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# Chitosan coatings in the maintenance of strawberry quality during refrigerated storage

# Revestimentos de quitosana na manutenção da qualidade de morango durante o armazenamento refrigerado

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**ABSTRACT**

This study aimed to assess the use of chitosan coatings in the maintenance of postharvest quality of strawberries during refrigerated storage. The strawberries were selected, immersed in chitosan solution, packed and stored at 5°C for 20 days. The effect of different chitosan concentrations (0.5% and 1.0% w/v) on the qualitative aspects of the fruits was analyzed during storage. The parameters assessed were: weight loss, pH, titratable acidity, soluble solids, total sugars, reducing and non-reducing sugars and ascorbic acid. It was observed that refrigerated storage, associated with chitosan coatings, was effective in maintaining postharvest quality of strawberries, given that chitosan coatings delayed changes in weight loss, soluble solids, total sugars, reducing and non-reducing sugars, titratable acidity, ascorbic acid and pH during 20 days of storage. The chitosan concentration of 1.0% (w/v) was the most promising in maintaining quality, as well as preserving the fruits for more than 10 days under refrigeration.

**Keywords:** chemical composition, chitosan coating, preservation, postharvest, *Fragaria x ananassa*.

**RESUMO**

Este trabalho teve como objetivo avaliar o uso de revestimentos de quitosana na manutenção da qualidade pós-colheita de morangos durante o armazenamento refrigerado. Os morangos foram selecionados, imersos em solução de quitosana, acondicionados e armazenados a 5° C por 20 dias. Os efeitos de diferentes concentrações de quitosana (0,5% e 1,0% p/v) em alguns aspectos qualitativos dos frutos foram analisados durante o armazenamento. Os parâmetros avaliados foram: perda de peso, pH, acidez titulável, sólidos solúveis, açúcares totais, açúcares redutores e não redutores e ácido ascórbico. Observou-se que o armazenamento refrigerado, associado aos revestimentos de quitosana, foi eficaz na manutenção da qualidade pós-colheita dos morangos, visto que os revestimentos de quitosana retardaram as alterações na perda de peso, sólidos solúveis, açúcares totais, açúcares redutores e não redutores, acidez titulável, ácido ascórbico e pH durante 20 dias de armazenamento. A concentração de quitosana de 1,0% (p/v) foi a mais promissora na manutenção da qualidade, além de preservar os frutos por mais de 10 dias sob refrigeração.

**Palavras-chave**: composição química, revestimento de quitosana, preservação, pós-colheita, *Fragaria x ananassa*.

## 1 INTRODUCTION

Strawberry (*Fragaria x ananassa*) is a pseudo-fruit with pleasant flavor and aroma, besides a high nutritional value, being widely accepted by consumers. However, strawberries are highly perishable, susceptible to moisture loss, injuries and post-harvest deterioration, in

addition to the high susceptibility to the attack by rot-causing pathogens, especially fungi *Botrytis cinerea* and *Rhizopus stolonifer* (Nagpala et al., 2016).

The use of low temperatures is essential for pre-cooling, storage, long-distance transportation and marketing of strawberries. However, for long storage periods, only the reduction in temperature is not sufficient to maintain quality; it is necessary to associate other techniques to avoid losses(Chitarra and Chitarra, 2005). Thus, it is necessary to use cooling in strawberry postharvest, associated or not with packaging in controlled atmosphere, not only to contribute to quality maintenance, but also to reduce losses.

Fruits are excellent sources of vitamins and other nutrients. However, they have a short post-harvest life. Thus, they often exhibit high pesticide residue levels, in comparison to other food groups (Chen et al., 2011). This is a major problem, since this type of food is essential for a healthy diet.

Therefore, the use of edible coatings is an alternative technology which has been increasingly widespread and evaluated, as a viable procedure to extend fruit shelf life. These coatings are not intended to replace the use of conventional packaging materials or eliminate the use of refrigeration, but to present a functional and adjuvant performance, helping preserve texture and nutritional value, reducing gas exchange and excessive water loss (RIBEIRO et al., 2005).

Thus, the use of natural, biodegradable and non-toxic compounds, derived from animals or plants, with antifungal action, or those which induce natural resistance in plants, has been highlighted in recent years (Bautista-Baños et al, 2006), resulting in further studies which show the use of natural promising coatings in fruit postharvest.

Among the several natural compounds investigated, chitosan has currently aroused great scientific interest. The polymers chitin and chitosan have been extensively studied due to the high potential for the application in food, pharmaceutical and cosmetic industries, as well as in agriculture. These polymers consist of linear chains of the carbohydrates 2-acetamido-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose, linked by β (1-4) glycosidic bonds, and can be distinguished by their solubilities in aqueous 1.0% acetic acid solution. Chitin insoluble, with a number ≥ 40% N-acetyl-D-glucosamine, since soluble polymers are called chitosan (Fráguas et al., 2015). Due to its ability to form films and its antimicrobial properties, chitosan has been widely used for fruit preservation(Bautista-Baños et al, 2006).

Several studies have investigated the use of chitosan in the maintenance of fruit quality parameters (El Ghaouth et al., 1991a; El Ghaouth et al., 1991b; ; El Ghaouth et al., 1997; Fang et al., 1994; Jiang et al., 2001; Velickova et al., 2013).The ability of chitosan to control diseases caused by microorganisms in strawberries has also been reported (El Ghaouth et al., 1992). However, most studies describing effects of chitosan coating on the quality of fruits involved poorly characterized chitosans. Besides, properties of chitosan films from these samples were not previsously assessed, such as water vapour permeability and mechanical properties (tensile strength and elongation at break).

Thus, further studies should be conducted in order to assess the use of chitosan in postharvest, the best preparation of this coating and its influence on the quality of strawberries and important information is lacking on the influence of molar mass and degree of acetylation of chitosan on the water vapour permeability and mechanical properties.

Given the above, the objective and novelty of this study was to assess the effect of different chitosan concentrations and its films, previously, characterized as regards the properties of water vapor permeability and mechanical properties and then the chitosan samples were applied in postharvest, in the quality maintenance of strawberries during refrigerated storage.

## 2 MATERIAL AND METHODS

Strawberries (*Fragaria x ananassa*) cv. Camino Real were harvested in the early hours of the day, in a commercial orchard located in the municipality of Itutinga MG, with 910m altitude and 21º18’45” South latitude and 44º41’15” longitude. After harvest, the fruits were transported in suitable packaging to the Biochemistry Laboratory, in the Chemistry Department, Universidade Federal de Lavras (UFLA) in Lavras-MG; 360 fruits were selected in relation to average size, fully red maturity stage and absence of defects.

A completely randomized design (CRD) was used in a factorial design (3x5), with three treatments: a) Control (uncoated); b) 0.5% w/v chitosan coatings and c) 1.0% w/v chitosan coatings; fiveanalysis times, corresponding to days 0, 5, 10, 15 and 20, with 3 replications of 8 fruits for each treatment.

Commercial chitosan (trade name Chitoclear®), acquired by PrimexEhf Iceland, was used as a coating. This sample was used by Fráguas et al. (2015) for preparation and characterization of chitosan edible films, aiming the subsequent application of these films in fruit coatings. Average molar mass (kDa) determined by gel permeation chromatography

with a refractive index detector (GPC-IR), degree of acetylation (%) determined by potentiometric titration, average thickness (μm) and grammage (g × m-2) were described by Fráguas et al. [6]. Water vapour transmission rate (WVTR), water vapour permeability (WVP) and mechanical properties of chitosan films, tensile strength (MPa) and elongation at break (%) were described by Fráguas et al. (2015). All parameters are listed in Table 1.

Table 1. Parameters of commercial chitosan sample and its film described by Fráguas et al. (2015)

| **Average molar mass Mw (kDa)** | **Degree of acetylation (%)** | **Average thickness (μm)** | **Grammage (g × m-2)** | **WVTR 1 (g × day-1 × m-2)** | **WVP2 (g × μm × m-2 × day-1 × mmHg-1)** | **Tensile strength (MPa)** | **Elongation at break (%)** |
|---|---|---|---|---|---|---|---|
| 245 ± 2 | 6,3 ± 0.1 | 52,43 ± 5,34 | 4,28 ± 0,69 | 174,49 ±1,76 | 367,26 ± 38,03 | 87,15 ±7,14 | 2,78 ±0,13 |

**1**Water vapour transmission rate (WVTR) and 2water vapour permeability (WVP).

For the preparation of the coating, chitosan solutions at concentrations of 0.5% and 1.0% w/v, which were dissolved in 1.0% v/v acetic acid and homogenized with a mixer (Walita), were prepared.

The purchased fruits (360) were selected and disinfected, according to Vargas et al.(2006), by immersion in 1.0% sodium hypochlorite solution, followed by rinsing by immersion in distilled water. Subsequently, they were dried at room temperature. After drying, the fruits were separated in three batches, which were coded and hung on brackets: two batches received treatment by manual immersion of the fruits in the respective coating solutions. After drying, the fruits were removed from the brackets, weighed, packed in Polyethylene Terephthalate (PET) trays and stored at 5°C in a BOD refrigerator, for 20 days. Analyses started immediately after drying of the coating (day zero), and every five days until the end of the storage period. The same was done for the control fruits.

The results were submitted to analysis of variance (ANOVA), using the statistical program Sisvar (Ferreira, 2011)and, when significant, the means were submitted to polynomial regression analysis.

Weight loss was determined from the weight difference calculation of the experimental units observed between the time of experiment installation (day zero), and each storage period. Therefore, the fruits were weighed in a semianalitical scale (Precision, PR1000) and the results were expressed as percentage (%).

After weighing, the fruits were cut into pieces and 5g of the sample was ground in a polytron in 50 mL of distilled water, and this homogenate was used to determine pH, titratable acidity and soluble solids, according to the Association of Official Analytical Chemists AOAC(2005). The pH was determined using a Marconi digital pHmeter, the content of total soluble solids (°Brix), by using a digital refractometer (PR101 - ATAGO Company Ltd., Tokyo, Japan) and total acidity was determined by the volume of the NaOH solution (0.1N) required for the sample (10mL juice in 90mL distilled water) to reach pH 8.1. The results for titratable acidity were expressed as percentage of citric acid (%).

For the determination of total, reducing and non-reducing sugars, extraction was carried out according to the Lane-Eynon method AOAC(2005)and dosing according to the Somogyi technique, adapted by Nelson (1944).The absorbances were measured using a spectrophotometer in a computerized plan, and the results were expressed in mg sugar 100 g-1 pulp.

Ascorbic acid was extracted in 50 mL oxalic acid (0.5%) added with 0.1 g Kieselguhr; therefore, 5g of the previously ground sample was homogenized by shaking for 15 minutes in an orbital shaker (Tecnal), and the extract was then filtered in cellulosic paper. The contents of ascorbic acid, expressed in mg ascorbic acid 100 g-1 pulp, were determined by the colorimetric method(Strohecker and Henning, 1967).

## 3 RESULTS AND DISCUSSION

There was a significant interaction between treatments and days of storage for all studied parameters ($p<0.05$), and the results are shown with curves and regression equations.

### 3.1 WEIGHT LOSS (%)

Regardless of the treatment (control fruits and fruits coated with 0.5% and 1.0% w/v chitosan), there was a significant weight loss during the days of storage (Fig. 1). However, weight loss was more pronounced in the fruits of the control treatment than in those observed in the fruits of other treatments. The data of weight loss shown in Figure 1 were adjusted by a linear fit and the curve inclination in the equation is physically interpreted as

the rate of weight loss per day of storage (%/day). The faster rate of weight loss during storage of fruits was observed at control (1.15%/day), compared with fruits treated with chitosan 245 kDa at concentrations 0.5% and 1.0%, whose rates of weight loss were 1.09 and 0,93%/day, respectively. The lower rates of weight loss were observed on fruits coated with chitosan 245 kDa at concentration 1.0% (0,93%/day). The reduction in weight loss rate of fruits control compared with fruits coated with chitosan can be explained by its filmogenic and barrier properties. Yoshida et al. (2008) reported that chitosan films of different molar weights had different water vapour transmission rates, tensile strength and elongation at break. Regarding fruit firmness, Tavares et al. (2017) observed that the coatings with chitosan 245 kDa, mainly in the concentration of 1.0%, contributed to the maintenance of the firmness of the fruit pulp, thus corroborating with results of weight loss (Fig. 1).

According to Duan et al. (2011), water migration to the environment is the main cause of weight loss during storage; therefore, the fresh weight loss in stored fruits occurs (Malgarim et al., 2006a)due to water eliminated by transpiration and metabolic breathing processes. In a refrigerated environment, the lower temperature reduces the metabolism of the fruit, which consequently results in a lower weight loss (Antunes and Filho, 2003).

However, even if stored under refrigeration, uncoated fruits showed 12.25% weight loss on the tenth day of storage. According toMalgarimet al.(2006a) the commercially tolerated weight loss for strawberries is 6%. Above this value, fruit quality is already compromised. On the other hand, fruits treated with chitosan (0.5 and 1.0% w/v) associated with refrigeration, showed losses of 4.85% and 4.96%, respectively, meaning that the chitosan coating was effective in attenuating weight loss for up to 10 days of storage.

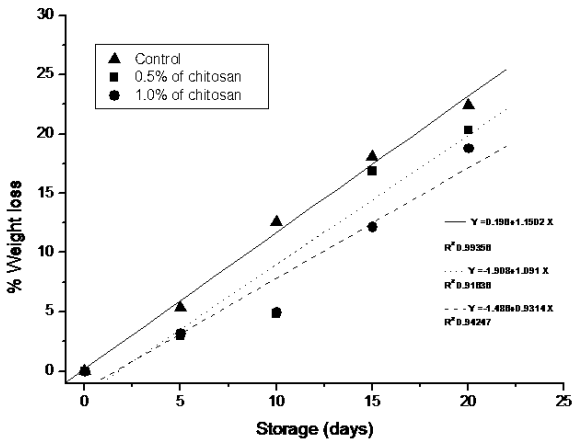


Figure 1- Curves and weight loss regression equations of strawberries coated with chitosan and stored under refrigerated temperature (5°C) for twenty days.

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Hernández-Muñoz et al.(2008) observed a lower weight loss in strawberries coated with chitosan, especially at the concentration of 1.5%. The results found in this study show that, at concentrations of 0.5 and 1.0% w/v, chitosan was effective in reducing water loss, reflecting a lower weight loss and higher fruit conservation.

## 3.2 PH AND TITRATABLE ACIDITY (TA)

There was an increase in the pH of the fruit throughout the storage period, for fruits of the three treatments (Fig. 2a). However, fruits coated with chitosan at 1.0% w/v showed lower pH values, indicating that these fruits had a lower metabolism than that of fruits of other treatments. Thus, it can be inferred that the treatment with chitosan 1.0% w/v minimized the increase in pH, decreased fruit metabolism, slowing the consumption of organic acids which, consequently, extended the storage period.

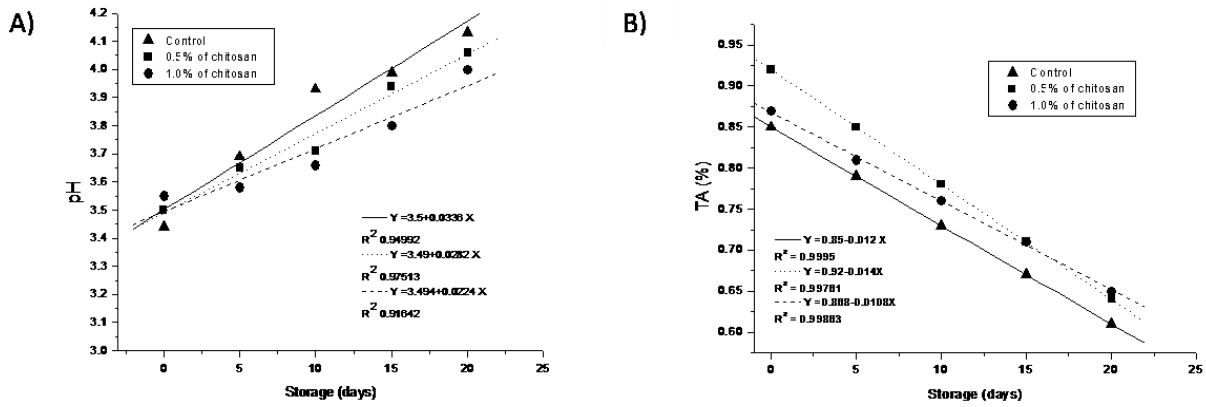


Figure 2- Curves and regression equations of pH and titratable acidity for strawberries coated with chitosan and stored under refrigerated temperature (5°C) for twenty days

Hernández-Muñoz et al.(2008) and Perdones et al.(2012)also observed an increase in pH over the storage period of strawberries coated with chitosan.

The pH behavior of the fruits observed in this study is in accordance with the observed titratable acidity (TA) (Fig. 2b). Thus, as the storage period was extended, there was a reduction in the contents of organic acids in all tested treatments, which resulted in the reduction in TA. On the fifth day of storage, higher TA decreases were observed in the treatment with 0.5% chitosan and in the control samples (8.60% and 7.06%, respectively), when compared with the fruit treated with 1.0 % chitosan (6.89%).

The decrease in TA is associated with the consumption of organic acids in the respiratory process(Maftoonazadet al., 2008), due to ripening. Therefore, it can be inferred that strawberries with 1.0% w/v chitosan had their normal ripening process slowed.

Similar results were found by Souza et al.(2011)and Ali et al.(2011), who observed smaller decreases in TA for mango and papaya, respectively, treated with chitosan, as well as Velickova et al. (2013) who observed a lower decrease in TA for strawberries coated with chitosan associated with beeswax.

## 3.3 TOTAL SOLUBLE SOLIDS (TSS)

It was possible to observe an increase in the levels of TSS for all treatments during storage (Fig. 3).

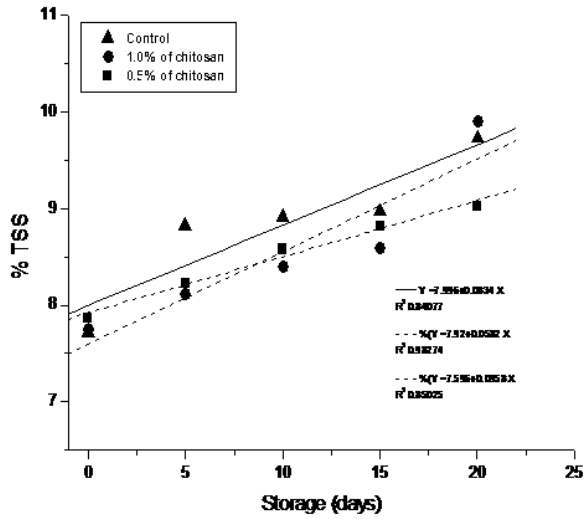


Figure 3- Curves and regression equations of total soluble solids (TSS) for strawberries coated with chitosan and stored under refrigerated temperature (5°C) for twenty days

The fruits coated with 0.5% w/v chitosan had lower levels of TSS (4,71,0%, 9.16% and 12,21,0% and 14.76%), compared to the uncoated fruits (14.25%, 15,41,0%, 16.19% and 26.04%) over storage (Fig. 4), demonstrating the effectiveness of this coating in the retention of the increase in TSS.

The increase in TSS can be explained by weight loss, concentrating the solids, or as a result of hydrolysis of other materials of the cell wall, making the reserves accumulated during the formation and development of these solids in soluble sugars(Antunes&Filho, 2003).

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3.4 TOTAL, REDUCING AND NON-REDUCING SUGARS

During storage, the increase in the levels of total sugar occurred in all treatments (Fig. 4a). This increase may be due to the degradation of cell wall carbohydrates with the release of bound sugars during ripening. The increase of 18.61% in total sugars in uncoated samples (10th day) was three times higher than those observed in fruits coated with 0.5 and 1.0% w/v (6.74 and 6.16%, respectively). Thus, at both concentrations, the chitosan used in this study was effective in maintaining fruit quality for a longer time.

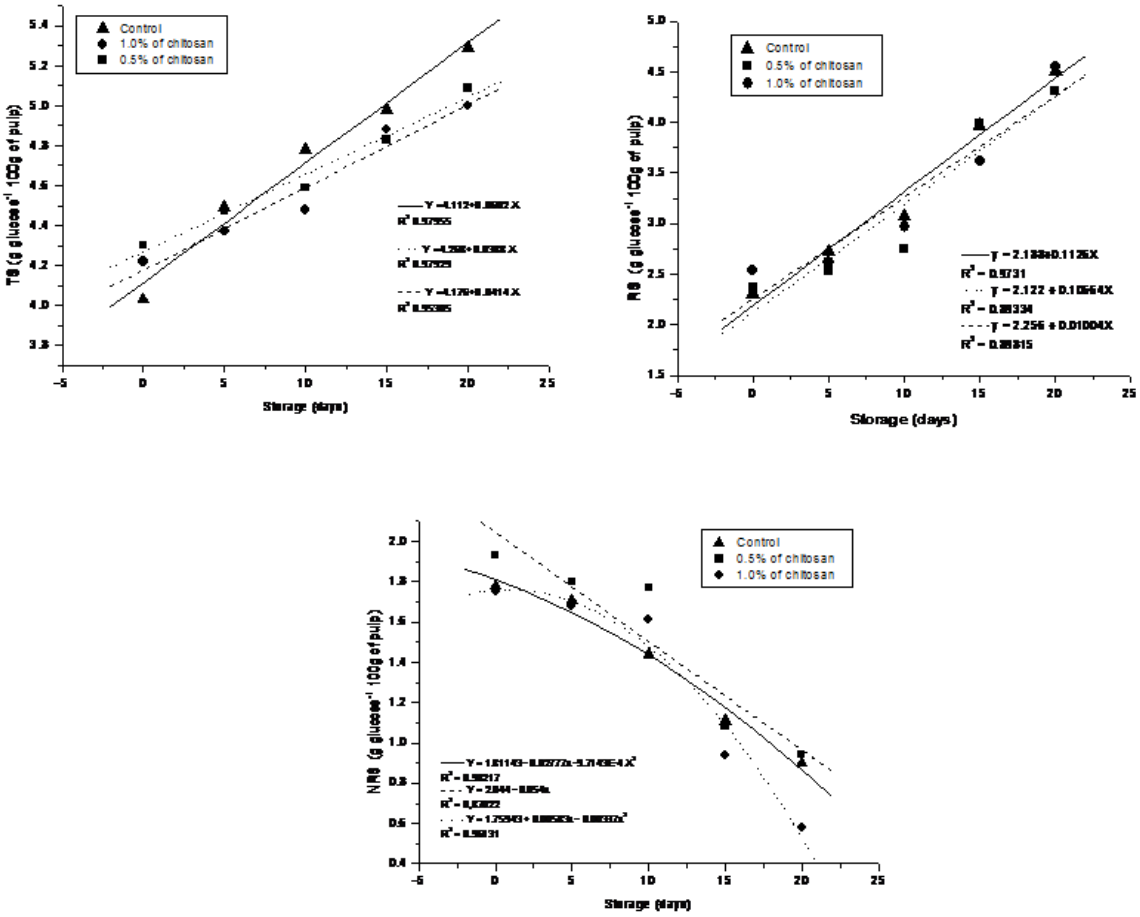


Figure 4- Curves and regression equations of total sugar (TS), reducing sugar (RS) and non-reducing sugar (NRS) for strawberries coated with chitosan and stored under refrigerated temperature (5°C) for twenty days

The contents of reducing sugars (RS) increased throughout storage, both in fruits coated with 0.5% w/v and 1.0% w/v chitosan, and in the control fruits (Fig. 4b). However, this increase was higher in the control fruits, with 94.80% RS at the end of storage, while the fruits treated with chitosan, at concentrations of 0.5 and 1.0%, showed contents of 81.86%

During storage, non-reducing sugars (NRS) decreased in all treatments (Fig. 4c). This behavior was expected since, during ripening, sucrose, a non-reducing sugar, is hydrolyzed to reducing sugars, which justifies the increase (Fig. 4b).

During storage, it was possible to observe decreases in NRS for all treatments, which were more pronounced in the control fruits than in those coated with different concentrations of chitosan.

The decrease in the contents of NRS occurred in the control samples indicates that these fruits showed a higher metabolism than those treated with chitosan, which suggests that the coatings attenuated metabolic reactions in the fruits, resulting in lower decreases in non-reducing sugars until the tenth day of storage.

### 3.5 ASCORBIC ACID

The loss of ascorbic acid in all treatments over storage was observed (Fig.5). In general, strawberries treated with chitosan coatings had a lower loss of ascorbic acid, when compared to the control.

The concentration of 1.0% w/v chitosan in coatings resulted in a lower loss of ascorbic acid, which is probably due to a lower oxidation of this compound in fruits. At the end of storage (days 15 and 20), it was possible to observe losses ranging from 30.2% to 39.5% ascorbic acid in the control fruits, values higher than those observed in the other treatments with chitosan, at 0.5% (21.0% to 28.5%) and 1.0% (20.0% to 27.0%). The values observed in strawberries (cv. Camino Real) were higher than those found by Cardoso et al.(2012)in cv. Diamante (62.5 mg 100 g-1) and cv. Camarosa (54.5 mg 100 g-1), reported by Malgarim et al. (2006b).

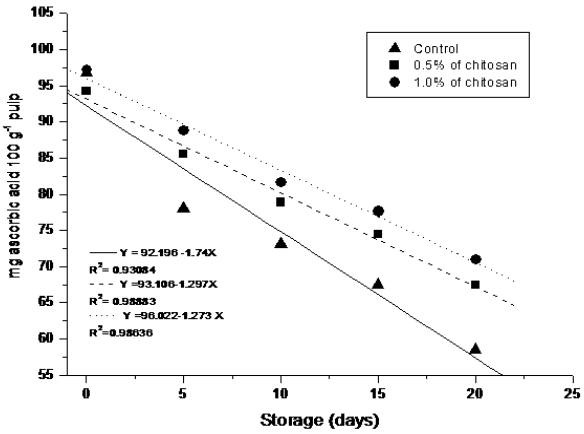


Figure 5- Curves and regression equations of ascorbic acid in strawberries coated with chitosan and stored under refrigerated temperature (5°C) for twenty days

## 4 CONCLUSION

Chitosan coatings at concentrations 0.5% and 1.0% are promising in the reduction in the metabolism of strawberries. However, the coating with 1.0% w/v chitosan is more efficient than that at 0.5% w/v, and retains strawberry ripening and acts as a protective barrier to excessive water loss. Chitosan coatings associated with cooling can be used to improve the commercial quality strawberries, enabling a shelf life of up to 10 days.

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