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Quality Parameters of "Champagne" Loquat Fruit as Affected by Calcium Chloride and Chitosan Treatments

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Abstract: "Champagne" Loquat fruits (*Eriobotrya japonica* Lindl*)* were harvested at firm ripe stage from a commercial orchard located in Ismailia Governorate, Egypt in 2017 and 2018 seasons. The fruits were treated with 1) Distilled water (control) 2) 0.75% Chitosan coating solutions for 20 sec. 3) 1.0 % Chitosan coating solutions (w/v) for 20 sec. 4) 2 % $CaCl_2$ solution for 2 min 5) 3 % $CaCl_2$ solution for 2 min to study the effect of these treatments on quality parameters of loquat fruits. The fruits were placed in one-layer soft plastic boxes and stored at 7°C + 90-95% RH for 4 wks. After cold storage the treated fruits kept for 3 days at 20°C + 60-65% RH to simulate market condition. Weight loss, chilling injury, browning index, Firmness (N), soluble solids content (SSC), Acidity, Vitamin C, Total phenolics, antioxidant activity and overall acceptability were assessed. The chitosan coating and $CaCl_2$ treatments significantly diminished weight loss and inhibited browning of fruits during cold storage when compared with the untreated fruits. All treatments significantly retained maximum firmness and increased SSC%, Vitamin C, total phenolics and antioxidant activity. Generally, the data illustrated that chitosan coating and $CaCl_2$ treatments followed by cold storage at 7 °C significantly maintained fruit quality and extended fruit life.

Key words: *Loquat-Quality · C0ld storage · Chitosan coating · $CaCl_2$ · Antioxidant activity*

INTRODUCTION

Loquat (*Eriobotrya japonica* Lindl) is subtropical evergreen fruit tree. Fruits has a nutritional value and health benefits (good source of minerals and carotenoids) while the kernel is rich in protein and carbohydrates [1]. The fruit is used either fresh or processed (juice or jam). It is grouped as Non-climacteric fruit having a very short shelf life. The fruit is very perishable and sensitive to mechanical injure. Loquat fruit is also a good source of natural antioxidants such as polyphenols, carotenoids and flavonoids [2].

Chilling injury (pulp woodiness, adhesion of peel to the pulp, leathery and juiceless pulp and internal browning) occurs in loquat fruit when they are stored at temperatures lower than 5°C [3-6].

Chitosan is a polycationic biopolymer created artificially by chemical deacetylation of chitin which is found in arthropod exoskeletons. Useful impacts of this biopolymer as an edible coating have been decided for many produces such as strawberry fruit and papaya fruit [7, 8]. The chitosan coating diminishes moisture transfer, respiration rate and browning appearance which related to the reduction of lipoxygenase (LOX) and PPO activities [9, 10].

Calcium (Ca_2^+) contributing to the linkages between pectic substances within the cell walls [11]. It is an essential element as well as playing a potential role in preserving the postharvest quality of fruit and vegetables [12, 13]. Ca_2^+ ion increases the cohesion of cell-walls [11]. It has a vital role in delaying senescence symptoms and fruit ripening [14].

In this study, Chitosan coating and $CaCl_2$ were used to determine the postharvest quality attributes of loquat fruit during cold storage at 7°C and subsequent shelf life at 20°C.

MATERIALS AND METHODS

This experiment was conducted during two successive seasons of 2017 and 2018 on "Champagne" Loquat (*Eriobotrya japonica* Lindl) fruits. The fruits were harvested at firm ripe stage from a commercial

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orchard located in Ismailia Governorate, Egypt. It was transported immediately to postharvest lab, Horticulture Department, Suez Canal University. The fruits were screened for optimum ripe stage, freshness, free from defects and any mechanical injury. Sound fruits were clipped and washed with distilled water then air dried. A lot of 1000 fruits divided randomly into five groups each one contained 200 fruit and were treated by soaking as follows:

- Distilled water (control)
- Chitosan coating solutions 0.75% (w/v) for 20 sec.
- Chitosan coating solutions 1.0 % chitosan (w/v) for 20 sec. [15].

Chitosan coating solutions was prepared as a described by Petriccione *et al*. [16].

- 2 % $CaCl_2$ solution for 2 min.
- 3 % $CaCl_2$ solution for 2 min. [17, 18]

All treated fruits were allowed to dry at 20°C for 2 hr, each of the previous group had 200 fruits divided into two unequal groups (80 labeled fruit), individually weighed and used for weight loss determination, chilling injury incidence and browning index. Then, 120 fruits were used for quality evaluation, (Firmness, soluble solids content, Acidity, Ascorbic acid content, Total phenolic compound, Antioxidant activity and overall acceptability. The fruits were placed in one-layer soft plastic boxes and stored at 7°C + 90-95% RH for 4 weeks [18, 19]. Fruit samples (24 fruits from each treatment) were removed from the cold storage for quality evaluation at 1, 2, 3 and 4 weeks of storage. Ten fruits were analyzed directly from the cold storage while the rest were kept for 3 days at 20 °C + 60-65% RH to simulate market condition and fruit quality estimation.

Weight Loss: Labeled fruits were weighed individually at each sampling time (one week). Weight loss was expressed as a percentage of fruits original fresh weight according to the following Eq.:

$$Weight\ loss\ \% = \frac{Initial\ weight - sample\ weight\ (after\ storage)}{Initial\ weight} \times 100$$

Evaluation of Chilling Injury: After each of the 1st, 2nd, 3rd and 4th weeks of cold storage at 7°C and 90-95 % RH fruits were evaluated for chilling injury severity according to the following scale: 0 = sound (no symptoms), 1 = slight (a few symptoms), 2 = moderate (symptoms covering up to 30% of the fruit) and 3 = severe (extensive symptoms covering more than 30% of the fruit) [20]. Symptoms appeared as pulp woodiness, adhesion of peel to the pulp, leathery and juiceless pulp and internal browning [6].

Browning Index: At weekly intervals browning index were assessed by the extension of browning area as described by Wang *et al*. [21] and Attiq Akhtar *et al.* [18] using 80 fruits for the following scale: 0= no browning; 1=less than 25% browning; 2= 50% browning; 3= 75% browning; 4= more than 75% browning. The browning index was calculated using the following Eq.:

$$Browning\ Index = \left(\frac{1xN1 + 2x\,N2 + 3x\,N3 + 4x\,N4}{4XN}\right) \times 100.$$

where, N = total number of fruits observed and N1, N2, N3 and N4 are the number of fruits showing the different degrees of browning. Fruit was considered unacceptable for consumption if it had browning grades of 3 -4.

Fruit Firmness: Was measured from two opposite sides by peeling the fruit at two equatorial sites then using a hand Magness Taylor pressure tester equipped with an 8mm plunger tip, data expressed in Newton (N).

Soluble Solids Content (SSC): Was measured in fruit juice by using ATTAGO hand refractometer at 20°C and expressed as percent.

Titratable Acidity: Was determined in fruit juice by using 0.1 NaOH in the presence of phenolphthalein until pH 8.0 and expressed as malic acid percent. It was calculated by using the following Eq.:

$$\%TA = \frac{(ml\ NaOH\ used)\ (Normality\ of\ NaOH)\ (Equivalent\ wt.\ of\ malic\ acid)}{(wt.of\ sample)(vol.of\ aliquot\ taken)}$$

Ascorbic acid (Vitamin C): Was determined in fruit juice as mg Ascorbic acid / 100 ml juice by titration with 2,6 dichlorophenol-indophenol solution in the presence of oxalic acid solution [22].

Total Phenolic Compounds: Were determined using Folin-Ciocalteau reagent and absorbance was read at 760 nm. The values were expressed as mg of Gallic acid / 100 g fresh weight [23].

The Antioxidant Activity: The samples were analyzed by using DPPH assay according to the procedures of Gadow *et al*. [24] and Maisuthisakul *et al*. [25]. Diluted sample extract (100 mL, prepared at 5 different concentrations and provided 10-90% inhibition for DPPH radical) was added into 4 mL of freshly prepared DPPH (2,2-diphenyl-1-picryhydrazyl radical) solutions (6 × 10-5 M in MeOH). The mixtures were shaken and kept in the dark at room temperature for 30 min. Absorbance values of the final solutions were estimated at 515 nm using a spectrophotometer (UNICO UV/Visible 2100, USA) versus a control solution (80% MeOH, in place of sample, in DPPH solution). The antioxidant activity of the samples was expressed as percentage inhibition of the DPPH radical, which was calculated by using the following Eq.:

$$I\ \%\ (\text{inhibition percentage}) = \frac{Ac - As}{Ac} \times 100$$

where, *Ac* and *As* are the absorbance values of the control and test samples, respectively. The sample extract concentration providing 50% inhibition (EC_{50}) of the DPPH radical was calculated by plotting the concentration versus inhibition %.

Overall Acceptability: Estimation of general appearance by browning index: The scale used was: 5 = absence of symptoms, 4 = slight occurrence, 3 = moderate, 2 = severe and 1= extremely high. Evaluation was done by a panel of ten assessors weekly during cold storage at 7°C.

Statistical Analysis: The experimental design was completely randomized blocks [26]. Groups of four replicates per treatment for the cold storage period were established. The data analyzed using the Co-Stat program version 3 (Co.Hort. Software) and treatments means were statistically compared using the Duncan's [27] multiple range test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Weight Loss Percentage: It was gradually increased during cold storage and the maximum loss was observed for the control fruit (Table 1; 4.8 and 4.3% in both seasons, respectively). However, all Chitosan and Calcium chloride treatments significantly ($P \leq 0.05$) inhibited weight loss (ranged from 2.0 and 2.7%). No significant different in weight loss among all treatments except for the control fruits. The same trend was detected at 20°C for 3 days shelf life in both seasons (Table 2). Calcium application is effective in membrane functionality and maintenance, which resulted in reducing weight loss of loquat fruit [28]. Also, El-Shiekh [17] revealed that postharvest sprayed

Table 1: Weight loss%, browning index and Firmness (N) of "Champagne" loquat fruits as affected by Chitosan and Calcium chloride treatments after cold storage at 7°C for 4 weeks in 2017 and 2018 seasons

Treatments	Weight loss%	[b] Browning index	Firmness (N)
		Season 2017	
At harvest	---	---	3.8 c
Control	[a] 4.8 a	4.4 a	3.9 c
Chitosan 0.75	2.7 b	1.7 b	5.4 b
Chitosan 1	2.2 b	1.0 b	5.6 b
2% $CaCl_2$	2.6 b	1.8 b	5.4 b
3% $CaCl_2$	2.3 b	1.1 b	6.2 a
		Season 2018	
At harvest	---	---	3.7 c
Control	4.3 a	4.6 a	4.2 b
Chitosan 0.75	2.5 b	1.6 b	5.5 a
Chitosan 1	2.0 b	1.7 b	5.6 a
2% $CaCl_2$	2.3 b	1.5 b	5.5 a
3% $CaCl_2$	2.1 b	1.3 b	6.1 a

[a] The values within a column with different letters are significantly different at $P \leq 0.05$ according to the Duncan's multiple range test.

[b] BI= 0= no browning; 1=less than 25% browning; 2= 50% browning; 3= 75% browning; 4= more than 75% browning.

Table 2: Weight loss%, browning index and Firmness (N) of "Champagne" loquat fruits as affected by Chitosan and Calcium chloride treatments after cold storage at 7°C for 4 weeks plus 3 days at 20°C in 2017 and 2018 seasons

Treatments	Weight loss%	[b] Browning index	Firmness (N)
At harvest	---	---	3.8 c
		Season 2017	
Control	[a] 8.8 a	4.8 a	3.6 c
Chitosan 0.75	3.3 b	1.9 b	5.1 b
Chitosan 1	3.3 b	1.3 b	5.0 b
2% $CaCl_2$	3.9 b	1.9 b	5.2 b
3% $CaCl_2$	3.8 b	1.3 b	5.8 a
		Season 2018	
At harvest	-	-	3.7 c
Control	9.3 a	4.7 a	3.5 c
Chitosan 0.75	3.4 c	1.9 b	5.1 b
Chitosan 1	3.1 c	1.3 b	5.3 b
2% $CaCl_2$	4.3 b	1.7 b	5.2 b
3% $CaCl_2$	4.2 b	1.3 b	5.9 a

[a] The values within a column with different letters are significantly different at $P \leq 0.05$ according to the Duncan's multiple range test.

[b] BI= 0= no browning; 1=less than 25% browning; 2= 50% browning; 3= 75% browning; 4= more than 75% browning.

"Le Conte" pear trees with calcium chloride significantly decreased fruit weight loss under cold storage conditions. Mahajan and Dhatt [29] found the same results when treated Asian pear with postharvest calcium chloride during cold storage. Chitosan forms semi-permeable film that regulates gas exchange, reduces water loss and slows fruit ripening. Since Chitosan is used as a coating, respiration rate and water loss are reduced in general for many horticultural commodities [15].

Chilling Injury: Major chilling injury symptoms were pulp woodiness, adhesion of peel to the pulp, leathery and juiceless pulp and internal browning [6]. Few of these symptoms were first apparent after 3 weeks of cold storage at 7°C for control loquat fruit at low level and was relatively increased by the end of the cold storage. But, all remaining treatments had no chilling injury symptoms, till the end of storage (data not shown).

Browning Index (BI): The browning index of all fruits increased towards the end of cold storage. There were significant ($P \leq 0.05$) differences among control treatment and all other treatments. A wide range of external Browning among control and other treatments (Figs. 1 and 2) was noticed. The index percentages were 43%, 16%, 13%, 16% and 12% for control, Chitosan 0.75, Chitosan 1, CaCl 2% and CaCl 3%, respectively, as average of two seasons. In the same direction, Tables 1 and 2 indicated a significant difference in Browning index values among all treatments. Hodges [30] concluded that injury of Oxidative membrane allows the normal separation of PPO enzyme and oxidizable substrates (polyphenols) to be mixed, leading to browning. This work indicated that $CaCl_2$ treatments resulted in lower BI compared to the control fruits. This was in agreement with Poovaiah [31] and Picchioni *et al.* [32] who decided that calcium helps to keep membrane steadiness. As for Chitosan treatments, Chong *et al.* [33] attributed the significant decrease in Browning index of the cold-stored fruits by chitosan coating to the inhibition the PPO activities and LOX which are involved in browning.

Firmness (N): From Tables 1 and 2 it can be noticed that the initial fruit firmness at harvest was 3.8 and 3.7 N in both seasons, respectively and it was increased significantly ($P \leq 0.05$) at the end of cold storage and subsequent shelf life at 20°C in all treated fruits. Except control treatment, no significant difference was assessed. At the end of 4 weeks of cold storage, the final firmness for control fruits were 3.9 and 4.2 (N) and after shelf life period were 3.6 and 3.5 (N). On the contrary, a significant inhibition of fruit softening was found in chitosan coating and Calcium chloride treatments, the maximum significant values were recorded with CaCl 3% of 6.2 and 6.1(N) at the end of cold storage and of 6.2 and 6.1 (N) after shelf life period. Retaining firmness in calcium-treated fruits may be attributed to calcium accumulation in the cell walls resulting in simplifying cross-linking of pectic

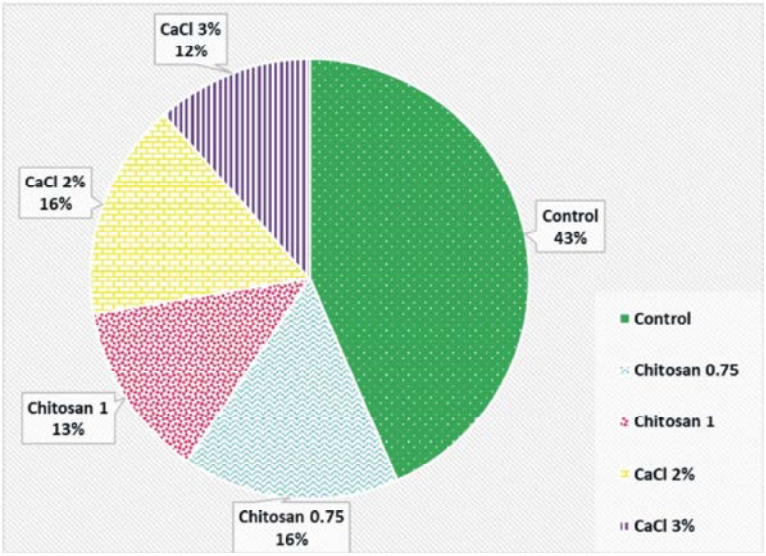

Fig. 1: Overall acceptability expressed as browning index of "Champagne" loquat fruits as affected by Chitosan and Calcium chloride treatments after cold storage at 7°C for 4 weeks. The percentages as average of two seasons

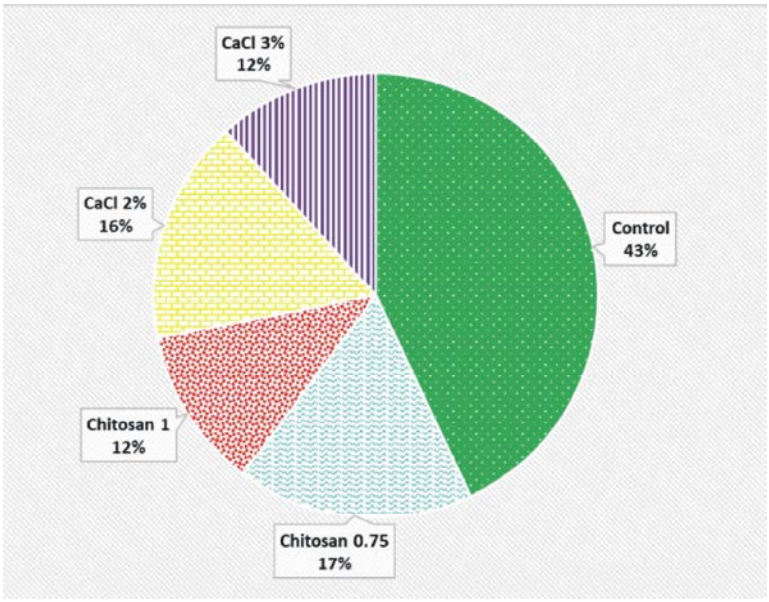

Fig. 2: Overall acceptability expressed as browning index of "Champagne" loquat fruits as affected by Chitosan and Calcium chloride treatments after cold storage at 7°C for 4 weeks plus 3 days at 20°C. The percentages as average of two seasons

polymers, which increases wall resilience and cell cohesion [34]. These findings came in line with those found by Shuiliang *et al.* [35] and Attiq Akhtar *et al.* [18]. Also, Pareek *et al.* [1] revealed that coating loquat fruit with Chitosan certainly kept the flesh firm.

Soluble Solids Content (SSC): The initial SSC percentage was 8.9 and 9.2 in the two seasons, respectively (Tables 3 and 4). A general trend of a significant ($P \leq 0.05$) increase in SSC% in all treatments was noticed at the end of cold storage period and then

Table 3: Soluble solids content SSC, titratable acidity TA, Vitamin C, total phenols and antioxidant activity of "Champagne" loquat fruits as affected by Chitosan and Calcium chloride treatments after cold storage at 7°C for 4 weeks in 2017 and 2018 seasons

Treatments	SSC%	Acidity%	Vitamin C%	[b] Total phenols%	[c] EC50 values b (mg mg-1 DPPH)
			Season 2017		
At harvest	[a] 8.9 b	0.90 b	24.4 c	88.0 d	48.8 c
Control	11.6 a	0.44 a	30.2 b	100.0 c	56.7 b
Chitosan 0.75	12.0 a	0. 42 a	40.6 a	113.0 b	66.2 a
Chitosan 1	11.8 a	0. 43 a	40.8 a	114.0 b	67.8 a
2% $CaCl_2$	11.8 a	0.42 a	40.0 a	112.8 b	68.0 a
3% $CaCl_2$	11.8 a	0.44 a	40.9 a	120.8 a	68.1 a
			Season 2018		
At harvest	9.2 b	0.88 b	22.8 c	90.0 d	47.8 c
Control	11.8 a	0.42 a	32.8 b	101.5 c	53.4 b
Chitosan 0.75	12.2 a	0.43 a	40.5 a	123.8 b	66.5 a
Chitosan 1	11.8 a	0.43 a	40.9 a	123.1 b	67.8 a
2% $CaCl_2$	12.0 a	0.44 a	40.5 a	123.5 b	67.6 a
3% $CaCl_2$	12.1 a	0.44 a	41.1 a	126.4 a	68.9 a

[a] The values within a column with different letters are significantly different at $P \leq 0.05$ according to the Duncan's multiple range test.
[b] (mg /100 g fresh weight)
[c] EC50 of Trolox (an antioxidant vitamin E derivative) was determined as 0.12 ± 0.01 mg mg-1 DPPH

Table 4: Soluble solids content SSC, titratable acidity TA, Vitamin C, total phenols and antioxidant activity of "Champagne" loquat fruits as affected by Chitosan and Calcium chloride treatments after cold storage at 7°C for 4 weeks plus 3 days at 20°C in 2017 and 2018 season

Treatments	SSC%	Acidity%	Vitamin C%	[b] Total phenols%	[c] EC50 values (mg mg-1 DPPH)
			Season 2017		
At harvest	[a] 8.9 c	0.90 a	24.4 a	88.0 c	48.7 c
Control	10.6 b	0.50 b	33.8 b	110.0 b	58.9 b
Chitosan 0.75	11.4 a	0. 48 b	41.2 a	123.0 a	66.7 a
Chitosan 1	11.8 a	0. 50 b	41.5 a	125.0 a	68.9 a
2% $CaCl_2$	11.3 ab	0.49 b	40.6 a	122.8 a	68.2 a
3% $CaCl_2$	11.5 a	0.50 b	42.1 a	125.8 a	69.2 a
			Season 2018		
At harvest	9.2 c	0.88 a	22.8 c	90.0 c	47.8 c
Control	10.8 b	0.49 b	34.5 b	112.5 b	55.7 b
Chitosan 0.75	11.6 ab	0.50 b	40.4 a	128.6 a	66.5 a
Chitosan 1	11.8 a	0.48 b	42.5 a	127.9 a	67.8 a
2% $CaCl_2$	11.4 ab	0.50 b	41.0 a	126.7 a	68.5 a
3% $CaCl_2$	11.8 a	0.50 b	42.3 a	129.2 a	69.4 a

[a] The values within a column with different letters are significantly different at $P \leq 0.05$ according to the Duncan's multiple range test.
[b] (mg /100 g fresh weight)
[c] EC50 of Trolox (an antioxidant vitamin E derivative) was determined as 0.12 ± 0.01 mg mg-1 DPPH

fluctuated during subsequent shelf life conditions but continued to be higher than at harvest. No significant difference among all treatments in SSC% was observed. This increment in SSC % is a result of starch conversion to sugar and therefore transformation of sugars to carbon dioxide and H_2O [36]. The results of this work consistent with those found for other varieties by Ding *et al.* [2]; Lin *et al*. [3] and Zheng *et al.* [4].

Titratable Acidity (TA): High fruit acidity considered the most important factor affecting in loquat fruit quality and its commercial value [1]. At harvest, Malic acid concentration was 0.90 and 0.88 % in the two seasons, respectively, then decreased significantly at the end of storage and shelf life periods. No significant ($P \leq 0.05$) difference was noticed among all treatments in malic acid concentration (Tables 3 and 4). So, both treatments of Chitosan coating or Calcium dips were not effective in reducing acidity at used concentrations [2]. Ball [37] stated that acidity reduction was attributed to anaerobic respiration and therefore, the occurrence of fermentation or break up of acids to sugars in fruits during respiration. In this work it seems that Calcium and Chitosan treatments did not have any significant effect on fermentation. These results seem to be comply with the previous studies of Cai *et al.* [6] and Attiq Akhtar *et al.* [18].

Ascorbic Acid (Vitamin C): It is clear from Tables 3 and 4 that there was a significant ($P \leq 0.05$) increase in Vitamin C in all treatments both after cold storage or shelf life periods. Vitamin C concentrations at harvest were 24.4 and 22.8. Whilst it reached (30.2 and 32.8), (40.6 and 40.5), (40.8 and 40.9), (40.0 and 40.5) and (40.9 and 41.1) for Control, Chitosan 0.75, Chitosan 1, CaCl 2% and CaCl 3% at the end of cold storage period in the two seasons, respectively. No significant difference was noticed among all treatments in Vitamin C in the two seasons. There was a significant difference between the control and the other treatments. Ascorbic acid is an important parameter of nutrient quality and because of its oxidation, it is very sensitive to degradation [38]. Both treatments had a significant effect on maintaining ascorbic acid content in loquat fruit. This may due to delaying the oxidation of Vitamin C. these findings are in the same direction of Ruoyi *et al.* [39] data on peaches and Attiq Akhtar *et al* [18] on loquat fruits.

Total Phenolic Compounds: Total phenolic content in both control and all treatments significantly increased during storage period. The maximum significant values were obtained by CaCl 3% treated fruit (120.8 and 126.4) after cold storage period in the two seasons, respectively. But, after 3 days at ambient temperature, no significant different among treatments except for the control which scored the lowest values of 100.0 and 101.5 in both seasons, respectively (Tables 3 and 4). The data reported herein agreed with the results of Cordenunsi *et al.* [40] on strawberry and with Ding *et al.* [5] and Cao *et al*. [41] on loquats.

Antioxidant Activity: The changes in antioxidant activity of loquat fruits over storage time and shelf life period is shown in Tables 3 and 4. All treatments significantly increased the antioxidant activity and maintained higher levels of it after cold storage period at 7°C and subsequent shelf life at 20°C. There were significant differences among the control and all other treatments in antioxidant activity in both season of the experiment. The treated fruits exhibited the highest phenolic content. DPPH is generally used for the assessment of antioxidant activity. The stronger activity of DPPH in loquat fruit stored at 7°C is radical scavenging may be due to the higher phenolic level [41]. Storage at cold temperatures kept the overall phenolic and DPPH radical scavenging content higher.

Overall Acceptability: it is quite evident from (Figs. 1 and 2) that the Browning index (BI) percentages of the control fruits recorded the highest levels of 43% followed by Chitosan 0.75 and 2%$CaCl_2$ achieved the same percentage of 16% then Chitosan 1 and 3% $CaCl_2$ recorded 13% and 12% respectively, this percentages as average of two seasons. Fig.1 represents the browning index percentages after cold storage at 7°C for 4 weeks. The data presented in Fig 2 showed the same percentages of Browning index after shelf life at 20°C for 3 days. Both results, after cold storage or shelf life periods had the same trend.

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