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Effects of Aloe-pectin coatings and osmotic dehydration on storage stability of mango slices

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Abstract

Mango (*Mangifera indica* L.) is one of the most important tropical fruits with inimitable taste, unique flavor, fragrance, and therapeutic traits. It is the national fruit of Pakistan and is commonly called the king of fruits, however, it is very challenging to protect the keeping quality of the fruit. To enhance the shelf life and overcome the microbial count, edible biodegradable (aloe-pectin) coatings were applied on minimally processed (osmotically treated) fresh-cut mango slices. Pre-treatment of Osmotic Dehydration (OD) was performed at 45 °C for 3 hours by 55 °Brix solutions of sucrose and glucose, respectively. The coating solution was made by aloe vera gel with combination of 0.2% $CaCl_2$ (w/v) and 0.5% pectin (w/v). Total Fungal Count (TFC) (yeast and mold growth), Moisture Contents (MC), and Total Soluble Solids (TSS) of minimally processed (osmo-coated) mango slices were analyzed during 15 days of storage at 5 °C. A minimum increase in Total Fungal Count (TFC) from 1.00 to 2.53 Log CFU/g, TSS from 25.30 to 27.16 °Brix, and a decrease in moisture contents from 62.43 to 60.65% was observed in double-coated with osmo-sucrose treated (osmotic dehydration in 55˚Brx sucrose solution before coating) samples from 0 to 15 days respectively. However, significant changes in the TFC, moisture contents, and TSS, were observed in double-coated with osmo-glucose treated (osmotic dehydration in 55 °Brix glucose solution before coating) samples after 10 days of storage. According to the results of the current study, the aloe-pectin coating can significantly reduce the TFC, decrease the TSS, and minimize the moisture loss during storage, when used after osmo-sucrose pre-treatment.

Keywords: Mango; Sucrose; Glucose; Coating; Aloe vera; Preservation; Osmotic dehydration.

Highlights

- The mucilaginous gel from inner colorless *hydroparenchymatic* tissues of aloe vera leaf, calcium chloride (0.2% W/V), and pectin (0.5% W/V) was used in the formulation of the edible biodegradable coating
- Microbial growth could be suppressed by single and double-layered aloe-pectin coatings and pre-treatment of osmotic dehydration
- Shelf life of minimally processed fresh-cut mango slices in refrigeration storage can be enhanced by edible biodegradable (aloe-pectin) coatings and osmotic dehydration

1 Introduction

Mango (*Mangifera indica* L.) is an indispensable fruit crop in warm and humid areas of the world, with an estimated yield of 45 million tons (Food and Agriculture Organization Corporate Statistical Database, 2017). Pakistan ranked fifth position in mango production after India, China, Thailand, and Indonesia in the world (Food and Agriculture Organization of the United Nations, 2011). However, due to a lack of advanced processing and post-harvest practices, 30 to 40% of the mangoes are wasted annually; on the other hand, Pakistan contributes only 2.78% of share of global export (Khan, 2010; Azam et al., 2013). Various drying methods are used for mango pulp preservation for later uses in the home industry, but are less marketable due to faded color, undesirable texture, and cooked flavor (Rincon & Kerr, 2010; Torres et al., 2006).

Osmotic Dehydration (OD) has obtained substantial recognition in recent years, thus being a pre-treatment. It involves fruit and vegetable immersion in hypertonic solutions to slightly reduce water content and improve solid contents (Özkan-Karabacak et al., 2022). It is the best pre-treatment to develop intermediate moisture foods of the best quality and sensory properties (Gomez et al., 2022). In addition, it is also an economical process because it can be performed at ambient temperature and conserves the cellular integrity of fruit and vegetables, leading to better color, flavor, and nutrients (Heredia et al., 2012).

Edible coatings can be applied before OD to reduce the undesirable solid gain during osmotic dehydration (Rahman et al., 2020), and after OD to study the quality of the product during storage (Šuput et al., 2022). Edible coatings have the potential to extend shelf life by preventing the deterioration caused by cutting, slicing, and peeling of fruits, and act as semi-permeable membranes which reduce oxidation caused by gases exchange and lower moisture loss and provide a similar effect as modified atmospheric packaging, hence extend the keeping quality of fresh-cut fruits (Galus et al., 2020; Nicolau-Lapena et al., 2021).

Paidari et al. (2021) reviewed numerous types of coating materials such as polysaccharides, protein, fruits, vegetables, and essential oils, etc., and concluded that in near future artificial packaging is more likely to be replaced by edible coatings. To increase the shelf life of minimally processed fruits, nano-composite materials have a coating potential with germicidal effects (Lloret et al., 2012). The development of metal nano-particle conjugates with biopolymer-based coatings is also an emerging trend, to increase the physicochemical and permeability characteristics of coating materials (Jafarzadeh et al., 2021).

In recent years, the consumers' requirements for consumption of natural preservatives have increased, leading scientists to isolate and use new bioactive compounds from plant sources. These bioactive compounds prevent the enzymatic and non-enzymatic browning and microbial infestation owing to their antioxidant and antimicrobial properties. Carvacrol from *Origanum vulgare* L. and menthol from peppermint were used in polysaccharide-based coating and proved to be an effective antimicrobial coating on polyethylene film (Jahdkaran et al., 2021).

Due to the presence of numerous polysaccharides and functional compounds in aloe vera gel composition, it can be used as an edible coating for slightly processed fruits (Pugh et al., 2001; Suriati, 2022). Various analyses have been conducted on the utilization of aloe vera gel- based coatings on guava, blackberry, apple, white button mushroom, and strawberries (Arrubla Vélez et al., 2021; Kumar et al., 2018; Mirshekari et al.,

2019; Rehman et al., 2020; Suriati & Suardani, 2021). Edible nano-coating of aloe vera gel could effectively enhance the shelf life of fresh-cut mangosteen and mango (Suriati et al., 2020; Suriati et al., 2021). Aloe vera gel was proclaimed to efficiently kill or appreciably decrease or eradicate the proliferation of microbes such as *Staphylococcus aureus*, *Klebsiella pneumoniae, Streptococcus pyogenes, Pseudomonas aeruginosa, Escherichia coli, Propionibacterium acne, Helicobacter pylori,* and *Salmonella typhi* due to its component of pyrocatechol, cinnamic acid and p-coumaric acid (Lawrence et al., 2009; Ullah et al., 2016).

The present work was conducted to evaluate the outcome of osmo-pretreated, aloe-pectin coating (control, single and double layered) on the Total Fungal Count (TFC), moisture contents, and TSS of minimally processed fresh-cut mango slices through refrigeration storage (5ºC) of 15 days. Analyses were performed at intervals of 0, 5, 10, and 15 days.

2 Materials and methods

The current study was designed to estimate the keeping quality of mango slices under different treatment conditions. All the chemicals and reagents of analytical grade were purchased from the sole distributors of Merk. Mango variety of Black Chounsa (Late Chounsa) at harvest maturity stage was pulled off from the MRI Multan and carried to the laboratory in stilted cardboard boxes, kept under controlled environments for 5 days (60 ± 2 RH, 25 ± 2 ºC) and the mango fruits were sorted out visually for color, texture, and bruises. Aloe vera plants were taken from the National Herbarium of Pakistan. This research was accomplished in the microbiology laboratories of Pir Mehr Ali Shah Arid Agriculture University, and the National Agriculture Research Center Islamabad, in Pakistan.

2.1 Sample preparation

The mango fruits were cleansed with chlorinated (calcium hypochlorite 4 ppm) water to remove dust and dirt, then held under the fan to dry up unnecessary surface water. A sharp knife was used to manually peel and slice the fruits into approximately 0.8 ± 2 cm thickness, 7 ± 0.5 cm long, and 3 ± 0.5 cm wide. For blanching, the fresh cut mango slices were dipped in boiling water for 30 seconds and then immersed in ice cold water for 30 seconds to stop cooking.

2.2 Coating preparation

Coating preparation was carried out by the process described by Ramachandra & Rao (2008) with a few changes. Mature aloe vera leaves were harvested, washed, and peeled the base, tips, and spikes of the leaves. The skin was separated, and aloe vera flesh (*hydroparenchyma*) was obtained (Figure 1b). The flesh was then rinsed with distilled water and blanched by loading on perforated metal strainer and immersed in hot water (100 ºC) for 4 minutes, *hydroparenchyma* to water ratio was set at 1:6. The blanched colorless *hydroparenchyma* was stirred and the gel was obtained. To remove the anthraquinones, the gel was filtered through the active carbon paper. Pectin solution of 0.5% (W/V) with calcium chloride of 0.2% (W/V) was prepared in distilled water. Aloe vera gel (50%) + pectin solution was prepared for coating and named aloe-pectin coating solution. The aloe-pectin solution was then pasteurized at 85 ºC for one minute in bench mounted HTST pasteurizer (Armfield FT43 Laboratory pasteurizer).

2.3 Single layer coating

Mango slices were laden on a stainless-steel mesh screen, immersed for 2 minutes in the coating solution, then drained and put in a hot air oven or tray dryer (Armfield, Ringwood U.K) at 65 ºC with air velocity of 1.5m/s for 15 minutes to fix the coating layer. Process of coating and drying is shown in Figure 1.

2.4 Double layer coating

After the first coating was fixed, the process of immersion in aloe-pectin coating and drying (in a try dryer) was repeated in the same way as done for the single layered coating.

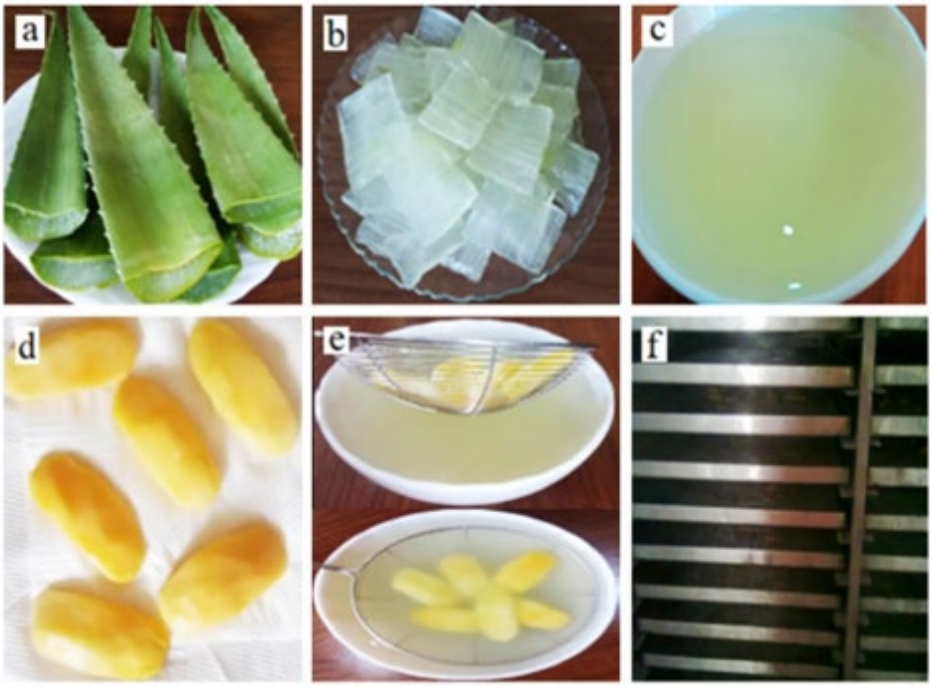

Figure 1. Photograph of aloe-vera leaves (a), inner parenchyma of aloe-vera leaves (b), aloe-pectin solution (c), osmotically treated mango slices (d), coating application (e), dehydration of coating in hot air oven (f).

2.5 Osmotic dehydration

The osmotic solutions of 55 °Brix glucose and sucrose were prepared by adding up citric acid (0.5%) and sodium metabisulphite (0.25%) in distilled water at 80 °C with continuous stirring. Mango samples were osmotically dried in glucose (55 °Brix) and sucrose (55 °Brix) solutions respectively, the ratio between the mass of mango samples to osmotic solvent was set at 1:20 to prevent the consequential thinness of the osmotic solution as a result of mass exchange phenomena of osmotic dehydration. Osmotic dehydration was conducted at 45 °C for 3 hours in separate stainless-steel bowls. After osmotic dehydration surplus osmotic solution from the surface of samples was gently rinsed with distilled water and superficial water was eliminated by ambient air current for one hour. The osmo-coated (osmotically dried and coated) and control mango slices were packed in polyethylene (high-density polyethylene) bags (16.51 cm x 14.9 cm) of 0.09 mm thickness and stored at refrigeration temperature. Figure 2 shows the flow diagram of OD and coating process. Samples were randomly analyzed for the TFC, moisture contents, and TSS for 0, 5, 10, and 15 days of intervals for 15 days.

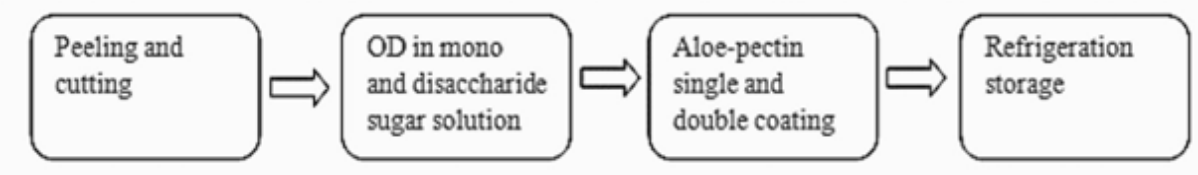

Figure 2. Flow diagram of osmo-coating process.

2.6 Microbial analysis

Minimally processed fresh-cut mango slices were investigated for *coliforms, salmonella spp*. by the serial dilution method. Serial dilution was made in the ratio of 1:10 and 1ml of each dilution and then this serial dilution was dropped into the plates of nutrient agar and incubation was conducted at 28-30˚C for 24 hours. The salmonella spp. and Coliforms were examined after osmo-coating (osmotic dehydration and coating) process to estimate the sanitary processing. Whereas the yeast and mold count were investigated by direct plate method as described by Downes & Ito (2001) throughout the storage with 0, 5, 10, and 15 days intervals.

2.7 Moisture contents

The moisture content of mango slices stored in refrigeration was calculated by oven drying technique at regular intervals of five days as designated by AOAC (Association of Official Analytical Chemists, 2000). Randomly selected mango slices from each treatment were weighed (5 grams) on electrical balance (Electronic Balance, FX-40, Canada). The samples were then put in a hot air oven at 105 ± 5 °C for successive dehydration till constant weight.

2.8 Total soluble solids

Total Soluble Solid (TSS) of mango slices were calculated by the method of AOAC (Association of Official Analytical Chemists, 2000). Composite juice was extracted from the mango slices to check on digital refractometer (PAL-3®, ATAGO Japan).

2.9 Statistical analysis

Results are shown in the mean standard deviation. Factorial design (2 x 4 experimental levels) was applied for the analysis of data, LSD is used for separation of mean significant values at $p \leq 0.05$ by using “Statistics 8.1”.

3 Results and discussion

Susceptibility of mango slices for TFC (yeast and mold count) was investigated for different treatments of coatings (control, single and double coated) at 5 °C for 15 days. According to our knowledge, concerning yeast and mold counts, there are no specific safety limits for the minimally processed fresh-cut fruits. However, the growth of these microbes is a worrying question because when they overreach the limit of Log 5 CFU/g, they can affect the product appearance and produce off odour due to sugar fermentation resulting in volatile esters, organic acids, and ethanol in sliced fruits (Rojas-Graü et al., 2007). Moreover, it was also reported that when yeast and mold exceed the limit of 10^6 CFU/g they may result in excreting the toxic materials, since this assessment is considered an acceptable limit during the storage study of edible products (Lee et al., 2003).

The propagation of microbes in the edible product is not only objectionable to consumers’ well-being but also declines the sensory acceptance of the food products. To assess the efficiency of hygienic practices of processing, the samples were analyzed for *E. coli* group and *Salmonella ssp.* directly after osmo-coating treatment. No evidence of *Salmonella spp* and *E. coli.* was reported in both without coating (control) and osmo-coated (osmotically dehydrated and coated) samples.

At the start of storage, the colony count of all the samples was very less as shown in Table 1, however, from the 5th day and onward the increasing trend in colony count was observed reaching a maximum of 6.23 Log CFU/g and Log 4.23 CFU/g in control osmo-glucose (OD in glucose) and osmo-sucrose (OD in sucrose) treatments respectively on 15th day.

The Result showed a significant difference between treatments means of control (without coating) and double coated samples. The TFC of 4.29 Log CFU/g and 8.92 Log CFU/g was observed in double coated and control osmo-glucose treated (OD in glucose) samples respectively, whereas 2.53 and 6.97 Log CFU/g was calculated in double coated and control osmo-sucrose treated samples. So, it may be inferred that the application of aloe-pectin coatings, even if not restrain the yeast and mold growth, may decrease them significantly (Table 1). Aloe vera gel-based coating when used in combination with an antioxidant and the gelling agent has been reported to improve the quality of fresh-cut kiwi fruit (Passafiume et al., 2020).

From the previous study, it is proved that aloe vera is effectual against the pathogenic fungi of plants (*Penicilliam digitatum, Botrytis cinerea, Alternaria alternate*). The antifungal activity of aloe vera gel is established by its ability to inhibit germination and suppress mycelial propagation, due to its additional functional constituents with antifungal activity (Valverde et al., 2005; Zapata et al., 2013).

An Increasing growth trend of Log CFU/g was seen in control samples from 0 to the 15th day, but colony count has not exceeded the acceptable limit till the tenth day of storage, because organic acid (citric acid, ascorbic acid) dip in food increases proton incorporation in food which results in increasing the acidity and lowering the pH (Oms-Oliu et al., 2010; Yang et al., 2019). In the present study, 0.5% citric acid was used in an osmotic solution (OS). Mango slices were dipped in OS for three hours due to the absorption of organic acid (citric acid) cations along with the small sugar particles increased samples acidity resulting in lowering the pH values.

So, the propagation of pathogenic microbes in samples was hindered as the pH decreased below the optimum range (3.0 to 7.5) required for their expansion (Botondi et al., 2021). The yeast and mold count were not exceeded by the safety limit (more than 5CFU/g) till the tenth day in control samples as well, because of osmotic treatment of a hypertonic solution of glucose and sucrose-containing citric acid (0.5%) and sodium metabisulphite (0.25%). A significantly lower fungal count of 2.53 Log CFU/g was calculated in double coated with osmo-sucrose treatment and a higher of 4.29 Log CFU/g was calculated in double coated with osmo-glucose treatment at the end of preservation. Whereas no significant difference was observed in single coated (3.18 Log CFU/g) and double coated with osmo-sucrose treated samples (2.53 Log CFU/g) and double coated (4.29 Log CFU/g) and single coated with osmo-glucose (5 Log CFU/g) treated samples at the end of storage. The higher fungal count was seen in osmo-glucose treated samples (osmotically dehydrated in glucose) might be due to the collection of higher exuded moisture in the packaging along the storage.

Table 1. Yeast and mold counts (Log CFU/g) without coating as well as coated and osmotically treated mango slices during refrigeration storage.

Sugar type	Storage days	Without coating	Single coating	Double coating
Glucose	0	$1.97 \pm 0.15^{e, A}$	$1.72 \pm 0.25^{cd, A}$	$1.80 \pm 0.39^{bc, A}$
	5	$3.88 \pm 0.29^{cd, A}$	$2.94 \pm 0.20^{bcd, A}$	$2.15 \pm 0.51^{bc, A}$
	10	$5.07 \pm 0.36^{c, A}$	$3.53 \pm 0.63^{ab, AB}$	$3.04 \pm 0.85^{ab, B}$
	15	$8.92 \pm 0.47^{a, A}$	$5.00 \pm 0.34^{a, B}$	$4.29 \pm 0.92^{a, B}$
Sucrose	0	$1.42 \pm 0.61^{e, A}$	$1.27 \pm 0.51^{d, A}$	$1.00 \pm 0.37^{c, A}$
	5	$2.92 \pm 0.33^{de, A}$	$2.57 \pm 0.73^{bcd, A}$	$1.84 \pm 0.18^{bc, A}$
	10	$4.85 \pm 0.40^{c, A}$	$2.80 \pm 0.19^{bcd, AB}$	$2.11 \pm 0.27^{bc, B}$
	15	$6.97 \pm 0.52^{b, A}$	$3.18 \pm 0.84^{bc, B}$	$2.53 \pm 0.81^{bc, B}$

Similar small letters shown column wise (among storage days) and similar capital letters shown row wise (among coating treatments) are not significantly different at $p \leq 0.05$.

Different osmotic solutions have different potentials of mass exchange. The high solute intake and water loss observed in osmo-glucose treated samples are due to the higher osmotic pressure of glucose, due to the lower molecular weight of glucose (180 molecular weight) compared to sucrose (342 molecular weight) thus resulting in double the molecular concentration of glucose particles in same solution concentration, hence resulted in higher osmotic pressure and effective water loss and solid gain (Panagiotou et al., 1999). A significant ($p \leq 0.05$) variation in Moisture Content (MC) and TSS was observed in glucose and sucrose osmo-treated (osmotically dehydrated)

samples at zero days, respectively. This variation at zero days resulted in higher water loss and solid gain in glucose OD, so the remaining MC was lower and TSS was higher compared to sucrose OD.

According to Castelló et al. (2009), the impact of dehydration level was coupled with turgor loss, cellular damage, and shrinkage of product tissue. So, in the present study, the higher dehydration level and solute uptake in glucose OD might cause damage to the cellular structure which resulted in comparatively higher moisture loss from tissues along with storage days. The decrease in MC is not significant during 10 days of storage in double coated with glucose osmo-treated samples but a significant decrease in MC was seen on the 15th day of storage (Table 2). A slight but continuous moisture loss along with the storage days might have washed out the outer protective layers of coatings (aloe-pectin) toward the end of storage.

Table 2. Moisture contents (%) of osmotically treated control and aloe-pectin coated mango slices during refrigeration storage.

Sugar type	Storage days	Without coating (control)	Single coating	Double coating
Glucose	0	$58.01 \pm 0.57^{bc, A}$	$57.89 \pm 0.63^{cd, A}$	$57.92 \pm 0.17^{b, A}$
	5	$55.95 \pm 0.55^{d, A}$	$57.04 \pm 0.48^{d, A}$	$57.12 \pm 0.23^{b, A}$
	10	$53.07 \pm 0.86^{e, B}$	$54.68 \pm 0.30^{e, AB}$	$56.27 \pm 0.90^{b, A}$
	15	$50.01 \pm 0.98^{f, B}$	$51.75 \pm 0.72^{f, AB}$	$53.68 \pm 0.23^{c, A}$
Sucrose	0	$62.46 \pm 0.96^{a, A}$	$62.40 \pm 0.11^{a, A}$	$62.43 \pm 0.13^{a, A}$
	5	$61.58 \pm 0.73^{a, A}$	$62.13 \pm 0.29^{a, A}$	$62.30 \pm 0.29^{a, A}$
	10	$59.56 \pm 0.44^{b, B}$	$60.73 \pm 0.82^{ab, AB}$	$61.94 \pm 0.52^{a, A}$
	15	$57.71 \pm 0.51^{c, B}$	$59.60 \pm 0.79^{bc, AB}$	$60.65 \pm 0.41^{a, A}$

Similar small letters shown column wise (among storage days) and similar capital letters shown row wise (among coating treatments) are not significantly different at $p \leq 0.05$.

The decrease in moisture content along with the storage might be due to the surface water evaporation, respiration, transpiration and due to wounding stress during minimal processing operations. A significant variation in MC of 50.01 and 53.68% was calculated in control and double coated with osmo-glucose treated samples, and 57.71 and 60.65% was calculated in control and double coated with osmo-sucrose treated samples at the end of storage.

The moisture content is better preserved in coated samples, in our coating composition we used pectin, aloe vera gel and $CaCl_2$, however, it could be noted that pectin was a good moisture loss barrier (Ferrari et al., 2013). Polysaccharide composition of aloe vera gel and hygroscopic nature of coating resulted in effective moisture barrier between fruit and outer environment (Allegra et al., 2019; Morillon et al., 2002). $CaCl_2$ helps to maintain the better texture of cell wall and strengthen the barrier properties (Nasution et al., 2015).

Table 3. Total soluble solids (°B) of osmotically treated control and aloe-pectin coated mango slices during refrigeration storage.

Sugar type	Storage days	Without coating (control)	Single coating	Double coating
Glucose	0	$29.7 \pm 0.31^{d, A}$	$29.59 \pm 0.42^{cd, A}$	$29.68 \pm 0.07^{b, A}$
	5	$31.89 \pm 0.45^{c, A}$	$30.67 \pm 0.19^{bc, A}$	$30.01 \pm 0.29^{b, A}$
	10	$33.76 \pm 0.62^{b, A}$	$31.98 \pm 0.29^{b, AB}$	$31.19 \pm 0.34^{ab, B}$
	15	$36.13 \pm 0.34^{a, A}$	$33.81 \pm 0.56^{a, AB}$	$32.39 \pm 0.22^{a, B}$
Sucrose	0	$25.25 \pm 0.38^{e, A}$	$25.19 \pm 0.08^{e, A}$	$25.30 \pm 0.46^{c, A}$
	5	$26.93 \pm 0.20^{e, A}$	$26.32 \pm 0.75^{e, A}$	$26.15 \pm 0.29^{c, A}$
	10	$28.95 \pm 0.27^{d, A}$	$28.23 \pm 0.64^{d, AB}$	$26.87 \pm 0.02^{c, B}$
	15	$30.36 \pm 0.12^{cd, A}$	$28.85 \pm 0.42^{d, AB}$	$27.16 \pm 0.13^{c, B}$

Similar small letters shown column wise (among storage days) and similar capital letters shown row wise (among coating treatments) are not significantly different at $p \leq 0.05$.

A non-significant increase in TSS was observed in double coated with osmo-sucrose treated samples along with the storage days, however, a slow increase in TSS (Table 3) and decrease in MC (Table 2) was observed in double-coated with osmo-glucose treated samples which showed non-significant variation among samples from 0 to 10 days. While a significant difference was observed between double coated and control samples from 10 to 15 days in both osmo-glucose and osmo-sucrose treated samples. This increase in TSS along with the storage time may be caused by saturation that occurs due to moisture evaporation, leading to an increase in solute concentrations. The higher increase in TSS in control samples was due to higher evaporation of moisture and conversion of organic acids into sugars. A Similar increase in TSS was observed by Arrubla Vélez et al. (2021) in samples without coated in blackberries during refrigeration storage for 19 days. It is also reported that along with the preservation of water evaporation, it could be observed that edible coatings may reduce the permeability of CO_2 and O_2 which are usually end products of degradation of complex sugars during storage, hence leading to the minimum increase in TSS (Pinzon et al., 2020).

4 Conclusion

In the present study, the shelf life of control mango slice samples was limited to 5 to 10 days in osmo-glucose and osmo-sucrose treated samples, respectively. This may occur because after these days the TFC of samples exceeded acceptable limits, whereas the single-coated and double-coated samples remain acceptable for 10 days in osmo-glucose pretreatment. Regarding osmo-sucrose single and double coating samples, both remained acceptable for 15 days of storage. The coating treatment improved the stability of moisture contents, and TSS and decreased the fungal count. OD reduces the product's initial moisture contents and in combination with aloe-pectin coatings, it is effective in increasing storage stability for 15 days. The OD in combination with other mild treatments like coating and refrigeration storage can extend product shelf life.

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