Alternative Approach for Extending Shelf life of Orange Fruits and Prevent Deterioration by *Penicillium digitatum* the Cause of Green Mold

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Abstract: One of the most serious problems of marketing orange fruits is postharvest diseases because of rapid fruits deterioration during handling, transportation and storage. Edible coating of fruits is used to improve their shelf life and quality. Recently, there has been increased interest in using essential oils, chitosan, wax, and yeast as an edible coating material through antifungal activity, moisture loss prevention, control respiratory rate and maturation development. In case of orange fruits, essential oils (clove, neem, jojoba, rosemary, mint, and aloe vera) at concentration 4% for each oil, chitosan solution at 1 gm /litter, yeast 11 gm /litter and wax coated fruits survived the storage period of 21 days at room temperature whereas all the uncoated controls decayed within 7-10 days. The results showed that all treatments maintained the quality properties of the orange fruit juice (T.S.S, pH, acidity). The best treatments for prolonging the shelf life of fruits and maintaining the weight of fruits during the storage period were wax, yeast, jojoba, clove, and neem oils. Thus, essential oils, wax, chitosan, yeast as edible coating in fruits, would be an innovation and interesting means for commercial application and alternative to the use of postharvest chemical treatments and extending of shelf life of fruits.

Keywords: citrus, chitosan, wax, yeast, quality, TSS

INTRODUCTION

Citrus is one of the most popular and widely grown fruit crops in the world. Citrus and its products are a rich source of vitamins, minerals, and dietary fiber that are essential for overall nutritional well-being. These important fruit crops are cultivated throughout the tropical and subtropical regions and hold an important economic position in the global fruit industry, with global production exceeding 98 million tons (United States Department of Agriculture, 2021). During the postharvest stage, including handling, shipping, storing, and marketing, fruits are subject to a series of biotic or abiotic stresses, and fruit decay and risks to food safety caused by postharvest fungal diseases are some of the most serious problems. Green mold and blue mold, caused by *P. digitatum* and *P. italicum*, respectively, are the two most important postharvest diseases in all citrus production areas. Postharvest green mold, which is the main factor resulting in citrus fruit decay, leads to huge economic losses worldwide every year and can account for up to 90% of the total citrus postharvest losses, especially in arid zones and subtropical climates (Marcet *et al.,* 2012). The edible coatings enriched with essential oils have been developed as an alternative and eco-friendly approach to control postharvest decay and maintain the quality and shelf life of citrus fruits. Accordingly, the objective of this research is to investigate essential oils as amended edible coatings for replacing the chemical fungicides and increase the shelf life of orange fruits.

MATERIALS AND METHODS

Collection samples of orange fruits: Infested orange fruits (rotted fruits) were collected from different local markets in Ismailia and Port Said governments, then transferred to the Plant Pathology laboratory, Agricultural Botany department, Faculty of Agriculture Suez Canal University-Ismailia, for isolation and identification of the causal pathogen of citrus green mold disease.

Isolation and Identification of the pathogen: Samples of rotted orange fruits were washed well with tap water for 1 minute, and washed with distilled water, small parts of decayed fruit, specifically from the area between the infected and healthy parts were cut then surface-sterilized in sodium hypochloride (0.5%) for 30 seconds and washed two times with sterilized distilled water. Then dried on sterilized filter papers, three parts were transferred onto potato dextrose agar medium (PDA) in plates. Plates were incubated for 7 days at 25 °C and purified by single spore method. The isolates were cultured and maintained on PDA slants kept in a refrigerator for further studies.

Coating fruits with different materials EOs, wax, chitosan, and mixture: Orange fruits were divided into ten equal groups, each group comprised of six fruits /treatment. The tested treatments were as following: immersing in sterilized distilled water (control), immersing in essential oils at (4%) concentration for each oil, chitosan solution at concentration (1 gm /1 litter), and yeast at (11 gm/1 litter) for 20 minutes. All fruits were air dried, then put into plastic bags retained high humidity (85-90%) and stored at room temperature. Samples were taken weekly during storage period to estimate physical, chemical fruit properties and reducing decay.

Fruit weight loss percentage (%): Fruits were weighted at the beginning of the experiment and at the end of each storage.

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Weight loss %= $\frac{(Initial\ weight - Final\ weight}{Initial\ weight} \times 100$

Fruit Decay %: Fruit decay % was determined by counting the number of decayed fruits at the end of the experiment and expressed as a percentage of fruit decay according to the following equation:

Fruit decay % = $\frac{NO.of\ decayed\ fruits}{initial\ NO.of\ stored\ fruits} \times 10$

Total soluble solids in the fruit juice (TSS): TSS was determined using a hand refractometer, a drop of orange juice was placed on refractometer and the reading was recorded and briefly expressed in percentage according to Association of Official Analysis Chemists (A.O.A.C, 2005).

Estimate Titratable acidity of orange juice (TA): A phenolphthalein indicator is added to a fresh portion of orange juice sample. Then, the sample is titrated with a sodium hydroxide solution until the indicator color changed at the endpoint pH of 8.3. This titration includes both strong and weak acid species and is a measure of the total acidity.

Acidity = $\frac{Volume\ of\ NaOH \times moles\ of\ NaOH \times moles\ of\ citric\ acid}{Volume\ of\ sample \times 1000} \times 100$

pH of orange juice was estimated using pH meter.

RESULTS

Fruit weight loss and Fruit Decay percentage (%) Data in Table (1) physiological loss in weight (PLW) was significantly increased in all the treatments with the advancement of the storage period and the increasing trends in the weight loss percentage was found Minimum percentage of PLW was observed in the fruits treated with wax in all the weeks during storage and the losses ranged from 0 % in the 1st week to 0.6 % in the 4th week , whereas maximum weight loss was recorded in the fruits which treated with mint at a rate of 5.13% ,the highest treatment had an effect on weight loss, followed by fruits treated with clove oil, which recorded a rate of 2.17% during the storage. The decay loss was noticed from the second week of the storage in untreated fruits (control) and it was appeared from the third week in fruits which treated with rosemary, aloe vera and chitosan which recorded a rate of 50%. The minimum decay loss was noticed in the fruits which treated with wax, yeast (Saccharomyces cerevisiae), clove, mint, neem and jojoba oil. The maximum decay loss was observed in the fruits were treated with rosemary, aloe vera and chitosan recorded a rate of 100% at the end of storage.

Table 1: Effect of essential oils, chitosan, yeast, and wax on the loss of weight and decay in orange fruits

Treatments	Physiological loss in weight (%)				Decay loss (%)			
	Weeks of storage				Weeks of storage			
	1	2	3	4	1	2	3	4
Control	1.17	—	—	—	0.00	**100.0**	—	—
Neem	0.71	1.42	1.42	2.13	0.00	0.00	0.00	0.00
Mint	0.93	2.16	2.47	5.13	0.00	0.00	0.00	0.00
Clove	1.51	2.11	2.41	2.71	0.00	0.00	0.00	0.00
Jojoba	0.29	0.88	1.18	1.76	0.00	0.00	0.00	0.00
Rosmary	1.06	2.13	—	—	0.00	0.00	**100.0**	**100.0**
Aloe vera	1.11	1.66	—	—	0.00	0.00	**100.0**	**100.0**
Yeast	1.17	1.46	2.05	2.05	0.00	0.00	0.00	0.00
Wax	0	0.6	0.6	0.6	0.00	0.00	0.00	0.00
Chitosan	1.35	2.77	—	—	0.00	0.00	**100.0**	**100.0**

***(-): No sample of orange fruits**

Total soluble solid (T.S.S): Data in Table (2) showed a significant decrease in total soluble solid (T.S.S) of orange juice samples during postharvest period (three weeks) regardless of kind of coating especially in the first week no different significant apparent between all treatments and control.

Acidity: Data in Table (3) indicated that all treatments were increased the (TA) in orange juice during the postharvest storage (three weeks). from data, treatments can divide into three groups, first group contain mint, aloe vera and clove 0.85 ,0.84 and 0.83 of citric acid. Second group contain chitosan, jojoba, neem and wax 0.73 ,0.72 ,0.72 and 0.72 from citric acid. And the third group contain yeast and rosemary, it's contained citric acid by 0.63 and 0.57 comparing of the control (Non – coating fruits) contains 0.67.

Table (2): Effect of essential oils, chitosan, yeast (*S. cerevisiae*) and wax on the total soluble solid (T.S.S)

Treatment	Storage period		
	1st week	2nd week	3rd week
Clove	12.22 ab	12.00 bcd	10.41 b
Neem	13.24 a	12.25 bc	11 b
Jojoba	13.17 a	11.33 d	12 b
Mint	12.22 ab	12.2 bc	11.5 b
Aloe vera	13.51 a	12.41 b	10.85 b
Chitosan	11.62 b	12.25 bc	12.08 b
Saccharomyces cerevisiae	13.00 ab	13.25 a	13.5 a
Rosemary	13.35 a	12.41 b	11.41 b
Wax	13.33 a	12.41 b	11.25 b
Control	12.73 ab	11.5 cd	10.5 b

Acidity: Data in Table (3) indicated that all treatments were increased the (TA) in orange juice during the postharvest storage (three weeks). from data, treatments can divide into three groups, first group contain mint, aloe vera and clove 0.85 ,0.84 and 0.83 of citric acid. Second group contain chitosan, jojoba, neem and wax 0.73 ,0.72 ,0.72 and 0.72 from citric acid. Also, the third group contain yeast and rosemary, it's contained citric acid by 0.63 and 0.57 comparing of the control (Non – coating fruits) contains 0.67**.**

pH of orange juice: Data in Table (4) show a significant increase of the pH values of orange juice during postharvest period (three weeks) regardless of coating materials, but the best treatments were chitosan, wax, yeast, clove, mint and jojob.

Table (3): Effect of essential oils, chitosan, yeast (Saccharomyces cerevisiae) and wax on the acidity

Treatment	Storage period		
	First week	Second week	Third week
Aloe vera	0.84 a	0.616 e	0.64 ef
Clove	0.833 a	0.733 c	0.936 b
Jojoba	0.72 bc	0.47 f	1.02 a
Mint	0.853 a	0.73 c	0.8 d
Neem	0.723 bc	0.616 e	0.63 ef
Rosmary	0.57 e	0.86 b	0.64 ef
Chitosan	0.73 b	0.92 a	0.87 c
Wax	0.72 bc	0.71 c	0.64 ef
Saccharomyces cerevisiae	0.63 d	0.67 d	0.71 e
Control	0.673 c	0.66 d	0.61 f

Table 4 Effect of essential oils, chitosan, yeast, and wax on the pH of orange juice

Treatment	Postharvest period		
	First week	Second week	Third week
Neem	3.81 c	3.62 b	3.64 a
Aloe vera	3.66 e	3.82 a	3.52 b
Mint	3.63 f	3.62 b	3.43 d
Jojoba	3.78 d	3.44 d	3.23 f
Clove	3.62 f	3.63 b	3.32 e
Rosmary	4.06 a	3.45 d	3.47 c
Saccharomyces cerevisiae	3.94 b	3.63 b	3.53 b
Chitosan	3.75 d	3.37 e	3.22 f
Wax	3.52 g	3.51 c	3.42 d
Control	3.75 d	3.58 b	3.32 e

DISCUSSION

Edible coatings are become common methods because of their safe environmental friendly properties and active ingredients as compared to other types of packing and chemical treatments. The essential oils can be used as natural additives in fruits because of their antifungal, antibacterial, ant carcinogenic and antioxidant properties. Therefore, understanding the effective concentrations and combinations of essential oils amended coatings and their antagonistic effects against different postharvest pathogens will have significant importance to enhance the quality and longevity of fruits. In this research, edible coatings used, essential oils (mint, clove, jojoba, *Aloe vera*, neem, rosmary, chitosan, yeast and wax) caused significant effect on reducing weight loss, decay of orange fruits and improved the chemical parameters (TA, TSS, pH and color) comparing with untreated fruits. Also, they increased the shelf life of fruits for four weeks whereas,untreated fruits (control) were from 7-10 days .These results are in agreement with Du Plooy *et al.,* (2009) who reported that carnauba wax formulated with EOs from the plants *Lippia scarberrina* (2500 ul/l) and *Mentha spicata* (2500 ul/l) significantly reduced the incidence of *P.digitatum* on Tomango orange and didn't affect the juiciness, pH and TSS contents whereas reduced the moisture loss and lesion development on Tomango orange. Faten *et al.,* (2010) observed good results with a combination of citral (4 ml or 5 ml /litre) and chitosan (6 gm or 8 gm /litre) for the control of lime fruits sour rot caused by *Geotrichum candidum*. In addition, Chafer (2011) noticed that application of chitosan wax coating enriched with tree oil was found effective against *P. digitatum* and reduced the weight loss and firmness but other quality parameters (TSS, TA juice percentage and color) remain unchanged on Navel Powell orange. The application of essential oils (limon grass, clove, eucalyptus and neem) significantly inhibited the mycelial growth of *P. digitatum* and *P. italicum* and were able to remain the postharvest quality parameters like weight loss, firmness ,TSS ,TA and vitamin c ,also reducing ethylene production and respiration rate Jhalegar *et al.,* (2014, and 2015). Fan *et al*., (2014) reported that the application of citral with wax significantly improved vitamin C content and antioxidants enzyme activities such as catalase, dismutase, superoxidase and peroxidase on *Ponkan mandarin* while the effect was minor on pH ,coloration index and total soluble solids. Also, they noticed that the antifungal activity of *P. digitatum* was significantly inhibited by citral. Essential oils (thymol and carvacol) enriched with wax treated on lemon fruits showed significant improvement in qualitative attributes (weight loss, firmness and color) Castillo *et al*., (2014). Fruit senescence accompanied by rapid respiration and ethylene production was positively correlated with antioxidant enzymes such as catalase, superoxidase and peroxidase Lemoine et al., (2010), Shao *et al*.,(2013) which found to increase by the application of EOs with wax (Fan *et al*., 2014). The eythylene production and respiration rate have a linear positive relationship with postharvest decay Jhalegar *et al.,* (2014 and 2015) as the fungus is responsible to increase the ethylene production and respiration rate Cristescu *et al.,* (2002). Application of EOs reduced the postharvest ethylene production and respiration rate assuring better quality and storability of fruits Valero *et al*., (2006) and Sharafi *et al*., (2011). Additionally, edible coating with essential oils may have a positive effect on TSS and TA content as the EOs form a thin film around the fruit peel surface and modify the microclimate of fruits there by reducing moisture loss, respiration rate and ethylene production Martínez *et al*., (2006) and Jhalegar *et al.*, (2014). Therefore, Essential oils, chitosan, wax, yeast as edible coating of fruits be an innovation and interesting means for commercial application and alternative for using of postharvest chemical treatments and extending of shelf-life fruits.

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طرق بديلة لإطالة فترة حفظ ثمار البرتقال ووقايتها من التدهور بالفطر *Penicillium digitatum* مسبب العفن الاخضر

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تعد خسائر الثمار فى مرحلة مابعد الحصاد واحده من اكثر المشاكل التسويقيه لثمار البرتقال ، بسبب التدهور السريع الذى يحدث اثناء التداول والشحن والتخزين . حيث يتم تغليف الثمار للحفاظ على جودتها وإطالة فترة التخزين . زاد الاهتمام مؤخرا لاستخدام تغليف الثمار بالزيوت الطبيعيه والشيتوزان والشمع والخميره بسبب قدرة هذه المواد على مكافحة النشاط الفطرى وتنظيم معدلات التنفس والتطور فى نضج الثمار . تم استخدام الزيوت الطبيعيه مثل القرنفل والنيم والجوجوبا والروزمارى والنعناع والصبار *Aloa vera* لتغطية ثمار البرتقال ، حيث تم استخدام تركيز 4% / لتر لكل زيت من الزيوت المستخدمه ، ومحلول الشيتوزان عند 1 جم / لتر والخميره عند تركيز 11جم/ لتر بالاضافه لتغليف الثمار بالشمع النقى . وقد بلغت فترة تخزين الثمار 21 يوم فى درجة حرارة الغرفه ، بينما الثمار الغير معامله حدث لها التلف خلال 7–10 ايام .

أظهرت النتائج ان جميع المعاملات حافظت على خصائص الجوده لعصير البرتقال مثل نسبة المواد الصلبه الكليه للعصير والاس الهيدروجيني وكذلك نسبة الحموضه (حمض السيتريك) . كانت افضل هذه المعاملات والتى اطالت فترة التخزين هى معاملات الشمع والخميره والشيتوزان وزيت الجوجوبا والقرنفل والنيم . لذا فان استخدام الزيوت الطبيعيه والشمع والشيتوزان والخميره يعد واحده من الوسائل الهامه للتطبيقات التجاريه وكبديل للمعاملات الكيميائيه التى تستخدم لاطالة فترة تخزين الثمار .