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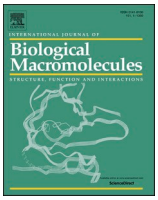

Development of hyaluronic acid based polysaccharide-protein composite edible coatings for preservation of strawberry fruit

Sawsan A. Al-Hilifi [a,*], Rawdah M. Al-Ali [a], Le N.M. Dinh [b], Yin Yao [c], Vipul Agarwal [b,*]

[a] Department of Food Science, College of Agriculture, University of Basrah, Iraq
[b] Cluster for Advanced Macromolecular Design (CAMD), School of Chemical Engineering, University of New South Wales, Sydney, NSW 2052, Australia
[c] Mark Wainwright Analytical Centre, University of New South Wales, Sydney, NSW 2052, Australia

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ABSTRACT

With the growing demand for extending the shelf-life of perishable goods such as fruits and vegetables, there is continued interest towards the development of edible coatings derived from natural sources. To avoid rapid dissolution, water insoluble polysaccharide such as chitosan has been widely explored. In this work, we developed robust hyaluronic acid-based edible polysaccharide-protein coatings by combining it (hyaluronic acid) with chitosan and gelatin to introduce additional antioxidant properties. This work is the first example of using hyaluronic acid in edible coatings for fruit preservation. The effect of developed edible composite coatings on the quality of coated strawberries was investigated over a 15 day storage period with 3-day examination intervals. The obtained results revealed hyaluronic acid dose-dependent improvement in intrinsic properties of coated strawberries including weight loss, pH, titratable acidity (TA) and total solids content (TSS). Furthermore, the inclusion of hyaluronic acid significantly enhanced the antioxidant properties of developed edible coatings as measured using total phenolic content, change in ascorbic acid content and DPPH assay prolonging the shelf-life of coated strawberries.

1. Introduction

Globalisation of the food industry has led to wider accessibility of fresh produce across the globe making it possible to obtain fruits and vegetables all year round. This accessibility requires long distance transportation of fresh produce from farms which can be in different countries across the globe to consumers in different parts of the world. To withstand prolonged transportation time from farms to supermarkets, fresh produce needs to be protected against damage and fouling and to extend its shelf-life. Furthermore, there is a growing push to minimise postharvest loss of fresh produce by extending their shelf-life. [1] To this end, edible coatings remain the most effective strategy with cost effectiveness and no harmful health implications. [2–4] The effectiveness of edible coatings has been attributed to reducing weight loss, change in pH and other quality attributes by minimizing lipid peroxidation, altering the respiration rate and preservation of color and texture. [5–9]

Strawberries (*Fragaria Xananassa*) are one of the most popular summer fruits with considerable health benefits owing to the presence of natural antioxidants including polyphenols and anthocyanin, vitamins and amino acids. [10] However, they are highly perishable with very short post-harvest shelf-life due to their vulnerability to mechanical injury, physiological deterioration, water loss, fungal decay and high respiration rate. [5,11,12] Traditional approach to extend postharvest strawberry shelf-life is to use cold storage conditions and exposure to modified atmospheres with elevated CO_2 levels. [13] However, due to only short-term benefits and associated costs of cold storage and reduction in taste with prolonged exposure to elevated levels of CO_2, alternate strategies are desired. To this end, edible coatings have been explored with the majority focussing on using natural biopolymers and polysaccharides in particular chitosan, which has emerged as a primary component in edible coatings due to the reasons mentioned above. [10,12–18] Furthermore, lately use of natural antioxidant and antimicrobial constituents are used in edible coatings to provide additional benefit with prolonged safety. [19,20] In terms of natural antioxidants, chitosan is the most widely explored polysaccharide in edible coatings due to its additional antimicrobial properties. [15,21] Coatings produced using chitosan and its blending with other components have been shown to both extend the shelf-life of fruits and enhance intrinsic antioxidants profile of coated fruits. [10,13,22,23] Another benefit of using

* Corresponding authors.
E-mail addresses: sawsan.hameed@uobasrah.edu.iq (S.A. Al-Hilifi), agarwalvipul84@gmail.com (V. Agarwal).

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chitosan in edible coatings is its low to no solubility in pure water ensuring no peeling or dissolution of coatings from fruits during storage and transport. Recently, there has been a growing interest in blending chitosan with other natural constituents such as natural oils, and biopolymers to overcome problems of low mechanical and thermal stability and high sensitivity to humidity with prolonged exposure observed in neat chitosan coatings and simultaneously improving its overall preservation performance. [15,20,22,24,25]

In this study, we blended chitosan with gelatin and hyaluronic acid to produce natural edible coatings. To the best of our knowledge, hyaluronic acid has never been explored in edible food coatings despite being used in the cosmetics industry and extensively studied in biomedical science. The obtained blended coatings were investigated for their physicochemical and fruit preservation effectiveness using strawberries as a model fruit. Hyaluronic acid was selected for its natural antioxidant properties and biocompatibility [26,27] with the hypothesis that its (hyaluronic acid) inclusion will significantly enhance the properties of produced edible coatings. We observed a significant preservation and shelf-life of strawberries with increasing concentrations of hyaluronic acid in produced edible coatings. The obtained results are significant in the field and envisioned to disrupt the status quo inspiring further research and eventual commercial use of hyaluronic acid in edible coatings.

2. Materials and methods

2.1. Materials

Hyaluronic acid was extracted in our laboratory from local potatoes. Gelatin (from bovine) was purchased from Merck (Germany), and chitosan (91.3 % degree of deacetylation), 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, Folin-Ciocalteu reagent, oxalic acid, 2,6-dichloroindophenol, gallic acid (GA) were obtained from Sigma (Germany).

2.2. Extraction of the hyaluronic acid

Hyaluronic acid (HA) was extracted using a previously described method [28] with some minor modifications. Potatoes were obtained from a local market in the city of Basrah, Iraq. Potatoes were washed, cleaned, and cut into slices, and dried at 40 ± 2 °C for 48 h in an oven. The dried potato slices were ground to a fine powder using a grinder (MX-KM5070, Panasonic, Malaysia) and stored in airtight containers until further use. Potato powder (500 g) was mixed with acetone (250 mL), stirred for 1 h and filtered to remove any fat. The obtained powder was then incubated with a chloroform:methanol mixture (2,1, 300 mL) for 24 h at 25 °C. The solvent mixture was removed by filtration and the filtrate was dried for 20 min at room temperature to obtain a dried powder. Next, a digestion buffer (100 mM sodium acetate pH 5.0, 5.0 mM cysteine and 5.0 mM disodium EDTA) was prepared. The chloroform:methanol treated dried powder was digested in the prepared buffer for 48 h at 4 ± 2 °C before centrifugation at 4000 rpm for 25 min. The obtained supernatant was discarded, and the pellet was washed once with sodium chloride (3 mL, 2.0 M) and absolute ethanol. Following which, the pellet was incubated in absolute ethanol for 24 h at −16 °C and then centrifuged at 4000 rpm for 25 min. After centrifugation, the supernatant was removed, and the pellet was suspended and washed with 80 % ethanol. The solution was centrifuged again at 4000 rpm for 25 min, the supernatant was then discarded and the pellet was dried at 25 °C for 24 h. The obtained dried powdered hyaluronic acid was stored at 4 ± 2 °C prior to further use.

2.3. Film preparation

The chitosan: gelatin: hyaluronic acid (CS:G:HA) films were prepared using a previously described method [29] with some minor modifications. Polymer and polysaccharide solutions were prepared by dissolving 1 wt% CS in acetic acid (1 *v*/v in water), and 1 wt% G and 0.01 wt% HA separately in water. The obtained solutions were mixed in the following proportions: 7:2:1 (CS:G:HA); 6:2:2 (CS:G:HA); 5:2:3 (CS:G:HA) and 5:5 (CS:G) and labelled as 7CS:2G:1HA, 6CS:2G:2HA, 5CS:2G:3HA and 5CS:5G, respectively. After that the obtained solutions were divided into two equal aliquots, with one half poured into 15 mL petri dishes and left to dry to obtain standalone composite coatings for characterisation and the second half was used to coat strawberries.

2.4. Fourier transform infrared spectrometer (FTIR)

FTIR was conducted using a Bruker ATR-FTIR (Germany). Measurements were conducted on standalone composite coatings with an average of 32 scans and a wavelength ranging from 400 to 4000 cm^{-1} at a resolution of 4 cm^{-1}.

2.5. Thermogravimetric Analysis (TGA)

TGA analysis was conducted using a TA Instruments TGA Q5000. Standalone composite coatings of 2 to 5 mg were loaded in a platinum sample holder heated at a temperature ramp of 20–30 to 800 °C with a heating rate of 10 °C/min under an air atmosphere with a flow rate of 25 mL/min.

2.6. Scanning electron microscopy (SEM)

SEM was conducted on standalone composite coatings using a FEI Nova NanoSEM 450 FE-SEM operating at an accelerating voltage of 5 kV. The standalone coatings were sputter coated with platinum prior to imaging.

2.7. Fresh Fruit

Fresh strawberries were purchased from a local market in Basrah, Iraq. Ripe strawberries of roughly homogeneous size (~30 g each by weight), and free from mechanical injuries were used in the experiments. The fruits were carefully washed with distilled water and left to dry at laboratory temperature for approximately 10–15 min before coating.

2.8. Coating application and storage conditions

Strawberries were primed for coating by dividing them into five groups each containing 50 fruits. Strawberries from each group were completely immersed for 2 min in respective coating solutions (7CS:2G:1HA, 6CS:2G:2HA, 5CS:2G:3HA and 5CS:5G). 5CS:5G coating and uncoated fruits were used as control groups. After immersion, fruits were placed on a metal clip and the excess coating solution was allowed to drip off and dry for 1 h at 25 ± 2 °C. Uncoated control fruits were treated the same way without any coating. After drying, all coated fruits were preserved in cork boxes and stored at 5 ± 2 °C in a refrigerator. The coated fruit quality assessment of each group was evaluated regularly at 0, 3, 6, 9, 12 and 15 days of storage in refrigerated conditions.

2.9. Total soluble solids (TSS), pH, titratable acidity (TA)

Strawberry fruit juice of fruits from each group was used to determine TSS, pH and TA. Fruit juice was obtained by mashing 25 g of fruit pulp in 100 mL of distilled water and the washed mixture was filtered using Whatman paper. TSS was estimated using a digital refractometer (A87117, Bellingham, UK) and the data were expressed as °Brix. The TA of the fruits were determined by titrating obtained strawberry juice (10 mL in 40 mL distilled water) with 0.1 N NaOH using phenolphthalein as an indicator until a permanent pink color appeared, and it was expressed as a percentage of citric acid equivalent based on fresh fruit weight. The pH value of juice samples was measured using a digital pH meter (pH-

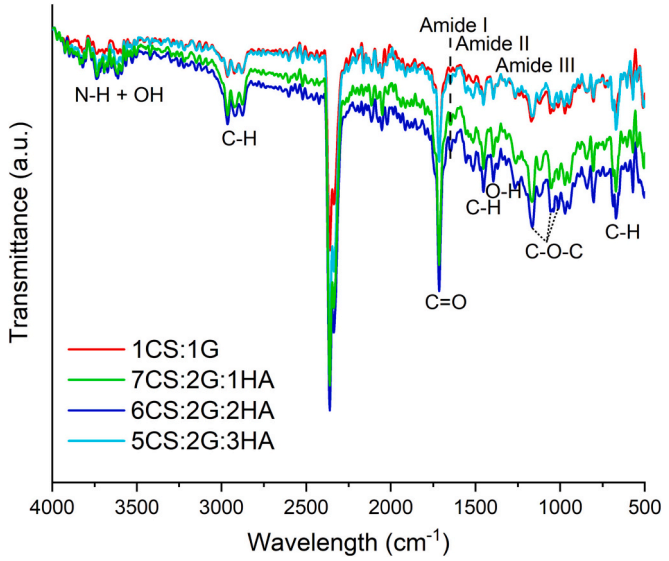

Fig. 1. Characterisation of composite films using FTIR. CS stands for chitosan, G for gelatin and HA for hyaluronic acid. All ratios are weight ratios between the different components used to prepare coatings.

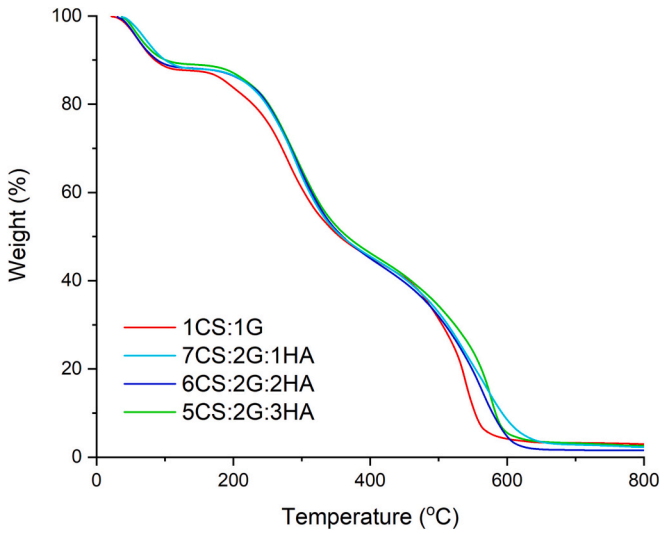

Fig. 2. TGA analysis of composite films. CS stands for chitosan, G for gelatin and HA for hyaluronic acid. All ratios are weight ratios between the different components used to prepare coatings.

EMCO-256071, Japan) at an ambient temperature.

2.10. Weight loss

To determine weight loss, coated and uncoated fruits before and after storage were weighed. The change in weight with storage time was recorded and presented as a percentage ($n = 5$).

2.11. Determination of total phenolic content (TPC)

The total phenolic content was measured by the Folin-Ciocalteu method using gallic acid as a standard. At specific time points, fruit extract was obtained by mashing the fruit in water and filtering the obtained slurry using a Whatman paper. The fruit extract (0.5 mL) was diluted in water and mixed with the Folin-Ciocalteu reagent (0.5 mL) followed by sodium carbonate solution (10 %) after 1 min and subsequently incubated for 30 min at 35 °C. The absorbance of the solution was measured at 750 nm. Total phenols content was expressed as mg of gallic acid equivalent in strawberry fruit (mg GAE/mL). All experiments were performed in three replicates.

2.12. Determination of ascorbic acid

To determine the amount of ascorbic acid, at each specific time point, 25 g of fruit pulp was mashed with oxalic acid (1 % in water, 200 mL). 10 mL of the mashed fruit oxalic acid mixture was filtered and the obtained filtrate (10 mL) was titrated against 2,6-dichloroindophenol (0.25 g/L in distilled water) to obtain a pink endpoint (color should persist for ≥15 s). The results obtained were expressed as mg of ascorbic acid per 100 mL sample. Each group of samples was measured three times and data is presented as an average.

2.13. Antioxidant activity

The 2,2-diphenyl-1-picryhydrazyl (DPPH) assay was used to measure the antioxidant activity of uncoated and coated fruits. Briefly, 1 mL of 0.01 % DPPH was mixed with 1 mL of fruit extract (prepared by mashing 25 g of fruit pulp and distilled water). The mixed solution was kept in the dark for 30 min at room temperature, and then the absorbance of the change in color of the mixture was measured at 517 nm.

2.14. Statistics

The results for coating performance on coated fruits are expressed as mean ± standard deviation and analyzed using one-way analysis of variance (ANOVA). Significance was evaluated using a Bonferroni *posthoc* analysis and set at 95 % confidence ($p < 0.05$).

3. Results and discussion

3.1. Physicochemical characterisation of composite coatings

3.1.1. FTIR

The structural determination of different constituents used to prepare standalone composite coatings prepared in petri dishes was conducted using a FTIR (Fig. 1). We observed no prominent differences between the spectra of different coatings. This could be due to the similarity in chemical structure of the two polysaccharides – chitosan and hyaluronic acid, and protein gelatin. In all samples, we observed multiple characteristic peaks for polysaccharides, for example, bands at 1165, 1057, and 1010 cm^{-1} ascribed to C-O-C stretching vibrations, [30] and proteins – three characteristic amide bands at ~1640, 1550 and 1260 cm^{-1} which can be attributed to C═O stretching vibrations (amide I), N—H bending vibrations and C—N stretching vibrations (amide II) and C—N and C—O stretching vibrations, N—H and O-C-N bending vibrations (amide III), and C═O band at ~1710 cm^{-1}. [31,32] The prominent bands at 670, ~1450 and 2960 cm^{-1} can be assigned to C—C stretching, C—H deformation and CH_2 stretching vibrations contributed from both polysaccharides (chitosan and hyaluronic acid) and gelatin protein. The otherwise noisy bands between 3740 and 3610 cm^{-1} can be ascribed to the combination of O—H and N—H stretching vibrations. Taken together, we postulate that FTIR data validate the presence of all three constituents in hyaluronic acid-based coatings. It should be noted that it is difficult to isolate signals from each constituent due to the presence of similar functional groups in the two polysaccharides (chitosan and hyaluronic acid) and the protein (gelatin). Furthermore, in the case of chitosan and gelatin composite coatings, the presence of hydrogen bonding has been proposed and we hypothesise the possibility of similar interactions with the inclusion of hyaluronic acid in developed edible coatings. The potential hydrogen bonding interactions can be attributed to the presence of different polar functional groups including hydroxyl, carboxyl, carbonyl and amines in our ternary composite coatings.

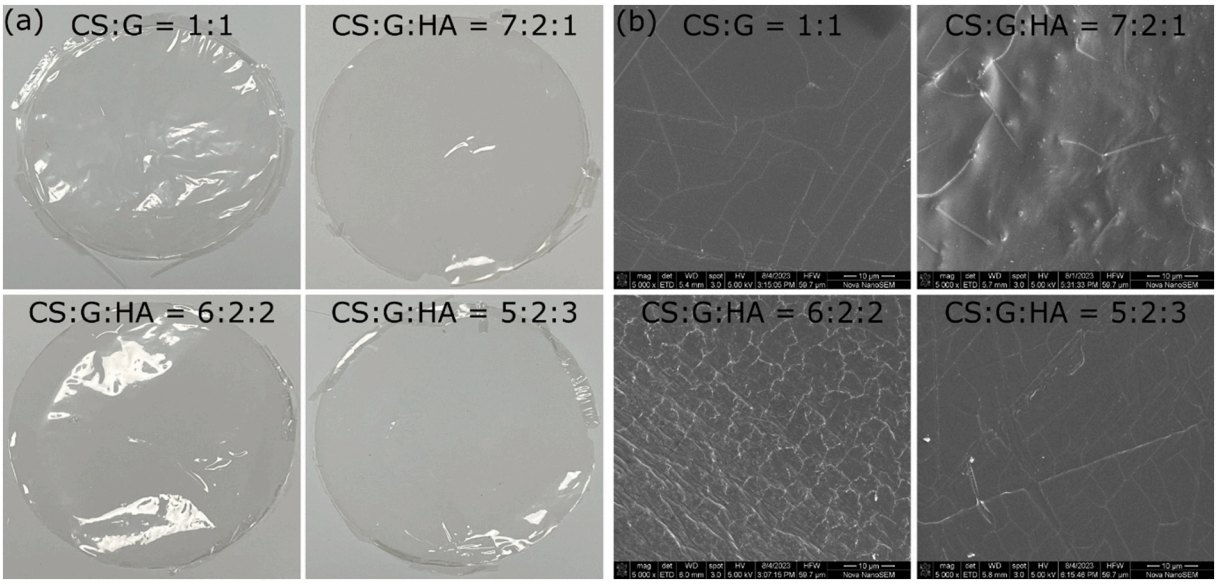

Fig. 3. Representative (a) optical and (b) SEM images of different composite films. CS stands for chitosan, G for gelatin and HA for hyaluronic acid. All ratios are weight ratios between the different components used to prepare coatings. Scale bar in (b) 10 μm.

3.1.2. TGA

Thermal stability of the prepared coatings was determined using thermogravimetric analysis (TGA) particularly to investigate any potential deterioration of thermal properties with the inclusion of hyaluronic acid in developed edible coatings. The three-stage decomposition process was observed in all samples – (i) room temperature – 100 °C, (ii) 200–350 °C and (iii) 450–600 °C (Fig. 2). The first decomposition stage of around 10–12 % weight loss at ~100 °C was caused by the loss of adsorbed or molecular water molecules. [33,34] The loss was largest albeit not significant in the case of 1CS:1G (~12 %) control coating compared to HA containing coatings (CS:G:HA – 7:2:1, 6:2:2 and 5:2:3). The second decomposition observed between 200 and 350 °C of approximately 40 % can be attributed to the degradation of polysaccharide and protein functional groups, and final degradation 450 and 600 °C resulting in almost complete loss can be ascribed to the degradation of carbon backbone of polysaccharide and protein used in coatings. We observed no significant differences and no noticeable trend between different coatings. Overall, all coatings were fairly stable at

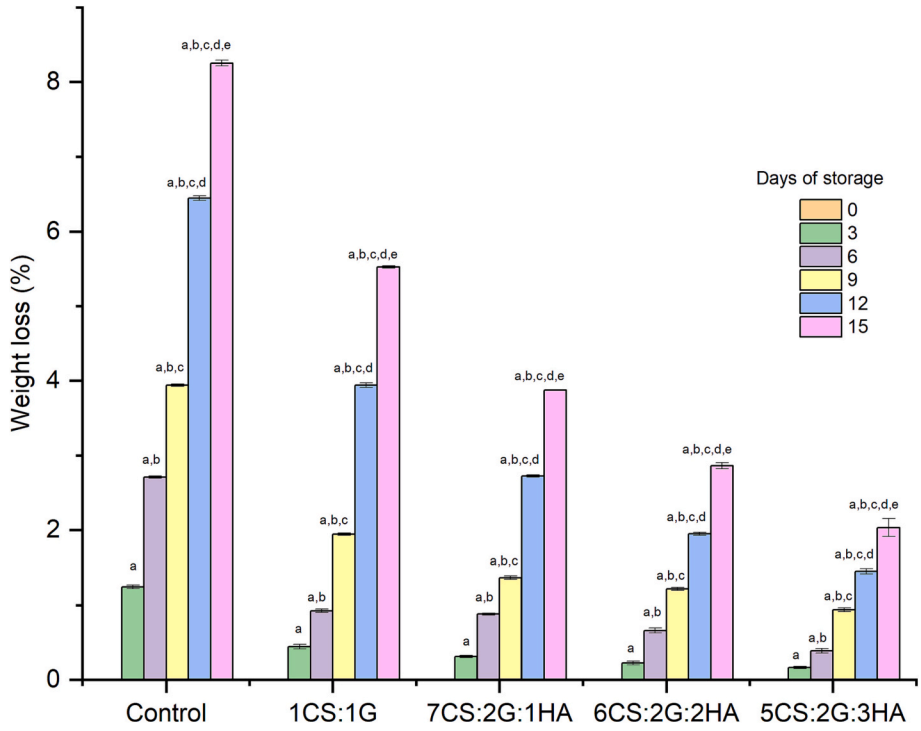

Fig. 4. Effect of edible coatings on weight loss of strawberries over different storage times. CS stands for chitosan, G for gelatin and HA for hyaluronic acid. All ratios are weight ratios between the different components used to prepare coatings. Data are presented as mean ± SD ($n = 3$) (error bars are significantly smaller than the data points). Values with different letters on the top of bars are significantly different ($p < 0.05$) and determined by using a Bonferroni *posthoc* test in a one-way ANOVA analysis—a, b, c, d, e are relative to coated fruits at day 0, 3, 6, 9, 12 and 15 days of storage, respectively.

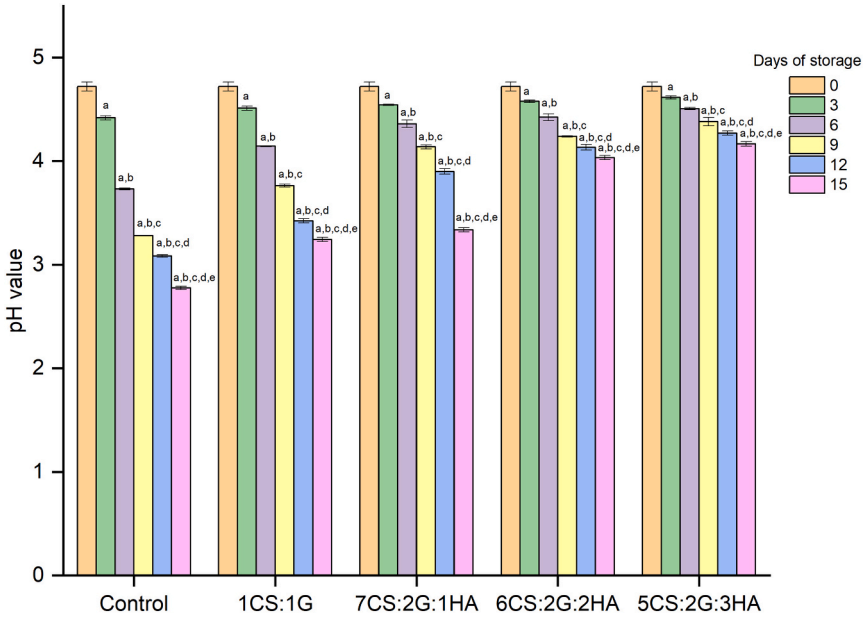

Fig. 5. Effect of edible coatings on pH of strawberries over different storage times. CS stands for chitosan, G for gelatin and HA for hyaluronic acid. All ratios are weight ratios between the different components used to prepare coatings. Data are presented as mean ± SD ($n = 3$) (error bars are significantly smaller than the data points). Values with different letters on the top of bars are significantly different ($p < 0.05$) and determined by using a Bonferroni *posthoc* test in a one-way ANOVA analysis—a, b, c, d, e are relative to coated fruits at day 0, 3, 6, 9, 12 and 15 days of storage, respectively.

room temperature and demonstrated behaviour similar to different previously reported coatings [34,35] highlighting that inclusion of hyaluronic acid did not induce any detrimental impact on thermal properties of produced edible coatings. Also, no hyaluronic acid concentration dependent change in thermal behaviour was observed on the thermal properties of coatings.

3.1.3. Optical and electron imaging

We also prepared coatings in a petri dish to showcase their appearance upon drying. Fig. 3a shows the images of dried coatings prepared

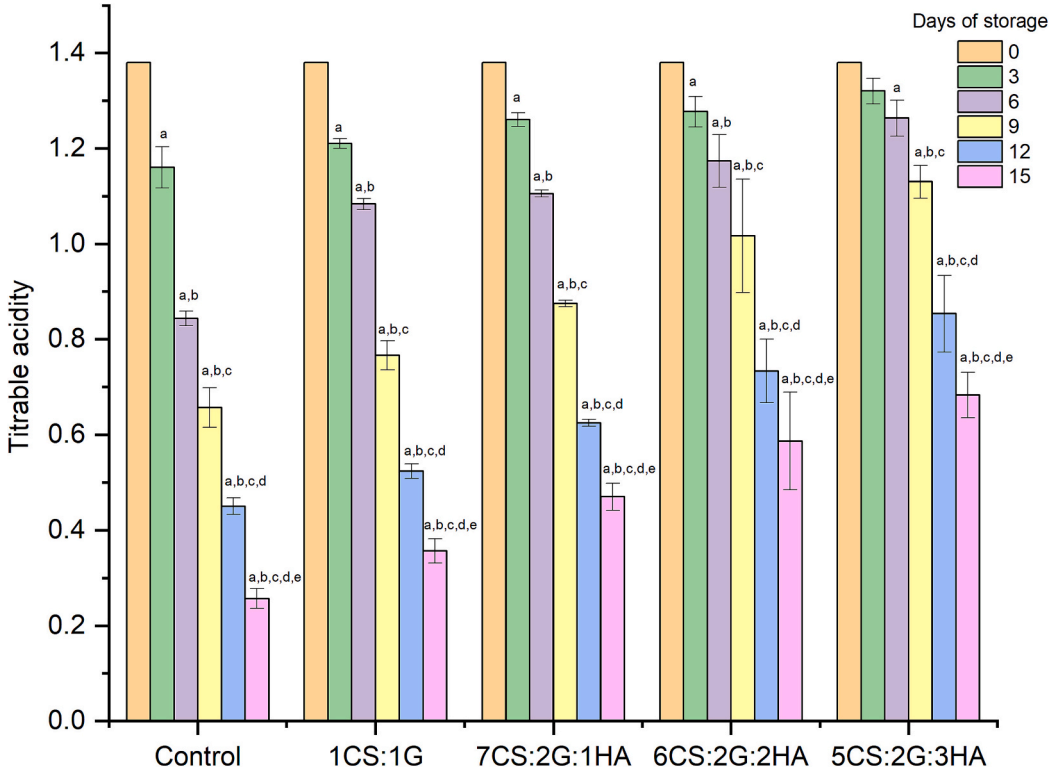

Fig. 6. Effect of edible coatings on titratable acidity of strawberries over different storage times. CS stands for chitosan, G for gelatin and HA for hyaluronic acid. All ratios are weight ratios between the different components used to prepare coatings. Data are presented as mean ± SD ($n = 3$). Values with different letters on the top of bars are significantly different ($p < 0.05$) and determined by using a Bonferroni *posthoc* test in a one-way ANOVA analysis—a, b, c, d, e are relative to coated fruits at day 0, 3, 6, 9, 12 and 15 days of storage, respectively.

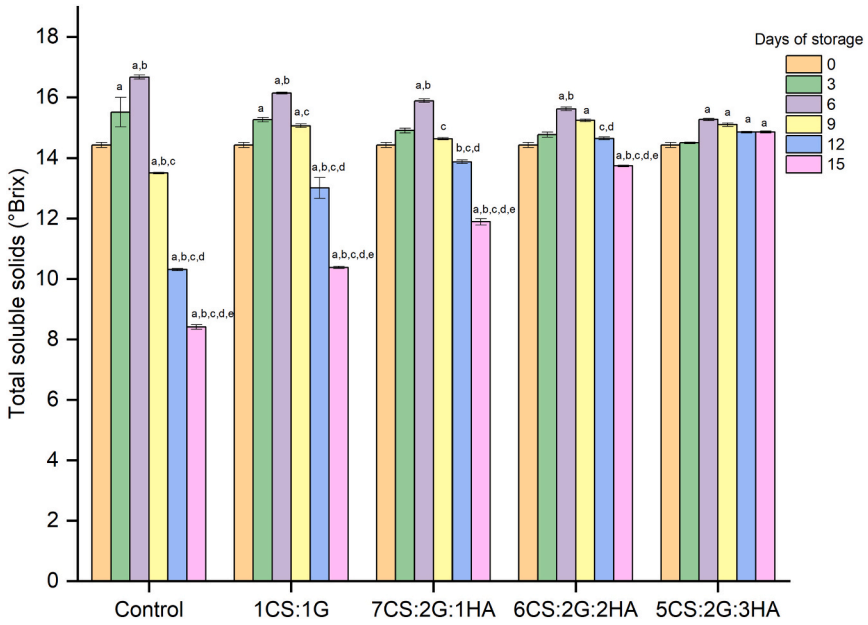

Fig. 7. Effect of edible coatings on total soluble solids (TSS) of strawberries over different storage times. CS stands for chitosan, G for gelatin and HA for hyaluronic acid. All ratios are weight ratios between the different components used to prepare coatings. Data are presented as mean ± SD ($n = 3$). Values with different letters on the top of bars are significantly different ($p < 0.05$) and determined by using a Bonferroni *posthoc* test in a one-way ANOVA analysis—a, b, c, d, e are relative to coated fruits at day 0, 3, 6, 9, 12 and 15 days of storage, respectively.

and peeled from a petri dish. The films prepared on petri dishes were quite thin making it almost impractical to determine their thickness. All coatings appear transparent with a glossy surface. We observed no noticeable differences in terms of consistency or thickness between different coatings with and without the inclusion of hyaluronic acid. Subsequently, we also performed SEM imaging on samples used for optimal imaging. SEM images revealed a homogenous surface with visible cracking (Fig. 3b). The observed cracking on the surface has been postulated to be caused by the SEM sample preparation method and ultrahigh vacuum used for sputter coating and during imaging. Similar surface features and possible cracking have been previously reported for chitosan/pectin/essential oil- and *aloe vera* gel/carnauba wax-based coatings. [19,20]

3.2. Effect of composite edible coatings on postharvest quality of strawberries

3.2.1. Weight loss

Fruits such as strawberries are particularly vulnerable to degradation and weight loss with time caused by the potential loss of moisture due to their very thin and highly permeable skin. Fig. 4 shows the weight loss of uncoated and coated fruits over 15 days of storage time. We observed a significant weight loss (8.25 %) in uncoated control samples over the entire storage period of 15 days. Coated fruits exhibited significantly lower weight loss compared to uncoated control fruits ($p < 0.05$). Amongst the coated samples, chitosan+gelatin (1CS:1G) coated samples exhibited significantly higher weight loss compared to hyaluronic acid containing coatings (CS:G:HA – 7:2:1, 6:2:2 and 5:2:3), particularly after 6 days of storage. At day 15 of storage, 1CS:1G coated fruits had an average weight loss of 5.5 % followed by 7CS:2G:1HA (3.9 %), 6CS:2G:2HA (2.9 %) and 5CS:2G:3HA (2 %). Based on the weight loss trend, it can be deduced that increasing concentration of hyaluronic acid in coatings preserved fruits against weight loss during the 15 days of storage. This could be attributed to the interactions between hyaluronic acid, chitosan and gelatin leading to the formation of a semi-permeable layer on the fruit surface preventing moisture loss mediated weight loss.

3.2.2. pH

pH is an important indicator of fruit properties with an increase in pH typically indicative of fruit ripening and oxidation over time. Fig. 5 shows the change in pH of uncoated and coated strawberries during the 15 days storage period. We observed a reduction in pH under all conditions (uncoated and coated strawberries). The most significant reduction was observed in the case of uncoated control fruits reaching the minimum value of 2.8 at day 15 of storage. The increase in acidity is associated with fruit decay during the 15 days storage period. In the case of coated strawberries, fruits coated with 1CS:1G coating exhibited the most significant reduction in pH compared to hyaluronic acid containing coatings (7CS:2G:1HA, 6CS:2G:2HA and 5CS:2G:3HA). This difference in pH could be explained by the combination of fruit decay during storage, and antioxidant and protective properties of hyaluronic acid. The significant drop in pH in both uncoated control and 1CS:1G coated fruits could also be explained by the dramatic weight loss observed under these conditions which could in turn concentrate the remaining solvent content. In the case of hyaluronic acid containing coatings, reduction in pH was inversely correlated with the amount of hyaluronic acid in composite coatings with minimum change observed for 5CS:2G:3HA during the 15 days storage period.

3.2.3. Titratable acidity (TA)

The TA values are associated with the amount of organic acid in fruits which is also a marker of change in intrinsic fruit acidity with time. The TA value for uncoated and coated fruits was reduced with storage time (Fig. 6). We observed a clear trend in the change in TA with maximum reduction observed in uncoated control fruits followed by 1CS:1G, 7CS:2G:1HA, 6CS:2G:2HA and 5CS:2G:3HA. The significantly higher reduction in the uncoated fruits control compared to coated fruits ($p < 0.05$) could be attributed to the significant weight loss mediated by the restriction of the respiration rate during the 15 days storage period. [36] This notion is supported by the trend in the weight loss data and pH values. We observed a slower reduction at higher hyaluronic acid containing coatings (6CS:2G:2HA and 5CS:2G:3HA) compared to the remaining coatings. Based on this data it can be deduced that inclusion of hyaluronic acid in edible coatings can reduce the respiration rate of coated fruits (strawberries) and consequently inhibit the release of

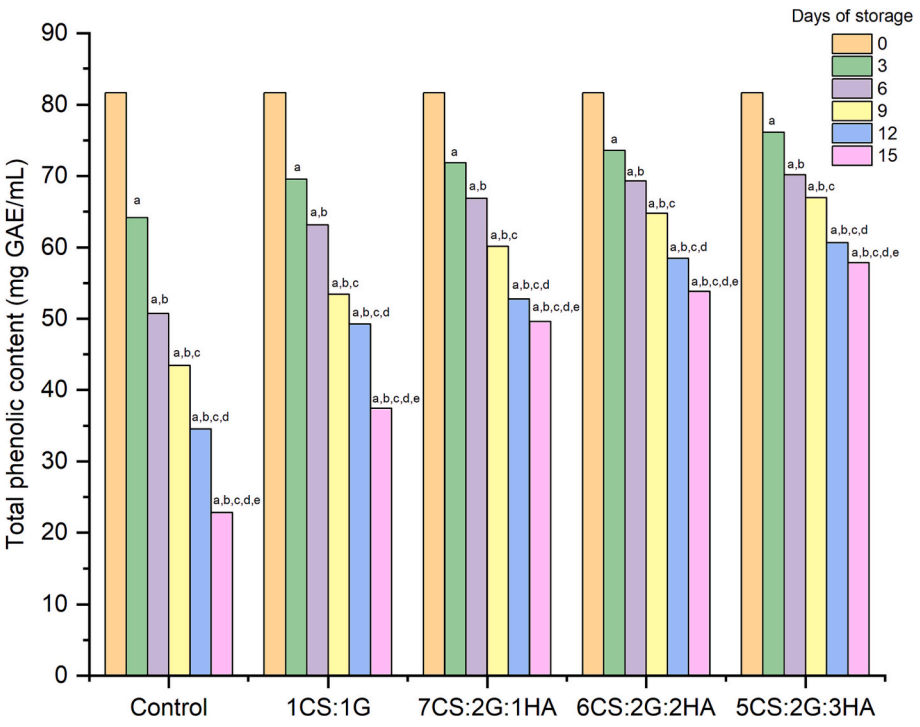

Fig. 8. Effect of edible coatings on total phenolic content of strawberries over different storage times. CS stands for chitosan, G for gelatin, HA for hyaluronic acid and GAE stands for gallic acid equivalent. All ratios are weight ratios between the different components used to prepare coatings. Data are presented as mean ± SD ($n = 3$) (error bars are significantly smaller than the data points). Values with different letters on the top of bars are significantly different ($p < 0.05$) and determined by using a Bonferroni *posthoc* test in a one-way ANOVA analysis—a, b, c, d, e are relative to coated fruits at day 0, 3, 6, 9, 12 and 15 days of storage, respectively.

organic acids. The observed restricted change in TA values of coated strawberries compared to uncoated strawberries in our work is in agreement with a previous report on quaternized catechol-functionalized chitosan coated strawberries. [24]

3.2.4. Total soluble solids (TSS)

The TSS is composed of organic acids and polysaccharides present in fruits which are associated with flavour and indicative of ripening degree. Under storage, TSS tend to increase due to the hydrolysis of polysaccharides into simple sugars and other soluble compounds. [24] Fig. 7 shows the change in TSS values of uncoated and coated strawberries over the 15 days storage period. In all samples, we observed a significant increase in TSS values until day 6 of storage followed by different degrees of reduction in TSS values depending on the coating type until 15 days of storage ($p < 0.05$). The rate and extent of increase in TSS values followed the trend uncoated control >1CS:1G > 7CS:2G:1HA > 6CS:2G:2HA > 5CS:2G:3HA. The control uncoated fruits reached the maximum TSS of 16.67 ± 0.07°Brix (day 6). The observed increase in TSS in all coatings could be postulated to a combination of natural fruit ripening processes and hydrolysis of polysaccharides until day 6. The lower rate and extent of TSS increase until day 6 in coated fruits could also be attributed to the prevention of hydrolysis of innate polysaccharides compared to uncoated control. Although polysaccharides (chitosan and hyaluronic acid) present in polysaccharide-based coatings used in this study (1CS:1G, 7CS:2G:1HA, 6CS:2G:2HA and 5CS:2G:3HA) can contribute to the observed reduction in the increase of TSS values in coated fruits.

After 6 days of storage, in the case of uncoated control fruits, a significant almost linear reduction in TSS was observed reaching the lowest value of 8.40 ± 0.08°Brix at day 15 of storage (Fig. 7). In the case of coated fruits, the reduction of TSS was more gradual where a relatively greater reduction (in TSS) was observed in 1CS:1G, 7CS:2G:1HA coated samples. Furthermore, TSS values only dropped below the starting value (TSS at day 0) after ~10 days of storage in 1CS:1G, 7CS:2G:1HA coated samples and at ~13 days of storage in 6CS:2G:2HA. At day 15, 1CS:1G coated fruits exhibited lower TSS (10.38 ± 0.04°Brix) than 7CS:2G:1HA coated fruits (11.89 ± 0.10°Brix) indicating the protective role of hyaluronic acid in these coatings. Notably, marginal change was observed in 5CS:2G:3HA coated strawberries over the entire 15 days storage period (14.42 ± 0.06°Brix at day 0 to 14.86 ± 0.04°Brix at day 15). This protective role of hyaluronic acid can also be deduced from the values of TSS measured at day 15 which exhibit direct correlation (greater amount of TSS with increasing amount of hyaluronic acid in edible coatings – 5CS:2G:3HA (14.86 ± 0.04°Brix) > 6CS:2G:2HA (13.73 ± 0.03°Brix) > 7CS:2G:1HA (11.89 ± 0.10°Brix)). The initial increase in TSS values until day 6 of storage could be attributed to the fruit getting mature during storage, while the subsequent reduction in TSS values (after day 6) can be explained by the fruit decay. Taken together, TSS analysis indicated that the hyaluronic acid containing edible coatings maintained fruit freshness over the 15 days study period due to potential reduction in hydrolysis of polysaccharides and fruit maturation. Similar initially increasing and subsequently reducing TSS trend has been reported previously in short hot water treated cucumbers. [37]

3.2.5. Total phenolic content (TPC)

The TPC is indicative of antioxidant activity mediated by the phenolic compounds with redox and free radical scavenging properties found in fruit constituents. Berries including strawberries contain natural antioxidants including flavonoids and ascorbic acid (vitamin C). These natural antioxidants (flavonoids and polyols) function through the glutathione pathway. [38] TPC tend to decrease with storage time which could also contribute to the decay of strawberries over time. [39] We observed a reduction in TPC under all conditions both uncoated and coated strawberries over the 15 days storage period (Fig. 8), although the rate of TPC reduction was significantly lower in coated fruits compared to uncoated control counterparts. In the case of coated fruits, 1CS:1G coated fruits exhibited the most rapid decline in TPC content compared to hyaluronic acid containing coatings (7CS:2G:1HA,

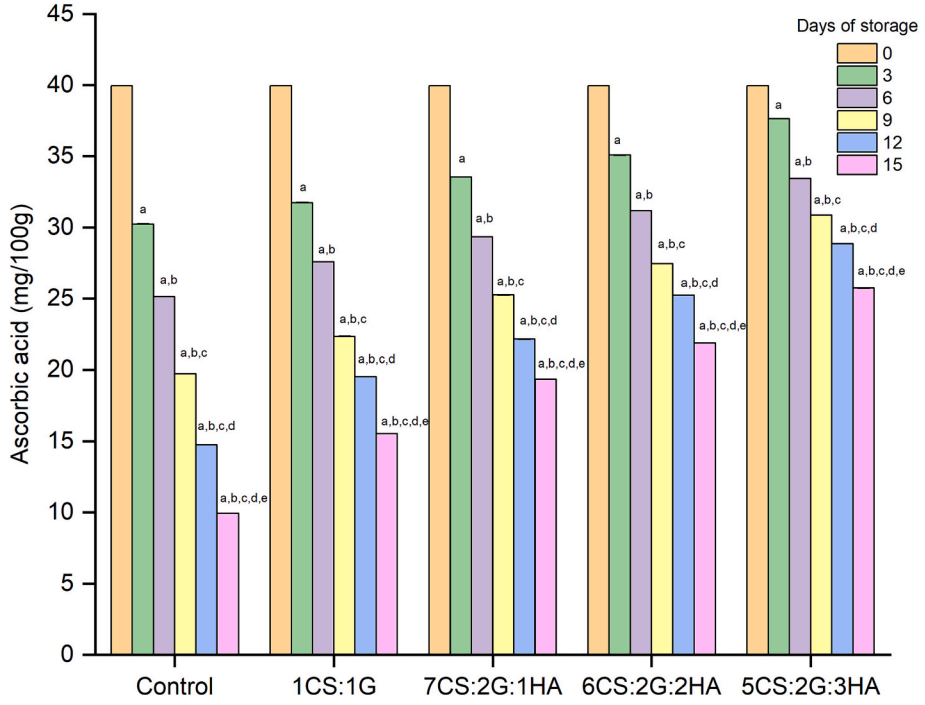

Fig. 9. Effect of edible coatings on ascorbic acid content of strawberries over different storage times. CS stands for chitosan, G for gelatin and HA for hyaluronic acid. All ratios are weight ratios between the different components used to prepare coatings. Data are presented as mean ± SD ($n = 3$) (error bars are significantly smaller than the data points). Values with different letters on the top of bars are significantly different ($p < 0.05$) and determined by using a Bonferroni *posthoc* test in a one-way ANOVA analysis—a, b, c, d, e are relative to coated fruits at day 0, 3, 6, 9, 12 and 15 days of storage, respectively.

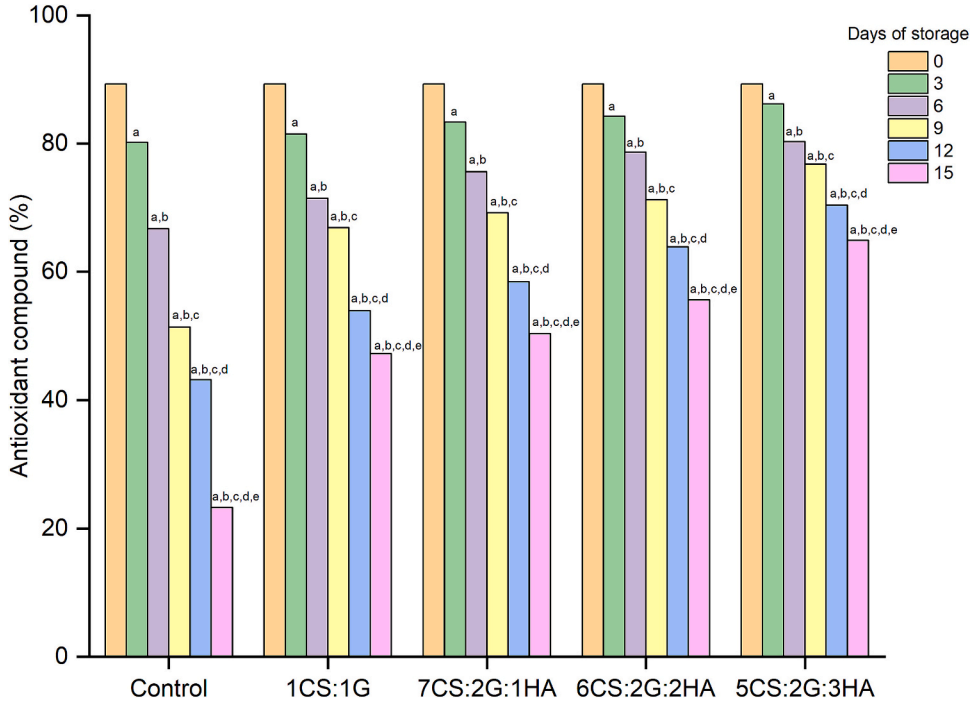

Fig. 10. The total antioxidant (DPPH) activity of different coatings. CS stands for chitosan, G for gelatin and HA for hyaluronic acid. All ratios are weight ratios between the different components used to prepare coatings. Data are presented as mean ± SD ($n = 3$) (error bars are significantly smaller than the data points). Values with different letters on the top of bars are significantly different ($p < 0.05$) and determined by using a Bonferroni *posthoc* test in a one-way ANOVA analysis—a, b, c, d, e are relative to coated fruits at day 0, 3, 6, 9, 12 and 15 days of storage, respectively.

6CS:2G:2HA and 5CS:2G:3HA). Furthermore, in the case of hyaluronic acid containing coatings, we observed an inverse linear correlation between the hyaluronic acid amount and reduction in TPC amount with storage time while the lowest reduction in TPC was observed in 5CS:2G:3HA (Fig. 8). The obtained indicate the protective role of hyaluronic acid in edible coatings.

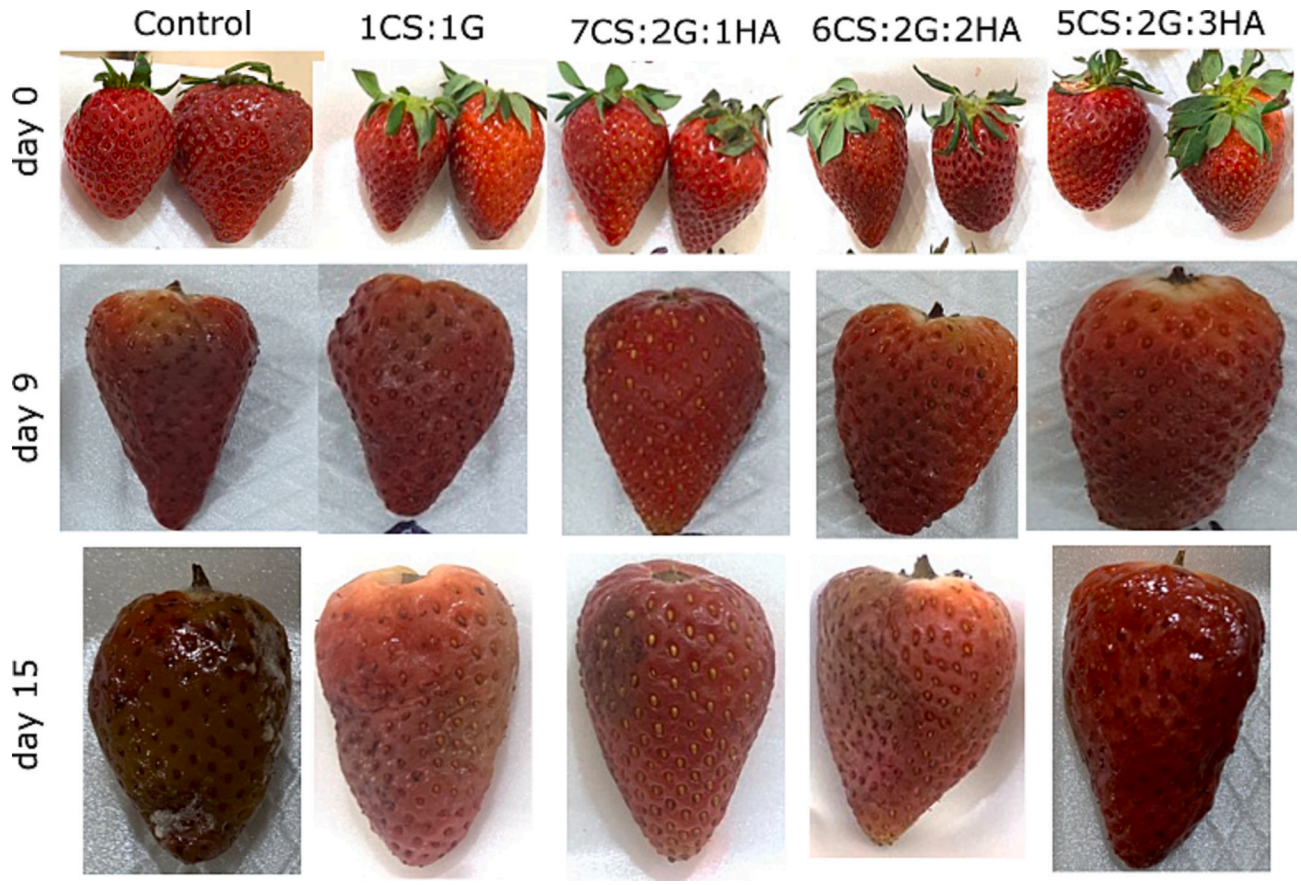

Fig. 11. The representative optical images of uncoated control and coated strawberries at different time points (0, 9 and 15 days after coating). White mold and brown discoloration indicate fruit damage. CS stands for chitosan, G for gelatin and HA for hyaluronic acid. All ratios are weight ratios between the different components used to prepare coatings. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2.6. Amount of ascorbic acid

The ascorbic acid (vitamin C) is a natural antioxidant [40] which is also found in strawberries. Ascorbic acid content tends to reduce during storage. [5] We also observed a reduction in ascorbic acid amount in both uncoated and coated strawberries in this study (Fig. 9). The uncoated fruits exhibited rapid and most significant reduction in ascorbic acid amount reaching the lowest value of 9.9 ± 0.04 mg/100 g after 15 days of storage compared to the coated counterparts. In the case of coated fruits, hyaluronic acid containing coatings (7CS:2G:1HA, 6CS:2G:2HA and 5CS:2G:3HA) performed significantly better than the 1CS:1G coating control in preserving the rate of decline of ascorbic acid. In the case of hyaluronic acid containing coatings, we observed a hyaluronic acid concentration dependent response in terms of preventing the loss of ascorbic acid from coated strawberries. The reduction in ascorbic acid was lowest in 5CS:2G:3HA coated strawberries. Similar to TPC analysis, ascorbic acid data highlight the preservation properties of hyaluronic acid in edible coatings.

3.2.7. Antioxidant capacity DPPH radical scavenging activity assay

To further corroborate the TPC and ascorbic acid analysis of edible coatings, we conducted the well-established DPPH assay to determine the overall antioxidant capacity of fabricated coatings. In a simple.

colorimetric method, DPPH (2,2′-diphenyl-1-picrylhydrazyl radical) assay is used to determine the free radical scavenging potential of a material. [22,41,42] In a typical DPPH assay, antioxidant species in the sample react with DPPH radicals causing a change in color from violet to yellow. The quantification of the intensity of the changed color is then used to quantitate the antioxidant efficiency of the test sample. Fig. 10 shows the DPPS activity of different coatings over the 15 days storage period. Similar to other antioxidant assays (TPC and ascorbic acid), drastic and significant reduction was observed in antioxidant amounts in uncoated strawberries compared to coated fruits. Further, in coated samples, hyaluronic acid containing coatings (7CS:2G:1HA, 6CS:2G:2HA and 5CS:2G:3HA) performed significantly better than the 1CS:1G coating control which could be explained by the strong intrinsic antioxidant properties of hyaluronic acid [26,27]. Also, hyaluronic acid concentration dependent response in terms of preventing loss of antioxidant activity during storage was obtained for hyaluronic acid containing coatings. The superior performance of 1CS:1G coating over uncoated control can be attributed to the intrinsic free-radical scavenging property of chitosan present in the edible coating. Taken together, it can be concluded that the additive effect of the two naturally antioxidant materials (chitosan and hyaluronic acid) impart hyaluronic acid containing coatings (7CS:2G:1HA, 6CS:2G:2HA and 5CS:2G:3HA) highly protective towards fruit maturation and decay compared to controls (1CS:1G and uncoated fruits).

3.2.8. Images of coated fruits

The change in the physical appearance of coated fruits is shown in Fig. 11. It can be seen that fruit quality diminished during the storage period with control fruit starting to decay by day 9 and showing signs of full decay with widespread mold growth observed on day 15 of storage. Comparatively, in the case of best performing 5CS:2G:3HA coating, coated fruits show low to no sign of mold growth even after 15 days of storage indicating the preservation quality of the coating.

4. Conclusions

The globalisation of the food industry has led to the shipment of fruits and vegetables from farms to plates across continents. Even within counties most time there is a considerable transportation time needed to transfer fresh produce from farms to markets. Different strategies have been employed and developed over the years to prolong the shelf life of perishable food items in particular fresh fruits. One of the key strategies is the use of edible coatings. In this study, composite natural polysaccharide and protein-based coatings were developed and explored as edible coatings for the preservation of strawberries over a 15 day storage period. The objective of this study was to explore a natural antioxidant polysaccharide, hyaluronic acid to prepare composite coatings with a hypothesis that inclusion of a natural antioxidant will preserve the coated fruits against decay during storage and prolong their shelf-life. From analytical characterisation using FTIR we determined the presence of hyaluronic acid in developed coatings. Furthermore, the inclusion of hyaluronic acid in composite coatings caused no detrimental impact on the thermal stability and consistency of produced edible coatings as adjudged from TGA, and optical and electron imaging. We observed significant improvements in coating performance in terms of weight loss, changes in pH, titratable acidity and total soluble solids towards extending the shelf-life of strawberries with the inclusion of hyaluronic acid in coatings. Compared to uncoated fruit control, coated fruits maintained their properties with extended shelf-life whereas for the best coating (5CS:2G:3HA) weight loss was only ~2 % compared to over 8 % in uncoated control after 15 days of storage. Similarly, the change in pH was only 0.55 compared to 2, titratable acidity was ~0.7 compared to 1.12, and total soluble solids was ~0.44°Brix compared to ~6°Brix between 5CS:2G:3HA (best performing coating) and uncoated control. Comparison between different coatings revealed hyaluronic acid amount dependence on coating performance with minimal weight loss, change in pH, titratable acidity and total phenolic content observed for best performing 5CS:2G:3HA coatings (containing the highest amount of hyaluronic acid). Finally, the inclusion of hyaluronic acid also significantly improved the antioxidant performance of edible coatings in a dose dependent manner as measured using total phenolic content, ascorbic acid amount and DPPH assay. Overall, we found that the inclusion of an antioxidant component can significantly improve edible coating performance and extend the shelf-life of perishable fruits such as strawberries. We envision further exploration of this strategy in using hyaluronic acid towards the development of commercial edible coatings.

CRediT authorship contribution statement

Sawsan A. Al-Hilifi: Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Rawdah M. Al-Ali:** Writing – review & editing, Validation, Investigation, Formal analysis. **Le N.M. Dinh:** Writing – review & editing, Validation, Investigation, Formal analysis. **Yin Yao:** Writing – review & editing, Validation, Resources, Investigation, Formal analysis. **Vipul Agarwal:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

Vipul Agarwal reports financial support was provided by National Health and Medical Research Council. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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