

The Role of Chitosan and Selenium in Improving Physico-Chemical Quality and Reducing External Chilling Injury During Low Temperature Storage of "Fuerte" Avocado

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Abstract: Fuerte avocado (*Persea americana* Mill.) fruit is sensitive to chilling injury (CI) when exposed to low temperatures. High lipid content in avocado pulp makes it prone to oxidation, resulting in rancidity and subsequent producing undesirable flavours and losing quality during storage. Mature pre-climacteric avocados were subjected in 2017 and 2018 seasons to pre storage treatments with sodium selenate solution (Se) at 0.05, 0.1, 0.15 and 0.2mg/L and chitosan solution at 0.5, 1.0, 1.5 and 2.0% as dipping for 5- minutes and the untreated fruits were investigated, comparing to the control and commercial treatments. All treatments except commercial treatment were cold stored at 5°C for one or two weeks followed by 5 days at 18°C as simulation for marketing condition, whilst commercial treatment was stored at 10 °C for two weeks and 5 days at 18°C. The fruits were analyzed for physico-chemical quality parameters viz. physiological loss in weight, firmness, total soluble solids, titratable acidity, salute leakage, total chlorophyll and total phenolic compound. Changes in respiration rate and ethylene production were also evaluated. The application of Se at 0.15 and 0.2 mg as well as chitosan at 0.5 and 1.0% were effective in minimizing or preventing chilling injury symptoms and delayed the onset of climacteric peaks of respiration and ethylene production. The delay was associated with reductions in fruit softening and cell membrane permeability as expressed by salute leakage. Also, alleviated a reduction in the chlorophyll content was obtained. Conclusion, overall, the results support that the Se at 0.15 and 0.2 mg was the most effective for alleviation of CI and extending the marketing life of avocado fruit under low temperature storage compared with the control or commercial treatments.

Key words: Avocado • Chilling injury • Cold storage • Sodium selenate • Chitosan • Storability

INTRODUCTION

Avocado (*Persea americana* Mill.) is a noticeable tropical fruit with high marketing ability. The fruit is reported to show some health-promoting properties because of its high concentrations of monounsaturated fatty acids and a major amount of beneficial healthy compounds like tocopherols (vitamin E), sterols and folate [1]. It's also contains a significant source of vitamins A, B and C and minerals such as potassium, phosphorus, magnesium, iron and a rich source of fiber and antioxidants [2, 3].

It is well known that avocado is a climacteric fruit, the ripening period takes in a short time. Ethylene production is limited when avocado is on the tree, but once the fruits is cutting off the tree ethylene production

is triggering , fruits will ripe within 5 to 7 days loading to difficulty in maintaining the resembling fresh condition both in nutritional values and sensory quality [4, 5].

Avocado production areas round the world are typical remote from their overseas markets. Usually these major delay between harvesting and the arrival of the fruit to the place of consumption, throughout this period fruit could ripen. Arriving the fruit for domestic markets may additionally be stored on at times of over production [6]. Thus, there's a necessity to develop suitable in storage technology to delay ripening and supply quality produce for both domestic and export markets [7, 8]. Although the storage period and marketing life of avocados could be prolonged by lowering the storage temperature, a cold injury occurs at temperatures below 7°C. This is a key factor affecting the quality of stored avocados [9].

Selenium is an essential trace element for both humans and animals. Se becomes toxic with high concentrations due to it is replacing sulfur in proteins [10-12] Se is of tremendous interest for many aspects of biomedicine, biochemistry and environmental science [13-15]. Moreover, Se is used for getting a lot of bioactivity and safety for chemical pesticide [16, 17]. Selenium sulfide and sodium selenite have been tested for inhibition of several pathogens [18]. The used of Se at low concentrations as potential alternatives to artificial fungicides for control of plant diseases, may reduce the potential hazardous impact on the environment and human health [19]. Additionally selenium is an important element related with the antioxidant activity [20]. It has effective in delaying plant senescence and a few antioxidative losses because of increased activity of glutathione peroxidase [19]. Fruits treated with selenium maintained total soluble solids, sugar to acid and firmness fruit [21]. It's has been shown to be effective in decreasing the production of ethylene, consequently improving the quality and therefore the market -life in lettuce and chicory and tomato [22, 23]. It's a positive impact on plant protection against abiotic stress in plants at low concentrations wherever it acts as an antioxidant [24].

Selenium is related to the oxygen-sulfur-tellurium group and plants typically modify transfer from soil to the food chain though than not been valued as an essential plant nutrient [25]. Also Se regulates antioxidants and reactive oxygen species preventing absorption of heavy metals, rebuilds cell membrane and chloroplast structures, maintaining the photosynthetic system, control mineral absorption and distributes in antioxidant systems, an increases cell balance [26]. Furthermore, Se led to enhancing resistance toward oxidative stresses and improving both mineral nutrients and vitamin E concentration [27].

Chitosan, a high molecular weight cationic polysaccharide and in theory be used as a preservative coating material for fruits. The additionally is coating safe and shows antifungal activity against many fungi [28]. So, (due to its ability to make a permeable film) chitosan coating may be expected to modify the internal atmosphere furthermore to decrease transpiration losses in fruits [29]. In their researches, Zhang and Quantick [30]; Varasteh *et al.* [31] and Ibukunoluwa *et al.* [32] indicated that chitosan coating had the potential to prolong the storage life and manage decay of strawberry, tomato, peach, pear, kiwifruit and litchi. Chitosan, has a linear polysaccharide consisting of linked 2-amino-deoxy- β -D-

glucan, is that a deacetylated derivative of chitin, which is the second most abundant polysaccharide found in nature after cellulose [33, 34].

Chitosan has been found to be non-toxic, biodegradable, biofunctional and biocompatible and is reported to has strong antimicrobial and antifungal activities [35]. It creates a film on fruit and vegetable surfaces and reduces respiration rate by regulating the permeability of carbon dioxide and oxygen. The 3-NH groups of chitosan can prevent the spread of harmful germs, thus effectively controlling fruit decay. Given these excellent properties of chitosan it has been successfully used our several postharvested fruits, vegetables or their fresh-cut samples [36, 37]. Recently, several reports involving chitosan coating focus mostly on fruit and vegetable varieties or chitosan-based compound coatings they demonstrated that chitosan-treated plants are less susceptible to stress due to unfavorable conditions, such as dehydration, salinity, low or high temperatures [38, 39]. However, chitosan and its derivatives have an ability to inhibit the growth of yeasts, molds and bacteria. However, the applying of chitosan is limited because of its insolubility at neutral pH. To overcome this problem and to enhance solubility of chitosan, completely different acids were used to decrease pH values below pH 6 to improve chitosan solubility [40]. Also, chitosan stimulates important processes of plants on each level of biological organization, from single cells and tissues, through physiological and biochemical processes, to changes on the molecular level associated with expression of genes [41, 42].

Therefore, the aim of this study was to determine the effects of postharvest application of, chitosan and selenium treatments on the changes in physico-chemical quality parameters related to avocado quality during prolonged storage.

MATERIALS AND METHODS

Avocado (*Persea americana* Mill.) cv. "Fuerte" fruits (190- 220 g) were harvested at their commercial harvest maturity in the mid-November, during 2017 and 2018. The fruits were harvested at early morning and directly transferred to the post harvest laboratory in Department of Horticulture, Faculty of Agriculture, Ain Shams University, Cairo, Egypt. The fruits were in good appearance and initially had a firmness of 10.7 - 11.2Kg /force and soluble solids content which ranged from

7.1 to 7.6 %, were uniform in size, shape and maturity and free from defects. The fruits were sorted, dry cleaned and randomized divided into different treatments. Then avocado fruits were washed with chlorinated water (chlorox 0.05% Sodium hypochlorite was used) then air dried followed by immersion in Biohealth fungicide at 2.5 g/l concentration as fast dipping for all fruits then air dried and wrapped in silky paper. Fruits were weighed for recording their initial fresh weight and divided among the following treatments:

- Dipping fruits in 0.05, 0.1, 0.15 and 0.2 mg/L of sodium selenate Na_2SeO_4 solution for 5-minutes at ambient temperature.
- Dipping fruits in 0.5, 1.0, 1.5 and 2.0% of chitosan solution for 5-minutes at ambient temperature.
- Untreated fruits

All the above mentioned treatments for avocado fruits were stored for one or two weeks at $5 \pm 1^\circ\text{C}$ followed by holding for 5 days at $18 \pm 2^\circ\text{C}$ for monitoring and recording the impact of different treatments on the ripening process and quality after transferring fruits from cold storage.

- Control (Commercial treatments): fruits were stored at $10 \pm 1^\circ\text{C}$ for one or two weeks before transferred to ripening room at $18 \pm 2^\circ\text{C}$. (This condition is simulation for commercial storage). Fruits were physically and chemically analyzed after one or two weeks of cold storage at $5 \pm 1^\circ\text{C}$ or $10 \pm 1^\circ\text{C}$ and 5 days at $18 \pm 2^\circ\text{C}$. The fruits of each treatment were arranged in 3 replicates and all were packed in carton boxes with dimensions of (40X30X10 cm) and each replicate contained 4 fruits (12fruits /box).

Fruit Physical Analysis

Visual Chilling Injury Symptoms: Symptoms of chilling injury (CI) were visually assessed upon removal of the fruits from cold storage and threshold. The degree of CI severity, based on external damage, such as surface pitting, water-soaked areas and abnormal ripening, was determined in (15 fruits), sample of each treatment, according to Lederman *et al.* [43], the modified classifications were as follow:

Scale 1 = Fruit of free CI symptoms (0% surface discoloration)

Scale 2 = Fruits of very slight CI symptoms (0-25% surface discoloration)

Scale 3 = Fruits of slight CI symptoms (25-50% surface discoloration)

Scale 4 = Fruits of moderate CI symptoms (50-75% surface discoloration)

Scale 5 = Fruits of severe CI symptoms (more than 75% surface discoloration);

The CI index was calculated according to the following formula:

$$\text{CI index} = \frac{\text{Injury level} \times \text{Number of fruits at the level}}{\text{Total number of fruits in the treatment}}$$

Percentage of Fruit Weight Loss (WL%): Fruits initial weight was recorded before chilling treatments and fruit weight loss was calculated by weighing the same fruits at the end of cold storage durations at $5 \pm 1^\circ\text{C}$ and the end of subsequent storage at $18 \pm 2^\circ\text{C}$. (after 7 days) using the following formula:

$$\text{WL \%} = \frac{\text{Fruit initial weight} - \text{fruit weight at each sampling date}}{\text{Fruit initial weight}} \times 100$$

Percentage of Discarded Fruits: Fruits which showed any sign of decay development by chilling termination and during the subsequent storage period were counted and the percentage of decayed fruits was calculated on the basis of fruits number.

Fruit Firmness (Kg/f): Fruit firmness was determined by peeling the fruit at two equatorial sites and firmness was measured by means of a Wagner® Fruit Firmness Tester, model FT-327, equipped with an 8 mm plunger tip. Values were expressed in kilo gram force (kg/f) after treatments and immediately after cold storage and the days subsequent storage at $18 \pm 2^\circ\text{C}$.

Fruit Chemical Analysis

Total Soluble Solids (T.S.S %): Was determined by hand refractometer.

Titrateable Acidity (TA %): Was determined according to the Official Methods of Analysis [44]. Titrateable acidity was expressed as percentage of citric acid (g citric acid/100 ml juice).

L-Ascorbic Acid (mg / 100 g Fresh Weight): The L-ascorbic acid content (V.C) was determined and expressed as mg/100 g fresh weight following the methods by A.O.A.C., [44].

Solute Leakage (% EC Leakage): Ten grams disks of the fruits tissues were placed in a 100 ml glass beaker containing 30 ml of deionized water and magnetic stirred for 15 min. Electrical conductivity (EC) of the stirred solution was measured using electrical conductivity meter. Stirred solution of each beaker was then replaced by equal volume (30 ml) of deionized water for homogenizing the disks in a blender and the aliquot was then used for measuring EC level as previously described. Percentage of solute leakage was then calculated as EC leakage using formula of Mirdehghan *et al.* [45] as following:

$$\text{EC leaking \%} = \frac{\text{EC of stirred solution}}{\text{EC of stirred solution} + \text{EC of homogenized disks}} \times 100$$

Total Chlorophyll (mg/g Fresh Weight): Total chlorophyll in avocado fruits was determined by using the protocol devised Nagata and Yamashta [46].

Determination of Total Phenolic Content (mg/100g Fresh Weight): Total phenolic contents of the fruit extracts was measured using the modified colorimetric Folin-Ciocalteu method [47].

Respiration Rate (mg CO₂/kg fruits/h.): Carbon dioxide that produced by fruits was determined 10 hrs after treatments and after being (stored for one or two week and 5 days at 18 ±2°C). The air-flow was passed through concentrated NaOH, to insure that air-flow is CO₂ free, before passing into 1-liter jar fruit container (fruit ambient) one fruit/jar was considered as one replicate. The out-coming air-flow was then passed into 100 ml NaOH of 0.1 N for 1 h. Such solution was then titrated against 0.1 N HCl [44] and CO₂ levels produced by the fruits were then calculated as mg CO₂/kg fruits/h.

Ethylene Production Measurements (uL C₂H₄/Kg Fresh Weight Fruit /hr): Levels of ethylene production from fruits treated with 1.0 % Chitosan & 0.15mg/L Selenium & untreated and control) were analyzed after 10 hrs finished from treatments and after (from being stored for one or two week and 5 days at 18 ±2°C). Fruits were incubated in glass jars of 1000 ml volume with a capacity of one fruit per jar. After 2 hrs of incubation, one ml gas sample was with drawn from each jar head space and injected in a Varian Gas Chromatography model Vestia-6000 with a Flame Ionization Detector (FID) for determining the level of ethylene production. Data were then recorded as uL C₂H₄ /Kg fresh fruit weight /hr.

Statistical Analysis: The obtained data in the two studied seasons were conducted in a Completely Randomized Design (CRD) with three replications. The obtained data were subjected to analysis of variance (ANOVA) using MSTAT-C software (MSTAT Michigan University East Lansing). Duncan multiple rang test (LSR) was performed to determine any significant difference among various treatments. p<0.05 was selected as decision for significant differences) according to Steel *et al.* [48].

RESULTS AND DISCUSSION

Physical Determinations

Chilling Injury (Scale): Results tabulated in Table (1) showed that, appearing of chilling injury symptoms was greatly affected with different treatments and advanced in storage days. Dipping "Fuerte" avocado fruits in sodium selenate solution at 0.15 or 0.20 mg was effective in improving storability of the fruits at 5°C with free CI symptoms (1. 0 scale). Commercial fruit stored at 10°C for one or two weeks was similar to the fruits stored at 5°C after pre storage treatments with 1% Chitosan application which recorded very slight CI symptoms (0-25% surface discoloration). Moderate chilling injury was obtained with 0.5% Chitosan and 0.05 mg sodium selenate treatments. On the other hand, untreated "Fuerte" avocado fruits or treated with Chitosan at 2.0 % exhibited (1.50 & 1.33) scale after one week and (2.75 & 2.42) scale after two weeks storage at 5°C. These finding results were nearly similar for the both studied seasons. However, chilling injury symptoms was developed at 18±2°C (marketable period for 5 days) but the positive effect to mentioned treatment still appear.

It is well known that low temperature stresses render the commodity more susceptible to postharvest pathogens and sensitivity chilling injury [49, 50]. In this respect, Se can protect the plant cell from oxidative damage by antioxidant defenses, also has a beneficial role in plants subjected to various abiotic stresses, chilling injury, metals accumulation and drought stress [51-54].

The great effect to chitosan findings in this research is attributed to that chitosan coatings act as barriers, thereby restricting water transfer and protecting fruit skin from mechanical injuries, as well as sealing small that wounds and thus delaying dehydration [55]. This might be due to chitosan, has an ability to that develop a modified system for the exchange of gases and improves the ability of enzymes activity during storage [56].

Table 1: Effect of chitosan and selenium dipping treatments on chilling injures (scale) of "Fuerte" avocado fruits during storage at 5 ± 1°C for one or two weeks followed by marketable life 5 days at 18 ± 2°C during 2017 and 2018 seasons

Treatments	2017 season				2018 season			
	Cold storage for one week		Cold storage for two weeks		Cold storage for one week		Cold storage for two weeks	
	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C
Chitosan 0.5 %	1.00 b	1.16 d	1.33 cd	1.83 cd	1.00 b	1.00 c	1.16 c	1.75 de
Chitosan 1.0 %	1.00 b	1.00 d	1.00 d	1.67 de	1.00 b	1.00 c	1.00 a	2.08 c
Chitosan 1.5 %	1.26 ab	1.92 ab	2.00 bc	2.92 ab	1.67 a	1.92 a	2.25 b	2.75 bc
Chitosan 2.0 %	1.33 a	2.08 ab	2.42 ab	3.25 a	1.42 a	2.25 a	3.16 a	3.67 a
Selenium 0.05mg/L	1.00 b	1.67 bc	1.83 bc	2.42 bc	1.16 ab	1.42 bc	1.92 b	2.83 bc
Selenium 0.10mg/L	1.00 b	1.33 cd	1.67 cd	2.08 cd	1.00 b	1.00 c	1.33 c	1.57 de
Selenium 0.15mg/L	1.00 b	1.00 d	1.00 d	1.00 e	1.00 b	1.00 c	1.00 c	1.00 e
Selenium 0.20mg/L	1.00 b	1.00 d	1.00 d	1.00 e	1.00 b	1.00 c	1.00 c	1.16 e
Untreated	1.50 a	2.25 a	2.75 a	3.50 a	1.58 a	1.75 ab	2.42 b	3.33 ab
Control	1.00 b	1.00 d	1.00 d	1.33 de	1.00 b	1.00 c	1.00 c	1.67 de

Values followed by the same letter (s) are not significantly different at 5% level

Control: Untreated fruit stored at 10±1°C for 7 days followed by Marketable Life 5 days at 18 ± 2°C (commercial)

Untreated fruit stored at 5±1°C for 7 or 15 days followed by Marketable Life 5 days at 18 ± 2°

Table 2: Effect of chitosan and selenium dipping treatments on fruit weight loss % of "Fuerte" avocado fruits during storage at 5 ± 1°C for one or two weeks followed by marketable life 5 days at 18 ± 2°C during 2017 and 2018 seasons.

Treatments	2017 season				2018 season			
	Cold storage for one week		Cold storage for two weeks		Cold storage for one week		Cold storage for two weeks	
	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C
Chitosan 0.5 %	1.78 cd	2.57 cd	2.87 de	4.95 d	1.68 cd	2.42 de	2.64 de	4.65 de
Chitosan 1.0 %	1.98 cd	3.11 bc	3.32 bc	5.12 d	1.89 bc	2.92 c- e	3.12 bc	4.81 de
Chitosan 1.5 %	2.41 ab	4.06 ab	3.45 bc	6.18 b	2.17 ab	4.18 ab	3.62 b	6.49 bc
Chitosan 2.0 %	2.72 a	4.64 a	4.17 a	7.17 a	2.45 a	4.39 a	4.34 a	7.85 a
Selenium 0.05mg/L	1.77 c-e	2.91 cd	3.13 cd	5.70 bc	1.59 cd	3.16 cd	3.00 cd	5.87 c
Selenium 0.10mg/L	1.67 de	2.66 cd	2.67 de	5.31 cd	1.46 d	2.39 de	2.48 ef	4.99 d
Selenium 0.15mg/L	1.42 e	2.28 d	2.31e	4.39 e	1.38 d	2.14 e	2.17 f	4.13 e
Selenium 0.20mg/L	1.46 e	2.17 d	2.64 e	4.62 de	1.32 d	2.05 e	2.46 ef	4.48 e
Untreated	2.35 ab	3.97 ab	3.81 ab	6.83 a	2.12 ab	3.57 a-c	3.43 bc	6.15 bc
Control	2.13 bc	3.56 bc	3.16 cd	5.64 c	1.92 bc	3.27 b-d	2.84 de	5.09 d

Values followed by the same letter (s) are not significantly different at 5% level

Control: Untreated fruit stored at 10±1°C for 7 days followed by Marketable Life 5 days at 18 ± 2°C (commercial)

Untreated fruit stored at 5±1°C for 7 or 15 days followed by Marketable Life 5 days at 18 ± 2°

Weight Loss %: Generally, an increase in weight loss % was recorded with increasing time of cold storage followed by 18 ± 2°C for days (Table 2). It is clear that fruits treated with high selenium concentrations (0.15&0.20 mg) Se led to a reduction in weight loss% regardless of duration and storage temperature. On the contrary, treated fruits with high chitosan concentrations (1.5 and 2.0 %) were exhibited the highest values of weight loss % compared with other treatments or control (commercial Storage). At the end of marketable life (5 days at 18 ± 2°C) after chilling fruits for two weeks untreated fruits and treated with chitosan at 1.5 & 2.0 % exhibited highest percentage of weight loss.

Sodium selenate at 0.15 and 0.20 mg treatments led to takes reduction in the weight loss % followed by Chitosan at 0.5 & 1.0 % with significant difference among them.

The obtained results are in harmony with those of Hernandez-Munoz *et al.* [57] who reported that chitosan coatings act as barriers, thereby restricting water transfer and protecting fruit skin from mechanical injuries, as well as sealing small wounds and thus delaying dehydration. Also Zhao and McGrath [10] and Winkel *et al.* [14] reported that Se plays roles in conferring oxidative stress tolerance by enhancement of the antioxidant defense system in plants.

Table 3: Effect of chitosan and selenium dipping treatments on discarded fruits (%) of "Fuerte" avocado fruits during storage at 5 ± 1°C for one or two week followed by marketable life 5 days at 18 ± 2°C during 2017 and 2018 seasons

Treatments	2017 season				2018 season			
	Cold storage for one week		Cold storage for two weeks		Cold storage for one week		Cold storage for two weeks	
	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C
Chitosan 0.5 %	0.0 b	0.0 c	8.33 bc	16.67 de	0.0 b	0.0 b	16.67 b	16.67c
Chitosan 1.0 %	0.0 b	0.0 c	0.0 c	25.0cd	0.0 b	0.0 b	0.0 c	16.67 c
Chitosan 1.5 %	0.0 b	16.67 b	25.0 a	41.67 b	8.33 ab	16.67 a	33.34 a	50.0 a
Chitosan 2.0 %	16.67 a	33.34 a	25.0 a	58.33 a	8.33ab	25.0 a	25.0 ab	50.0 a
Selenium 0.05mg/L	0.0 b	16.67 b	16.67ab	33.34 bc	0.0 b	16.67 a	33.34 a	41.67 ab
Selenium 0.10mg/L	0.0 b	0.0 c	0.0 c	16.67 de	0.0 b	0.0 b	16.67 b	33.34 b
Selenium 0.15mg/L	0.0 b	0.0 c	0.0 c	0.0 e	0.0 b	0.0 b	0.0 c	0.0 d
Selenium 0.20mg/L	0.0 b	0.0 c	0.0 c	0.0 e	0.0 b	0.0 b	0.0 c	8.33 cd
Untreated	8.33 ab	25.0 ab	16.67ab	41.67 b	16.67 a	25.0 a	25.5 ab	50.0 a
Control	0.0 b	0.0 c	0.0 c	8.33 e	0.0 b	0.0 b	0.0 c	8.33 cd

Values followed by the same letter (s) are not significantly different at 5% level

Control: Untreated fruit stored at 10±1°C for 7 days followed by Marketable Life 5 days at 18 ± 2°C (commercial)

Untreated fruit stored at 5±1°C for 7 or 15 days followed by Marketable Life 5 days at 18 ± 2°

Table 4: Effect of chitosan and selenium dipping treatments on fruit firmness (Kg/ force) of "Fuerte" avocado fruits during storage at 5 ± 1°C for one or two weeks followed by marketable life 5 days at 18 ± 2°C during 2017 and 2018 seasons

Treatments	2017 season				2018 season			
	Cold storage for one week		Cold storage for two weeks		Cold storage for one week		Cold storage for two weeks	
	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C
Chitosan 0.5 %	8.6 cd	6.0 cd	6.1 cd	3.7cd	8.6 cd	6.0 b	6.1 bc	4.1 c
Chitosan 1.0 %	8.7cd	6.5 bc	6.5 bc	4.1 bc	8.6 cd	6.6 b	6.8 b	4.4 bc
Chitosan 1.5 %	8.1 de	5.2 e	5.5 de	2.8 e	8.0 de	5.0 c	5.0 d	2.1 ef
Chitosan 2.0 %	7.6 e	4.7 e	5.1 e	1.2 f	7.4 e	4.4 c	4.1 e	1.5 f
Selenium 0.05mg/L	8.8 cd	6.2 b	6.0 cd	3.6 d	9.0 bc	6.4 b	6.3 bc	3.2 d
Selenium 0.10mg/L	9.2 bc	6.8 b	6.7 bc	4.2 bc	9.3 bc	6.2 b	6.3 bc	3.6 cd
Selenium 0.15mg/L	10.5 a	8.1 a	7.6 a	4.6 ab	10.1 a	8.4 a	7.6 a	4.9 ab
Selenium 0.20mg/L	9.8 ab	8.4 a	7.1 ab	5.1 a	9.4 ab	8.0 a	7.6 a	5.4 a
Untreated	8.0 de	5.4 de	5.2 e	2.0 e	8.2 d	5.0 c	5.2 d	2.4 e
Control	8.6 cd	6.6 bc	5.8 d	3.6 d	8.1 de	6.0 b	5.6 cd	3.7 cd

Values followed by the same letter (s) are not significantly different at 5% level

Control: Untreated fruit stored at 10±1°C for 7 days followed by Marketable Life 5 days at 18 ± 2°C (commercial)

Untreated fruit stored at 5±1°C for 7 or 15 days followed by Marketable Life 5 days at 18 ± 2°

Discarded Fruits (%): An evident increase in discarded fruits (%) was observed after marketable life at 18 ± 2°C especially with the fruit chilled for two weeks irrespective of the used treatments was detected (Table 3). Slight differences were noticed among treatments of Se at 0.15 and 0.20 mg as well as 0.5 % chitosan and the commercial treatment exhibited the least values of discarded fruit until the end of marketable period (5days) at 18 ± 2°C after chilled fruits for one or two weeks during both seasons. On the other side, untreated fruits and 2.0 % chitosan treated fruits recorded the highest percentage of

discarded fruits 41.67 & 58.33 %) and (50.0. & 50.00%) without significant differences between them, after 5 days at 18 ± 2 °C with chilled fruit for two weeks during both seasons respectively.

The beneficial effect demonstrated in this work could be explained due to El-Ghaouth *et al.* [29] who suggested that chitosan induces chitinase, a defense enzyme, which catalyzes the hydrolysis of chitin, a common component of fungal cell walls. Fruit treated with chitosan showed no CI, during the whole storage period, this could be due to antibacterial membranes that produced from a mixture of

hydrolyzed starch that causes the semi permeable barrier in cell wall which prevents spores entering in the cell wall [57]. In this concern, selenium decreased fungal incidence in harvested fruits by affecting intracellular plasma membrane of pathogens [14].

Fruit Firmness (Kg/ Force): A clear decrease in fruit firmness of "Fuerte" avocado fruits during storage periods (either at cold storage or marketable life at $18\pm 2^{\circ}\text{C}$ with all used treatment was observed (Table 4). Selenium application at 0.20 mg slows fruit firmness loss (reduced of fruit softness) during the subsequent storage at $18\pm 2^{\circ}\text{C}$., slight differences between this treatment and selenium application at 0.15 mg (5.1, 4.6, 5.4 and 4.9 Kg/force) during both seasons after 5 days at $18\pm 2^{\circ}\text{C}$ for the subsequent two weeks of cold storage respectively. Low concentrations of both substances (Se at 0.05 & 0.1 mg and Ch at 0.5 & 1.0 %) recorded moderate values of firmness that are similar to commercial storage treatment, during both seasons after 5 days at $18\pm 2^{\circ}\text{C}$ subsequent two weeks of cold storage. A sharp decrease in fruit firmness were noticed with untreated and chitosan treated fruits at 1.5 and 2.0 % without significant differences between them.

Initial Sample Recorded 11.2 and 10.7 kg/ Force at First and Second Season: The obtained data are in harmony with McCollum and McDonald [58] and Jesus and Yahia [59] who found that fruit was firmness decreased in chilled fruits due to the membranes damage. Additionally, Pezzarossa *et al.* [60] and Zhu *et al.* [23] reported that, firmness was higher in selenium-treated compared with the control at harvest time and after storage which occurred because of less ethylene production.

Se could delay the softening of peach fruits during storage due to the antioxidative property, also se delayed senescence there by delaying the reduction in fruit firmness during storage [60, 22]. Chitosan coating provides beneficial effects on flesh firmness. It is thought that this is due to coating which caused a reduction in cell wall degradation which in turn maintained cell turgidity and protected structure of cell wall [61].

Fruit Chemical Analysis

Total Soluble Solids (TSS%): Generally, it could be noticed that total soluble solids % (Table 5) were increased with the advances in cold storage durations regardless of pre-storage treatments. The differences between treatments were appeared during their presence

in the temperature at $18\pm 2^{\circ}\text{C}$. The highest T.S.S% were obtained with Se-treated fruits at 0.2 mg during both seasons. A reduction in total soluble solids was observed with untreated and chitosan treated fruits at 2.0 % which were more sensitive to chilling injury (previously discussed) that recorded low values at the end of days at $18\pm 2^{\circ}\text{C}$ after chilled fruits of two weeks at $5\pm 1^{\circ}\text{C}$ during first and second seasons.

It is well know that, soluble solids influence the taste of the products and are represented as acids, salts, vitamins, amino acids, sugars and other substances that important for food quality. Therefore, the use of Se improved the flavor of fruit when applying the element in the form of selenate [60].

Titrateable Acidity %: As shown in Table (6) selenium treatment at 0.20 mg exhibited an evident decrease in acidity amounting to (0.37 and 0.34 %) during both seasons after 5 days at $18\pm 2^{\circ}\text{C}$ subsequent the two weeks respectively, which is similar to its optimal storage of fruits (commercial storage) at $10\pm 1^{\circ}\text{C}$. No significant differences were found between chitosan at 0.5 & 1.0 % and selenium at 0.05 & 0.1 mg treatments which gave moderate values of acidity.

On the other hand, fruits treated with chitosan at 1.5 & 2.0% and the untreated chilled fruits for two weeks showing symptoms of CI (previously discussed) as well as a significant increase was observed in titrateable acidity (TA) compared to the other treatments and control which reached to 0.95% at first season and 0.90 % at second season. The inhibition of such enzymes as a resulted of CI may resulted in the accumulation of their substances which are acidic metabolic substances. The accumulations of such middle substances resulted in the subsequent development of CI symptoms. Since most of these middle substances are acids, it is logic therefore, to assume that higher acidity level will be paralleled to the fruits having symptoms of CI. It is suggested, therefore, that acidity increment is an accurate indicator for fruit CI. This phenomenon is unique, practical and need to be tested on other fruit species sensitive to CI, especially tropical and sub-tropical fruits.

The obtained data could be explained by Liu *et al.* [21], who reported that selenium is an important component of antioxidant enzymes, such as glutathione peroxidase, thioredoxin reductase and iodothyronine deiodinases and several selenoproteins or selenoenzymes. However Pezzarossa *et al.* [62] and Malorgio *et al.* [22] showed that Se plays a significant role in protecting cells against oxidative damage from

Table 5: Effect of chitosan and selenium dipping treatments on treatments on total soluble solids (TSS%) of "Fuerte" avocado fruits during storage at 5 ± 1°C for one or two weeks followed by marketable life 5 days at 18 ± 2°C during 2017 and 2018 seasons

Treatments	2017 season				2018 season			
	Cold storage for one week		Cold storage for two weeks		Cold storage for one week		Cold storage for two weeks	
	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C
Chitosan 0.5 %	7.2 bc	7.6 bc	7.5 ab	7.9 bc	7.7 ab	8.0 bc	7.9 bc	8.3 cd
Chitosan 1.0 %	7.3 ab	8.1 ab	7.7 ab	8.0 b	7.9 ab	8.3 ab	8.1 ab	8.5 bc
Chitosan 1.5 %	6.6 d	6.0 d	6.6 c	5.2 d	6.8 c	6.2 d	6.9 d	5.0 ef
Chitosan 2.0 %	6.4 d	6.0 d	6.1 c	4.5 e	6.8 c	6.1 d	6.3 e	4.6 f
Selenium 0.05mg/L	7.1 bc	7.1 c	7.2 b	7.4 c	7.7 ab	7.5 c	7.6 c	7.8 d
Selenium 0.10mg/L	7.2 bc	7.6 bc	7.5 ab	7.9 bc	7.6 b	8.0 bc	8.0 c	8.4 bc
Selenium 0.15mg/L	7.3 ab	7.9 ab	7.7 ab	8.3 ab	7.8 ab	8.5 ab	8.2 ab	9.0 ab
Selenium 0.20mg/L	7.5 ab	8.0 ab	8.0 a	8.4 ab	8.1 ab	8.6 a	8.6 a	9.0 ab
Untreated	6.8 cd	6.4 d	6.4 c	5.4 d	7.0 c	6.5 d	6.0 e	5.6 e
Control	7.8 a	8.3 a	7.9 a	8.6 a	8.2 a	8.8 a	8.5 a	9.2 a

Values followed by the same letter (s) are not significantly different at 5% level

Control: Untreated fruit stored at 10±1°C for 7 days followed by Marketable Life 5 days at 18 ± 2°C (commercial)

Untreated fruit stored at 5±1°C for 7 or 15 days followed by Marketable Life 5 days at 18 ± 2°

Initial sample recorded 7.12 and 7.56 % at first and second season

Table 6: Effect of chitosan and selenium dipping treatments on titratable acidity (TA%) of "Fuerte" avocado fruits during storage at 5 ± 1°C for one or two weeks followed by marketable life 5 days at 18 ± 2°C during 2017 and 2018 seasons

Treatments	2017 season				2018 season			
	Cold storage for one week		Cold storage for two weeks		Cold storage for one week		Cold storage for two weeks	
	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C
Chitosan 0.5 %	0.63 b	0.58 b	0.57 c	0.46 c	0.59 b	0.49 bc	0.56 c	0.47 c
Chitosan 1.0 %	0.61 bc	0.56 b	0.54 cd	0.44 c	0.58 bc	0.47 bc	0.55 cd	0.43 c
Chitosan 1.5 %	0.67 a	0.75 a	0.80 a	0.87 a	0.66 a	0.77 a	0.81 a	0.86 ab
Chitosan 2.0 %	0.69 a	0.77 a	0.73 ab	0.95 a	0.69 a	0.75 a	0.76 ab	0.90 a
Selenium 0.05mg/L	0.63 b	0.54 bc	0.57 c	0.47 c	0.61 b	0.53 b	0.57 c	0.46 c
Selenium 0.10mg/L	0.61 bc	0.55 bc	0.55 cd	0.46 c	0.60 b	0.47 bc	0.55 c	0.42 cd
Selenium 0.15mg/L	0.60 bc	0.51 cd	0.50 de	0.40 cd	0.55 c	0.45 cd	0.51 d	0.36 de
Selenium 0.20mg/L	0.61 bc	0.48 d	0.51 de	0.37 d	0.57 bc	0.44 cd	0.48 de	0.34 e
Untreated	0.65 ab	0.71 a	0.68 b	0.79 b	0.61 b	0.73 a	0.72 b	0.81 b
Control	0.56 c	0.45 d	0.47 e	0.33 d	0.53 c	0.40 d	0.45 e	0.30 e

Values followed by the same letter (s) are not significantly different at 5% level

Control: Untreated fruit stored at 10±1°C for 7 days followed by Marketable Life 5 days at 18 ± 2°C (commercial)

Untreated fruit stored at 5±1°C for 7 or 15 days followed by Marketable Life 5 days at 18 ± 2°

Initial sample recorded 0.71 and 0.67 % at first and second season

reactive oxygen species and reactive nitrogen species. Titratable acidity was decreased linearly with increasing period of storage. This might be due to the conversion of acids into salts and sugars and their further utilization in cold storage respiration and metabolic processes [62].

L-Ascorbic Acid: It is clear from (Table 7) that L-ascorbic acid content was decreased with the advances in storage period for treated and untreated fruits. This finding could be attributed to the conversion of L-ascorbic acid to

dehydroascorbic acid and decreasing the active form of ascorbic. An evident decline in the rate of L-ascorbic acid content in chilled fruits treated was noticed for two weeks than chilled fruits for one week. Chitosan at 2.0 % treated fruits contained less values of L-ascorbic acid than the others. This finding is correlated to the previously mentioned about acidity and T.S.S where this treatment recorded less fruit quality characters. The reduction in L-ascorbic acid in these fruits was decreased from 19.5 mg to 9.7 mg/100 g fresh weight (50.2 % losses) in the first

Table 7: Effect of chitosan and selenium dipping treatments on L-ascorbic acid content (mg/100g fresh weight) of "Fuerte" avocado fruits during storage at 5 ± 1°C for one or two weeks followed by marketable life 5 days at 18 ± 2°C during 2017 and 2018 seasons

Treatments	2017 season				2018 season			
	Cold storage for one week		Cold storage for two weeks		Cold storage for one week		Cold storage for two weeks	
	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C
Chitosan 0.5 %	17.5 c	15.5 c	15.7 c	12.9 c	16.3 b	15.0 c	14.8 d	13.6 c
Chitosan 1.0 %	17.3 c	16.1 b	16.5 b	14.8 b	16.3 b	15.3 bc	15.6 bc	14.1 bc
Chitosan 1.5 %	16.1 d	14.0 d	13.2 e	11.1 d	15.7 cd	13.7 d	11.7 e	10.8 de
Chitosan 2.0 %	16.4 d	14.1 d	12.8 e	9.7 e	15.3 d	12.5 e	11.8 e	10.3 e
Selenium 0.05mg/L	17.7 bc	15.7 bc	15.5 e	13.5 c	16.2 b	14.9c	15.2 cd	14.0 bc
Selenium 0.10mg/L	17.6 c	16.3 b	16.7 bc	15.0 b	16.3 b	15.2 bc	15.6 bc	14.4 b
Selenium 0.15mg/L	18.7 a	17.0 a	17.0 ab	16.8 a	17.4 a	16.1 a	16.7 a	15.7 a
Selenium 0.20mg/L	18.6 a	17.2 a	17.5 a	16.4 a	17.5 a	15.7 ab	16.3 ab	15.7 a
Untreated	16, 8 d	15.3 c	14.8 d	11.3 d	16.0 bc	14.3 d	12.3 e	11.5 d
Control	18.2 ab	16.9 a	16.5 b	15.2 c	17.2 a	15.7 ab	15.3 cd	14.1 bc

Values followed by the same letter (s) are not significantly different at 5% level

Control: Untreated fruit stored at 10±1°C for 7 days followed by Marketable Life 5 days at 18 ± 2°C (commercial)

Untreated fruit stored at 5±1°C for 7 or 15 days followed by Marketable Life 5 days at 18 ± 2°

Initial sample recorded 19.5 and 17.9 mg/100 g fresh weight at first and second season

season and from 17.9 mg to 10.3 mg/100 g fresh weight (42.45 % losses) in the second season. In contrast selenium at 2.0 mg treatment exhibited high values of L-ascorbic acid (from initial sample to the end of subsequent days of two weeks at cold storage) also L-ascorbic acid decreased from 19.7 to 16.8 mg (13.7% in the first season) and from 17.9 to 15.0 (12.3% in the second season), with the commercial storage treatment, it recorded moderate values of L-ascorbic acid (22.8 and 21.2 %) during the two studied seasons.

Ascorbic acid carries out a number of non-antioxidant functions in the cell. It has been implicated in the regulation of cell division, cell cycle progression and plays a role in antioxidant defenses. The ascorbic acid synthesis, was decreased with advancement of storage period, due to the activity of oxidation of ascorbic acid by oxidizing enzymes like ascorbic acid oxidase (ascorbinase), peroxidase, catalase and polyphenol oxidase [63, 64]. Coatings serve as a protective layer and control the permeability of O₂ and CO₂, thus decreased the autoxidation of ascorbic acid. Higher level of ascorbic acid in chitosan-coated fruit might reflect on the low oxygen permeability, slow down the respiration rate, which reduced the activity of the enzymes involved in the oxidation of ascorbic acid and delays the deteriorative oxidation reaction of ascorbic acid in the fruit [65]. Moreover, the selenium modifies the plant redox balance probably due to increasing activities of antioxidant enzymes [24]. The gradual increase in total ascorbate content is in agreement with the antioxidant activities of

the Se fortified fruits. Ascorbate is the major antioxidant in every plant cell compartment that gradually increases the antioxidant activities [66].

Solute Leakage % (EC): It is observed from Table (8), that there was an evident increase in solute leakage with advanced in storage days with all treated or untreated fruits. The increase in solute leakage with increasing fruit life could be considered a reason for appearing as chilling injury of avocado fruits. It is also noted that treated fruits with selenium at higher concentrations (0.15 and 0.20 mg) give lower values of solute leakage(24.6 & 26.1%) in 2017 season and 22.8 & 24.5%) in 2018 season after the end marketable days 18 ± 2°C after chilled fruits for two weeks, respectively The reduction in solute leakage explained the ability of selenium treated fruits to chilling injury, followed by the fruits treated with 1.0 % chitosan, which gave similar values of optimal storage 30.9 and 28.8 (control treatment). On the contrary, fruits treated with 2.0 % chitosan exhibited the highest values of solute leakage (49.5% in first season and 47.2% in second season) at the end marketable days at 18 ± 2°C after chilled fruits for two weeks respectively.

Generally, cell membrane is a chilling sensitive component of the fruit cell. Several metabolic enzymes are membranes bound. When fruit are chilled, membrane deteriorate and a sudden drop in the activity of membrane bound enzyme will be obtained. As a result, the substrate of such enzymes (middle metabolites) will accumulate causing chilling injury development.

Table 8: Effect of chitosan and selenium dipping treatments on solute leakage % (EC) of "Fuerte" avocado fruits during storage at 5 ± 1°C for one or two weeks followed by marketable life 5 days at 18 ± 2°C during 2017 and 2018 seasons

Treatments	2017 season				2018 season			
	Cold storage for one week		Cold storage for two weeks		Cold storage for one week		Cold storage for two weeks	
	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C
Chitosan 0.5 %	10.9 c	26.3 b	21.5 c	38.7 c	9.8 c	24.0 c	20.0 c	36.0 c
Chitosan 1.0 %	11.2 c	24.5 c	19.7 de	32.5 de	10.2 c	23.5 c	19.3 c	27.2 e
Chitosan 1.5 %	14.1 ab	29.5 a	25.5 b	43.6 b	13.0 b	28.1 ab	23.9 b	42.3 b
Chitosan 2.0 %	14.6 a	30.0 a	29.1 a	49.5 a	14.5 a	30.9 a	30.0 a	47.2 a
Selenium 0.05mg/L	10.7 c	26.1 b	20.5 d	34.0 d	10.0 c	24.5 c	24.1 b	35.2 c
Selenium 0.10mg/L	9.5 c	23.7 c	20.1 d	35.2 d	8.6 d	22.5 c	20.0 c	31.6 d
Selenium 0.15mg/L	8.7 d	19.4 d	17.1 ef	24.5 f	8.6 d	20.2 d	16.1 d	22.8 f
Selenium 0.20mg/L	9.1 d	18.7 d	16.3 f	26.1 f	8.7 d	19.2 d	16.0 d	23.5 f
Untreated	13.4 b	27.7 b	26.4 b	41.2 bc	12.5 b	26.6 b	27.8 a	40.3 b
Control	10.5 c	19.4 d	18.2 e	30.9 e	10.2 c	19.0 d	17.6 cd	28.8 de

Values followed by the same letter (s) are not significantly different at 5% level

Control: Untreated fruit stored at 10±1°C for 7 days followed by Marketable Life 5 days at 18 ± 2°C (commercial)

Untreated fruit stored at 5±1°C for 7 or 15 days followed by Marketable Life 5 days at 18 ± 2°

Initial sample recorded 7.38 and 8.14 % (EC) at first and second season

Table 9: Effect of chitosan and selenium dipping treatments on total chlorophyll (mg/g fresh weight) of "Fuerte" avocado fruits during storage at 5 ± 1°C for one or two weeks followed by marketable life 5 days at 18 ± 2°C during 2017 and 2018 seasons

Treatments	2017 season				2018 season			
	Cold storage for one week		Cold storage for two weeks		Cold storage for one week		Cold storage for two weeks	
	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C
Chitosan 0.5 %	1.76 bc	1.35 c	1.47 cd	0.96 bc	1.61 c	1.24 c	1.35 cd	0.88 d
Chitosan 1.0 %	1.70 c	1.32 c	1.51 cd	0.98 b	1.60 c	1.34 b	1.43 bc	0.90 cd
Chitosan 1.5 %	1.56 d	1.08 d	1.30 e	0.84 de	1.51 d	1.02 d	1.20 e	0.81 e
Chitosan 2.0 %	1.55 d	1.12 d	1.16 f	0.76 e	1.43 d	1.03 d	1.17 e	0.77 e
Selenium 0.05mg/L	1.75 bc	1.30 e	1.41 d	0.90 cd	1.60 c	1.33 b	1.27 de	0.92 cd
Selenium 0.10mg/L	1.84 ab	1.46 b	1.57 bc	1.02 b	1.76 ab	1.36 ab	1.48 b	0.98 bc
Selenium 0.15mg/L	1.87 a	1.49 ab	1.63 ab	1.17 a	1.74 ab	1.43 a	1.51 b	1.04 ab
Selenium 0.20mg/L	1.90 a	1.56 a	1.71 a	1.26 a	1.80 a	1.40 ab	1.67 a	1.11 a
Untreated	1.61 d	1.17 d	1.23 ef	0.81 e	1.49 d	1.16 c	1.20 e	0.73 e
Control	1.77 bc	1.33 c	1.45 d	0.98 b	1.70 b	1.24 c	1.32 d	0.95 b

Values followed by the same letter (s) are not significantly different at 5% level

Control: Untreated fruit stored at 10±1°C for 7 days followed by Marketable Life 5 days at 18 ± 2°C (commercial)

Untreated fruit stored at 5±1°C for 7 or 15 days followed by Marketable Life 5 days at 18 ± 2°

Initial sample recorded 1.96 and 1.84 mg/100g fresh weight at first and second season

With chilling injury, membrane permeability was considerably increased due to its deterioration allowing more solute leakage. Electrolyte leakage was increased significantly in chilled fruits [67, 68]. After coating with chitosan, the increase in the cell membrane permeability and MDA content can be restrained and the cell membrane of post-harvest fruit and vegetable may maximally execute normal physiological function. Chitosan is a promising tool for preventing postharvest oxidative damage during cold storage [27, 69]. Se plays important roles in the maintenance of cell membrane structure and cell integrity under stress [70].

Total Chlorophyll (mg/100g Fresh Weight): Chlorophyll content was gradually decreased within cold storage in treated or untreated fruits (Table 9). Chlorophyll values were also decreased significantly when chilling avocado for two weeks. Se had a significant effect on total chlorophyll of avocado fruits compared to the untreated avocado or the other treatments. It was noted that, concentration of se at 0.15, 0.2 mg and 1% of chitosan were the most effective treatment in maintaining the level of chlorophyll gradient and achieving the highest value of chlorophyll. In contrast the high concentrations of chitosan (1.5 and 2.0%) caused a significant reduction in chlorophyll content.

Table 10: Effect of chitosan and selenium dipping treatments on total phenols (mg /100 g fresh weight of "Fuerte" avocado fruits during storage at 5 ± 1°C for one or two weeks followed by marketable life 5 days at 18 ± 2°C during 2017 and 2018 seasons

Treatments	2017 season				2018 season			
	Cold storage for one week		Cold storage for two weeks		Cold storage for one week		Cold storage for two weeks	
	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C
	Chitosan 0.5 %	0.174 d	0.289 c	0.302 c	0.414 c	0.164 c	0.284 c	0.275 cd
Chitosan 1.0 %	0.185 c	0.276 c	0.258 d	0.428 c	0.170 bc	0.266 c	0.273 d	0.390cd
Chitosan 1.5 %	0.197 ab	0.341 b	0.489 a	0.608 a	0.189 a	0.316 b	0.237 a	0.543 b
Chitosan 2.0 %	0.201 a	0.367 a	0.441 b	0.584 a	0.185 a	0.372 a	0.474 ab	0.600 a
Selenium 0.05mg/L	0.188 bc	0.285 c	0.326 c	0.469 b	0.166 c	0.260 cd	0.455 c	0.430 c
Selenium 0.10mg/L	0.179 cd	0.260 d	0.264 d	0.374 d	0.173 bc	0.259 cd	0.304 d	0.352 d
Selenium 0.15mg/L	0.160 e	0.249 d	0.213 e	0.331 e	0.149 e	0.232 de	0.248 e	0.301 e
Selenium 0.20mg/L	0.155 e	0.231 e	0.241de	0.357 de	0.153 e	0.217 e	0.194 e	0.295 e
Untreated	0.206 a	0.359 ab	0.251 b	0.579 a	0.181 ab	0.357 a	0.426 b	0.534 b
Control	0.183 c	0.284 c	0.317 c	0.436 c	0.166 c	0.258 cd	0.304 c	0.407 c

Values followed by the same letter (s) are not significantly different at 5% level

Control: Untreated fruit stored at 10±1°C for 7 days followed by Marketable Life 5 days at 18 ± 2°C (commercial)

Untreated fruit stored at 5±1°C for 7 or 15 days followed by Marketable Life 5 days at 18 ± 2°

Initial sample recorded 0.102 and 0.093 mg at first and second season

The untreated fruit recorded the least values of chlorophyll content which give 0.81 and 0.73 mg/100g F.wt compared to 1.26 and 1.26 mg/100g F.wt the fruits treated with 0.2 mg Se in the end of marketing after two weeks of cold storage in both seasons.

The obtained data results nearly similar to those of Han *et al.* [71] who reported that, (Se) alleviated a reduction in the chlorophyll content, potentially by promoting the absorption of mineral elements related to chlorophyll synthesis in the plants. Se may participate in the synthesis of chlorophyll precursors in the form of Se amino acids. Meanwhile, Germ *et al.* [72] mentioned that, (Se) has a promoting influence on chlorophyll content and this effect depends on soaking time in selenium. This may be due to that Se could stimulate the respiration rates and the flow of electrons in respiratory chain and accelerate the chlorophyll biosynthesis.

Total Phenolic Content (mg/100g Fresh Weight):

Total phenols as shown in Table (10) was increased in fruits with increasing and prolonging periods storage days in both seasons. These compounds were increased with untreated fruit and chitosan at 1.5 & 2.0 % with no significant differences among them since they recorded 0.579 & 608 & 0.584 and 0.534 & 0.543 & 0.600 mg/100g fresh weight after the end marketable life period at 18 ± 2°C and after chilled fruit for two weeks in both seasons. Also the results show that the low values of total phenols in marketable life at 18 ± 2°C after cold

storage for two weeks (0.357&0.331 and 0.295 & 0.301 mg / 100g fresh weight) were obtained with fruits treated by selenium at 0.20 and 0.15 mg in 2017 and 2018 seasons. No significant differences were observed between fruit treated with chitosan at 0.05 & 1.0 % and commercial storage treatment which gave values ranged from 0.414 to 0.436 and from 0.389 to 0.407 mg /100g fresh weight at the end in marketable life at 18 ± 2°C after cold storage for two weeks and during both seasons.

Increase phenolic compounds in tissues during chilling treatments may be partially due to chilling adaptation as defense mechanisms and also to mediate these stresses. It is assumed that freezing temperatures with distribution of cell membranes may trigger the release of oxidative and hydrolytic enzymes that would destroy the antioxidants probably, deactivating these enzymes avoid the loss of phenolics and therefore lead to the increase of total phenolics content [73]. Chitosan may inhibit the activity of polyphenol oxidase, which is involved in the process of phenolic compound degradation [74]. This might due that chitosan, which has an ability to develop a modify system for the exchange of gases and improves the ability of enzymes activity during the storage [36].

Respiration Rate (mg CO₂/kg-hr): Respiration rate, (Table 11) was decreased at the end of cold storage periods (one or two weeks) with all used treatments compared with commercial storage of the fruits.

Table 11: Effect of chitosan and selenium dipping treatments on respiration rate (mg CO₂/Kg fruit /hr.) of "Fuerte" avocado fruits during storage at 5 ± 1°C for one or two weeks followed by marketable life 5 days at 18 ± 2°C during 2017 and 2018 seasons

Treatments	2017 season				2018 season			
	Cold storage for one week		Cold storage for two weeks		Cold storage for one week		Cold storage for two weeks	
	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C
Chitosan 0.5 %	34.15 cd	85.11 cd	49.53 c	116.4c-e	32.10 cd	78.30 e	47.29 c	115.9cd
Chitosan 1.0 %	31.34 d	91.26 c	42.30 d	110.4 ef	30.82 de	83.38de	49.71 c	112.7cd
Chitosan 1.5 %	40.76 b	101.67 b	63.60 b	147.3 b	37.10 bc	92.52bc	63.14 b	151.8 b
Chitosan 2.0 %	41.00 b	114.51 a	67.22 b	158.7 a	40.56 b	104, 2a	66.01 b	160.3 a
Selenium 0.05mg/L	32.15 ed	86.20 cd	46.93cd	123.4 c	31.15 de	87.23 cd	50.88c	118.5 c
Selenium 0.10mg/L	30.12 de	80.91 de	42.41d	112.5 de	33.95 cd	78.43 e	42.91de	105.8 de
Selenium 0.15mg/L	25.11 e	71.34 f	35.13e	109.9 ef	26.25 e	68.47 f	38.66ef	103.3 ef
Selenium 0.20mg/L	23.63 e	76.41 ef	35.21e	104.2 f	27.03 e	70.87 f	33.96 f	97.1 f
Untreated	38.27 bc	106, 11 b	64.50 b	146.1 b	39.11 b	94.26 b	61.33b	149.4 b
Control	53.67 a	93.15 c	76.17 a	120.6 cd	46.06 a	80.37 de	79.88 a	122.8 c

Values followed by the same letter (s) are not significantly different at 5% level

Control: Untreated fruit stored at 10±1°C for 7 days followed by Marketable Life 5 days at 18 ± 2°C (commercial)

Untreated fruit stored at 5±1°C for 7 or 15 days followed by Marketable Life 5 days at 18 ± 2°

Initial sample recorded 31.19 and 27.55 mg CO₂/Kg fruit /hr at first and second season

Table 12: Effect of chitosan and selenium dipping treatments on ethylene production uL C₂H₄ /Kg fresh weight fruit /h of "Fuerte" avocado fruits during storage at 5 ± 1°C for one or two weeks followed by marketable life 5 days at 18 ± 2°C during 2017 and 2018 seasons

Treatments	2017 season				2018 season			
	Cold storage for one week		Cold storage for two weeks		Cold storage for one week		Cold storage for two weeks	
	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C	Out of storage	5 days at 18 ± 2°C
Chitosan 1.0 %	10.59 bc	30.78 b	11.64 c	36.05 b	11.63 c	31.15 b	14.52 c	38.81 b
Selenium 0.15 mg/L	12.84 b	32.57 b	14.73 b	42.58 a	14.77 b	36.64 a	19.08 b	44.11 a
Untreated	8.63 c	18.69 c	8.11 d	16.47 c	7.15 d	17.82 c	10.13 d	18.95 c
Control	19.96 a	36.07 a	25.32 a	47.29 a	20.13 a	38.52 a	27.59 a	46.34 a

Values followed by the same letter (s) are not significantly different at 5% level

Control: Untreated fruit stored at 10±1°C for 7 days followed by Marketable Life 5 days at 18 ± 2°C (commercial)

Untreated fruit stored at 5±1°C for 7 or 15 days followed by Marketable Life 5 days at 18 ± 2°

Initial sample recorded 10.47 and 12.39 uL C₂H₄ /Kg fresh weight fruit /h at first and second season

Sodium selenate treatments at 0.15 and 0.2 mg led to a reduction in respiration rate after one and two weeks of cold storage and after 5 days holding at the (18 ± 2°C) (109.9 and 104.2 mg CO₂/kg fruit /hr.) at first season and (103.3 and 97.1 mg CO₂/kg fruit /hr.) at second season after 5 days at 18 ± 2°C for two weeks chilling fruit respectively. However, treated fruits with chitosan at 0.5 & 1.0 % and sodium selenate at 0.05 and 0.1 mg gave values similar to the commercially fruits during marketable life at 18 ± 2°C. Untreated fruits and those treated with chitosan at 1.5 & 2.0 % exhibited a sharp increase in respiration rate after removal from cold storage and during

holding at 18 ± 2°C ranging from 146.1 to 158.7 and 149.4 to 160.3 mg CO₂/kg fruit/hr at the first and second seasons respectively after two weeks at 5 ± 1°C and subsequent 5 days at 18 ± 2°C.

The obtained results were nearly similar to those explained by Sabehat *et al.* [75] who demonstrate that the rate of oxidation relative to glycolysis might lead to the accumulation of an intermediate respiratory substances at toxic levels or fermentation causing chilling injury development. Fruit treated with chitosan and selenium showed no CI, during the whole storage period, this could be due to antibacterial membranes produced from a

mixture of hydrolyzed starch that causes the semipermeable barrier in cell wall which prevents spores entering in the cell wall [68]. The decreased respiration rate which might be due to reduction oxygen supply on the fruit surface [76]. Also, Du *et al.* [77] reported that application of chitosan coating inhibited respiration rates of fruits.

Ethylene Production (uL C₂H₄ /Kg Fresh Weight Fruit /hr.): Data tabulated in Table (12) show the effect of different treatments on ethylene production of "Fuerte" avocado fruits after one and two weeks of cold storage at 5±1°C and subsequent 7 days at 18 ±2°C. Generally, ethylene production was decreased during cold storage period with all used treatments compared with the commercial fruits. At the end of cold storage (two weeks), selenium treatment at 0.15 mg caused a gradual increase in ethylene production of initial sample and during subsequent days of cold storage. An evident increase in ethylene production was observed during days of storage at 18 ±2°C with all used treatments. Selenium treatments achieved the highest values of ethylene production (42.58 and 44.11 uL/kg fruit fresh weight/hr) during 2017 and 2018 seasons at the end of subsequent days after two weeks of chilled fruit at 5 ±1 °C respectively. On the contrary, the untreated fruits recorded the least values of ethylene production .A sharp decrease in ethylene production during cold storage was recorded which means that fruits failure in ripening, in the end of subsequent days after two weeks of chilled fruit at 5 ±1 °C respectively.

Ethylene production and the capacity to convert ACC to ethylene were not enhanced by storage temperatures, which cause chilling injury to the peel [43]. Lower biosynthesis ethylene action inside the package during storage is dependent on the action of this plant growth regulator [61, 78]. Se is effective in delaying ripening and senescence, respectively, through a decrease in ethylene biosynthesis and enhancing the antioxidant defence system [24, 23]. The application of Se at 0.15 & 0.2 mg as well as chitosan at 0.5 & 1.0 edible coating could extend the marketable life at 18 ± 2°C of fruits up to avocado several days. In general, treating avocado samples could delay ripening process that lowering respiration rate and ethylene production which slowing the ripening process. The least fresh weight loss and the longest shelf life, TSS values, enhanced the fruits quality and maintained the physiological responses including chilling injury and fruit rot as compared to the untreated samples.

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