Int.J.Curr.Microbiol.App.Sci (2018) *7*(7): 3293-3300

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 7 Number 07 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.707.383

Chitosan and $CaCl_2$ Coatings on Physicochemical and Shelf Life of Strawberry Fruits (*Fragaria x ananassa* Duch.)

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Keywords

$CaCl_2$, Chitosan, Edible coatings, Strawberry, Shelf-life, Spoilage

Article Info

Accepted:
24 June 2018
Available Online:
10 July 2018

A B S T R A C T

The present study was measured the effects of chitosan and $CaCl_2$ treatments on shelf-life of strawberry fruits under ambient condition for 3 days. The coated treatments had significantly reduced the loss of weight and firmness of fruits. The coated strawberries had retained higher TSS, ascorbic acid and anthocyanin. Among the coating treatments, 1.5% chitosan was most effective ($P < 0.01$) in maintaining higher ascorbic acid, TSS and titratable acidity. Chitosan coating also reduced the microbial load compared to other treatments. These results indicate that edible coatings have potential as a means to reduce postharvest spoilage in strawberry fruit.

Introduction

The strawberry (*Fragaria x ananassa* Duch), is one of the most consumed berries in the world and one of the important fruit crops cultivated worldwide. 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 microorganisms. Many preservation methods have been used to extend the shelf life and improve the quality of strawberry, 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 color, flavor, taste and texture therefore; the use of natural edible materials to control physiological processes draws increasing interest (Pelayo *et al.*, 2003).

Edible coatings are thin layers of edible material applied on to the product surface in addition to or as a replacement for natural protective waxy coatings. They are used to

extend the shelf life of fruits and vegetables and are environment friendly. These can also be safely eaten as part of the product and do not add unfavorable properties to the food stuff such as chitosan, calcium chloride, *etc*. Chitosan, a modified, natural carbohydrate polymer has attracted attention as a potential food preservative due to its antimicrobial activity against a wide range of food borne filamentous fungi, yeast, and bacteria (Sagoo *et al.,* 2002; Manoj *et al.,* 2016). Similarly, postharvest dips in concentrated solutions of $CaCl_2$ have been used to improve firmness in blue berries and it could result in more efficient calcium translocation to the fruit tissues than foliar applications. The suitability of chitosan and $CaCl_2$in enhancing the shelf life of strawberry fruits is studied in this study.

Materials and Methods

Strawberry fruits (*Fragaria x anannasa* Duch.), cv. 'Camarosa', obtained from Mahabaleshwar, Maharastra State was used in the experiment. They were selected based on uniformity of size, shape, color and maturity. The strawberry fruits were treated with edible coatings such as chitosan and $CaCl_2$.Chitosan, 1.0 and 1.5 % w/v were prepared by dispensing the solutions of glacial acetic acid respectively in 100ml (v/v) warm water (50°C). The solution was heated and agitated constantly for 12 hours using hot plate magnetic stirrer. The pH of the solution was adjusted to 5.6 with 1 N NaOH. Strawberry fruits were dipped in 1.0 and 1.5% solutions as per the treatments for 1 min and were air dried at room temperature. Similarly, for $CaCl_2$ treatments, ten and five grams of calcium chloride were dissolved separately in 1000ml of distilled water to obtain 1.0 and 0.5 per cent calcium chloride solution. Strawberry fruits were dipped either in 0.5 or 1.0 per cent solutions as per the treatments for 10 min and were air dried at room temperature. The edible coatings were compared against untreated control. There were five treatments, each replicated four times and 500 g fruits were used for each replication.

The observations such as physiological loss in weight, firmness, respiration rate, total soluble solids (TSS), pH, titratable acidity, ascorbic acid content and total anthocyanin content and sensory evaluation were recorded at 1, 2 and 3 days of storage. The physiological loss in weight was measured by using electronic weighing balance (Model: Essae, DS-852, Teraoaka Ltd.). Firmness (Kg per cm^2)was measured by using texture analyzer (TA HD+, Stable Microsystems, UK) equipped with a 50 kg load cell. The respiration rate (mg CO_2 $kg^{-1}h^{-1}$)was measured by taking known volume of strawberry fruits, enclosed in a hermetic container for specified time and head space gas concentration of CO_2 was measured by piercing the probe of an auto oxygen/carbon dioxide analyzer (Make: Quantek, Model: 902D Dual track) into the container through the septa fixed on the lid of container and direct reading was noted down from the instrument screen. The content of total soluble solids (TSS) was determined with the help of digital hand refractometer and expressed as degree Brix (°B). Care was taken that the prism of the refractometer was washed with distilled water and wiped dry before every reading (Anon., 1984).Digital pH meter (Model number PB 3001) was used to measure the pH of the product samples. The temperature was kept constant while taking sample observations. The total titratable acidity (per cent) of strawberry fruits was determined by visual titration method as explained by Cohen (1971).Ascorbic acid content (mg $100g^{-1}$) of strawberry fruits were determined by modified method using 2, 6-dichlorophenol indophenol sodium salt described by AOAC, 2006.Total monomeric anthocyanin content (mg $100g^{-1}$) was quantified using a pH differential method described by Giusti and Wrolstad

(2001).Organoleptic evaluation of strawberry fruits was conducted on the basis of colour, aroma, taste, texture and overall acceptability by a panel of ten judges using a nine point Hedonic scale as described by Amerine *et al.*, (1965). The results were analyzed by following completely randomized design (CRD) as suggested by Panse and Sukhatme (1978).

Results and Discussion

The results about physiological loss in weight, firmness, respiration rate, total soluble solids (TSS), pH, titratable acidity, ascorbic acid content and total anthocyanin content and sensory evaluation were found to be varied among the treatments chitosan and $CaCl_2$, and their concentrations during storage at 1, 2 and 3 days of storage.

The physiological loss in weight had increased with prolongation of storage in all the treatments (Table 1). Significant differences were recorded between coated and uncoated fruits. Strawberry fruits treated with chitosan @1.5 per cent (T4) showed significantly less physiological loss in weight (2.24 and 5.79) as recorded at the end of 1st and 3rd days of storage, respectively, it is due to chitosan conferring a physical barrier to moisture loss and therefore retarding dehydration and fruit shriveling (Hernandez *et al.*, 2006).The controlled fruits sample (T5) lost maximum physiological loss in weight as recorded at different days of storage (8.46, and 13.27) under ambient condition.

There was a significant decrease in firmness of strawberry fruits due to different treatments throughout the storage. All the treatments coated with edible preservatives had showed higher firmness when compared to control (Table 1). Fruits coated with calcium chloride (1%) (T2) found to be significantly harder (1.40 and 1.35) on 1st and 3rd day of storage, respectively, which was on par with fruits treated with chitosan @ 1.5 per cent (1.39 and 1.31). The uncoated samples lost fruit firmness (1.24, 0.82) as recorded on 1st and 3rddays of storage respectively, under room temperature. The effect of $CaCl_2$ treatment in reduction of firmness loss of strawberries during storage may be due to the stabilization of membrane systems and formation of Ca-pectats, which increase the rigidity of the middle lamella and cell wall to increase resistance for polygalacturonase activity (Akhtar *et al.*, 2010; Madani *et al.*, 2016).

The respiration rate had increased in all the treatments as the storage period progressed (Table1).Strawberry fruits treated with chitosan @ 1.5 per cent (T4) showed minimum respiration rate (13.86 and 22.78 mg CO_2 $kg^{-1}h^{-1}$) on 1st and 3rd days of storage, respectively whereas, the controlled fruits showed maximum respiration rate as recorded at different days of storage (17.10 and 27.26mg CO_2 $kg^{-1}h^{1}$) under ambient condition. The lower respiration rate in chitosan treated fruit might be due to effect of chitosan as gas barrier. Similar result was reported by (Velickova *et al.*, 2013;Uliana *et al.*, 2014).

The total soluble solids (TSS) content of the fruits showed an increasing trend during storage period as presented in (Table 2).

The treatment, chitosan @ 1.5 per cent (T4) showed very less variation (5.85 and 5.91°B) in TSS content from the initial (5.5°B) at the end of first and third days of storage respectively, while, uncoated fruits showed maximum variation (5.95 and 6.43°B) after 1st and 3rd day of storage respectively under room temperature. Higher TSS in controlled treatment might be due to considerable loss of water by strawberry during storage time. (Sogvar *et al.*, 2016; Emamifar and Bavaisi, 2017; Nasrin *et al.*, 2017).

The pH of fruits showed significant variations due to treatments irrespective of coated and uncoated up to the end of storage (Table 2). Strawberry fruits coated with different coating material (T1 toT4) showed slight increase in pH between 3.82 and 3.89 from the initial 3.67 as against uncoated fruits (T5- 3.97 pH) at the end of third day of storage. The fruits coated with chitosan @ 1.5 per cent (T4) showed lowest pH (3.68 and 3.82) at the first and third days of storage respectively under ambient condition. The pH increased during storage in either untreated or treated fruits but it was greater in untreated fruits than those of the coated treatments (Gol *et al.*, 2013; Sogvar *et al.*, 2016).

Table.1 Effect of edible coatings on physiological loss in weight (PLW), firmness and respiration rateof strawberry fruits stored under ambient condition

Treatments	PLW (%)		Firmness (kg per cm^2)		Respiration rate (mg CO_2 kg^{-1} h^{-1})	
	1 DAS	**3 DAS**	**1 DAS**	**3 DAS**	**1 DAS**	**3 DAS**
T1- $CaCl_2$ @ 0.5%	2.26	6.09	1.35	1.30	15.60	24.66
T2 - $CaCl_2$ @ 1.0%	2.18	6.38	1.40	1.35	15.51	24.29
T3 - Chitosan @ 1.0%	2.39	6.02	1.35	1.14	15.05	24.03
T4 - Chitosan @ 1.5%	2.24	5.79	1.39	1.31	13.86	22.78
T5 - Control	8.46	13.27	1.24	0.82	17.10	27.26
S. Em ±	0.16	0.12	0.01	0.02	0.45	0.51
CD @ 1%	0.68	0.52	0.04	0.07	1.89	2.12

Note: DAS: Days after storage; Initial PLW: 0.0%; firmness: 1.46 (kg per cm^2) and respiration rate: 6.40 (mg CO_2 kg^{-1} h^{-1})

Table.2 Effect of edible coatings on total soluble solids (TSS), pH and titratable acidity (TA)of strawberry fruits stored under ambient condition

Treatments	TSS (°Brix)		pH		Titratable acidity (%)	
	1 DAS	**3 DAS**	**1 DAS**	**3 DAS**	**1 DAS**	**3 DAS**
T1- $CaCl_2$ @ 0.5%	5.90	6.13	3.76	3.89	1.13	0.99
T2 - $CaCl_2$ @ 1.0%	5.78	6.21	3.70	3.88	1.11	0.98
T3 - Chitosan @ 1.0%	5.73	6.03	3.71	3.86	1.18	1.08
T4 - Chitosan @ 1.5%	5.85	5.91	3.68	3.82	1.21	1.14
T5 – Control	5.95	6.43	3.83	3.97	1.01	0.85
S. Em ±	0.08	0.07	0.02	0.02	0.03	0.02
CD @ 1%	0.34	0.30	0.09	0.08	0.11	0.09

Note: DAS: Days after storage; Initial TSS: 5.5(°Brix); Initial pH: 3.67; Initial TA: 1.28

Table.3 Effect of edible coatings on ascorbic acid and anthocyanin content of strawberry fruits stored under ambient condition

Treatments	Ascorbic acid (mg $100g^{-1}$)		Anthocyanin (mg $100g^{-1}$)	
	1 DAS	**3 DAS**	**1 DAS**	**3 DAS**
T1- $CaCl_2$ @ 0.5%	37.00	33.50	8.59	9.36
T2 - $CaCl_2$ @ 1.0%	36.34	32.61	8.86	9.38
T3 - Chitosan @ 1.0%	37.91	35.18	8.21	9.26
T4 - Chitosan @ 1.5%	38.02	35.32	7.91	9.10
T5 - Control	34.98	29.16	9.41	10.87
S. Em ±	0.21	0.22	0.17	0.20
CD @ 1%	0.88	0.91	0.72	0.82

Note:DAS: Days after storage; Initial ascorbic acid content:40.11 (mg $100g^{-1}$); Initial anthocyanin content:7.09 (mg $100g^{-1}$).

Table.4 Effect of edible coatings on microbial population (CFU g^{-1}) of strawberry fruits

Treatments	Colour	Aroma	Taste	Texture	Overall acceptability
T1- $CaCl_2$ @ 0.5%	5.30	6.20	5.40	5.10	5.30
T2 - $CaCl_2$ @ 1.0%	5.50	6.00	5.50	5.50	5.50
T3 - Chitosan @ 1.0%	6.10	6.00	5.90	5.60	5.80
T4 - Chitosan @ 1.5%	6.25	6.20	6.25	6.25	6.25
T5 – Control	5.60	6.00	5.60	5.60	5.60
Initial scores	8.83	8.95	8.93	8.81	8.86

Note: ** Significant at 1 per cent level Initial TSS: 5.5(°Brix); pH: 3.67 and TA: 1.28% NS: Non significant
DAS: Days after storage

Table.5 Effect of edible coatings on sensory evaluation of strawberry fruits stored under ambient condition at 3rd day of storage

Treatments	Bacteria 10^3		Fungi 10^3		Yeast 10^3	
	2 DAS	**4 DAS**	**2 DAS**	**4 DAS**	**2 DAS**	**4 DAS**
T1- $CaCl_2$ @ 0.5%	14.75	31.50	14.00	63.50	18.75	96.50
T2 - $CaCl_2$ @ 1.0%	14.00	30.50	12.75	60.50	17.00	90.75
T3 - Chitosan @ 1.0%	9.75	16.50	9.00	19.50	13.00	31.50
T4 - Chitosan @ 1.5%	7.75	13.00	7.50	13.50	10.00	21.50
T5 – Control	24.50	59.50	25.50	110.50	29.50	124.50
S. Em ±	0.50	0.61	0.46	0.65	0.48	0.69
CD @ 1%	2.10	2.52	1.92	2.69	1.99	2.88

Note: DAS: Days after storage; Initial population of bacteria: $12.25x10^3$; yeast:$15x10^3$; fungi: $10.5x10^3$ (CFU g^{-1})

The titratable acidity had decreased from 1.28 % in all the treatments during storage (Table 2). Among the treatments, fruits coated with chitosan @ 1.5 per cent (T4) showed maximum titratable acidity (1.21, 1.14%) at the end of 1st and 3rd days of storage, respectively. Reduction in acidity may be expected as a result of metabolic changes in fruit or due to the use of organic acids in the respiratory process (Maftoonazad *et al.*, 2008). Maintaining titratable acidity in chitosan treated fruits might be due to reduction in metabolic changes of organic acid into carbon dioxide and water.

The ascorbic acid content of the strawberry fruits as influenced by various edible coatings showed variations. The ascorbic acid content had decreased with storage period regardless of the treatments and differed significantly (Table 3). The fruits coated with chitosan @ 1.5per cent (T4) maintained maximum ascorbic acid content of 38.02 and 35.32 mg $100g^{-1}$, on 1st and 3rddays of storage respectively, followed by was fruits coated with chitosan @ 1 per cents (T3). Ascorbic acid content of fruits had decreased during storage due to oxidizing enzymes like ascorbic acid oxidase, peroxidase, catalase and polyphenol oxidase (Singh *et al.*, 2005; Manoj *et al.*, 2016).

Significant increase in total anthocyanin content was observed in all the treatments during storage period (Table 3). Strawberry fruits treated with chitosan @ 1.5 per cent (T4) had significantly maintained minimum level of anthocyanin 7.91 and 9.10 mg $100g^{-1}$at the end of first and third days of storage, respectively, whereas, control fruits (T5) showed highest anthocyanin content of (9.41 and10.87 mg $100g^{-1}$) after 1st and 3rd day of storage, respectively, under ambient condition. The greater anthocyanin content presented by uncoated samples can be explained by the higher respiration rate, lead to higher metabolic activity, resulting in a greater pigment production (Garcia *et al.*, 2011).

The initial population of total bacteria, fungi and yeasts observed in strawberry fruits were12.25, 10.5 and $15x10^3$CFU g^{-1} respectively. The population showed variation due to treatment effects during storage (Table 4). The population had increased in strawberry fruits coated with calcium chloride whereas, the population had decreased in chitosan treated fruits at the end of 2nd day of storage. The strawberry fruits treated with chitosan @ 1.5 per cent (T4) exhibited the significantly lowest microbial population compared to other treatments. The untreated fruits (T5) contained the highest bacterial, fungal and yeast (59.50, 110.5 and $124.50x10^3$) colony forming units at the last day of storage respectively. The lowest microbial population in chitosan treated fruits was due to antimicrobial activity of this coting material (Habeeb *et al.*, 2007; Tajkarimi and Ibrahim, 2011).

A continuous decreasing trend was noticed for the sensory scores of strawberry fruits during 3 days of storage and the data is presented in (Table 5). Majority of the panelists gave preference score such as "Extremely good" during initial day and "Good" at the last day of storage. The maximum scores were received by chitosan @ 1.5 per cent (T4) at the last day of storage in all the parameters like colour, aroma, taste, texture and overall acceptability, for the fruits stored at ambient condition. This is might be due to that chitosan improved the sensory quality, protection of flavor, visual appearances, and inhibition spoilage (Manoj *et al.*, 2016).

In conclusion coating with chitosan @ 1.5 per cent was found to be the very best treatment to conserve, all biological, nutritional and

sensory characters of strawberry fruits. The edible coatings enhance the shelf life of strawberry fruits up to 3 days under ambient condition.

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How to cite this article:

Basir Ahmad Rahimi, T.H. Shankarappa, H.C. Krishna, S.K. Mushrif, K.R. Vasudeva G.K. Sadananda and Abdullah Masoumi. 2018. Chitosan and $CaCl_2$ Coatings on Physicochemical and Shelf Life of Strawberry Fruits (*Fragaria x ananassa* Duch.). *Int.J.Curr.Microbiol.App.Sci.* 7(07): 3293-3300. doi: https://doi.org/10.20546/ijcmas.2018.707.383