



Research Article

Effect of edible quinoa protein-chitosan based films on refrigerated strawberry (*Fragaria × ananassa*) quality



Carolina Valenzuela^{a,*}, Cristian Tapia^b, Luis López^b, Andrea Bunger^b, Víctor Escalona^c, Lilian Abugoch^{b,*}

^a Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, Chile

^b Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile

^c Facultad de Ciencias Agronómicas, Universidad de Chile, Santiago, Chile

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ABSTRACT

Background: Strawberries are non-climacteric fruits with a low respiration rate, but are subject to serious fungal deterioration during postharvest handling. The edible coatings based on chitosan (CH), quinoa protein-chitosan (Q/CH) and quinoa protein-chitosan-sunflower oil (Q/CH/SO) may provide a solution to this problem. Thus, in this work CH, Q/CH and Q/CH/SO were elaborated and applied to fresh strawberries, and its effect on the strawberries shelf life during storage for 15 d was evaluated by mold and yeast count, fungal decay, carbon dioxide rate, physicochemical properties, and sensory evaluation.

Results: On all analysis days, the strawberries coated with the film-forming CH, Q/CH and Q/CH/SO solutions presented a significant lower amount of mold and yeast growth than the uncoated strawberries. Coated strawberries with Q/CH/SO decreased the CO₂ emission rate by 60% compared to the uncoated strawberries. The color of the strawberries was not influenced by the films. There was no significant difference between the different coating groups and the uncoated group in the physicochemical parameters. Sensory analysis showed that the coating application retained the total sensorial quality.

Conclusions: Fresh strawberries coated with CH, Q/CH/SO and Q/CH edible films had longer shelf lives than uncoated fruits.

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1. Introduction

Edible films have been widely studied and used to coat strawberries, however, despite investigations in the field, a successful formula that will maintain its quality and increase its shelf life has still not been reached [1,2,3]. In the literature there are many works focused on to increase the shelf life of the strawberries, because they are sensory highly appreciated by consumers and also have large amounts of bioactive compounds. Strawberry fruit is non-climacteric and highly susceptible to fungal decay, mechanical injury, and water loss during storage [4,5]. Strawberries have a very short postharvest life (around 5 d at 0–4°C) that limits its commercialization and consumption [5]. The combination of refrigeration and the use of edible films can increase the postharvest shelf life of strawberries, however the major problems that need to be confronted are to prevent water loss and microbiological development, and keep their organoleptic properties during storage [4,6,7].

Chitosan (CH) has been used because it has a good film-forming and anti-fungal activity against several post-harvest pathogens, especially grey mold (*Botrytis cinerea*), which is one of the main causes of the deterioration and postharvest decay of strawberries [8]. However, the CH films have a hydrophilic nature and high water vapor permeability that gets worse with the use of plasticizers. Besides, according to Han et al. [9], and Vargas et al. [10], the CH solutions to cover strawberries produced astringent fruits that cause rejection by the sensory panel. To improve some properties of the CH edible films to cover strawberries, this has been blended with other polymers as polysaccharide [4,11], and hydrophobic components, which enhance the water vapor barrier properties [1,2,12]. However, the use of lipids to be effective must be made in high proportions, and this causes a bad taste and oily sensation [10,12,13].

Chenopodium quinoa Willd (quinoa) has been cultivated in the Andean region for several decades. Quinoa seeds are a complete food with high-nutritional value due mainly to their high content of good quality protein [14]; which has been sparsely studied as edible coating materials. Quinoa protein (Q) was shown to be a good biopolymer to form composite edible films in blend with CH yielding mechanically resistant films without the use of a plasticizer [15]; and the addition of low levels of SO to the quinoa protein-chitosan (Q/CH) films improved the water vapor permeability as a result of hydrophobic interactions

* Corresponding authors.

E-mail addresses: cvalenzuelav@u.uchile.cl (C. Valenzuela), labugoch@uchile.cl (L. Abugoch).

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[16]. To our knowledge, the formation/application of composite edible film based on proteins extracted from quinoa flour to evaluate the shelf life of strawberries has not been reported previously. Therefore, the aim of this work was to evaluate the effectiveness of CH, Q/CH and quinoa protein-chitosan-sunflower oil (Q/CH/SO) edible films to improve strawberry fruit storability.

2. Materials and methods

2.1. Materials

Fragaria × ananassa (strawberries) were obtained from Hortifrut S.A, Chile, and were taken to the laboratory keeping cold chain, and covering experiments were carried out on the same day. Quinoa flour (free saponins) was supplied by “Cooperativa Las Nieves” in the VI Region of Chile. CH ($\leq 1\%$ insoluble matter and viscosity $>400 \text{ mPa}\cdot\text{s}$, 1% in acetic acid at 20°C) was obtained from crab shells (Sigma-Aldrich, USA). SO was purchased from Camilo Ferrón S.A, Chile ($\geq 700 \text{ g kg}^{-1}$ oleic acid), and Tween 80 (T_{80}) was purchased from Comercial Montero Ltda., Chile.

2.2. Preparation of coating solutions

The coating solutions were prepared according Valenzuela et al. [16]. Solution of CH at 2% w/v in citric acid 0.1 M was prepared. The quinoa flour was suspended in distilled water (18% w/v), and the pH was adjusted to pH 8 with 1 M NaOH. This suspension was stirred for 60 min and centrifuged at $21,000 \times g$ for 30 min at 15°C . The supernatant obtained (quinoa protein) was denominated Q. The soluble protein content of the Q was measured according to Bradford's method and reported by Valenzuela et al. [17] as $7.5 \pm 0.4 \text{ mg protein mL}^{-1}$.

The Q/CH blends were prepared by mixing solutions of Q and CH at 0.1 Q/CH ratio using a blade homogenizer (Bosch MSM6A3R 750w, China). The pH of the mixtures was adjusted to 3.0 with 1 mol/L citric acid, and stirring was continued for 30 min. Then, the Q/CH was blended with 2.9% w/v of SO and T_{80} at 0.6% w/v with a high-speed Ultraturrax (Silverson L4R Machines, UK) for 10 min at 10,000 rpm. Then, CH was incorporated into the blend by mixing with a blade homogenizer for 10 min at 1000 rpm. The pH was adjusted to 3.0 with citric acid. The coating solutions were made on the same day as the application on strawberries.

2.3. Coating application on strawberries

Twelve kg of strawberries were used for each treatment condition, and all analyses were performed in triplicate. Only fruits with over 75% surface red color that were uniform in size and did not have visible mechanical damage or fungal infection were selected for the study. Fresh fruits were randomly assigned to 4 treatments: (1) uncoated group, which did not have any manipulation, (2) CH coated group, (3) Q/CH coated group, and (4) Q/CH/SO coated group. The fruits in each coating group were immersed into the coating solutions for 1 min, the excess of the film-forming solutions were drained, and the coated strawberries were dried in a chamber with a forced-air dryer overnight ($4\text{--}5^\circ\text{C}$ and 80% RH). The strawberries were packed into commercial clamshell, and they were stored in a refrigerated chamber at $0 \pm 0.5^\circ\text{C}$ and 90% RH. Three replications per treatment were analyzed for shelf life analysis on d 0, 5, 10, and 15 of storage.

2.4. Film formation on strawberries surface

Prior to the shelf life analysis, the formation and thickness of the coatings on the surface of the strawberries at 0 and 15 d were observed. Then, 100 g of strawberries from each treatment was frozen

at -18°C for 24 h, and samples of frozen tissue surface sections (thickness $\approx 0.1 \text{ mm}$) were collected and examined with an optical microscope (AxioStar plus Carl Zeiss, USA). The thickness was measured with the software AxioVision v. 4.8, USA.

2.5. Mold and yeast count, and fungal decay

The microbiological analyses were performed in triplicate according to the Official Standard Method [18]. For the yeast and mold count, 10 g of strawberries were cut into small pieces and suspended in 90 mL of peptone water. The suspension was mixed in a blender (Seward Stomacher 400 Lab System, Norfolk, UK) for 5 min. Serial dilutions (10^{-1} , 10^{-2} and 10^{-3}) of the strawberry homogenates were plated on the surface of selective media (Potato Dextrose Agar, OXOID, UK), and the uninverted plates were incubated at 25°C for 5 d. Mold and yeast counts were expressed as logarithm colony forming units per gram of strawberries (log CFU/g).

Fungal decay was visually inspected in 20 strawberries per treatment. A fruit was considered to be infected when visible contamination was observed (development of mycelium on the fruit surface, brown spots and a softening of the injured zone). The results were expressed as the percentage of fruits infected. Two strawberries were randomly selected from each treatment on d 0, 5, 10 and 15 to photograph the development of spoilage during the storage period using a digital camera (Sony DSC-HX1, Japan).

2.6. Carbon dioxide emission rate (CO_2)

To test the CO_2 emission rate, 250 g of strawberries was placed in 500 mL hermetically sealed glass containers and was stored with the lids open at 0°C and 90% RH. After 1 h of enclosure, 1 mL of air sample was extracted from the headspace and was analyzed by gas chromatography for CO_2 (Hewlett Packard 5820, USA).

2.7. Physicochemical properties

2.7.1. Color

The color of the strawberry surface was measured with a colorimeter (Hunter Lab system, Model Miniscan 2.0/45, USA) using the Hunter Lab color scale.

2.7.2. Weight loss

Two hundred grams from each treatment was weighed, just after air-drying at the beginning and during storage period. The results were expressed as the percentage of loss compared to the initial weight.

2.7.3. Firmness

The firmness of the strawberries was determined using the method proposed by Hernández-Muñoz et al. [7], with a universal tensile testing machine (LLOYD, Model LR5K, UK) controlled by DAPMAT v. 3.0 software. The firmness was reported as the peak force and was expressed in newtons (N). Firmness was measured as the maximum penetration force determined with a 1 mm diameter metal probe. The penetration depth was 5 mm. Twenty-five fruit for each treatment were sliced into halves and each half was measured to 1 mm of central zone (“strawberry shoulder”).

2.7.4. pH, soluble solid content (SSC), titratable acidity (TA), and maturity index (MI)

Five strawberries from each group were ground and filtered. Measurements of pH were carried out using a pH meter (pH-537, KFW Microprocessor, USA). SSC were measured by a digital refractometer (PR1; Atago, Co. Ltd, Japan) at $25 \pm 2^\circ\text{C}$. TA was determinate according AOAC (method 942.15) [19], and was expressed as g of citric acid per 100 g of fruit. MI was calculated as the quotient of SSC and TA.

2.8. Sensory evaluation

The sensory total quality (STQ) of the uncoated and coated strawberries was performed at 0, 4 and 9 d of storage. The STQ (external and internal color, appearance, aroma, flavor, texture) was evaluated by 14 trained assessors, according to a 9-point-Karlsruhe scale. Samples were considered acceptable if the mean value of the sample was equal to or above a score of 5. The total quality was obtained by weighing the sensory parameters by the following percentages according to importance: 15% external color, 10% internal color, 20% appearance, 15% aroma, 20% flavor, and 20% texture.

2.9. Statistical analysis

StatGraphics plus (v. 5.1) was used for all of the statistical analyses. Analysis of variance (ANOVA) and significance of differences between the means of Tukey's tests ($P < 0.05$) were used to determine significance.

3. Results and discussion

3.1. Film formation on strawberries surface

The coatings were found adhered to the surface of the strawberries during the entire storage time (Fig. 1). The coating that formed over surface presented a continuous appearance, which confirmed that the application technique was effective. This experiment is important because none of the publications reviewed in the literature report this aspect. The thickness range (indicated as T in Fig. 1) was similar for all coatings, fluctuating from 442 to 692 μm . On d 15, no variations in the thickness of the coatings were observed; however, a reddish coloration had developed in the films, possibly by pigment migration from the strawberries into the coatings (Fig. 1, d 15). This phenomenon was more intense in the strawberries that were coated with the CH film due to its higher hydrophilicity [15].

3.2. Reduction in the mold and yeast count and in the fungal decay

The importance of this study lies in the quantification of the log CFU/g of the uncoated and coated strawberries because similar

experiments monitored the antimicrobial effect of the coating on fungal decay by qualitative determination through simple observation, and microbiological counts were only performed in a few studies [11,20]. The evolution of the mold and yeast infection during the 15 d storage of the coated and uncoated strawberries is shown in Fig. 2. On all analysis days, the strawberries coated with the film-forming CH, Q/CH and Q/CH/SO solutions presented a significantly lower amount of mold and yeast growth than the uncoated strawberries; however, the mold and yeast reduction was more evident in the strawberries that were coated with CH (Fig. 2a). The mold and yeast counts reported by this study were similar to the counts for strawberries coated with CH described by Ribeiro et al. [11]. The lower effectiveness of the Q/CH and Q/CH/SO coatings is due to the ionic and hydrophobic interaction between CH-Q and CH-Q-SO, respectively [16], which reduces the availability of the reactive amino groups of the CH to the antimicrobial properties.

On d 5, the CH coating prevented all fungal damage to the fruit, 10% of the uncoated fruit was damaged and 3.3% of the strawberries coated with the Q/CH and Q/CH/SO blends were damaged. On d 10 and 15, the coating treatments reduced the damage to the fruits, mainly in the fruits covered with CH film. On the d 15, the coated strawberries showed 18.3%, 36.7% and 50% damage for CH, Q/CH and Q/CH/SO coatings groups, respectively, while the uncoated strawberries showed approximately 100% damage (Fig. 2b).

On d 5 of storage, the uncoated strawberries exhibited brown spots (denoted with an arrow in Fig. 2c), which is indicative of the onset of a fungal infection [21], while the coated strawberries did not exhibit injuries. On d 10, the uncoated strawberries showed the development of mycelium on the tip of the fruit (denoted with a circle in Fig. 2c). In the strawberries coated with Q/CH/SO, the observed fungal infection was focused (denoted with an arrow in Fig. 2c), and the other samples exhibited no lesions. At the end of the storage period all strawberry samples had fungal contamination; however, the infection in the coated fruits was more focused than in the uncoated fruits, which had infections that covered most of the surface of the fruit. Only small, focalized fungal growth was observed in strawberries that were coated with CH.

Other authors have demonstrated similar results in strawberries coated with CH [1,3,20]; because of the antimicrobial capacity of CH, especially against the fungi and yeast spoilage of strawberries [8]. CH

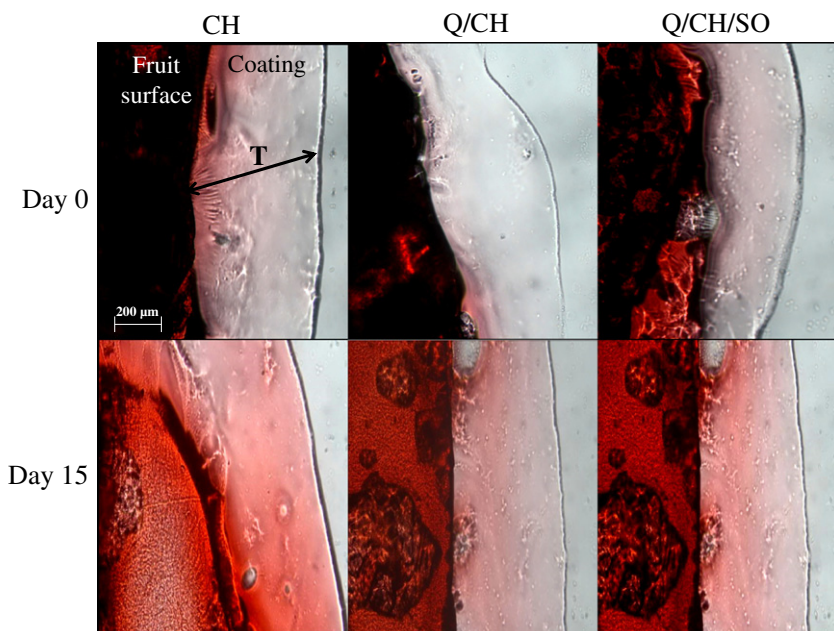


Fig. 1. Edible films formation of CH, Q/CH, and Q/CH/SO on strawberries surface at 0 and 15 d of storage.

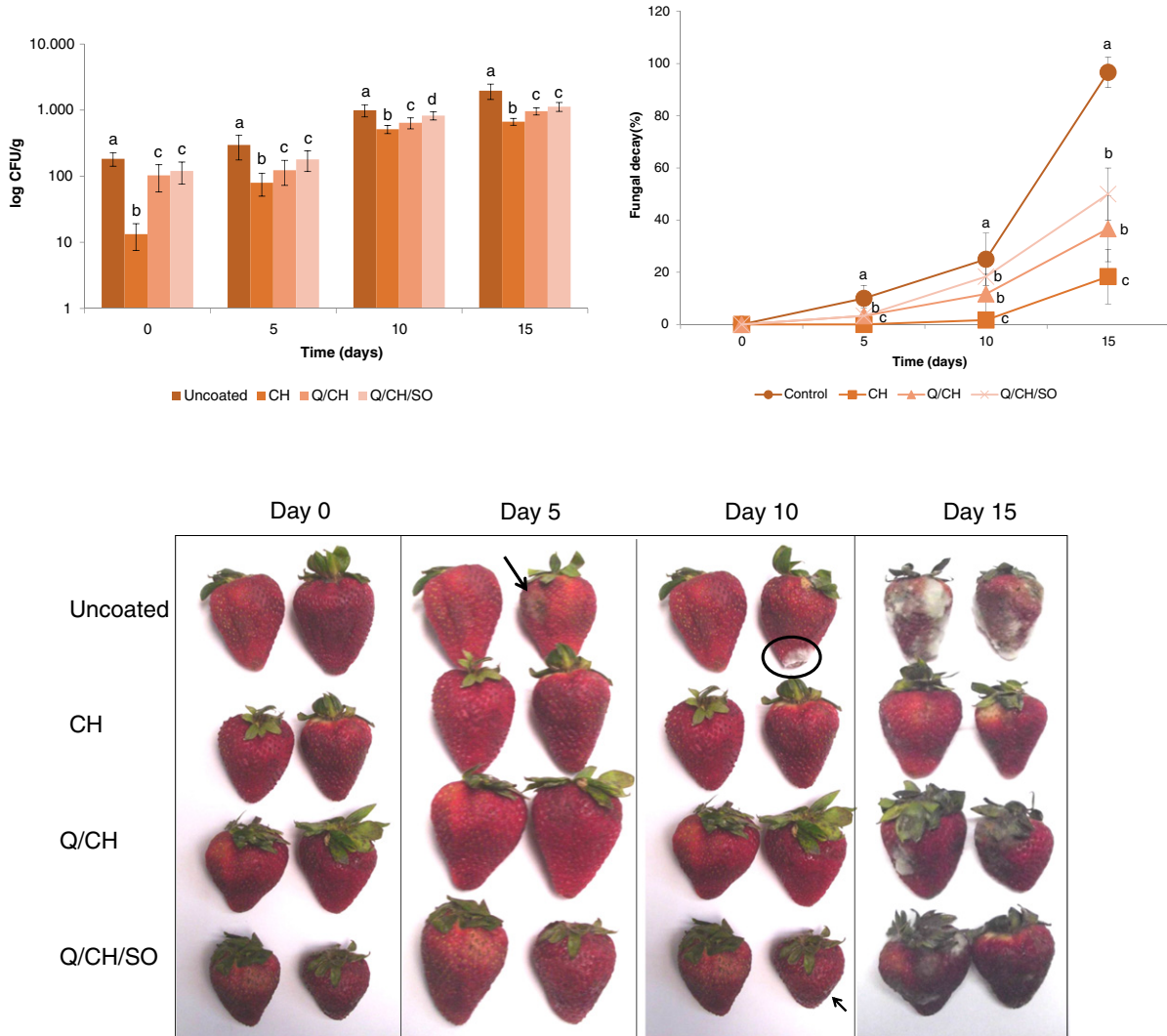


Fig. 2. Evolution of mold and yeast counts (a), fungal decay (b) and appearance (c) during the storage of uncoated and coated strawberries with CH, quinoa protein/chitosan Q/CH, and Q/CH/SO edible films. Different letters in each day show significant differences ($P < 0.05$).

potentially causes severe cellular damage in mold and yeast by altering the synthesis of fungal enzymes [22], inducing morphological changes, and causing structural alterations and molecular disorganization in fungal cells [23]. Fungal contamination on the surface of strawberries is a main reason that consumers do not purchase and consume this fruit, and coating the surface of strawberries with an edible film-forming solution can decrease this type of spoilage and increase the shelf life from 7 to 10 d for the uncoated fruits to approximately 12 d for fruits coated with CH, Q/CH or Q/CH/SO films.

3.3. Carbon dioxide emission rate (CO_2)

All of the coated strawberries presented a significantly lower CO_2 emission rate than the uncoated fruit; the coated strawberries with Q/CH/SO decreased the CO_2 emission rate by 60% compared to the uncoated strawberries. On d 5 of storage, Q/CH/SO was the only coating that maintained a significantly lower CO_2 emission rate. On d 10 and 15, no significant differences were observed between any of the samples (Fig. 3). During the last period of storage (10 to 15 d), all of the strawberry samples showed a CO_2 emission rate within the expected values (< 20 mg de CO_2 kg/h) for strawberries stored under refrigerated conditions ($0^\circ C$) [5]. The lower CO_2 emission rate observed from the coated strawberries compared with uncoated during the first storage stage (0 and 5 d) could be

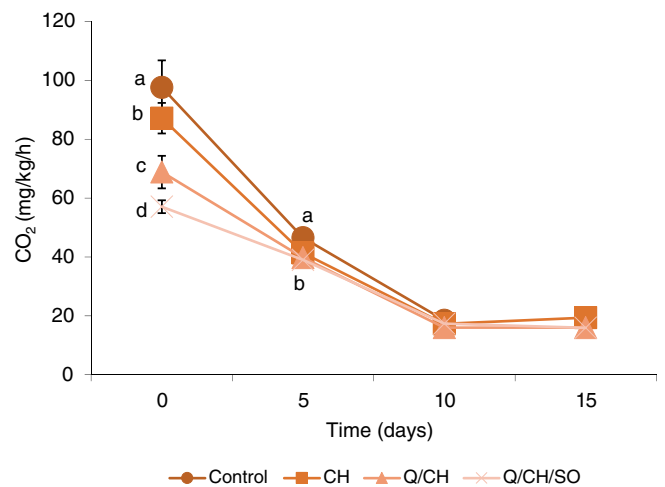


Fig. 3. Carbon dioxide (CO_2) emission rate of uncoated and coated strawberries with CH, Q/CH, and Q/CH/SO edible films, throughout storage. Different letters in each day show significant differences ($P < 0.05$).

caused by internal gas atmosphere modifications by the coating on the strawberries [24], a phenomenon that was also observed by Hernández-Muñoz et al. [7], Perdonés et al. [1], and Vargas et al. [10] in strawberries coated with CH. The additional lipid molecules in the Q/CH/SO formulation could explain the lower CO₂ emission rate from the strawberries with this coating [25]. Vargas et al. [10] also observed significantly reduced CO₂ emission rate in strawberries coated with CH-oleic acid edible coatings when the ratio of oleic acid in the films increased (1 and 2% v/v).

3.4. Physicochemical properties

The color of the strawberries was not influenced by the coatings (Fig. 4); this result was satisfactory because some studies have shown that application of emulsified film-forming solutions on strawberries can cause color changes and increase the opacity of the coated fruits [10]. The chroma and hue angle of the coated and uncoated samples did not change, although all strawberries exhibited significantly lower hue and chroma values on the last analysis day, as has been reported by other authors [1,10]. The decrease of the chroma indicates a change to less vivid colors, and the hue angle darkening of the strawberry skin is characteristic of oxidative browning reactions that are typical of fruit senescence [10].

Table 1 shows the weight loss evolution of uncoated and coated strawberries during storage. On d 5, the weight loss of the strawberries coated with Q/CH/SO was similar to the weight loss of the uncoated samples and was significantly lower than the weight loss of the samples coated with CH or Q/CH. On d 10 and

Table 1

Weight loss (respect control), firmness, pH, TA, SSC and MI of uncoated and coated strawberries with CH, Q/CH, and Q/CH/SO edible films.

Days	Uncoated	CH	Q/CH	Q/CH/SO
<i>Weight loss (%)</i>				
5	0.5 ± 0.1 ^{ax}	0.9 ± 0.2 ^{bx}	1.1 ± 0.3 ^{bx}	0.6 ± 0.1 ^{ax}
10	1.7 ± 0.2 ^{ay}	2.0 ± 0.2 ^{by}	1.7 ± 0.3 ^{ay}	1.7 ± 0.2 ^{ay}
15	2.4 ± 0.2 ^{az}	2.8 ± 0.2 ^{bz}	2.4 ± 0.2 ^{az}	2.4 ± 0.3 ^{az}
<i>Firmness (N)</i>				
0	1.01 ± 0.23 ^{ax}	1.07 ± 0.18 ^{ax}	1.02 ± 0.23 ^{ax}	1.04 ± 0.21 ^{ax}
5	1.00 ± 0.18 ^{ax}	1.03 ± 0.23 ^{ax}	1.01 ± 0.19 ^{ax}	1.02 ± 0.19 ^{ax}
10	0.99 ± 0.15 ^{ax}	0.98 ± 0.21 ^{ax}	0.89 ± 0.15 ^{ax}	0.95 ± 0.22 ^{ax}
15	0.65 ± 0.14 ^{ay}	0.63 ± 0.14 ^{ay}	0.65 ± 0.15 ^{ay}	0.62 ± 0.18 ^{ay}
<i>pH</i>				
0	3.26 ± 0.04 ^{ax}	3.27 ± 0.03 ^{ax}	3.22 ± 0.03 ^{ax}	3.28 ± 0.04 ^{ax}
5	3.29 ± 0.02 ^{ax}	3.24 ± 0.02 ^{ax}	3.26 ± 0.04 ^{ax}	3.26 ± 0.02 ^{ax}
10	3.25 ± 0.01 ^{ax}	3.26 ± 0.02 ^{ax}	3.24 ± 0.02 ^{ax}	3.24 ± 0.03 ^{ax}
15	3.44 ± 0.02 ^{ay}	3.40 ± 0.02 ^{ay}	3.46 ± 0.04 ^{ay}	3.47 ± 0.04 ^{ay}
<i>TA (g of citric acid/100 g)</i>				
0	1.08 ± 0.04 ^{ax}	1.11 ± 0.07 ^{ax}	1.07 ± 0.02 ^{ax}	1.10 ± 0.05 ^{ax}
5	1.05 ± 0.05 ^{ax}	0.99 ± 0.05 ^{ax}	0.98 ± 0.05 ^{ax}	1.03 ± 0.07 ^{ax}
10	0.92 ± 0.04 ^{ax}	0.91 ± 0.07 ^{ax}	1.00 ± 0.04 ^{ax}	0.93 ± 0.04 ^{ax}
15	0.81 ± 0.02 ^{ay}	0.71 ± 0.04 ^{ay}	0.82 ± 0.03 ^{ay}	0.78 ± 0.04 ^{ay}
<i>SSC (%)</i>				
0	10.8 ± 0.5 ^{ax}	9.8 ± 0.5 ^{ax}	10.0 ± 0.7 ^{ax}	10.2 ± 0.4 ^{ax}
5	11.0 ± 0.7 ^{ax}	10.5 ± 0.5 ^{ax}	11.1 ± 0.5 ^{ax}	10.8 ± 0.5 ^{ax}
10	11.0 ± 0.4 ^{ax}	11.0 ± 0.7 ^{ax}	11.4 ± 0.5 ^{ax}	11.2 ± 0.6 ^{ax}
15	12.5 ± 0.5 ^{ay}	12.5 ± 0.4 ^{ay}	12.8 ± 0.6 ^{ay}	12.7 ± 0.4 ^{ay}
<i>IM (SSC/TA)</i>				
0	9.4 ± 0.3 ^{ax}	9.0 ± 0.5 ^{ax}	9.1 ± 0.3 ^{ax}	10.1 ± 0.6 ^{ax}
5	10.4 ± 0.4 ^{ax}	11.0 ± 1.0 ^{ax}	11.0 ± 0.4 ^{ax}	11.0 ± 0.7 ^{ax}
10	12.4 ± 0.4 ^{ax}	12.3 ± 0.7 ^{ax}	12.8 ± 0.6 ^{ax}	12.9 ± 0.5 ^{ax}
15	13.8 ± 0.5 ^{ay}	13.6 ± 0.7 ^{ay}	13.8 ± 0.4 ^{ay}	14.1 ± 0.7 ^{ay}

Different letters show significant differences for treatments (a, b) and storage time (x, y, z) ($P < 0.05$).

15 of storage, the CH coated strawberries showed the highest weight loss. This behavior was expected because of the hydrophilic nature of CH, which interacts with water molecules in the environment and increases the flow of permeable water vapor [15,16]. The addition of 2.9% w/v of SO was insufficient to avoid minor levels of water loss. The weight loss of all strawberries (coated and uncoated) did not exceed the market limit (5%) [5].

Table 1 shows the firmness evolution of the uncoated and coated strawberries during storage. There was no significant difference between the different coating groups and the uncoated group in the loss of firmness. On d 15, the firmness of the coated and uncoated fruits decreased significantly due to senescence, which softens the fruits by pectin hydrolysis and depolymerization, degradation of the cell wall and cellular breakdown, causing a loss of fruit turgidity [26].

The pH, SSC, TA and MI parameters of the uncoated and coated strawberries during storage at 0 ± 0.5°C and 90% RH for 15 d are shown in Table 1. The pH, SSC, TA and MI were not affected by the coating treatments during storage, in agreement with Vargas et al. [10]. On d 15, the pH, SSC and MI increased, and the TA decreased significantly in the uncoated and coated fruit. These changes during the last period of storage were a result of fruit senescence, as was observed in the firmness analysis.

3.5. Sensory total quality

The STQ of the uncoated and coated strawberries ranged from 6.4 to 7.6 during the entire storage time. The STQ did not decrease until d 9 for any of the tested samples. No significant differences were found between the uncoated or coated strawberries nor between storage times. On d 4, differences in aroma indicated that the CH coating was a slightly lower rated quality than the Q/CH/SO coated and the uncoated samples, and on d 9, the appearance of the Q/CH group was

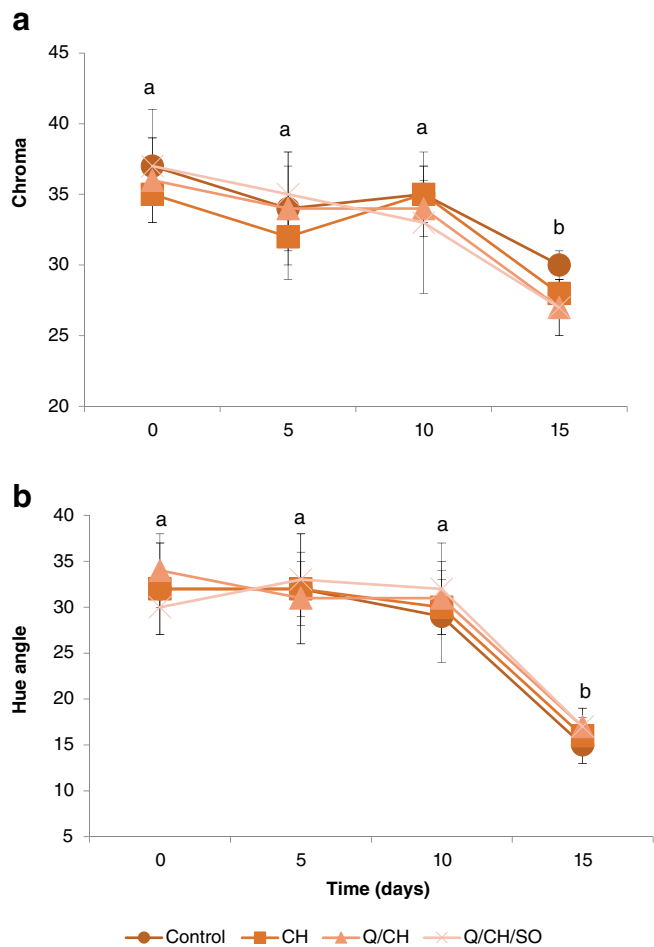


Fig. 4. Chroma (a) and hue angle (b) of uncoated and coated strawberries with CH, Q/CH, and Q/CH/SO edible films, throughout storage. Different letters in each day show significant differences ($P < 0.05$).

significantly lower rated than the CH group. The panelist did not detect the oiliness or astringency in the strawberries coated with Q/CH/SO that has been reported in fresh fruits coated with emulsified film-forming solutions with hydrophobic agents [10,12,13].

4. Conclusions

Fresh strawberries coated with CH, Q/CH/SO and Q/CH coatings had longer shelf lives than uncoated fruits. This effect is mainly due to the antifungal activity of CH, which remains when CH is combined with quinoa protein and sunflower oil. A sensory study assessed the consumer acceptance of the coated strawberries.

Conflict of interest

None.

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