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Effect of chitosan coating combined with postharvest calcium treatment on strawberry (*Fragaria* × *ananassa*) quality during refrigerated storage

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Abstract

Strawberries (*Fragaria* × *ananassa* Duch.) were coated with either 1% or 1.5% chitosan (CS) or chitosan combined with calcium gluconate (CaGlu). Following treatment, strawberries were stored at 10 °C and 70 ± 5% RH for one week. The effectiveness of the treatments in extending fruit shelf-life was evaluated by determining fungal decay, respiration rate, quality attributes and overall visual appearance. No sign of fungal decay was observed during the storage period for fruit coated with 1.5% CS (with or without the addition of CaGlu) or 1% CS + 0.5% CaGlu. By contrast, 12.5% of the strawberries coated with 1% CS lacking calcium salt were infected after five days of storage. The chitosan coating reduced respiration activity, thus delaying ripening and the progress of fruit decay due to senescence. Chitosan coatings delayed changes in weight loss, firmness and external colour compared to untreated samples. Strawberries coated with 1.5% chitosan exhibited less weight loss and reduced darkening than did those treated with 1% chitosan, independently of the presence or absence of CaGlu. However, addition of calcium to the 1% chitosan solution increased the firmness of the fruit. Coated samples had greater visual acceptability than had untreated fruits. The addition of calcium gluconate to the chitosan coating formulation increased the nutritional value by incrementing the calcium content of the fruit.

Keywords: Strawberry; Chitosan concentration; Calcium gluconate; Shelf-life; Refrigerated storage

1. Introduction

Strawberry (*Fragaria* × *ananassa*) is a highly perishable non-climacteric fruit. It must be harvested at full maturity to achieve maximum quality in terms of visual appearance (freshness, colour and absence of decay or physiological disorders), texture (firmness, juiciness and crispness), flavour and nutritional value (vitamins, minerals, dietary fibre and phytonutrients). Grey mold, caused by *Botrytis cinerea* Pers. Fr., is the most economically significant postharvest pathogen of strawberry fruits. Strawberry spoilage after harvest, can also occur by mechanical injury and desiccation.

Low storage temperatures and modified atmospheres with elevated CO_2 levels are common tools for avoiding, at least partially, mold growth and senescence, and extending fruit shelf-life (Manning, 1996). However, prolonged exposure of berries to high CO_2 concentrations can cause off-flavour development (Ke, Zhou, & Kader, 1994).

The use of synthetic chemical fungicides has been the main method for reducing postharvest disease. However, consumer concern over pesticide residues on foods, along with pathogen resistance to many currently used pesticides, has increased the need to find alternative methods for decay control. Recently, biologically active natural products have started to become an effective alternative to synthetic fungicides (Tripathi & Dubey, 2004).

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The preservation of fresh produce can also be achieved by the application of edible coatings. Several mechanisms are involved in extending the shelf-life of fruits and vegetables by film coatings. These include decreasing moisture loss and controlled gas (CO_2/O_2) exchange, resulting in reducing respiration rate. Edible films can also prevent mechanical injury produced during post-harvest handling and processing. Whey protein, calcium caseinate, gluten and polysaccharides, such as cactus mucilage or starch, have also been shown to exert beneficial effects on strawberry fruit quality when applied as coatings (Del-Valle, Hernández-Muñoz, Guarda, & Galotto, 2005; García, Martino, & Zaritzky, 1998; Tanada-Palmu & Grosso, 2005; Vachon, D'ApPrano, Lacroix, & Letendre, 2003).

Chitosan (poly β-(1,4)*N*-acetyl-D-glucosamine) polymer is industrially produced by chemical deacetylation of the chitin found in arthropod exoskeletons. This biopolymer can also be obtained directly from the cell wall of some plant-pathogenic fungi. Chitosan and its derivatives have been shown to inhibit the growth of a wide range of fungi and trigger defensive mechanisms in plants and fruits against infections caused by several pathogens. Chitosan possesses excellent film-forming properties and can be applied as an edible surface coating to fruits and vegetables. Chitosan coatings have been reported to limit fungal decay and delay the ripening of several commodities, including strawberry (El Ghaouth, Arul, Ponnampalam, & Boulet, 1991a; Han, Zhao, Leonard, & Traber, 2004; Ribeiro, Vicente, Teixeira, & Miranda, 2007). Preharvest chitosan sprays have been noted to be effective in controlling postharvest fungal infection in strawberries (Reddy, Belkacemi, Corcuff, Castaigne, & Arul, 2000).

Calcium ions perform multiple roles in plant cell physiology. They are important intracellular messengers, mediating responses to hormones, biotic and abiotic stress signals and a variety of developmental processes (Reddy & Reddy, 2004). They also play an essential role in the structural maintenance of membranes and cell walls. Calcium ions cross-link free carboxyl groups on adjacent polygalacturonate chains present in the middle lamella of the plant cell wall contributing to cell–cell adhesion and cohesion. Preharvest and postharvest treatments with calcium salts have been effective in controlling several physiological disorders, reducing the incidence of fungal pathogens and maintaining fruit firmness (Bakshi, Fa, Gs, & Ta, 2005). Foliar applications of calcium chloride have been reported to delay ripening and retard fungal growth on strawberries (Wojcik & Lewandowski, 2003). Postharvest treatments with calcium salts include dipping, vacuum and pressure infiltration which can be combined with other treatments. Calcium dips alone, or in combination with heat treatments or modified atmosphere, have resulted in improved strawberry shelf-life (Garcia, Herrera, & Morilla, 1996).

The aim of the present work is to study the effect of chitosan coatings combined with calcium gluconate on strawberry (*Fragaria* × *ananassa* cv. Camarosa) quality attributes during refrigerated storage. Strawberries were treated with 1% or 1.5% chitosan acetate solution, with or without the addition of calcium gluconate. Assessment of the treatments is based on their effects on fungal decay, respiration rate, quality attributes, and the visual appearance of strawberries stored for six days at 10 °C.

2. Materials and methods

2.1. Fruit material

Strawberry fruit (*Fragaria* × *ananassa* Duch. cv. Camarosa) were purchased from a local market. Fruits were harvested and shipped from Palos de la Frontera (Huelva, Spain) in a refrigerated truck on the previous day. Fruits were selected, based on uniformity of size, the absence of physical damage and fungal infection, and >75% of the surface showing red colour.

2.2. Edible coating formulations

Acetic acid, calcium gluconate and high molecular weight chitosan were purchased from Sigma Chemical Co (St. Louis, MO, USA). Coating solutions were prepared by dissolving 1% or 1.5% chitosan in 0.5% acetic acid solution. Chitosan coatings containing calcium gluconate were prepared by dissolving calcium salt at 0.5% or 0.75% in water prior to the incorporation of the acetic acid; chitosan was subsequently added to 1% or 1.5%, respectively.

2.3. Coating application

Strawberries were randomly distributed into five groups. Four groups were assigned to one of four treatments whilst the fifth group provided the untreated control. The treatments consisted in immersing fruits for 5 min in: (a) 1% chitosan acetate; (b) 1.5% chitosan acetate; (c) 1% chitosan + 0.5% calcium gluconate; and (d) 1.5% chitosan + 0.75% calcium gluconate solution. Fruits were allowed to dry for 2 h at 20 °C and were subsequently stored at 10 °C and 70 ± 5% RH.

2.4. Fungal decay

Fungal decay was visually inspected daily during the storage period. Strawberry fruits showing surface mycelial development were considered decayed. Results were expressed as the percentage of fruits infected.

2.5. Respiration rate

Respiration rate was determined by using the static method. Ten berries were placed in hermetically sealed 750 ml glass jars and kept at 10 °C. After 1 h of enclosure, a 100 μl sample was withdrawn from the headspace and analyzed for CO_2 using a gas chromatograph (Hewlett-Packard 5890 series II GC, Agilent Technology, Barcelona, Spain) equipped with a thermal conductivity detector (TCD) and

a Chromosorb 102 column (Restek, Tecknokroma, Barcelona, Spain). Helium was the gas carrier. Injector, oven and detector temperatures were 100, 32 and 100 °C, respectively. After sampling, jars were opened and strawberries were kept at 10 °C and 70% RH until the next measurement. CO_2 production was monitored each day for up to 6 days. Results were expressed in mg kg^{-1} h^{-1}. Three replicates of each treatment were analyzed.

2.6. Quality attributes

2.6.1. Weight loss

Strawberries were weighed at the beginning of the experiment just after coating and air-drying, and thereafter each day during the storage period. Weight loss was expressed as the percentage loss of the initial total weight. For each measurement, 25 fruits corresponding to each treatment were used and the experiment was performed in triplicate.

2.6.2. Texture analysis

Firmness was measured as the maximum penetration force (N) reached during tissue breakage, and determined with a 5 mm diameter flat probe. The penetration depth was 5 mm and the cross-head speed was 5 mm s^{-1} using a TA-XT2 Texture Analyzer (Stable Micro Systems, Godalming, UK), MA. Strawberries were sliced into halves and each half was measured in the central zone. Fruit firmness values were an average of 25 strawberries.

2.6.3. pH, titrable acidity (TA) and soluble solids content (SSC)

After firmness analysis, strawberries were cut into small pieces and homogenised in a grinder, and 10 g of ground strawberry was suspended in 100 ml of distilled water and then filtered. The pH and titrable acidity of the samples were assessed using a pH meter (pH-526; WTW Measurement Systems, Wissenschaftlich, Technische Werkstätten GmbH, Wellhelm, Germany) and titrated to pH 8.1 using 0.1 M NaOH. Titrable acidity was expressed as grammes of citric acid per 100 g of strawberry weight. The SSC was determined in the juice of ground strawberries by means of an Atago RX-1000 digital refractometer (Atago Co. Ltd., Tokyo, Japan) at 20 °C and expressed as a percentage. Measurements were done in triplicate.

2.6.4. Colour

Strawberry external colour was evaluated with a Hunter Labscan II colorimeter (Hunter Laboratory, Inc., Reston, VA). CIE $L^*a^*b^*$ coordinates were recorded using D65 illuminant and a 10° Standard Observer as a reference system. L^* is lightness, a^* (−greenness to +redness) and b^* (−blueness to +yellowness) are the chromaticity coordinates. The a^* and b^* values were converted to chroma ($C = (a^{*2} + b^{*2})^{1/2}$) and hue angle ($h = \tan^{-1}(b^*/a^*)$). Four readings were taken at different locations on each strawberry, using a total of 25 fruits from each treatment. Measurements were done in triplicate.

2.7. Sensory analysis

Sensory evaluation, based on general visual appeal, colour and visible structural integrity, was conducted using a 7-point hedonic scale. The scores were: like extremely (7); like very much (6); like moderately (5); neither like nor dislike (4), dislike moderately (3); dislike very much (2); and dislike extremely (1). Fruit scored above 4 was considered acceptable. Sensory evaluation was performed by 40 members of an untrained panel. Twenty-five fruits from each treatment were evaluated on the initial day and on days 2 and 6. Only fruits lacking signs of fungal decay were evaluated.

2.8. Calcium determination

Fifty strawberries for each treatment were lyophilized and mineralized by the dry-ashing procedure described in AOAC (Method 975.03, 1990). Calcium contents were analyzed by atomic absorption spectrophotometry using a Perkin–Elmer 3300 spectrophotometer (Perkin–Elmer Hispania S.A., Barcelona, Spain). Measurements were done in triplicate and calcium content was reported as a percentage of the dry weight.

2.9. Statistical analysis

Statistical analysis of the results was performed using a one-way analysis of variance (ANOVA). Means were separated using the Tukey test ($P < 0.05$) (SPSS commercial software, SPSS Inc., Chicago, IL). The data were analyzed and graphically plotted using Sigma-plot software (Systat Software Inc., Richmond, CA).

3. Results and discussion

3.1. Loss of fruit due to visible fungal growth

Uncoated strawberries showed signs of fungal decay after the third day of storage at 10 °C (Fig. 1). After six days of storage, 33.5% of uncoated fruit was infected by molds while no sign of fungal decay could be detected by visual inspection of fruits coated with 1.5% chitosan or 1.5% chitosan + 0.75% CaGlu. Of the fruit coated with 1% chitosan, 12.5% was observed to be infected on the sixth day of storage.

The capacity of chitosan coating to inhibit the growth of several fungi has been shown for a wide variety of harvested commodities. According to the work carried out by El Ghaouth, Arul, Grenier, and Asselin (1992a) on two postharvest pathogens, Botrytis cinerea and Rhizopus stolonifer, the antimicrobial activity of chitosan on strawberries appears to be related to the ability of this biopolymer to cause severe cellular damage to the mold and interfere in the secretion of polygalacturonases rather than its ability to induce plant defence enzymes.

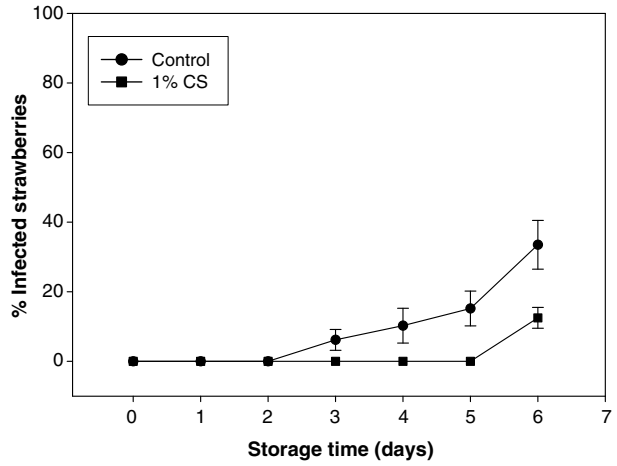

Fig. 1. Percentage of infected strawberries as a function of storage time at 10 °C for control and 1% CS coated samples. Vertical bars indicate standard deviation.

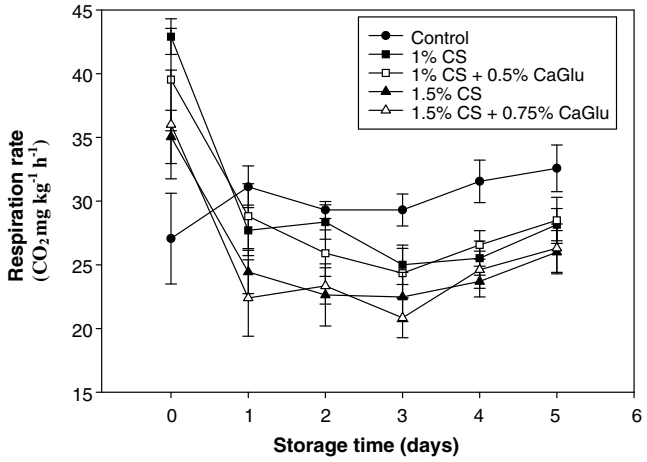

Fig. 2. Respiration rate of control and chitosan-coated strawberries as a function of storage time at 10 °C. Vertical bars indicate standard deviation.

The concentration of chitosan in the coating solution affects the fungal decay of the fruit. A concentration of 1.5% inhibits fungal growth during the storage period whereas fungal decay was observed in fruit coated with a 1% chitosan solution. It can be expected that an increase in the viscosity of the chitosan coating solution will increase the content of chitosan adhered to the fruit surface and the uniformity of the coating. Studies carried out by Cisneros-Zevallos and Krochta (2003), on Fuji apples coated with hydroxypropyl methylcellulose, showed that the dry coating load on the fruit surface is a function of the viscosity, draining time and solid concentration of the coating solution. The effect of the coating solution concentration on the dry matter adhered to the fruit surface has been observed to be more pronounced for high viscosity hydrocolloids. The present study was carried out with high molecular weight chitosan, which could explain the differences observed in the extent of fungal decay between fruits dipped in 1% and 1.5% chitosan.

The combined treatment of 1% chitosan solution and 0.5% calcium gluconate inhibited the fungal decay of fruit during the storage period. The incorporation of calcium ions in fruit tissue promotes new cross-links between anionic homogalacturonans, strengthening the cell wall and particularly the middle lamella which is responsible for holding cells together. Thus, increasing the stability of the cell wall and middle lamella by calcium treatment can be expected to improve strawberry resistance to enzymes caused by fungal pathogens.

3.2. Respiration rate

Fig. 2 shows the effect of chitosan coatings on the CO_2 production of strawberries stored at 10 °C for six days. On the first day of storage, chitosan coating had a stimulatory effect on the respiration rate, a phenomenon which has been reported previously for strawberries (El Ghaouth et al., 1991a) and tomatoes (El Ghaouth, Ponnampalam, Castaigne, & Arul, 1992b). After the first day of storage, CO_2 production was lower for coated strawberries than for the control with differences becoming more noticeable after the third day of storage. Between the coating concentrations applied, respiration rate was lower for fruit coated with 1.5% chitosan but no clear differences were found between samples coated with or without the addition of CaGlu.

The respiration pattern for coated fruit differed from that of untreated fruit. The latter showed a slight increase in CO_2 production at the end of the storage period, which could be associated with fruit damage and fungal decay. For coated strawberries, the respiration rate showed a minimum on the third storage day and returned to values similar to those obtained after the first day. Internal gas atmosphere modification has been suggested to be the cause of reduced CO_2 production by coated fruits. In this regard the gas barrier properties and permselectivity (the ratio of P_{CO_2}/P_{O_2} permeation coefficient) of the edible coating applied to the skin surface and their dependence on relative humidity and temperature will play an important role in the changes in endogenous O_2 and CO_2 levels. It is well known that excessive restriction of gas exchange can lead to anaerobiosis and the development of off-flavour. Chitosan coating has been reported to modify the internal atmosphere of tomatoes (El Ghaouth et al., 1992b), Japanese pear (Du, Hiroshi, & Iwahori, 1997) and apples (Gemma & Du, 1998) by depletion of endogenous O_2 and a rise in CO_2 without achieving anaerobiosis.

3.3. Weight loss

Fruit weight loss is mainly associated with respiration and moisture evaporation through the skin. The thin skin of strawberry fruits makes them susceptible to rapid water loss, resulting in shrivelling and deterioration. The rate at which water is lost depends on the water pressure gradient between the fruit tissue and the surrounding atmosphere,

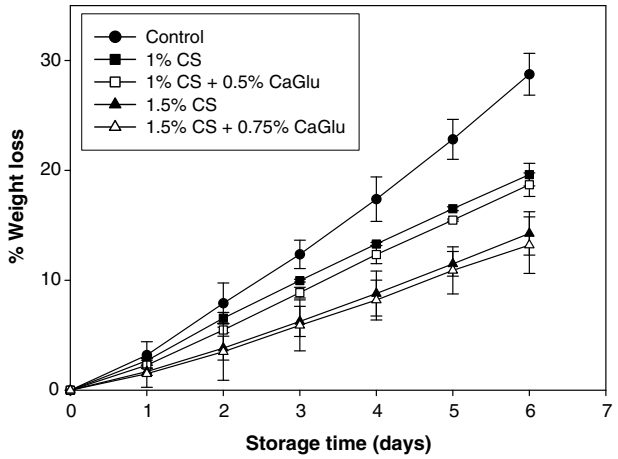

Fig. 3. Loss of weight of strawberries as a function of storage time at 10 °C. Vertical bars indicate standard deviation.

and the storage temperature. Low vapour pressure differences between the fruit and its surroundings and low temperature are recommended for the storage of strawberries. Dehydration will also cause increase in surface-wounded fruit. Edible coatings act as barriers, thereby restricting water transfer and protecting fruit skin from mechanical injuries, as well as sealing small wounds and thus delaying dehydration. Fig. 3 shows weight loss during storage (10 °C and 70 ± 5% RH) of uncoated fruit compared to fruit coated with 1% and 1.5% chitosan, with and without the addition of CaGl. All samples demonstrated a gradual loss of weight during storage. Throughout storage, the loss of weight of uncoated fruit was significantly greater than that of coated fruit. At the end of storage, untreated strawberries showed 28.7% loss in weight, whereas the weight losses of samples coated with 1% and 1.5% chitosan were 19.6% and 14.2%, respectively. The greater viscosity of the 1.5% chitosan solution likely results in a coating of greater thickness, further reducing moisture loss. As can be observed in Fig. 3, incorporation of CaGlu into the film-forming solution did not have any significant effect on weight loss reduction of strawberries. Apart from strawberry fruit, chitosan coatings have been effective at controlling water loss from other commodities, including cucumber and pepper (El Ghaouth, Arul, Ponnampalam, & Boulet, 1991b) and longan fruit (Jiang & Li, 2001). The addition of lipids and surfactants improves the moisture retention of the coating, although it also causes undesirable effects on the sensory quality of fruit. Chitosan has been reported to be more effective at delaying weight loss in banana and mango (Kittur, Saroja, & Habibunnisa Tharanathan, 2001) and strawberries (Ribeiro et al., 2007) than are starch and cellulose derivatives.

3.4. Firmness

Texture is a critical quality attribute in the consumer acceptability of fresh fruit and vegetables. Strawberry is a soft fruit that suffers a rapid loss of firmness during ripening which contributes greatly to its short postharvest life and susceptibility to fungal contamination. Fruit texture properties are affected by cell turgidity and the structure and composition of the cell wall polysaccharides. The biochemical basis of strawberry softening is not clear. Strawberry softening has been associated with the degradation of the middle lamella of cortical parenchyma cells, resulting in a dramatic increase in pectin solubilisation, with slight changes in pectin molecular weight and (Koh & Melton, 2002)small decreases in the content of hemicelluloses.

Fig. 4 shows the changes in flesh firmness of control and treated fruit during the storage period of six days at 10 °C and 70 ± 5% RH. All the samples presented similar initial flesh firmness values ($P > 0.05$). Chitosan coatings exerted a beneficial effect on fruit firmness such that, by the end of the storage period, all the treatments gave rise to fruit with higher flesh firmness values than untreated fruit ($P < 0.05$). Indeed, chitosan treatment retained the initial flesh firmness of fruit and significant differences were only found on the sixth day of storage. By contrast, uncoated fruit lost its firmness gradually during the storage period. Significant differences were noted between 1% and 1.5% chitosan coating treatments, higher values for flesh firmness being found for fruit coated with 1.5% chitosan. The beneficial effect of the elevated chitosan concentration on firmness has also been reported for tomato (El Ghaouth et al., 1992b), peach, Japanese pear, kiwifruit (Du et al., 1997) and 'Murcott' tangor (Chien, Sheu, & Lin, 2007).

Addition of CaGlu to the coating formulation increased the flesh firmness values of fruit. By the end of the storage period, fruit treated with 1% chitosan + 0.5% CaGlu gave flesh firmness values similar to those treated with 1.5% chitosan, with or without the added calcium salt. The uptake of exogenous calcium ions by ripe strawberry fruit has been related to an increase in the proportion of ionically bound pectins (Lara, García, & Vendrell, 2004), thus contributing to the maintenance of cell-to-cell adhesion and the stability of the cell wall, both of which contribute to fruit firmness.

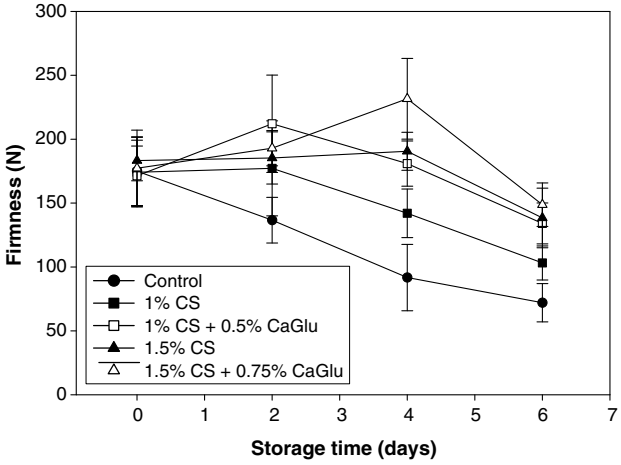

Fig. 4. Effect of chitosan coatings on the firmness of strawberries stored at 10 °C. Vertical bars indicate standard deviation.

3.5. External colour

Colour is an important factor in the perception of strawberry fruit quality. Fig. 5a–c shows the changes in surface colour of strawberries stored at 10 °C and 70 ± 5% RH for one week, as given by L^*, hue angle and chroma. Coating treatments did not impart significant changes in initial colour coordinates of fruit. The L^* parameter is an indicator of fruit darkening. As can be observed in Fig. 5a, all the samples showed decreasing L^* values with storage time. Uncoated fruit was significantly ($P < 0.05$) darker than coated fruit throughout the storage period. The chitosan concentration of the coating solution gave rise to significant differences in fruit colour. By the end of the storage period, L^* had decreased by around 27% for control fruit and by around 18% and 7% for fruit coated with 1% and 1.5% chitosan, respectively. Incorporation of CaGlu in the coating formulation did not exert any additional effect on delaying fruit darkening.

Changes in the chroma value of the strawberry surface during storage are presented in Fig. 5b. Fruit developed a less vivid colouration, as evidenced by lower values of chroma. The reduction in chroma values was significantly greater for uncoated fruit, and significant differences with respect to initial values were found after the second day of storage ($P < 0.05$). As regards coated fruit, no significant differences were found among samples treated with different concentrations of chitosan and CaGlu. Changes in the chroma of coated fruit with storage time were slight and only became significant at the end of the storage period. Chroma was reduced by around 30% for control and 10% for coated fruit.

The hue angle of uncoated strawberry began to decrease after the second day of storage and at the end of the storage period the decline was 32%. The hue angle of coated fruit did not show any significant change during storage.

Colour changes in harvested, fully red, ripe strawberries occur progressively during storage. Fruit darkens, skin colour becomes less chromatic and surface browning develops. Less red skin and darkening due to oxidative browning reactions have been found to be more marked in ripe strawberries that suffer greater moisture loss during storage (Nunes, Brecht, Morais, & Sargent, 2005). The control of moisture loss by chitosan coatings contributes to minimizing external colour changes in fully ripe strawberries. Along with water loss, colour changes in strawberry fruit are greatly influenced by storage temperature. It is thus to be expected that colour differences between control and coated strawberries be more accentuated in fruit stored at higher temperatures.

3.6. pH, titratable acidity (TA) and soluble solids content (SSC)

The pH of uncoated samples stored at 10 °C and 70 ± 5% RH increased slightly during storage ($P < 0.05$) while no significant differences were observed in the variously coated

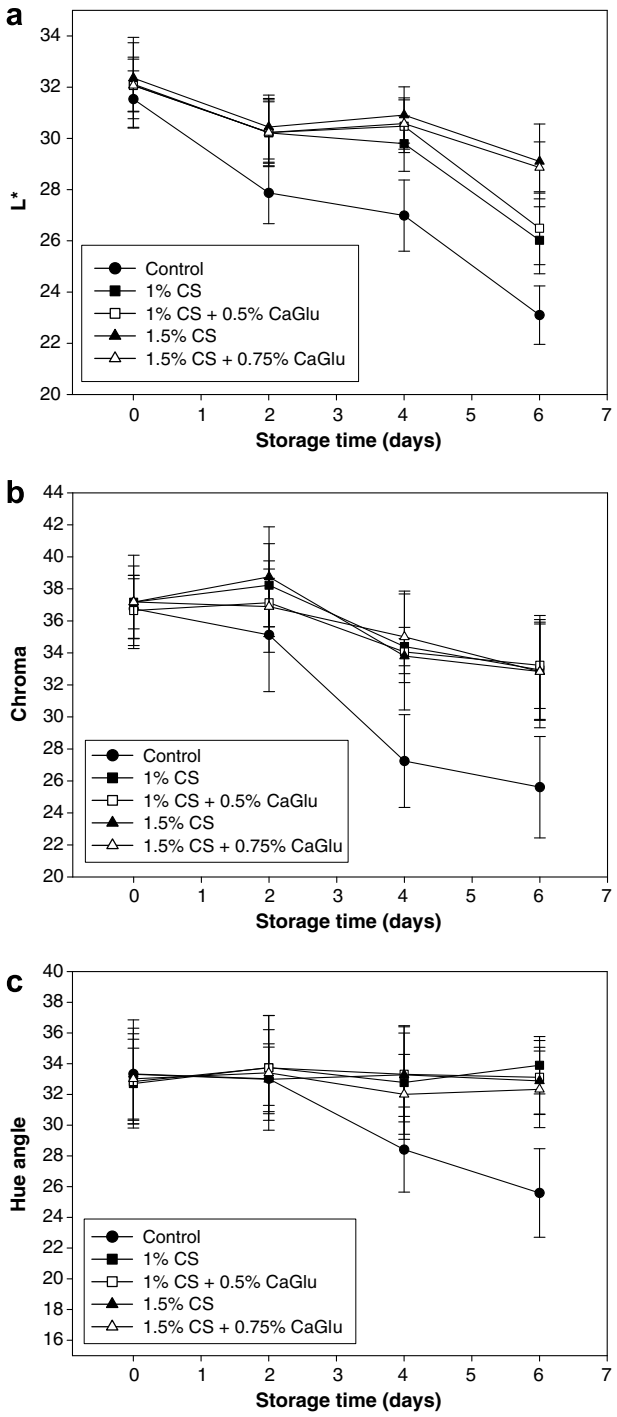

Fig. 5. External colour evolution (a) lightness, (b) chroma and (c) hue angle of control and chitosan-coated strawberries stored at 10 °C. Vertical bars indicate standard deviation.

samples (Fig. 6a). No significant changes were observed in the TA of coated fruit throughout the storage period. The TA of uncoated fruit fell slightly toward the end of the storage period but differences with respect to coated samples were not significant (data not shown). The small differences found in pH and TA during storage between uncoated and coated strawberries could be related to the greater loss of water by uncoated samples since TA is given as a percentage of citric acid per strawberry wet weight.

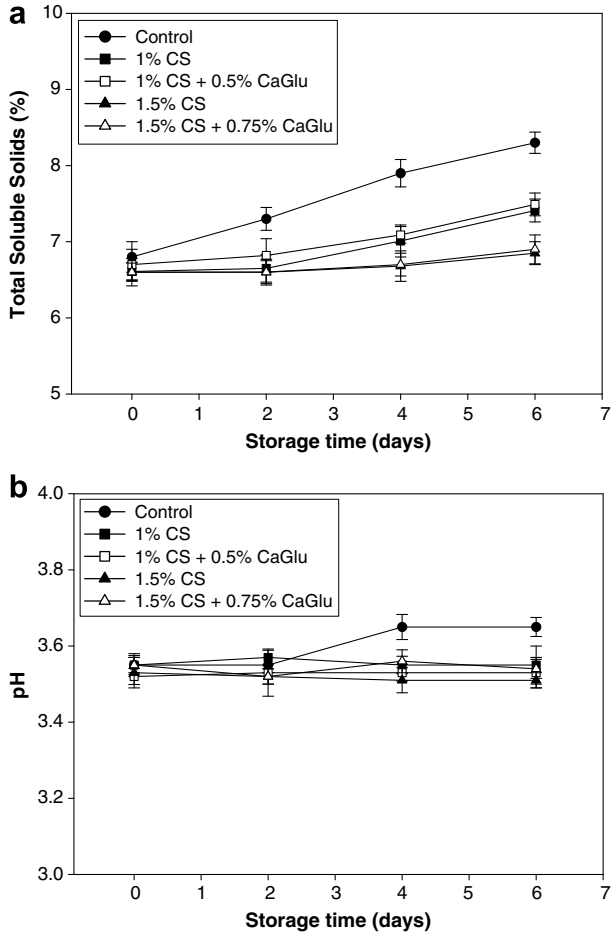

Fig. 6. Changes in (a) pH and (b) soluble solids content of strawberries as a function of storage time at 10 °C. Vertical bars indicate standard deviation.

Changes in the SSC of strawberries over storage time are shown in Fig. 6b. The SSC of control fruit increased with storage time whilst coated strawberries experienced only a very slight increase after the second day of storage. Regardless of the addition of CaGlu, samples coated with the higher concentration of chitosan showed a lower increase in SSC. Tanada-Palmu and Grosso (2005) have also reported an increase in SSC in control and gluten-coated strawberries stored at 7–10 °C and 60–80% RH. It can be expected that soluble solids content increases during strawberry ripening and decreases in mature fruit due to respiration. A plausible explanation for the observed increment in SSC is the considerable loss of water suffered by strawberries during storage. Indeed, the greater changes in SSC occurred in those strawberries which suffered the greatest water loss. The solubilization of the cell wall polyuronides and hemicelluloses in mature strawberry might also contribute to the increase in SSC.

3.7. Visual acceptance

Sensory data for strawberries stored for one week at 10 °C and 70 ± 5% RH are presented in Table 1. Initially

Table 1
Overall visual appearance of strawberries after 0, 2 and 6 days of storage at 10 °C

Treatments	Overall visual appearance (1–7)		
	Day 0	Day 2	Day 6
Control	6.0 ± 0.6a	4.1 ± 0.6a	3.0 ± 0.6a
1% CS	6.5 ± 0.6b	5.1 ± 0.7b	4.2 ± 0.7b
1% CS + 0.5% CaGlu	6.5 ± 0.6b	5.4 ± 0.7b	4.3 ± 0.6b
1.5% CS	6.6 ± 0.6b	5.0 ± 0.8b	4.1 ± 0.7b
1.5% CS + 0.75% CaGlu	6.5 ± 0.5b	5.5 ± 0.6b	4.3 ± 0.5b

Means within columns with different letters are significantly different ($P < 0.05$).

(day 0), consumers showed a preference for coated fruit, as shown by the higher mean acceptance scores obtained. Treatments did not alter strawberry colour and the greater acceptance for coated fruit could be due to the glossy appearance imparted by the coating. Chitosan coating concentration did not significantly alter the appearance of fruit and incorporation of CaGlu had no effect on the glossiness and transparency of the coating. Throughout the storage time, all the fruit showed a lost of visual acceptance. However, after the second and sixth days, consumers showed a significant preference for coated fruit which obtained mean acceptance scores between the categories "like moderately" and "neither like nor dislike" on the hedonic scale. By the sixth day of storage uncoated fruit fell below the limit of acceptability. The greater visual acceptance for coated strawberries by consumers correlates with the lower levels of dehydration and darkening experienced by them during storage.

3.8. Calcium content

The calcium content of strawberries was 2001 ± 98 ppm Ca (g kg^{-1}dry matter). The incorporation of CaGlu into the coating solution increased the calcium content by 17% for strawberries coated with 1% chitosan and by 35% in fruit coated with 1.5% chitosan. This increase in Ca content when a higher concentration of chitosan was applied can be related to the greater viscosity of the 1.5% chitosan solution and the greater solid content expected on the fruit surface. Calcium chloride is commonly employed as a firming agent to improve the texture of fresh and processed fruit, and it has also been reported to delay the fungal decay of several commodities, including strawberries. It is use, however, is limited, since it imparts a bitter and salty taste and also gives rise to other flavour changes in fruit. Alternatives to the use of calcium chloride are calcium lactates and gluconates which impart a more neutral taste without altering fruit flavour (Luna-Guzman & Barrett, 2000). The employment of organic acid salts of calcium as firming agents enhances the nutritional value of foods since organic calcium salts are more bioavailable than are the inorganic salts. Organic calcium salts are already employed in therapeutic applications, such as

nutraceutical dietary supplements and in the fortification of foods. In this regard, edible coatings represent an alternative means to enrich the nutritional value of foods.

In summary, chitosan coating was seen to delay fruit senescence and fungal decay of strawberry fruits stored at 10 °C and 70 ± 5% relative humidity. The beneficial effect of chitosan was enhanced when the polymer was applied at a greater concentration. CaGlu contributed to extending the shelf-life of strawberries by inhibiting fungal decay and maintaining fruit firmness when used in combination with a low concentration of chitosan. A chitosan concentration greater than 1% masked any effect of CaGlu. Sensory analysis of strawberries based on visual appeal showed that chitosan coatings delayed fruit senescence associated with colour changes and dehydration. Incorporation of CaGlu increased the nutritional value of fruit without altering visual appeal. Since chitosan application modified the respiration pattern of strawberry, further research is underway to evaluate the effect of this treatment on the strawberry flavour profile.

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