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Original Article

Development of chitosan edible coatings incorporated with clove essential oil nanoemulsions and its effect on shelf life of fresh-cut mangoes

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Abstract

A novel approach for the preservation of freshly cut mangoes is proposed using clove essential oil (CEO) nanoemulsions incorporated edible coatings. CEO nanoemulsions were fabricated with CEO in the range of 1 to 2.5% (v/v), while the surfactant concentration was fixed at 5% (v/v). The droplet size of prepared nanoemulsions was found in the range of 77.54 to 210.2 nm after size reduction by ultrasonication. CEO nanoemulsions showed a better antimicrobial activity against *E. coli* and *P. aeruginosa* in comparison to free oil. Chitosan films loaded with CEO nanoemulsions showed less tensile strength while maintaining elongation. Shelf life studies of freshly cut mangoes with edible coatings was conducted and changes in TSS content, titratable acidity and weight loss was determined during 15 days of storage at 4 °C. Sensory evaluation of the coated fruits on a five-point hedonic scale showed scores above three, suggesting overall acceptability of quality.

Keywords: essential oil, clove, nanoemulsions, edible coatings, edible films

1. Introduction

Due to the advent of fast paced lifestyle and increasing consumer awareness towards fresh and healthy food, the market of convenient ready-to-use fresh fruits and vegetables have grown rapidly (Ali, Yeoh, Forney, & Siddiqui, 2018). Minimal processing of fruits involves simple unit operations like sorting, washing, peeling, deseeding, cutting, etc. However, minimal processing of fruits leads to removal of their natural outer protective layers, exposing them to various physical, chemical and biological stresses (Finnegan & O'Beirne, 2015). Along with the nutritional and sensory aspects, people are also conscious about the safety of the food they consume.

The use of biodegradable environment friendly packaging is sought due to the growing burden on environment and increasing outreach for green packaging solutions. Functional edible packaging films incorporated with preservatives can counter all these problems for fresh-cut fruits (Yousuf, Qadri, & Srivastava, 2018). In edible coatings (ECs), a thin layer of food grade, non-toxic and biodegradable polymer (polysaccharide, protein, and lipid) is used to coat the fruit. This thin layer acts as a barrier to moisture, gases and microbes, thus enhancing the shelf life of the product and also maintaining its texture and physicochemical properties (Dhall, 2013). Chitosan has been widely used to coat fresh produce ranging from meat and poultry, fish and seafood to fruits and vegetables (Mujtaba *et al.*, 2019) due to its excellent gelling properties, emulsification ability and inherent bacteriostatic properties (Casariego *et al.*, 2008).

Nowadays people are focusing more about using natural ingredients in their food and thus becoming little careful towards the use of chemical preservatives. Edible films (EFs) can carry functional ingredients like natural antimicrobial agents to enhance shelf life of foods. Of late essential oils (EOs) have received substantial attention as potent antimicrobial agents. Eugenol, a major component of several EOs (clove, nutmeg and cinnamon) has antimicrobial

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activity against wide range of food borne microorganism (Hu, Zhou, & Wei, 2018). However, the incorporation of hydrophobic compounds like EOs having poor solubility in EFs is a difficult task. Despite of having excellent bioactive properties, the use of EOs in food systems as well as food packaging is not widespread due to their high volatility and thermal decomposition (Weiss, Gaysinsky, Davidson, & McClements, 2009). EOs needs to be encapsulated in some form of carrier in order to retain their properties and reduce their impact on the organoleptic properties of the food in contact.

Food grade nanoemulsions are emerging as a potential tool to encapsulate lipophilic bioactives. Nanoemulsions are true emulsion with mean droplet size ranging from 20 to 200 nm (McClements, 2011). Nano-emulsification of EOs prevents their potential degradation and provides increased bioavailability (Silva, Cerqueira, & Vicente, 2012). Keeping this in mind, the present study was aimed to incorporate different concentrations of clove essential oil nanoemulsion (CEO-N) in chitosan based ECs, and also to study the effect of clove essential oil (CEO) loaded ECs on physicochemical and sensory qualities of fresh cut mangoes during storage.

2. Material and Methods

2.1 Materials

Fresh ripe mango fruit (*Mangifera indica*) of the “Alphonso” variety were procured from a local market of South Delhi. Chitosan (98% deacetylation), Tween 80, PEG 400, acetic acid, glycerol and CEO were procured from a local distributor of Sigma-Aldrich, USA.

2.2 Preparation and characterization of nanoemulsions

CEO-N was formulated according to a pre-established method (Ghosh, Saranya, Mukherjee, & Chandrasekaran, 2013) with slight modifications. The primary coarse emulsions (O/W) were prepared using a magnetic stirrer. First step was the blending of the surfactant mix of Tween 80 and PEG 400 (in the ratio of 1:5 to comply with FDA permissible limits of Tween 80≤1% and PEG 400 is generally regarded as safe compound as food additives) with the CEO, followed by its addition to Milli-Q water drop wise with the help of a syringe. Four formulations were made with 1%, 1.5%, 2% and 2.5% of CEO, and 5% of surfactant mix. Next, the coarse emulsions were subjected to ultrasonic emulsification using a 20 kHz sonicator (LABMAN probe sonicator, India) with a maximum power output of 650 W. The energy input was provided through a 6 mm-diameter probe, with amplitude of 40% and 10 s pulse on and 10 s pulse off cycles for 5 min. The temperature was controlled by placing the sample container in a beaker filled with ice while ultrasonication.

The mean diameter and polydispersity index (PdI) of the nanoemulsions were determined using dynamic light scattering (DLS) technique employing a Zetasizer Nano ZS (Malvern instruments, Worcestershire, UK). All the samples were diluted 100 folds with distilled water before analysis to reduce the effect of multiple light scattering. The measurements were carried out at 25 °C with an equilibration time of 60 s.

The lowest size CEO-N was selected for antibacterial studies as a test formulation. The antibacterial assay was conducted by agar well diffusion method against two bacterial species, *E. coli and P. aeruginosa*, which are considered pathogenic for humans and infect fresh-cut produce on storage. 0.25 ml of test strains (10^6 CFU/ml) was as spread on to a nutrient agar plate. The solutions 100 µl each of CEO-N, free CEO and gentamicin (positive control) were introduced into each well (5 mm) of the plates. The plates were left for diffusion for one hour and then incubated at 37 °C for 24 hrs, then the diameter of inhibition zones (mm) were measured after 24 hrs and 72 hrs.

2.3 Preparation of coating solution and edible films

The chitosan coating solution was prepared with 2% (w/v) chitosan in 1% acetic acid. In this process, chitosan was blended with water on a hotplate magnetic stirrer at 40 °C for 10 min until it becomes transparent. Afterwards, glycerol (0.75 ml/g of chitosan) was added to the chitosan solution as a plasticizer. Thereafter, the CEO (concentration of 1.5%) nanoemulsion was added to the prepared chitosan solution, and the solution was stirred for 30 min to form a transparent solution. Following that, the coating solutions were subjected to ultrasonic emulsification using a 20 kHz probe sonicator with a maximum power output of 650 W. The energy input was provided through a 6 mm-diameter probe with amplitude of 40% and 15 s pulse on and 45s pulse off cycles for 6 min. The temperature was controlled by placing the sample container in a beaker with ice.

The coating solutions (30 ml) were cast in glass petri plates with dimension of 90 mm x 12 mm and then dried for 72 hrs in a hot air oven at 40 °C to prepare the films. Dried films (Ch-CEO-N films) were then peeled and stored in a desiccator (containing saturated magnesium nitrate solution) at 25°C and 75% relative humidity for conditioning until evaluation. The control film (Chitosan film) was made using the same procedure but without adding the CEO-N.

2.4 Characterization of edible films

The thickness, moisture content, solubility, swelling power, tensile strength (σ) and percentage of elongation at break (EAB) of the films were determined according to the method reported by Sun *et al.* (2017).

2.5 Treatment of fresh cut mangoes

Alphonso mangoes were brought from a local market. Purchase was made on visual and color characteristics (70–80% skin yellowness, ¾ ripe). The fruits were washed with tap water, and then with chlorinated water (100 ppm) for 5 min. The mangoes were peeled, destoned and cut into cubes of 2 cm x 2 cm x 2 cm size roughly. All the cut slices were mixed thoroughly in order to obtain a random distribution of fruit samples. Fresh cut fruits were then immersed in the coating solution, dripped on a wire mesh and allowed to dry for some time to obtain uniformly coated samples. Uncoated samples (mango slices without coating) were used as control.

2.6 Storage study of coated and uncoated mango slices

A batch of coated and uncoated samples was stored at 4 °C for 15 days. Various properties of both the batches were estimated at interval of 5 days starting from day 0 till day 15.

2.6.1 Weight loss study

For determining the weight loss, four mango slices in each replication for each treatment were marked before storage, and weighed using a digital balance. The same slices were weighed at the beginning of the experiment and at the end of each storage period. The results were expressed as the percentage loss of initial weight and calculated by the formula:

$$WL\ (\%) = \frac{W_i - W_f}{W_i} \times 100$$

where WL (%) is percentage weight loss, W_i is initial weight (g) of the sample, and W_f is final weight (g) of the sample

2.6.2 Total soluble solids and titratable acidity

Mango slices were blended and filtered through a muslin cloth. The resulting liquid was used for determination of TSS using Abbe's hand refractometer and titratable acidity (TA) following the method of Islam *et al.* (2013).

2.6.3 Sensory analysis

Ten semi trained panelists from Department of Food Technology, Jamia Hamdard evaluated the quality attributes of uncoated and coated mango slices. The evaluation was done in four phases, once on the first day after application of coating and then after 5, 10 and 15 days of storage at 4 °C. Attributes considered in the sensory evaluation were appearance, odor, and texture. A preference test based on five-point hedonic scale, where 5 = like extremely and 1 = dislike extremely was conducted. A score of three was taken as the lower limit of acceptability.

2.7 Statistical analysis

The data presented in the Table 3 were subjected to two sample t-test using Microsoft Excel. The data presented in Table 1, 2 and 4, and Figure 4 was subjected to one-way ANOVA (Analysis of Variance) using IBM SPSS Statistics, Version 20. The data were taken in three replicates for the analysis.

3. Results and Discussion

3.1 Characterization of nanoemulsions

Coarse emulsions appeared opaque and milky white, due to mean particle diameter greater than the wavelength of visible light (McClements, 2011). However, the nanoemulsions (Figure 1) showed homogeneous and fluid characteristic with a slightly bluish-white color, which can be caused by the Tyndall effect, distinctive for nanoemulsions. The combination of Tween 80 and PEG 400 yielded fairly transparent emulsions due to the hydrophilic nature of the cosurfactant. This observation is in consonance with results described for curcumin nanoemulsion (Ahmad *et al.*, 2020) and eugenol nanoemulsions (Ahmad *et al.*, 2018) prepared with the same combination of surfactants.

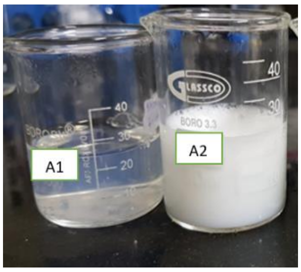

Figure 1. Coarse and nanoemulsions (O/W) of 1.5% clove essential oil (CEO). A2: Coarse emulsion; and A1: nanoemulsion after ultrasonication

Three out of four formulations were well within the nanometric range of <200 nm (Table 1; Figure 2). The smallest particle size (77.54 nm) was obtained in nanoemulsions with 1.5% of CEO, whereas largest droplet size (210.2 nm) occurred in nanoemulsions with 2.5% CEO. Wan *et al.*, (2020) prepared nanoemulsions using 1.5% clove oil, stabilized by 1% Tween 80, and obtained an average droplet diameter of 130.95±5.40 nm. CEO nanoemulsions with reduced droplet size were obtained at same concentration by modulating the surfactant-oil ratio. Previous studies also mentioned that oil-surfactant ratios with less proportion of oil and higher proportions of surfactant yielded good particle size distribution (Zhang, Zhang, Fang, & Liu, 2017). These small droplet diameters were achieved due to the presence of only liquid lipids in the formulations that were easily susceptible to size reduction on ultrasonication (Leong, Wooster, Kentish, & Ashokkumar, 2009). PDI is the indicator of the droplet stability and homogeneity in nanoemulsion values below 0.2 are acceptable for polymer-based nanoparticles (Danaei *et al.*, 2018). All the formulations showed a PDI ≤0.22 and single narrow peaks implying that, the nanoemulsion formed had uniformly size distributed droplets.

Table 1. Droplet diameters and polydispersity index (PdI) of nanoemulsions measured by Zetasizer Nano ZS

CEO (%)	Surfactant (%)	Z – Average (nm)	PdI
1 %	5 %	161.4 ± 0.35^b	0.220 ± 0.011^d
1.5 %	5 %	77.54 ± 0.10^a	0.193 ± 0.003^c
2 %	5 %	171.7 ± 0.70^c	0.082 ± 0.004^a
2.5 %	5 %	210.2 ± 0.85^d	0.143 ± 0.004^b

Values are represented as mean±standard deviation. Values followed by similar superscript letter do not differ significantly (p<0.05).

3.2 Antimicrobial activity

The nanoemulsion formulation with 1.5% CEO was selected for antibacterial testing. The antimicrobial assay revealed that nanoemulsions showed good inhibition zones

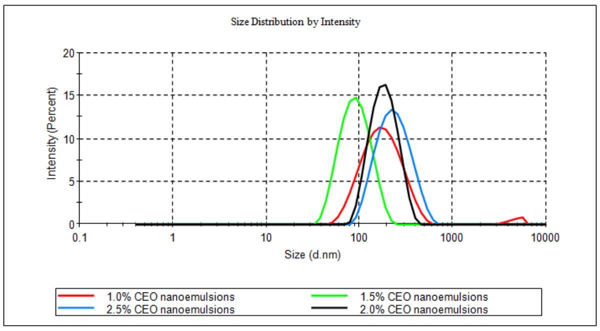

Figure 2. Size distribution by intensity of clove essential oil (CEO) nanoemulsions using Zetasizer Nano ZS

against all bacterial strains investigated (Table 2). It was observed that the inhibition zone (mm) of CEO-N was larger than that of free CEO against both the bacterial strains. Anwer, Jamil, Ibnouf, & Shakeel (2014) compared the antibacterial activity of clove oil nanoemulsion and free clove oil against a group of Gram positive bacteria and Gram negative bacteria (*Bacillus subtilis, Proteus vulgaris, Staphylococus aureus, Klebsiella pneumoniae and Pseudomans aeruginosa*). Free clove oil presented a higher MIC than the nanoemulsion for both gram-positive and negative bacteria, indicating that the nanoemulsification is able to increase the antimicrobial potential of clove oil (de Meneses *et al.*, 2019). However, the inhibition zones of nanoemulsions were smaller as compared to that of positive control (gentamicin). The inhibition zones of the nanoemulsion for *E. coli* increased significantly ($p<0.5$) after 72 hrs suggesting delayed release of EOs from the emulsion. Comparable behavior was cited in a study on oregano oil nanoemulsion against *L. monocytogenes.* Nanoemulsion lead to 2.83 log reduction after 3 hrs and the reduction continued by 3.05 and 3.44 logs after 24 hrs and 72 hrs, respectively (Bhargava, Conti, da Rocha, & Zhang, 2015). Similar observations were made for nanoencapsulation of thyme oil (Zhang *et al.*, 2016) and eugenol (Sharifimehr, Soltanizadeh, & Hossein Goli, 2019).

3.3 Physical properties of the films

Film thickness, density, moisture content, swelling power and solubility are the properties significant to understand the efficiency of a film as a barrier. The thickness of the chitosan films increased from 0.08±0.02 mm to 0.11±0.01 mm on addition of CEO-N. However, it can be called a stretch film, as ASTM D883 specifically defines *"film"* as having a nominal thickness of ≤ 0.25 mm. As the solute concentration increased in the film forming solution due to the CEO-N, it increased the dry matter content of the film and hence the increased thickness. Similar results for film thickness were found in the literature (Acevedo-Fani, Salvia-Trujillo, Rojas-Graü, & Martín-Belloso, 2015; Otoni *et al.*, 2016). Density of the film also decreased slightly on incorporation of CEO-N. The moisture content of chitosan films decreased on addition of nanoemulsions. Similar results were reported in the literature that the moisture may decrease due to a direct effect of hydrophobicity of essential oils on the ability of film to retain water (Ghasemlou *et al.*, 2013). Swelling power and solubility are important characteristics for food packaging films because they can affect resistance of film to moisture, especially in humid environments. Both swelling power and solubility of the chitosan films containing nanoemulsions were found to be less than the control films (Table 3). However, the difference was not very large because the hydrophobic groups causing the lowering of solubility and swelling power were encapsulated in the nanoemulsions.

Table 3. Physical and mechanical properties of chitosan clove essential oil nanoemulsion (Ch-CEO NE) films and chitosan (Ch) films as control

Properties	Ch film	Ch-CEO NE film
Thickness (mm)	0.08±0.02^{a}	0.11±0.01^{a}
Density (g/cm^{3})	1.54±0.01^{b}	1.46±0.01^{a}
Moisture content	16.80 ± 0.17^{b}	13.20±0.23^{a}
Solubility index (%)	21.80±0.57^{b}	19.80± 0.52^{a}
Swelling power (%)	236.30± 0.72^{b}	232.60±0.86^{a}
Tensile Strength (MPa)	19.22±1.37^{b}	15.44 ±1.48^{a}
Elongation at Break (%)	12.16± 2.21^{a}	18.73±1.16^{b}

Values are represented as mean±standard deviation. Values followed by similar superscript letter do not differ significantly ($p<0.05$).

3.4 Mechanical properties of the films

Tensile strength (TS) and elongation are parameters that link mechanical properties of films to their chemical organization. The tensile strength of the Ch-CEO-N films was found to be 15.44 ±1.48 MPa. The addition of CEO nanoemulsion to the film reduced its tensile strength (Table 3). Similar results are reported regarding chitosan films suggesting that incorporation of additives lowers TS values if they do not have polymer cross linking property, as it reduces cohesiveness between the chitosan molecules (Cagri, Ustunol,

Table 2. Antimicrobial activity (zone of inhibition) of clove essential oil (CEO) nanoemulsion, free clove essential oil and gentamicin (control)

Sample	Zone of Inhibition (mm)			
	E. coli		*P. aeruginosa*	
	24 hrs	72 hrs	24 hrs	72 hrs
CEO nanoemulsion	16.40±0.21^{b}	17.10±0.23^{b}	16.30±0.30^{b}	16.60±0.35^{b}
Free CEO	15.10±0.10^{a}	15.30±0.17^{a}	13.10±0.25^{a}	13.40±0.10^{a}
Gentamicin	22.00±0.10^{c}	22.00±0.10^{c}	25.20±0.20^{c}	25.20±0.20^{c}

Values are represented as mean±standard deviation. Values followed by similar superscript letter do not differ significantly ($p<0.05$).

& Ryser, 2004). However, the percentage elongation at break (EAB) significantly increased from 12.16±2.21% to 18.73±1.16% on addition of nanoemulsified clove oil. These effects are seen because nanoemulsified oils act as plasticizers reducing the film resistance while maintaining extensibility as suggested by studies on chitosan film containing nanoemulsified cinnamaldehyde (Chen *et al.*, 2016) and chitosan-gelatin films encapsulating various nanoemulsified bioactives (Pérez-Córdoba *et al.*, 2018).

3.5 Storage studies of coated fresh-cut mangoes

3.5.1 Percentage weight loss

Weight loss is a major determinant of storage life and quality of fresh cut fruits. The weight loss increased throughout the storage period in coated and uncoated fruits (Table 4, Figure 3). Water content of fruits post-harvest and post processing, is a significant parameter to assess their quality. Moisture losses lead to change in color, turgidity and firmness of fruits (Nunes & Emond, 2007). The Ch-CEO nanoemulsion coating limited the fruit weight loss compared to uncoated fruit, and a better effect on delaying the weight loss of fresh cut mangoes during the 15 days of storage thus maintaining their firmness and preventing shrinkage in the fruit slices attributing to better quality. At the end of the storage period (15th day) the weight loss in coated samples and uncoated samples was 10.27±0.50% and 19.86±0.85%, respectively. This stark difference can also be attributed to the nanoemulsified essential oil present in the edible coatings, which acts as moisture barrier. Similar results of reduction in weight loss and softening of fruit were observed in strawberries coated with pullulan films containing cinnamon oil (Chu *et al.*, 2020) and alginate films embedded with eugenol-citral combination (Guerreiro *et al.*, 2015).

3.5.2 TSS and TA

TSS and TA (%) are significant parameters for judging organoleptic quality of fruits. The TSS and TA estimate the soluble sugar and organic acid contents of fruits, respectively. The TSS of uncoated fruit increased from 13.06±0.004 °Brix to 13.33±0.004 °Brix with increasing storage time. This increase could be attributed to the decrease in respiration rate, deconstructed cell wall and the increase in dry matter due to water loss, as apparent in climacteric fruits like mangoes. However, the increase was slight due to low temperature storage. Chitosan coated samples showed gradual decrease in TSS (13.10±0.007 °Brix to 12.45±0.004 °Brix) on 15th day of storage at 4 °C due to reduction in metabolic activity as combined effect from low temperature storage and functional edible coating (Radi, Akhavan-Darabi, Akhavan, & Amiri, 2018). TA showed a significant decrease throughout cold storage in fresh cut mangoes, with lower values in uncoated fruit compared to Ch-CEO nanoemulsion coated fruits (Table 4). The TA (%) of the uncoated fruits decreased on storage from 0.83% to 0.57 %, while in coated fruits the decrease was comparatively lower and went only up to 0.68% at 15th day of storage. Previous studies have suggested that the higher acidity loss in uncoated fruits might imply the use of organic acids as substrates for respiratory metabolism and energy production during storage (Dubey *et al.*, 2019).

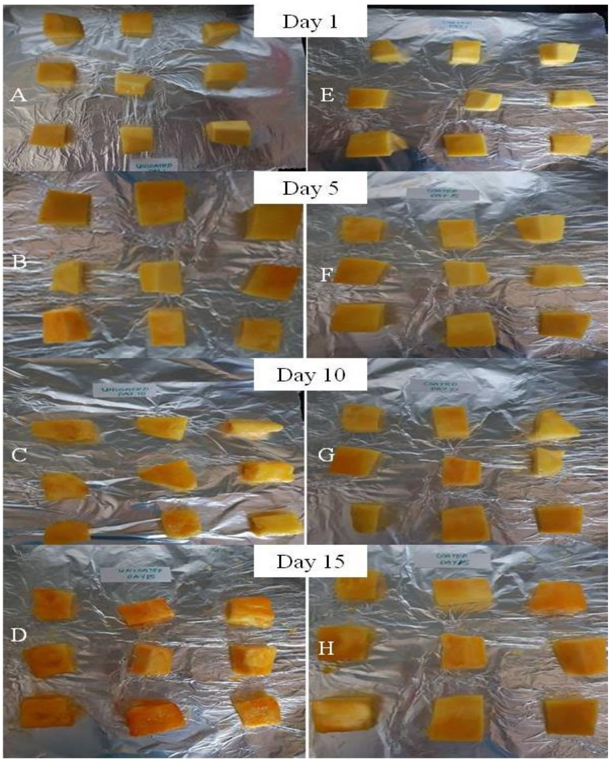

Figure 3. Visual appearance of the uncoated and coated mango pieces under different storage periods at 4 ºC. Uncoated (mango slices without any coating): A= Day 1, B=Day 5, C=Day 10 and D=Day 15; Coated (Mango slices coated with chitosan coating containing clove essential oil nanoemulsion): E= Day 1, F=Day 5, G=Day 10, and H=Day.

Table 4. Total soluble solids (TSS), titratable acidity (TA) and weight loss of the mango slices over a storage period of 15 days at 4 ºC

Storage	TSS (°Brix)		TA (%)		Weight Loss (%)	
	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated
Day 1	13.06±0.004^{a}	13.10±0.007^{c}	0.83±0.03^{d}	0.83±0.03^{c}	0	0
Day 5	13.16±0.004^{b}	13.12±0.005^{c}	0.78±0.02^{c}	0.81±0.03^{c}	7.95±0.31^{a}	5.92±0.29^{a}
Day 10	13.21±0.005^{c}	12.82±0.002^{b}	0.68±0.03^{b}	0.72±0.02^{b}	15.24±0.52^{b}	6.98±0.34^{a}
Day 15	13.33±0.004^{d}	12.45±0.004^{a}	0.57±0.03^{a}	0.68±0.03^{a}	19.86±0.85^{c}	10.27±0.50^{b}

Values are represented as mean±standard deviation. Values followed by similar superscript letter do not differ significantly ($p<0.05$). Uncoated: Mango slices without any coating; Coated: Mango slices coated with chitosan coating containing clove essential oil nanoemulsion.

3.5.3 Sensory evaluation

Sensory evaluation plays a major role in consumer acceptance of any product. In the study conducted, both the odor and texture sensory scores of mango slices fell quickly during storage. Ch-CEO nanoemulsion coatings delayed the drop in the quality, and extended the shelf life. Both the uncoated, as well as the coated mango fruit slices stored for five days were acceptable to the sensory panelists during the preference test. However, the uncoated fruit slices started deteriorating little before five days. The coated samples showed a lower score for odor on the first day, which can be attributed to the unfamiliar odor of chitosan and clove oil. When stored for more than five days, the control became unacceptable to the panelists, whereas the quality of the Ch-CEO nanoemulsion coated sliced fruit was retained. The edible coating on sliced mango improved its quality and prevented surface cracking and the leaking of juice (Figure 3). Overall, a score of three on a 5-point hedonic scale for the coated samples indicated acceptability by the sensory panelists.

4. Conclusions

Through this study, it was found that active chitosan films incorporated with nanoemulsified clove oil were effective in prolonging the shelf life of fresh cut mangoes stored at 4 °C for 15 days, while uncoated samples started deteriorating after five days of storage. Ultrasonic emulsification reduced the particles size to as low as 77.54 nm giving a very clear and freely flowing emulsion. The nanoencapsulation of clove oil showed significant improvement in its antimicrobial activity on *E. coli* and *P. aeruginosa* as depicted by the zone of inhibitions. Chitosan proved to be an excellent choice for the formation of edible films. Incorporation of nanoemulsions increased the extensibility of chitosan films but decreased the tensile strength of the film owing to its hydrophobic nature. CEO nanoemulsion changed the physical properties of the film like thickness, moisture content, swelling and solubility, but the changes were well within the range for the films to act efficiently as a protective coating.

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