Impact of chitosan-beeswax edible coatings on the quality of fresh strawberries (*Fragaria ananassa* cv Camarosa) under commercial storage conditions

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ABSTRACT

Chitosan-based edible coatings were used to prolong the shelf-life of strawberries stored at 20 °C and 35 −40% RH. Strawberries were coated with four different coating formulations (chitosan as monolayer, three layer coating consisting of separate beeswax–chitosan–beeswax layers, three layer coating where chitosan was crosslinked with sodium tripolyphosphate, TPP, and composite). The effectiveness of the coatings was evaluated by the changes of several parameters: fungal infection, weight loss, respiration rate, skin and flesh color, firmness, pH value, titratabale acidity, soluble solids content, reducing sugars content and sensory evaluation. The coatings, especially the three-layer coatings, significantly decreased the senescence and weight loss of the fruits. They modified the respiration rates of the strawberries and slowed down their metabolism as shown by the retention of the color and the texture of the tissue. Sensory evaluation of the coated strawberries showed that the chitosan and composite coatings gave better visual appearance and taste and were therefore more preferable by 90% of the judges than the three-layer coatings, even though the later had higher protective effect of the overall quality of the strawberries.

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1. Introduction

Attempts to reduce the crop losses and maintain the quality of the fresh fruits over extended period has been priority to all food producers. This is true both for fruits being directly sold for consumption or for fruits being subjected to further processing. For the consumers, the most important attributes of fresh fruits are flavor and appearance, as well as their safety and nutritional value.

Strawberries are one of the most popular summer fruits worldwide that are characterized with unique and highly desirable taste and flavor. They are rich in polyphenols and anthocyanins, vitamins and amino acids. The main characteristics related to the quality of the ripe strawberries are their texture, flavor (organic acids and soluble sugars content) and color (Campaniello, Bevilacqua, Sinigaglia, & Corbo, 2008; Koyuncu & Dilmaçünal, 2010). Due to their very active metabolism strawberries are highly perishable and have high physiological postharvest activities which lead to short ripening and senescence periods that make their marketing a challenge (Garcia, Martino, & Zaritzky, 1998). Loss of quality in this fruit is connected with its sensitivity to fungal infection and susceptibility to water loss, bruising, mechanical injuries and texture softening due to the lack of protective rind (Atress, El-Mogy, Aboul-Anean, & Alsanius, 2010).

Several preservation techniques such as refrigeration, synthetic chemical fungicides, modified atmosphere packaging, osmotic treatments, hypobaric treatments and heat treatments have been studied to extend the shelf life of fresh or minimally processed strawberries (An, Park, & Lee, 2009; Castello, Fito, & Chiralt, 2010; Harker, Redgwell, Hallet, Murray, & Carter, 2000; Vicente, Costa, Martinez, Chaves, & Civello, 2005; Wszelaki & Mitcham, 2000; Zhu & Zhou, 2007). In the last several years, edible coatings have been widely studied for preservation of fruits and vegetables. Their beneficial effects and easy handling made them broadly applicable to soft fruits as the berries. The main functional advantages attributed to use of edible coatings are slower respiration rate, extended storage periods, firmness retention and controlled microbial growth (Fan et al., 2009; Garcia, Pereira, de Luca Sarantopoulos, & Hubinger, 2010; Vu, Hollingsworth, Leroux, Salmieri, & Lacroix, 2011).

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Development of edible coatings has been based upon the use of polysaccharides, proteins, lipids or their combination in different ways. Polysaccharides and proteins are known to form films with good mechanical properties, but with poor permeability, while the lipids form brittle films but with improved permeability. Therefore, the newly developed coatings are focused on combining the polymer matrix with some hydrophobic component (Navarro-Tarazaga, Sothornvit, & Perez-Gago, 2008; Zúñiga et al., 2012).

Previous studies related to chitosan indicated its potential to prolong storage period and control decay of many fruits, including strawberries (Hernández-Muñoz, Almenar, Ocio, & Gavara, 2006; Lin, Du, Liang, Wang, & Yang, 2011; Vargas, Albors, Chiralt, & González-Martínez, 2006). The wide application of chitosan as edible coating material stem from its good biocompatibility, biodegradability, antibacterial and antifungal activity, membrane forming capacity and safety. The disadvantages of the chitosan films and coatings lay in its relatively high water vapor permeability and possibility to cause allergies to some people. To this date Wong, Gastineu, Gregorski, Tillin, and Pavlath (1992) added saturated fatty acids and fatty acid esters; Vargas et al. (2006) added unsaturated fatty acids and Perdones, Sanchez-Gonzalez, Chiralt, and Vargas (2012) added lemon essential oil to chitosan based coatings to improve their barrier properties to water vapor transfer. So far, there are no data reported on the combined effect of beeswax and chitosan coatings on the water vapor permeability. Furthermore, the vast majority of the published papers focused on the quality of coated strawberries during refrigerated storage (4–8 °C). Therefore, the first goal of the presented work was to study the effect of chitosan based mono- and three layered coatings modified with beeswax and sodium tripolyphosphate on the quality attributes of less investigated strawberry variety *Fragaria ananassa* cv Camarosa. Chitosan coatings could be prepared in a wide range of concentrations (Mitra & Dey, 2011). During preliminary laboratory testing the 0.8 g/100 g chitosan solution provided good adhesion to the fruit and it was chosen for further experiments. Three layered coatings (beeswax−chitosan−beeswax) were prepared to improve the water vapor permeability barrier of the original chitosan films and to avoid the direct contact of the hydrophilic chitosan films with the fruit and the atmosphere. The second goal was to evaluate the quality of coated strawberries at ambient temperature of 20 °C instead of refrigerated storage. Non-coated fruits and fruits coated with four different types of coatings were compared and analyzed in terms of fungal infection, weight loss, respiration rate, firmness retention, skin and flesh color development, titratable acidity, soluble solids, reducing sugar content and sensory evaluation.

2. Material and methods

2.1. Raw material and chemicals

Strawberries (*Fragaria ananassa* cv Camarosa) were purchased in a local supermarket, Continente, Lisbon, Portugal. All fruits were selected based on the same ripening stage (>75% red surface color), uniform size (height: 30 ± 2 mm; width: 27 ± 3 mm), absence of any physical damage and fungal infection. The strawberry composition was determined by the producer and it contained 0.7 g/100 g proteins, 7.0 g/100 g carbohydrates, 0.5 g/100 g fat, 46 mg/100 g vitamin C and 200 mg/100 g polyphenols. The energy value of the strawberries was 35 kcal or 146 kJ.

Beeswax was provided by the beekeeping center at the Instituto Superior de Agronomia in Lisbon, Portugal. All the other chemicals used in the experiments were of analytical grade and were purchased from Panreac Chimica S.A.U., Barcelona, Spain.

2.2. Beeswax characterization

2.2.1. Saponification value

An amount of 0.30 ± 0.01 g beeswax was weighed into a 10 ml screw-capped glass vial. Four milliliters of 4 mol/L aqueous NaOH solution was pipetted into the vial before it was closed and placed into an oven at 100 °C for 1 h. Next, the aqueous solution was poured into a beaker. About 5 ml of distilled water was used to rinse the vial and the water was later combined with the aqueous solution. Two drops of 1 g/100 g phenolphthalein TS was used as an indicator. This solution was titrated against a 0.5 mol/L aqueous HCl solution with manual shaking and the end point was carefully monitored. Blank analyzes were also performed. The saponification value (mg KOH/g) was calculated as follows:

$$\text{Saponification value} = 56.1 \times M(V_b - V_s/w) \tag{1}$$

where 56.1 is the molar mass of KOH in g/mol; M is the molarity of HCl solution in mol/L; V_b is the titrated volume of HCl solution for the blank in ml; V_s is the titrated volume of HCl solution for the sample in ml; and w is the weight of the sample in g.

2.2.2. Acid value

The beeswax (1 g) was weighed, placed in an Erlenmeyer flask, and 50 ml of chloroform were added. The flask was placed in an ultrasonic bath for about 5 min. Two drops of 1 g/100 g phenolphthalein solution were added to the solution before it was titrated with 0.05 mol/L NaOH in methanol. The titration was continued by manually shaking the flask until the indicator turned the clear solution a faint pink color. Blank analyzes were also conducted. The acid value was expressed as mg KOH/g, and calculated as follows:

$$\text{Acid value} = 56.1 \times M(V_b - V_s/w) \tag{2}$$

where 56.1 is the molecular weight of KOH in g/mol; M is the molarity of NaOH solution in mol/L; V_s is the titrated volume of NaOH in methanol used for the sample in ml; V_b is the titrated volume of NaOH in methanol used for the blank solution in ml; and w is the mass of the sample (g).

2.3. Preparation of the edible films

Chitosan solution (0.8 g/100 g) was prepared by dissolving 0.8 g of chitosan in 100 ml of acetic acid (1 ml/100 ml) with continuous stirring at room temperature (20 ± 2 °C). After, the complete dissolution of the chitosan, 0.2 g of glycerol and 0.2 g of Tween 80, were added and the solution was stirred for another 30 min. Afterward, the chitosan solution was centrifuged for half an hour at 16,900 g (Hermle Labortechnik, Wehingen, Germany) to separate all the impurities. The resulting solution was used as chitosan coating.

The cross-linking of the chitosan coating was carried with 0.5 g/100 g sodium tripolyphosphate, TPP, prepared as aqueous solutions by dissolving the salt in distilled water under vigorous stirring. The cross-linking lasted few minutes and then the excess of the cross-linking solution was blotted with absorbent paper.

For beeswax coatings, 0.5 g/100 g wax solution was prepared by melting 0.5 g of beeswax in 100 ml of ethanol heated to 70 °C. The wax solution was emulsified with 25% of Tween 80 with respect to the wax content.

The composite coating was prepared by mixing 100 ml of 0.8 g/100 g chitosan solution with 10 g of wax. The chitosan coating solution was prepared, as described previously, and then heated to 70 °C. The wax was melted in the heated solution and the whole mixture was homogenized for 2 min with Ultra-turrax homogenizer (Cole-Parmer, Vernon Hills, USA) at 8000 rpm.

The chitosan films were casted in a plastic casting container, ensuring that all air bubbles were removed from the viscous medium before casting. The same thickness of the films was controlled by casting 20 ml film forming solution in containers with standard diameter of 90 mm and height of 20 mm. Afterward they were dried at 40 °C for 48 h. The dried chitosan films were peeled off the container before further treatment. The cross-linking of the chitosan films was done by covering them with TPP. The cross-linking procedure lasted few minutes and then the excess of the cross-linking solution was poured out and the films were dried at 40 °C. The chitosan films were coated by dipping the films in the heated wax solutions and then letting the chitosan film coated on the both sides to dry at room temperature. The emulsion was casted in plastic casting containers and dried at 40 °C for 48 h to produce composite films. All the formulations of chitosan based films are listed in Table 1. The obtained films were conditioned in a desiccator at 50% relative humidity and 20 ± 2 °C for one week prior to analysis.

2.4. Film characterization

2.4.1. Thickness measurement

Thickness of the films was measured using a hand-held micrometer (Alves, Costa, & Coelhoso, 2010). Eight thickness measurements were taken at random positions on each film and the mean was calculated. For the multilayered films measurements were taken after each step and after applying each layer to evaluate the thickness of the separate layers. The mean value for the thickness was used in calculating the film water vapor barrier and mechanical properties.

2.4.2. Optical properties

Film color was determined by Dr Lange spectro-color colorimeter (HACH LANGE, Denver, USA) calibrated with a standard white and black tile. The CIE Lab scale was used, lightness, L, and chromaticity parameters a^* (red-green) and b^* (yellow-blue) were measured. Assays were performed by placing the film samples over the standard white tile. Samples were analyzed in five repetitions. The color differences (ΔE), chroma (C) and (h) values were calculated by the following equations:

$$\Delta E = \left(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}\right)^{1/2} \tag{3}$$

$$C = \left(a^{*2} + b^{*2}\right)^{1/2} \tag{4}$$

$$h_{ab} = \tan^{-1}\left(b^*/a^*\right) \tag{5}$$

where L^*, a^* and b^* values were the color parameters of the films, while the L^*_o, a^*_o and b^*_o values were the color parameters of the white tile.

2.4.3. Water absorption capacity

In order to determine the water absorption capacity or swelling degree of the films, three samples from each film were dried under vacuum for 48 h at 40 ± 1 °C as described in the ASTM D5229 method. After weighing, the samples were slowly immersed in water or 1 mL/100mL acetic acid (14.5 mL), with 0.5 mL of sodium azide solution (1 μg/100mL) to avoid the growth of fungi. The samples were taken out of the liquid, blotted with absorbent paper to remove the excess of water or acetic acid from the surface and weighed again. The procedure was repeated until reaching a constant mass in two consecutive measurements (during 4 days), and the absorption capacity of the films was calculated:

$$\text{absorption capacity} = \frac{m_s - m_i}{m_i} \tag{6}$$

where m_s is the mass of the immersed film (g) and m_i is the mass of the dried film (g).

2.4.4. Water vapor permeability

The water vapor permeability was measured gravimetrically according to the ASTM-96-80 protocol. The films were sealed with silicone to the top of a glass petri dish with a diameter of 5 cm filled with saturated solution of $BaCl_2 \times 2H_2O$ to provide relative humidity of 90%. The water vapor permeability was measured using films previously conditioned at a relative humidity of 54%. The sealed petri dishes were placed in a desiccator. The bottom of the desiccator was covered with saturated solution of $Mg(NO_3)_2 \times 6H_2O$. The temperature and the relative humidity inside the desiccator were set at 27 ± 2 °C and 44 ± 1%. A fan was used to promote the circulation of air inside the desiccator, in order to minimize the mass transfer resistance of the air boundary layer above the membrane. The temperature and relative humidity were measured over time using a thermohygrometer to obtain better control of the set conditions and to keep them constant. The water vapor molar flux (N) was determined by weighing the petri dish in regular time intervals for 24 h. The water vapor permeability was calculated as follows:

$$\text{WVP} = \frac{N \times \delta}{\Delta P_w} \tag{7}$$

where δ is the film thickness (m) and ΔP_w is the water vapor difference between both sides of the film.

2.4.5. Mechanical properties

The mechanical properties were studied using a TAXT2 (Stable Micro Systems, England). Tensile strength, TS, and elongation at break, E, were determined in accordance with ASTM D-882-91 (1996). Seven sample strips (20 × 70 mm) of the films were cut from the conditioned films. Force (N) and deformation were recorded during extension at 0.2 mm/s and with an initial distance of 50 mm between the grips clamping the films. TS was

Table 1
Composition and coding of the chitosan-based edible films.

Sample code	Type of film	Chitosan (g/100 g)	TPP:Chitosan ratio	Glycerol (g/100 g)	Tween 80 (g/100 g)	Wax (g/100 g)	Original film thickness (μm)	Wax coating weight (mg/cm²)[a]	Wax coating thickness (μm/side)[b]
B	Monolayer film	0.8	/	0.2	0.2	/	52	/	/
C	Wax coated, multilayer film	0.8	/	0.2	0.2	0.50	52	2.50	1.5
D	Crosslinked, wax coated, multilayer film	0.8	0.3:1	0.2	0.20	0.50	48	2.50	2.5
E	Composite film	0.8	/	0.2	0.2	10	60	/	/

[a] Wax weight on both sides of the chitosan film.
[b] Wax thickness per side of the chitosan film.

calculated by dividing the maximum load by the initial cross-sectional area of the sample and expressed in MPa. *E* was calculated as a ratio of the elongation at the point of sample rupture to the initial length of a sample as a percentage. The Young's modulus, calculated as ratio between the tensile strength and elongation in the elastic deformation region of the stress–strain curve, was also expressed in MPa. The thickness of the chitosan films was 52 μm, while the thickness of the cross-linked chitosan films was 48 μm (Table 1).

2.5. Sample treatment

Total mass of 8 kg strawberries were randomly divided into five groups, each group containing 120 strawberries. The group A was used as a control and it consisted of fresh strawberries, without any treatment. The group B consisted of chitosan coated strawberries. The coating was applied by dipping the each fruit in the coating solution for 30 s. The fruits were held by the stem during dipping to avoid any handling injuries. After the dip, the fruits were placed upside down on a plastic support (20 × 15 × 10 cm) with open squares like a wire mesh to allow the settling of the coating. Next group, C, consisted of three-layered coating comprised of wax-chitosan-wax coating. In the group D, strawberries were coated with wax, followed by chitosan layer crosslinked with sodium tri-polyphosphate, and one more wax layer. The three-layer coating of the strawberries in the groups C and D were formed by firstly dipping the strawberries into the melted solution of beeswax, then after 5 min the strawberries were dipped in the chitosan solution and at the end the fruits were dipped again in the melted beeswax solution. The strawberries in the last E group were coated with composite coating. Fruits were allowed to dry for 2–4 h at 20 °C depending on the coating. A summary of the sample treatment is given in Table 2. After the settling of the coating the fruits were randomly selected and placed in perforated (6 perforations with 10 mm diameter on the bottom of each box) plastic boxes (capacity: 250 g) in one layer for each replicate. Seven replicates were used per treatment. Packaged fruits were stored at 20 °C temperature and 35–40% relative humidity for 7 days.

2.5.1. Coating microscopic examination

Microscopic examination was performed to evaluate coating uniformity and adherence to the strawberries' surface. Samples from each treatment (chitosan, beeswax, beeswax–chitosan–beeswax and composite coatings) and uncoated controls were analyzed at room temperature. The coating thickness was imaged using an Optical Microscope (Axiostar Plus, Carl Zeiss Light Microscopy, Germany) illuminated with a pre-centered 20 W halogen lamp. Slices from the surface of the coated strawberries were cut using a stainless steel blade. Sample surfaces of coated strawberry

pieces were observed with 10× magnification lens (numerical aperture 0.25) and the images were analyzed and recorded with software AxioVision 4.8 (Carl Zeiss Light Microscopy, Germany).

2.5.2. Fruit visual infection

The strawberries were examined for mold during storage. Fruits from all groups were visually inspected for any signs of mycelia development on the fruit surface. Infected strawberries were characterized as moldy, with brown spots and softening of the infected area. Forty five fruits were used for each measurement and three batches of forty five fruits were prepared for each group, total of 135 strawberries. The results were expressed as the percentage of infected fruit from the starting 135 strawberries.

2.5.3. Weight loss

To determine weight loss, the same strawberries were weighed at the beginning of the experiment after the settling of the coating and thereafter each day during the storage period. Weight loss was expressed as the percentage loss of the initial total weight. Ten fruits in three repetitions were used to evaluate the weight loss of every group.

2.5.4. Respiration rate

In order to measure the respiration rate a closed system was used. Strawberry samples (around 115 g) were placed in 1.02 L hermetic glass jars with a septum in the lid for sampling gas in the headspace at different sampling times, over period of 44 h. The jars were stored at ambient temperature of 20 °C. Gas sampling was carried out every 30 min, in the first 5 h, then after 90 min till the 9th h and once per day till 44 h by means of a needle probe connected to a gas analyzer (CheckMate 9900 PBI Dansensor, Ringsted, Denmark). The needle probe was pushed through the septum and the headspace gas analyzer based on a mini-IR spectrophotometer recorded the CO_2 content. Three replicates were performed for each coating treatment. The respiration rate was calculated by using the following equation:

$$\text{Respiration rate} = \frac{\Delta CO_2}{100} \cdot V_{\text{headspace}} \cdot \frac{1000}{m} \cdot \frac{60}{t} (\text{ml } CO_2/\text{kgh}) \quad (8)$$

where *m* is mass of strawberry (g); $V_{\text{headspace}}$ is the empty volume of the hermetic jar (ml); ΔCO_2 is the difference between the initial and final concentration of CO_2 and *t* is sampling time (min).

2.5.5. Skin and flash color evaluation

The color of strawberry skin and inside flesh was evaluated on control and treated strawberries using a colorimeter (Konica Minolta, Sensing, Spectro photo meter CR-300, Osaka, Japan) to obtain L^* (lightness), a^* (redness) and b^* (yellowness) values as mean of 10 readings. Surface color was measured by placing the whole fruits against the colorimeter, while for the inside flesh, the strawberries were cut in halves and measured. The total color difference ΔE, as well as the chroma value, *C*, and hue angle, h_{ab}, were calculated using the Equations (3)–(5).

2.5.6. Measurement of fruit firmness

The firmness of the fresh, control, and coated strawberries was measured at 20 °C using a texture analyzer (TA-XT2i of Stable Micro Systems, Godalming, England) with a 5 kg load cell. The cutting method according to Velickova et al. (2011), was slightly modified. In brief, crosshead speed was set at 1 mm/s and the penetration depth was 20 mm. A single, whole, strawberry was placed on a flat platform and a sharpened 5 mm diameter cork borer adapted to fit the texture analyzer was used to cut through the strawberry tissue from one side to another, penetrating to 55% of the total fruit width.

Table 2
Coding and treatment of the strawberries.

Sample code	Sample treatment			
	Type of sample	Layer	Type of coating	Treatment time
A	Control	/	None	No treatment
B	Coated	1	Chitosan	30 s dipping, 2–4 h drying
C	Coated	3	Wax-chitosan-wax	30 s dipping, 5 min drying
				30 s dipping, 2–4 h drying
				30 s dipping, 5 min drying
D	Coated	3	Wax-chitosan-TPP-wax	30 s dipping, 5 min drying
				30 s dipping, 2–4 h drying
				30 s dipping, 5 min cross-linking
				30 s dipping, 5 min drying
E	Coated	1	Composite	30 s dipping, 2–4 h drying

The textural parameter measured on the resulting force–distance curves was firmness and the mean values of six replicates, expressed in *N*, were reported. The coating effect on the surface and core firmness was calculated as

$$\text{Coating effect} = \ln(F_c/F_r) \tag{9}$$

where F_c is the maximum force of the coated fruit and F_r is the maximum force of the raw fruit.

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

After the measurement of the firmness, the strawberries cut into small pieces were homogenized for 1 min at high speed using a hand-held blender. The variety Camarosa, being a larger fruit, provided a sufficient fruit mass for all the chemical analyzes. The pH of the strawberry puree was measured with a calibrated pH meter. For the chemical analyzes 5 g of the homogenized strawberry puree was suspended in 50 ml of hot distilled water (80 °C) for 30 min and then filtered. This solution was titrated to pH 8.1 using 0.1 mol/L NaOH. The total titrable acidity of the diluted puree was calculated using the formula:

$$\text{Titratable acidity} = \frac{V(\text{NaOH}) \cdot 0.1 \cdot 0.064}{m_{\text{aliquote}}} \cdot 100 (\%) \tag{10}$$

Where $V(\text{NaOH})$ is the ml of NaOH spent for titration, 0.1 is the molarity of the NaOH solution, 0.064 is conversion factor for citric acid and m_{aliquot} is the mass of the aliquot sample taken for analysis. The soluble solids content was determined in the juice of blended strawberries by means of an Atago PAL-1 digital refractometer at 20 °C and expressed as a percentage. All measurements were done in triplicate.

2.5.8. Reducing sugar content

For the determination of reducing and total sugar content, 5 g of the homogenized strawberry puree was suspended in 50 ml of hot distilled water (80 °C) for 30 min and then filtered. The content of reducing sugars was determined using a reducing chemical reagent, 3,5-dinitrosalycilic acid solution following the procedure described by Miller (1959).

2.5.9. Sensory analysis

Sensory evaluation of the strawberries was carried out in a standardized test room, in the sensory laboratory of the Department of Food Science and Technology, ISA/UTL, 3 days after the coating application. Fresh strawberries were used as control samples. Samples were given to each judge in a completely randomized order, served on white saucers and labeled with three digit random numbers. The sensorial attributes of the fruits such as appearance, in terms of shininess, presence of aftertaste, sweetness, overall acceptance and intention to buy were evaluated by seven trained judges. The panel of trained judges consisted of food engineers among which 2 men and 5 women in the age range from 30 to 65 years. The attributes were ranked on 6-points intensity scale where the score for the absence of gloss, absence of aftertaste, no sweetness, no acceptance and no intention to buy was (1), while (6) was given to samples with very high gloss, strong aftertaste, extremely high sweetness, very high overall acceptance and definite intention to buy. An average score of 3.5 and above was considered as limit for acceptability for all the attributes, except the presence of aftertaste, where score below 3.5 was considered acceptable. After the attributes ranking the judges were asked to order the samples according to their preference in decreasing order.

2.6. Statistical analysis

Descriptive statistics and one-way analysis of variance (ANOVA) was performed on the instrumental and sensory parameters to evaluate significant differences among the samples at 95% confidence interval according to Tukey's test using Minitab 15 statistical software. The variables that showed high significance were used in the Principal Component Analysis (PCA) to identify a reduced number of principal components that sufficiently explain most of the information in the starting data. This analysis was a multilinear modeling method of pattern recognition which shows the relationship between the objects (groups) on the basis of their distribution in the multidimensional space described from all the variables and also made it possible to determine which variables are principally responsible for the separation of the objects. The data obtained from all analysis (sensory, chemical and mechanical properties) of the strawberries were also analyzed by means of multivariate analysis employing hierarchical cluster analysis. The method used was Ward's method. The distances between samples were calculated using square Euclidean distances.

3. Results and discussion

3.1. Characterization of the edible films

The chitosan films were easy to peel from the casting container. They were transparent, homogenous, thin, flexible and easy to handle. The addition of the glycerol and Tween 80 to the chitosan solution resulted in films with a slightly yellow appearance. The beeswax, used in these studies, was a yellowish waxy solid with density of 850 kg/m^3 at room temperature, acid value of 16.12 ± 1.2 mg KOH/g and saponification value of 92.01 ± 1.56 mg KOH/g. The melting point of the wax was around 61 °C. The four types of the prepared edible films and their properties are presented in Table 3. The use of the waxy coating to the chitosan films resulted in multilayered films with mat look and decreased transparency, while the composite films had a more intensive yellow-brownish color. The applied cross-linking procedure made the films glossier, harder and slightly brittle.

The thickness of the edible films is very important for the physical properties of the films. The average thickness values for all chitosan-based films were from 48 to 60 μm. The cross-linked chitosan films exhibited reduced thickness, while the wax coated films had slightly increased thickness. The thickness of the composite films was similar to the one of pure chitosan films.

The water absorption capacity of the films was higher in acetic acid than in distilled water. The degree of absorption capacity of chitosan films in distilled water was $1.21 \pm 0.247\%$ which is similar to the results for absorption capacity of chitosan films in distilled water of 1.73% reported by Mayachiew, Devahastin, Mackey, and Niranjan (2010). A higher water absorption capacity was observed for the multilayered films, when compared to the single chitosan films. This increase might be due to water entrapped in small pores between the film and the wax layers. The composite films showed a significantly lower absorption capacity in water compared to the other films. The presence of the beeswax as a component in the composite films prevents the water absorption due to its hydrophobic nature.

The optical properties of the edible films are also important for the consumer's acceptability of the product. Transparent films are less likely to change the appearance of the strawberries. All films exhibited a yellowish color, which is reflected on a hue value near 90°. The addition of wax, in the multilayered films and the composite films, did not change significantly the hue value of the edible films. The calculated values for overall color change (ΔE) revealed

Table 3
Parameters of the edible films.

Sample code	Film thickness[a]	Absorption capacity[b]		Optical properties[c]			Water vapor permeability[d]	Mechanical properties[e]		
	(μm)	Water (%)	Acetic acid (%)	E	C	h°	(mol m/m² s Pa)	Tension (MPa)	ε (%)	Young's modulus (MPa)
B	52 ± 7	1.2 ± 0.3	2.8 ± 0.4	15.6 ± 0.5	17.0 ± 0.5	82.2 ± 2.6	4.15×10^{-11}	10 ± 2.1	24 ± 2.1	42 ± 3.8
C	55 ± 2	4.6 ± 0.7	5.9 ± 0.9	20.8 ± 0.6	23.1 ± 0.7	84.0 ± 2.5	2.66×10^{-11}	11 ± 1.8	20 ± 2.9	56 ± 6.8
D	48 ± 7	4.6 ± 0.2	6.9 ± 1.0	15.2 ± 0.4	17.4 ± 0.5	83.0 ± 2.5	3.26×10^{-11}	23 ± 2.2	5 ± 0.7	425 ± 18.8
E	60 ± 7	0.7 ± 0.1	4.3 ± 1.0	19.0 ± 0.6	21.2 ± 0.6	85.4 ± 2.5	1.66×10^{-11}	1 ± 0.1	25 ± 2.5	4 ± 0.7

[a] Number of replications, $n = 8$.
[b] $n = 3$.
[c] $n = 5$.
[d] $n = 3$.
[e] $n = 7$.

the color differences among the four different edible films. The values of ΔE were low, but still revealed the mat look and loss of shininess of the color wax coated chitosan films.

The water vapor permeability of the chitosan films was expected to be reduced by the modification of the edible films with cross-linking, coating and preparation of composite films. The results confirmed that the wax coating decreased the permeability of the initial chitosan films (B) from 4.15×10^{-11} mol m/m² s Pa to 2.66×10^{-11} and 3.66×10^{-11} mol m/m² s Pa for wax coated films (C and D). Similar results of improved barrier properties by application of lipid layers were reported by several other authors, using different waxes, such as beeswax, paraffin wax and carnauba wax as a coating of the polysaccharide edible films (Debeaufort & Voilley, 1995; Martin-Polo et al., 1992). The composite films (E) presented water vapor permeability 2.5 times lower than that of the chitosan films (B).

From the analysis of the mechanical properties it was proven that different treatments resulted in films with different mechanical properties. The crosslinking of chitosan films produced considerably stiffer film with increased tensile strength at break (from 10 MPa for B to 23 MPa for D), decrease of elongation at break (from 24% for B to 5% for D) and increase of Young's modulus (from 42 MPa for B to 425 MPa for D). The beeswax coating of the chitosan film did not affect significantly ($p < 0.05$) the tensile strength at break, the elongation at break or the Young's modulus of the original chitosan films. The incorporation of the beeswax in the composite films formed very fragile and less stiff edible films.

3.2. Characteristics of the edible coatings

Microscopic analysis of the edible coatings is shown in Fig. 1. It may be observed that all coatings formed continuous layers and presented a good adhesion to the surface of the fruit. The coating thickness showed a good uniformity, but was quite affected by the coating formulation. While the thickness of the chitosan coating was 65 ± 1 μm, composite coatings were around 105 ± 2 μm, and the overall thickness of the multilayered coatings was 250 ± 4 μm. When the wax was applied at the fruits surface, a slight wax-tissue interpenetration may be perceived. This fact is present in the three layer coating (Fig. 1c), and confirmed when a single wax layer was applied for illustration (Fig. 1b).

3.3. Evaluation of the strawberry infection

Since strawberries are known to be highly perishable fruits their shelf life was defined as the time elapsed between the coating application and the appearance of fungal infection (Tanada-Palmu & Grosso, 2005). The signs of strawberry infection in the control strawberries (A) appeared only after one day of storage. The coated strawberries, on the other hand exhibited delayed mycelia growth as shown in Fig. 2. Mold infection in the coated strawberries was noticeable after the third day of storage, but it was still significantly ($p < 0.05$) lower than the infection of the control fruits. At the end of the 7th day of storage, 98% of control fruits were infected. The mold infection incidence of the chitosan coated fruits (B) and fruits
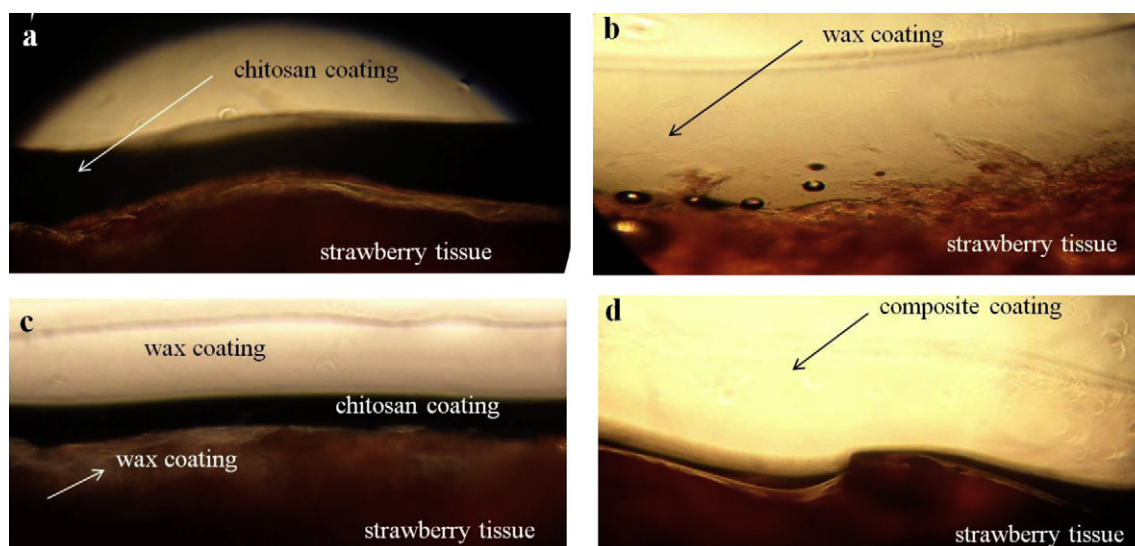

Fig. 1. Fresh strawberries coated with edible coatings (cross-sectional view) for (a) chitosan, (b) beeswax, (c) beeswax–chitosan–beeswax and (d) composite coating. Images were obtained using light microscope Axiostar Plus, Carl Zeiss, 10× magnifications.
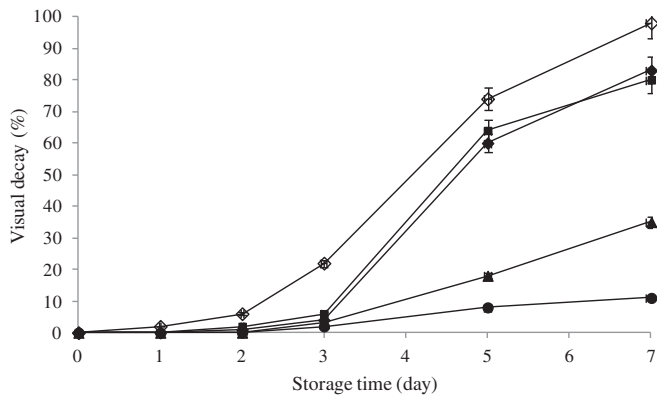
Fig. 2. Percentage of infected strawberries as a function of storage time at 20 °C and 35–40% RH for control (open diamonds, ◇), chitosan (full squares, ■), beeswax–chitosan–beeswax (full triangles, ▲), beeswax–chitosan–TPP–beeswax (full circles, ●) and composite coated samples (full diamonds, ◆). Vertical bars represent the standard deviation ($n = 3$).
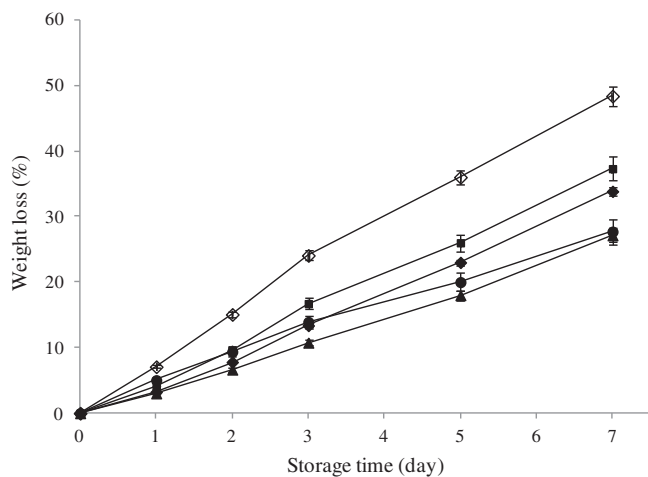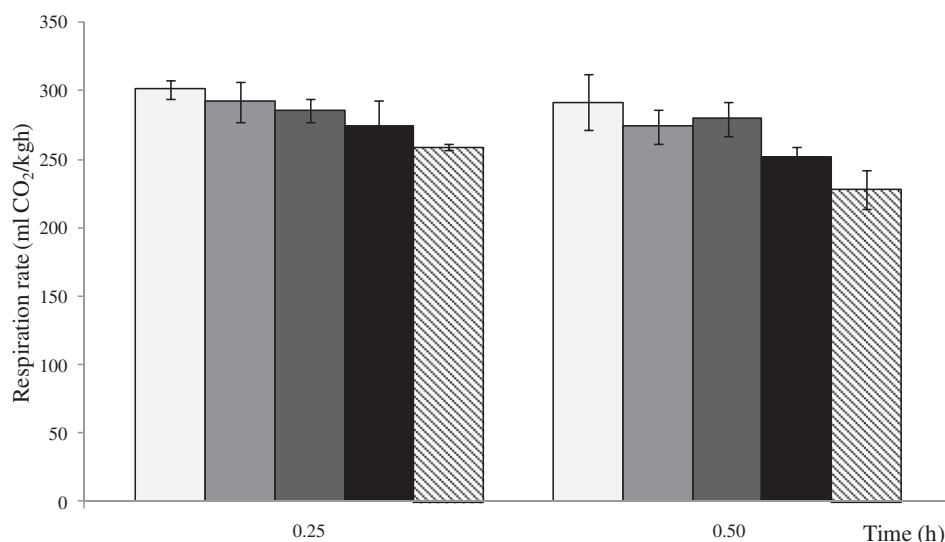

Fig. 3. Loss of weight of strawberries as a function of storage time at 20 °C and 35–40% relative humidity for control (open diamonds, ◇), chitosan (full squares, ■), beeswax–chitosan–beeswax (full triangles, ▲), beeswax–chitosan–TPP–beeswax (full circles, ●) and composite coated samples (full diamonds, ◆). Vertical bars represent the standard deviation ($n = 3$).

treated with composite coatings (E) were around 80%, while the infection of the three-layer coated strawberries (C and D) was 35 and 11%, respectively. These results agree with findings of Vargas et al. (2006), who reported that the presence of lipid components enhanced the antimicrobial properties of chitosan. Hernández-Muñoz, Almenar, Del Valle, Velez, and Gavara (2008) proved that the antimicrobial activity of chitosan on strawberries was related to its ability to cause severe cellular damage to the molds and interfere in the secretion of polygalacturonases rather than its ability to induce plant defense enzymes.

3.4. Weight loss

Strawberry fruits are highly susceptible to rapid loss of water which results in fruit shrinkage and weakening of the tissue due to their very thin skin. Therefore, the weight loss was followed over the storage period of 7 days to evaluate the effects of the coatings. All fruits exhibited a continuous weight loss over the storage period (Fig. 3). Nevertheless, after the second day of storage, weight loss for all coated strawberries was significantly lower ($p < 0.05$) than those of the control fruits. At the 7th day of storage at 20 °C, the weight loss was 11% lower for chitosan coated fruits compared to control samples which exhibited 48% loss of the initial weight. Obviously chitosan acted as physical barrier to moisture loss and constrained the dehydration and shrinkage of the fruits. The addition of beeswax as a separate coating layer or as a component in the composite coating exhibited higher reduction in the loss of weight which was 15–20% lower than the control fruits for the composite (E) and three-layered coatings (C and D), respectively. Similar results for improved barrier properties due to the presence of the lipid component were reported for gluten based coatings modified with beeswax, stearic and palmitic acid and for hydroxypropyl methylcellulose coatings modified with beeswax (Navarro-Tarazaga et al., 2008; Tanada-Palmu & Grosso, 2005).

3.5. Respiration rate

It is known that the storage at elevated temperatures enhances the respiration process of the fruits. The respiration of the control and coated strawberries was followed over period of time to evaluate the effect of the coatings on the respiration rate of strawberries at 20 °C. The respiration rate was calculated and only the
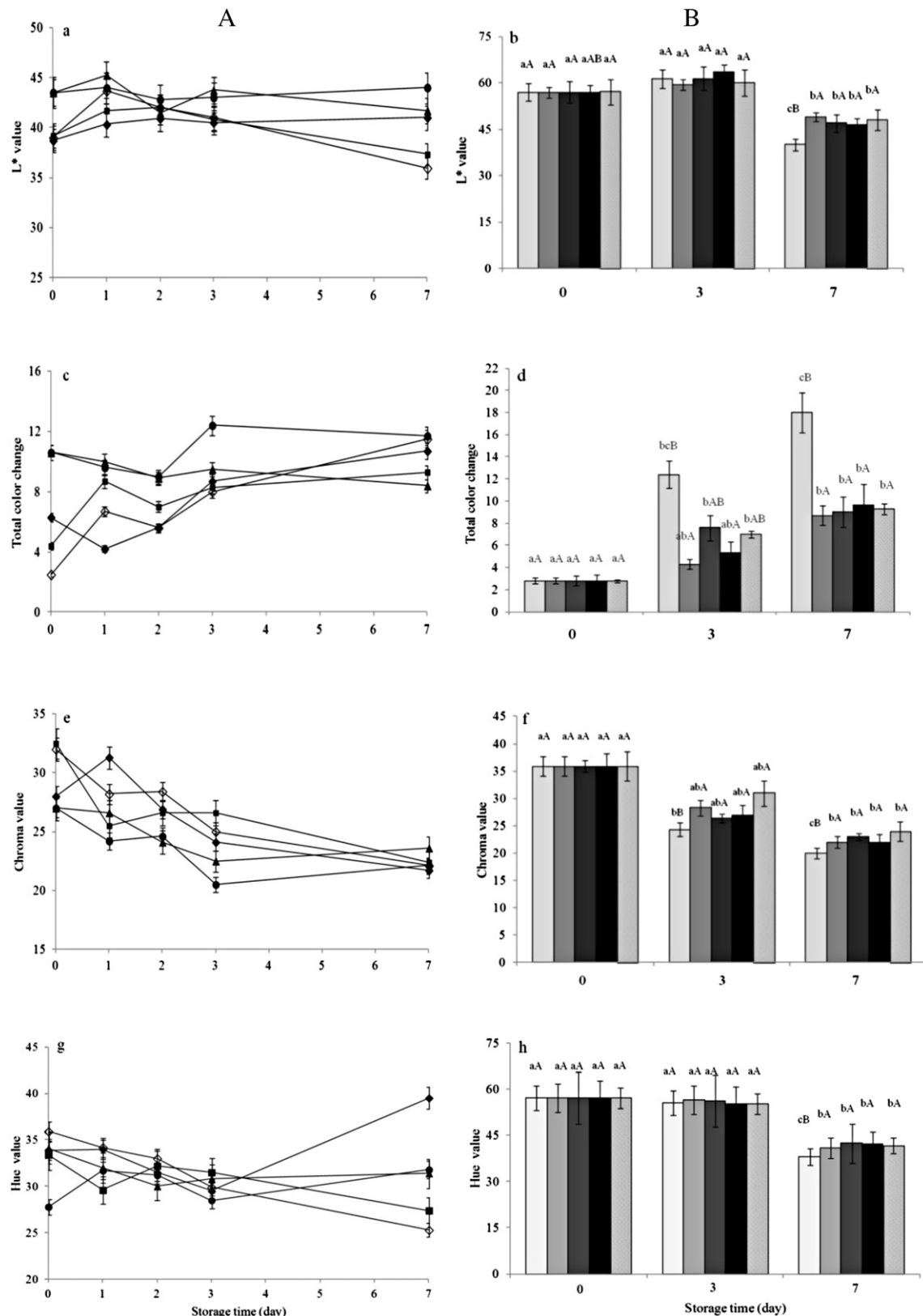

Fig. 4. Respiration rate of strawberries in terms of CO_2 as a function of time and coating formulation. Control (white bars), chitosan (light grey bars), beeswax–chitosan–beeswax (dark grey bars), beeswax–chitosan–TPP–beeswax (black bars) and composite coated samples (patterned bars). Vertical bars represent the standard deviation ($n = 3$).

Fig. 5. Effect of the coating on the skin (A) and flesh (B) color of strawberries stored at 20 °C. The vertical bars indicate the standard deviation (*n* = 9). Column A: control (open diamonds, ◇), chitosan (full squares, ■), beeswax–chitosan–beeswax (full triangles, ▲), beeswax–chitosan–TPP–beeswax (full circles, ●) and composite coated samples (full diamonds, ◆). Column B: control (white bars), chitosan (light grey bars), beeswax–chitosan–beeswax (dark grey bars), beeswax–chitosan–TPP–beeswax (black bars) and composite coated samples (patterned bars). The bars with different small letters differ significantly due to storage time (*p* < 0.05); the bars with different capital letters differ significantly among coatings (*p* < 0.5).

results from the first half an hour, until the saturation point with CO_2, were given in Fig. 4. The ANOVA analysis showed that the time and coating formulation affected the respiration rate significantly ($p < 0.05$). It was evident that the coatings controlled the exchange of the gases between the fruit and the environment due to their different permeability to gases like CO_2 and O_2. Incorporation of the beeswax in the coating significantly reduced the respiration rate of the strawberries, which is in agreement with the reported effect of lipids on the gas barrier properties of polysaccharide films (Vargas et al., 2006). The values of 291.7 ml CO_2/kgh for the control samples (A) and 274.3 (B); 279.7 (C); 252.1 (D) and 227.9 (E) ml CO_2/kgh for the coated samples obtained after 0.5 h are comparable with the results reported by Lima et al. (2010) when working with sliced and then coated apples and mangoes stored at 20 °C. The modification of the gas balance in the microenvironment of the fruits led to their decreased respiration and hence better preservation.

3.6. Skin and flash color development

Changes in the skin and flash color of the control and coated strawberries were evaluated by measuring the luminosity (L^*), total color change (ΔE), hue angle and chroma during 7 days of storage at 20 °C. Values for the strawberry skin are presented in Fig. 5, A column, while the values for the inside flesh are presented in Fig. 5, B column. After the coating application the strawberries coated with beeswax had slightly higher L^* values due to the whitish and mat look of the beeswax coating. As it could be seen, there was a slight visual color difference between the beeswax coated and the other samples. There were no significant changes ($p > 0.05$) of the luminosity of the samples until the third day of the storage. Only at the end of the storage the control and chitosan coated sample showed significant darkening of the skin ($p < 0.05$). The inside flash also developed darker color at the end of the storage period in all samples, and the control sample was significantly darker than the rest of the fruits.

The total color change of beeswax coated samples was significantly higher ($p < 0.05$) at the beginning, but was kept more or less constant over the storage. The color change of the control samples and samples coated with chitosan and composite coatings increased with time. The control fruits exhibited four and six-fold higher values of the total color change at the third and seventh day of storage, respectively. In contrast, the color change of the coated fruits increased only three times at the end of the storage. There were no significant differences ($p > 0.05$) in terms of hue or chroma due to the coatings. The chroma and hue values of the strawberry skin decreased over time about 30% for the control and between 10 and 23% for differently coated samples, which could be attributed to the drying of the surface. The inside flash on the other hand did not change significantly at the beginning. The control samples always had lower values for the chroma and hue when compared to the coated fruits. There were no tissue damages and lesions of the inside flash of the coated strawberries until the third day of storage at 20 °C. The most noticeable color changes during storage occurred in the control samples due to the advanced atmospheric dehydration. The absence of the coating, which acted as protective barrier, accelerated the senescence of the fruit, causing loss of the intracellular liquid and tissue collapse (Figs. 2 and 3). The storage temperature of 20 °C, compared to refrigerated storage, increased the respiration of the fruit and gave rise to some enzymatic processes that caused darkening of the strawberries. The evidence for this was found in the lower chroma (less vivid) and hue angle (less red). The senescence delay, evidenced by the decrease in color changes, which demonstrated the effectiveness of the coatings was reported for the chitosan-oleic acid edible coatings (Vargas et al., 2006); alginate coatings (Fan et al., 2009) and soy protein and wheat gluten coatings (Atress et al., 2010).

3.7. Fruit firmness

Strawberries soften considerably during ripening due to degradation of the middle lamella of the cell wall of cortical parenchyma cells (Perkins-Veazie, 1995). Therefore the texture of the strawberry is a very important quality parameter. The changes of the surface and core firmness of the control and treated strawberries were determined with cutting method (Velickova et al., 2011). In the beginning of the curve there was a rapid increase of the force up to a yielding point, where strawberries were slightly deformed and compressed without any cutting of the tissue (Fig. 6a). The end of first peak indicated the point of internal fracture and has been taken as the parameter to measure surface firmness. The firmness of the strawberries core, also known as pith, is given by the second peak on the curve. The effect of the different coatings on the surface and core firmness of the strawberries is presented in Fig. 6b. The three-layer coating of the strawberry with beeswax-crosslinked chitosan−beeswax led to significant softening of the strawberry surface ($p < 0.05$), while the other coating formulations did not cause significant softening of the surface when compared to the fresh strawberries. The strawberry core, on the other hand, exhibited different behavior. The coating application did not cause any softening of the inside flash. Furthermore, the three-layer coating of beeswax−chitosan−beeswax even
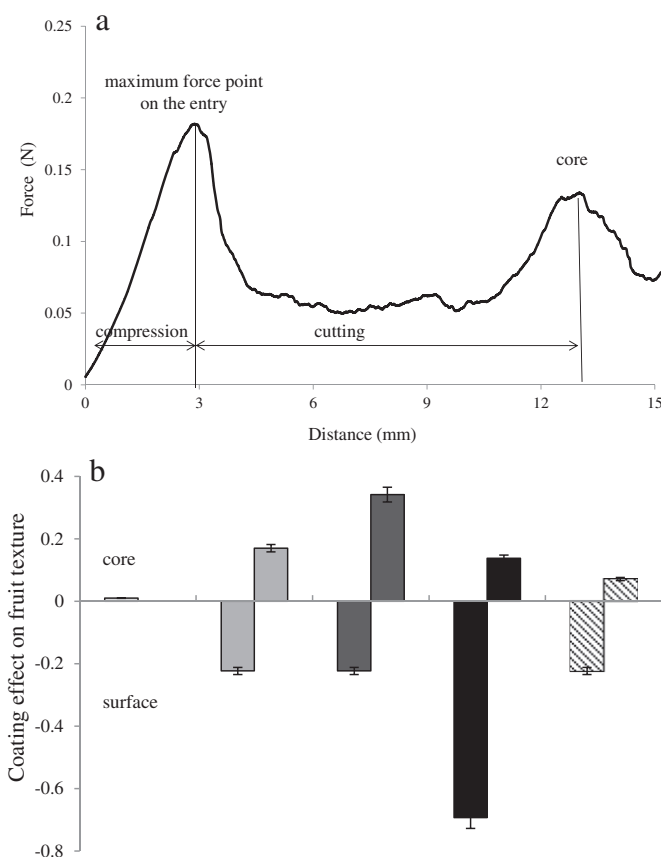

Fig. 6. Force-displacement curve obtained from the cutting test (a) and coating effect on the strawberry texture (b). Negative values indicate softening and positive values hardening compared with fresh strawberry. Error bars represent the standard deviation ($n = 6$). Control (white bars), chitosan (light grey bars), beeswax−chitosan−beeswax (dark grey bars), beeswax−chitosan−TPP−beeswax (black bars) and composite coated samples (patterned bars).
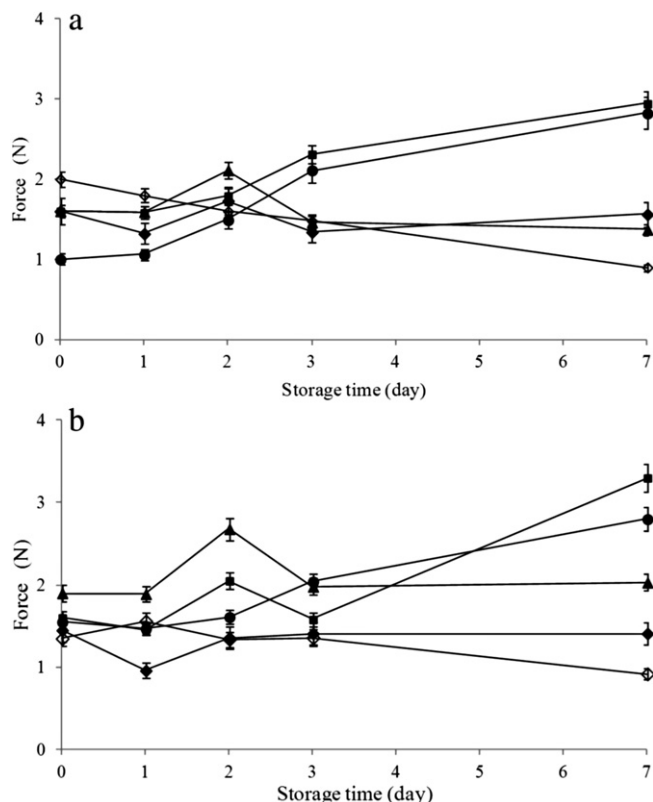
Fig. 7. Effect of the coating on the surface (a) and core (b) firmness of strawberries stored at 20 °C. The vertical bars indicate the standard deviation ($n = 6$). Control (open diamonds, ◇), chitosan (full squares, ■), beeswax–chitosan–beeswax (full triangles, ▲), beeswax–chitosan–TPP–beeswax (full circles, ●) and composite coated samples (full diamonds, ◆).

induced slight increase of the firmness, probably due to the increased temperature of the beeswax coating solution. It has been reported that heat treatments could increase the firmness and delay the softening in different fruits (Vicente et al., 2005).

The changes of the strawberry firmness between the control and coated fruits during 7 days of storage at 20 °C are given in Fig. 7. All samples had similar initial firmness values at $p > 0.05$ level as measured by the cutting test. The control or non-coated fruits exhibited liner decrease in the both surface and core firmness over the storage period. These kinds of changes could be attributed to

the higher water loss and fungal infection which led to more pronounced tissue senescence and cell wall breakdown. All coating formulations kept the firmness of the whole coated strawberry constant during the storage period. Improvement of the flesh firmness of coated fruits was related to the reduced fruit metabolism (Fig. 4). The beneficial effect of the coating applications on the strawberry texture were reported for coatings prepared from cactus mucilage (Del-Valle, Hernández-Muñoz, Guarda, & Galotto, 2005); chitosan–oleic acid coatings (Vargas et al., 2006) and chitosan coatings in combination with calcium dips (Hernández-Muñoz et al., 2008).

3.8. Chemical analysis

The data from the chemical analysis of the control and coated strawberries during storage time are given in Table 4. In all samples, the initial pH of 4.4 of strawberries decreased during storage, yielding in significant differences at the end of the storage period. Still, there were no significant differences among the pH values of the variously coated fruits at the end of the storage period. The end pH values for coated strawberries of 3.7; 3.7; 3.9 and 4 for beeswax–chitosan–TPP–beeswax (D), composite (E), chitosan (B) and beeswax–chitosan–beeswax (C) coating, respectively, were still above the average reported values for ripe strawberry of 3.3 (Green, 1971). The results for the organic acids, the second most important component of the strawberry flavor were in agreement with the pH values. The percentage of the titratabale acidity was between 0.3 and 0.6 for the differently coated samples during the storage period. These values are close to the lowest values mentioned in the literature, between 0.6 and 2.3% (Montero, Mollá, Esteban, & López-Andréu, 1996). Titratabale acidity significantly decreased ($p < 0.05$) as a function of the storage time for all coating applications that were studied. The control samples on the other hand, exhibited slightly higher value for the titrable acidity after the 7th day of storage. The decrease in the titrable acidity indicated the maturity development and was also reported by Garcia et al. (1998) for strawberries coated with starch-based coatings.

The soluble solids content of coated samples remained constant until the third day of storage and showed no significant difference ($p > 0.05$) among the treatments. Only the soluble solid content of the control strawberries was higher and differed significantly from the rest of the fruits, caused the considerable loss of water during storage at 20 °C. At the end of the storage four of the samples (control strawberries and composite and three-layer coated fruits) had decreased content of the soluble solids due to the respiration.

Table 4
Soluble solids, pH value, titratable acidity, maturity index and reducing sugars contents of control (A) and coated strawberries (B–E) stored at 20 °C.

	Days	A	B	C	D	E
pH	0	4.4 ± 0.1[aA]	4.4 ± 0.1[aA]	4.4 ± 0.1[aA]	4.4 ± 0.1[aA]	4.4 ± 0.1[aA]
	3	4.0 ± 0.1[bA]	4.0 ± 0.1[bA]	4.2 ± 0.1[aA]	4.2 ± 0.1[aA]	4.0 ± 0.1[abA]
	7	3.6 ± 0.1[bcA]	3.9 ± 0.1[bA]	4.0 ± 0.1[abA]	3.7 ± 0.1[bA]	3.7 ± 0.1[aA]
Titratable acidity (g citric acid/100 g)	0	0.5 ± 0.01[aA]	0.5 ± 0.01[aA]	0.5 ± 0.02[aA]	0.5 ± 0.01[aA]	0.5 ± 0.01[aA]
	3	0.6 ± 0.02[abA]	0.4 ± 0.01[bB]	0.4 ± 0.01[bB]	0.4 ± 0.01[bC]	0.5 ± 0.02[bC]
	7	0.6 ± 0.02[bA]	0.4 ± 0.01[bB]	0.3 ± 0.01[cC]	0.4 ± 0.01[cB]	0.5 ± 0.02[bD]
Soluble solids (g/100g)	0	7.0 ± 0.8[aA]	7.0 ± 0.7[aA]	7.0 ± 0.6[aA]	7.0 ± 0.8[aA]	7.0 ± 0.2[aA]
	3	7.7 ± 1.2[aA]	7.1 ± 0.8[aB]	6.3 ± 0.4[aB]	6.8 ± 0.6[aB]	6.2 ± 0.5[aB]
	7	6.5 ± 0.6[aA]	7.5 ± 0.6[aB]	5.0 ± 0.2[bC]	6.3 ± 0.5[aA]	5.4 ± 0.4[bC]
Reducing sugars (g/100g)	0	3.5 ± 0.1[aA]	3.5 ± 0.1[aA]	3.5 ± 0.1[aA]	3.5 ± 0.1[aA]	3.5 ± 0.1[aA]
	3	1.4 ± 0.1[bcA]	1.5 ± 0.1[bcAB]	1.3 ± 0.1[bcA]	1.5 ± 0.1[bcAB]	1.2 ± 0.1[cA]
	7	1.9 ± 0.1[bcA]	1.5 ± 0.1[bcA]	1.8 ± 0.1[bB]	1.6 ± 0.1[bAB]	1.4 ± 0.1[cA]
Maturity index (°Brix/% acid)	0	14.0 ± 0.4[aA]	14.0 ± 0.4[aA]	14.0 ± 0.4[aA]	14.0 ± 0.4[aA]	14.0 ± 0.4[aA]
	3	12.4 ± 0.3[bA]	17.6 ± 0.5[bB]	15.8 ± 0.4[abA]	17.0 ± 0.3[bA]	12.4 ± 0.4[abA]
	7	10.8 ± 0.3[aA]	18.9 ± 0.6[cB]	16.7 ± 0.5[bB]	15.8 ± 0.3[cBC]	10.8 ± 0.3[aA]

Mean values and standard deviation ($n = 3$). B: chitosan; C: beeswax–chitosan–beeswax; D: beeswax–chitosan–TPP–beeswax; E: composite coating. Different small letters within a column indicate significant differences due to storage time ($p < 0.05$). Different capital letters within a row indicate significant differences among coatings ($p < 0.05$).

The reducing sugar concentration of the three-layer and composite coated sample was the lowest, suggesting that the incorporation of the beeswax in the coating treatments slowed down the conversion of reducing sugars in the strawberries. Considering that an acceptable strawberry flavor is achieved with a minimum of soluble solids of 7% and a maximum of titratable acidity of 0.8%, or maturity index above 6, all the coated fruits were acceptable for consumption due to their much higher maturity index than the average values quoted in the literature (Manning, 1996).

The results demonstrated that the coatings prolonged the shelf-life of the strawberries by slowing down the metabolism and delaying their ripening as indicated by the retention of the firmness, color, loss of titratable acidity, total soluble solids content and retarded production of reducing sugars.

3.9. Sensory analysis

All attributes were subjected to principal component analysis. The eigenvalues were used to identify the number of principal components to be retained in order to comprehend the data structure. There was a drastic change after the second eigenvalue, meaning that the first two components explained 70% of the variance of information contained in the sensory data set. The projections of the original variables in the subspace of the principal components are depicted in Fig. 8. It could be seen that the sensory acceptance of the strawberries and the intention to buy them was positively correlated with the luminosity or the visual appearance of the fruits (Fig. 8a). On the other side, they were negatively correlated with the presence of the aftertaste and hence decreased aroma in the samples. The value for the sweetness of the fruits was more distant from both axes and closer to the their center, indicating its low contribution to the discriminating groups and helping us to find out which variables contributed the most to the difference among the groups. From the chemical analysis, it was shown (Fig. 8b) that the total soluble solids as well as the total titratable acidity, were positively correlated with the acceptance of the fruits, while the low pH and reducing sugars content would have negative impact on the fruit's acceptance. The L^* value of the fruit's surface color seemed to have low contribution to the discriminating groups (Fig. 8c). Since the red and vivid color of the strawberries usually came from the chroma and hue values, those were the variables that contributed the most to the difference among the groups. It was shown that the hue and chroma values together with the fruit's firmness were positively correlated with the acceptance, which accompanied the findings from the sensory tests that the acceptance mainly depends on the visual appearance of the strawberries.

Furthermore, the sensory evaluation of the control and coated strawberries was analyzed trough one-way analysis of variance taking into account the effect of the different coating on the quality attributes such as luminosity, aroma, sweetness, aftertaste, overall acceptance and intention to buy (Fig. 9). The judges evaluated the coatings C and D significantly less ($p < 0.05$) luminous or shiny than the rest of the samples. In contrast to the results reported by Han and Gennadios (2005), the composite coating did not reduce the gloss of the strawberries. Chitosan could show astringent taste due to its dissolution in an acidic medium. This astringent taste was detected in sensory evaluations of strawberries carried out on the very day of film application by Deblieguere, Vermeulen, and Debreve (2004) and Vargas et al. (2006). This was not observed in the present study, since the sensory analysis was performed on the third day of storage. The aroma of the samples coated with three-layer coatings (C and D) was significantly different ($p < 0.05$) when compared to the control fruits and strawberries coated with just chitosan or composite coating. The change of the aroma in the

samples C and D was accompanied by the presence of an aftertaste in the same samples, most probably due to the alcoholic solution for the beeswax coating. The panelists detected an untypical alcoholic aroma in the samples coated with the beeswax coating, but did not report any presence of oily aroma. Furthermore, composite coatings, where the lipid component was dissolved in heated chitosan solution did not exhibit any aftertaste or strange aroma. From the results for the overall acceptance and the intention to buy it was evident that the judges showed significant preference for the chitosan and composite coated fruit which obtained mean acceptance scores above 3 on the 6-point intensity scale. When asked to order
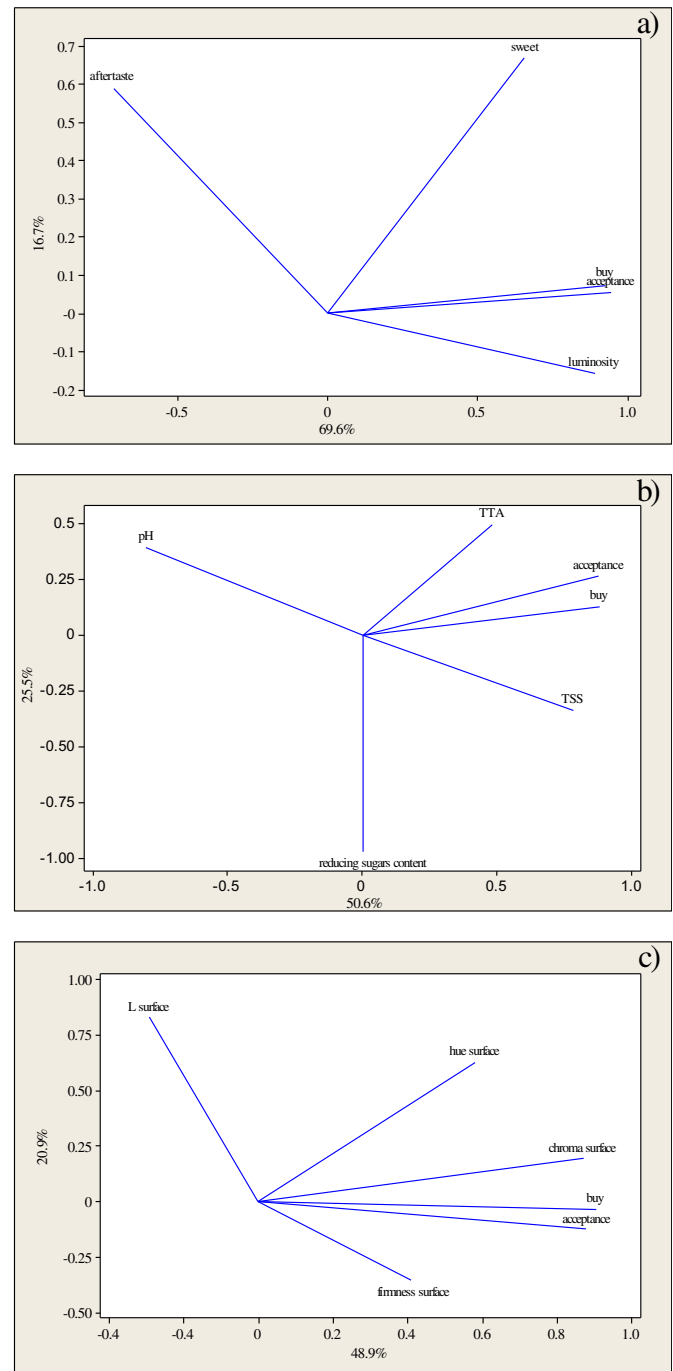

Fig. 8. The loading plots of the first two principal components of the sensory (a), chemical (b) and mechanical (c) variables.
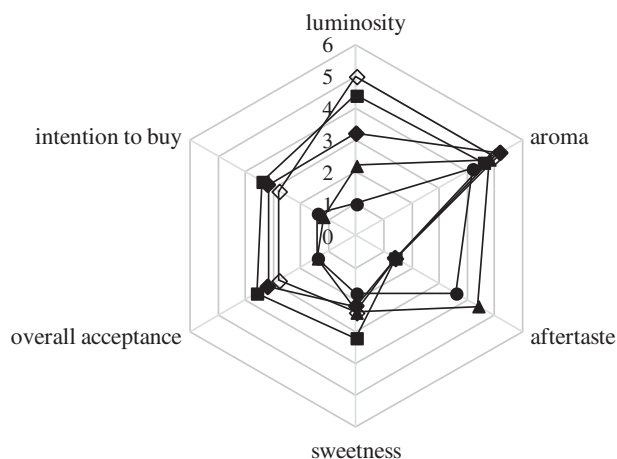
Fig. 9. The average sensory scores of the strawberry fruits. Control (open diamonds, ◇), chitosan (full squares, ■), beeswax–chitosan–beeswax (full triangles, ▲), beeswax–chitosan–TPP–beeswax (full circles, ●) and composite coated samples (full diamonds, ◆). The score above 3.5 is acceptable for luminosity, aroma, taste, overall acceptance and intention to buy, while score bellow 3.5 is acceptable for the aftertaste. A 6-point scale was used for the sensory scores.

the fruits according to their intention to buy, the panelists organized the different fruit groups in the following order: B > E ≥ A > D > C.

At the end, a cluster analysis was used to identify the similar groups according to all measured parameters within the strawberry samples. As can be seen from Fig. 10, the first division of the fruit samples is made into two clusters 1 and 2. Cluster 1 grouped together the control and monolayer coated strawberries and it is composed of two subclusters, 1′ and 1″. Subcluster 1′ contained the control strawberries and strawberries coated with composite film, while subcluster 1″ represented the strawberries coated with chitosan film. The three-layer coated strawberries on the other hand, which had separate wax layers in the coating, belonged to the cluster 2 and were distinguished by the significantly lower sensory acceptance of the panel group. Cluster analysis confirmed the sensory analysis results that the chitosan and composite coating could be used to coat strawberries since their taste was not perceptible by the judges and they still enhanced the shelf life of the strawberries compared to the control samples. On the other hand, the judges disliked the appearance and the taste of the three-

layer waxy coatings, even though these coatings were the best for maintaining the quality of the strawberries during the whole storage period. The higher overall acceptance of the coated strawberries by the trained judges was probably due to the lower levels of dehydration and darkening of the coated strawberries.

4. Conclusion

The chitosan and composite coatings were transparent, while the three-layer coatings were more mat and slightly white. The chitosan based coatings prolonged the storage period of strawberries for seven days at temperature of 20 °C and relative humidity of 53% and slowed down their senescence process compared to uncoated strawberries. The addition of beeswax as separate layer or as a component in the composite coating showed beneficial effect against fungal infection, weight loss reduction, and respiration rate, retention of the firmness and color, as well as retention of the titratable acidity, pH, soluble solids and sugars. The sensory evaluation of the strawberries showed that fruits coated with chitosan (0.8 g/100 g) and composite coating (0.8 g/100 g chitosan and 10 g/100 g beeswax emulsion) had higher overall acceptance and intention to buy. The judges disliked the sensory properties of the three-layer waxy coatings, even though they maintained the best the quality of the strawberries during the storage period.

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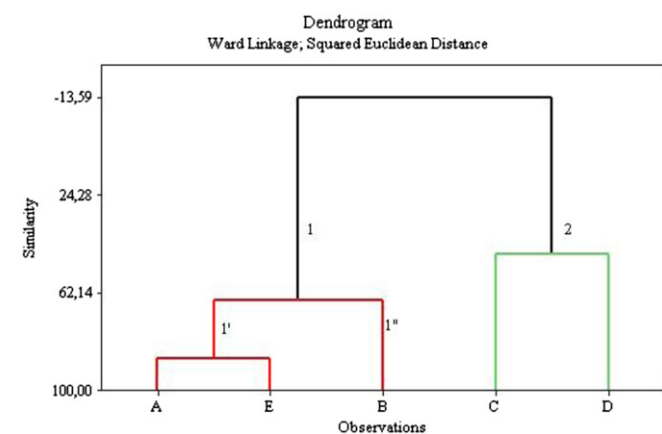
Fig. 10. Dendogram showing clustering of all strawberry samples based on the color, firmness, chemical and sensory analysis of the strawberries. Control (A), chitosan (B), beeswax–chitosan–beeswax (C), beeswax–chitosan–TPP–beeswax (D) and composite (E) coated fruits.

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