Postharvest Quality of Shattered Grapes Coated with Chitosan and Stored under Refrigeration

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Abstract: The main problems of postharvest preservation of fine table grapes are the rotting, the dehydration of the stalk, the browning and shattering of the berries, causing losses and impairing the quality of the products. Aiming to improve the use of shattered grape berries, and consequently the producer’s income, the present study evaluated the postharvest quality of fine table grapes of different varieties of Vitis vinifera L., shattered berries, coated with different concentrations of chitosan and stored for 56 days under refrigeration. The experiment was conducted in a completely randomized design in a factorial arrangement consisting of three factors: cultivars (Italy and Rubi), chitosan concentrations (control, 0.5%, 1.0% and 1.5%) and conservation periods (0, 7, 14, 21, 28, 35, 42, 49 and 56 days), with four replications. Physical and chemical analyses were carried out every seven days. The physical analysis considered the weight loss, color, rot incidence and appearance of the fruit. Chemical analyses evaluated the levels of soluble solids (SS), titratable acidity (TA), ratio (SS/TA) vitamin C and pH. The coating with chitosan has increased the shelf life by up to 14 days for both cultivars, with the best results observed with grapes treated with 1.5% chitosan for cv. Rubi, and chitosan at 1.0 and 1.5% for cv. Italy.

Keywords: Vitis vinifera L., chitosan, storage, refrigeration, postharvest

INTRODUCTION
The grape is one of the most important fruit in Brazil, with a production around 1.3 million tons in an area of 73,203 mil hectares. Among the states producers of grapes stands out the Paraná representing 8.3% of Brazilian grape production, with 99.3 thousand tons and 10.34% of planted area in Brazil, with 5,603 hectares (IPARDES, 2005). According to IPARDES (2005), the grape is the main fruit produced in Paraná State, with 23.32% fruit production of the state. Since it is perishable the grape is susceptible to damage from different origins. The leading problems in the postharvest storage of fine table grapes are the rooting, the dehydration of the stalk, the browning and shattering of the berries, which lead to losses and impair the quality. The rooting and the shattering of grapes may occur due to the inadequate handling of the fruit, whereas the dehydration of the stalk and the browning of the berries owe physiological disorders, the effect of ripening and senescence (Mattiuiz et al., 2004).

The application of different postharvest treatments that may be combined with the grape production is necessary to reduce losses and keep the quality of the product for a longer period. This favors the technological, economic, environmental and social development, which are embedded in the grape production chain.

The minimum processing aims to meet these requirements, making available fresh products that are sold clean, conveniently, and can be prepared and consumed in a shorter time (Cantwell, 1995). In grapes, the minimum processing is an interesting option that enables to highlight the berries with good quality, from bunches not suitable for commercialization owing the shattering or defective berries (Mattiuiz et al., 2004).

The biodegradable coatings applied in the postharvest stage contribute to reduce the use of pesticides, which is in line with the global concern considering food safety and environmental preservation (Campos et al., 2012). The coatings or coverings regulate the gas exchange between the product and external environment; the loss of water vapor that results in loss of mass, and control the loss of volatile compounds responsible for the fruit flavor (Chitarra & Chitarra, 2005). The use of low temperatures may assist in extending the postharvest shelf life as a basis for additional methods for the preservation of fruit.

In order to add value to products, increase the use of the production, reduce the postharvest losses, enhance the effectiveness in the management of grape waste, and improve thus the income of producers of fine table grapes, the present study aimed to examine the postharvest quality of shattered fine table grapes of different varieties of Vitis vinifera L., placed on trays of polyethylene terephthalate (PET) for 56 days under refrigeration.

MATERIAL AND METHODS
Fruit of cultivars Itália and Rubi were purchased at a property located in the municipality of Marialva – Paraná State, at approximately 600 m altitude, latitude
23°25’32.6"S and longitude 51°48’42.1”WO. Fruit were harvested 145 days after pruning – in the crop of January/July 2011 – in the conditions for marketing, at late afternoon, in the cooler hours. Fruit were selected and standardized as for the size, color, shape, point of ripeness and sanity, and placed in cardboard boxes. Then were quickly and carefully taken to the Food Biochemistry Laboratory of the State University of Maringá, Campus in Maringá, Paraná State. In laboratory, fruit were kept under refrigeration at 12 °C, until the experiment in the next morning.

In the laboratory, grapes were again selected and standardized as for the size, color, shape, point of ripeness and sanity. Bunches were cleaned by immersion in chlorinated water at 300 mg chlorine.L⁻¹ for 5 minutes and kept in a cold chamber at 12 °C for 12 hours. People trained and with adequate protection have shattered bunches and rinsed the berries with chlorinated water (20 mg chlorine.L⁻¹). Then, grapes were placed on plastic sieves to drain excess of water. After this, chitosan was applied at different concentrations (0.5; 1.0 and 1.5 %) by immersing fruit in the solution with the desired concentration and berries were thus placed on trays for drying.

It was used two trays with 100 grams of grape for each treatment, placed in trays of polyethylene terephthalate (PET), transparent with lid, and capacity of 200 grams (Neoform® N-90), after applying the coating. Units containing 100 grams were stored under refrigeration (10 ± 1 °C). Plastic boxes were labeled according to treatment (chitosan concentration, cultivar and day of preservation), and number of repetition.

Physico-chemical analyses were performed every seven days. For physical analysis it was evaluated the loss of fresh weight through a semi-analytical balance (Bel engineering Mark 2200), expressed in percentage; the color through a Minolta CR 400b colorimeter, expressed in luminosity (L), hue angle (h°) and chroma (C); rot incidence expressed in percentage; appearance of fruit by assigning grades, where: 4 (excellent) = turgid berry without fungi, regular color; 3 (good) = dull grape, without fungi, regular color; 2 (acceptable) = dull grape, without fungi, dark color; 1 (poor) = wilted berry, with fungi and dark color. For chemical analyses, it was evaluated the content of soluble solids through a portable refractometer (Atago, Pocket pal-1) expressed in °Brix; titratable acidity, by titration with 0.1 M NaOH, expressed in percentage of tartaric acid (Cia et al., 2010); total soluble solids/total titratable acidity ratio; vitamin C by titration with 2.6- sodium dichlorophenol indophenol expressed in mg of ascorbic acid.100g⁻¹ (Carvalho et al., 1990); and pH (pHmeter Hanna Instruments, pH 300).

The experiment was conducted in a completely randomized design in a factorial arrangement consisting of three factors: (2 x 4 x 9); cultivar (cv. Itália and Rubi); concentrations of the chitosan biofilm (0,5; 1,0 e 1,5 %) plus the control; and storage period (0; 7; 14; 21; 28; 35; 42 and 56 days). It was used two trays of a hundred grams for each treatment, of which three berries were taken to determine the color (lightness, hue angle and chroma).

The results obtained in the physical and chemical analyses were subjected to analysis of variance (ANOVA) and F-test, and mean values were compared by the Tukey’s test at 5% probability, and results obtained with the weight loss monitoring were subjected to a regression analysis.

RESULTS
During the storage period, the weight loss was low for both chitosan concentrations as for the two cultivars. The low values are probably due to the combined effects of storage temperature with the packaging, and consequent change in the atmosphere inside. This fact besides promoting a delay in the respiratory activity of the material, prevented a great vapor pressure deficit and hence the loss of water by the product.

A significant difference was observed when compared the tested treatments (Figure 1; cultivars, chitosan concentrations, and storage periods). Lower losses were detected for the cv. Itália, with a mean of 9.03%, while for the cv. Rubi (Figure 1A) the loss was 11.70%. Comparing the different concentrations of chitosan with the storage period, the most effective treatment was achieved with chitosan at 0.5%, with losses of 10.36%, while the control, chitosan at 1.0 and 1.5% have had losses of 12.66, 11.98 and 11.78%, respectively. Oliveira et al. (2012) examining mulberries coated with chitosan, has observed excellent results in the fruit preservation, compared with the control.

For the cultivar Itália, the most effective treatments consisted of using chitosan at 0.5 and 1.5%, with losses of 8.33 ad 8.56%, respectively (Figure 1B).
One of the major quality attributes, which interferes with the purchase decision of the consumer, is the appearance of the fruit. The deterioration in the appearance of tested materials has evolved similarly. According to the Figure 2A, fruit without treatment with chitosan have remained excellent for consumption up to the 35th storage day, whereas the treatments with chitosan at 0.5 and 1.0%, remained in excellent conditions up to the 42nd day. The best treatment in the analysis of the appearance was reached using chitosan at 1.5%, in which fruit remained under good condition for the consumption up to the 49th day. Usually minimally processed fruit present short shelf life, restricted to a few days. Mattiuz et al. (2004) investigated the minimum processing in seedless grape cv. BRS Morena and Seleção 8, and have preserved the fruit for 36 and 24 days respectively, without using any type of biofilm.
at 2.5 °C. The Figure 2B shows that the control kept the fruit under conditions for consumption up to the 42nd storage day. The most efficient treatments for the appearance of the fruit was with chitosan at 1.0 and 1.5%, maintaining good conditions for consumption up to the 56th day, followed by chitosan at 0.5%, with preservation up to the 49th day. These results are partially in accordance with those found by Campos et al. (2011) and Han et al. (2005) who had good results of appearance of strawberries, with fruit coated with chitosan at 1.0%.

Figure 2: Evolution of fruit appearance, cultivar Rubi (A) and Itália (B), coated with different concentrations of chitosan and stored for 56 days under refrigeration.

A major problem found in the preservation of minimally processed fruit is the rot incidence. The Figure 3 illustrates that treatments with chitosan were effective to inhibit the development of fungi, maintaining the fruit without rot for 14 days longer than the control. Also there was lower rot incidence in the cv. Itália than in the cv. Rubi. Teixeira et al. (2001) obtained commercial viability for 7 days for
minimally processed Formosa papaya stored at 3 °C. Durigan & Sargent (1999) verified a gradual reduction in the appearance of minimally processed Cantaloupe melon, also attaining an adequate period for commercialization of up to 7 days. According to Cantwell & Suslow (2002), the operations involved in the preparation of minimally processed vegetable and fruit, generally, reduce the shelf life thereof, once they lead to physiological changes which result in impaired appearance.

In the Table 1 it can be seen that for the cv. Rubi there was no significant difference in the color during the storage period, maintaining the same color up to the end of 56 days. Similar values were found by Cia et al. (2010) who analyzed grape cv. Niágara Rosada, stored under refrigeration with different types of packaging, and observed that none of packaging systems affected the color, compared to baseline values. The Table 2 indicates that the control differed from the other treatments, with a dark greenish-yellow color ($L$: 36.58; $h^\circ$: 113.56 and $C$: 11.20), while for all chitosan concentrations, fruit presented a light greenish-yellow color. Similarly Miguel et al. (2009) analyzed grapes coated with sodium alginate at 1% and observed a darker greenish-yellow color ($L$ = 37.19; $h^\circ$ = 114.06; $C$ = 12.45) than other treatments. During the storage period, no significant difference was found for the hue angle and chroma, but there was a reduction in the luminosity value.
Figure 3: Evolution of rot incidence of fruit of cultivar Rubi (A) and Italia (B), coated with different concentrations of chitosan and stored for 56 days under refrigeration.

Table 1: Results of chemical and color evaluation for the minimally processed cv. Rubi coated with different concentrations of chitosan and stored for 56 days under refrigeration (10°C; n=4).

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Volume 3 - February 2014 (2)
### Table 1: Effect of chitosan concentration on color evaluation for the minimally processed cv. Italia

<table>
<thead>
<tr>
<th>Variable</th>
<th>pH</th>
<th>TA</th>
<th>SS</th>
<th>SS/ATT</th>
<th>Vit C</th>
<th>Color</th>
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</table>

CV (%) 10.04

n = number of repetitions, TA = titratable acidity (%Tartaric acid); SS = soluble solids (°Brix); SS/ATT = ratio; Vit C = vitamin C (mg ascorbic acid.100 g⁻¹); L = luminosity; $h^\theta$ = hue angle; C = chroma. Mean values followed by the same lower case are not significantly different by Tukey’s test (p<0.05).

The pH was not significantly affected during the storage period of the grape, cv. Rubi (Table 1). The application of chitosan has determined the increase in pH, which is due to the effect of filmogenic solutions, corroborating the results of Fontes (2005) in minimally processed apple coated with chitosan. In general, the pH has decreased throughout the storage period. These same results were observed by Cenci (1994), with a reduction in the pH of the Niágara Rosada grape stored under refrigeration. In the Table 2, cv. Italia, it can be seen that up to the 21ª day, the pH of all treatments has reduced. From this data on, there was an increase in pH, and by the end of the period, higher values were found for fruit treated with chitosan at 1.0%, followed by chitosan at 1.5%, and 0.5%.

The total titratable acidity was not influenced by the application of chitosan in different concentrations, maintaining thus practically constant values, with only small variations in the results, during the 56 storage days.

Table 2: Results of chemical and color evaluation for the minimally processed cv. Italia coated with different concentrations of chitosan and stored for 56 days under refrigeration (10°C; n=4).

<table>
<thead>
<tr>
<th>Variable</th>
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<th>TA</th>
<th>SS</th>
<th>SS/ATT</th>
<th>Vit C</th>
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</table>

http://www.ijSciences.com  Volume 3 - February 2014 (2)
There was no statistical difference during the grape storage period for the two cultivars, neither between chitosan concentrations. Only on the 35th day, the lower values of total titratable acidity were found in the control and in the grapes treated with chitosan at 0.5%. The values of total titratable acidity ranged between 1.50% and 1.89% of tartaric acid. Meneguel et al. (2008) worked with coatings in mulberry cv. Comanche and verified that the acidity was not influenced by coatings and remained practically constant throughout the 18 storage days. Mattiuz et al. (2004) for the analysis on total titratable acidity, registered that the BRS Morena was less acidic, with 0.56% of tartaric acid, compared to the Seleção 8 (0.86%). Our results were superior to those found by Mattiuz et al. (2004), possibly owing the lowest values of soluble solids.

The initial values of soluble solids content were around 12.15 and 12.40 °Brix and during the storage period there was a small reduction, ranging from 11.68 to 12.08 °Brix.

The results listed in Tables 1 and 2 pointed out no significant difference during the storage period, or between different chitosan concentrations, for the two cultivars. Antunes, Duarte-Filho and Souza (2003) observed a reduction in the content of soluble solids for fruit of the cultivars Brazos and Comanche during the storage period at 20 °C.

According to Oliveira et al. (2013), a slight decrease of SS during the storage is because sugars and acids are used as respiratory substrate, reducing thus their reserves. Sousa (2007) analyzing the cv. Arapaho harvested in early June, registered 13.0 °Brix and harvested in late June, 11.0 °Brix, during the storage, with reduction in SST in both cases, with final SST 11.5 and 10.6 °Brix, respectively.

The total soluble solids/total titratable acidity ratio is a very important parameter in determining the fruit quality. The quantification of this ratio is related to the balance between sugar and acid in the fruit, being an important indicative of flavor (Chitarra & Chitarra, 2005). There was no significant difference in this ratio during the storage period. This result was also detected between different chitosan concentrations, for both cultivar Rubi and cultivar Italia, with values between 5.32 and 7.63. Mattiuz et al. (2004) have examined varieties of seedless grapes and found higher values, and attributed the lower values to the low value of total soluble solids and high value of total titratable acidity.

Regarding the content of ascorbic acid, it was verified that the concentration of edible film and storage day have positively influenced only until the 14th day, with higher values in the treatments with chitosan. During the storage period, there was only significant difference in the last two periods of storage, with lower values of ascorbic acid content. These results were similar to those found by Detoni et al. (2005) who observed a decrease in the ascorbic acid content in grapes stored at 1, 14 and 24 °C. Moreover, Miguel et al. (2009) have verified an upward trend in the content of ascorbic acid along the storage period, indicating a maintenance of the quality of the grapes. In this study, the application of chitosan as edible film for coating grapes was not effective in preventing the diffusion of oxygen into the product, degrading thus this acid.

CONCLUSIONS
In summary, minimally processed grapes coated with chitosan, combined with refrigeration (10 °C), are viable by extending the shelf life when compared with uncoated fruit.

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