EVALUTATION OF ACTIVE EDIBLE COATING CONTAINING ESSENTIAL OIL EMULSIONS TO EXTEND SHELFLIFE OF ORANGE WASHINGTON NAVEL ORANGE FRUITS

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ABSTRACT

Background: Contamination of the skin of fruits and vegetables is responsible for increases the possibilities of the spoilage. Edible coatings with antimicrobial agents can extend the shelf life of fruits and vegetables.

Methodology: Edible coating agents (pectin and chitosan) individual or mixed with emulsions of lemon grass and thyme oils were prepared at different concentrations, and were evaluated for their antimicrobial activity, and used for coating orange fruits (Washington Navel Orange), which stored at 10°C and 95% relative humidity for 90 days. Changes in weight loss, firmness, Decay rate, respiration rate, pH, Total Soluble Solids (T.S.S.), titratable acidity, chlorophyll and carotenoids, ascorbic Acid (AsA) and microbial load were determined.

Results: Coating solutions containing emulsion enhanced chemical and microbial properties of fruits. Weight loss was decreases compared to uncoated samples (25 *vs* 3%), Control treatment fruits without coating showed higher decay ratereaching 15% after 90 days of storage. Coating the

Vol. (51); Iss. (7); No. (5); May. 2022
ISSN 1110-0826
ONLINE ISSN 2636 - 3178

fruits with pectin or chitosan and their emulsion with lemon grass oil reduced the decay rate to the level of 3% to 6%. Firmness value was improved in the treated samples. Orange fruits coated with the chitosan or chitosan /emulsion recorded the lowest respiration rate (10.3 to 10.44 mgCO_2 /kg. h) at the end of cold storage. Coating the fruit with pectin or chitosan emulsion reduced the losses of ascorbic acid (vitamin C) to about 34%.
Conclusion: chitosan-pectin / emulsion coating increased the shelf life and enhanced different properties of orange fruits.
Key words: orange, emulsion, edible coating, antimicrobial.

INTRODUCTION

Fruits and vegetables are important component of daily diet. They are in high request from most of the population. They have a lot of vitamins, essential minerals, antioxidants, bioflavonoids, dietary fibers and flavor compounds. Due to perishablenature of fruits and vegetables, microorganism and insects easily attack them beside respiration and transpiration processes, which lead to reduce their quality (Tiwari 2014). In addition to this, spoilage of fruits and vegetables is caused due to external and internal factors, mainly O_2, CO_2, ethylene gas, temperature and other stress factors (Freitase*t et al.* 2016). In addition, the skin of fruits and vegetables is capable for defilement of the tissue, which increases the conceivable of the deterioration through some factors such as browning, off-flavor and texture breakdown. This decrease in the quality of fruits and vegetables expose the consumer to risk due to the presence of pathogenic microbes (Akinmusire 2011). Fruits and vegetableswere deteriorated due to postharvest losses. Decay of fruits and

Vol. (51); Iss. (7); No. (5); May. 2022
ISSN 1110-0826
ONLINE ISSN 2636 - 3178

vegetables comes about in a diminish in commercial value and parts of harms are caused to the producer. It has been found that different preservation methods are used to reduce the decay, increase shelf life and retain the nutritional value of fresh fruits (Duan *et al.* 2011). Recently, edible coatings have been broadly studied for preservation of fruits and vegetables. Edible film, coating is characterized as any thin material utilized for wrapping or coating food materials to extend shelf life of the fruits or vegetables, which may be consumed together or removed before consumption. (Bonilla *et al.* 2012). They can increasethe quality of food products by decreasing the physical, chemical and microbiological deteriorations such as moisture loss, enzymatic browning reactions, microbial spoilage and lipid oxidation (Erkmen and Bozoglu 2016). Chitosan is a polysaccharide obtained by deacetylation of chitin, which is extracted from the exoskeleton of crustaceans and fungal cell walls. Chitosan has been broadly utilized in films and coatings due to its ability to inhibit the bacterial and fungal pathogens growth (Muzzarelli *et al.* 2012). In addition, toits common natural antimicrobial property and null toxicity, chitosan contains endless potential that can be used safelyin the food industry (Xing, *et al.* 2011). Pectin could be a complex bunch of polysaccharides in which D-galacturonic acid is a vital constituent. Pectin forms gels and is naturally found in plants and fungi. This property has made pectin a very imperative in jellies, jams, marmalades, and confectionaries, in addition to edible coatings and films (Han, 2003).Essential

Vol. (51); Iss. (7); No. (5); May. 2022
ISSN 1110-0826
ONLINE ISSN 2636 - 3178

oils have phenolic components known to have antimicrobial activity and some are Generally Recognized As Safe (GRAS), substances and thus might be utilized in food to prevent post-harvest growth ofpathogenic fungi and bacteria (Erkmen and Bozoglu2016).Lemon grass (*Citrus limetta*) belong to Rutaceae family, Lemon grass oil is a natural origin, which was safely to human and environment and can be used as an elective substitute for chemical pesticides. Result of chemical contents proved that gerarial, neral, β-myrcene and limonene are fundamental composition of lemon grass oil (Chanthaphon *et al*.2008). Thyme (*Zataria multiflora*) is an aromatic plant of the Lamiaceae family. This leaves and stems have essential oils, tannins, saponins and antiseptic material (Grigore *et al.* 2010). Important and active components of this plant are thymol and carvacrol. (Rota *et al.* 2008). In Egypt orange is considered one of the foremost vital traded crops. In Egypt the major citrus species, representing about 80% of the entire developed citrus zone. Egypt's main orange varieties include Navel and Valencia orange (Wally and Akingbe, 2020).

The aim of the present work was to extend the shelf life of oranges fruits by using essential oil emulsion that loaded on pectin and chitosan as an edible coating and evaluate their effects on the quality and safety characteristics of orange. This would provide useful information contribute to increasing export opportunities for agricultural crops.

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ONLINE ISSN 2636 - 3178

MATERIALS AND METHODS

Raw materials and microbial culture:

Chitosan with molecular weight 200000 Da and pectin (Low methoxyl pectin 106) were purchased from Acrosorganics and Mefad Company,Egypt, respectively. Tween 80 was purchased from Qualikems Fine Chem Pvt., India. Glycerol was obtained from Adwik company, Egypt. Lemongrass and thyme were obtained from the Medicinal and Aromatic Plants Department at the Agricultural Research Center in Egypt. Orange fruits (Washington Navel Orange) were obtained from private farm in Qalubia Governorate. Three pathogenic bacterial strains namely, *Escherichia coli* 0157 HTCC 25922, *Salmonella typhiumurium* ATCC 25566, *Staphylococcus aureus* ATCC 29737 and two fungal strains, *Alternaria alternate* and *Penecillium spp.* were procured from Agricultural Microbiology Department, Fac. of Agriculture, Ain Shams University, Cairo.

Extraction of essential oils:

Extraction of lemon grass oil and thyme oil were carried out by steam distillation method using Clevenger-type apparatus for 3–4 h. Vapors were condensed on a cold surface using condenser attached to it. The condensed essential oil was separated from the continuous phase based on differences in density and immiscibility and then, it was collected and dried over anhydrous sodium sulphate. The extracted oil was stored in brown bottle at low temperature (4 ± 1 °C) until used (AOAC 2007).

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ISSN 1110-0826
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Separation and identification of essential oils components:

Essential oil samples components were separated and identified using Gas Liquid Chromatography (GLC) as reported by Bunzen *et al*. (1969). Gas chromatographic analysis of lemon grass oil the major components of the oil are the two aldehydes gerarial (48.73%) and β- citral (37.58%), while the major components of Thyme oil were the alcohol Thymol (28.57%) and the ϒ-Terpinene (16.49%).

Preparation of Emulsion:

The emulsions were produced by the emulsion inversion point method at room temperature (approximately 25 °C) and according to the method reported by Forgiarini, *et al.* (2001) with some modification. First, the oil phase was prepared by mixing each oil (10%) with Tween 80 surfactant (10%) using mechanical stirrer (500 rpm) for 15 min. The aqueous phase (55% water and 25% glycerol) was then added to the oil phase dropwise while the system was mechanically stirred (500 rpm). After completion of the addition of the aqueous phase to the oil phase, the resulting dispersion was continued to be stirred for 30 min and homogenized by (VIRTIS TEMPEST VIRTISHEAR 302968 HOMOGENIZER) at 6000 rpm for 10 min.

Preparation of coating solution:

- Chitosan coating solutions (2% w/v) were dispersed in an aqueous solution of (1% v/v) glacial acetic acid according to Patricia *et al*. (2004), using 1% glycerol as plasticizer.

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- High ester pectin powder (6% w/v) was dissolved in distilled water at room temperature, mechanically stirring (500 rpm) for 20 min until being fully solubilized using 1% glycerol as plasticizer.

Coating dispersions, (emulsions/pectin and emulsions/ chitosan) were made by mixing each of the pectin solution and chitosan solution separately with the prepared emulsion solution in a ratio of 1:1 and mechanically stirred (500 rpm) for 10 min. Six coating were prepared: Pectin solution(P), pectin with emulsion of lemon grass oil at different concentration (PL),pectin with emulsion of thyme at different concentration (PT), chitosan solution (CH), chitosan with emulsion of lemon grass oil at different concentration (CHL)and chitosan emulsion with thyme at different concentration (CHT) (Abdou *et al*. 2018).

Determination of antimicrobial activity: -

Antimicrobial test was performed for used edible coating; chitosan, pectin individual or mixed with lemongrass or thyme oils at different concentrations (1.25,2.5,5%) against pathogenic bacteria and fungi using well diffusion test (Girian *et al*. 2013) as following : 100 µl of standard inoculum of indicator strain was inoculated into 100 ml of specific medium for culture, *E. coli* on MacConkey agar, *Salmonella* on Bismuth sulfite agar and *Staphylococcus* on Baird Parker potassium Tellurite egg yolk agar media, however, *Alternaria* and *Penicillium* on potato dextrose agar at 37^0 C for 48 hrs.. Subsequently, the medium containing test culture was poured into a petri

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ONLINE ISSN 2636 - 3178

dish and allowed to solidify.100 µl of tested film solution was fed into wells broadly sterile cork borer (8mm diameter). All plates were incubated at 37^0 C/48 hrs for tested pathogenic bacteria and 5 days for tested fungi. The diameter of inhibition zones was measured. All the experiments were performed in triplicate. (Jianu *et al.* 2012).

Preparation of oranges fruit:

Orange fruit was immediately transported to the laboratory after harvesting, washed under tap water and washed by sodium hypochlorite solution (100 mg/kg) for 10 min to reduce the microbial load and then surface dried at room temperature (25 ± 1 ◦C). The orange fruits that were free of any signs of mechanical damage or fungal decay were selected and standardized size, shape and color. The orange fruits were randomly divided into five groups then coated by dipping in the coating solution (P, PL, CH and CHLsolution) for 2 min; the control group was left uncoated. After drying, all fruits were stored in cartons individually at 10°C and 95% relative humidity for 90 days.

METHOD OF ANALYSIS

A-Microbiological analysis:

Ten grams of fresh fruit peels were mixed with 90 ml ringer solution into a sterile conical flask and homogenized for 5-10 min. The sample was diluted seven times (10^{-1}-10^{-7}). The plate count technique was used to calculate the total viable count for total bacteria, total mold and yeast in orange samples

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(Giménez *et al.* 2003), through storage period (zero time, 18, 36 ,54, 72 and 90 days). *Salmonella*, *Coliforms* and *Staphylococci*, were enumerated on bismuth sulphite, MacConkey and Baird Parker potassium Tellurite egg yolk agar media at 37^0 C for 48 hrs, respectively at the end of storage period (90 days) using plate count technique. All samples were tested in triplicate and colonies were counted and expressed as CFU/g.

B-Physicochemical Parameters of Orange fruits:

1-Weight Loss:

Weight loss was determined by single fruit weighting, using following Equation:

Weight loss (%) = $(Wi - W_0)/W_0 \times 100$ ---------- (1)

Where W_0 and W_i are the initial and final weight (g), respectively, of the same fruits.

2-Total Soluble Solid (TSS):

The TSS of juice extracted from the coated and control fruits was measured using a RA-250WE Brix-meter (Atago, Tokyo, Japan) and the results were expressed as a percentage**.**

3-Ascorbic Acid (AsA):

AsA of extracted juice was determined by titration with 2,6-dichlorophenol indophenol and expressed as mg per 100 ml juice (mg/100 ml) (Ranganna 2008).

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4-Decay Rate: -

Decay rate was visually evaluated using the same 120 fruits per treatment and 3 replicate, and expressed as the percentage of rotten fruits. For this test, 360 fruits from each coating treatment were used and evaluated on days 0, 18, 36, 54, 72 and 90 (Chen *et al*. 2019).

5-Respiration Rate: -

The respiration rate was measured by using oxygen analyzer (Witt oxybabyhead space gas analyzer)

expressed as mg CO_2 kg^{-1} h^{-1} fresh weight (FW) and calculated by using the following equation:

Respiration rate = $\Delta CO_2/100$ X V headspace X 1000/m X t /60----- (2)

Where m is the mass of oranges (g), V isheadspace (the empty volume of the jar mL), and ΔCO2 is the difference between the initial and final concentration of CO_2, and t is the recorded time (min.) (Chen *et al*. 2016).

6-Firmness:

Fruit firmness was determined using Brookfield CT3 Texture analyzer (6 mm diameter probe) on the opposite surfaces of each fruit, data was given in Newton.

7-pH:

pH was measured using digital pH meter (UB-10, DENVER INSTRUMENT Company, America) according to (AOAC 2007).

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8-Titratable Acidity (TA):

Titratable acidity was determined by titration of orange juice with 0.1 N NaOH solution and expressed as percentage of citric acid (AOAC 2007).

9-Chlorophyll and carotenoids:

Chlorophyll A and B and carotenoids were determined in fruit pulp by spectrophotometer, (JENWAY, UK). Absorptions were recorded at wavelengths of 662, 644 and 440 nm and then calculated by using formula of Nagata and Yamashita (1992).

10- Sweetness ratio: -

Sweetness ratio was calculated by dividing the TSS value through the total acid content (El-Mahdy *et al.* 2017).

Statistical Analysis:-

The research relied on the description and quantitative analytical method, where some statistical methods were used, such as the arithmetic mean and conducting an analysis of variance test for different levels of morality to find out the moral difference between the averages by using SPSS v.20.

RESULTS AND DISCUSSION

1-Antimicrobial activity of chitosan and pectin emulsions:

Fig (1) shows the inhibitory effect of different coating agent on *E. coli* ,*Staphylococcus aureus, Salmonella typhimurium*, *Alternaria alternata* and *Penicillium* spp. presented by the diameter of inhibition zone. Generally, it was observed that chitosan has high antagonistic effect in comparing to pectin against

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tested cultures. Thyme oil in the coating agent led to elevate its antimicrobial activity. The results indicated that CHL or CHT at 5% recorded the highest values of inhibition zone followed by pectin mixtures by the same essential oil emulsion at 5%. The corresponding values were 2.97, 3.2 mm against *E.coli* and 2.06, 2.7mm against *Staphylococcus aureus* with CHL (5%) and CHT (5%) respectively. *Salmonella typhimurium* was affected by CHL and CHT at 5% and PT at 2.5% and 5% giving the highest values being 1.87, 1.3, 1.3 and 1.47 mm in respective order. The fungal cultures were highly affected by CHL (5%) and PL at 2.5 and 5% recorded the maximum values of inhibition zone being 1.97, 1.8, 1.97 mm against *Penicillium* spp. and 2.7, 2.0, 2.5mm against *Alternaria alternate*, respectively. The effect of essential oil containing coating increase with concentration increases. Individual chitosan or pectin treatment gave the lowest effect against all tested cultures comparing to the other treatments. The results show that the most efficient coating treatments were CHL at 5% and PL at 5%, which gave a broad effect on tested cultures. The potent effect of the essential oil incorporated in the coating formulation depended on its concentration. Chitosan is a natural biopolymer with antimicrobial activity that has the property to form edible films and coating (Mo *et al.* 2007). It has exhibited high inhibition activity against a wide range of spoilage microorganisms (mold, yeasts, bacteria) .Essentials oil have hydrophobic properties and are able to penetrate to mitochondria and cell content leakage be caused by dissolving lipids of bacteria cell wall (Lopoz-Romero *et al.* 2015).The obtained result agree with those Raphael and Meimandi (2017) who reported that

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chitosan combined with thyme or oregano essential oil exhibited inhibition effect against *E.coli* and *Staphylococcus aureus.*The strong antibacterial effects was recorded by a combination of chitosan enriched with thyme essential oil while the inhibition of chitosan was very low. The same results were shown against *Aspergillus niger* and *Alternariaalternata* (Rodriguez-Nunez *et al*. 2012), who demonstrated a larger bactericidal effect of chitosan coating emulsion against G^{-ve} bacteria (*Salmonella typhimurim*) than G^{+ve} bacteria (*Staphylococcus aureus)*. These results are consistent with data given in the present study. Tajidin *et al.* (2012) reported that the chemical compound predominantly present in thyme oil that has antifungal activity is thymol, eugenol, menthol and Linalool (Dambolena *et al*. 2012). The antimicrobial effects of thyme essential oil analyzed is related to the presence of phenolic compounds (thymol and terpene hydrocarbons) (Rota *et al.* 2008). Shanjun *et al.* (2020) reported that the biologically active component of lemongrass essential oil is citral and other compounds. The antifungal activity of citral is linked to its lipophilic behavior and its ability to disrupt the fungal cell membrane (Tao *et al.* 2014), in addition, Debonne *et al.* (2021) reported that the antimicrobial activity of lemongrass essential oil is stronger than thyme essential oil, which agree with the result in this study. Based on the aforementioned results the following experiments for coating orange fruits were carried out using only emulsion of lemon grass oil as antimicrobial agent due to its high anti- microbial effect.

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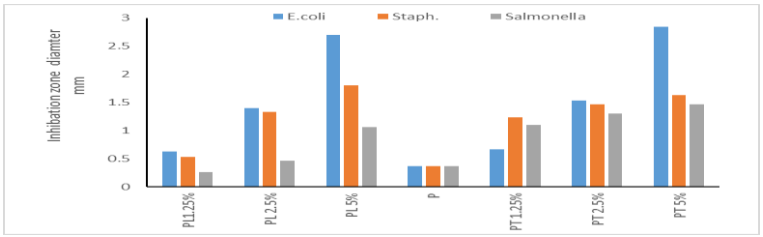

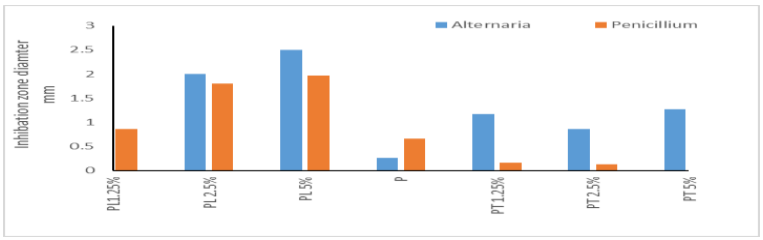

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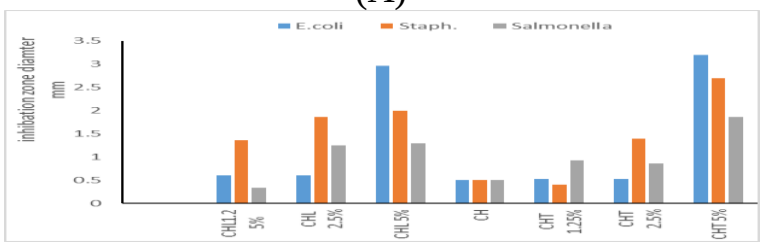

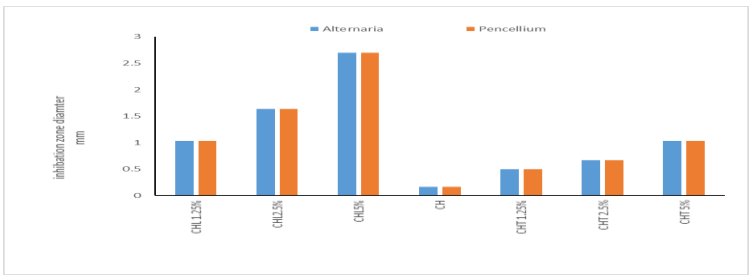

(B)

Fig. (1) Antimicrobial activity of pectin (A) and chitosan (B) coating solution combined with emulsions of lemon grass oil and thyme oil on the growth of some microorganism.

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2-Evaluation the effect of coating agent on microbial load:

Fig.(2-A) shows the count of total bacteria loaded on the orange surface presented as log CFU/g, to evaluate the microbiological quality of the orange during storage period (90 days). Before the application of the formulation, bacterial count on orange was 3.9log CFU/g. The orange samples showed gradually decrease of bacterial count during storage period with all coating treatments. The control treatment, (orange sample without any coating) recorded the highest count (9.4 log CFU/g) after 90 days of storage period followed by pectin treatment whereas the orange sample treated by pectin with lemon grass oil or chitosan with or without lemon grass oil recorded the lowest viable count. Fig (2-B) shows the total count of mold and yeast (log CFU/g) on orange samples during 90 days of storage period. Immediately before the application of the formulation, the count of yeast and mold was 1.7 log CFU/g. The bacterial count increased gradually during storage period reached to 7.44 log CFU/g when applied without coating – agents but in the case of chitosan (CH) and CHL at 5%, the count of yeast and mold was ranged between 6.24 to 6.52 log CFU/g during 90 days. Fig (5-C) presented the count of *E.coli* ,*Staphylococcus aureus* and *Salmonella typhimurium*on orange sample at the end of storage period. It was observed that *Salmonella typhimurium* was not detect with chitosan (CH) and CHL (5%). The lowest values of *Salmonella typhimurium* was achieved by PL treatment giving 1.3 log CFU/g. The most effect treatment was CHL followed by PL comparing to

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control treatment. At the end of storage, the survival of *Staphylococcus aureus* showed a significant decrease when applied with coating- forming agents. The highest values of *E.coli* was recorded on control orange samples being 4.9 log CFU/g whereas no detect on orange samples coating with CHL. Chitosan only decreased the count of *E.coli*. to 1.69 log CFU/g. Pectin(P) or PL decreased the count of *E. coli*. The highest effect was achieved by CHL which showed an absence of total *E. coli* and *Salmonella typhimurium* on the treated samples and lowest count of, *Staphylococcus aureus* at the end of storage. The foremost critical postharvest diseases of fresh orange fruits are caused by filamentous fungi- chitosan and pectin edible coating diminish microbial load. Panebianco *et al* (2014) reported the antifungal effects of chitosan were related to fungal toxic activity against the pathogens. Chitosan at 2% was applied to oranges inoculated by *Penicillium digitatun* or *P.italicum* and it was noticed that the disease incidence and severity were significantly lower on coating samples than on uncoated (Zeng *et al.* 2010).

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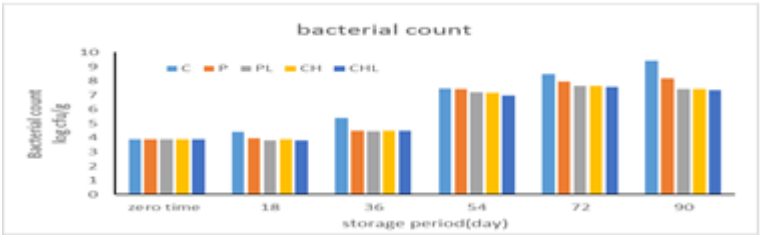

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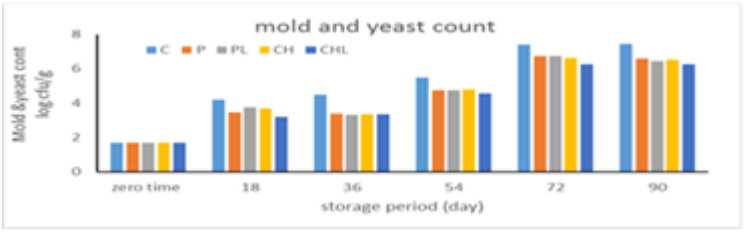

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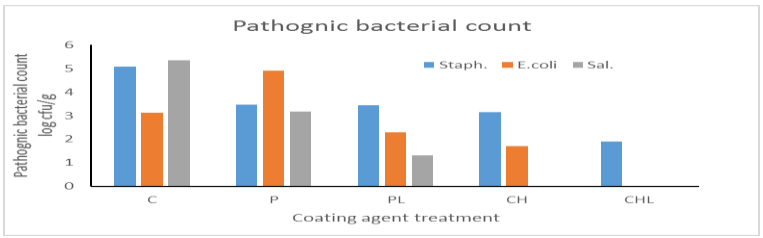

(C)

Fig. (2) Effect of chitosan and pectin coating solution and combined with emulsions of Lemon grass oil 5% on enumerated mesophilic bacteria (A), yeasts and mold (B) during storage period and pathogenic bacteria at the end of storage period (C) of fresh orange fruits at 10^0C.

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3 – Changes in physical parameter of orange fruits during storage:

3-1-Weight loss: -

Fig. (3-a) shows the weight loss of orange fruits treated with different coatings during 90 days of cold storage. As seen, the untreated control samples recorded 25.6% weight loss at the end of storage period (90 days). However, the control fruit sample were totally infected and covered with mold cells after 18 and 36 days of storage as seen in Figure (3-a). Coating the fruits with pectin or chitosan as well as their emulsion with lemon grass oil drastically reduced the weight loss to only 3 and 4.98% at the end of cold storage period.

3-2- Decay rate:-

Decay rate could be expressed as percentage of the infected fruit during storage.Figure (3-b) show the percentage of the infected and discarded orange fruits during storage. Control treated fruits show higher decay rate reaching 15% after 90 days of storage. On other side, coating the fruits with pectin or chitosan and their emulsion with lemon grass oil reduced the decay rate to the level of 3% to 6%. The results show that chitosan emulsion was an efficient treatment in reducing the fungal infection and rotting of the orange fruits.

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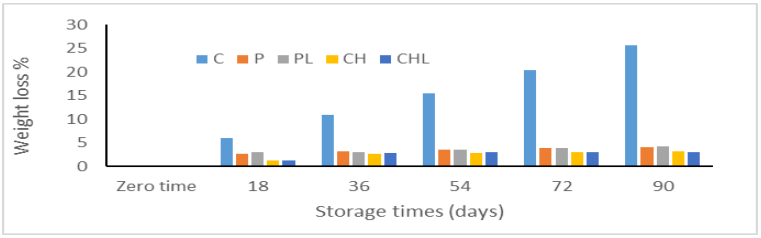
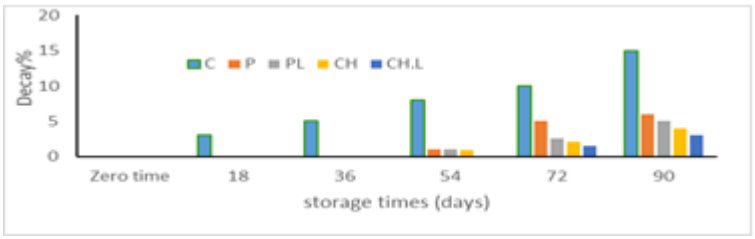


(a)



(b)

Fig. (3): Effect of different coating materials on weight loss % (a) and decay %(b) of Navel oranges fruits during storage at 10^0 C: -

3-3-Firmness:

Firmness is a major characteristic for the quality of orange fruits during storage period. As seen in Table (1), the average firmness value of the fresh orange fruits was 61.22 Newton (N). Untreated control samples were rapidly decayed and their peels were softened during storage. The firmness value was decreased by 40% after 18 days of storage and this decrease in firmness was continued until reaching only 10.95 N at the end of storage period (90 days). All coating treatments did reduce the firmness deterioration, since the loss in firmness was only 8.5% after 18 days of storage. The maintenance of the firmness was sustained during the whole period of all storage. The final loss

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ONLINE ISSN 2636 - 3178

in firmness of pectin or chitosan coated samples was 31% while the loss in control sample reached 82% at the end of cold storage. Final firmness losses in orange samples coated with pectin and chitosan emulsions was 35.3 and 19.8%, respectively the firmness loss could be referred to activity of pectolytic enzymes such as polyglocturonase and pectin methyl esterase, which decompose and degrade pectin substances in the peel. The results prove that coating orange skin with chitosan or pectin, in the present work, is essential for keeping quality and firmness of orange fruits during storage. The obtained result agree with this of Kheder (2017). They reported firmness value of 72 N for fresh and coated Valencia orange fruit at begin of cold storage. The loss in firmness was about 10-15% at the end of storage period (90 days at 5^0C). This clear that reducing the storage temperature from 10^0 C (in the present work) to 5^0 C will sustain the firmness of the orange fruits due to the drastic reduction in the enzyme activity. In addition, change the puncture probe diameter from 8 to 5 mm will reduce the necessary force by 50-60 % due to reduced penetration area on the surface of orange skin.

3-4- pH value:

pH value of the fresh fruits was 3.76. During storage (Table1), the pH-values were principally, increased and recorded higher values. Control and uncoated fruits recorded the highest pH-value (4.48 and4.49) after 90 days of cold storage. On other side, orange fruits coated with pectin, pectin emulsion, chitosan and chitosan emulsion recorded the lowest change in pH-values,

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since the recorded values were in the range of 4.2 to 4.29 at the end of cold storage period (90 days). The increased pH-value could be referred to the loss in acidity as the result of involvement of organic acid in the respiration process of the fruits. These results agree with Radi *et al.* (2017).

3-5-Respiration rate:

Respiration could be measured either through measurement of the evolved heat or the produced CO_2. Table (1) gives the respiration rate ($mgCO_2$ / kg. h) for Navel orange fruits coated with different emulsions and stored for 90 days at 10^0 C. As seen, the respiration rate at zero time recorded 19.7 to 19.78 $mgCO_2$ / kg.h. This means that coating did slightly reduce the respiration intensity of the tested fruits. However, during cold storage the respiration rate was reduced due to the consumption of the soluble sugar and/or reducing the rate of respiration itself. However, the respiration rate of control fruits was still high (15.52 mg CO_2 /kg. h) even after 90 days of storage. On other side, all coated fruits samples recorded lower respiration rate, which also further decreased with the progress of storage period. Orange fruits coated with the chitosan or chitosan emulsion recorded the lowest respiration rate (10.3 to 10.44 $mgCO_2$ /kg. h) at the end of storage. It could be concluded that the sample coated with chitosan or chitosan emulsion led to reduction in gas exchange, which results in depressing the rate of respiration. These results agree with Chen *et al*. (2019).

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Table (1): Changes in physical parameter of orange fruits during storage at 10^{0}C

Days		0	18	36	54	72	90
	Firmness (N)						
C		61.22^{a}	37.29^{k}	29.78^{m}	23.76^{n}	18.57^{o}	10.95^{p}
CH		61.22^{a}	56.54^{bc}	51.01^{e}	49.91^{ef}	45.25^{h}	42.99^{i}
CHL		61.22^{a}	57.54^{b}	56.01^{bcd}	55.91^{cd}	54.51^{d}	49.1^{f}
P		61.22^{a}	56.04^{bcd}	55.71^{cd}	45.91^{gh}	34.95^{l}	34.59^{l}
PL		61.22^{a}	56.04^{bcd}	55.91^{cd}	46.91^{g}	44.95^{h}	39.59^{j}
	P≤0.05			LSD =1.616			
	pH value						
C		3.76k	3.92hij	3.97hi	4.33bcd	4.4abc	4.49a
P		3.76k	3.85ijk	3.89hijk	4.12efg	4.27cd	4.42ab
PL		3.76k	3.8jk	3.85ikg	4.11fg	4.28cd	4.29bcd
CH		3.76k	3.8ijk	3.84ijk	4.01gh	4.25de	4.29bcd
CHL		3.76k	3.8Jk	3.82kg	3.99gh	4.11fg	4.2def
	P≤0.05			LSD=0.1331			
	Respiration rate mg $CO_2\,Kg^{-1}\,h^{-1}$						
C		19.70^{a}	17.58^{c}	15^{c}	13.54^{b}	15.82^{b}	15.52^{c}
CH		16.36^{f}	14.11^{f}	10.56^{f}	9.82^{e}	10.09^{e}	10.3^{lmn}
CHL		16.6^{lmn}	14.32^{jk}	10.64^{j}	9.93^{g}	9.673^{q}	10.44^{op}
P		17.16^{kl}	14.52^{ml}	11.29^{d}	10.91^{op}	12.28^{q}	12.86^{q}
PL		17.14^{h}	15.07^{d}	11.57^{nop}	10.81^{mno}	12.73^{h}	12.28^{i}
	P≤0.05			LSD=0.4481			

C: control, P: pectin solution, PL: pectin and emulsions, CH: chitosan solution, CHL: chitosan and emulsion. Each treatment triplicated in two ways ANOVA.

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4- Changes in chemical parameters of Navel orange fruits during cold storage:

The chemical parameter include: changes in Total Soluble Solids (TSS), change in Titratable Acidity (TA), change in ascorbic acid content, respiration rate and change in fruit pigments (chlorophyll and carotenoids):

A- Change in Total Soluble Solids (TSS):

The TSS value of the fresh orange fruits was 14.2^0 brix Table (2), which is slightly higher but still in the range given by Eleryan (2015), Kheder (2017) and Kassim *et al*. (2020) for Navel oranges (10 to 14%) depending on the climate condition in the growing country. During cold storage, the TSS value of the control (uncoated fruits) was increased to 18.10. Brix at the end of the storage period (90 days). TSS values were also increased in the coated fruits but with lower intensity than that of control sample. The reason for the increased TSS value could be referred to the loss in weight (moisture content) of the fruits during storage, which results in concentration of soluble content in less amount of water. Coated sample, especially those coated with chitosan or chitosan emulsion showed the lowest change in TSS value during storage period, which comply with the result of weight loss given in the present work. Actually, the mass of total soluble solid should decrease during the cold storage as the result of sugar consumption for the respiration processes. However, the loss of fruit weight compensates the actual loss in sugar masscaused by the action of hydrolytic enzymes. On other side, Plasido *et al.*

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(2016) mentioned that sugar build up occurs in both juice and flavedo of the fruit.

B- Change in Titratable Acidity (TA):

As seen in Table (2), the acidity content of the fresh orange fruit was 0.76% which agree with the value given by Eleryan (2015) and kaseem *et al*. (2020) (0.55 to 0.84%). On contrary to the behavior in the total soluble solid, the total acidity decreased with the progress of the storage period. In the control fruits, the total of TA content was reduced by more than 50% at the end of storage period, while coated fruit sample showed lower losses during the storage period, especially those coated with chitosan emulsion, which showed the loss level of only 10.5% at the end of storage period.

From biochemical view, organic acids could also be involved in the respiration processes, which lead to reduction in organic acid content of the fruit. These results agree with those of Kassem *et al.* (2020). They reported losses up to 70% of TA during storage of Navel orange.

C- **Change in sweetness degree (TSS/ acid ratio) during storage of orange fruits: -**

The ratio between Total Soluble Solids (TSS) and acids represent the sweetness degree of the fruits and, consequently, their palatability by the consumer. As seen in Table (2), the TSS/ acid ratio of the control fruits was 51.7, while those coated with pectin, chitosan or their emulsions recorded lower TSS/acid ratio in the range of 21.6 to 27.6. These results agree with this

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of Eleryan (2015), who reported TSS/acid ratio at 31.23 for coated and cold stored orange fruits. The high sweetness degree of the control orange samples could be referred to the high loss in weight (moisture content), the higher concentration ratio of the remaining soluble solids and the intensive use of acids in respiration process, which reduces the final acid content. On other hand, coating did reduce the moisture losses, keeping acidity from sharing respiration process, which results in moderate TSS/acid ratios at the end of storage period.

D- **Change in ascorbic acid content in the Navel orange during storage: -**

As seen in Table (2), ascorbic acid content in the fresh orange fruit was 35.8 mg/100g pulp, which agree with the value reported by Eleryan (2015), (35.12mg/100g), but lower than those reported by kheder *et al.* (2017) (55.65mg/100g). Control uncoated orange fruits loosed about 80% of their vitamin C content after 90 days of cold storage. Coating the fruit with pectin or chitosan emulsion reduced the losses to about 34%; especially those fruit coated with chitosan emulsion. It should be mentioned that vitamin C content is one of most important factors in the nutrition evaluation of orange juice.

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Table (2): Changes in chemical parameters of Navel orange fruits during cold storage:

Days	**0**	**18**	**36**	**54**	**72**	**90**
Total Soluble Solids TSS Brix						
C	14.2^{m}	15.2^{egh}	15.9^{de}	16.5^{c}	17.2^{b}	18.1^{a}
P	14.2^{m}	14.8^{hIuk}	15^{hij}	15.5^{ef}	15.9^{de}	16.3^{cd}
PL	14.2^{m}	14.6^{ijklm}	14.8^{hijk}	15^{hig}	15.2^{egh}	15.5^{ef}
CH	14.2^{m}	14.5^{jklm}	14.7^{ijkl}	14.9^{hij}	15.2^{egh}	15.4^{fg}
CHL	14.2^{M}	14.3^{LM}	14.4^{KLM}	14.5^{jklm}	14.6^{ijkm}	14.7^{ijkl}
P≤0.05				LSD=0.4999		
Titratable Acidity (TA %)						
C	0.76^{a}	0.57^{n}	0.5^{o}	0.45^{p}	0.4^{q}	0.35^{r}
P	0.76^{a}	0.68^{efg}	0.66^{hig}	0.62^{kl}	0.6^{lm}	0.59^{mn}
PL	0.76^{a}	0.71^{bcd}	0.69^{edf}	0.65^{hij}	0.63^{jk}	0.62^{kl}
CH	0.76^{a}	0.71^{bcd}	0.71^{bcd}	0.67^{fhg}	0.65^{hij}	0.64^{ikj}
CHL	0.76^{a}	0.73^{b}	0.72^{bc}	0.7^{cdl}	0.69^{def}	0.68^{efg}
P≤0.05				LSD=0.0216		
Sweetness ratio(TSS/TA)						
C	18.7^{q}	26.7^{f}	31.2^{d}	36.7^{c}	43^{b}	51.7^{a}
P	18.7^{q}	21.8^{kl}	22.7^{il}	25^{g}	26.5^{f}	27.6^{e}
PL	18.7^{q}	20.6^{no}	21.5^{klm}	23.1^{i}	24.1^{h}	25^{g}
CH	18.7^{q}	20.5^{no}	20.7^{mno}	22.2^{jk}	23.5^{hi}	24.1^{h}
CHL	18.7^{q}	19.6^{p}	20^{op}	20.7^{mno}	21.2^{lmn}	21.6^{kl}
P≤0.05				LSD=0.8436		
Ascorbic acid (mg ascorbic /100g pulp)						
C	35.8^{a}	25.9^{fg}	20.3^{i}	17.2^{k}	10.67^{n}	7.05^{o}
P	35.8^{a}	30.12^{bc}	27.9^{e}	25.3^{g}	15.45^{l}	14.07^{m}
PL	35.8^{a}	30.12^{bc}	28.98^{d}	26.3^{f}	16.15^{l}	16.1^{l}
CH	35.8^{a}	30.59^{b}	29.48^{de}	28.9^{d}	25.95^{fg}	18.9^{j}
CHL	35.8^{a}	30.7^{b}	30.15^{bc}	29.01^{d}	27.65^{e}	23.5^{h}
P≤0.05				LSD=0.8804		

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C: control, P: pectin solution, PL: pectin and emulsions, CH: chitosan solution, CHL: chitosan and emulsion, TSS: Total Soluble Solid, TA: Titratable Acidity. Each treatment triplicated in two ways ANOVA

a) Change in chlorophyll and carotenoid pigment contents during cold storage of orange Fruits: -

Pigments of peel and pulp of orange fruits undergo changes in type and concentration of the individual pigment due to physiological and biochemical activities during storage. Fig. (6) shows the change in the content of chlorophyll and carotenoid in Navel orange fruit pulp as affected by the type of coating and the period of cold storage. As seen, the pigment concentrations in the pulp of fresh fruits were 0.087, 0.189 and 0.69 mg/L, respectively for chlorophyll a, chlorophyll b and total carotenoids. The obtained results show that the level of chlorophyll a and b decreased, with some fluctuation during the cold storage period accompanied with gradual increase in content of total carotenoids. The level of chlorophyll loss and carotenoids development depends on the coating type of the fruit and the storage conditions (Temperature and time). In principle, chlorophyll a and b disappear completely at the end of cold storage (90 days). On other side, the concentration of carotenoid was increased by 1.56 to 2.83 folds at the end of cold storage. According to Placido *et al*. (2016) and Vilfvert *et al.* (2022), the accumulation and biosynthesis of carotenoids has been observed during fruit storage and cold storage turn this change slowly than those stored under

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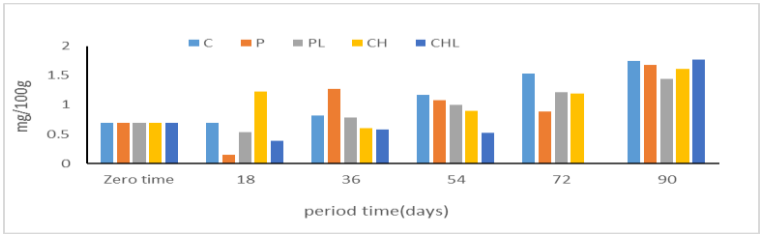
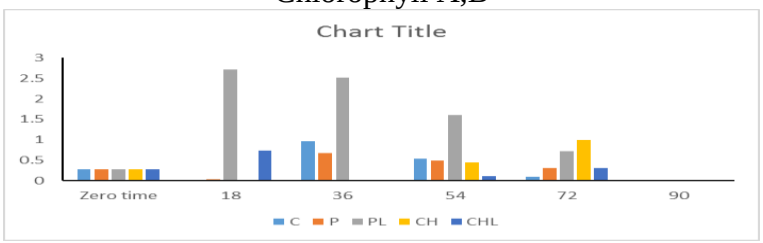
atmospheric condition. These also reported that the chlorophyll content decreased throughout the storage as the result of their degradation, which could be attributed to at the activity of the enzyme chlorophyllase during storage.



Chlorophyll A,B



Carotenoid

Fig. (6): change in Chlorophyll A, B and carotenoids (mg/L) pigments during cold storage of coated Navel oranges: -

CONCLUSION

The results showed that using essential oils in edible coating for orange (Washiington Navel orange) lower the loss in the values of physical characteristics (weight loss, decay and respiration rate), and extending the shelf life due to their inhibitory effect on microorganisms. In addition,

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significantly higher values of ascorbic acid and carotenoids were observed during the storage period. Coatings also ensured better maintenance in relation to soluble solids, ascorbic acid and total titratable acidity showing the positive effect of coatings.

The best treatment was chitosan loaded/lemongrass oil emulsion (CHL), which had the best results. Accordingly, it is recommended to treat fresh orange fruits with edible coating solution of chitosan loaded/lemongrass oil emulsion due to its antimicrobial properties. This result will provide useful information for export companies to mitigate the fruits quality during shipment and when marketing the product.

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تقييم الاغطية النشطة القابلة للاكل التي تحتوي علي مستحلبات زيوت عطرية لاطالة صلاحية ثمار البرتقال

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المستخلص

المقدمة: تتلوث ثمار الخضراوات والفاكهة عن طريق السطح الخارجي لها.

الهدف: هدفت هذه الدراسة الي اعداد واستخدام اغطية قابلة للاكل مع مواد مضادة للميكروبات تعمل علي زيادة فترة صلاحية الفاكهة.

المنهجية: إعداد أغشية من الكيتوزان والبكتين منفردين وأخري مع الزيوت العطرية (زيت حشيشة الليمون – زيت الزعتر) بنسب مختلفة لتقيم تأثيرها علي النشاط الميكروبي وتغطية ثمار البرتقال لزيادة فترة صلاحيتها . تم حفظ البرتقال لمدة ٩٠ يوم عند ١٠ °م ورطوبة نسبية ٩٥ % . وأثناء فترة التخزين تم اختبار :الجودة الميكروبية والتغير في الوزن , الصلابة , نسبة الثمار التالفة , درجة

Vol. (51); Iss. (7); No. (5); May. 2022
ISSN 1110-0826
ONLINE ISSN 2636 - 3178

الحموضة , نسبة المواد الصلبة الذائبة , الحموضة الكلية , الكلورفيل , الكاروتينات وحمض الاسكوربيك.

النتائج : محلول الكيتوزان مع مستحلب حشيشة الليمون حسن من الخواص الميكروبية والكيميائية للبرتقال وكانت نسبة الفقد في الوزن , نسبة الثمار التالفة , الصلابة ومعدل التنفس في الثمار أعلي من مثيلتها التي لم يتم معاملتها أو التي تم معاملتها بالبكتين , البكتين وزيت حشيشة الليمون والكيتوزان اما معاملة الكيتوزان مع زيت حشيشة الليمون فقد سجلت اقل تغيرات لهذه المعاملات .

الملخص: الكيتوزان والبكتين مع زيت حشيشة الليمون تزيد من صلاحية البرتقال .

التوصيات: يوصي باستخدام الكيتوزان مع زيت حشيشة الليمون بتركيز ٥% للتغطية علي ثمار الفاكهة عند حفظها علي درجة حرارة ١٠° م ورطوبة نسبية ٩٥ % لاطالة مدة صلاحية البرتقال اثناء تسويقه.

الكلمات المفتاحية: البرتقال، الأغطية النشطة القابلة للاكل ،مضادات الميكروبات، فترات الصلاحية.