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Influence of Postharvest Application with Chitosan and some Natural Plant Extracts on Storage Life and Quality Attributes of Navel Orange Fruits during Cold Storage

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ABSTRACT

Chitosan and some natural plant extracts were tested to maintain post harvest quality of Navel orange. The treatments included control (distilled water), chitosan at 2% and three aqueous plants extracts namely; olive water at 4%, rosemary at 4% and lemongrass at 3%.The fruits were stored for 12 weeks at 5±1°C and 90% RH during two successive seasons of 2015 and 2016. Fruits evaluated every 3 weeks to determine the changes in fruit quality characteristics during cold storage. Results indicated that all treatments helped to minimize the deterioration of quality Navel orange in all stored periods of cold storage. All treatments decreased weight loss, decay and delayed the changes in percentage of juice, total soluble solids, titratable acidity, carotene content and vitamin C compared with untreated fruits especially rosemary extract at 4% exhibited the best results in preserving fruit quality during the storage, thus prolongs postharvest shelf life during shipping and marketing also safe on human health and environmental.

Key words: Navel orange, chitosan, aqueous extract, olive water, rosemary, lemongrass, cold storage.

Introduction

Navel orange expose to fungal diseases; green, blue mould caused by *(Penecillium digitatum* Pers. Sac *and Penecillium italicum* Whemer*)* and Sour rot caused by (*Geotrichum candidum* Link.) (Palou *et al*., 2001; Ladaniya, 2008) which often cause post-harvest decay of fruit and represents major losses during harvest, storage and exportation. So, orange fruits are often treated with chemical fungicides to reduce the incidence of postharvest diseases in various fruits. However, the application of synthetic chemical fungicides to control postharvest diseases often results in chemical residues on fruits that may affect human health, environmental pollution. On the other hand, the increasing demand for more 'natural' food has led to search for alternative antimicrobial agents within the food industry (Guynot *et al*., 2005). Recently, non-chemical methods such as the use of gamma rays, UV light, heat therapy and the use of natural ingredients such as plant extracts, which in most cases have antimicrobial, fungicidal antioxidant and bio-regulating properties (Asghari *et al*., 2009) have been developed in the storage process of horticultural products to control decay and prolong storage life of post-harvest (Tripathi *et al*., 2008).

Chitosan, a natural biopolymer has been reported to enhance resistance against many fungal diseases including *Penicillium digitatum*, *Penicillium italicum* of fruits and vegetables when applied as either a pre- or postharvest treatment (Riad *et al*., 2015). In addition, chitosan can be directly antimicrobial and has been shown to interfere with the germination and growth of several phytopathogenic fungi (Ben-Shalom *et al*., 2003). Furthermore, various investigators have demonstrated that treatment with chitosan has the potential to inhibit decay and hence prolong the

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cold storage life of Navel orange fruits. (Janparvar *et al*., 2014; EL-Eleryan, 2015; Youssef *et al*., 2015; Ahmed *et al*., 2016).

Many studies showed that antifungal and antimicrobial effect of plant extracts, i.e. leaf extracts, have been reported to inhibit different plant diseases (Ghassan *et al*., 2013) and also, against several species of fungi including both human and plant pathogenic types (Roller, 2003). Olive (*Olea europaea* L.) leaves aqueous extract are rich in phenolic substances, which considered an important antioxidant and potent antimicrobial activities against bacteria, fungi and mycoplasma (Korukluoglu *et al*., 2008; Darsanaki *et al*., 2012, Abd– Alla, *et al*., 2013). Rosemary (*Rosmarinus officinalis*) is rich in bioactive compounds, has antioxidative (Ramírez *et al*., 2004; Peng *et al*., 2005) and antimicrobial properties (Lawrence, 2000; Rasooli *et al*., 2008) which can be important in reducing post-harvest losses of the frequency range of interest and enhance the storage life. Lemongrass (*Cymbopogon citratus* L.) was reported to be antifungal activity against several plant pathogens (Hyun *et al*., 2015) and reduced the incidence diseases.

The target of this study was examining the effect of chitosan and three aqueous plant extracts namely; olive water, lemon grass and rosemary on storage life and quality attributes of Navel orange fruits during cold storage.

Material and Methods

Fruit material:

Mature Navel orange fruits (*Citrus sinensis* L. Osbeck) were harvested from 20 years old trees budded on Sour Orange (*Citrus aurantium* L.) rootstock in a private orchard at Beheria, Governorate during two successive seasons 2015 and 2016. Fruit harvested at full color stage and total soluble solids (10.57& 10.53%), titratable acidity (0.89& 0.95 %), juice ratio (60.04& 58.37 %), carotene (0.143 & 0.135 mg/100 ml juice) and ascorbic acid (56.99& 55.43 mg/100ml juice) in the first and second seasons, respectively. Fruit immediately transported to the laboratory, of Agriculture Development Systems (ADS) project at Faculty of Agriculture, Cairo University. Fruits uniform in shape, weight, color, size and absence of defects.

Treatments:

Selected fruits were washed with running tap water, air dried and subjected to the following treatments; dipping in chitosan 2 % , olive extract 4%, lemongrass extract 3 %, rosemary extract 3 % for 3 minutes and control (Dipping in tab water only), then left for 2 h at room temperature to be dried. Fruits were packed in carton boxes (3 Kg each and measures 43×33×9 cm) in one layer and stored at 5±1°C and 90-95% relative humidity (RH) for 12 weeks. Each treatment (60 fruit) was replicated thrice and each replicate (20 fruit). Three groups were used for each treatment as box to determine decay, the second to determine weight loss and the third for fruit physical and chemical analysis.

Preparation of chitosan solution:

Chitosan solution (2 %, w/v) was prepared by dissolving 2 g chitosan powder in 100 ml glacial acetic acid (Ghasemnezhad *et al*., 2010) with continuous stirring.

Preparation of olive water extract:

An aqueous extract of olive leaves (*Olea europaea* L. cv. Picual) was prepared by putting dried leaves (4g) in boiling water (100 mL). The mixture was boiled for 30 min and then filtered using Whatman's sterile filter paper and kept in the dark for phenolic compounds analysis and dissolved in water (Pereira *et al*., 2007).

Preparation of rosemary and lemon grass aqueous extract:

Rosemary (*Rosmarinus officinalis*) and Lemongrass (*Cymbopogon citratus*) leaves were thoroughly washed and dried at room temperature. The methods of (Moumene *et al*., 2015; Rusin *et al*., 2016) were used to prepare the extracts with some modifications. Plant extracts were prepared using 3 g of plant material in 100 ml hot distilled water 70°C. After 1 h, the mixture was boiled for 30 minutes and subsequent filtering through cheesecloth.

Determination of physical and chemical properties:

Physical and chemical properties were evaluated every 3 weeks from the beginning to the end of storage period (12 weeks).

Weight loss percentage:

The difference between the initial weight of the fruits at the beginning of storage and that recorded at the date of sampling was translated as weight loss percentage and calculated as follows:

$$\text{Weight loss \%} = \frac{\text{Weight at the date of sampling (g)}}{\text{Initial weight of the fruits (g)}} X\ 100$$

Decay percentage:

The percentage of disordered fruits included all of the spoiled fruits resulted from rots, fungus, bacterial and pathogens were assessed and the defects were calculated as follows:

$$\text{Decay \%} = \frac{\text{No. of fruit decay}}{\text{No. of fruit at the beginning of storage}} X\ 100$$

Percentage of juice:

Fresh fruits were ground in an electric juice extractor for freshly prepared juice, then juice weight and percentage of juice was evaluated.

Total Soluble Solids (TSS) %:

Soluble solids content was measured using Digital rafractometer PR32 (0.32% Atago Palete ATago.CO .LTD. Japan).

Titratable acidity (%):

Total acidity (expressed as malic acid %) was determined by titrating 5 ml of the extracted juice against 0.1 N of NaOH using phenolphethalin indicator according to (AOAC, 2000).

Carotene content (mg/100 ml juice):

Carotene content of fruits juice was extracted by direct dipping of 10 gm of blended fruit pulp into solution containing (40 ml acetone, 60 ml hexane and 0.1 g Mg Co_3 and blended for 5 minutes. It was determined by colorimeter according to (AOAC, 2000).

Ascorbic acid content (vitamin C) mg/ 100 ml juice:

It was determined by titration against 2, 6 dichlorophenolendophenol and using 2% oxalic acid solution as substrate described by (AOAC, 2000).

Statistical Analysis:

The treatments were arranged as experiment in a randomized complete design. All data were subjected to statistical analysis according to the procedures reported by Snedecor and Cochran (1990) and means were compared by Duncan's multiple range tests at the 5 % level of probability according to Duncan (1955) in the two seasons of experimentation.

Results and Discussion

Physical properties:

Weight loss percentage:

Results in Table 1, cleared that weight loss percentage increased with extending cold storage period. Highest losses of weight were obtained at the end of storage period (Davarynejad *et al*., 2013). Normally, this occurs during the fruit storage due to respiratory process, the transference of humidity and some processes of oxidation (Ayranci and Tunc, 2003). After 12 weeks of storage, rosemary extract recorded the lowest significant percentage of fruit weight loss (7.85, 8.20 %) in both seasons, respectively as compared with the control which had the highest percentage of weight loss (11.95% and 10.39%) in the first and second, respectively. The reducing value of weight loss in Navel orange fruits at different period's storage was related with fruit postharvest treatments by all natural extracts and chitosan compared with control in both seasons. From these results, the decreasing of weight loss may be attributed to controlling of decay and its damages through the biological activity of olive water, rosemary, lemongrass extracts and chitosan against postharvest diseases and make a thin film surrounding the fruit peel and induced a modification of atmosphere around the fruits.

Table 1: Effect of postharvest treatments with chitosan and some natural plant extracts on weight loss % of Navel orange fruits stored at 5±1°C and 90% RH during 2015 and 2016 seasons.

Treatments	Storage periods (weeks)			
	3	6	9	12
	Season 2015			
Chitosan at 2 %	2.35c	5.59b	8.34b	11.52a
Olive water at 4%	3.18b	6.10b	8.10b	10.56b
Rosemary extract at 4%	1.74c	3.89c	5.52c	7.85c
Limongrass extract at 3%	3.19b	6.36b	8.32b	10.18b
Control	4.26a	7.19a	9.13a	11.95a
	Season 2016			
Chitosan at 2 %	2.03bc	4.42bc	6.49bc	8.94bc
Olive extract at 4%	2.48ab	5.20a	7.33ab	9.52b
Rosemary extract at 4%	1.70c	3.74c	6.01c	8.20c
Limongrass extract at 3%	2.81a	5.09ab	6.95ab	9.26b
Control	2.03bc	5.10ab	7.56a	10.39a

Means within a column with different letters are significantly different at P<0.05.

Fruit decay percentage:

Table (2) showed that no decay before 6 weeks of cold storage. All treatments reduced the percentage of decay than control fruits. After 12 weeks of storage, fruits treated by olive extract exhibited the least decay percentage in the first season, while no significant difference between treatments in the second season.

This decreasing in decay percentages of treated samples probably due to increase defense by treatments on surface fruits and its effects on delaying pathogenic infection as result to main components in extracts e.g. carnosol, carnosic acid, carnosol, rosmarinic acid and phenolic di-terpenoids in rosemary extract (Ibrahim and Ebady, 2014), the phenolic compounds; caffeic acid, verbascoside, oleuropein, luteolin 7-O-glucoside, rutin, apigenin 7-O-glucoside and luteolin 40-O-glucoside in olive leaf extract (Pereira *et al*., 2007; Hendel *et al*, 2016) and alkanoids, saponins,

flavonoids, tannins, anthraquinones, steroids and phenols in Lemon grass extract (Asaolu *et al*., 2009). The different extracts tested in the present study contain phenol compounds that have a great antifungal activity and could affect the enzymes responsible for spore germination of fungi and have also been recognized as bioactive components (Tabassum and Vidyasagar, 2013; Abd-El Wahab, 2015). Besides, the positive effects of treatment with chitosan on control postharvest diseases and reduce decay percentages (Youssef *et al*., 2015; Ahmed *et al*., 2016) , this leading to improve storability and shelf life of Navel orange.

Table 2: Effect of postharvest treatments with chitosan and some natural plant extracts on decay % of Navel orange fruits stored at 5±1°C and 90% RH during 2015 and 2016 seasons.

Treatments	Storage periods (weeks)			
	3	6	9	12
	Season 2015			
Chitosan at 2 %	0.00	16.00a	33.00a	35.00c
Olive extract at 4%	0.00	0.00b	16.00b	33.00d
Rosemary extract at 4%	0.00	16.00a	33.00a	35.00c
Limongrass extract at 3%	0.00	16.00a	33.00a	36.00b
Control	0.00	16.00a	33.00a	37.00a
	Season 2016			
Chitosan at 2 %	0.00	0.00b	0.00c	16.00b
Olive extract at 4%	0.00	0.00b	16.00b	16.00b
Rosemary extract at 4%	0.00	0.00b	16.00b	16.00b
Limongrass extract at 3%	0.00	0.00b	16.00b	16.00b
Control	0.00	16.00a	33.00a	35.00a

Means within a column with different letters are significantly different at P<0.05.

Juice percentage:

Juice percentage of stored fruits as shown in Table 4 decreased significantly with the extended of storage period during 2015 & 2016 seasons. It is reached the minimum values at the end of storage period (12 weeks) as previously detected by Pradeep *et al*. (2016). There were significant differences between all treatments in both seasons. Rosemary extract treated fruits had the highest value in percentage of juice; meanwhile untreated fruits had the lowest value of juice % as compared with the other treatments during 2015 and 2016 seasons.

Table 4: Effect of postharvest treatments with chitosan and some natural plant extracts on juice % of Navel orange fruits stored at 5±1°C and 90% RH during 2015 and 2016 seasons.

Treatments	Storage periods (weeks)				
	0	3	6	9	12
	Season 2015				
Chitosan at 2 %	60.04 a	51.53c	50.11c	44.63d	42.79d
Olive extract at 4%	60.04 a	50.00e	49.59d	47.35c	46.72b
Rosemary extract at 4%	60.04 a	57.53b	55.63a	49.19b	47.30a
Limongrass extract at 3%	60.04 a	64.79a	54.96b	53.78a	44.94c
Control	60.04 a	50.59d	44.49e	40.71e	34.05e
	Season 2016				
Chitosan at 2 %	58.37 a	53.81c	48.89e	47.21d	43.27c
Olive extract at 4%	58.37 a	57.47a	54.77a	47.30c	40.51d
Rosemary extract at 4%	58.37 a	54.89b	52.13b	51.30a	47.59a
Limongrass extract at 3%	58.37 a	52.16e	50.94c	49.73b	44.69b
Control	58.37 a	53.28d	50.69d	45.50e	37.27e

Means within a column with different letters are significantly different at P<0.05.

Total soluble solids percentage (TSS %):

Results presented in Table (5) cleared that percentage of total soluble solids were gradually increased with extend of storage period during 2015 & 2016 seasons. It is reached the maximum

values at the end of storage period (12 weeks) as previously reported by (Shaaban *et al*., 2015; Thanaa *et al*., 2016). There were significant differences between almost tested treatments. After 12 weeks of storage, fruits treated with chitosan recorded the highest values (12.55 and 12.97 %) during 2015 and 2016 seasons, respectively. On the other hand, control treatment showed the lowest total soluble solids percentage (10.03 and 11.17%) during the two seasons, respectively. The effect of chitosan treatment on soluble solid contents 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 (Yonemoto *et al*., 2002; Dong *et al*., 2004). These results are in harmony with (Youssef *et al*., 2015; EL-Eleryan, 2015; Ahmed *et al*, 2016) whose found that Navel orange fruits treated with chitosan showed the significant increase in total soluble solids percentage over the storage periods.

Table 5: Effect of postharvest treatments with chitosan and some natural plant extracts on total soluble solids % of Navel orange fruits stored at 5±1°C and 90% RH during 2015 and 2016 seasons.

Treatments	Storage periods (weeks)				
	0	3	6	9	12
	Season 2015				
Chitosan at 2 %	10.57a	9.60c	11.03c	10.73a	12.55a
Olive extract at 4%	10.57a	10.10b	11.50b	11.00a	12.47a
Rosemary extract at 4%	10.57a	9.13d	12.00a	10.83a	12.13b
Limongrass extract at 3%	10.57a	9.60c	9.53d	10.33b	11.00d
Control	10.57a	11.70a	11.80a	10.83b	10.03d
	Season 2016				
Chitosan at 2 %	10.53a	10.00bc	10.50c	11.37b	12.97a
Olive extract at 4%	10.53a	9.60c	10.93b	12.13a	12.47b
Rosemary extract at 4%	10.53a	10.10b	12.50a	10.83cd	12.57b
Limongrass extract at 3%	10.53a	10.00bc	9.30d	11.00c	11.97c
Control	10.53a	11.20a	11.03b	10.80d	11.17d

Means within a column with different letters are significantly different at P<0.05.

Titratables acidity percentage:

Table 6 revealed that, titratables acidity percentage was decreased with extended storage period (12 weeks) in both seasons. Chitosan and all extracted compounds delayed the decreasing of fruit titratable acidity at the end of storage period compared with untreated fruits which gave the highest value of titratables acidity rate (0.77 and 0.72 %) in both season, respectively. The statistically analysis, showed that chitosan treatment more effective on decreasing titratable acidity percentages (0.60 %) than that other treatments during the first season of study. These results are in line with those obtained by (Scalon *et al*., 2012, Plácido *et al*., 2016); they found that treated fruits with chitosan decreased titratables acidity during storage period. This might be due to chitosan formation a semi-permeable layer regulate gas exchanges on fruit surface and inhibiting respiration (Shiri *et al*., 2013), thus reduce production of compounds responsible for acidity (organic acids) in fruits and release hydrogen ions, contributing to decrease fruit titratables acidity and delay the senescence stage progress.

Carotene content (mg/100 ml juice):

The carotenoid pigments are the main factors determining the color of orange juice and also have pro-vitamin A activity. The total carotene content was very small and less than 5 percent of the total carotenoids (Ting, 1961). In non-climacteric fruits, like citrus, chemical variations are not very significant, as after the harvest, these fruits do not present considerable alterations in the content of carotene (Buruger *et al*., 1956) reported that the Washington Navel had about 1.0 mg carotene per liter of juice. As clear in Table 7, carotene contents of stored Navel orange fruits were not significantly affected by treatments after storage period (9 and12 weeks). Rosemary and limongrass extracts exhibited the highest values in the first season and Limongrass extract in the second season, while the control treatment exhibited the lowest values in the first and second season.

Table 6: Effect of postharvest treatments with chitosan and some natural plant extracts on titratable acidity % of Navel orange fruits stored at 5±1°C and 90% RH during 2015 and 2016 seasons.

Treatments	Storage periods (weeks)				
	0	3	6	9	12
	Season 2015				
Chitosan at 2 %	0.89 a	0.83a	0.73b	0.63c	0.60c
Olive extract at 4%	0.89 a	0.87a	0.87a	0.70a-c	0.70a
Rosemary extract at 4%	0.89 a	0.83a	0.77ab	0.67bc	0.66b
Limongrass extract at 3%	0.89 a	0.87a	0.80ab	0.73ab	0.70a
Control	0.89 a	0.87a	0.83ab	0.77a	0.77a
	Season 2016				
Chitosan at 2 %	0.95a	0.82ab	0.77a	0.67a	0.62a
Olive extract at 4%	0.95a	0.77ab	0.75a	0.69a	0.67a
Rosemary extract at 4%	0.95a	0.92a	0.75a	0.71a	0.64a
Limongrass extract at 3%	0.95a	0.79ab	0.79a	0.75a	0.71a
Control	0.95a	0.72b	0.86a	0.79a	0.72a

Means within a column with different letters are significantly different at P<0.05.

Table 7: Effect of postharvest treatments with chitosan and some natural plant extracts on carotene content (mg/100 ml juice) of Navel orange fruits stored at 5±1°C and 90% RH during 2015 and 2016 seasons.

Treatments	Storage periods (weeks)				
	0	3	6	9	12
	Season 2015				
Chitosan at 2 %	0.143a	0.109a	0.095a	0.040a	0.037a
Olive extract at 4%	0.143a	0.067b	0.060b	0.041a	0.037a
Rosemary extract at 4%	0.143a	0.050b	0.052b	0.038a	0.038a
Limongrass extract at 3%	0.143a	0.114a	0.083a	0.043a	0.038a
Control	0.143a	0.057b	0.047b	0.044a	0.032a
	Season 2016				
Chitosan at 2 %	0.135a	0.067ab	0.046a	0.044a	0.034a
Olive extract at 4%	0.135a	0.084a	0.057a	0.039a	0.035a
Rosemary extract at 4%	0.135a	0.058ab	0.039a	0.037a	0.035a
Limongrass extract at 3%	0.135a	0.067ab	0.047a	0.046a	0.045a
Control	0.135a	0.050b	0.040a	0.037a	0.032a

Means within a column with different letters are significantly different at P<0.05.

Ascorbic acid content (mg/ 100ml juice):

Ascorbic acid (vitamin C) degradation with advance cold storage duration, because unstable under the inclement storage conditions in terms of light, temperature, humidity and diseases (Sajad *et al*., 2011). It is obvious from these results shown in Table 8 that, all tested treatments succeeded to inhibit damage which causing oxidation of ascorbic acid in Navel orange during storage in both seasons. Rosemary extract treatment proved the most efficient in maintaining the highest level of vitamin C (45.16 and 46.87 mg /100ml juice) during the two seasons, respectively. Meanwhile, control treatment scored the least values (43.42 and 43.17 mg /100ml juice) in the first and second seasons, respectively. The maximum retention of vitamin C in fruits treated with rosemary extract may be due to the antioxidant activities in rosemary extracts (carnosol, carnosic acid, rosmarinic acid, rosmanol and rosemaridiphenol phenolic compounds) (Hendel *et al*., 2016), which causing reduce the oxidation of ascorbic acid in the fruits. While, the minimum of vitamin C in control fruits probably due to physiological disorders, decay and weight loss which led to rapid the oxidation reaction in vitamin C, so decreased and showed the lowest level in control fruits.

Table 8: Effect of postharvest treatments with chitosan and some natural plant extracts on ascorbic acid (mg/100ml juice) of Navel avel orange fruits stored at 5±1°C and 90% RH during 2015 and 2016 seasons.

Treatments	Storage periods (weeks)				
	0	3	6	9	12
	Season 2015				
Chitosan at 2 %	56.99a	50.08c	48.30b	45.95c	44.92a
Olive extract at 4%	56.99a	52.55a	51.79a	49.42a	44.90a
Rosemary extract at 4%	56.99a	50.53bc	48.72b	47.93b	45.16a
Limongrass extract at 3%	56.99a	51.36b	48.59b	47.70b	44.67a
Control	56.99a	49.79c	48.93b	47.18b	43.42b
	Season 2016				
Chitosan at 2 %	55.43a	49.37c	48.73b	46.92b	44.53bc
Olive extract at 4%	55.43a	49.92bc	48.65b	45.52c	44.03c
Rosemary extract at 4%	55.43a	53.95a	45.29c	51.05a	46.87a
Limongrass extract at 3%	55.43a	50.75b	50.13a	47.35b	44.91b
Control	55.43a	49.91bc	49.34ab	46.84b	43.17d

Means within a column with different letters are significantly different at P<0.05.

Conclusion

In recent years, the need to application of natural materials as alternative to synthetic chemicals to control qualitative and quantitative losses of stored fruits that occur as a result of postharvest diseases. Our results revealed that the chitosan and aqueous extracts of olive, rosemary and lemongrass more effective than untreated fruits in keeping quality of Navel orange during the storage. All treatments led to decrease weight loss, decay and delayed the changes in percentage of juice, total soluble solids, titratable acidity, carotenoids and vitamin C compared with untreated fruits especially rosemary extract. Therefore, it may be concluded that dipping Navel orange fruits in rosemary aqueous extract at 4% for 3 minutes before cold storage preserve fruit quality during the storage, thus prolongs postharvest life during marketing also safe on health and environment.

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