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ORIGINAL PAPER

Effects of Antimicrobial Edible Coating of Thymol Nanoemulsion/Quinoa Protein/Chitosan on the Safety, Sensorial Properties, and Quality of Refrigerated Strawberries (*Fragaria* × *ananassa*) Under Commercial Storage Environment

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Abstract

The effectiveness of the edible coating with thymol nanoemulsion on the safety, sensorial properties, and quality of refrigerated strawberries was investigated under commercial storage conditions. Spontaneous emulsification was used to obtain the thymol nanoemulsion that was included on quinoa protein/chitosan coatings. During the entire storage time, strawberries coated with thymol-antimicrobial packaging had a lower fungal and yeast load compared with the controls (uncoated and coated with quinoa protein/chitosan). The flavour and aroma of the coated strawberries was initially affected, although this sensory appreciation was improved from the fifth day of storage and showed similar scores than the controls, and presenting better aroma score at day 12 of storage. Furthermore, the shelf life of the thymol nanoemulsion-coated strawberries increased in 4 days, unlike that in the both controls. Further, the application of these biocoatings on strawberries significantly decreased the weight loss relative to that in the control, during 16 days of storage at 5 °C and 90% relative humidity, and did not alter the quality parameters (pH, titrable acidity, and percentage of soluble solids). These results suggest that the application of thymol/nanomulsion-loaded edible films is an effective strategy to increase the shelf life of highly perishable products such as strawberries.

Keywords Thymol nanoemulsion · Antimicrobial coating · Safety · Sensorial · Quality properties · Strawberries

Introduction

Moulds are prevalent in the environment, and they can proliferate easily on fresh foods and trigger fungal decay, especially in delicate fruits with a short shelf life such as strawberries (Emamifar and Mohammadizadeh 2015; Valenzuela et al. 2015; Tournas and Katsoudas 2005). The postharvest life of strawberries is around 5 days at 0–4 °C. The use of edible coating has been shown to increase the postharvest shelf life of strawberries; however, resolving some critical parameters such as water loss and fungal development and retaining the sensory properties of strawberries during storage are necessary (Pagliarulo et al. 2016; Valenzuela et al. 2015; Gol et al. 2013). At present, the use of natural additives has increased globally.

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Thymol (Thy) is a natural essential oil, which has been classified as "generally recognized as safe" (CFR 2015); it has numerous beneficial properties and is used in various applications such as an aromatic spice in food preparations or an antioxidant and antifungal agent against *Botrytis cinerea* (Marchese et al. 2016; Abbaszadeh et al. 2014; Campos et al. 2011; Burt 2004). However, the use of Thy as an antifungal additive has some drawbacks such as strong odour, low water solubility, and high volatility, decreasing its antimicrobial activity over time (Shah et al. 2012; Mastromatteo et al. 2010; Ben Arfa et al. 2006). Because Thy has a remarkable potential as a natural antifungal additive, studies have focused on efforts to mitigate these drawbacks. Systems to encapsulate Thy are required to improve flavour and increase its food preservation capacity as an effective antifungal agent against *B. cinerea* (Robledo et al. 2018; Celebioglu et al. 2017; Su and

Zhong 2016). Different methods have been used to encapsulate Thy; recent studies have used systems based on hydrophobic products such as nanoemulsions that incorporate Thy as a lipophilic ingredient inside the aqueous food matrix, leading to the retention of the antifungal activity at 100 ppm (Robledo et al. 2018; Celebioglu et al. 2017; Li et al. 2017). Acevedo-Fani et al. (2017) and Fu et al. (2016) point out that the use of delivery systems as antimicrobial nanoemulsions have been tested mainly by antimicrobial properties and they need to be verified in real food systems. Thy nanoemulsion has been considered to be a good primary packaging material for fruits (Robledo et al. 2018). However, studies on the application of this material in fruits are not sufficient to confirm its effectiveness with reduction in volatility and strong odour, improvement of food safety, and thus increase in the shelf life of fruits (Li et al. 2017; Ma et al. 2016; Su and Zhong 2016). This study aimed to investigate the efficacy of the antimicrobial coating of thymol nanoemulsion/quinoa protein/chitosan applied to fresh strawberries and evaluate its effect on the antifungal, sensory, and physicochemical properties under commercial storage conditions.

Materials and Methods

Materials

Thymol was acquired from Sigma-Aldrich, USA (≥ 99.5%, T0501). Miglyol 812 was obtained from Sasol Germany GmbH (Witten, Germany); it corresponded to medium-chain triglyceride (MCT) oil. The surfactant Tween 80 was purchased from Commercial Montero Ltda, Chile Oxoid Ltd. (Hampshire, UK). Milli-Q ultrapure water was used as the aqueous phase for all nanoemulsion (NE) preparations. Quinoa flour (saponin-free) was supplied by "Cooperativa Las Nieves" of the VI Region of Chile. Chitosan from crab shells (ref. 448877) origin was obtained from Sigma-Aldrich. Reagents and culture media for microbiological analysis were obtained from Oxoid (Hampshire, England). Fresh strawberries (*Fragaria* × *ananassa* Duch. Cv. "Albion") used in the experiments were commercially acquired into packed commercial clamshells from Hortifrut Commercial S.A., Santiago, Chile, and stored for up to 24 h at 5–7 °C and 90% relative humidity until use.

Thymol Nanoemulsion Preparation

The Thy nanoemulsion (Thy/Ne) was prepared using the spontaneous method previously described by Robledo et al. (2018) and Chang et al. (2013). The surfactant was first mixed with the oil phase (OP) by magnetic stirring (500 rpm) for 30 min. The Thy/Ne was then mixed with (surfactant + OP) in the aqueous phase by using an infusion pump (kdScientific, model KDS-210, USA) at an injection rate of 0.5 mL/min, whilst stirring at 1200 rpm and room temperature (approximately 25 °C). The surfactant used was (Tween 80)/oil (Miglyol 812) at a ratio of 1:1, and OP with 45% Thy + 55% MCT. Thy/Ne had a droplet diameter of < 200 nm and polydispersity index of 0.197 ± 0.006; it was measured using a Zetasizer Nano ZS-20 (Malvern Instruments, USA) according to Robledo et al. (2018).

Preparation of Coating Solutions

Coating solution with Thy/Ne was prepared according to Robledo et al. (2018); quinoa flour was suspended in distilled water (1:5 *w*/*v*) with 10% of Ne, and the pH was adjusted to 11. This suspension was then centrifuged at 24,000×*g* for 30 min at 15 °C in order to obtain the aqueous protein extract. A solution of 2 g of chitosan in 100 mL of 0.1 M citric acid was then prepared. The protein extract and chitosan solution were mixed at a ratio of 1:1 by using a blade homogeniser. The pH of the mixture was adjusted to 3.0 with 1 M citric acid.

Coating solution without Thy/Ne was prepared in the same way as that containing Thy/Ne, but without the incorporation of Thy/Ne (Robledo et al. 2018; Abugoch et al. 2016).

Strawberry Coating and Storage Conditions

Fresh fruits were selected based on firmness, mechanical damage absence, and colour uniformity. The strawberries were then washed with tap water containing 10 g/L sodium hypochlorite, rinsed with tap water, and dried at room temperature. They were randomly divided into three groups: one strawberry group was without coating (S), the second one was coated with quinoa protein/chitosan edible coating (Sc), and the last was coated with Thy/Ne quinoa protein/chitosan edible coating solution (Sc-Thy). Samples were immersed into the coating solutions for 3 min, the excess of the film-forming solutions were drained, and the coated strawberries were dried in an open oven with a forced-air dryer at room temperature (± 20 °C) for 20 min (Robledo et al. 2018).

Approximately 18–20 kg of strawberries was used; after the selection process, around 6 kg strawberries was used in each treatment group, and approximately 100 g samples of control and coated strawberries were placed per clamshell. The strawberries were packed into commercial clamshells and stored in a commercial refrigerated chamber at ± 5 °C and 90% relative humidity for 0, 5, 10, and 16 days. Three clamshells per treatment (S, Sc, and Sc-Thy) and per measurement parameter were prepared, and each measurement was performed in triplicate.

Biopolymeric Cover Formation on Strawberry Surface

The formation of coating on the strawberry surfaces was ascertained according to Valenzuela et al. (2015) at the beginning and end of the experiments. Frozen surface tissue sections of strawberry were examined under an optical microscope (Axiostar plus Carl Zeiss, USA). The thickness was measured using software AxioVision 4.8 (USA).

Mould and Yeast Count and Fungal Decay

Mould and yeast counts (MYCs) were obtained according to the Official Standard Method (NCh2734.Of2002). Ten grams of each sample was suspended in 90 mL of peptone water (0.1% *v*/*v*) with continuous stirring for 2 min. The serial dilutions of the homogenates (1 mL) were plated on the surface of the selective media (potato dextrose agar, Oxoid), and the plates were incubated at 28 °C for 5 days. Counts were expressed as log CFU/cm^2. The counts/cm^2 were determined by using the data previously determined from the weight, diameter, and external surface of strawberries.

Samples for the MYCs and fungal decay inspection were carried out at 0, 5, 10, and 16 days. A fruit was considered to be infected when visible contamination was observed. The results were expressed as the percentage of fruits infected.

Sensory Evaluation

The sensory quality of strawberries was checked as per the Karlsruhe test (Valenzuela et al. 2015; Ibarra et al. 2012; Wittig 1981) adapted to the strawberry profile (Valenzuela et al. 2015). The quality of strawberries was analysed by eight trained judges (one man and seven women in the age range of 24 to 26 years) who were food engineers. The following parameters were evaluated: colour (internal and external), aroma, texture, taste, and general appearance. Quality parameters were evaluated between 1 and 9 points (Valenzuela et al. 2015; Velickova et al. 2013; Ibarra et al. 2012); the test scores the quality of strawberries was as follows: I. typical: 9 = excellent, 8 = very good, and 7 = good; II. tolerable: 6 = satisfactory, 5 = regular, and 4 = sufficient; and III. undesirable: 3 = deficient, 2 = bad, and 1 = very bad. The minimum score for acceptable total sensory quality of strawberries was 5, as per the Karlsruhe scale (Ibarra et al. 2012; Wittig 1981). The overall sensory quality (OSQ) was obtained by using the sensory parameters of Valenzuela et al. (2015), considering 15% external colour, 10% internal colour, 20% appearance, 15% aroma, 20% flavour, and 20% texture. The flavour attribute was evaluated until day 5 in order to protect the health risks of the panellists. All sensory evaluations of strawberries were carried out in a standardised test room in the sensory laboratory of the Department of Food Science University of Chile.

Physicochemical Properties

Weight Loss The weight of each box was measured using a balance (RADWAG; WTB 3000, Poland) daily. The results were expressed as the percentage of loss compared to the initial weight.

Firmness The firmness of strawberries was determined using the method proposed by Hietaranta and Linna (1999) by using a universal tensile testing machine (LLOYD, Model LR5K, England). A 100-N load cell was used to measure firmness, and the maximum penetration force (N) was determined using a 1-mm-diameter metal probe. The penetration depth was 5 mm; the speed was 5 mm s^{-1}. The firmness was reported as the maximum peak force and expressed in newton.

pH, Soluble Solid Content, Titrable Acidity, and Maturity Index The pH was measured using a pH meter (Jenway, 370, RPC). The soluble solid content (SSC) was measured using a digital refractometer (PR1; Atago, Co. Ltd., Japan) at 25 ± 2 °C. Titrable acidity (TA) was determined according AOAC 942.15 (1995) and was expressed as grams of citric acid per 100 g of fruit.

Statistical Analysis

GraphPad Prism 6 software was used for all statistical analyses. Analysis of variance and significance of differences between the means of Tukey's multiple range tests at the *p* level of 0.05 were used to determine significance.

Results and Discussion

Adherence of Thy Nanoemulsion-Polymeric Coating on Strawberry Surface

After the coatings were applied, a homogeneous film was formed over the surface of strawberries and was conserved throughout the storage time. Coatings were continuous both for Sc and Sc/Thy/Ne (Fig. 1). Further, the coating remained adhered to the surface of strawberries even after 16 days of storage (Fig. 1). The thicknesses of the coatings on the surface of the strawberries measured from the microphotographs were similar: 0.224 ± 0.020 for the coating without Thy/Ne and 0.245 ± 0.016 mm for that with Thy/Ne. However, at the end of storage, their thicknesses were 0.162 ± 0.033 mm (Sc) and 0.151 ± 0.018 mm (Sc-Thy/Ne). After 16 days of storage, the thickness of the coatings was reduced, likely because of the loss of moisture during storage. Valenzuela et al. (2015) did not observe any changes in the thickness of the chitosan/quinoa protein/oil coatings on day 15 of storage; however, they noted remarkable water loss during strawberry storage.

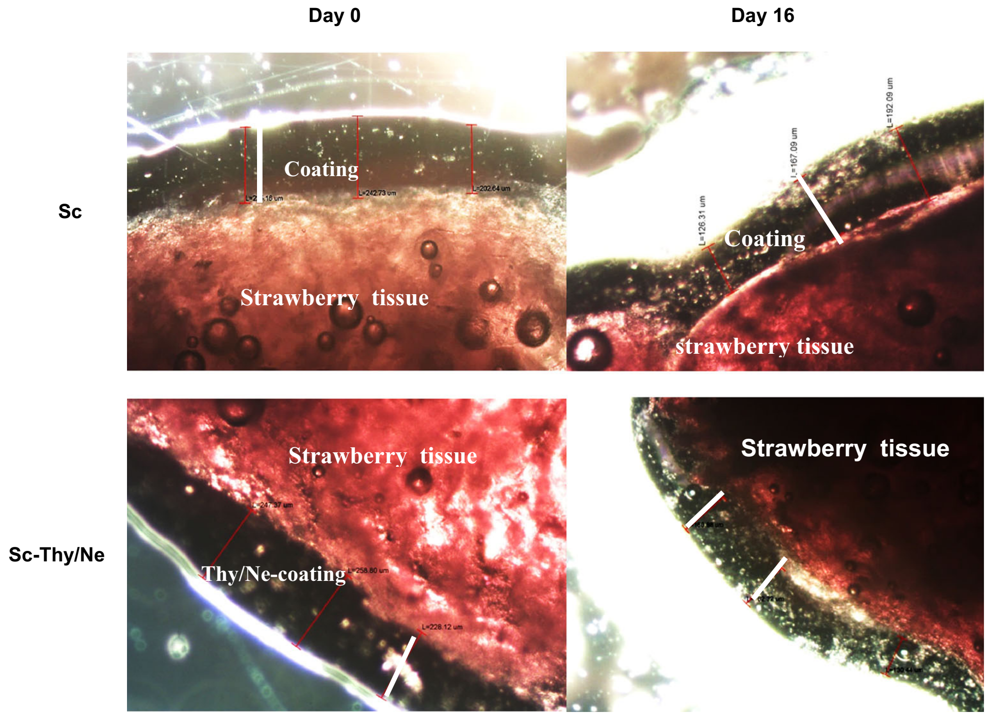

Fig. 1 Coating formation of quinoa protein-chitosan (Sc) and thymol-nanoemulsion-quinoo protein-chitosan (Sc-ne) on strawberries cv. "Albion" surface at 0 and 16 days of storage at ± 5 °C (cross-sectional view; ×10 magnifications)

Antifungal Activity of Thymol Coating Applied to Strawberries

During the storage time, the strawberries coated with Thy-antimicrobial packaging (Sc-Thy/Ne) had a lower MYC than control strawberries (S) and those coated without Thy/Ne (Sc; Fig. 2). On day 0 of storage, MYC was significantly lower ($p < 0.05$) in the strawberries coated with Thy/Ne quinoa protein/chitosan than in the control strawberries; this could be attributed to the immediate antimicrobial effect of the chitosan and Thy present in the coatings. At day 5, the MYCs of Sc-Thy/Ne increased slightly, but was not higher than that of S or Sc. On day 10, the MYCs of the control strawberries increased and that of Sc-Thy/Ne decreased, both significantly, whereas the those of Sc did not change significantly (with respect to that on day 5). The MYCs were significantly different amongst all treatments ($p < 0.05$) and were the highest in control and the lowest in Thy-Ne-coated strawberries. At the end of storage, the MYCs of the Sc-Thy/Ne group were significantly lower than those of S and Sc, which did not show significant differences between them ($p > 0.05$). The lower effectiveness of Sc that contained chitosan could be attributed to the interaction between quinoa protein and chitosan biopolymers (Abugoch et al. 2011; Valenzuela et al. 2015); however, the use of Thy/Ne increased the safety of strawberries. Robledo et al. (2018) reported that the fungal inhibitory effect of Thy

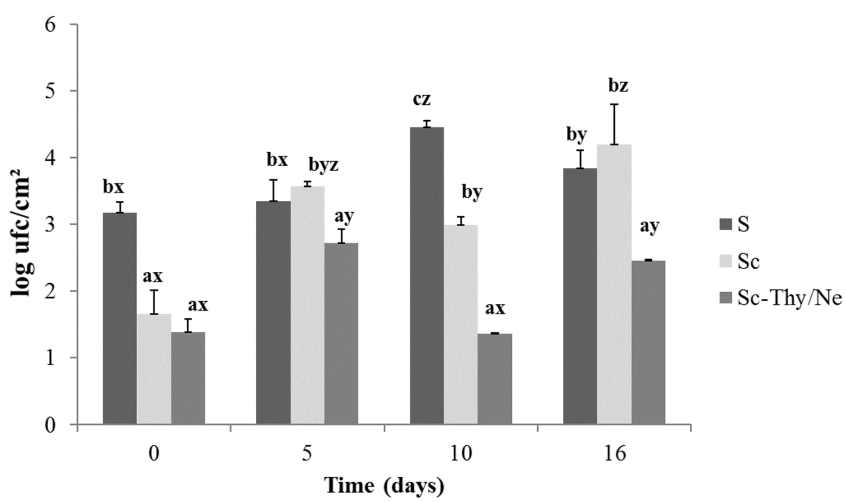

Fig. 2 Evolution of mould and yeast counts during the storage of uncoated (S), coated with quinoa protein/chitosan (Sc), and Thy-nanoemulsion quinoa protein/chitosan (Sc-Thy/Ne) strawberries cv. "Albion" storage at ± 5 °C. Different letters show significant differences ($p < 0.05$) between treatments (*a*, *b*) and storage time (*x*, *y*, *z*)

nanoemulsion/quinoa protein/chitosan coating might be related to the controlled release of Thy from the film to the fruit (they showed that 33.0% of Thy were released from Thy/Ne).

The results of fungal decay are shown in Fig. 3. Until day 10, Sc-Thy/Ne coating prevented all fungal damage to the fruit (Fig. 3b): 41.3% of the control strawberry were damaged, and 33.4% of the fruits coated without Thy/Ne were damaged. On day 16, the S and Sc showed 100% of damage, whereas Sc-Thy showed 58.3% of damage. Thus, on day 16, the Thy nanoemulsion coating treatment reduced the damage to 41.7% in the fruits (Fig. 3b). Strawberries that were coated with Thy nanoemulsion/quinoa protein/chitosan showed localised fungal growth, because of the antimicrobial capacity of Thy nanoemulsion, especially against spoilage caused by fungi and yeast and mainly against *B. cinerea* (Robledo et al. 2018). Antifungal effects afforded by Thy nanoemulsion/quinoa protein/chitosan coating on the surface of strawberries can decrease this type of damage and can increase the shelf life by 10 days or more, owing to the release of Thy during the storage time (Robledo et al. 2018), and thus protecting strawberries from fungal infection. Atress et al. (2010) found that using soy or wheat gluten/thymol to coat strawberries (0 °C and 90–95% RH), the microbial growth could be reduced.

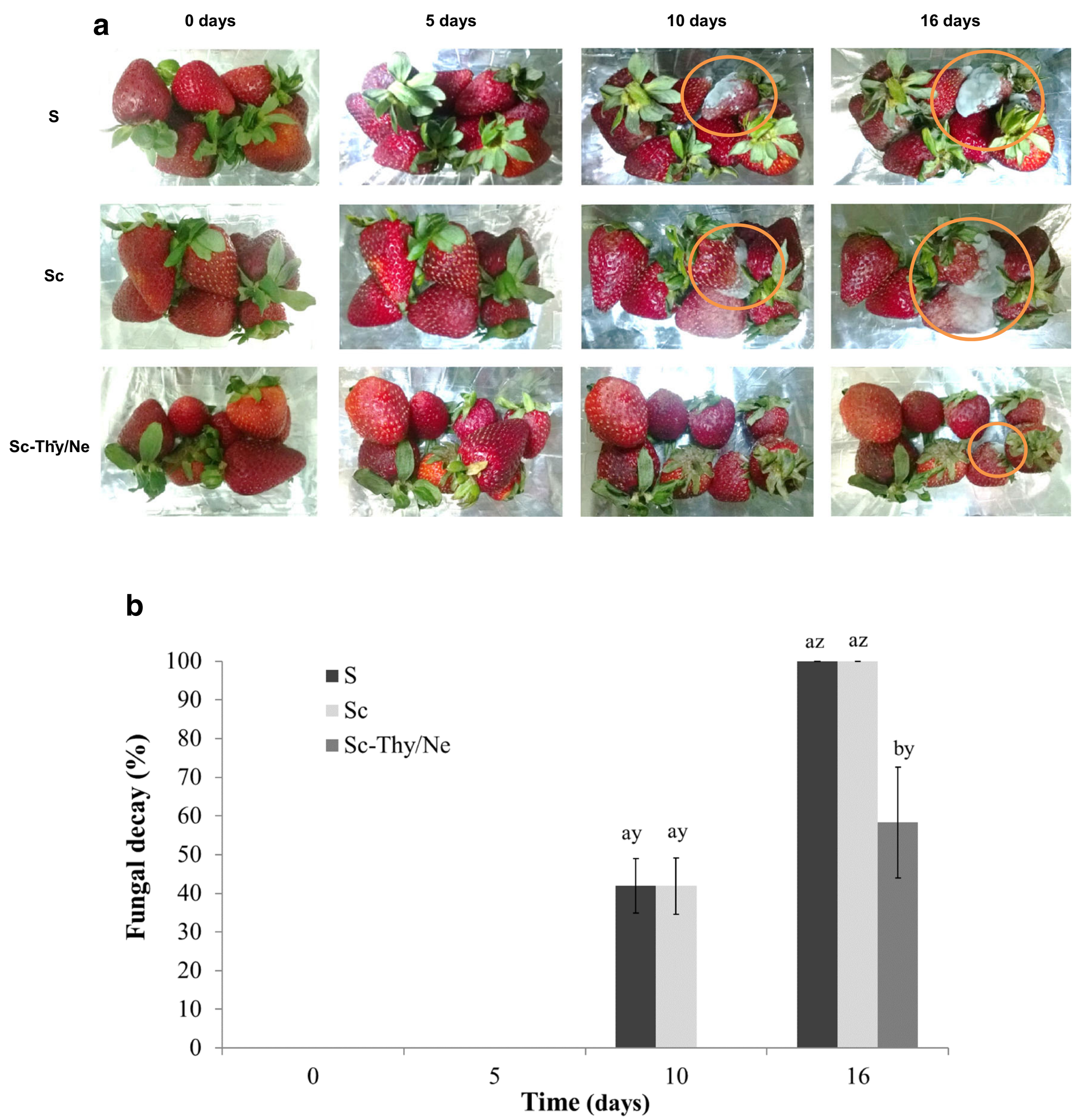

Fig. 3 **a** Appearance of uncoated (S) and coated strawberries cv. "Albion" with quinoa protein/chitosan coated (Sc), and Thy-nanoemulsion quinoa protein/chitosan (Sc-Thy/Ne), throughout storage at 5 °C. **b** Fungal decay (%) of uncoated (S) and coated strawberries with quinoa protein/chitosan coated (Sc), and Thy-nanoemulsion quinoa protein/chitosan (Sc-Thy/Ne), throughout storage at 5 °C. Different letters show significant differences ($p < 0.05$) between treatments (*a*, *b*) and storage time (*y*, *z*)

Sensory Analysis

The OSQ of the uncoated and coated strawberries is shown in Fig. 4a. No significant differences in OSQ were found between the uncoated or coated strawberries, and it did not decrease until day 5 for any of the tested samples; however, only Sc-Thy/Ne had an acceptable overall quality (score more than that of commercial limit value, 5.2; Valenzuela et al. 2015) at day 12 of refrigerated storage; this OSQ value of 5.2 could have been higher, since the sensory flavour parameter was not included in this overall value. On the other hand, Nasrin et al. (2017) found on day 12 of storage that overall acceptability the uncoated strawberries had a low scores (dislike), whereas the strawberries coated with chitosan obtained scores as dislike slightly. Conversely, only aroma and flavour, amongst all sensory parameters evaluated, showed significant differences ($p < 0.05$). The aroma (Fig. 4b) of Sc-Thy/Ne was significantly different from those of S and Sc ($p < 0.05$) at day 1, and its sensory score was 5.1 ± 1.3, which is the commercial limit; however, after 5 days, this value changed remarkably to 6.8 ± 0.5, possibly could be attributed to the panellists could not detect the presence of Thy. As observed in Fig. 4b, there was no significant aroma differences amongst treatments on day 12. The flavour (4.3 ± 1.7) of strawberries in the Sc-Thy/Ne group at the beginning of the storage was significantly different ($p < 0.05$) from that of S and Sc (Fig. 4c); this can be attributed to the presence of Thy-nanoemulsion, which was detected on day 1 of storage. However, at day 5, the sensory panel did not perceive its presence and scored it similar to the other samples (S and Sc). This effect could be related to the controlled release of Thy from the coating to strawberries (Gupta and Variyar 2016; Ramos et al. 2016). Robledo et al. (2018) showed that 33.0% of Thy are released from the nanoemulsion after 6 days; therefore, this release is probably related to Thy volatility (Celebioglu et al. 2017).

Physicochemical Properties

The weight loss evaluation of uncoated and coated strawberries during storage is shown in Fig. 5. During the storage time, the weight loss of the Sc-Thy/Ne was similar to that of Sc samples and was significantly ($p < 0.05$) lower than that of uncoated samples. This behaviour could be explained by the protector effect of the biopolymeric edible coating. The effect of storage intervals and different coatings (Sc-Thy/Ne; Sc) had significant effects on weight loss of fruit. Weight loss at 5, 10, and 16 day of storage varied considerably. The maximum weight loss occurred in control at the 16th day of storage (36.1%), and that for coated strawberries, at the same time, was significantly lower ($p < 0.05$) (Fig. 5); Hernández-Muñoz et al. (2008) found on day 7 of storage (10 °C and 70 ± 5% RH) that uncoated strawberries showed a 28.7% loss in weight, although the weight loss of fruits coated with chitosan around 19.6%. Conversely, the use of coatings significantly ($p < 0.05$) reduced weight loss at different storage periods. Compared to that in control, weight loss in fruit samples treated with Sc-Thy/Ne and Sc was 11.8 and 12.8% at the tenth day of storage. Nasrin et al. (2017) found that on day 9 of storage, uncoated strawberries showed a 12% loss in weight, whereas the weight loss of samples coated chitosan were around 10%, respectively, when stored at 6 ± 1 °C and 50 ± 5% relative humidity. However, the addition of Thy/Ne to the edible coating conferred antifungal effects, but did not influence the loss of water.

The firmness values of S, Sc, and Sc-Thy/Ne during storage are shown in Table 1. The firmness decreased in all samples during the storage period, and this observation agreed with other studies (Valenzuela et al. 2015; Villarreal et al. 2008). The loss of firmness is related to different factors such as water loss and fungal attack, amongst others (Gol et al. 2013). When Thy/Ne coating was used, this loss did not follow a clear pattern; however, no significant difference was noted between the different Sc-Thy/Ne and the uncoated group between 5 and 10 days. On day 16, the firmness of all samples decreased significantly ($p < 0.05$), and Sc-Thy/Ne more than the others ($p < 0.05$). Nevertheless, according to Vargas et al. (2006), strawberries at this time are not marketable due to the reported shelf life of 5 days when storage between 0 and 4 °C. The observed strawberry firmness loss could be related mainly to fungal attack especially between day 10 and 16 of storage (see Figs. 2 and 3). Fungi probably degraded the cell wall of the fruits between the abovementioned days of refrigerated storage, producing a softening that leads to the loss of firmness in all strawberries samples (Ordentlich et al. 1988; Ebel and Mithöfer 1998). The pH of Sc-Thy/Ne, Sc, and S stored at refrigeration conditions ranged from 3.4 to 3.2 (Table 1), showing a slight decrease during storage. Significant differences were only observed in the SC-Thy/Ne at day 10 showing a light increase. The pH values in general do not present great changes during strawberries refrigerated storage (Vargas et al. 2006; Valenzuela et al. 2015). On the other hand, the percentages of TA in all samples were between 0.8 and 1.1% during 16 days of storage. These values agree with those reported by Chisari et al. (2007), Montero et al. (1999), and Valenzuela et al. (2015), which are between 0.7 and 1.3%. The initial TA of strawberries for the control samples (S and Sc) and Sc-Thy/Ne were 1.08, 0.86, and 0.99%, respectively. In addition, with respect to the decrease in TA of Sc-Thy/Ne during storage, these results are consistent with those reported by Chisari et al. (2007), Vargas et al. (2006), and Atress et al. (2010), in which a decrease in TA of strawberries was observed during storage (9 or 10 days), probably due to ripening of the fruit. Changes in the SSC of strawberries over storage time are shown in Table 1.

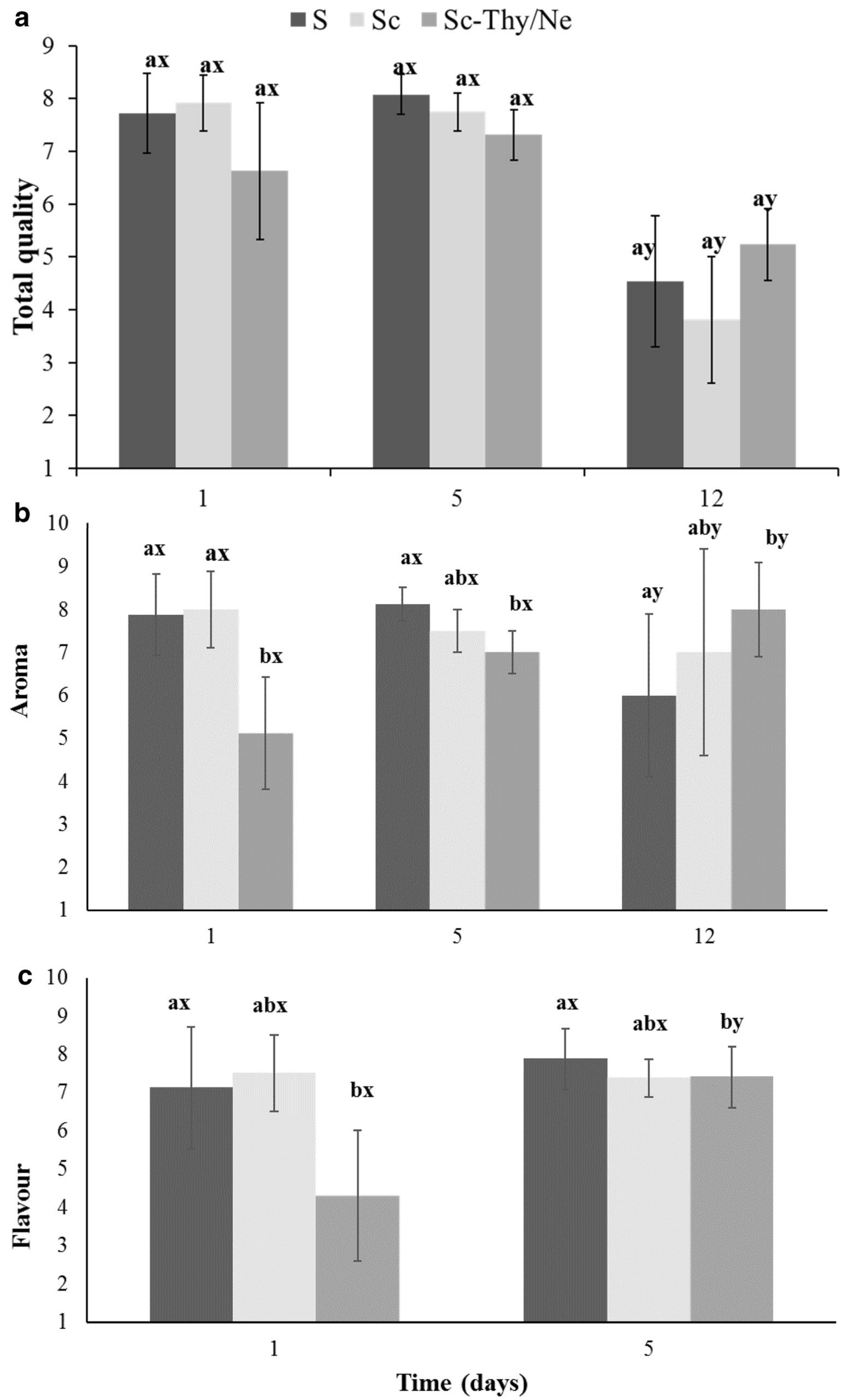

Fig. 4 **a** Sensory total quality of uncoated (S) and coated strawberries cv. "Albion" with quinoa protein/chitosan (Sc), thymol/nanoemulsion/quinoa protein/chitosan, and Sc-Thy/Ne coatings. **b** Average scores of aroma obtained from the panel for S, Sc, and Sc-Thy/Ne coatings. **c** Average scores of flavour obtained from the panel for S, Sc, and Sc-Thy/Ne coatings. During refrigerated storage at ± 5 °C. Different letters show significant differences ($p < 0.05$) between treatments (*a*, *b*) and storage time (*x*, *y*)

SSC were not affected by the coating treatments during 10 days of storage; this was consistent with the findings of Valenzuela et al. (2015) and Vargas et al. (2006). On day 16, the SSC of Sc-Thy/Ne had a mild increase, from 9.5 to

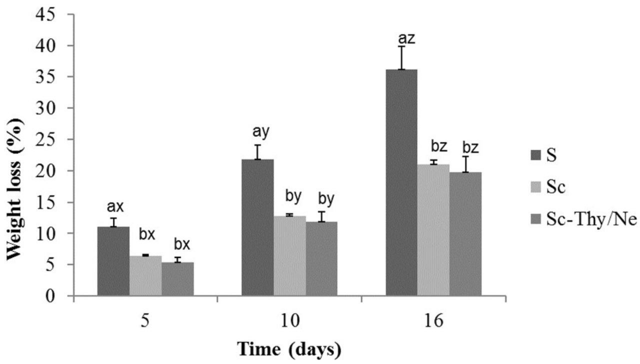

Fig. 5 Weight loss (%) of uncoated (S) and coated strawberries cv. "Albion" with quinoa protein/chitosan (Sc), and thymol/nanoemulsion/quinoa protein/chitosan (Sc-Thy/Ne), throughout storage at ± 5 °C. Different letters show significant differences ($p < 0.05$) between treatments (*a*, *b*) and storage time (*x*, *y*, *z*)

10.3; however, these values are agreeing with the literature (Vargas et al. 2006; Chisari et al. 2007). In general, reported values from literature for shelf life of strawberries are between 7 and 10 days (Hernández-Muñoz et al. 2008; Chisari et al. 2007). Our results were shelf life of 16 days, which could confirm that the senescence of the fruit has a fungic dependence (Ordentlich et al. 1988; Ebel and Mithöfer 1998).

Table 1 Firmness, pH, soluble solid content (SSC), and titrable acidity (TA) of uncoated (S) and coated strawberries cv. "Albion" with quinoa protein/chitosan (Sc), and thymol-nanoemulsion quinoa protein/chitosan (Sc-Thy/Ne), during refrigerated storage at ±5 °C

Time (days)	S	Sc	Sc-Thy/Ne
Firmness (N)			
0	1.25 ± 0.68 ax	1.45 ± 0.63 abx	0.93 ± 0.34 cx
5	1.56 ± 0.47 ax	1.50 ± 0.76 ax	1.40 ± 0.61 ay
10	1.22 ± 0.60 abx	1.47 ± 0.87 ax	0.92 ± 0.55 bx
16	0.74 ± 0.55 ay	0.56 ± 0.43 aby	0.41 ± 0.20 bz
pH			
0	3.30 ± 0.08 ax	3.36 ± 0.13 ax	3.40 ± 0.11 ax
5	3.26 ± 0.07 ax	3.23 ± 0.04 ax	3.27 ± 0.10 ax
10	3.23 ± 0.09 ax	3.25 ± 0.07 ax	3.43 ± 0.03 bx
16	3.15 ± 0.06 ax	3.25 ± 0.18 ax	3.25 ± 0.07 ax
TA (g of citric acid/100 g)			
0	1.08 ± 0.09 bx	0.86 ± 0.03 bx	0.99 ± 0.09 aby
5	0.96 ± 0.06 ax	0.98 ± 0.01 axy	0.94 ± 0.04 ay
10	1.11 ± 0.02 ax	1.35 ± 0.63 axy	0.80 ± 0.03 ax
16	1.07 ± 0.10 abx	1.16 ± 0.10 by	1.04 ± 0.07 aby
SSC (%)			
0	9.33 ± 1.21 ax	8.80 ± 0.92 ax	9.53 ± 0.81 ax
5	9.80 ± 1.05 ax	9.05 ± 0.05 ax	9.58 ± 0.51 ax
10	8.90 ± 1.37 ax	9.10 ± 0.79 ax	8.78 ± 0.60 ax
16	8.27 ± 0.57 ax	8.67 ± 0.81 ax	10.25 ± 0.88 bx

Different letters show significant differences ($p < 0.05$) between treatments (a, b) and storage time (x, y)

Conclusions

The incorporation of Thy/Ne to quinoa protein/chitosan coatings showed antifungal activity in strawberries under commercial refrigerated conditions of storage, which could be mainly attributed to Thy/Ne, and its protective effect was retained at least for 10 days under the storage condition. This antimicrobial coating also contributed to the sensory properties of strawberries, especially aroma and flavour, from day 5, and maintained this behaviour until day 12 of storage of Sc-Thy/Ne. Furthermore, it managed to increase the shelf life of coated strawberries at 4 days, relative to that in the control.

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