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Candelilla Wax-Based Edible Coatings: A Novel Strategy to Prolong Shelf Life of *Cucumis Sativus*

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Abstract
This research work was done to investigate and critically evaluate the impacts of Candelilla wax-based edible composites, on the weight loss percentage, firmness, brix, color change, and pH of *Cucumis sativus* fruits. The coatings under investigation consisted of ethanol-based and water-based plant leaf extracts of *Toona ciliate, Melia azedarach*, *Swietenia macrophylla, azedirachta indica*, and *Chukrasia tabularies* belonging to family *meliaceae* (each 4% (w/w) incorporated into Candelilla wax (16%), while uncoated fruits served as control. Control and coated samples of *Cucumis sativus* were placed separately in zip bags with 3 to 4 micro-perforations and stored at 22 ± 2 ºC and 70-80 % humidity levels for 18 days. An analysis of variance (ANOVA) test was used to investigate how candelilla wax composite coatings and time of storage affected the qualitative attributes of cucumber. It was investigated that all coatings delayed the ripening processes and reduced the weight loss percentage in the coated samples, as compared to the control (uncoated) samples. The firmness of the untreated samples exhibited a significant decline over time. The application of the edible coating effectively delayed the softening of *C. sativus* throughout the 18-day storage period. Coated *C. sativus* samples were relatively better preserved during the 18-day. Overall, the utilization of candelilla wax composite coatings enhanced the physico-chemical quality attributes and potentially extended the shelf life of cucumbers for up to 18 days.

Keywords: *Cucumis sativus*; Composite wax coating, pH, firmness, color, weight loss.

1. Introduction

Currently, the global population is rapidly increasing, particularly in developing countries, which also have encountered food insecurity and food safety issues. This poses significant challenges in meeting the food requirement of up to 70%. (Kumar & Kalita (2017).Horticultural products have the potential to solve the confronting issues by increasing revenue, nutritional standards, and the production of fruits and vegetables. Food availability and accessibility can be enhanced by reducing postharvest losses (Sharma et al., 2019). Literature has demonstrated that around 50% of fresh fruits and vegetables are wasted in developing countries before their consumption (Ahmad et al., 2015). Fresh produce is highly susceptible to spoilage due to temperature and microbial activity.

Microbes are a key contributor to fresh produce deterioration, and food contamination, leading to food borne diseases (Khare et al., 2018).To ensure the quality attributes of fresh produce, it is necessary to prevent it from microbial activity throughout transportation and storage. Several methods such as chemical treatment, low temperature, temperature storage, high irradiation, and modified atmospheric packaging (MAP) are employed to enhance the nutritional quality and shelf life of the fresh produce. However, the techniques have to be handled with care, as they result in an undesirable reduction in the nutritional value of the produce (Mohapatra et al., 2013).

Cucumis sativus (Cucumber), which belongs to the family *Cucurbitaceous* is an important vegetable that contains 95% water, 3.6% carbohydrate, and0.65% protein (Bahnasawy*et al*., 2014). Globally, its production reaches up to 40, 000 tons annually. Cucumber quality declines rapidly after post-harvesting due to the reason of water loss resulting in shriveling and yellow peel color and shortened shelf life. Postharvest technology aims to delay the ripening process in vegetables. Changes in peel color are due to ripening and senescence processes in the vegetables.

Recently, edible coatings, thin layers applied to fruits and vegetables, have served multiple functions by regulating moisture exchange, and gas levels, and preventing deterioration in these produced items. They play crucial roles in the enhancement of the shelf life of fresh produce while being safe for consumers with no adverse health issues (Bhattarai & Adhikari 2023). Their primary objective is to mitigate risks like shrinkage and weight loss, thereby improving the overall appearance. Additionally, they contribute to reducing the occurrence of various disorders (Aayush et al., 2022). Furthermore, the coatings effectively act as a water barrier and enhance the shelf life of fruits and vegetables.

A variety of treatments are employed to maintain the post-harvest quality of fresh produce, such as controlled atmosphere packaging, modified atmosphere packaging, and fungicide treatment. By regulating the gaseous composition and temperature these techniques produced a controlled environment thus ensuring that fresh produce retained their appearance, texture flavor, and color. However, it is important to note that these methods have disadvantages, such as being economically less applicable and generating issues of chilling injury and harm to public health (Ochoa et al., 2011).

In these days edible coatings have emerged as one of the most promising solutions to address these inherent challenges. They play a pivotal role for enhancement in shelf life of fresh produce, without any toxic and harmful effects thus ensuring their safety for consumption. Generally, edible coatings are formulated from carbohydrates, proteins, lipids, or combinations thereof. Among the various options, carbohydrate-based coatings, like cornstarch, are frequently employed due to their favorable properties. Cornstarch is absorbent, odorless, tasteless, colorless, non-toxic, andsemi-permeable to gases (Timilsena et al., 2017). It is economical for post-harvest packaging and acts as a protective material with fabulous mechanical characteristics(Belay et al., 2023).

Additionally, herbal extracts such as aloe vera, tulsi, neem, marigold, and their combinations also gain prominence in the preparation of edible coatings. These herbal extracts serve not only as antimicrobial and antioxidant agents but also as effective preservatives, making them aptly referred to as herbal edible coatings. Despite their novelty, so far none has been reported as the edible coating material for prolonging the shelf life and maintaining the quality attributes of fresh produce.

Incorporating natural ingredients and cutting-edge technology in various materials and new approaches improves banana postharvest quality. Researchers are creating sustainable and cost-effective solutions to meet growing fresh produce demand using a multidisciplinary approach. This is crucial for food supply chain security and resilience(Alikhani, 2014).

To bridge the research gap, this work investigates the family *Meliaceae* common plant (*Melia azedarach*, *Toona ciliate*, *Chukrasia tabularies*, *Azedirachta indica*, and *Swietenia macrophylla* species leaves aqueous and ethanolic extract in combination with Candelilla wax composites as a protective coating material for the *Cucumis sativus* samples. The parameters such as weight loss% age, firmness, brix, color change, and pH of coated samples and control were studied in the selected period and stored at 22± 2 temperature. The prepared edible composites have been proven to an effective, and stable against the postharvest spoiling agents. To our knowledge, these plant species' edible composites are still not utilized as coating materials. Other fruits such as *Tragacanth* gum (Apple) (Gutiérrez-Pacheco *et al*., 2020), *Candelilla* (Avocado), gelatin, carboxymethyl cellulose; soy protein isolates (SPI) (Cherry) (Yaman et al., 2002), zein (Corn)(Kumar *et al*., 2021), rosemary oil with mucilage solution (Mango) (Ghosh & Katiyar 2022)and chitosan (Mushroom) (Eissa, (2007) are tested and analyzed.

2. Materials and method

2.1. Materials

Fresh, mature *Cucumis sativus* (cucumbers) of the same size, color, and age were purchased from a local market in Lahore (Pakistan). The cucumbers were washed in running water to remove dust particles. Further, they were disinfected by dipping in 0.05% solution of sodium hypochlorite (NaClO) for 3 min and then washed again with distilled water followed by air drying at room temperature.

For aqueous-based plant leaves extract, fresh plant leaves were obtained from the Botanical Garden (Lahore). The collected leaves were properly cleaned, thoroughly washed with distilled water, and then shade-dried. Dry material is then powered into fine particles through the mechanical grinder. Subsequently, the powder was boiled for 30 min in 500 ml of distilled water. The solutions were filtered through a vacuum pump. The samples were re-filtered at lower pressure. To reach a final concentration of 1 g/mL of bioactive reagents, the filtered samples were placed in an oven subjected to a process of evaporation for concentration. The concentrated extracts were then sterilized at 121°C for 20 min and kept in a refrigerator at 4ºC until further usage.

2.2 Methods

2.2.1 Preparation of ethanol-based plants leaves extract

In the initial phase, the Soxhlet apparatus was assembled with 40 g of dried plant leaf samples in a thimble ensuring uniformity. Over 7 hours 350 mL of ethanol were used for extraction. Subsequently, the resulting crude extract was filtered to eliminate solid particles and other impurities. The purified filtrate underwent further analysis to assess its composition and properties. To concentrate the extract, the ethanol was removed using a specialized rotary evaporator. This step aimed to produce a potent and manageable extract for subsequent experiments. The entire process involved precise handling of plant samples, meticulous purification, and concentration, ensuring the quality and consistency of the final extract.

2.2.2Synthesis of edible wax composite coatings

A homogenized mixture of the edible coating composites was prepared by heating a mixture of 4% (w/w) plant extract, and 16% (w/w) Candelilla wax in 80% of distilled water (w/v). The plant extract, Candelilla wax, and distilled water were measured and mixed in an appropriate container by maintaining a consistent stirring using magnetic stirrers. The temperature was gradually increased to a predetermined level, ensuring it stayed within the specified limit for the materials. Controlled heating caused the Candelilla wax to melt and disperse evenly in the aqueous phase of the emulsion.

This thermal treatment resulted in a uniform blend, where plant extract and Candelilla wax particles were uniformly distributed throughout the water phase. After 30 min of heating and agitation, close monitoring was conducted to ensure proper emulsification free from visible phase or clustering. This emulsion was ready for use in coating applications based on experimental design requirements.

2.2.3 Experimental design

The *Cucumis sativuswas* tested by coatings of Candelilla wax composites, which included control (uncoated), as well as those treated with water-based plant leaf extracts from *Melia azedarach* (MaCWH), *Toona ciliate* (TcCWH), *Chukrasia tabularies* (CtCWH), *Azedirachta indica* (AiCWH), and *Swietenia macrophylla* (SmCWH). Further, five distinct ethanol-based candelilla wax treatments were applied to *Cucumis sativus* samples such as *Melia azedarach* (MaCWE), *Toona ciliate* (TcCWE), *Chukrasia tabularies* (CtCWE), *Azedirachta indica* (AiCWE), and *Swietenia macrophylla* (SmCWE). Each *Cucumis sativus* sample was immersed in its respective treatment solution for 5s and subsequently air-dried at room temperature for 15 min.

2.2.4 Applications of Candelilla wax composites and storage

The *Cucumis sativus* samples were coated with all synthetic composites based on water and ethanol extract including uncoated used as a control. Each batch of *Cucumis sativus* underwent a 5 s long individual coating procedure. The control (uncoated) and coated samples were kept at 22 ± 2 °C with a humidity level between 70 - 80 %throughout 18 days. Throughout the study, parameters such as change in pH, color, firmness, Brix and weight loss percentage were recorded and analyzed to determine the efficacy of these edible composite coatings to slow down the rate of ripening process.

2.2.5Physiochemical analysis of *Cucumis sativus*

2.2.5.1 Weight loss

At the beginning of the experiment, *Cucumis sativus* were weighed on a digital scale (model Delta range, Switzerland).The fruits were tagged and separated at the same time. The *Cucumis sativus* were weighed at 0, 3, 6, 9, 12, 15, and 18 days to investigate the weight loss % in coated cucumbersby comparing them with uncoated (control). The initial and final weights were measured while the weight loss % was calculated based on this formula;

$$W_L\,(\%) = \frac{Wi - Wf}{Wf} \times 100 \qquad (1)$$

where:W_L, W_i,and $W_f$represent the weight loss, initial weight, and final weight of the samples.

2.2.5.2 pH

During 18 days of study, thepH of the *Cucumis sativus* edible composites coated samples with control was determined. The pH values were evaluated using a pH meter (model Hanna model – HI 8424 Origin Romania). The juice was obtained from crushed cucumber and then an electrode was used to analyze the pH values of each coated and control samples. The recorded pH variations underwent posthoc analysis using the *ANOVA* test.

2.2.5.3 Color

A color reader model CR-10 Plus Konica Minolta in Japan was used to detect the color of the cucumber fruit, which consisted of the colors L*(white-black), a*(red-green), and b*(yellow-blue). Readings were taken at random from three different fruits that were chosen at random from each replication during each phase.

2.2.5.4 Firmness

To measure the firmness of fruit *Cucumis sativus* samples with and without treatment, *Imada* texture analyzer FRTS was used. The firmness of the fruit was measured at two distinct locations. The average of the readings at each site produced values (kg), which were then translated into Newtons (N).

2.2.5.5 Brix evaluation

The 18-days of Brix readings were collected to evaluate the efficacy of edible composite coatings. Brix levels measure the sugar content in fruits, providing important information about their flavor attributes. Total soluble solids content (Brix) was measured using a portable refractometer (ATC, China).The total soluble solid (Brix) value for cucumber was evaluated by placing the drop of juice of crushed fruit onto the Brix refractometer.

2.2.6 Statistical Analysis

The experiment involved a completely randomized design (CRD), and physicochemical quality attributes were measured at 0, 3, 6, 9, 12, 15, and 18 days of storage. Data analysis utilized one-way

ANOVA followed by Tuckey post hoc test through SPSS software, One-way ANOVA test was utilized to assess the significance of pH, firmness, and TSS changes among different treatments at SPSS software (Version 27, IBM, NY, USA, 2020) were used to perform the statistical analyses of the measured data.

3. Results and discussion

The quality of the *Cucumis sativus* samples was determined by measuring the weight loss, pH, color, firmness and brix (total soluble solid) evaluation.

3.1 Effect of composite edible coatingson weight loss and firmness

Figure 1a illustrates the coating of the composite material based on the water and ethanol extract to evaluate the weight loss and firmness of *Cucumis sativus.* There were variations in weight loss observed in *Cucumis sativus* stored at 22± 2 °C and 70-80 % humidity levels for 18 days.The weight loss of the control group exhibited a significant increase and reached up to 10 % as the storage duration extended up to 18 days.

In contrast, *C. sativus* samples coated with *Candelilla* wax edible composite prepared with ethanol and water exhibited comparatively less weight loss throughout the storage period. Their weight loss showed a gradual increase over storage time. The coatings sample weight loss, controlled (9.60%), MaCWH (5.30%), AiCWH (5.90%), SmCWH (5.47%), TcCWH (5.10%), CtCWH (5.20%), CtCWE (6.55%), TcCWE (6.09%), SmCWE (6.90%), MaCWE (6.60%) and AiCWE (4.37%) respectively. The basic mechanism of the slow weight loss was due to the coating materials that actas a protective barrier against moisture evaporation. Further, the coating material is a semi-permeable barrier gas (O_2 and CO_2), solute molecules, and oxidation rate(Gutiérrez-Pacheco et al., 2020; Van Doan et al., 2018; Ghosh & Katiyar 2022).

Further, the firmness of the controlled fruits significantly decreased, while the coatings samples had a higher firmness value in storage days (Figure 1b-c).At the end of the storage (18 days), the control had the lowest firmness, while the coated samples have maximum firmness values. However, all the coated samples showed stability in their firmness. When results of the overall storage period of each coated and controlled samples were compared using one-way ANOVA followed by Tukey test, averages firmness values (19.75-16.71) were seen ($p = 0.0029$). However, water and ethanol composites coated samples showed average firmness ofMaCWH(18.15 N), AiCWH (17.4 N), SmCWH (19.01 N), TcCWH (18.62 N), CtCWH (18.68 N), CtCWE (19.06 N), TcCWE (19.71 N), SmCWE (17.33 N), MaCWE (17.98 N) and AiCWE (16.71 N) respectively.

The softness of *C. sativus* occurred due to changes in the chemical composition of the cell membranes and intracellular materials. The biological reactions preceded by the enzyme (hydrolases) played a key role in the hydrolysis of pectin and starch.

Furthermore, the ripening of *C. sativus* was due to the depolymerization of the pectin chain that was accompanied by the activities of the polygalacturonase and pectinesterase (Yaman, Ö &Bayoındırlı 2002). The enzyme's activities slowed down due to low O_2 and high CO_2 levels, which helped maintain firmness during the storage period (Emragi et al., 2022). The respiration rate of *Cucumis sativus* coated samples decreased and retarded the softening and firmness to enhance the shelf life.

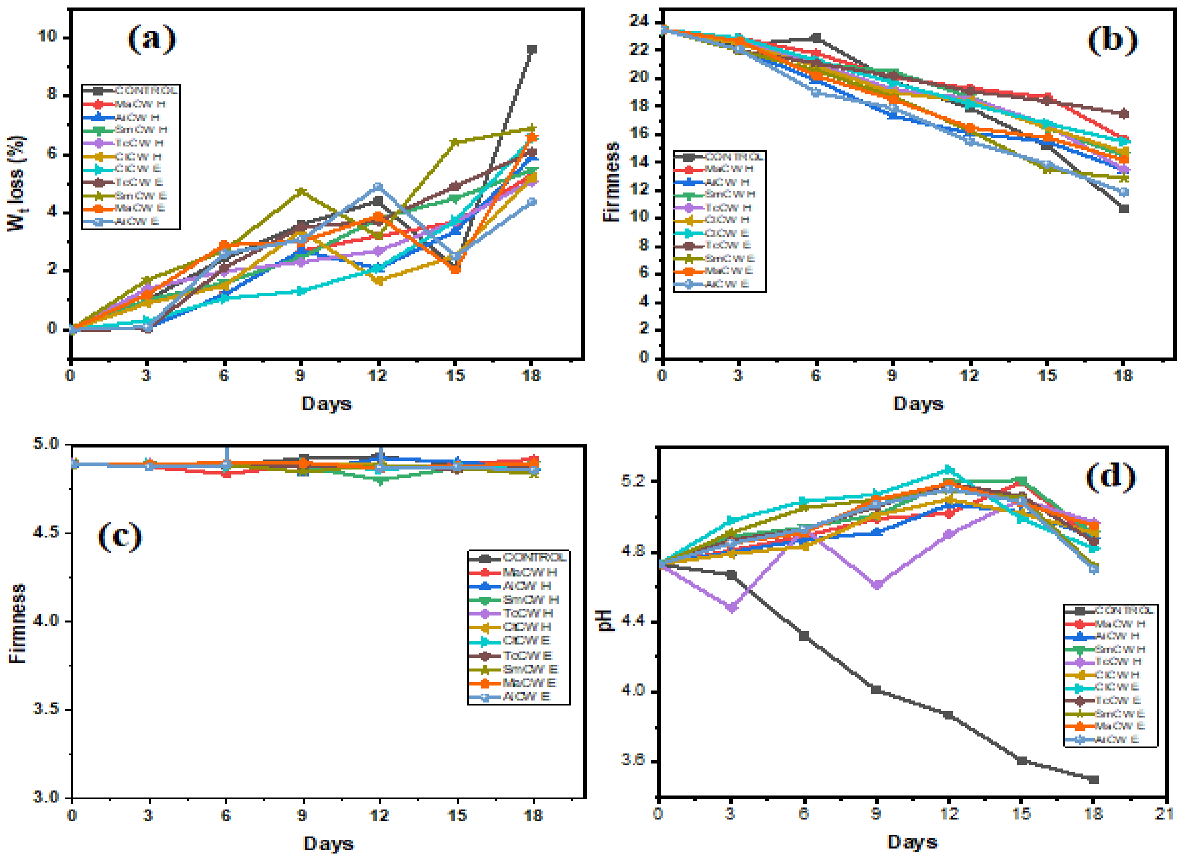

Figure 1. Effect of coatings on *Cucumis sativus* on weight loss and firmness on storage time at 25 °C

3.2*Coating effects on the pH, and bioactive properties of Cucumis sativus*

Figure 1d presents the effects of coatings on pH, acidity, total phenolic content (TPC), and DPPH radical inhibition activity of *C. Sativus* samples coated after being stored at 22 °C for 18 days. The pH of the *C. sativus* was significantly affected by the coating treatment and the duration of storage ($p<0.05$; *Anova* test). The application of coating increased the pH levels in both fresh and stored *Cucumis sativus* samples. The highest pH of the controlled (4.73) and the sample CtCWE (5.3) were recorded, which was coated, while the lowest pH values were observed in the control (3.5) and coated TcCWH (4.48) during storage.

Overall, there is similarly, studies involving *C. sativus* coatings such as modified corn starch/gelatin films (Kumar et al., 2021) and latex coatings with calcium oxide nanoparticles (Cid-López et al., 2021)reported elevated pH levels. During storage, the pH of the untreated control sample progressively decreased due to the metabolic conversion of sugars into acids (Maleki et al., 2018). In contrast, the pH of the coated cucumber samples initially increased and peaked at day 14 of storage due to the utilization of organic acids in respiration metabolism. Subsequently, the pH decreased with extended storage time due to the conversion of sugars into organic acids (Sarker et al., 2021). Similar observations on pH increases in both fresh and stored coated *C. sativus* samples have been reported in various studies (Bakliwal et al., 2019). The p value (0.0142) of one-way ANOVA is less than 0.05, suggesting the significant difference. One way ANOVA with post-hoc Tuckey HSD test would likely identify which of the pairs of treatments are significantly different from each other.

3.3 Effect of edible coatings on the surface color of *Cucumissativus*

Figure 2 displays the change in surface color attributes (L*(white-black), a*(red-green), and b*(yellow-blue) for both uncoated and coated *C. sativus* samples throughout storage period. The coating treatment and the duration of storage exhibited distinct effects on the color ($p \leq 0.05$; t-test).The lightness values of coated *C. sativus* were consistently the highest across all storage intervals.

These values decreased to their lowest levels in *Cucumis sativus* uncoated and subsequently increased in the samples with coated composites. The diminished lightness of the coated *C. sativus* was attributed to the opacity and darker color of the functional coating materials. Over the course of storage, the lightness values of all *C. sativus* samples decreased to their lowest points by the end of the storage period.

Similarly, earlier studies have reported lower L* values in the coated fruits, as compared to their uncoated counterparts. This was due to the opacity and color of the coating materials (Mohammadi et al., 2015). Furthermore, a reduction in L* values for both coated and uncoated fruits has been observed during storage at various temperatures and durations.

The red-green (a*) values of *C. sativus* was subject to the influence of both the coating treatment and the duration of storage ($p<0.05$; *t-test*). For fresh *Cucumis sativus* samples,red-green of uncoated samples surpassed that of their coated counterparts and the red-green values decreased. This decline in red-green, resulting from the application of functional materials, was attributed to the darker color imparted by the ingredients. As the storage period extended to 18 days, the red-green values of *C. sativus* significantly diminished. However, the changes in red-green were less pronounced in the coated samples. This suggests that the coating materials used in this study had a stabilizing effect on the color. The reduction in green color during storage could be attributed to the enzymatic degradation of chlorophyll, a process that the coating helps counteract by altering the surface environment of the *C. sativus*, thereby preventing oxidative and enzymatic browning.

The findings aligned with previous reports, which noted a decrease in red-greenness for both coated and uncoated cucumbers during storage with the coated samples exhibiting fewer color changes, as compared to the uncoated ones. The yellow-blue (b*) values of *Cucumis sativus* exhibited varying responses to both the application of functional coatings and the duration of storage. In fresh *Cucumis sativus* the uncoated sample displayed the highest yellowblue value, which decreased to its lowest in the samples of edible coated composites (*Anova* test).

In stored *Cucumis sativus* samples, the highest yellow-blue values were observed in those coated with the Candelilla Wax-based edible coatings, while the lowest values were found in the uncoated samples. Overall, the functional coatings of the Cucumis sativus with Candelilla-wax based coatings have a positive impact on their color attributes to enhancing consumer acceptance of the product.

3.4Effects of coating on moisture content, water activity, and soluble solids of *Cucumis sativus*

Figure 2d presents the effects of coating on moisture content, water activity, and total soluble solids (TSS) (Brix evaluation) of *C. sativus*, considering the impact of coating and storage. Regardless of the treatment and storage duration, all the samples exhibited high moisture content, exceeding 90%. Notably, the coating treatments effectively preserved the moisture content of the *C. sativus*with coated samples consistently showing higher moisture content, as compared to their uncoated counterparts (*Anova test*). However, with prolonged storage, the moisture content experienced a significant reduction, reaching its lowest levels by day 18 for all samples.

The data variations showed that the usage of composite wax coatings might cause a shift in sugar concentration in*C. sativus*. Throughout storage duration, Brix changes (1.5-3.8) were modest in response to the edible composite coverings placed on *C. sativus*. The findings made it clear that coatings consistently affected the sugar content of the *C. sativus*. Other *C. sativus*quality indicators might be affected over time by the edible composite coverings.

The improved moisture retention in coated *C. sativus*samples was attributed to the chitosan coating, whether or not it contained functional ingredients. This coating served as a barrier against water evaporation, effectively suppressing transpiration (Hashim et al., 2018). The decrease in moisture content during extended storage can be due to the natural processes of evaporation and transpiration that commonly occur when storing fruits/vegetables, driven by fluctuations in temperature and humidity (Saha et al., 2016).

In line with the findings, previous research indicated that coating treatments reduced moisture loss during the cold storage of cucumbers (Li et al., 2018). Additionally, the increased weight loss observed in fruits and vegetables during storage can be linked to moisture loss. Several studies have supported the idea that coating treatments reduce weight loss in cucumber samples during storage under various conditions (Hashim et al., 2018; Fan et al., 2019).

The water activity of the cucumber samples remained unaffected by both the coating treatments and the duration of storage. However, there was a minor and statistically insignificant decrease in water activity during cold storage. Notably, all cucumber samples stored at 4°C had a water activity level above 0.960, indicating their vulnerability to spoilage by mesophilic microbes unless adequately preserved. The decrease in water activity observed during storage was linked to the reduction in moisture content that occurred during the cold storage of cucumbers. Fresh *Cucumis sativus* are known to be susceptible to decay and spoilage by microorganisms due to their high moisture content (95%) and water activity (0.96). This needs special processing treatments and preservation conditions (Berger et al., 2010).

Total soluble solids (TSS) exhibited a significant increase in *Cucumis sativus* coated with Candelilla-wax based coatings, as compared to the untreated control or samples. The lowest TSS value was found in untreated control samples stored for 18 days, while the highest TSS value was observed in the fresh cucumber samples treated Candelilla-wax based coatings. This suggests that the functional ingredients in the Candelilla-wax based coatings contributed to the enhancement of TSS in the *Cucumis sativus* samples.

The increased TSS in functionally coated *Cucumis sativus* can be attributed to the presence of reducing sugars in coating materials. Additionally, coating *Cucumis sativus* with Candelilla-wax based coatings might reduce the respiration rate, thereby maintaining higher TSS, as compared to uncoated samples. Similarly, prior reports indicated that *Cucumis sativus* coatings with chitosan with substances such as salicylic acid (Zhang et al., 2015) and carbon dots as functional ingredients resulted in higher TSS levels than uncoated samples. During storage, a concurrent reduction in TSS was observed as the storage duration extended to 18 days, reaching its lowest value at the end of the storage period. This reduction in TSS during cold storage is likely due to the senescence of the *Cucumis sativus* samples. One-way ANOVA (p=1.37534) test of the measured data of Brix (TSS) did not show any significant difference in the Candelilla wax based edible coatings but in the controlled samples minimum TSS level (1.5) of different samples were measured at 18 day.

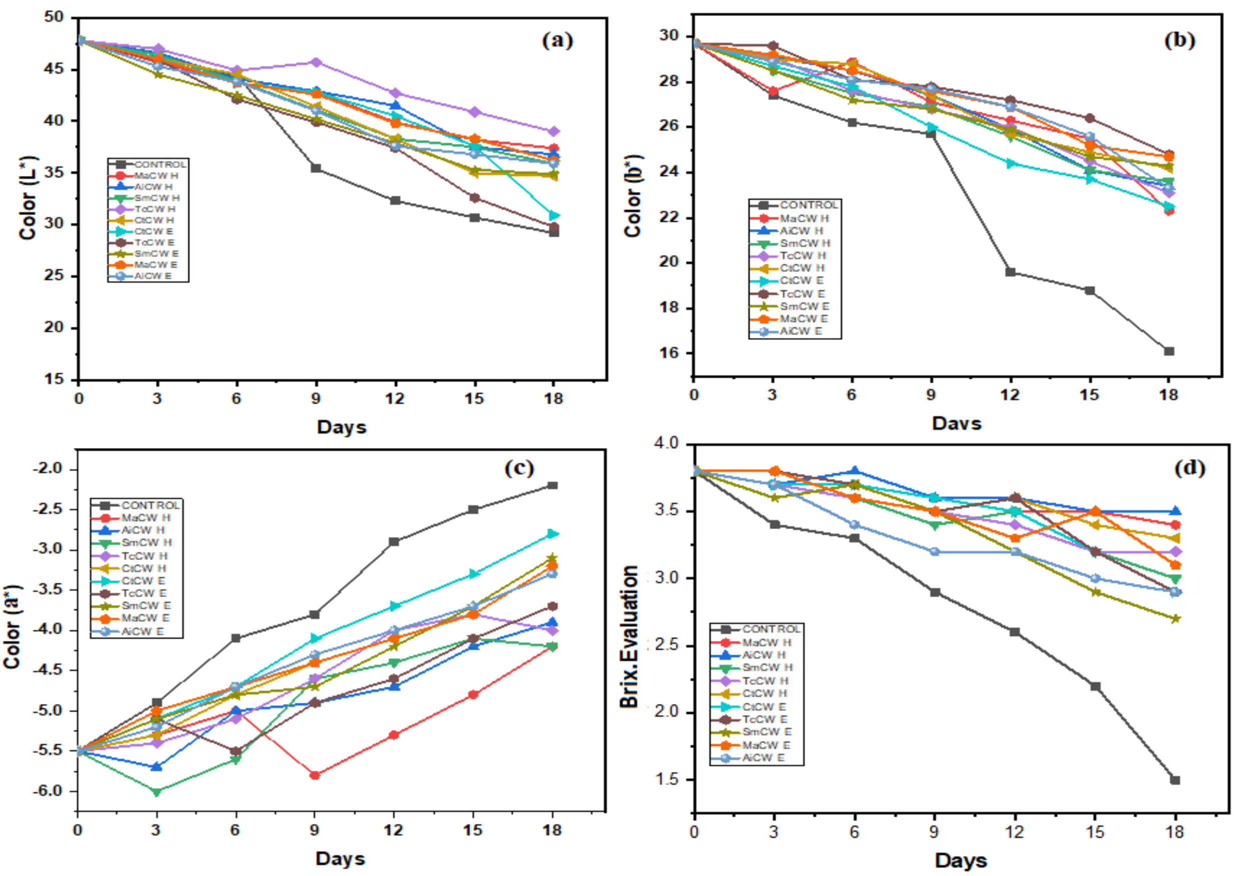

Figure 2. Color changes during the storage days (a-c);Brix evaluation (d)

4. Conclusion

This study has revealed that both coating treatment and storage at 22± 2 °C had diverse impacts on the physico-chemical, nutritional, and health-related qualities of *Cucumis sativus*. The application of functional coatings resulted in an increasing level of total soluble solids, firmness, sensory evaluation, and shelf life and maintains the pH weight loss in the *Cucumis sativus* edible coated samples. Nevertheless, as the storage duration extended to 18 days, there was a decline in total soluble solids, firmness, moisture content, pH and color in controlled samples. Overall, the utilization of functional coatings enhanced the physico-chemical quality attributes and potentially extends the shelf life of the *Cucumis sativus* for up to 18 days when being stored at 22± 2 ºC.

Authors statement

Tabassum Munir is responsible for overall investigation

Ayesha Mohyuddin is responsible for data collection and supervision and project administration

Muhammad Amjad is responsible for resources

Declaration of competing interests

☒The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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