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USE OF CHITOSAN AND ESSENTIAL OILS TO CONTROL BIOTIC AND NONBIOTIC AGENTS OF BELL PEPPER FRUITS

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ABSTRACT: Bell pepper (*Capsicum annuum* L.) fruits are appreciated in the food industry for its high content of bioactive molecules. However, vegetable fruits are a highly perishable food and might be attacked by phytopathogenic origin, which decreases its quality. The application of edible coatings by chitosan and essential oils [(*Rosmarinus officinalis* L. and *Cymbopogon citratus* L.] have been considered an innovative strategy to reduce postharvest losses and tended shelf life of pepper. This study carried out through 2018 and 2019 summer seasons to describe the effect of different concentrations of chitosan, citronella essential oil and rosemary essential oil on the bell pepper at refrigeration conditions (8 ± 1 °C), physicochemical characterizes and micro biotical properties were evaluated after 7, 14, 21, 28 and 35 days of storage and shilf life periods. Generally, no decay was noticed and good general appearance of pepper fruits treated with 1.0% cetronella essential oil after 28 days of cold storage in both seasons. The results showed that the treated fruits have higher acceptance and quality than the control, being more effective with cetronella essential oil compared to chitosan and rosemary essential oil. It is noticeable that chitosan and cetronella essential oil resulted in an increase in the shelf-life of bell pepper up to 28 days. Furthermore, all dipping treatments under current study increased fruit firmness, total chlorophyll content, Vit. C and TSS characteristics than the control. In addition, treated bell pepper fruits by chitosan or essential oils delayed the loss of physicochemical and fungal growth, due to protection against the microbial development of aerobic mesophylls, molds, and yeasts.

Key words: Chitosan; Essential Oils; *Rosmarinus officinalis*; *Cymbopogon citratus*; Shelf-Life; Bell Peppers

INTRODUCTION

Bell pepper (*Capsicum annuum* L.) is the most commonly used fruits of family *Solanaceae* with excellent a high content of ascorbic acid and other vitamins such as vitamin A and E, nutritive value and whole range of vitamin B complex. Its consuming and request have increased due to the increase of population **(Martinez *et al.*, 2005)**. Bell pepper fruit, due to its short shelf life, is susceptible to flaccidity, wilting, shriveling, fungal diseases, and decay **(Lownds *et al.*, 1994)**. Postharvest quality of sweet pepper is affected by several factors like moisture loss and chilling injury that break down the quality of pepper fruits during postharvest processes **(Maalekuu *et al.*, 2002)**. Peppers are produce very low ethylene levels at 0.1 to 0.2 μlkg^{-1} h^{-1} at 10 and 20°C (50 and 68°F), respectively. Fresh peppers can be kept for 15 -21 days at 7°C (45 °F) with 90 to 95 % RH. Storage-life can be extended another week by packaging in moisture-retentive films at 7 to 10°C **(Groos *et al.*, 2016).** Previously, the role of different chitosan and essential oil of aromatic plants dipping treatments has been investigated on many fruit crops. **Hussein *et al.* (2015)** reported that chitosan treatment preserved fruit texture and reduced decay happening during all the storage periods. Thus, chitosan at 0.5 or 1% are promising treatments for decreasing chilling injury and maintaining quality of sweet peppers at 5 °C and 90-95 RH%.

Essential oils treatment have been utilized as antimicrobial as postharvest treatment effective in prolonging shelf life of vegetable fruits without affecting their sensual attributes **(Ding and Lee, 2019). Also, Abbasi *et al.* (2017)** found that using 12% arabic gum coating with cinnamon essential oil significantly decreased weight loss and decay incidence with less increase in total soluble solids and sugar percentage of pepper cv. Yolo Wonder. The

genus *Cymbopogon* of the *Poaceae*family has been investigated for its pharmacological potential. The essential oil of the leaves of *C. citratus* is commonly used in perfumes, the production of cosmetics, and as an insect repellent. The major chemical constituents are geraniol, citral, citronellal, and citronellol **(Chanthai *et al.*, 2012).** Studies have demonstrated the antiviral **(Aini *et al.*, 2006)**, antibacterial **(Innsan *et al.*, 2011)**, and antifungal activities **(Chanthai *et al.*, 2012)** of this oil. Rosemary (*Rosmarinusofficinalis* L.) is a species member of the Labiatae family, is a woody herb **(Zargari, 1990)** cultivated mainly for essential oil production. Rosemary is well cultivated in Egypt and available throughout the year. **Bakhtiarizade and Souri (2019)** indicated that the least total acids of fruits were recorded in 500 ppm rosemary oil treatment. The significant maximum vitamin C content was recorded in fruits in control compared with both concentration (500 and 1000 ppm) of rosemary oil.

Sweet pepper fruits are generally very susceptible to decay during postharvest; however, application of chemicals and fungicides could face consumer with health risk. Aromatic plant essential oils can significantly decrease postharvest decay of pepper fruits. The effects of citronella and rosemary oils have not been yet studied on sweet pepper fruit decay. So, in the present study essential oil from these two different aromatic plants as well as chitosan were used and evaluated for their possible preventing effects on enhancing fruit quality and decreasing decay of sweet pepper fruits cv. Top Star.

MATERIALS AND METHODS

Fruit samples

The experiments were conducted at Postharvest Laboratory and Agricultural Microbiology Laboratory, Horticulture Department and Agricultural Microbiology Department, Faculty of Agriculture, Zagazig University, Egypt, during 2018 and 2019 summer seasons. Sweet pepper fruits (Top Star F1) were obtained from private Farm, Al-Khattara region, Sharkia Governorate, Egypt. The fruits were harvested with a short calyx (1 cm long) and uniformed size at green ripe stage in the second week of March in both seasons. The source of Top Star F1 (type Japanese) was Gaara Company- Import and Export, Cairo, Egypt. Fruits packed in carton boxes and transported directly to the Post-Harvest Laboratory.

Essential oils extraction

The rosemary (*Rosmarinus officinalis* L.) and cymbopogon (*Cymbopogon citratus* L.) herbs were obtained from The Experimental Farm of Medicinal and Aromatic plants, Research Department in El-Qanater ELkhairia, HRI, ARC, Egypt. The essential oil was isolated by hydro-distillation according to **Guenther (1961)**.

Gas Chromatography analysis of Cetronella and Rosemary essential oil

The gas chromatography (GC) analysis of the essential oil samples was carried out in the Laboratory of Medicinal and Aromatic Plants Research Department, Horticulture Research Institute, ARC using Ds Chrom 6200 Gas Chromatograph apparatus, fitted with a capillary column BPX-5, 5 phenyl (equiv.) polysillphenylene-siloxane 30 × 0.25 mm ID × 0.25µ film. The temperature program varied in the range 70˚-200˚C, at a rate of 10˚C/min. Flow rates of gases were nitrogen at 1 ml/min, hydrogen at 30 ml/min and 330 ml/min for air. Detector and injector temperatures were 300˚ C and 250˚ C respectively. The identification of the compounds was done by matching their retention times with those of authentic samples injected under the same conditions. Chemical oil components of citronella and rosemary essential oils were presented in Table 1.

Table 1. The chemical oil components of citronella and rosemary essential oils

Citronella essential oil		Rosemary essential oil	
Major components	**%**	**Major components**	**%**
Myrcene	17.80	α-pinene	13.56
P-Cymene	0.36	Camphene	3.16
Limonene	1.42	β-pinene	3.32
Linalool	2.85	Limonene	19.15
Citronellol	8.55	1,8 cineol	2.09
Geraniol	30.28	Linalool	0.60
Citronellal	37.87	Camphor	24.87
Gentanyl acetate	4.32	α-terpineol	2.03
β-caryophyllene	0.28	Borneol	10.53
		Eugenol	1.92
		β-caryophyllene	3.10

***In vitro* antimicrobial activity of chitosan, cetronella and rosemary essential oil**

Antimicrobial activity of chitosan and essential oils (*Rosmarinus officinalis* & *Cymbopogon citratus*) were investigated by disc assay method **(Deans and Ritchie, 1987)** against Gram-positive bacteria (i.e. *Bacillus subtilis*) and Gram-negative bacteria (i.e. *Escherichia coli*) as well as against fungi (i.e. *Alternaria*). *Alternaria* strains used in this study were kindly obtained from Department of Plant Pathology, Faculty of Agriculture, Zagazig University, Egypt. The fungus strain was isolated from the sweet pepper fruits (Top Star). The strain was routinely grown in potato dextrose agar (PDA) at 25 °C for 72 h and were maintained in PDA at 4 °C. The pure cultures of the bacterial strains were subculture on Mueller Hinton broth (MHB, M1657, India) for 24 h at 37 °C. The bacterial suspension was adjusted to a density of bacterial cells of 1.2×10^8 cfu/mL. A sterile swab immersed in this bacterial suspension was used to inoculate the entire surface of Mueller-Hinton Agar (MHA: M173, India) plates. Discs of 6-mm diameter were used in this study. About 30 µL of each Chitosan of different concentrations (0, 0.5 and 1.0 mg/mL) diluted in acetic acid (1.0%) while, Citronella essential oil and Rosemary essential oil of different concentrations (0, 0.5 and 1.0 mg/mL) diluted in dimethylsulfoxide DMSO (CH3)2 SO were transferred onto each disc of all plates then, plates were incubated for 24 h at 37°C in the case of bacteria and at 25 °C for 72 h for the fungs , the inhibition zones were measured in millimeters. A positive control was prepared with solution of 0.1% imazalil. All experiments were done in three replicates.

Experimental design

This investigation have been planned to record the influence of dipping treatments (control, chitosan at 0.5 and 1 %, citronella essential oil at 0.5 and 1 ml/l and rosemary essential oil at 0.5 and 1 ml/l) to prolong storage and enhancing the quality of sweet pepper fruits during different cold storage periods (7, 14, 21, 28 and 35 days) and shelf life period (after 4 day).

Treatments

1- Dipping in tap water (control).

2- Dipping in chitosan at 0.5 %.

3- Dipping in chitosan at 1 %.

4- Dipping in citronella essential oil at 0.5 ml/l.

5- Dipping in citronella essential oil at 1 ml/l.

6- Dipping in rosemary essential oil at 0.5 ml/l.

7- Dipping in rosemary essential oil at 1.0 ml/l.

These treatments were arranged in complete randomized design as simple experiment. Each treatment was divided into 3 replicates, uniform pepper fruits were randomly taken for each replicate. Pepper fruits were immersed for 2 mints in different solutions of chitosan, citronella and rosemary oils as well as tap water (control) and dried at 20-22 °C for 2 hrs. and packaged in sealed low-density polyethylene bags (35.5 × 20 cm) then stored at 90-95 % relative humidity (RH) and 8 ± 1°C for different periods (7, 14, 21, 28 and 35 days). Samples of each treatment were randomly taken weekly intervals for five weeks. After each storage period some of these fruits were subjected to conditions of 18-20 °C and 60- 70% RH for four days as a shelf life. Fruits number required to this work were 12 fruits × 3 replicates= 36 fruits for each storage period × 5 storage periods = 180 fruits × 7 dipping treatments = 1260 fruits at the beginning of the experiment (Zero time), the following data (fruit firmness, tirtratable acidity, total soluble solids and total chlorophyll content) were measured to compare fruit quality after different storage periods. Chitosan (2-Amino-2-deoxy-beta-D-glucosasmine) solution was prepared by dissolving a proper amount of Chito–Care®, an Egyptian commercial product of chitosan, in 0.5 % acetic acid, then added fresh water to obtain the two concentrations (0.5 and 1%).

Data recorded

- Fresh weight loss (FWL%) according **(AOAC, 2007)**.

- Fruit firmness (g/cm^2): It was determined on (Model FD 101).

- Fruits decay percentage **(El-Mougy *et al*., 2012)**.

- Total chlorophyll (a+b): **(Wettestein, 1957)**.

- Ascorbic acid (mg/100ml juice): The method described by **AOAC (2007)**.

- Total soluble solids (TSS) of juice (Brix°): It was determined by using a hand refractometer as Brix degree.

- General appearance (GA): It was evaluated by using a scale from 1-9 with: 9= excellent, 7= good, 5= fair, 3= poor, 1= unsalable and fruits rating 5 or below were considered unmarketable **(Shehata *et al*., 2013)**.

Microbiological Quality

These evaluates were achieved in duplicate, for the count of the total bacterial count, total fungi count, and total yeasts count, on days 0, 7, 14, 21, 28 and 35 of the treatments under kept at 8±1 °C. During these storage, sweet pepper (*Capsicum annuum* L.) of approximately 10 g of mass were taken; subsequently, they were homogenized for 10 min in 90 mL of sterilized peptone water (0.1 % *w/v*). The homogenate was serially diluted from 10^{-1} to 10^{-5}, and after that 1.0 mL of each solution was poured into plates on the surface of the medium containing Potato Dextrose Agar (PDA) with 10% tartaric acid (p/v) for the test of molds and yeasts, and Plate Count Agar

(PCA) for total bacterial count, and was incubated for a period of 3 days. The total bacterial count was performed in accordance with ISO 4833 using the plate colony counting technique at 30 °C. The results were reported as logarithm colony-forming units per gram (Log CFU/g) of total bacteria. Mold and yeast counts were performed in accordance with ISO 7954, based on the plate colony counting technique at 25 °C. The results were reported as log UFC/g of molds and yeasts.

Statistical Analysis

The statistical layout of this experiment was completely randomized block design. Data were analyzed according to **Snedecor and Cochran (1980)**. The means were compared using computer program of Statistix version 9 **(Analytical software, 2008)**.

RESULTS AND DISCUSSION

In vitro antimicrobial of citronella essential oil and rosemary essential oil

In vitro testing of the chitosan, citronella oil and rosemary oil showed that they all possessed inhibitory effects against Gram positive bacteria (*B. subtilis*), Gram negative bacteria (*E. coli*) and the fungus of *Alternaria* sp. in the following order of efficacy: chitosan > citronella > rosemary (Table 2). The chitosan inhibition ranged from 15 to 23 mm for all the tested microorganisms. All three-antimicrobial material (chitosan, citronella essential oil and rosemary essential oil) possessed antimicrobial properties (Table, 2). The major components found in citronella essential oil was citronellal (37.87%) and geraniol (30.28%) (Table 1); it is a phenolic. Phenols are also known to interact with membrane proteins and periplasmic enzymes, leading to deformations in the membrane structure and functions. The minor constituent of rosemary essential oil was eugenol (1.92%) (Table 1), which belongs to the phenylpropene group with hydroxyl group. Eugenol treatment was shown to alter cell wall structure and cell membrane of yeast *Saccharomyces cerevisiae*, resulting in the release of cellular content **(Bennis *et al*., 2004)**. Its antimicrobial activity is linked to its ability for nonspecific permeabilization of cytoplasmic membrane and interaction with proteins, which results in increased transport of ATP and potassium out of the cells **(Walsh *et al*., 2003)**. The lack of these phenolic compounds (i.e. geraniol) in the rosemary essential oils could explain its relatively lower antifungal activity in comparison to citronella. However, volatiles such as geraniol found in the citronella essential oil in this study (Table 1), possess antifungal activity. Their mode of action also involves penetration and disruption of the membrane structure of microorganisms **(Sivakumar and Bautista-Banos, 2014)**. In addition, the lipophilic characteristic of major components of essential oils could aid in their ability to penetrate cellular and mitochondrial membrane structures. This action leads to membrane disruption, cytoplasmic leakage, cell lysis and death **(Sanchez-Gonzalez *et al*., 2011)**. **Hyldgaard *et al*. (2012)** suggested that the antimicrobial activity of essential oil is not entirely dependent on the presence of the major compounds, but is rather a collective synergism of both the major and minor compounds. For example, geraniol a major constituent of citronella essential oil in this study (Table 1), is an acyclic monoterpene and a key precursor compound of other volatiles such as citronellol **(Davidovich-Rikanati *et al*., 2007)**. It has been shown that geraniol can induce cell cycle arrest and apoptosis in various cells **(Chen and Vilijoen, 2010)**, and have effective antimicrobial activity **(Suppakul *et al*., 2003)**. Thus, the inhibitory efficacy of the essential oils could be considered a function of the composition, the function groups present and the synergistic interaction between major and minor constituents of the essential oils. Chitosan is an antimicrobial in the dissolved state **(Wang *et al*., 2011)**. To achieve antimicrobial activity the positively charged amino groups of the chitosan monomer units, which can react with the anionic groups of the microbial cell surface must be freely available **(Wang *et al*., 2011; Salarbashi *et al*., 2014)**.

Table 2. The antimicrobial effect of chitosan, citronella and rosemary essential oils

	Minimum Inhibitory zone (mm)		
Dipping treatments	*Bacillus subtilis*	*Escherichia coli*	*Alternaria sp.*
Control (tap water for 2min.)	0.0	0.0	0.0
Chitosan at 0.5 %	20.15±0.09d	19.17±0.16d	15.15±0.09d
Chitosan at 1 %	23.31±0.13c	21.17±0.22c	17.31±0.13c
Citronella oil at 0.5 ml/l	19.67±0.21b	16.15±0.19b	21.17±0.21b
Citronella oil at 1 ml/l	22.14±0.09d	21.17±0.16d	22.55±0.09d
Rosemary oil at 0.5 ml/l	13.38±0.13c	13.41±0.22c	17.18±0.13c
Rosemary oil at 1 ml/l	14.17±0.21b	14.61±0.19b	21.17±0.21b

Values are means of three replicates ± standard error. Values in the same column with a different superscript letter differ significantly at $P \leq 0.05$

Fresh weight loss percentage (FWL %)

Effect of dipping treatments during cold storage

The data described in Table 3 indicate that dipping pepper fruits in solutions of chitosan, citronella and rosemary essential oils had significant influence on fresh weight loss during cold storage periods compared to control. FWL % of fruits increased with prolonging the storage periods for all treatments under study. The best treatments in decreased fresh weight loss % were dipping pepper fruits in chitosan at 1 and 0.5 % as well as citronella at 1 ml/l compared to the other ones under study during cold storage periods. Previous experiments utilizing eugenol, thymol or menthol vapors showed benefits due to decreased weight loss in cherries and grapes **(Martinez-Romero *et al*., 2005 and Serrano *et al*., 2005)**. Similarly, chitosan and cinnamon oil coating decreased the weight loss of jujube fruits during 60 days of storage at 4°C **(Xing *et al*., 2015)**.

Table 3. Influence of dipping treatments on fruit weight loss (FWL) percentage of sweet pepper during cold storage periods during 2018 and 2019 seasons

Dipping treatments	Cold storage periods (days)				
	7	14	21	28	35
	2018 season				
Control (tap water for 2min.)	0.24	0.55	0.96	1.28	1.52
Chitosan at 0.5 %	0.15	0.18	0.40	1.15	1.30
Chitosan at 1 %	0.13	0.14	0.48	0.86	1.16
Citronella oil at 0.5 ml/l	0.16	0.31	0.39	1.08	1.27
Citronella oil at 1 ml/l	0.15	0.20	0.45	0.91	1.14
Rosemary oil at 0.5 ml/l	0.19	0.48	0.69	1.21	1.45
Rosemary oil at 1 ml/l	0.18	0.34	0.54	1.04	1.38
LSD at 5 %	0.02	0.05	0.05	0.07	0.07
	2019 season				
Control (tap water for 2min.)	0.91	1.11	1.09	1.78	2.61
Chitosan at 0.5 %	0.63	0.49	0.92	1.67	1.30
Chitosan at 1 %	0.28	0.48	0.88	1.13	1.34
Citronella oil at 0.5 ml/l	0.67	0.88	1.01	1.67	1.16
Citronella oil at 1 ml/l	0.32	0.68	0.86	1.12	1.22
Rosemary oil at 0.5 ml/l	0.75	0.90	1.13	1.59	1.88
Rosemary oil at 1 ml/l	0.68	0.84	1.04	1.76	2.03
LSD at 5 %	0.10	0.08	0.04	0.13	0.08

Effect of dipping treatments during shelf life periods

Data of both seasons in Table 4 demonstrate that, after four days of shelf life, dipping in solutions of chitosan at 1% and citronella essential oil at 1 ml/l reflected a significant effect on fresh weight loss percentage of pepper fruits after different storage periods. The lowest values in FWL% were observed with the same two treatments at 7 and 14 days then the values generally began to increase to 35 days.

Chitosan forms a semi-permeable film that regulates the gas exchange and reduces transpiration losses and fruit ripening is slowed down. Also, respiration rate and hence water loss is reduced **(Bautista–Banos *et al*., 2006)**. In addition, **Haffez *et al*. (2016)** reported that fresh - cut cantaloupe fruits dipped in chitosan at 2000 ppm was the most effective treatments for reduced loss of texture. Also, **Mohamed *et al*. (2019)** found that chitosan at 1% significantly reduced weight loss. Furthermore, the lemongrass essential oil shows a wide spectrum of biological activities. High antibacterial and remarkable antifungal activities make the lemongrass oil a potential food and fruits preservative **(Majewska *et al*., 2019)**.

Table 4. Influence of dipping treatments on fruit weight loss (FWL) percentage of sweet pepper fruits after 4 days shelf life during 2018 and 2019 seasons

Dipping treatments	Cold storage periods + 4days shelf life				
	7+4	14+4	21+4	28+4	35+4
	2018 season				
Control (tap water for 2min.)	10.89	13.11	13.98	15.15	20.88
Chitosan at 0.5 %	9.06	11.76	12.32	13.37	15.98
Chitosan at 1 %	7.61	8.87	10.79	11.54	13.11
Citronella oil at 0.5 ml/l	9.80	12.23	12.76	13.92	16.24
Citronella oil at 1 ml/l	8.08	8.96	10.71	11.71	13.31
Rosemary oil at 0.5 ml/l	10.06	12.46	12.78	14.07	17.01
Rosemary oil at 1 ml/l	8.76	11.84	12.20	13.57	14.71
LSD at 5 %	0.22	0.32	0.31	0.42	0.72
	2019 season				
Control (tap water for 2min.)	14.84	15.94	18.53	19.99	34.75
Chitosan at 0.5 %	12.65	13.82	15.46	16.48	21.17
Chitosan at 1 %	10.65	12.08	13.78	14.84	19.68
Citronella oil at 0.5 ml/l	12.97	14.03	15.88	17.12	23.38
Citronella oil at 1 ml/l	10.92	11.84	13.85	14.67	19.89
Rosemary oil at 0.5 ml/l	13.42	13.66	16.16	18.07	24.16
Rosemary oil at 1 ml/l	13.06	12.05	14.27	16.47	21.64
LSD at 5 %	0.46	0.52	0.45	0.59	0.78

Fruit firmness (g/cm^2)

Effect of dipping treatments during cold storage

As shown in Table 5 that, all dipping solutions treatments significantly increased fruit firmness of sweet pepper compared to control in both seasons. At 21 days of cold storage, dipping pepper fruits in chitosan at 1 % and citronella essential oil at 0.5 ml/l in the first season and chitosan at 1 % and rosemary essential oil at 0.5 ml/l in the second season recorded the highest values of fruit firmness. Moreover, at 28 days of cold storage, dipping pepper fruits in chitosan at 1 % and rosemary essential oil at 0.5 ml/l in the first season as well as rosemary essential oil at 0.5 ml/l and chitosan at 0.5% in the second season gave the highest values in this concern compared to the other ones under study. It was found by **Lambert *et al*. (2001)** that the essential oils (due to their hydrophobic nature) affect the partitioning the lipids of the plasma membrane and changing its integrity, permeability and the inorganic ion equilibrium. Also, the faster reduction in texture in control samples might also be due to accelerated ripening process during storage periods which mainly occurs because of degradation of the middle lamella of the cell wall **(Díaz–Mula*et al*., 2011 and Abd Elwahab *et al.*, 2014).**

Effect of dipping treatments during shelf life periods

The data given in Table 6 suggest that, during shelf life period, dipping sweet pepper fruits in chitosan, citronella and rosemary essential oils at any rate increased the fruit firmness (g/ cm^2) compared to control (dipping in tap water) in both seasons, in most cases. Generally, at 28 days of cold storage + 4 days of shelf life, dipping sweet pepper fruits in chitosan at 1 % and citronella essential oil at 1 ml/l in the first season as well as citronella essential oil at 1 ml/l and rosemary essential oil at 0.5 ml/l in the second one gave the highest values in fruit firmness compared to the other ones under study in both seasons. Also, **Youwei and Yinzhe (2013)** reported that after coating with chitosan on the surface of post-harvest fruit and vegetable, higher firmness is remained. Likewise, **Ibrahim *et al*. (2017)** showed that coating fruits by chitosan, lemongrass and thyme oils-maintained firmness, total acidity, ascorbic acid and overall acceptability of strawberry fruits.

Table 5. Influence of dipping treatments on fruit firmness (g/cm^2) of sweet pepper during cold storage periods during 2018 and 2019seasons

Dipping treatments	Cold storage periods (days)					
	0	7	14	21	28	35
	2018 season					
Control (tap water for 2min.)	590.0	573.3	514.0	503.3	468.7	455.3
Chitosan at 0.5 %	590.0	642.0	594.0	573.3	570.7	551.3
Chitosan at 1 %	590.0	703.3	644.0	616.7	642.0	534.7
Citronella oil at 0.5 ml/l	590.0	628.0	596.7	576.7	554.0	543.3
Citronella oil at 1 ml/l	590.0	630.7	575.3	544.0	552.7	503.3
Rosemary oil at 0.5 ml/l	590.0	626.7	614.7	555.3	581.3	476.7
Rosemary oil at 1 ml/l	590.0	628.7	626.7	558.3	537.3	523.3
LSD at 5 %		39.0	44.6	21.7	40.7	45.2
	2019 season					
Control (tap water for 2min.)	610.0	648.7	649.3	573.3	569.3	552.7
Chitosan at 0.5 %	610.0	744.7	712.0	688.0	678.7	646.7
Chitosan at 1 %	610.0	744.7	724.7	706.7	670.7	597.3
Citronella oil at 0.5 ml/l	610.0	719.3	710.7	683.3	655.3	634.0
Citronella oil at 1 ml/l	610.0	730.7	687.3	663.3	648.0	610.0
Rosemary oil at 0.5 ml/l	610.0	722.0	676.7	693.3	682.0	640.0
Rosemary oil at 1 ml/l	610.0	724.7	698.7	669.3	672.0	614.0
LSD at 5 %		51.3	47.4	38.9	40.4	61.6

Table 6. Influence of dipping treatments on fruit firmness (g/cm^2) of sweet pepper fruits after 4 days shelf life during 2018 and 2019 seasons

Dipping treatments	Cold storage periods + 4 days shelf life					
	0+4	7+4	14+4	21+4	28+4	35+4
	2018 season					
Control (tap water for 2min.)	590.0	542.7	474.0	476.0	420.7	384.0
Chitosan at 0.5 %	590.0	594.7	574.7	536.0	468.0	471.3
Chitosan at 1 %	590.0	676.0	625.3	579.3	553.3	484.0
Citronella oil at 0.5 ml/l	590.0	571.3	576.0	555.3	462.0	450.7
Citronella oil at 1 ml/l	590.0	640.0	553.3	530.7	538.7	474.7
Rosemary oil at 0.5 ml/l	590.0	570.7	569.3	519.3	485.3	424.0
Rosemary oil at 1 ml/l	590.0	584.0	572.7	518.0	496.7	474.0
LSD at 5 %		20.1	25.5	16.0	16.2	26.9
	2019 season					
Control (tap water for 2min.)	610.0	618.0	626.7	523.3	528.7	511.3
Chitosan at 0.5 %	610.0	678.7	672.7	566.0	554.0	544.7
Chitosan at 1 %	610.0	728.0	694.0	621.7	582.7	567.3
Citronella oil at 0.5 ml/l	610.0	657.3	654.7	568.0	569.7	557.3
Citronella oil at 1 ml/l	610.0	711.3	645.3	620.0	604.7	566.7
Rosemary oil at 0.5 ml/l	610.0	659.0	624.7	628.0	594.0	542.7
Rosemary oil at 1 ml/l	610.0	686.0	673.3	618.0	574.7	584.0
LSD at 5 %		17.91	23.5	12.6	24.9	18.3

Fruit decay percentage

Effect of dipping treatments during cold storage

The data illustrated in Table 7 reveal that, decay percentage increased with the prolongation of cold storage periods. Since, all dipping treatments (chitosan at 1% and citronella essential oil at 1ml/l in the first season as well as both treatments plus citronella essential oil at 0.5 ml/l or rosemary essential oil at 1 ml/l in the second one) did not appear any decayed pepper fruits until 28 days of cold storage compared to the other treatments under study. These findings may be due to the continuous biochemical and chemical changes in fruits such as transformation of complex compounds to simple forms that more liable to fungal infection. However, the natural antimicrobial compound such as an essential oil could be a promising approach for controlling postharvest decay in fruit while also reducing the risk of fungicide usage in fruit preservation **(Maqbool *et al*., 2011)**. In another study utilizing sweet pepper (*Capsicum annum* L.), it was pointed out that chitosan and cinnamon oil coating could reducing decay (below 5%) and maintained sensory acceptability of sweet pepper for 35 days at 8°C **(Xing *et al*., 2011)**.

Table 7. Influence of dipping treatments on decay percentage of sweet pepper fruits during cold storage periods during 2018 and 2019 seasons

Dipping treatments	Cold storage periods (days)				
	7	14	21	28	35
			2018 season		
Control (tap water for 2min.)	0.00	0.00	0.00	9.73	20.27
Chitosan at 0.5 %	0.00	0.00	0.00	4.39	7.23
Chitosan at 1 %	0.00	0.00	0.00	0.00	3.90
Citronella oil at 0.5 ml/l	0.00	0.00	0.00	1.48	2.77
Citronella oil at 1 ml/l	0.00	0.00	0.00	0.00	1.30
Rosemary oil at 0.5 ml/l	0.00	0.00	0.00	2.33	2.83
Rosemary oil at 1 ml/l	0.00	0.00	0.00	1.73	2.23
LSD at 5 %	NS	NS	NS	0.89	0.70
			2019 season		
Control (tap water for 2min.)	0.00	0.00	0.00	12.47	14.43
Chitosan at 0.5 %	0.00	0.00	0.00	3.50	6.43
Chitosan at 1 %	0.00	0.00	0.00	0.00	1.33
Citronella oil at 0.5 ml/l	0.00	0.00	0.00	0.00	1.47
Citronella oil at 1 ml/l	0.00	0.00	0.00	0.00	2.40
Rosemary oil at 0.5 ml/l	0.00	0.00	0.00	1.67	4.03
Rosemary oil at 1 ml/l	0.00	0.00	0.00	0.00	1.60
LSD at 5 %	NS	NS	NS	0.12	0.37

Effect of dipping treatments during shelf life periods

It is quite clear from the data in Table 8 that, at four days of shelf life, dipping pepper fruits in solutions of citronella essential oil at 1ml/l in the first season as well as citronella essential oil at 0.5 or 1 ml/l and rosemary essential oil at 1 ml/l in the second one had significant effect on decay percentage at 28 + 4 days of cold storage. Most of the researches on the inhibition of strawberries post-harvest fungal pathogens by essential oils focus on *in vitro* conditions **(Nabigol and Morshedi, 2011)**. The effect of several essential oils and their antimicrobial compounds were reported in extending the shelf life and inhibiting decay of fruits **(Taghavi *et al*., 2018).**

Table 8. Influence of dipping treatments on decay percentage of sweet pepper fruits after 4 days shelf life during 2018 and 2019 seasons

Dipping treatments	Cold storage periods + 4 days shelf life				
	7+4	14+4	21+4	28+4	35+4
	2018 season				
Control (tap water for 2min.)	0.00	0.00	0.00	14.69	27.00
Chitosan at 0.5 %	0.00	0.00	0.00	5.73	7.46
Chitosan at 1 %	0.00	0.00	0.00	3.33	5.33
Citronella oil at 0.5 ml/l	0.00	0.00	0.00	4.56	4.50
Citronella oil at 1 ml/l	0.00	0.00	0.00	0.00	1.83
Rosemary oil at 0.5 ml/l	0.00	0.00	0.00	3.70	3.17
Rosemary oil at 1 ml/l	0.00	0.00	0.00	2.50	5.37
LSD at 5 %	NS	NS	NS	0.51	0.98
	2019 season				
Control (tap water for 2min.)	0.00	0.00	0.00	16.60	23.63
Chitosan at 0.5 %	0.00	0.00	0.00	6.70	8.80
Chitosan at 1 %	0.00	0.00	0.00	2.41	2.93
Citronella oil at 0.5 ml/l	0.00	0.00	0.00	0.00	4.60
Citronella oil at 1 ml/l	0.00	0.00	0.00	0.00	5.90
Rosemary oil at 0.5 ml/l	0.00	0.00	0.00	3.23	9.43
Rosemary oil at 1 ml/l	0.00	0.00	0.00	0.00	4.37
LSD at 5 %	NS	NS	NS	0.36	0.52

Total chlorophyll content (mg/100 g as fresh weight)

Effect of dipping treatments during cold storage

Data presented in Table 9 demonstrate that, in most cases, dipping treatments in different solutions in cold storage periods increased total chlorophyll content (a+b) in sweet pepper fruits compared to dipping fruits in tap water (control) during the two consecutive seasons. In general, total chlorophyll content gradually decreased with the advance of cold storage for all dipping treatments. Dipping fruits in solutions of citronella and rosemary essential oils each at 1ml/l in the first season as well as citronella essential oil at 1 ml/l and chitosan at 1 % in the second one gave the highest values in total chlorophyll content (a+b) at 21 days of cold storage.

Effect of dipping treatments during shelf life periods

From the data in Table 10 it is clear that, total chlorophyll content (a+b) in sweet pepper fruit tissues gradually decreased with the advance of cold storage + shelf life periods for all dipping solution treatments. In addition, dipping fruits in solutions at different shelf life periods increased total chlorophyll content in pepper fruits when compared with control (dipping in tap water). Dipping fruits in solutions of citronella essential oil at 1 ml/l and chitosan at 1 % gave the highest values in total chlorophyll content at 21 days of cold storage and after four days of shelf life during the two seasons. Moreover, color is one of the main fruit attributes for consumer acceptance and during ripening there is a degradation of chlorophyll **(Serrano *et al*., 2008)**.

Ascorbic acid content (Vit. C as mg/100 ml juice)

Effect of dipping treatments during cold storage

Table 11 shows that, in most cases, all dipping treatments significantly increased ascorbic acid content in sweet pepper fruits especially at 35 days of cold storage compared to control (dipping in tap water for 2 min.) in both seasons. The highest values in this connection were obtained by the treatment of dipping fruits in citronella essential oil at 1 ml/l during the first and second seasons compared to the other treatments under study. In addition, essential oil coatings are applied to enhance the quality and extend the shelf-life of fruit by decreasing moisture loss and respiration rate. An edible coating can have a positive influence on the surface of fruit by incorporating functional ingredients, such as antioxidants, colorants, antimicrobials and flavors **(Valencia-chamorro *et al*., 2012)**.

Table 9. Influence of dipping treatments on total chlorophyll content (mg/100g as fresh weight) of sweet pepper fruits during cold storage periods during 2018 and 2019 seasons

Dipping treatments	Cold storage periods (days)					
	0	7	14	21	28	35
	2018 season					
Control (tap water for 2min.)	340.0	313.7	294.3	257.7	227.7	210.3
Chitosan at 0.5 %	340.0	321.3	292.7	264.0	232.7	205.0
Chitosan at 1 %	340.0	325.0	307.0	264.3	228.3	216.3
Citronella oil at 0.5 ml/l	340.0	318.0	292.0	253.3	218.0	200.7
Citronella oil at 1 ml/l	340.0	325.7	308.3	270.3	225.7	211.7
Rosemary oil at 0.5 ml/l	340.0	313.7	301.0	262.7	230.3	210.0
Rosemary oil at 1 ml/l	340.0	314.0	298.0	268.0	241.7	221.7
LSD at 5 %		2.9	7.9	3.3	3.9	3.9
	2019 season					
Control (tap water for 2min.)	380.0	342.0	322.3	275.0	226.0	216.7
Chitosan at 0.5 %	380.0	360.0	318.3	280.3	243.7	225.3
Chitosan at 1 %	380.0	371.3	348.0	304.0	256.0	233.7
Citronella oil at 0.5 ml/l	380.0	337.7	327.7	278.0	235.0	221.0
Citronella oil at 1 ml/l	380.0	335.0	323.3	307.0	259.3	232.3
Rosemary oil at 0.5 ml/l	380.0	344.3	314.0	289.3	237.0	219.3
Rosemary oil at 1 ml/l	380.0	352.7	343.7	299.0	254.7	228.0
LSD at 5 %		9.1	3.2	4.3	5.5	3.4

Table 10. Influence of dipping treatments on total chlorophyll content (mg/100g as fresh weight) of sweet pepper fruits after 4 days shelf life during 2018 and 2019 seasons

Dipping treatments	Cold storage periods + 4 days shelf life					
	0+4	7+4	14+4	21+4	28+4	35+4
	2018 season					
Control (tap water for 2min.)	340.0	298.7	270.0	180.3	164.0	148.7
Chitosan at 0.5 %	340.0	287.0	278.0	187.0	170.3	153.3
Chitosan at 1 %	340.0	297.7	282.0	220.0	177.0	165.7
Citronella oil at 0.5 ml/l	340.0	302.0	271.7	205.7	186.7	146.0
Citronella oil at 1 ml/l	340.0	307.3	289.3	223.7	193.0	164.7
Rosemary oil at 0.5 ml/l	340.0	301.7	280.3	193.0	174.7	141.0
Rosemary oil at 1 ml/l	340.0	299.3	269.3	188.0	185.0	150.0
LSD at 5 %		3.8	4.4	7.8	3.0	4.9
	2019 season					
Control (tap water for 2min.)	380.0	309.3	291.0	168.0	172.0	138.3
Chitosan at 0.5 %	380.0	325.0	283.0	202.3	190.7	161.7
Chitosan at 1 %	380.0	322.7	326.0	226.3	188.0	159.3
Citronella oil at 0.5 ml/l	380.0	317.3	296.3	214.3	179.3	153.0
Citronella oil at 1 ml/l	380.0	331.7	287.7	229.7	187.3	169.3
Rosemary oil at 0.5 ml/l	380.0	320.0	277.7	207.3	173.7	151.3
Rosemary oil at 1 ml/l	380.0	338.7	317.0	221.0	178.0	163.0
LSD at 5 %		6.2	3.6	4.6	1.8	2.7

Table 11. Influence of dipping treatments on vitamin C content (mg/100ml juice) of sweet pepper during cold storage periods during 2018 and 2019 seasons

Dipping treatments	Cold storage periods (days)					
	0	7	14	21	28	35
			2018 season			
Control (tap water for 2min.)	91.00	60.73	46.65	34.37	31.74	28.99
Chitosan at 0.5 %	91.00	81.65	47.58	35.33	33.33	31.65
Chitosan at 1 %	91.00	68.66	48.30	36.10	33.77	31.61
Citronella oil at 0.5 ml/l	91.00	75.03	47.30	41.87	33.65	31.06
Citronella oil at 1 ml/l	91.00	79.87	48.65	43.32	36.26	31.86
Rosemary oil at 0.5 ml/l	91.00	72.88	49.27	37.66	33.65	31.26
Rosemary oil at 1 ml/l	91.00	78.11	51.25	40.04	36.26	31.86
LSD at 5 %		5.43	3.24	1.66	1.57	0.92
			2019 season			
Control (tap water for 2min.)	102.00	80.47	64.28	45.54	42.65	37.58
Chitosan at 0.5 %	102.00	82.31	67.65	56.97	50.62	47.06
Chitosan at 1 %	102.00	91.55	72.41	55.30	48.10	44.98
Citronella oil at 0.5 ml/l	102.00	90.63	69.76	55.69	46.41	43.73
Citronella oil at 1 ml/l	102.00	96.85	74.32	57.95	44.72	42.43
Rosemary oil at 0.5 ml/l	102.00	96.22	65.98	57.47	48.78	44.91
Rosemary oil at 1 ml/l	102.00	89.43	70.87	54.55	49.55	46.47
LSD at 5 %		5.61	5.16	4.70	1.64	1.53

Effect of dipping treatments during shelf life periods

Table 12 pointed out that, after 4 days of shelf life, ascorbic acid (Vit. C) content gradually decreased with the advance of cold storage and after shelf life periods for all dipping treatments. all dipping solution treatments significantly increased Vit. C content of sweet pepper compared to control in both seasons. At 28 days of cold storage + 4 days of shelf life, dipping fruits in citronella or rosemary essential oils at 1 ml/l in the first season as well as chitosan at 0.5 % and rosemary essential oil at 1 ml/l in the second one recorded the highest values in ascorbic acid content compared to the other treatments in both seasons. In this concern, **Montero *et al.* (2015)** stated that the maximum values of radish ascorbic acid were obtained from the concentration of 0.2 % essential oil of *Origanum vulgare* solution.

Total soluble solids (TSS as Brix°)

Effect of dipping treatments during cold storage

Data of both seasons in Table 13 show that, dipping treatments of pepper fruits had no significant influence on TSS content during 14 and 35 days of cold storage in first season and 21 days of cold storage in the second one. Furthermore, TSS increased with the prolonging storage periods up to 28 days, after that at 35 days TSS content in sweet pepper fruits decreased. The best treatments in increase TSS were that dipping pepper fruits in chitosan at 1 % and citronella essential oil at 0.5% compared to the other ones at 28 days of cold storage in both seasons.

Effect of dipping treatments during shelf life periods

Results under discussion in Table 14 indicate that, dipping of sweet pepper fruits in chitosan as well as citronella and rosemary essential oils had no significant effect on TSS content during 7+4, 14+4, 21+4 and 28+4 cold storage periods+ 4 days shelf life in the first season and 7+4 and 21+4 periods only in the second one. However, the highest values in TSS at 28+4days were achieved by dipping pepper fruits in chitosan at 0.5 % and rosemary essential oil at 0.5 % in the first season as well as chitosan at 0.5 % and citronella essential oil at 0.5 % in the second season. **Ahmed (2017)** reported that the decline in TSS contents in sweet pepper fruits after 35 days of cold storage and shelf life periods might be attributed to internal using of sugar for sub sustaining the essential physiological processes like respiration and other change associated with repining. In the same time, **Abdullah and Srour (2019)** found that total soluble solids of sweet pepper fruits decreased with the prolongation of storage till the end of storage period in both seasons.

Table 12. Influence of dipping treatments on vitamin C content (mg/100ml juice) of sweet pepper fruits after 4 days shelf life during 2018 and 2019 seasons.

Dipping treatments	Cold storage periods + 4days shelf life					
	0+4	7+4	14+4	21+4	28+4	35+4
	2018 season					
Control (tap water for 2min.)	91.00	56.65	42.62	33.21	31.82	26.96
Chitosan at 0.5 %	91.00	60.11	46.15	35.30	32.27	27.54
Chitosan at 1 %	91.00	62.22	46.30	39.18	31.87	28.11
Citronella oil at 0.5 ml/l	91.00	65.55	43.67	38.99	32.14	27.32
Citronella oil at 1 ml/l	91.00	71.34	45.65	40.66	34.16	28.05
Rosemary oil at 0.5 ml/l	91.00	70.73	45.08	36.37	32.55	27.88
Rosemary oil at 1 ml/l	91.00	73.48	48.82	39.34	34.16	28.50
LSD at 5 %		2.22	0.54	0.64	0.59	0.59
	2019 season					
Control (tap water for 2min.)	102.00	71.71	59.73	39.80	39.69	31.19
Chitosan at 0.5 %	102.00	75.25	60.59	49.54	46.66	34.73
Chitosan at 1 %	102.00	83.20	68.98	48.87	46.07	35.92
Citronella oil at 0.5 ml/l	102.00	79.91	70.36	51.96	44.64	34.29
Citronella oil at 1 ml/l	102.00	86.55	71.23	50.66	41.58	36.09
Rosemary oil at 0.5 ml/l	102.00	88.92	62.49	49.77	46.45	33.59
Rosemary oil at 1 ml/l	102.00	78.16	65.99	48.54	50.12	36.01
LSD at 5 %		0.88	1.43	1.35	0.93	0.43

Table 13. Influence of dipping treatments on total soluble solids (TSS as Brix°) of sweet pepper during cold storage periods during 2018 and 2019 seasons

Dipping treatments	Cold storage periods (days)					
	0	7	14	21	28	35
	2018 season					
Control (tap water for 2min.)	4.20	4.60	5.17	5.30	4.57	3.70
Chitosan at 0.5 %	4.20	4.60	5.47	5.43	5.43	2.97
Chitosan at 1 %	4.20	4.73	5.13	5.77	5.93	2.83
Citronella oil at 0.5 ml/l	4.20	4.47	5.37	5.93	5.90	2.60
Citronella oil at 1 ml/l	4.20	4.63	5.23	5.67	5.60	2.70
Rosemary oil at 0.5 ml/l	4.20	4.50	5.40	5.73	5.70	3.13
Rosemary oil at 1 ml/l	4.20	4.37	5.17	5.47	5.20	3.13
LSD at 5 %		0.18	N.S.	0.63	0.49	N.S.
	2019 season					
Control (tap water for 2min.)	4.40	4.70	4.60	5.60	5.30	3.10
Chitosan at 0.5 %	4.40	4.87	5.23	6.07	5.93	3.50
Chitosan at 1 %	4.40	4.77	5.33	5.77	5.97	3.30
Citronella oil at 0.5 ml/l	4.40	5.10	5.30	5.57	6.17	2.23
Citronella oil at 1 ml/l	4.40	5.17	5.20	5.63	5.63	2.73
Rosemary oil at 0.5 ml/l	4.40	5.07	4.97	5.70	5.87	3.40
Rosemary oil at 1 ml/l	4.40	5.17	5.30	5.47	5.63	4.43
LSD at 5 %		0.16	0.25	N.S.	0.53	1.06

Table 14. Influence of dipping treatments on total soluble solids (TSS as Brix°) of sweet pepper fruits after 4 days shelf life during 2018 and 2019 seasons

Dipping treatments	Cold storage periods + 4 days shelf life					
	0+4	7+4	14+4	21+4	28+4	35+4
			2018 season			
Control (tap water for 2min.)	4.20	5.33	5.57	6.30	6.33	3.87
Chitosan at 0.5 %	4.20	5.17	5.60	6.00	6.40	3.03
Chitosan at 1 %	4.20	4.77	5.30	6.13	6.30	2.60
Citronella oil at 0.5 ml/l	4.20	5.03	5.60	6.17	6.27	2.33
Citronella oil at 1 ml/l	4.20	4.87	5.47	6.17	6.27	2.60
Rosemary oil at 0.5 ml/l	4.20	5.33	5.53	5.97	6.37	3.33
Rosemary oil at 1 ml/l	4.20	5.17	5.43	5.60	5.80	3.07
LSD at 5 %		N.S.	N.S.	N.S.	N.S.	1.38
			2019 season			
Control (tap water for 2min.)	4.40	5.17	5.33	5.87	5.77	3.23
Chitosan at 0.5 %	4.40	5.03	5.60	6.30	6.37	3.60
Chitosan at 1 %	4.40	4.90	5.53	6.00	6.30	3.53
Citronella oil at 0.5 ml/l	4.40	5.20	5.57	5.83	6.43	2.33
Citronella oil at 1 ml/l	4.40	5.27	5.40	5.87	5.93	2.87
Rosemary oil at 0.5 ml/l	4.40	5.20	5.20	5.97	6.13	3.57
Rosemary oil at 1 ml/l	4.40	5.023	5.53	5.87	6.13	4.40
LSD at 5 %		N.S.	0.36	N.S.	0.54	1.34

General appearance (GA)

Effect of dipping treatments during cold storage

It is evident from the obtained data in Table 15 that, from zero time till 14 days of cold storage, dipping sweet pepper fruits in all solution even control gave excellent general appearance (GA) in the first season as well as dipping fruits in tap water, chitosan at 1 %, citronella and rosemary essential oils each at 1 ml/l in the second one. All dipping solution treatments recorded a good general appearance of pepper fruits till 28 days of cold storage compared to control (dipping in tap water) in the first season, Whereas, dipping in chitosan at 0.5 and 1 % and citronella essential oil at 1 ml/l only recorded good GA of fruits in the second one in the same period of storage. Fruits treated by essential oils enhanced storage life of sweet pepper fruits by controlling their fungal rotting and had the best marketability that might be due to reduce decay and little change in quality characters of the fruits compared to control fruits, which help in decreasing loss in weight and maintaining changes in total soluble solids and has high texture and Vitamin C, so, delaying the ripening process with a minimum quality loss and longer market life after 28 days from cold storage. These results are in line with those found by **Abd El wahab (2015)**.

Effect of dipping treatments during shelf life periods

From data recorded in Table 16 it is obvious that, after four days of shelf life, general appearance of sweet pepper fruits decreased with the prolonging storage periods. Dipping fruits in different solution had significant effect on GA of fruits (except at 21 days of cold storage in the first season). Sweet pepper fruits recorded a good general appearance from 0-time till 28 cold storage + 4 days of shelf life in the first season and 21 cold storage + 4 days of shelf life in the second one. It is known that, consumers usually judge the quality of fresh-cut fruit on the basis of appearance and freshness at the time of purchase **(Kader, 2002)**. For chitosan treatment, **Raymond *et al.* (2012)** suggested that chitosan treatment improved external appearance of green pepper during storage at 5ºC.

Table 15. Influence of dipping treatments on fruit general appearance of sweet Pepper fruits during cold storage periods during 2018 and 2019 seasons

Dipping treatments	Cold storage periods (days)					
	0	7	14	21	28	35
			2018 season			
Control (tap water for 2min.)	9.00	8.33	7.77	6.78	4.77	3.11
Chitosan at 0.5 %	9.00	8.33	7.89	6.22	5.22	3.55
Chitosan at 1 %	9.00	8.44	8.11	5.99	5.89	3.11
Citronella oil at 0.5 ml/l	9.00	8.33	7.89	6.00	5.33	3.55
Citronella oil at 1 ml/l	9.00	8.44	8.22	6.33	6.11	2.78
Rosemary oil at 0.5 ml/l	9.00	8.44	7.66	6.22	5.55	3.33
Rosemary oil at 1 ml/l	9.00	8.55	8.00	6.78	6.00	3.77
LSD at 5 %		N.S.	N.S.	N.S.	0.87	0.60
			2019 season			
Control (tap water for 2min.)	9.00	7.77	7.00	6.22	4.55	3.55
Chitosan at 0.5 %	9.00	7.55	6.77	5.66	5.11	3.22
Chitosan at 1 %	9.00	8.33	7.33	6.33	5.44	3.89
Citronella oil at 0.5 ml/l	9.00	7.66	6.89	6.11	4.22	2.89
Citronella oil at 1 ml/l	9.00	8.44	7.33	6.66	5.33	3.88
Rosemary oil at 0.5 ml/l	9.00	7.44	6.77	5.22	4.22	2.77
Rosemary oil at 1 ml/l	9.00	7.88	7.33	6.22	4.77	2.88
LSD at 5 %		0.53	0.56	0.66	0.68	0.78

(GA) scale from 1-9 with: 9= excellent, 7= good, 5= fair, 3= poor, 1= unsalable

Table 16. Influence of dipping treatments on fruit general appearance of sweet pepper fruits after 4 days shelf life during 2018 and 2019 seasons

Dipping treatments	Cold storage periods + 4days shelf life					
	0+4	7+4	14+4	21+4	28+4	35+4
			2018 season			
Control (tap water for 2min.)	9.00	7.75	7.14	6.00	4.22	2.22
Chitosan at 0.5 %	9.00	7.79	7.00	5.44	4.55	3.00
Chitosan at 1 %	9.00	7.97	7.44	5.44	5.11	2.44
Citronella oil at 0.5 ml/l	9.00	7.54	7.44	5.55	4.66	3.22
Citronella oil at 1 ml/l	9.00	7.45	7.77	5.77	5.55	2.33
Rosemary oil at 0.5 ml/l	9.00	7.61	7.22	5.66	5.00	3.00
Rosemary oil at 1 ml/l	9.00	8.14	7.55	6.00	5.33	3.33
LSD at 5 %		0.36	0.47	N.S.	0.66	0.67
			2019 season			
Control (tap water for 2min.)	9.00	7.33	6.33	5.44	4.11	2.77
Chitosan at 0.5 %	9.00	6.89	5.44	5.11	4.44	2.89
Chitosan at 1 %	9.00	7.77	6.77	5.88	4.78	3.44
Citronella oil at 0.5 ml/l	9.00	6.89	6.44	6.33	3.66	2.22
Citronella oil at 1 ml/l	9.00	7.77	7.00	6.22	4.78	3.22
Rosemary oil at 0.5 ml/l	9.00	6.88	6.22	4.55	3.55	2.77
Rosemary oil at 1 ml/l	9.00	7.33	6.89	5.55	4.22	2.22
LSD at 5 %		0.51	0.66	0.70	0.68	0.91

(GA) scale from 1-9 with: 9= excellent, 7= good, 5= fair, 3= poor, 1= unsalable

Microbiological finding

Figure 1 represents the values of total fungi and total bacterial counts, expressed as Log UFC/g and Log CFU/g, respectively, during 7, 14, 21, 28 and 35 days of storage at 8 °C and (Zero time). It can be seen that all the treatments showed lower growth of total fungi and TBC compared to the control and were more effective those with chitosan (1%) and cirtonella essential oil (1 ml/l), in the following order citronella essential oil (1 ml/l) >chitosan (1%)> citronella oil (0.5 ml/l)> chitosan (0.5 %)> rosemary oil (1 ml/l) > rosemary oil (0.5 ml/l). The volatiles such as geranial (30.28%) found in the citronella essential oil in this study (Table 1), possess antifungal activity. Their mode of action also involves penetration and disruption of the membrane structure of microorganisms **(Sivakumar and Bautista-Banos, 2014)**. The statistical analysis indicated that there are significant differences ($p< 0.05$) in relation to the parameter of total fungi and total bacterial counts in the storage time and between the treatments applied in bell pepper. It can be observed that all coatings from chitosan and citronella essential oil, rosemary essential oil showed an increase in only one logarithmic unit of the total fungi and total bacterial count ($p \leq 0.05$) after 20 days of storage compared to the control, in which these counts increased three logarithmic units. This could be the result of essential oil and chitosan, mainly due to the presence of major and minor compounds in citronella oil and rosemary oil that could have an antibacterial effect such as citronella, linalool, p-cymene, β-myrcene, and trans-β-caryophyllene. In general, chitosan by itself is capable of reducing microbial growth.

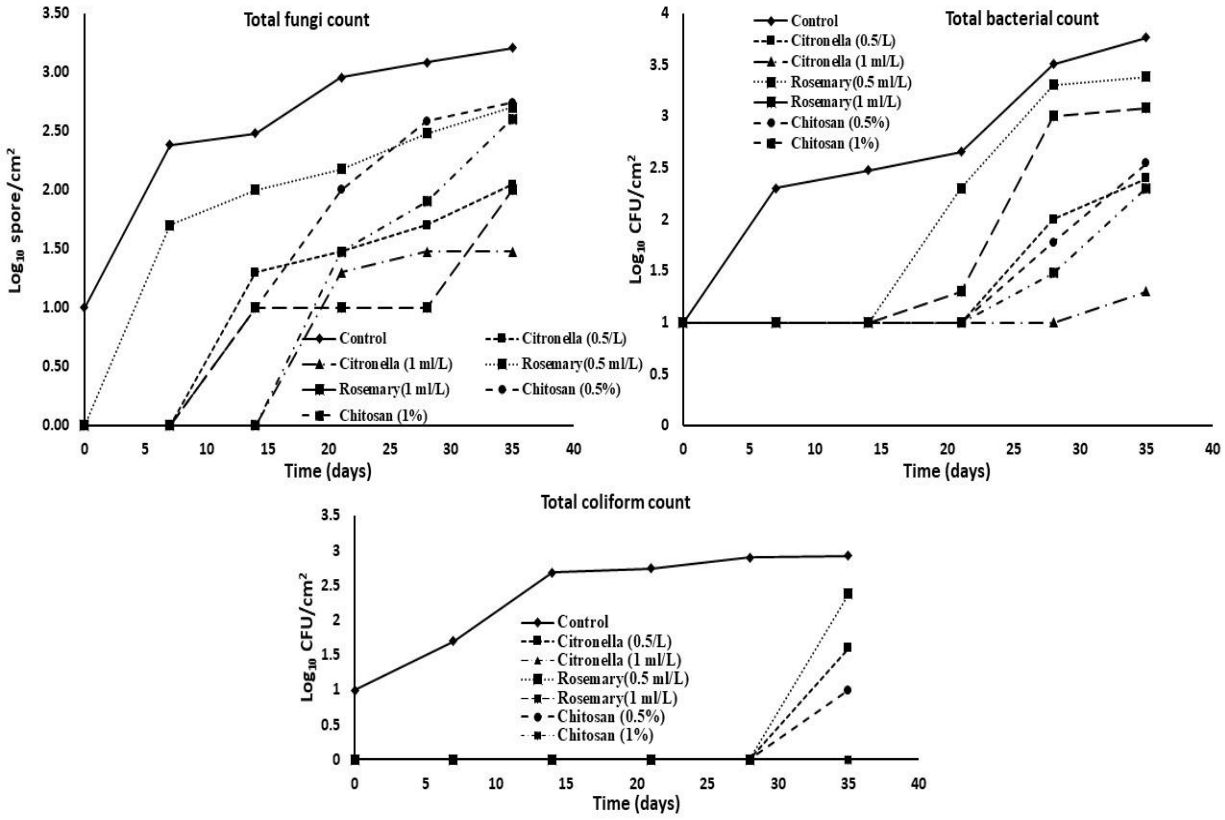

Figure 1. Effect of edible coatings by various concentrations of chitosan, citronella and rosemary essential oils on the microbial growth curves (total fungi, total yeast and total bacteria) associated to sweet pepper (*Capsicum annuum* L.) during cold storage for 35 days at 8±1^{0}C. All values for log number are mean values of three independent replicates.

Also, Figure 1 shows the effect of edible coatings of Chitosan, Citronella oil and Rosemary oil on the decrease in the growth of yeasts in bell pepper stored at 8 ± 1 °C. The statistical analysis indicated that there are significant differences ($p< 0.05$) in relation to the parameter of yeasts in the storage time and between the treatments applied to bell pepper. With respect to the control, it can be observed that the treatments significantly reduced ($p \leq 0.05$) the population of yeasts, resulting in more effective the treatments with chitosan, citronella oil and rosemary oil compared to the control. The population of yeasts reached to 3 Log

CFU/g after 15 days in the case of control while reached the same logarithmic unit in the case of treatment after 35 days. The increase in the population of yeasts between day 0 and 35 days in the treatments with a coating of chitosan (1%) and essential oils (1 ml/l) was under the detection limit; on the contrary, in the control treatment, this increase was of three logarithmic units. Different studies have shown that coatings based on chitosan and essential oils improved fruit quality and shelf-life due to the inhibitory properties of essential oils against pathogenic, food related fungi (**Grande-Tovar, *et al.*, 2018).** In fruit-life studies, the aim is to maintain the population of molds and yeasts below values of 7 log CFU/g, as was achieved with the treatments used here throughout 35 days of testing. However, in the production and supply chain, many factors influence the concentration of the initial microbiota, which is why this methodology is useful if it is applied in the early stages of fruit harvesting.

Conclusion

This study confirmed the variation in chemical profile of the selected Essential oils by GC. Furthermore, this study showed that the functional properties of chitosan films vary depending on the molecular weight and the type of chitosan used. From obtained results, citronella essential oil at 1 ml/l is a promising natural product for preserving stored fruits replacing synthetic additives which are associated with various adverse human health effects. However, no decay was noticed and general appearance of pepper fruits was good at 28 of cold storage in both seasons with dipping treatments of chitosan at 1% or citronella essential oil at 1ml/l rate.

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