

Original Research Article

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Calcium Chloride, Chitosan and Low Temperature Storage (7°C) Effect on Biochemical, PLW and Marketability of Strawberry cv. Camarosa

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ABSTRACT

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The trial was conducted in the year 2017 to observe the biochemical properties of strawberry cv. Camarosa by the pre-harvest application of chitosan, calcium chloride and low temperature storage (7 °C) condition. The maximum TSS was observed in T₁₁ (Chitosan @ 6 g/L + CaCl₂ @ 1.00% - 10.93°B). The maximum value of anthocyanin was recorded in T₄ (CaCl₂ @ 1.50% - 39.45 mg/100g) The least loss in physiological weight and have good shelf-life was recorded in T₁₂ (Chitosan @ 6 g/L + CaCl₂ @ 1.50% - 4.20%). At 7°C, the highest value of marketability percentage was found in T₁₁ (Chitosan @ 6 g/L + CaCl₂ @ 1.00% - 95.38%) which was at par with T₁₂, T₁₀ T₉, T₇, T₈ and T₅. Combined effect of calcium and chitosan resulted in delaying senescence, increasing shelf-life and firmness with higher fruit quality.

Introduction

Strawberry (*Fragaria x ananassa* Duch.) is a small fruit crop of great nutritional and medicinal values (Maas *et al.*, 1991) and is one of the most popular fruits among berries worldwide. The strawberry plant is herbaceous, perennial and having shallow root system, comes to flowering after about four months. Among the fruits, it gives the quickest return in the shortest time of plantation and is becoming popular in plain areas also as a fruit crop. The cultivated

strawberry is suitable for growing under different agro-climatic condition including sub-tropical regions.

Strawberries are extremely perishable and have a very short shelf-life and senescence period due to their susceptibility to mechanical injury, texture softening, physiological disorders and infections caused by several micro-organisms. The ripe red fruit of strawberry is interestingly thirst quenching and juicy. However, after harvesting its shelf-life is very short under ambient temperature

due to its thin skin and soft texture when ripe. This results in the loss of water and a high risk to infections from disease.

Chitosan is a linear amino polysaccharide consisting of glucosamine and N-acetylglucosamine units, which can be extracted from the exoskeleton of crustaceans, such as shrimps, crab and pinfish. The use of chitosan has been approved by the Environment Protection Agency (EPA) for fruits and vegetables. Chitosan appears to play a dual keen function, first by interfering directly with fungal growth and also by activating several biological processes in plant tissues. In addition, due to its polymeric nature chitosan can form films permeable to gases. Hence, chitosan has the potential as an edible antifungal coating material for the post-harvest produce and delay senescence.

Growers need to produce high-quality fruit that has the maximum possible storage or shelf-life to be competitive in the market place. Application of calcium is related to the firmness of the fruit by increasing the strength of cell wall, which in turn improves shelf-life (Van-Buren, 1979). Calcium is one of the major mineral element determining the fruit quality which has multiple roles associated with the plant cells. Pre-harvest Ca treatments used to increase Ca content of the cell wall were effective in delaying senescence, resulting in firmer and enhance fruit quality (Serrano *et al.*, 2004; Kluter *et al.*, 2006 and Raese and Drake, 2006). Foliar Ca applied to strawberries has been shown to delay fruit harvest, decrease incidence of fruit rot and improve fruit firmness (Cheour *et al.*, 1990; Singh *et al.*, 2007; Wojcik and Lewandowski, 2003).

After harvest, refrigeration is most commonly used to slow decay in strawberries and maintain quality (El Ghaouth *et al.*, 1991; Maas, 1980; Nunes *et al.*, 2002). Various

numerous preservation methods have been used to extend the shelf life and enhance the quality of strawberry fruits, such as freezing (Marina *et al.*, 2015), heat treatment (Vicente *et al.*, 2005), controlled atmospheres (Harker *et al.*, 2000), gamma irradiation (Peerzada *et al.*, 2012) and chemical treatments (Castello *et al.*, 2010). However, some of these methods have adverse effects on flavour, taste, color and texture resulting in decline of the consumer acceptability in the market. Therefore; the use of natural edible materials to control physiological processes draws increasing interest (Pelayo *et al.*, 2003).

Materials and Methods

Experimental site, cultivar and cultivation

In experiment was conducted in the Department of Horticulture (Fruit and Fruit Technology), Bihar Agricultural College, Sabour, Bhagalpur, Bihar. Among the various cultivars 'Camarosa' variety was taken for the experimental study. 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 foliar application of chitosan and calcium in combination as well as alone in nature of application. Varying concentrations of chitosan (5g/L and 6g/L), calcium chloride (@ 0.5%, 1.0% and 1.5%) and their combinations were applied to each treatment. Chitosan was dissolved in 0.1 N HCl solution and undissolved substances and impurities were filtered. Calcium chloride was taken as a source of Calcium which was dissolved into

water to make solution. The chemicals were sprayed until the uniform deposition of solution on the plants especially the fruits surface. Plants sprayed with the water were taken as a control treatment in each replication. The experiment was done to observe the effects of treatments on storage and the quality of strawberry fruits. The foliar application of chemicals was made 10 days prior to harvesting. After harvesting of the uniform standard size ripen fruits were taken for biochemical analysis and storage condition at 7°C.

Location and climate

Bihar Agricultural College, Sabour is situated between 25°15'40" North longitude 87°2'55" East Latitude with an elevation of 45.72 meters above the mean sea level in the heart of the vast alluvial Gangetic plains of North India, South of River Ganga. The climate of Sabour is semi-arid, subtropical with hot desiccating summer, cold but frost less winter with an average annual rainfall of about 1150 mm precipitating mainly in between middle of June to middle of October. The overall distribution regarding various details of meteorological observations was recorded on monthly basis for maximum and minimum temperature, rainfall, relative humidity and wind velocity from December, 2017 to April, 2018 and were collected from agro-meteorological observatory, Bihar Agricultural College, Sabour, Bhagalpur have been presented in Table- 1.

Physiological loss in weight

The initial weight of fruits under each treatment was recorded replication wise at the time of storage. The weight of the same fruits under each treatment was recorded at 3 days interval and difference in weight was recorded. The cumulative weight loss was calculated in per cent on the basis of initial fruit weight. The weight loss of fruit was

determined during post harvest storage; fruits were weighed at different sampling intervals. Then weight loss was calculated by using the following formula.

$$\text{PLW (\%)} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100.$$

Total Soluble Solids

Total soluble solids in °Brix was recorded with the help of digital refractometer. Fully ripe fruits of each treatment were taken and few (2-3) drops of juice from 5 fruits was taken separately and dropped in the clean glass on the prism base of the refractometer. Then pressed 'ON' button and took the reading displayed on the screen of digital refractometer. The mean of TSS of the taken fruits were taken as TSS of the respective treatments.

Anthocyanin

Aliquots (5.00 g) of the homogenized strawberry samples were dissolved in 25 ml methanolic hydrochloric acid (85:15) solution. The samples were kept for 24 hours at cool temperature (4-5°C) for the extraction of anthocyanin pigment. The flocculate was filtered off by a Whatman paper 1 and the absorbance of the resulting clear liquid was recorded at 535nm in Spectrophotometer (Model: Systronics 118). Anthocyanin content was calculated by using the following formula

$$\text{Anthocyanin (mg/100g pulp)} =$$

$$\frac{\text{OD (abs 535\AA)} \times \text{volume of solution} \times 100}{\text{Weight of sample} \times 98.2} \times 100$$

Marketability

Healthy and marketable fruits were separated treatment wise under replication on the day of observation and percentage of marketable

fruits was calculated on the basis of initial weight of fruits. Marketability of fruit was determined on the basis of firmness, colour and appearance of fruits.

Market ability (%) = 100 – spoiled fruit (%).

Statistical analysis

A randomized complete block design with 12 treatments and three replications were used in this study. Ten fruiting plants were used as an experimental unit. Data were subjected to analysis of variance. Arcsine transformation was applied on percentage data. The analysis of data was done in DMRT form. Post-harvest analysis was done at 3 days interval at low temperature storage condition at 7°C. The application of calcium chloride, chitosan and their combinations was made at pre-harvest level.

Results and Discussion

Physiological loss in weight

The soft nature of strawberry fruit makes it extremely perishable, which results that it hardly remains fresh at room temperature for around 2 days. Low temperature storage (at 7°C) has helped to reduce the PLW as well as the pre-harvest spraying of chemicals also have positive effect which can be noted from the treated fruits and the control one.

With the reference from the data reported in table 2 demonstrate that treatment chitosan @ 6g/L + CaCl₂ @ 1.5% treated to be the superior one in reducing the physiological loss in weight (T₁₂ – 4.20 %) as compared to the control (T₁ – 7.69 %). The loss in fruit weight was mainly due to evaporation and transpiration loss of water; and somewhere dry matter lost by respiration. 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 (Ribeiro *et al.*, 2007). Chitosan coating has been reported as an effective material in controlling water loss from other commodities. Calcium helps to maintain the turgidity of the cell and enhance the cell wall composition maintaining more firmness time. Serrano *et al.*, (2004), Hafez and Haggag (2007) and Mahmoud (2008) had realized that the loss in fruit weight during storage of sapotas, peaches and nectarines, apples and peaches, respectively was greatly reduced due to pre-harvest sprays of calcium in the form of calcium chloride at 0.3-7.5%.

Total soluble solids

The loss of texture is one of the main factor which limits quality and post-harvest shelf-life (Figuerire *et al.*, 2012). Table 3 shows that the TSS was changed over the storage period. On the 4th day of low temperature storage, we could observe a slight increase in the TSS of all the treatments which ranged from T₁ – 8.62°B to T₁₁ – 11.25°B. However, on the 7th day, deteriorating changes and decline in TSS was recorded in each treatment. Nevertheless, considering that an acceptable strawberry flavor is achieved with a minimum TSS of 7 % (Manning, 1996); they were not of the best quality for consumption.

Pre-harvest spraying of calcium and chitosan results to the activation of number of enzymes which might have been stimulated the physiological processes in terms of hydrolyzed starch and polysaccharides. Metabolic activity during the change of available starch, organic acid into soluble sugars and enhanced solubility of insoluble starch and protein present in the cell wall and middle lamella, thus TSS might have been increased. Qureshi *et al.*, (2013) on strawberry found similar results with respect to TSS.

Anthocyanin content

According to the literature, the biosynthesis pathway for anthocyanin is still operative after strawberry harvest and storage at low temperatures does not inhibit this process (Holkroft and Kader, 1999; Kalt and Macdonald, 1996). The amount of anthocyanin is important for the maturity determination and attractiveness. With reference from the data obtained from table 4 low temperature not only lowered the rate of pigment synthesis but also the ultimate level of anthocyanin. Pelargonidin and cyanidin glycosides are the principle pigments found in the strawberry.

Fruits produced by the plants sprayed with chitosan and calcium chloride exhibited the

highest total anthocyanin (T_4 – 39.45 mg/100gm pulp) content with statistically significant difference from the total anthocyanin content from the control (T_1 – 37.21 mg/100gm pulp) plants. Calcium treatment has been found to increase colour formation of strawberry fruits by affecting phenylalanine ammonia lyase and tyrosine ammonia lyase activities, thus justifying partly the more anthocyanin content of the fruits deriving from the plants treated with calcium. Similarly, chitosan treated fruits might have stimulated the metabolic activity. It could also be the effect of higher sink strength and/or assimilate supply, thus providing more substrate for anthocyanin formations. Saavedra *et al.*, (2016) and Xu *et al.*, (2014) found similar results with respect to anthocyanin on strawberry.

Table.1 Weather condition prevailing during experiment period (December 2017 to April 2018)

Date	Temperature (°C)		Relative Humidity (%)		Rainfall (mm)
	Max.	Min.	07.00 A.M.	02.00 P.M.	
02 Dec. – 08 Dec.	23.0	11.7	96.0	72.0	00.0
09 Dec. – 15 Dec.	18.7	8.0	97.0	75.0	00.0
16 Dec. – 21 Dec.	23.3	8.2	95.0	59.0	00.0
23 Dec. – 31 Dec.	23.0	10.0	96.0	74.0	00.0
01 Jan. – 07 Jan.	20.9	8.6	98.0	76.0	00.0
08 Jan. – 14 Jan.	21.3	8.0	96.0	61.0	00.0
15 Jan. – 21 Jan.	22.7	6.0	93.0	48.0	00.0
22 Jan. – 28 Jan.	25.1	8.2	91.0	59.0	12.4
29 Jan. – 04 Feb.	22.2	8.0	98.0	63.0	00.0
05 Feb. – 11 Feb.	25.8	7.9	89.0	51.0	00.0
12 Feb. – 18 Feb.	26.6	9.5	95.0	46.0	00.0
19 Feb. – 25 Feb.	28.4	11.5	86.0	44.0	00.0
26 Feb. – 04 Mar.	29.2	10.5	83.0	36.0	00.0
05 Mar.-11 Mar.	28.9	12.9	84.5	53.2	3.2
12 Mar.-18 Mar.	28.4	11.9	83	49.5	0.6
19 Mar.-25 Mar.	30.3	16.4	87.5	56.1	5.9
26 Mar.-01 Apr.	31.5	21.5	94.2	67.4	0
02 Apr.-08 Apr.	32.9	21.7	92.5	64.2	0

Table.2 Effect of pre-harvest application of calcium chloride and chitosan on PLW in storage condition at 7 °C

Treatments	1 st day	4 th day	7 th day	Pooled
Control	0.00	9.88 ^a	13.20 ^a	7.69 ^a
0.50% CaCl ₂	0.00	7.56 ^b	10.45 ^b	6.00 ^b
1.00% CaCl ₂	0.00	7.31 ^c	10.00 ^c	5.77 ^c
1.50% CaCl ₂	0.00	6.99 ^d	9.27 ^d	5.42 ^d
Chitosan 5 g/L	0.00	7.05 ^d	8.73 ^e	5.26 ^e
Chitosan 5 g/L + 0.50% CaCl ₂	0.00	6.70 ^e	8.65 ^e	5.12 ^f
Chitosan 5 g/L + 1.00% CaCl ₂	0.00	6.23 ^f	8.41 ^f	4.88 ^g
Chitosan 5 g/L + 1.50% CaCl ₂	0.00	6.07 ^g	8.08 ^g	4.71 ^h
Chitosan 6 g/L	0.00	6.58 ^e	8.17 ^g	4.91 ^g
Chitosan 6 g/L + 0.50% CaCl ₂	0.00	6.11 ^{fg}	7.75 ^h	4.62 ⁱ
Chitosan 6 g/L + 1.00% CaCl ₂	0.00	5.53 ^h	7.58 ⁱ	4.37 ^j
Chitosan 6 g/L + 1.50% CaCl ₂	0.00	5.21 ⁱ	7.39 ^j	4.20 ^k
C.D.(p=0.05)	0.00	0.141	0.142	0.063

Table.3 Effect of pre-harvest application of calcium chloride and chitosan on TSS in storage condition at 7 °C

Treatments	1 st day	4 th day	7 th day	Pooled
Control	8.11 ^d	8.62 ^c	8.14 ^c	8.29 ^f
0.50% CaCl ₂	9.45 ^c	10.23 ^b	9.99 ^b	9.89 ^e
1.00% CaCl ₂	10.21 ^{ab}	10.93 ^{ab}	10.62 ^{ab}	10.59 ^{ab}
1.50% CaCl ₂	10.22 ^{ab}	10.25 ^b	10.04 ^{ab}	10.17 ^{cde}
Chitosan 5 g/L	10.21 ^{ab}	10.45 ^b	10.32 ^{ab}	10.33 ^{bcd}
Chitosan 5 g/L + 0.50% CaCl ₂	9.88 ^{bc}	10.30 ^b	10.01 ^{ab}	10.06 ^{de}
Chitosan 5 g/L + 1.00% CaCl ₂	10.38 ^{ab}	10.67 ^{ab}	10.32 ^{ab}	10.46 ^{bc}
Chitosan 5 g/L + 1.50% CaCl ₂	10.35 ^{ab}	10.60 ^{ab}	10.35 ^{ab}	10.43 ^{bcd}
Chitosan 6 g/L	10.48 ^{ab}	10.65 ^{ab}	10.12 ^{ab}	10.41 ^{bcd}
Chitosan 6 g/L + 0.50% CaCl ₂	10.44 ^{ab}	10.67 ^{ab}	10.03 ^{ab}	10.38 ^{bcd}
Chitosan 6 g/L + 1.00% CaCl ₂	10.88 ^a	11.25 ^a	10.66 ^a	10.93 ^a
Chitosan 6 g/L + 1.50% CaCl ₂	10.49 ^{ab}	10.84 ^{ab}	10.25 ^{ab}	10.53 ^{bc}
C.D.(p=0.05)	0.717	0.730	0.665	0.381

Table.4 Effect of pre-harvest application of calcium chloride and chitosan on anthocyanin in storage condition at 7 °C

Treatments	1 st day	4 th day	7 th day	Pooled
Control	37.123	38.07 ^e	36.43	37.21 ^e
0.50% CaCl ₂	38.623	39.39 ^{bcde}	37.39	38.47 ^{bcd}
1.00% CaCl ₂	39.190	40.16 ^{abcd}	37.55	38.96 ^{abc}
1.50% CaCl ₂	39.653	40.99 ^a	37.73	39.45 ^a
Chitosan 5 g/L	38.323	38.91 ^{de}	36.33	37.85 ^{de}
Chitosan 5 g/L + 0.50% CaCl ₂	38.810	39.79 ^{abcd}	37.44	38.68 ^{abcd}
Chitosan 5 g/L + 1.00% CaCl ₂	39.483	40.37 ^{abc}	37.44	39.10 ^{ab}
Chitosan 5 g/L + 1.50% CaCl ₂	39.663	40.62 ^{ab}	37.50	39.26 ^{ab}
Chitosan 6 g/L	38.457	39.09 ^{cde}	36.69	38.08 ^{cde}
Chitosan 6 g/L + 0.50% CaCl ₂	38.867	39.85 ^{abcd}	36.97	38.56 ^{abcd}
Chitosan 6 g/L + 1.00% CaCl ₂	39.327	40.23 ^{abcd}	37.10	38.88 ^{abc}
Chitosan 6 g/L + 1.50% CaCl ₂	39.423	40.48 ^{abc}	37.43	39.11 ^{ab}
C.D.(p=0.05)	-	1.415	-	0.907

Table.5(a) Effect of pre-harvest application of calcium chloride and chitosan on marketability in storage condition at 7 °C

Treatments	1 st day	4 th day	7 th day	Pooled
Control	100.00	81.98 ^e	65.98 ^d	82.65 ^e
0.50% CaCl ₂	100.00	89.58 ^d	78.42 ^c	89.33 ^d
1.00% CaCl ₂	100.00	92.67 ^c	80.46 ^{bc}	91.04 ^c
1.50% CaCl ₂	100.00	92.67 ^c	80.92 ^{bc}	91.19 ^c
Chitosan 5 g/L	100.00	100.00 ^a	83.64 ^{ab}	94.54 ^a
Chitosan 5 g/L + 0.50% CaCl ₂	100.00	94.61 ^b	83.89 ^{ab}	92.83 ^b
Chitosan 5 g/L + 1.00% CaCl ₂	100.00	100.00 ^a	83.94 ^{ab}	94.64 ^a
Chitosan 5 g/L + 1.50% CaCl ₂	100.00	100.00 ^a	83.67 ^{ab}	94.55 ^a
Chitosan 6 g/L	100.00	100.00 ^a	84.88 ^{ab}	94.96 ^a
Chitosan 6 g/L + 0.50% CaCl ₂	100.00	100.00 ^a	85.57 ^a	95.19 ^a
Chitosan 6 g/L + 1.00% CaCl ₂	100.00	100.00 ^a	86.15 ^a	95.38 ^a
Chitosan 6 g/L + 1.50% CaCl ₂	100.00	100.00 ^a	85.98 ^a	95.32 ^a
C.D.(p=0.05)	0.00	1.281	4.439	1.442

Table.5(b) Effect of pre-harvest application of calcium chloride and chitosan on marketability in storage condition at 7 °C

Treatments	1 st day	4 th day	7 th day	Pooled
Control	85.94	64.90 ^e	54.35 ^e	19.21 ^d
0.50% CaCl ₂	85.94	71.17 ^d	62.35 ^d	19.76 ^{cd}
1.00% CaCl ₂	85.94	74.31 ^c	63.82 ^{cd}	20.01 ^{bcd}
1.50% CaCl ₂	85.94	74.30 ^c	64.11 ^{bcd}	20.06 ^{bcd}
Chitosan 5 g/L	85.94	85.94 ^a	66.16 ^{abc}	19.87 ^{cd}
Chitosan 5 g/L + 0.50% CaCl ₂	85.94	76.63 ^b	66.36 ^{abc}	20.09 ^{bcd}
Chitosan 5 g/L + 1.00% CaCl ₂	85.94	85.94 ^a	66.43 ^{abc}	20.35 ^{abc}
Chitosan 5 g/L + 1.50% CaCl ₂	85.94	85.94 ^a	66.21 ^{abc}	20.19 ^{bc}
Chitosan 6 g/L	85.94	85.94 ^a	67.15 ^{ab}	20.18 ^{bc}
Chitosan 6 g/L + 0.50% CaCl ₂	85.94	85.94 ^a	67.68 ^a	20.56 ^{abc}
Chitosan 6 g/L + 1.00% CaCl ₂	85.94	85.94 ^a	68.16 ^a	21.19 ^a
Chitosan 6 g/L + 1.50% CaCl ₂	85.94	85.94 ^a	68.03 ^a	20.83 ^{ab}
C.D.(p=0.05)	-	1.278	3.187	0.926

Note: Arcsine transformed data of marketability of strawberry fruits.

Marketability

Strawberry is very delicate and fancy fruit which cannot tolerate slight mechanical injuries. Referring to the effect of pre-harvest application of chemicals and stored condition, obtained data during post-harvest observation shows significant difference. At 7°C storage condition, the average marketability over the period of storage of 7 days range from 82.65% to 95.38%. Data obtained in table 5(a) reveals that the highest value of marketable fruits were found with application of chitosan @ 6 g/L + CaCl₂ @ 1.00% (T₁₁-95.38 %) while the lowest was seen in control (T₁ – 82.65%).

Several studies report that marketability of fruits is unacceptable below 88%. Chitosan acts as a barrier which decreased the respiration rate of fruits and reduced the water loss. It also helps to escape or provide disease resistant. Calcium serves as a catalytic metal which enhance the cell wall composition of fruit and maintain the firmness for a longer period and also increase the shelf-life. Wojcik

and Lewandowski (2003) and Singh *et al.*, (2009) found similar results with respect to marketability of strawberry fruits (Table 5b).

In conclusion, the findings of the present study have shown that, in general, CaCl₂ and chitosan especially as a foliar application have a positive boom effect on post-harvest quality and reduced physiological loss in weight in ‘Camarosa’ cultivar of strawberry. TSS, anthocyanin and marketability were relatively higher in treated strawberry fruits. The results suggest that these chemicals applications could be used to extend shelf-life and work as a promising alternative as an environment friendly compounds. Several other concentration and new chemicals in combination with in-focusing organic nature of fruit may be the ahead light view for further experimental research.

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