could be declared that control treatment achieved the least fruit acidity after the first period of post- storage shelf life as compared with using chitosan and antioxidants combinations, especially chitosan plus salicylic acid, which caused the greatest content of acidity. The content of acidity tended to decline significantly after the second period of post-storage shelf life in all treatments. At the end of post- storage shelf life, untreated apples still contained the least acidity in comparison with other treated ones. Furthermore, the highest acidity was obtained by the combination of chitosan plus salicylic acid. In addition, using chitosan with antioxidants added an advantage in keeping higher acidity in comparison with the sole applications of each component in a consistent manner during both seasons.

The response of vitamin C content in "Anna" apples to postharvest-applied treatments was presented in Table 8. Regardless of cold storage and post-storage shelf life periods, the results indicated that there was a significant reduction in vitamin C by the control treatment while the highest content of such vitamin was found by chitosan plus salicylic acid treatment either during cold storage or after post- storage shelf life in a consistent manner. The sole applications of ascorbic and citric acids were identical in their effect on vitamin C content to that in the chitosan combinations during cold storage in both seasons. Vitamin C of chitosan- treated apples was similar to that of citric acid- treated ones during cold storage in both seasons and after post- storage shelf life, only in the second season.

The influence of cold storage and post-storage shelf life periods on vitamin C content of "Anna" apples was shown in Table 8. The results revealed that the greatest vitamin C content was recorded at the initial time (zero) and this content significantly declined as the storage advanced to reach the least content after the third month of cold storage. Similar trend was noticed for the effect of post- storage shelf life periods on vitamin C content in a consistent manner during both seasons.

Vitamin C was also influenced by the interaction between postharvest-applied treatments and cold storage periods. The results tabulated in Table 8 indicate that all treatments after the first month of cold storage had the same content of vitamin C which did not change relative to the initial content in the two seasons, except the control and chitosan treatments since they caused a significant reduction in such vitamin. With the progress of cold storage period to the second month, it could be found that vitamin C content significantly changed in all treatments. At this time, control, chitosan and citric acid treatments resulted in great reduction in such vitamin as compared with the ability of salicylic acid plus chitosan treatment in keeping, relatively, high content of vitamin C. After the third month, the least content of vitamin C was recorded in untreated apples while the highest vitamin was found in apples treated with chitosan plus either ascorbic or salicylic acid. Chitosan was identical in its influence on vitamin C to that of citric acid treatment.

Concerning the interaction between treatments and post-storage shelf life periods, the results revealed that after the first period of post-storage shelf life, the greatest content of vitamin C was observed in all applied formulations of chitosan plus antioxidants, the individual treatments of ascorbic and salicylic acids relative to the control. After the second period of post-storage shelf life, vitamin C was significantly decreased by the control and chitosan treatments as compared with chitosan plus antioxidants and the sole application of salicylic acid treatments. By the end of post- storage shelf life, chitosan plus antioxidants were able to achieve a great content of vitamin C relative to the control treatment which caused the lowest content of such vitamin. Furthermore, chitosan showed a similar effect on vitamin C to that obtained by the sole application of citric acid.

Anthocyanin was also influenced with postharvest-applied treatments. The results tabulated in Table 9 show that there was a noticeable increase in anthocyanin content with chitosan plus either ascorbic acid or salicylic acid treatments in a consistent manner during cold storage and after post-storage shelf life periods. Moreover, the lowest content of anthocyanin was obtained with control and chitosan treatments during both seasons. Chitosan plus salicylic acid was capable of increasing anthocyanin content in "Anna" apples than application of each compound alone.

Table 8. Effect of the postharvest-applied treatments, time factor and their interactions on vitamin C content of "Anna" apple fruits during cold storage and after post- storage shelf life periods in the two seasons of 2016 and 2017

Treatment (T)	Vitamin C (mg/ 100 ml juice)								
	Cold storage period (P) (month)					Post-storage shelf life period (P) (5 days)			
	0	1	2	3	Mean	1	2	3	Mean
	2016 season								
Control	8.19	7.81	7.04	5.94	7.25	7.42	6.31	5.58	6.44
Chitosan	8.19	7.88	7.21	6.23	7.38	7.55	6.56	5.91	6.67
Citric acid	8.19	7.93	7.33	6.42	7.47	7.62	6.74	6.11	6.82
Ascorbic acid	8.19	7.99	7.41	6.57	7.54	7.70	6.87	6.27	6.95
Salicylic acid	8.19	7.99	7.46	6.66	7.58	7.74	6.94	6.38	7.02
Chitosan plus citric acid	8.19	8.00	7.53	6.78	7.63	7.77	7.03	6.50	7.10
Chitosan plus ascorbic acid	8.19	8.00	7.56	6.84	7.65	7.80	7.09	6.61	7.17
Chitosan plus salicylic acid	8.19	8.06	7.66	7.05	7.74	7.89	7.24	6.82	7.32
Mean	8.19	7.96	7.40	6.56	-	7.69	6.85	6.27	-
NLSD	T= 0.1182		P= 0.1020		T*P= 0. 2881	T= 0. 1145	P= 0. 0870	T*P= 0.2471	
	2017 season								
Control	8.72	8.30	7.44	6.63	7.77	7.85	7.05	6.21	7.04
Chitosan	8.72	8.39	7.59	6.92	7.91	7.95	7.22	6.56	7.24
Citric acid	8.72	8.45	7.72	7.04	7.98	8.06	7.39	6.68	7.38
Ascorbic acid	8.72	8.48	7.84	7.18	8.06	8.16	7.52	6.84	7.51
Salicylic acid	8.72	8.50	7.91	7.28	8.10	8.21	7.60	6.95	7.59
Chitosan plus citric acid	8.72	8.56	8.00	7.40	8.17	8.30	7.71	7.09	7.70
Chitosan plus ascorbic acid	8.72	8.60	8.10	7.54	8.24	8.38	7.82	7.24	7.81
Chitosan plus salicylic acid	8.72	8.68	8.25	7.75	8.35	8.49	8.00	7.48	7.99
Mean	8.72	8.49	7.86	7.22	-	8.17	7.54	6.88	-
NLSD	T= 0.1893		P= 0.1112		T*P= 0.3141	T= 0.1954	P= 0.1328		T*P= 0.3721

Table 9. Effect of the postharvest-applied treatments, time factor and their interactions on anthocyanin content of "Anna" apple fruit peel during cold storage and after post-storage shelf life periods in the two seasons of 2016 and 2017

Treatment (T)	Anthocyanin (mg/100 g)								
	Cold storage period (P) (month)					Post-storage shelf life period (P) (5 days)			
	0	1	2	3	Mean	1	2	3	Mean
	2016 season								
Control	17.25	18.00	19.01	19.10	18.34	18.08	19.07	19.13	18.76
Chitosan	17.25	18.16	19.21	19.34	18.49	18.21	19.28	19.40	18.96
Citric acid	17.25	18.22	19.39	19.53	18.59	18.30	19.46	19.61	19.12
Ascorbic acid	17.25	18.27	19.47	19.69	18.67	18.37	19.58	19.80	19.25
Salicylic acid	17.25	18.30	19.55	19.82	18.73	18.42	19.69	19.96	19.36
Chitosan plus citric acid	17.25	18.47	19.77	20.07	18.89	18.63	19.92	20.23	19.59
Chitosan plus ascorbic acid	17.25	18.68	20.04	20.39	19.09	18.85	20.21	20.57	19.88
Chitosan plus salicylic acid	17.25	18.72	20.16	20.54	19.17	18.90	20.35	20.79	20.01
Mean	17.25	18.35	19.58	19.81	-	18.47	19.69	19.94	-
NLSD	T= 0.2547		P= 0.1741		T*P= 0.4941	T= 0.4628		P= 0.2243	T*P= 0. 6341
	2017 season								
Control	17.87	18.63	19.63	19.70	18.96	18.66	19.65	19.77	19.36
Chitosan	17.87	18.83	19.90	19.98	19.15	18.88	19.98	20.09	19.65
Citric acid	17.87	18.90	20.02	20.19	19.25	18.96	20.10	20.34	19.80
Ascorbic acid	17.87	18.97	20.13	20.37	19.33	19.08	20.25	20.56	19.96
Salicylic acid	17.87	19.05	20.35	20.65	19.48	19.18	20.40	20.73	20.10
Chitosan plus citric acid	17.87	19.25	20.60	20.80	19.63	19.40	20.68	21.04	20.37
Chitosan plus ascorbic acid	17.87	19.50	20.90	21.24	19.88	19.67	20.99	21.43	20.69
Chitosan plus salicylic acid	17.87	19.57	21.05	21.45	19.98	19.76	21.10	21.69	20.85
Mean	17.87	19.09	20.32	20.55	-	19.19	20.39	20.71	-
NLSD	T= 0.2447		P= 0.1802		T*P= 0.5632	T= 0.2856	P= 0.1894		T*P= 0.5351

The effect of cold storage and post-storage shelf life durations was remarkable where anthocyanin content showed a continuous increase starting from the first month of cold storage and continued with the progress of cold storage and post- storage shelf life periods in both seasons.

The effect of the interaction between treatments and cold storage periods on anthocyanin content of "Anna" apple peel was shown in Table 9. The results confirmed that anthocyanin content significantly increased in all applied treatments after the first month of cold storage relative to the initial value. At this time, the greatest content of anthocyanin was generally obtained in apples treated with chitosan plus antioxidants as compared with the least content achieved by the control. After the second month of cold storage, it could be observed that anthocyanin content in untreated apples was similar to that content of those treated with the individual applications of citric acid and ascorbic acid in a consistent manner in the two seasons. The last month of cold storage revealed that there was a noteworthy higher content of anthocyanin in apples treated with all formulations of chitosan plus antioxidants than control. The least content of anthocyanin was achieved by citric acid, chitosan and control treatments, besides the differences among them were not significant in both seasons.

Similar pattern was noticed concerning the interaction effect of treatments and post-storage shelf life periods. The results in Table 9 reveal that using chitosan in combinations with antioxidants was more effective on increasing anthocyanin content than the sole application of chitosan and control which caused lower anthocyanin content. This trend continued with the progress of post-storage shelf life periods to achieve the highest amount of anthocyanin after the third period with all applied combinations of chitosan and antioxidants, while the significant reduction was observed by chitosan and control treatments. Moreover, ascorbic acid and salicylic acid resulted also in higher anthocyanin content relative to the control.

The influence of postharvest treatments on carotene content of "Anna" apples was shown in Table 10. The results declared that the greatest carotene content was achieved by chitosan plus salicylic acid treatment while the lowest was found by control, regardless cold storage and post- storage shelf life periods, in a consistent manner in the two seasons. In addition, using chitosan with either citric acid or ascorbic acid caused a marked increase in carotene content in comparison with the sole treatments of each component. Furthermore, citric acid was able to cause a similar effect on carotene content to that obtained with the individual applications of chitosan and ascorbic acid. Salicylic acid-treated fruits had similar carotene content to that found with those treated with ascorbic acid. This trend of results was consistent during cold storage and after shelf life periods in the two seasons.

With regard to the influence of cold storage and post- storage shelf life durations, the results in Table 10 reveal that carotene content tended to increase gradually after the first month of cold storage and after the first period of post-storage shelf life, besides this increase continued with the advancement of both durations to achieve the greatest content at the last periods of whether cold storage or post-storage shelf life. These results were consistent in the two successive seasons.

Carotene content was also affected with the interaction between applied treatments and cold storage periods. The results in Table 10 indicate that all applied treatments achieved a significant increase in carotene content after the first month of cold storage relative to the initial content with the exception of control, chitosan and citric acid treatments which did not cause a significant increase in such content. After the first month, the highest increase in carotene was obtained with chitosan plus antioxidants as compared to other treatments. Control in both seasons, chitosan and citric acid treatments in the second season did not cause a significant increase after the second month of cold storage and had lower carotene content in comparison with chitosan plus all antioxidants treatments. By the end of cold storage, it could be reported that the noticeable increase of carotene was found by all treatments, except ascorbic acid treatment in the second season, while the least content was obtained in untreated fruits. Furthermore, applying chitosan plus citric acid or ascorbic acid was capable of increasing carotene content

Table 10. Effect of the postharvest-applied treatments, time factor and their interactions on carotene content of "Anna" apple fruit peel during cold storage and after post-storage shelf life periods in the two seasons of 2016 and 2017

Treatment (T)	Carotene (mg/100 g)								
	Cold storage period (P) (month)					Post-storage shelf life period (P) (5 days)			
	0	1	2	3	Mean	1	2	3	Mean
	2016 season								
Control	3.60	3.71	3.93	4.96	4.05	3.79	4.08	5.09	4.32
Chitosan	3.60	3.72	4.09	5.36	4.19	3.81	4.31	5.50	4.54
Citric acid	3.60	3.84	4.13	5.49	4.27	3.90	4.40	5.64	4.65
Ascorbic acid	3.60	3.89	4.21	5.67	4.34	3.99	4.53	5.82	4.78
Salicylic acid	3.60	3.95	4.33	5.89	4.44	4.09	4.68	6.05	4.94
Chitosan plus citric acid	3.60	4.25	4.66	6.29	4.70	4.40	5.04	6.46	5.30
Chitosan plus ascorbic acid	3.60	4.53	4.98	6.66	4.94	4.71	5.40	6.85	5.65
Chitosan plus salicylic acid	3.60	4.90	5.39	7.18	5.27	5.14	5.87	7.38	6.13
Mean	3.60	4.09	4.47	5.94	-	4.23	4.79	6.09	-
NLSD	T= 0.1392	P= 0.0881		T*P= 0.2491		T= 0.1658	P= 0.0780	T*P= 0.2231	
	2017 season								
Control	4.15	4.23	4.58	5.65	4.65	4.40	4.74	5.82	4.98
Chitosan	4.15	4.41	4.78	6.09	4.86	4.47	4.99	6.26	5.24
Citric acid	4.15	4.49	4.86	6.27	4.94	4.58	5.10	6.44	5.37
Ascorbic acid	4.15	4.95	6.49	4.57	5.04	4.69	5.26	6.67	5.54
Salicylic acid	4.15	4.66	5.11	6.75	5.17	4.71	5.44	6.95	5.70
Chitosan plus citric acid	4.15	4.98	5.49	7.19	5.45	5.05	5.83	7.39	6.09
Chitosan plus ascorbic acid	4.15	5.27	5.83	7.60	5.71	5.39	6.22	7.80	6.47
Chitosan plus salicylic acid	4.15	5.66	6.27	8.15	6.06	5.77	6.43	8.39	6.86
Mean	4.15	4.83	5.43	6.53	-	4.88	5.50	6.96	-
NLSD	T= 0.1408	P= 0.0973		T*P= 0.4524		T= 0.2415	P= 0.0924	T*P= 0.2632	

as compared with the sole application of each component. Chitosan treated apples had similar carotene content to that obtained with those treated with citric acid in the two seasons. Similar trend was observed with the interaction between postharvest applied-treatments and post-storage shelf life periods in a consistent manner during both seasons.

DISCUSSION

It was obvious that chitosan, antioxidants and their combinations used as postharvest treatments were capable of reducing the deterioration of various physical and chemical characteristics during cold storage and after post- storage shelf life in addition to keeping fruit quality and extending its storability, marketability and shelf life. Since, all applied treatments mitigated the incidence of fruit decay and decreased fresh fruit weight loss. Furthermore, the treatments reduced electrolyte leakage of fruits, hindered the loss of fruit firmness and were successful in maintaining the physical and chemical properties such as acidity, vitamin C, TSS and total sugars, besides increased carotene and anthocyanin contents, relative to the control treatment. Apple is a climacteric fruit that increases the production of ethylene at maturity. The results of the present study were in line with **Yao and Tian (2005), Pila *et al.* (2010), Shafiee *et al.* (2010), Aghdam *et al.* (2011), Wei *et al.* (2011), Khademi *et al.* (2012), Ali *et al.* (2013), Razavi *et al.* (2014) and Abd El-Wahab (2015)** on salicylic acid. Furthermore, the obtained results partially were in agreement with **Awad (2013) and Abd El-Wahab (2015)** on citric and ascorbic acids.

The best effects of the applied treatments in terms of their ability to mitigate the incidence of decay, maintain fruit quality, extend fruit storability and post-storage shelf life are mainly due to the role of chitosan component in inhibiting spore germination, germ tube elongation and mycelial growth of several pathogens (**El-Ghaouth *et al.*, 1992; Chien and Chou, 2006; Eweis *et al.*, 2006; Liu *et al.*, 2007; Hernández-Lauzardo *et al.*, 2010**). Moreover, **Liu *et al.* (2007)** added that chitosan was also capable of damaging the plasma membranes in spores of *Penicillum expansum* and *Botrytis cinerea*. However, the reasons for the antimicrobial effect of chitosan remain controversial. There are two hypotheses as follows: The polycationic chitosan consumes the electronegative charges on cell surfaces, consequently the cell permeability is changed, this interaction causes the leakage of intracellular electrolytes and proteinaceous constituents. Chitosan enters fungal cells and adsorbs the essential nutrients, which inhibit or reduce the synthesis of mRNA and protein (**El-Ghaouth *et al.*, 1997; Chen *et al.*, 1998; Jia *et al.*, 2001; Avadi *et al.*, 2004; Chen *et al.*, 2005**). In addition, chitin induces chitinase activity which may have many biological roles including the antifungal activity (**Dahiya *et al.*, 2006; Gohel *et al.*, 2006; Li, 2006**). Chitosan can also induce host resistance by increasing the activities of several defense-related enzymes such as chitinase and β- 1, 3-glucanase in many fruits (**Fajardo *et al.*, 1998; Zhang and Quantick, 1998; Romanazzi *et al.*, 2000; Meng *et al.*, 2010**). In the same trend, chitosan elicits the responses of plant defense by activating pathogenesis related gene functions, such as chitinase (**Mauch *et al.*, 1984; Benhamou and Thériault, 1992**), chitosanase, β-glucanase and lignin (**Notsu *et al.*, 1994**) besides callose formation (**Kauss *et al.*, 1989**). **Zhang *et al.* (2011)** emphasized that chitosan could inhibit postharvest diseases by indirect inducement of defense related enzymes (POD, PPO, PAL and GLU). **Varasteh *et al.* (2012)** found that chitosan coating before cold storage delayed anthocyanin and color degradations in the pomegranate arils.

The use of citric and ascorbic acids retarded the deterioration of fruit quality and prolonged fruit storage age and its shelf life might be due to its ability to catch or chelate the free radicals and thus could result in extending the shelf life of plant cells (**Rao *et al.*, 2000**). In addition, citric, ascorbic and salicylic acids could reduce fruit transpiration leading to minimize weight loss, consequently improving storability (**Abd El-Monem *et al.*, 2013; Mohamed and Mehdi, 2014; Abd El-Wahab, 2015**). Moreover, antioxidants may give fruits a defense against oxidative stress, thus keeping its vitamin (**Abd El-Wahab, 2015**). Moreover, salicylic acid could be able to retard the physiological disorders due to its positive effect on the control of postharvest diseases (**Khademi *et al.*, 2012**) by simulating the activities of antioxidant enzymes (catalase, glutathione peroxidase, chitinase, β- 1, 3- glucanase, dismutase and superoxidase) which led to decrease free radical level and lipid peroxidation, thus increases the resistance against diseases, especially *Penicillium expansum* (**Zainuri *et al.*, 2001; Huang *et al.*, 2008; Zhang *et al.*, 2008; Gerailoo and Ghasemnezhad, 2011**). The effects of salicylic acid on delaying fruit ripening may also be due to decreasing ethylene production (**Mohammadi and Aminifard, 2013**) by suppression ACC synthase and ACC oxidase activities, in addition to increasing superoxide free radical and lipoxyenase activity so retarding the climacteric rise in ethylene, fruit ripening and relative electrical conductivity beside senescence will be delayed (**Zhang *et al.*, 2003**). The reduction occurred in the contents of TSS and total sugars by salicylic, ascorbic and citric acids may be related to the slow respiration, high level of antioxidant enzymes and defense mechanisms from high ripening during storage (**Awad, 2013; Abd El-Wahab, 2015**).

Furthermore, anthocyanin content of apple peel was increased by salicylic acid which may be attributed to enhancing antioxidant potential of fruits and simulating phenylalanineammonia-layase thus triggering the phenyl propanoid-flavonoids pathways leading to an increase in anthocyanin (**Dokhanieh *et al.*, 2013**). Moreover, there was an increase in peel carotene content with the advancement of cold storage periods. These results may be in line with those found by **Abd El-Wahab (2015)**.

In addition, the best results were clearly obtained by all applied combinations of chitosan plus citric, ascorbic or salicylic acids, than the sole applications of each component. This may be due to the synergistic effect between chitosan and other used antioxidant acids.

Conclusion

Finally, from the presented results, it could be concluded that the addition of chitosan to citric, ascorbic and salicylic acids was more effective than the individual application of each component on retarding the incidence of fruit decay and loss of firmness as well as maintaining other physical and chemical quality characteristics consequently, prolonging the storage and shelf life of “Anna” apples.

Recommendation

From the results of this investigation, it could be recommended that dipping “Anna” apples in a combination including 2% chitosan plus 2mM salicylic acid was effective on retarding the occurrence of decay, retarding the loss of firmness, reducing weight loss and electrolyte leakage beside keeping other physical and chemical quality characteristics of fruits, thus prolonging its storage and shelf life of “Anna” apple fruits. Therefore, this is an easy to apply, new, cheap and safe strategy that can ensure an increase of the growers income.

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إطالة العمر التخزيني و فترة العرض لثمار التفاح "آنا" باستخدام الشيتوزان وبعض مضادات الأكسدة الطبيعية

نيفين محمد نبيه ناجي

قسم البساتين (فاكهة) - كلية الزراعة - جامعة دمنهور- مصر

أُجريت هذه الدراسة في موسمين متعاقبين ٢٠١٦-٢٠١٧ في مزرعة بساتين خاصة بمنطقة أبو حمص بمحافظة البحيرة، مصر لتقييم تأثير معاملات بعد القطف التي اشتملت على: الشيتوزان بتركيز ٢%، حامض الستريك بتركيز ٢٠٠ جزء في المليون، حامض الأسكوربيك بتركيز ٢٠٠ جزء في المليون، حامض الساليسيلليك بتركيز ٢ مللي مول، الشيتوزان مع حامض الستريك، الشيتوزان مع حامض الأسكوربيك، الشيتوزان مع حامض الساليسيلليك عند نفس التركيزات السابقة علي التلف، الصلابة بالإضافة إلى خصائص الجودة الأخرى لثمار التفاح "آنا" أثناء التخزين على ٢±١°م درجة مئوية ورطوبة نسبية ٩٠- ٩٥% لمدة ثلاثة أشهر وبعد فترة إختبار حياتها على درجة حرارة الغرفة لمدة خمس أيام، أوضحت النتائج أن نسبة تلف الثمار، فقد الوزن الطازج، التسرب الإليكتروليتي، السكريات الكلية، المواد الصلبة الذائبة (TSS)، محتوى الأنثوسيانين والكاروتين قد إزدادت بينما إنخفض كلاً من الصلابة، فيتامين سي والحموضة مع تقدم فترة التخزين المبرد وفترة العرض، علاوةً على ذلك، أدت المعاملات إلي تقليل فقد الصلابة تضمن والتسرب الإليكتروليتي، وكانت أكثر فاعلية في حفظ جودة الثمار الفيزيائية والكيماوية مقارنة بمعاملة الكنترول، أثبتت الدراسة أن إضافة الشيتوزان إلى مضادات الأكسدة كانت أكثر فاعلية من المعاملات الفردية من كل مركب في تقليل حدوث التلف، وحفظ صلابة الثمار، تقليل فقد الوزن والتسرب الإليكتروليتي بالإضافة إلي جودة الثمارالفيزيائية والكيماوية كذلك أطالت فترة التخزين ومدة العرض علي حرارة الغرفة لثمار التفاح "آنا" خاصة مخلوط الشيتوزان مع حامض الساليسيلليك وبناءً على ذلك، فهذه الإستراتيجية سهلة التطبيق، جديدة، غير مكلفة وآمنة وتؤدي إلى زيادة قابلية ثمار التفاح "آنا" للتسويق وبالتالي زيادة دخل المزارعين.

المحكمـــون:

١- أ.د. أحمـد محمـد عبـده عيسى أستاذ الفاكهة – كلية الزراعة بالشاطبي – جامعة الإسكندرية.

٢- أ.د. سيد مجدي عثمان الحفناوي أستاذ الفاكهة المتفرغ – كلية الزراعة – جامعة الزقازيق.