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Pre-harvest spray of chitosan, calcium chloride and low temperature storage (7 ^{0}C) effect on biochemical attributes of strawberry cv. Camarosa

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Abstract
The present study aimed to measure the effects of chitosan and $CaCl_2$ treatments on biochemical attributes of strawberry fruits under refrigerated condition (at 7°C). The pre-harvest sprayed treatments had significantly reduced the TA and spoilage per cent. Results showed an increased in total sugar and total antioxidant activity during the storage period of fruits. Most efficient treatment for TA, total antioxidant activity and spoilage was found with chitosan @ 6 g/L + $CaCl_2$ @ 1.00% (T_{11} - 0.63%, 21.19 µmol TE g^{-1} FW and 4.61% respectively). The value of total sugar was observed maximum with chitosan @6 g/L + $CaCl_2$ @ 1.50% (T_{12} - 6.24%) while the lowest was noted in control (T_1- 3.69%). Chitosan sprays reduced post-harvest fungal rot, decreases respiration rate and maintained the keeping quality of the fruit compared with control. Calcium chloride serves as a source of calcium which modifies the cell wall composition in comparison to the control treatment and enhances the fruit quality especially by maintaining firmness.

Keywords: Strawberry, chemicals, ascorbic acid, spoilage, titrable acidity, antioxidant activity

Introduction
Strawberry is an attractive, nutritious, luscious and tasty fruit with a distinct and pleasant aroma. It occupies a unique place among cultivated berry fruits. It is mainly consumed as fresh. Jam and syrup are also made from strawberry. All cultivated varieties are octaploid in nature. Fruit matures rapidly, ripening occurs in 30 to 50 days after flowering. The quality of strawberry fruits comprises mainly appearance (colour, shape, size, free from decay), flavor, firmness and nutritional value. On the other part, several bio-chemical (ascorbic acid, sugar, antioxidant, titrable acidity etc.) parameters also determine the acceptability of consumer. An increase in anthocyanin content is accompanied by decrease in firmness and chlorophyll content. The accumulation of anthocyanin coincides with the induction of the activities of phenylalanine ammonialyase and uridine diphosphate glucose, flavanoid – glucosyltransferase enzymes (Given *et al.* 1988) [11].

Strawberry fruits contain ascorbic acid in the predominant form of vitamin C. Sucrose is the primary source of glucose and fructose present in strawberry. Berries are one of the main source of ellagic acid in our diet, a hydroxybenzoic acid normally present as polymer (ellagitannin) or glycosylated derivative, which has been shown to have anti-carcinogenic action ((Parr & Bolwell, 2000; Toma´s-Barbera´n & Clifford, 2000) [18, 26]. The amount of ascorbic acid ranged from 47 to 80 mg/100g of fresh fruits. There is an increased concern among consumers about the potentially harmful health effects of chemical residues (Klein and Lurie, 1991) [12], and development of chemical tolerance in post-harvest pathogens (Spotts and Cervantes, 1986) [25]. Thus, alternative approaches are necessary to maintain the quality and enhance the shelf-life of strawberries.

Chitosan, a high molecular weight b-(1,4)-glucosamine polymer, is an important structural component of the cell wall of some plant-pathogenic fungi, especially Zygomycetes (Bartnicki-Garcia, 1970) [4]. These characteristics allow chitosan to be considered a non-toxic, biodegradable and biocompatible product (Azevedo *et al.*, 2007) [2]. Calcium is the most important mineral element determining fruit quality. The multiple roles of Ca are associated with the plant cell. Soluble Ca is involved in protein phosphorylation via Ca-Cal- modulin binding. Pre-harvest Ca treatments used to increase Ca content of the cell wall were effective in delaying senescence, resulting in firmer and higher fruit quality (Serrano *et al.*, 2004; Kluter *et al.*, 2006 and Raese and Drake, 2006) [22, 13, 20].

Chitosan being a natural compound has a high potential in suppressing ethylene production and fungal decay in harvested fruits. In addition, calcium chloride has helped to maintain the firmness by modifying cell wall composition and delaying the senescence. Thus, by the application of these chemicals increases the post-harvest life of strawberry fruits. The aim of the present study was to evaluate the effect of chitosan and calcium chloride on strawberry fruits to extend the shelf-life and to study the biochemical parameters.

Materials and Methods

Experimental site, cultivar and cultivation

The fresh strawberry plants of 'Camarosa' cultivar were chosen for the experimental study at the division of horticulture at the Department of Fruit & Fruit technology, Bihar Agricultural University (25º15'40" North longitude 87°2'55" East Latitude with an elevation of 45.72 meters), Sabour, Bhagalpur in Bihar. The plants were planted in double row raised bed method in field at a spacing of 45 cm x 30 cm in field. The beds were covered with plastic mulch and poly tunnels imposition was given during the first week of December to first fortnight of February to protect the plants from severe frost.

Spraying of chemicals

The experiment was carried to examine the effect of pre-harvest foliar application of chitosan and calcium in combination as well as alone. Varying concentrations of chitosan (@ 5g/L and 6g/L), calcium chloride (@ 0.5%, 1.0% and 1.5%) and their combinations were applied to different treatment. 0.1 N HCl solution was used to dissolve the chitosan and undissolved substances were filtered. Calcium chloride was taken as a source of Calcium which was dissolved into water to make solution. The chemicals were sprayed after the fruit set until the uniform deposition of solution on the plants especially the fruits surface was made. Plants sprayed with the water were taken as a control treatment in each replication. The experiment was done to observe the effects the treatments had on storage at 7℃ and assessing the bio-chemical attributes of strawberry fruits at an interval of 2 days. The foliar application of chemicals was made 10 days prior to harvesting and uniform standard size ripen fruits was taken for study.

Titrable acidity

The titrable acidity is determined by titrating the juice against standard alkali solution (0.1 N NaOH). 2 g of sample of pulp was taken in distilled water and crushed the pulp was homogenized and diluted up to 100 ml with distilled water. 10 ml aliquot of diluted sample was pipette out and transferred in 250 ml beaker. 1-2 drops of phenolphthalein indicator was added to the solution. The juice of conical flask was titrated against 0.1 N NaOH solution. The alkali was added drop by drop to the conical flask with constant stirring until the end point was reached with disappearance of pink colour. The percentage of acidity was calculated from the following formula.

$$\text{Titrable acidity (\%)} = \frac{\text{Volume of .01 N NaOH} \times 64}{\text{Weight of juice taken} \times 1000} \times 100$$

Total sugar

It was determined by Lane and Eynon (1923) [15] copper titration method. 5 ml of each Fehling's solution was taken into 250 ml conical flask and 25 ml of water was added. Prepared standard invert sugar solution was taken in a 50 ml burette. 10 ml of standard invert sugar solution was added in the conical flask containing mixture of Fehling's solution. Then conical flask containing the mixture was heated and it was boiled for 2-3 minutes on low flame and invert sugar solution was added from the burette rapidly. Then further quantity of sugar solution added to the conical flask, which turned the solution colour of conical flask into light blue. 2 to 3 drops of methylene blue indicator was added and the titration was completed within 2-3 minutes by adding further quantity of invert sugar solution. At the end point a brick red precipitate developed.

$$\text{Total sugar (\%)} = \frac{\text{Factor} \times \text{Dilution} \times 100 \times 100}{50 \times \text{Titre value} \times \text{Wt. of Sample}}$$

$$\text{Factor for Fehling's solution} = \frac{\text{Titre (Burette Reading)} \times 2.5}{\text{(g of invert sugar) } 1000}$$

Ascorbic acid

2 g of crushed sample was taken in a conical flask and adjust volume up to 100 ml with 3% HPO_3 solution was added. This solution was kept it for 10-15 minutes and then filtered it. 10 ml filtrate solution was titrated with standard dye 2, 6-Dichlorophenol-indophenol to a pink end point appearance. Titrated rapidly and made a preliminary determination of the titre value. The ascorbic acid content of the sample calculated by the following formula:

$$\text{Ascorbic acid (mg/100g FW)} = \frac{\text{Titre value} \times \text{Dye factor} \times \text{Volume make up}}{\text{Weight of sample} \times \text{Volume of sample taken}} \times 100$$

$$\text{Dye factor} = \frac{1}{\text{Titre value (burette)}}$$

Total Antioxidant activity

Cupric reducing antioxidant capacity (CUPRAC) assay was performed according to the method of Apak *et al*. (2004). For this, 100 µl of sample aliquot and 1 ml each of copper (II) chloride solution, Neocuproine solution, ammonium acetate buffer solution and distilled water were mixed in a test tube. The tubes were then capped and after 1 h, the absorbance was recorded at 450 nm in a spectrophotometer, against a reagent blank. The antioxidant capacity was estimated by using following formula and expressed as µmol Trolox equivalent g^{-1} FW.

$$\text{Total antioxidant capacity} = \frac{\text{O.D.} \times 4.1 \times \text{volume made up} \times 1000 \times 100}{\text{Weight of sample} \times 1.67 \times 10000 \times 0.1} \times 100$$

Spoilage

The loss due to infestation of disease, shriveling and other deformity was considered as decay loss. On each day of observation, the spoiled or decayed fruits were separated treatment wise under all the replication. The fruit so obtained were weighed and decay loss in terms of percentage of decayed fruits on the each day of observations was calculated. The spoiled fruits on the day observations will be separated in all treatments. The fruits so obtained will be weighed separately and the percentage of spoiled fruits on the day will be calculated by using following formulae

$$\text{Spoilage (\%)} = \frac{\text{Weight of spoiled fruits}}{\text{Original weight of fruits}} \times 100$$

Statistical analysis

A randomized complete block design with 12 treatments and three replications were used in this study and each treatment was considered as an experimental unit. Data were subjected to analysis of variance. Arcsine transformation was applied on percentage data prior to analysis and arcsine transformed data are also presented for spoilage of strawberry fruits. The analysis of data is in RBD in DMRT form. Post-harvest analysis was done at 2 days interval at low temperature storage condition at 7 ^{0}C. The application of calcium chloride, chitosan and their combinations was made at pre-harvest level.

Results and Discussion

Titrable acidity

Strawberry fruits are slightly acidic with sweet in taste. Citric acid is the main compound accounting for titrable acid. In regard to the effect of pre-harvest treatments and cold storage at 7 ^{0}C (Table 1) demonstrates that all pre-harvest treatments succeeded in reducing the titrable acidity (TA). The most efficient treatment with least value of TA was in chitosan @ 6 g/L + $CaCl_2$ @ 1.00% (T_{11} – 0.63%) in comparison to the control (T_1 – 0.71%) with maximum value. The increase in fruit acidity during the storage period may be due to the metabolic changes in fruits or due to the use of organic acids in respiratory process. The lowering of acidity in various treatments might be due to the involvement of growth substances at metabolic level in regulating vital physiological and biochemical processes seems to have decreased total acidity in fruits. Naradisorn *et al*., (2006) [17], Singh *et al*., (2009) [24] and Qureshi *et al.,* (2013) [19] were found similar results with respect to titrable acidity of strawberry.

Total Sugar

Referring to the effect of pre-harvest treatments, obtained data during post-harvest observation shows significant difference (Table 2). The increase pattern was observed in the fruit total sugar with a declining pattern on last day. The maximum total sugar percentage was found highest with chitosan @ 6 g/L + $CaCl_2$ @1.50% (T_{12} – 6.24%) which was at par with T_{11} (5.78%) while the lowest total sugar percentage was found in control (T_1 – 3.96%) at 7℃. Since, the fruit of strawberry does not have starch to support soluble sugar synthesis after harvest, this increase may be a consequence of cell-wall degradation. There are three possible carbon sources for soluble sugar synthesis after harvest: starch, organic acids and cell-wall disassembly. Strawberry fruit has insufficient starch (~0.1%) to support this synthesis; organic acids and cell-wall are the more likely sources. During cool-storage, cell-wall disassembly plays an important role in sugar accumulation. This supposition accords with no change in texture and no recovery of soluble sugar. Cordenunsi *et al.,* (2003) [3] was also found similar results with respect to total sugar of strawberry.

Ascorbic acid

It was observed that ascorbic acid of fruits was non-significant which progressively decreased under each treatment during the period of storage. However, the average data shown significant variation among the treatments (Table 3). The value of ascorbic acid ranged from T_1 (52.19 mg/100g) to T_4 (66.31 mg/100g). Shanmugavelu and Srinivasan (1973) [23] explained that increase in ascorbic acid content is probably due to catalytic influence of growth substances on biosynthesis of ascorbic acid from sugar. The increase in ascorbic acid content in various treatments might be due to the continued synthesis of glucose-6 phosphate through the growth and development of fruits which is thought to be precursor of vitamin-C.

Low temperature has a protective effect on ascorbic acid content in fruits in comparison with the room temperature of around 25℃. Minimum delay to expose to low temperature after harvest would slow down the oxidative degradation of the remaining ascorbic acid. Cordenunsi *et al.,* (2003) [3] was found similar results with respect to ascorbic acid of strawberry. The variation of total ascorbic acid throughout the storage period showed that the vitamin content can be affected not only by the cultivar, but also by the temperature (Cordenunsi *et al.,* 2005) [9].

Total Antioxidant activity

The statistical analysis of the data clearly indicates that the total antioxidant activity of strawberry fruits was non-significant during the storage analysis period of seven days (Table 4). However, the pooled data found to be significant in nature. The treatment with maximum value of total antioxidant activity was found in Chitosan @ 6 g/L + $CaCl_2$ @ 1.00% (T_{11} – 21.19 µmol TE g^{-1} FW) while the least value was observed in control (T_1 – 19.21 µmol TE g^{-1} FW). The antioxidant activity of strawberry fruits relies on their phenol and ascorbic acid content. Besides anthocyanins, other flavonoids, phenolic acids and vitamins can contribute to the protective effect against oxidative damage cells. Since, the antioxidant activity of individual dietary compounds cannot always be evaluated. The determination of the total antioxidant activity allows a more realistic evaluation of the potential protective effect of a food. The reasons for higher retention of total antioxidant activity may be explained by lowering losses of anthocyanins, ascorbic acid and tannins. Kulkarni *et al*. (2004) [14] reported that anthocyanins, ascorbic acid and phenolics are responsible for the antioxidant activity, either alone or in combination. Barman *et al*., (2011) [3] also reported that antioxidant capacity of plant produce is mainly contributed by the presence of pigments, vitamins and polyphenolic compounds.

Spoilage

Microbial growth typically follows a first order reaction kinetics with respect to microbial population. In this study, spoilage was not evaluated by either severity of infection or microbial population, but by weight percentage of infected berries or rate of disease incidence. With regard to the effect of the tested pre-harvest treatments, data reported in Table 5(a) that treatment chitosan @ 6 g/L + $CaCl_2$ @ 1.00% treated fruits showed to be the superior one with least spoilage of around 4.61% in T_{11} in comparison to control (T_1 - 17.34%). Li and Yu (2001) [16] recorded that chitosan coating often inhibits CO_2 production; consequently ethylene production of the commodity is also reduced. The infection process involves secretion of enzymes by the pathogens, which depolymerize the insoluble pectic polymers of the plant cell wall, leading to the tissue maceration (Bateman, 1968) [5]. Conway *et al.,* (1993) [8] reported the effect of Ca on apple in reducing decay and maintaining fruit quality is associates with maintaining cell wall structure by dealing or modifying chemical changes in cell wall composition. The other component to tissue

softening is loss of turgor pressure which falls with loss of water or desiccation due to transpiration and respiration (Bourne, 1983) [6]. The gained results of pre-harvest chitosan in reducing the decay go in line with findings of Romanazzi *et al.*, (2002) [21] on table grapes, Chien *et al.*, (2007) [7] on citrus and Yu *et al.*, (2007) [27] on apples

Table 1: Effect of pre-harvest application of calcium chloride and chitosan on TA in storage condition at 7 ^{0}C

Treatments	1st day	4th day	7th day	Pooled
Control	0.69 ^{a}	0.71 ^{a}	0.72 ^{a}	0.71 ^{a}
0.50% $CaCl_2$	0.67 ^{b}	0.68 ^{b}	0.70 ^{b}	0.68 ^{b}
1.00% $CaCl_2$	0.66 ^{bc}	0.67 ^{bc}	0.68 ^{cd}	0.67 ^{c}
1.50% $CaCl_2$	0.65 ^{cd}	0.67 ^{cd}	0.67 ^{de}	0.66 ^{d}
Chitosan 5 g/L	0.66 ^{bc}	0.67 ^{bc}	0.69 ^{c}	0.67 ^{c}
Chitosan 5 g/L + 0.50% $CaCl_2$	0.65 ^{de}	0.66 ^{cd}	0.68 ^{cde}	0.66 ^{de}
Chitosan 5 g/L + 1.00% $CaCl_2$	0.63 ^{f}	0.64 ^{f}	0.65 ^{gh}	0.64 ^{g}
Chitosan 5 g/L + 1.50% $CaCl_2$	0.64 ^{ef}	0.65 ^{ef}	0.66 ^{fg}	0.65 ^{f}
Chitosan 6 g/L	0.65 ^{de}	0.66 ^{de}	0.67 ^{ef}	0.66 ^{e}
Chitosan 6 g/L + 0.50% $CaCl_2$	0.63 ^{f}	0.64 ^{f}	0.66 ^{fg}	0.64 ^{fg}
Chitosan 6 g/L + 1.00% $CaCl_2$	0.63 ^{f}	0.63 ^{g}	0.64 ^{h}	0.63 ^{h}
Chitosan 6 g/L + 1.50% $CaCl_2$	0.64 ^{ef}	0.647 ^{f}	0.65 ^{gh}	0.64 ^{fg}
C.D.(p=0.05)	0.010	0.012	0.013	0.007

Table 2: Effect of pre-harvest application of calcium chloride and chitosan on total sugar in storage condition at 7 ^{0}C

Treatments	1st day	4th day	7th day	Pooled
Control	4.22 ^{e}	4.35 ^{e}	3.32 ^{g}	3.96 ^{h}
0.50% $CaCl_2$	4.31 ^{e}	4.51 ^{de}	3.99 ^{f}	4.27 ^{g}
1.00% $CaCl_2$	4.36 ^{de}	4.58 ^{de}	4.08 ^{f}	4.34 ^{efg}
1.50% $CaCl_2$	4.47 ^{de}	4.67 ^{de}	4.10 ^{f}	4.41 ^{efg}
Chitosan 5 g/L	4.33 ^{e}	4.52 ^{de}	4.12 ^{f}	4.32 ^{fg}
Chitosan 5 g/L + 0.50% $CaCl_2$	4.37 ^{de}	4.58 ^{de}	4.05 ^{f}	4.33 ^{fg}
Chitosan 5 g/L + 1.00% $CaCl_2$	4.54 ^{de}	4.70 ^{de}	4.23 ^{ef}	4.49 ^{ef}
Chitosan 5 g/L + 1.50% $CaCl_2$	4.77 ^{cd}	4.89 ^{d}	4.46 ^{de}	4.70 ^{d}
Chitosan 6 g/L	4.46 ^{de}	4.56 ^{de}	4.60 ^{d}	4.54 ^{de}
Chitosan 6 g/L + 0.50% $CaCl_2$	5.18 ^{c}	5.33 ^{c}	5.46 ^{c}	5.32 ^{c}
Chitosan 6 g/L + 1.00% $CaCl_2$	5.67 ^{b}	5.80 ^{b}	5.89 ^{b}	5.78 ^{b}
Chitosan 6 g/L + 1.50% $CaCl_2$	6.13 ^{a}	6.24 ^{a}	6.35 ^{a}	6.24 ^{a}
C.D.(p=0.05)	0.428	0.407	0.250	0.201

Table 3: Effect of pre-harvest application of calcium chloride and chitosan on ascorbic acid in storage condition at 7 ^{0}C

Treatments	1st day	4th day	7th day	Pooled
Control	59.95	50.82	45.81	52.19 ^{c}
0.50% $CaCl_2$	64.96	57.87	52.79	58.54 ^{b}
1.00% $CaCl_2$	67.01	61.89	58.64	62.51 ^{ab}
1.50% $CaCl_2$	71.05	66.37	61.51	66.31 ^{a}
Chitosan 5 g/L	61.37	57.21	53.51	57.36 ^{bc}
Chitosan 5 g/L + 0.50% $CaCl_2$	64.41	58.94	55.38	59.58 ^{b}
Chitosan 5 g/L + 1.00% $CaCl_2$	65.18	60.37	56.66	60.74 ^{ab}
Chitosan 5 g/L + 1.50% $CaCl_2$	65.65	60.85	57.69	61.40 ^{ab}
Chitosan 6 g/L	61.64	58.71	55.39	58.58 ^{b}
Chitosan 6 g/L + 0.50% $CaCl_2$	64.88	59.61	53.78	59.42 ^{b}
Chitosan 6 g/L + 1.00% $CaCl_2$	66.55	61.81	56.80	61.72 ^{ab}
Chitosan 6 g/L + 1.50% $CaCl_2$	66.83	62.27	57.20	62.10 ^{ab}
C.D.(p=0.05)	–	–	–	5.970

Table 4: Effect of pre-harvest application of calcium chloride and chitosan on total antioxidant activity in storage condition at 7 ^{0}C

Treatments	1st day	4th day	7th day	Pooled
Control	18.543	19.217	19.883	19.21 ^{d}
0.50% $CaCl_2$	19.050	19.833	20.420	19.76 ^{cd}
1.00% $CaCl_2$	19.320	20.173	20.540	20.01 ^{bcd}
1.50% $CaCl_2$	19.417	20.233	20.557	20.06 ^{bcd}
Chitosan 5 g/L	19.353	19.920	20.347	19.87 ^{cd}
Chitosan 5 g/L + 0.50% $CaCl_2$	19.603	20.090	20.600	20.09 ^{bcd}
Chitosan 5 g/L + 1.00% $CaCl_2$	19.920	20.353	20.780	20.35 ^{abc}
Chitosan 5 g/L + 1.50% $CaCl_2$	19.787	20.103	20.693	20.19 ^{bc}
Chitosan 6 g/L	19.777	20.177	20.600	20.18 ^{bc}
Chitosan 6 g/L + 0.50% $CaCl_2$	20.250	20.557	20.887	20.56 ^{abc}
Chitosan 6 g/L + 1.00% $CaCl_2$	20.747	21.143	21.687	21.19 ^{a}
Chitosan 6 g/L + 1.50% $CaCl_2$	20.660	20.753	21.077	20.83 ^{ab}
C.D.(p=0.05)	–	–	–	0.926

Table 5(a): Effect of pre-harvest application of calcium chloride and chitosan on spoilage in storage condition at 7 ^{0}C

Treatments	1st day	4th day	7th day	Pooled
Control	0.00	18.01 ^{a}	34.01 ^{a}	17.34 ^{a}
0.50% $CaCl_2$	0.00	10.41 ^{b}	21.57 ^{b}	10.66 ^{b}
1.00% $CaCl_2$	0.00	7.33 ^{c}	19.53 ^{bc}	8.95 ^{c}
1.50% $CaCl_2$	0.00	7.32 ^{c}	19.08 ^{bc}	8.80 ^{c}
Chitosan 5 g/L	0.00	0.00 ^{e}	16.36 ^{cd}	5.45 ^{e}
Chitosan 5 g/L + 0.50% $CaCl_2$	0.00	5.39 ^{d}	16.10 ^{cd}	7.16 ^{d}
Chitosan 5 g/L + 1.00% $CaCl_2$	0.00	0.00 ^{e}	16.05 ^{cd}	5.35 ^{e}
Chitosan 5 g/L + 1.50% $CaCl_2$	0.00	0.00 ^{e}	16.32 ^{cd}	5.44 ^{e}
Chitosan 6 g/L	0.00	0.00 ^{e}	15.11 ^{cd}	5.03 ^{e}
Chitosan 6 g/L + 0.50% $CaCl_2$	0.00	0.00 ^{e}	14.42 ^{d}	4.80 ^{e}
Chitosan 6 g/L + 1.00% $CaCl_2$	0.00	0.00 ^{e}	13.84 ^{d}	4.61 ^{e}
Chitosan 6 g/L + 1.50% $CaCl_2$	0.00	0.00 ^{e}	14.02 ^{d}	4.67 ^{e}
C.D.(p=0.05)	0.00	1.282	4.439	1.442

Table 5(b): Effect of pre-harvest application of calcium chloride and chitosan on spoilage in storage condition at 7 ^{0}C

Treatments	1st day	4th day	7th day	Pooled
Control	4.05	25.09 ^{a}	35.64 ^{a}	37.21 ^{e}
0.50% $CaCl_2$	4.05	18.82 ^{b}	27.65 ^{b}	38.47 ^{bcd}
1.00% $CaCl_2$	4.05	15.68 ^{c}	26.18 ^{bc}	38.96 ^{abc}
1.50% $CaCl_2$	4.05	15.69 ^{c}	25.88 ^{bcd}	39.45 ^{a}
Chitosan 5 g/L	4.05	4.05 ^{e}	23.84 ^{cde}	37.85 ^{de}
Chitosan 5 g/L + 0.50% $CaCl_2$	4.05	13.37 ^{d}	23.63 ^{cde}	38.68 ^{abcd}
Chitosan 5 g/L + 1.00% $CaCl_2$	4.05	4.05 ^{e}	23.56 ^{cde}	39.10 ^{ab}

Chitosan 5 g/L + 1.50% $CaCl_2$	4.05	4.05 ^{e}	23.78 ^{cde}	39.26 ^{ab}
Chitosan 6 g/L	4.05	4.05 ^{e}	22.84 ^{de}	38.08 ^{cde}
Chitosan 6 g/L + 0.50% $CaCl_2$	4.05	4.05 ^{e}	22.31 ^{e}	38.56 ^{abcd}
Chitosan 6 g/L + 1.00% $CaCl_2$	4.05	4.05 ^{e}	21.83 ^{e}	38.88 ^{abc}
Chitosan 6 g/L + 1.50% $CaCl_2$	4.05	4.05 ^{e}	21.97 ^{e}	39.11 ^{ab}
C.D.(p=0.05)	–	1.278	3.186	0.907

Note: Arcsine transformed data of spoilage of strawberry fruits.

Conclusion

As discussed above, lowering the storage temperature is an effective way to reduce the spoilage per cent and extend the strawberry shelf-life i.e. consumer acceptability for additional few days. Chitosan and calcium chloride also have dynamic role as a foliar pre-harvest spray in reducing the spoilage per cent and extending the shelf-life of strawberry fruits. The biochemical attributes were also has a positive boom effect prolonging the storage days of around seven days under treatment chitosan @ 6 g/L + $CaCl_2$ @ 1.00%. However, deterioration was noticed at last day of storage, so it may be concluded that around 6 days of storage is effective for strawberry fruits at 7 ^{0}C. Several concentrations with other chemicals also in combination with in-focusing organic nature of fruit and various packaging methods may be studied ahead.

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