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Postharvest Biology and Technology 20 (2000) 39–51

Postharvest Biology and Technology

www.elsevier.com/locate/postharvbio

Effect of pre-harvest chitosan sprays on post-harvest infection by *Botrytis cinerea* and quality of strawberry fruit

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Received 15 December 1999; accepted 23 April 2000

Abstract

The effect of pre-harvest sprays of chitosan on post-harvest decay and quality of strawberries stored at 3 and 13°C was investigated. Strawberry plants were sprayed with 2, 4 and 6 g l^{-1}, chitosan solutions as the fruit were turning red. A second spray was performed after 10 days. Fruit were picked 5 and 10 days after each spray. Harvested fruit from chitosan sprayed plants were challenged with *Botrytis cinerea*. Chitosan sprays significantly reduced post-harvest fungal rot and maintained the keeping quality of the fruit compared with control. The incidence of decay decreased with increased chitosan concentration and increased with storage period and temperature. The second spray of chitosan extended the protective effect against decay of fruit from subsequent picks. Fruit from chitosan sprayed plants were firmer and ripened at a slower rate as indicated by anthocyanin content and titratable acidity than berries from non-treated plants. Chitosan sprays were not phytotoxic at all the concentrations tested. Chitosan sprays at 6 g l^{-1} concentration performed twice, 10 days apart, protected the fruit from decay and kept the fruit quality at an acceptable level throughout the storage period of 4 weeks in fruit stored at 3°C. The protective effect of chitosan sprays was more pronounced for fruit from pick 1 than pick 2. Kinetic data on decay and ripening characteristics provided quantitative evidence that chitosan compensates for higher storage temperature and protects against deterioration of lower quality fruit from the second harvest.

Keywords: Strawberry; Chitosan; Pre-harvest spray; *Botrytis cinerea*; Post-harvest quality; Decay and ripening kinetics

1. Introduction

Strawberries (*Fragaria ananassa* Duchesne) are especially perishable fruit, being susceptible to mechanical injury, desiccation, decay and physiological disorders during storage. *Botrytis cinerea* and *Rhizopus* sp. are the two major storage pathogens of strawberry (Barkai-Golan, 1981; Mass, 1981), and the infection of fruit by *B. cinerea* can be traced to the infection of floral parts in the field (Powelson, 1960) or by contact with rotting berries. Control of *Botrytis* during storage can be

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0925-5214/00/$ - see front matter
PII: S0925-5214(00)00108-3

achieved by physical and chemical methods. Modified atmospheres with elevated CO_2 levels and low temperatures are effective in reducing the incidence of decay (Mass, 1981; Reyes and Smith, 1986; Brown, 1992). However, prolonged exposure of berries to high CO_2 concentrations can cause off-flavor development (Li and Kader, 1989; Ke et al., 1994) and low temperature alone is not an effective means of control (Brown, 1992). Although prophylactic field sprays with systemic benzimidazoles are effective in controlling post-harvest fungal infections (Dennis, 1975), there is an increased concern among consumers about the potentially harmful health effects of chemical residues (Klein and Lurie, 1991), and development of chemical tolerance in post-harvest pathogens (Spotts and Cervantes, 1986). Thus alternative approaches are necessary to maintain the marketable quality of strawberries.

Chitosan, a high molecular weight β-(1,4)-glucosamine polymer, is an important structural component of the cell wall of some plant-pathogenic fungi, especially Zygomycetes (Bartnicki-Garcia, 1970). It is also produced from the chitin components of arthropod exoskeletons; by deacetylation. Chitosan has been shown to have anti-fungal activity against a wide range of fungi (El Ghaouth et al., 1992a,b). Chitosan coating of harvested strawberries protected them from infection by *B. cinerea* and improved their quality (El Ghaouth et al., 1991a). Chitosan coating also reduced the incidence of decay of tomato, bell pepper and cucumber, delayed ripening of apple and tomato fruits and reduced the desiccation of pepper and cucumber (El Ghaouth et al., 1991c; Davis and Elson, 1994). Chitosan-induced glucanohydrolase activity in strawberries was inhibitory against *B. cinerea* (El Ghaouth et al., 1991b). The present study reports the effects of prophylactic pre-harvest chitosan sprays applied at intervals on the decay and keeping quality of harvested strawberries stored at 3 and 13°C.

2. Materials and methods

2.1. Cultivar

Runner roots of the cultivar Seascape were transplanted to 30 cm diameter plastic pots (three roots per pot) filled with potting mixture containing four parts by volume of pasteurized organic soil, one part of peatmoss and one part of perlite. The pots were randomized on green house benches, all within one large chamber. Plants were maintained at 25°C 12/12 h, light/dark cycles and watered at regular intervals. At the time of flowering plants were agitated to simulate bee transfer of the pollen for optimum fruit setting. A total of 63 plants per treatment in three replications of 21 each were maintained in a randomized complete block design (RCBD).

2.2. Chitosan sprays

Shrimp-shell chitosan was purchased from Nova-Chem Ltd. (Dartmouth, Nova Scotia, Canada) and ground into a fine powder. The purified chitosan was prepared by dissolving chitosan in 0.25 N HCl, and the undissolved particles were removed by centrifugation (15 min, $10\,000 \times g$ at 24°C). The solution was neutralized with 2.5 N NaOH to a pH of 8.0 to precipitate the chitosan. The precipitated chitosan was recovered by filtration, washed extensively with deionized water to remove salts and was subsequently lyophilized. Chitosan stock solution ($10\ g\ l^{-1}$) was prepared by dissolving chitosan in 0.05 N HCl and pH was adjusted to 5.6. Different chitosan concentrations of 2, 4, and 6 g l^{-1} were prepared in water. Plants were sprayed with chitosan solution from a hand sprayer when the fruit were just turning red. The spray was continued until the deposition of chitosan droplets was uniform on the fruit surface. A set of plants was also sprayed with sterile water as control. Sprays were repeated after an interval of 10 days.

2.3. Harvesting and storage

Fruit were harvested 5 and 10 days after each spray (designated as pick 1 and pick 2, respectively). Berries of uniform size, free of physical damage and fungal infection were selected. For quality evaluation, 30 fruit were randomly dis-

tributed into replicates of 10 fruit for each treatment, temperature, spray, pick and storage interval.

2.4. Inoculation of chitosan sprayed fruits

B. cinerea was isolated from infected strawberries and maintained on potato dextrose agar (PDA). Conidia of *B. cinerea* were recovered by flooding 2-week-old cultures with sterile water containing 0.1% (v/v) Tween 80 and filtering the mycelial suspension through three layers of sterile cheese cloth. The concentration of the conidial suspension was adjusted to 2×10^5 conidia per ml. Berries to be inoculated were transferred to plastic containers (in three replicates of ten each) with three layers of moistened blotters at the bottom. Fruit were inoculated individually with 20 μl of conidial suspension. The boxes were closed with perforated lids and subsequently transferred from ambient temperature to 3 or 13°C for storage. Strawberries were evaluated weekly for disease symptoms, and spoiled fruit were discarded to avoid secondary infection.

2.5. Assessment of quality

The effect of pre-harvest chitosan sprays on post-harvest quality of strawberries was assessed each week. A sample of seven to nine berries was randomly removed from each replicate and analyzed for firmness, titratable acidity and anthocyanin content. For firmness, berries were sliced into halves and each half was punch tested on a texture analyzer using a 4-mm flat plunger (Texture Technologies Corp., Scarsdale, NY). Acidity was determined using a 10 g aliquot of puree in 40 ml of deionized water and titrating with 0.1 N NaOH to an end point of pH 8.1. Titratable acidity was expressed as g of citric acid per l. Anthocyanins were extracted with acidified ethanol from a 2 g aliquot of homogenate according to the method of Fuleki and Francis (1968). Anthocyanin content was expressed as mg anthocyanin per g fresh weight of strawberry homogenate.

2.6. Experimental design and analysis

The experiment was a completely randomized $4 \times 2 \times 2 \times 4$ factorial design. The factors were chitosan concentration, number of sprays, number of picks and storage interval. The experiment was conducted twice, and within each repetition the treatment order was random. Analysis was carried out with triplicate data from each repetition. Data were pooled across number of sprays and picks (2 sprays $\times$ 2 picks) and S.E.M. were determined.

2.7. Kinetics of fruit quality deterioration/change

Shelf life based on a specific criterion of quality acceptability at a specified environmental condition can be estimated from kinetic models (Labuza, 1982):

$$-\frac{\mathrm{d}q}{\mathrm{d}t} = k[q]^n \tag{1}$$

where $[q]$ is any quality characteristic, t the time, k the rate constant, and n is the order of the quality deterioration/change. The application of chitosan is expected to modify the kinetics of the deterioration/change of strawberry quality during storage. Therefore, the rate of quality loss can be modeled as:

$$-\frac{\mathrm{d}q}{\mathrm{d}t} = (k_0 - k_\mathrm{i}c)[q]^n \tag{2}$$

where c is chitosan concentration, k_0 the rate constant at 0 chitosan concentration, and k_i is the inhibition constant at different chitosan concentrations. The value of n can range from 0 (zero order kinetics) up to 2 (second order) for various reactions. Integration of Eq. (2) yields Eqs. (3)–(5) for zero, first and second order kinetics, respectively.

$$[q] = [q_0] - (k_0 - k_\mathrm{i}c)t, \quad n = 0 \tag{3}$$

$$\ln[q] = \ln[q_0] - (k_0 - k_\mathrm{i}c)t, \quad n = 1 \tag{4}$$

$$\frac{1}{[q]} = \frac{1}{[q_0]} + (k_0 - k_\mathrm{i}c)t, \quad n = 2 \tag{5}$$

with

$$k_c = k_0 - k_i c \quad (6)$$

where k_c is the rate constant at different chitosan concentrations.

At zero chitosan concentration, (Eq. (2)) becomes,

$$-\frac{dq}{dt} = k_0[q]^n \quad (7)$$

On integration, Eq. (7) yields Eqs. (8)–(10) for zero, first and second order kinetics, respectively:

$$[q] = [q_0] - k_0 t, \quad n = 0 \quad (8)$$

$$\ln[q] = \ln[q_0] - k_0 t, \quad n = 1 \quad (9)$$

$$\frac{1}{[q]} = \frac{1}{[q_0]} + k_0 t, \quad n = 2 \quad (10)$$

Where $[q_0]$ is the value of the quality attribute at time 0. From the experimental data k_0, k_c and $k_i c$ can be determined.

The sensitivity of a food material or a reaction to temperature changes can be expressed by Q_{10}, the degree by which the process is accelerated by a rise of 10°C. Expressing a modified Q^*_{10} factor for various chitosan concentrations with respect to 0 chitosan concentration,

$$Q^*_{10} = \frac{k_c \text{ at } (T + 10°C)}{k_0 \text{ at } (T°C)} \quad (11)$$

Mean inhibition constant $\bar{k}_i$ is given by,

$$\bar{k}_i = \frac{\sum k_{i1}c_1 + k_{i2}c_2 + k_{i3}c_3 + \ldots}{\sum c_1 + c_2 + c_3 + \ldots} \quad (12)$$

2.8. Determination of kinetic parameters

The quality characteristics considered include decay, texture, anthocyanin and titratable acidity. An optimization routine uses the values of the independent variable (the t values) to predict the value of a dependent variable (the $[q]$ value). The routine uses the Marquardt–Levenberg algorithm (Marquardt, 1963) to find the parameters of the independent variable (t) that give the best fit between the model and the data.

The model used obeys the following differential equation:

$$\gamma \frac{d[q]}{dt} = k[q]^n \quad (13)$$

with the initial condition at, $t = 0$; $[q] = [q_0]$; with $\gamma = 1$, if $[q]$ increases with time and $\gamma = -1$, if $[q]$ decreases with time.

The agreement between experimental and predicted values was judged acceptable when the mean deviation was less than the mean experimental error.

3. Results

3.1. Efficacy of pre-harvest sprays of chitosan in controlling post-harvest decay and quality of fruits

The general trend of development of decay in strawberry fruit from chitosan sprayed plants and stored at 3 and 13°C is shown in Fig. 1. The decay of fruit decreased with increasing chitosan concentration, and increased with storage interval and temperature. The rate of decay development was higher in fruit stored at 13°C compared with 3°C at all chitosan concentrations tested.

The effect of chitosan spray on firmness of fruit during storage is shown in Fig. 2. The maintenance of texture was dependent on chitosan spray concentration, storage time and temperature. Chitosan concentration and storage interval played an important role in texture maintenance at both 3 and 13°C storage temperatures. The fruit texture was firmer with increasing chitosan concentration, and it decreased with storage temperature and time. The mean of fruit texture from week 0 to 4 across number of sprays and picks decreased from 3.4 to 1.1 N at 3°C compared with 3.5–1.3 N at 13°C. The fruit texture was firmer with increasing chitosan concentrations.

The effect of chitosan sprays on the evolution of anthocyanin in strawberry fruit during storage at 3 and 13°C is shown in Fig. 3. The anthocyanin development was dependant on chitosan spray concentration, storage temperature and time. The rate of pigment development was lower with increase in chitosan concentration and was higher with increases in storage temperature and duration. In general, the rate of increase in an-

thocyanin content was lower during the initial storage period of up to 2 weeks for all treatments. Anthocyanin content of fruit from control plants was higher than from chitosan treated plants.

The citric acid content of fruit from chitosan sprayed and control plants is shown in Fig. 4. The acidity of fruit was dependant on chitosan concentration, storage temperature and time. The citric acid content of fruit decreased with increase in storage temperature and time, and the rate of decrease was lower with increase in chitosan concentration.

3.2. Kinetics of fruit quality deterioration/change

The data of fruit quality parameters (decay, anthocyanin content, tissue texture and titratable acidity) of fruit pooled across two sprays (sprays 1 and 2) and two picks (picks 1 and 2) and two repetitions were linearly transformed and plotted against storage time to describe the changes in quality by kinetic models. Kinetic models representing the goodness of fit of the data of fruit stored at 3°C are shown in Figs. 5 and 6. The data for 13°C storage showed similar fit except

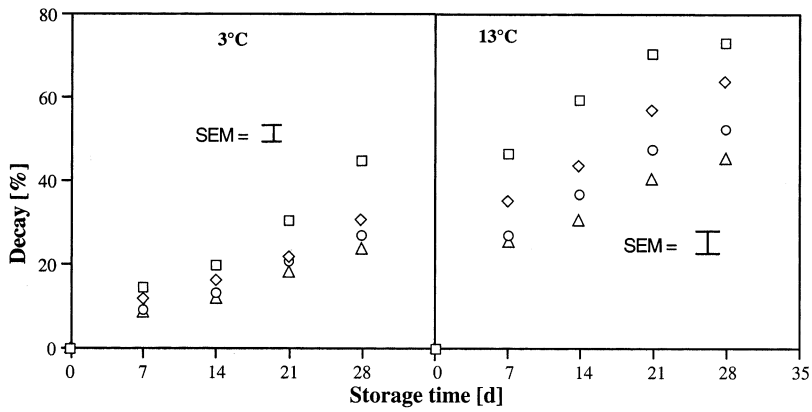

Fig. 1. Effect of pre-harvest chitosan spray treatments on the decay of strawberry fruit stored at 3 (S.E.M. ± 1.58) and 13°C (S.E.M. ± 2.28). Control (□), 2 g l^{-1} (◇), 64 g l^{-1} (○) and 6 g l^{-1} (△). Data were pooled across the number of sprays and picks and repetitions.

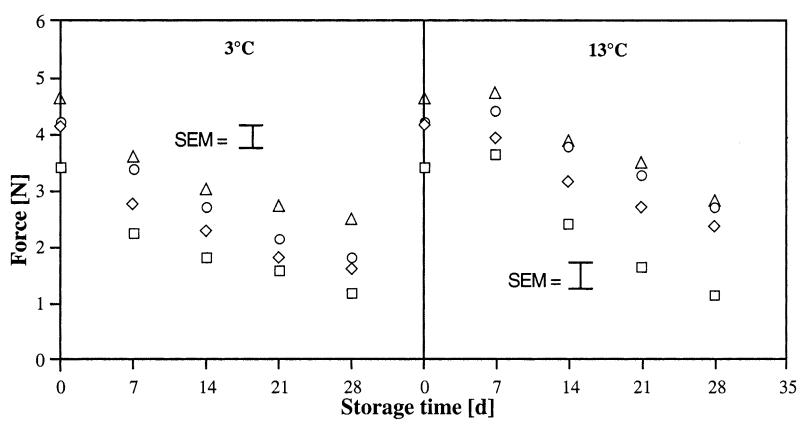

Fig. 2. Effect of pre-harvest chitosan spray treatments on the texture of strawberry fruit stored at 3 (S.E.M. ± 0.21) and 13°C (S.E.M. ± 0.18). Control (□), 2 g l^{-1} (◇), 4 g l^{-1} (○) and 6 g l^{-1} (△). Data were pooled across the number of sprays and picks and repetitions.

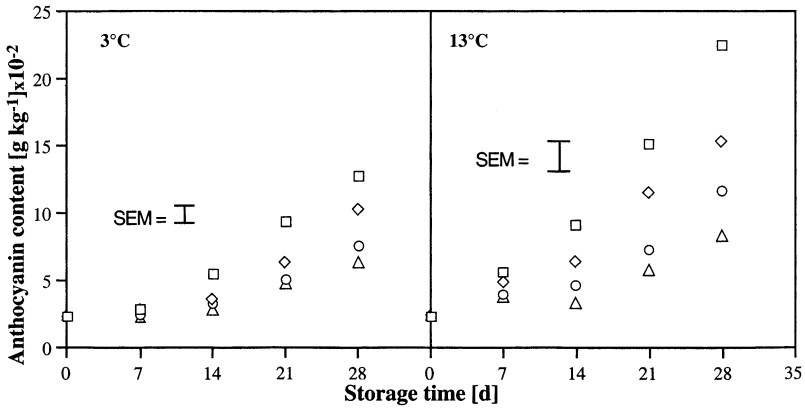

Fig. 3. Effect of pre-harvest chitosan spray treatments on anthocyanin content of strawberry fruit stored at 3 (S.E.M. $\pm 0.57 \times 10^{-2}$) and 13°C (S.E.M. $\pm 0.84 \times 10^{-2}$). Control (□), 2 g l^{-1} (◇), 4 g l^{-1} (○) and 6 g l^{-1} (△). Data were pooled across the number of sprays and picks and repetitions.

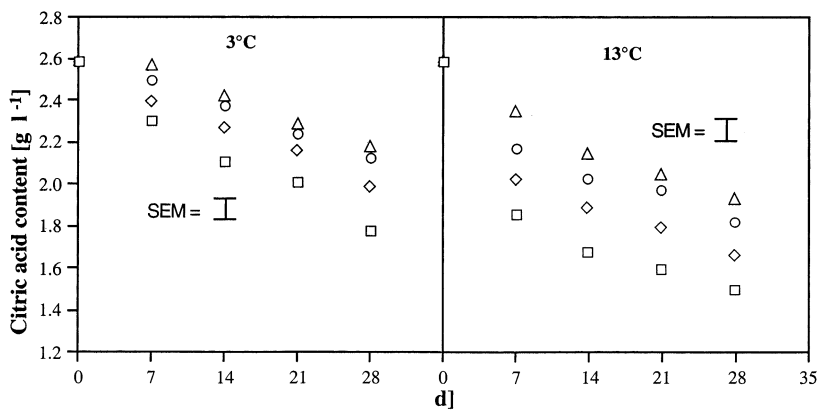

Fig. 4. Effect of pre-harvest chitosan spray treatments on titratable acidity of strawberry fruit stored at 3 (S.E.M. ± 0.038) and 13°C (S.E.M. ± 0.18). Control (□), 2 g l^{-1} (◇), 4 g l^{-1} (○) and 6 g l^{-1} (△). Data were pooled across the number of sprays and picks and repetitions.

that the changes in quality parameters were more pronounced. Linear regression coefficients (R^2) for the kinetic plots of quality parameters are given in Tables 1 and 2. Zero order reaction best described the progress of decay and anthocyanin development. First order reaction was appropriate for titratable acidity changes, whereas a second order reaction best described texture loss during storage.

Microbial growth typically follows first order reaction kinetics with respect to microbial population. In this work, decay was not evaluated by either severity of infection or microbial population, but by number of infected berries or rate of disease incidence. The infection process involves secretion of enzymes by the pathogen, which depolymerize the insoluble pectic polymers of the plant cell wall, leading to tissue maceration (Bateman, 1968). Tissue maceration is the key step for successful infection since it results in adequate supply of nutrients required for pathogen growth. Thus pathogenesis is ultimately an enzymatic process which determines the rate of infection. The general feature of enzyme catalyzed reactions is that, as the substrate concentration increases, the reaction order diminishes from first

Table 1
Rate constants k_0[a] and k_c[b], and modified Q^*_{10} factor for decay of strawberry fruit picked from plants sprayed with different concentrations of chitosan and stored at 3 and 13°C for 4 weeks

Quality factor	Chitosan (g l^{-1})	Spray 1						Spray 2						Regression coefficient[c]	
		Pick 1			Pick 2			Pick 1			Pick 2			R^2	
		3°C	13°C	Q^*_{10}	3°C	13°C	Q^*_{10}	3°C	13°C	Q^*_{10}	3°C	13°C	Q^*_{10}	3°C	13°C
Decay (per day)	0	1.58	6.00	3.8	1.65	6.86	4.2	1.33	6.71	5.0	1.67	7.00	4.2	0.978	0.906
Zero order	2	1.00	5.14	3.2	1.45	6.50	4.1	0.88	4.00	3.0	1.09	4.51	2.7	0.963	0.886
	4	0.82	3.43	2.2	1.41	3.86	2.4	0.64	3.11	2.3	1.09	3.30	2.0	0.984	0.937
	6	0.78	3.29	2.1	1.30	3.75	2.4	0.55	2.18	1.6	1.82	2.57	1.6	0.984	0.884

[a] k_0 is the rate constant at zero chitosan concentration.
[b] k_c is the rate constant at different chitosan concentrations.
[c] R^2 was determined from pooled data of spray 1 and 2 and pick 1 and 2 for a given storage temperature.

Table 2
Rate constants k_0[a] and k_c[b], and modified Q^*_{10} factor for quality factors of strawberry fruit picked from plants sprayed with different concentrations of chitosan and stored at 3 and 13°C for 4 weeks

Quality factor	Chitosan (g l^{-1})	Spray 1						Spray 2						Regression coefficient[c]	
		Pick 1			Pick 2			Pick 1			Pick 2			R^2	
		3°C	13°C	Q^*_{10}	3°C	13°C	Q^*_{10}	3°C	13°C	Q^*_{10}	3°C	13°C	Q^*_{10}	3°C	13°C
Anthocyanin (g kg^{-1} per day)	0	47	62	1.3	57	77	1.4	23	55	2.8	37	83	2.2	0.981	0.986
Zero order	2	39	42	0.9	44	54	0.9	18	43	1.9	22	57	1.5	0.920	0.982
	4	27	30	0.6	35	36	0.6	12	32	1.4	16	41	1.1	0.972	0.948
	6	22	24	0.5	28	27	0.5	11	25	1.1	16	28	0.8	0.978	0.943
Texture (N^{-1} per day)	0	14	23	1.6	22	35	1.6	16	28	1.8	32	41	1.3	0.983	0.934
Second order	2	6	17	1.2	18	28	1.3	8	18	1.1	16	26	0.8	0.975	0.961
	4	6	12	0.9	10	20	0.9	7	13	0.8	8	20	0.6	0.990	0.989
	6	4	10	0.7	10	15	0.7	6	10	0.6	8	15	0.5	0.987	0.963
Citric acid (kg m^{-3} per day)	0	14	23	2.1	24	29	1.2	13	25	1.9	15	30	2.0	0.997	0.955
First order	2	10	18	1.3	18	22	0.9	7	20	1.5	12	23	1.5	0.996	0.876
	4	8	10	0.7	14	17	0.7	5	15	1.2	8	17	1.1	0.977	0.858
	6	7	10	0.7	13	15	0.6	4	12	0.9	7	15	1.0	0.969	0.810

[a] k_0 is the rate constant at zero chitosan concentration.
[b] k_c is the rate constant at different chitosan concentrations.
[c] R^2 was determined from pooled data of spray 1 and 2 and pick 1 and 2 for a given storage temperature.

order to zero order. Since the substrates for macerating enzymes i.e. insoluble pectic polymers are not limiting, the reaction order should be 0, in accordance with our observation. Likewise anthocyanin synthesis is also an enzymatic process. The starter molecules for anthocyanin synthesis such as cinnamic, *p*-coumaric and caffeic acids are not limiting with tissue ripening (Harborne, 1964; Wardale, 1973).

Organic acids make a major contribution to the typical acidity of many unripe and ripe fruits. Citric and malic acids are the two major acids component of strawberry fruit (Haard, 1976). The loss of acidity which accompanies ripening appears to result, in part, from the use of the acids as respiratory substrates, via Krebs cycle. In addition, malate may also be decarboxylated by malic enzyme as shown in apples and pears (Hulme and Rhodes, 1971). Thus the rate of acidity loss is related to the concentration of organic acids at any time, in agreement with the observed first order kinetics.

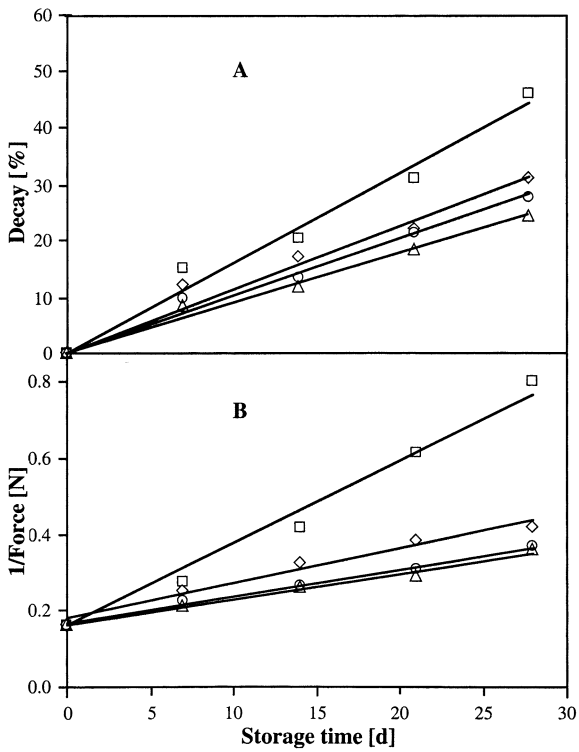

Fig. 5. Zero order kinetics for decay of strawberry fruit sprayed with chitosan before harvest and stored at 3°C (A). Second order kinetics for texture loss of strawberry fruit sprayed with chitosan before harvest and stored at 3°C (B). Control (□), 2 g l^{-1} (◇), 4 g l^{-1} (○) and 6 g l^{-1} (△). Data were pooled across the number of sprays and picks and repetitions. Lines represent the fit of the data to the kinetic model.

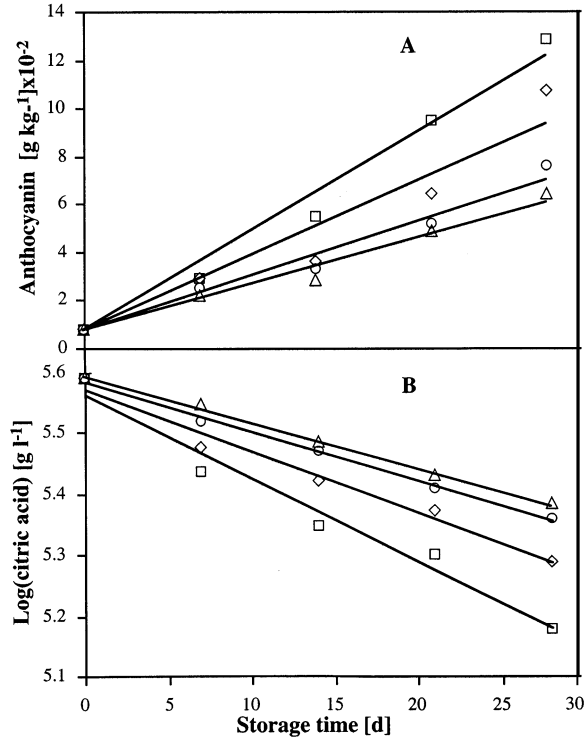

Fig. 6. Zero order kinetics for anthocyanin development of strawberry fruit sprayed with chitosan before harvest and stored at 3°C (A). First order kinetics for loss of titratable acidity of strawberry fruit sprayed with chitosan before harvest and stored at 3°C (B). Control (□), 2 g l^{-1} (◇), 4 g l^{-1} (○) and 6 g l^{-1} (△). Data were pooled across the number of sprays and picks and repetitions. Lines represent the fit of the data to the kinetic model.

Loss of texture is dependent on both cell wall degradation and loss of turgidity of the tissue. Cell wall degradation during ripening is an enzymatic process and follows increase in the activity of endogenous cell wall degrading enzymes such as polygalacturonase and cellulase. All cell wall polymers are susceptible to enzyme action if brought into contact with relevant enzymes. Exposure of cell wall associated pectin and cellulose to endogenous enzyme action is facilitated by free

radicals generated by aerobic respiration, which can participate in the derangement of the intact supramolecular structures of pectin (Kon and Schwimmer, 1977). Although the supply of substrate for these enzymes is not a limitation, their exposure to enzymes is limiting, and thus this process can be described by first order. 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). Since water loss from produce is typically of first order, it follows that the rate of loss of turgidity should also be first order. Thus loss of texture depends on two independent processes described by first order behavior, and therefore, it is expected to follow second order kinetics overall.

The rate constants of decay and ripening process indicators of stored fruit picked at two intervals following two chitosan sprays at concentrations between 0 and 6 g l^{-1} are listed in Tables 1 and 2. Pre-harvest chitosan treatments reduced the rate of both decay and ripening. Significant reduction in the rates was observed with 4 g l^{-1} chitosan concentration, beyond which the rates leveled off. At 4 g l^{-1} chitosan concentration, the rates were reduced typically from 40 to 60% at both 3 and 13°C storage temperatures. The decay rate constants were one order of magnitude higher than pigment development, and two orders of magnitude higher than texture loss and acidity. This suggests that strawberry fruit are quite susceptible to infection, more so at 13°C, and this is a major limiting factor for their preservation. Generally, the rates of decay and ripening were higher for pick 2 fruit than pick 1, and a double application of chitosan reduced these rates more effectively than a single application.

The rates of decay development and ripening were significantly lower at 3°C compared with 13°C storage temperature. The Q_{10}^{*} values for various quality characteristics at different chitosan concentrations with respect to zero chitosan concentration were calculated from the reaction rate constants at 3 and 13°C (Eq. (11)). The decay was more sensitive to temperature than the ripening parameters in general. The Q_{10}^{*} values for pigment development, texture loss or titratable acidity were typically < 2.0 but > 2.0 for decay (Tables 1 and 2). Chitosan treatment reduced the sensitivity of the fruit to higher storage temperature. For example, the Q_{10}^{*} values for decay decreased to values between 2.0 and 2.5 at 4 g l^{-1} concentration, while the Q_{10}^{*} values for ripening indicators decreased to ≈ 1.0.

The protective effect of chitosan can also be evaluated from the mean inhibition constants of chitosan for various fruit quality parameters. The mean inhibition constants (Eq. (12)) expressing the capacity of chitosan in controlling decay, anthocyanin accumulation, texture loss and titratable acidity of stored fruits are presented in Table 3. The protective effect of chitosan against decay and in delaying ripening was more pronounced at 13°C than at 3°C. Likewise, the follow-up spray was found more effective in protecting berries

Table 3
Mean inhibition constant (k_i) of chitosan in the decay and ripening characteristics (anthocyanin accumulation, texture loss and titratable acidity) of strawberry fruits picked from plants sprayed with different concentrations of chitosan and stored at 3 and 13°C for 4 weeks

Quality factor	Spray 1				Spray 2			
	Pick 1		Pick 2		Pick 1		Pick 2	
	13°C	3°C	13°C	3°C	13°C	3°C	13°C	3°C
Decay (m^3 kg^{-1} per day) $\times 10^{-2}$	17.8	51.2	6.6	53.0	16.0	90.4	16.7	88.4
Anthocyanin (g m^3 kg^{-1} per day) $\times 10^{-2}$	4.4	7.4	5.4	9.6	2.4	8.0	4.6	10.2
Texture (m^3 N^{-1} kg^{-1} per day) $\times 10^{-3}$	2.1	2.6	2.2	3.5	2.2	3.7	5.3	5.3
Citric acid (per day) $\times 10^{-3}$	1.4	2.5	2.2	2.7	1.8	2.4	1.5	2.9

from decay and in slowing down ripening. Chitosan offered stronger protection to fruits from pick 2, which are more perishable than pick 1 fruit. It is clear that chitosan compensates for a higher storage temperature and it offers protection by slowing decay and ripening. However, the protective effect is highly significant against infection.

4. General discussion

Pre-harvest fungicide sprays are undertaken to control the initial infection in the field and to obtain fruits free of infection (Aharoni and Barkai-Golan, 1987). Present study shows that prophylactic sprays of chitosan are effective in controlling the infection of *B. cinerea* in strawberries (Fig. 1). A previous study (El Ghaouth et al., 1991a) reported that dipping of strawberry fruit in chitosan solution protected the fruits from decay as effectively as dipping them in the fungicide, Rovrol®. Chitosan inhibits the growth of several fungi, induces chitinase activity, and elicits phytoalexins and defense barriers in the host tissues (Hirano and Nagao, 1989; El Ghaouth et al., 1992a,b, 1994, 1997). The control of decay in strawberries observed in this study could be attributed to either its fungistatic property, ability to induce defense enzymes and phytoalexins in plants, or a combination of these factors. Although the severity of decay was decreased significantly by chitosan sprays in pick 1, an increase in decay was observed with increase in storage time and subsequent picks. Decay was greater at 13°C than at 3°C. The lowered efficacy of chitosan sprays against fungal spoilage in pick 2 could be due to the higher susceptibility of second flush fruit to diseases. Browne et al. (1984) have shown that freshly harvested strawberries from the first harvest in each season have greater shelf-life compared with subsequent harvests since the first harvest is less susceptible to molds. The present study indicates that loss of chitosan spray effectiveness in subsequent harvests can be compensated by a follow-up spray at a maximum interval of 10 days.

The results from this study showed pre-harvest chitosan sprays also had a beneficial effect on flesh firmness, titratable acidity and in slowing the synthesis of anthocyanins in strawberries stored at both 3 and 13°C. This could be due to the formation of a chitosan film on fruit which can act as a barrier for O_2 uptake thereby slowing the metabolic activity, and consequently the ripening process. El Ghaouth et al. (1991b) have also observed retention of firmness, higher titratable acidity and reduced rate of anthocyanin production in chitosan coated strawberries. Coating of fruits with semipermeable films can retard ripening by modifying the internal CO_2, O_2 and ethylene levels (Lowings and Cutts, 1982). Chitosan coating is likely to modify the internal atmosphere without causing anaerobic respiration, since chitosan films are selectively more permeable to O_2 than to CO_2 (Bai et al., 1988). In addition, chitosan coating can reduce desiccation by providing a moisture barrier (El Ghaouth et al., 1991c). The suppressive effect on decay by chitosan can in part, be attributed to delaying the senescence process, since resistance to fungal infection can be greater as a result of slower senescence (Eckert, 1975). Our results showed that strawberry from chitosan sprayed plants maintained keeping quality for 4 weeks at 3°C.

Kinetic data on decay and ripening characteristics provide quantitative evidence that chitosan compensates for higher storage temperature and provides additional protection against deterioration of lower quality fruit from pick 2. It is difficult to coat strawberry fruit individually, but pre-harvest spraying is highly feasible. Many studies, including this one have shown the high potential of chitosan for preserving fresh fruits and vegetables, but translating this into practice requires optimizing chitosan concentrations for individual crops and improving chitosan coating formulations for integration with CA or MA during transport and storage of perishable commodities.

Acknowledgements

We acknowledge financial supports of Natural Sciences and Engineering Research Council (NSERC) of Canada-Strategic Grants Program

and of Conseil des Recherches en Pêche et en Agroalimentaire du Québec (CORPAQ).

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