

# Influence of zein and gelatin coatings on the postharvest quality and shelf life extension of mango (*Mangifera indica* L.)

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## Influence of zein and gelatin coatings on the postharvest quality and shelf life extension of mango (*Mangifera indica* L.).

**Abstract – Introduction.** Mango is the most economically important and nutritionally rich tropical fruit; it has high commercial value but a highly perishable nature; its sensitivity to postharvest diseases and physical injury limits its successful marketing. Postharvest losses in fruits are a serious problem because of rapid deterioration during handling, transport and storage. Edible films and coatings can be potentially used as an elective preservation technique to extend the shelf life of fruits. **Materials and methods.** The influence of zein and gelatin coatings on the physico-chemical characteristics, softening and antioxidative enzyme activities of mango fruits stored at  $(32 \pm 1) ^\circ\text{C}$  were evaluated at regular intervals of their storage period and compared with mango without coatings (control). **Results and discussion.** Zein and gelatin coatings seemed to have a beneficial impact on delaying the changes in weight loss, soluble solids, titratable acidity, pH, sugar content and total carotenoids. Zein and gelatin coatings resulted in the highest retention of ascorbic acid and phenolic content as compared with that of control. Zein and gelatin coatings delayed the ripening of mango fruit by suppressing the activity of softening enzymes such as polygalacturonase, pectin methyl esterase, cellulase and  $\beta$ -galactosidase. Zein 5% and gelatin 10% coatings maintained the highest induction of defense-related peroxidase enzymes, followed by gelatin 5% and zein 10% coatings. **Conclusion.** The application of zein 5% and gelatin 10% coatings could be used in delaying the ripening, maintaining the quality attributes and extending the shelf life of mango fruit during storage.

**India / *Mangifera indica* / fruits / keeping quality / edible films / zein / gelatin**

## Influence d'un enrobage de zéine et gélatine sur la qualité post-récolte et l'augmentation de la durée de vie de la mangue (*Mangifera indica* L.).

**Résumé – Introduction.** La mangue est le fruit tropical le plus important sur le plan économique et nutritionnel ; elle a une grande valeur commerciale mais est très périssable ; sa sensibilité aux maladies post-récoltes et aux blessures physiques limitent sa commercialisation. Les pertes post-récoltes des fruits sont un problème grave du fait de leur rapide détérioration au cours de la manutention, du transport et du stockage. Les films et revêtements comestibles peuvent être utilisés potentiellement comme une technique de conservation pour prolonger la durée de vie des fruits. **Matériel et méthodes.** L'influence de revêtements à base de zéine et gélatine sur les caractéristiques physico-chimiques, le ramollissement et l'activité des enzymes antioxydantes de mangues stockées à  $(32 \pm 1) ^\circ\text{C}$  a été évaluée à intervalles réguliers pendant leur période de stockage par rapport à des mangues non enrobées. **Résultats et discussion.** Les revêtements à base de zéine et gélatine ont semblé avoir un impact bénéfique pour retarder l'évolution de la perte de poids, des sucres solubles, de l'acidité titrable, du pH, des sucres et caroténoïdes totaux. Les revêtements de zéine et gélatine ont permis une meilleure rétention des teneurs en acide ascorbique et composés phénoliques par rapport à celle du témoin. Les revêtements à base de zéine et gélatine ont retardé le mûrissement des mangues en supprimant l'activité des enzymes de ramollissement tels que la polygalacturonase, la pectine méthyle estérase, la cellulase et la  $\beta$ -galactosidase. Les revêtements à 5 % de zéine et à 10 % gélatine ont maintenu la plus forte induction de l'enzyme peroxydase liée à la défense ; ils ont été suivis par les revêtements à 5 % de gélatine et 10 % de zéine. **Conclusion.** Des revêtements à 5 % de zéine et 10 % de gélatine peuvent être appliqués pour retarder le mûrissement, maintenir les caractéristiques de qualité et prolonger la durée de conservation de la mangue pendant son stockage.

**Inde / *Mangifera indica* / fruits / aptitude à la conservation / film comestible / zéine / gélatine**

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## 1. Introduction

Mango (*Mangifera indica* L.) belongs to the Anacardiaceae family [1]; it is the second most frequently cultivated tropical fruit worldwide. Mango fruit is one of the most important tropical fruits and it is known for its characteristic taste, aroma, flavor and health-promoting qualities, making it a common ingredient in new functional foods often labeled as “super fruits” [2]. Most of the world’s mango production is consumed raw as a dessert fruit and the rest of it is processed into diverse products such as puree, nectar, pickles, canned slices and chutneys [3]. Mango is a climacteric fruit with a high commercial value but the domestic and international trade of fresh mango has been limited by its highly perishable nature and its sensitivity to postharvest diseases and physical injury. The export of mango has become highly beneficial and, therefore, a longer shelf life is necessary for its successful marketing and consumer satisfaction [4].

In tropical fruits postharvest losses are a serious problem because of rapid deterioration during handling, transport and storage [5]. In developing countries, postharvest wastage of mango fruit occurs due to poor storage qualities and technologies. Several viable technologies have been developed by researchers for increasing the shelf life of mangoes, which include low temperature storage, controlled atmosphere and modified atmosphere or a combination of both [6]. Mango fruits stored in a modified atmosphere often show undesirable characteristics, *i.e.*, poor color and poor eating quality, and the storage of mangoes below 10 °C resulted in chilling injury manifested in grayish scald-like discoloration of the skin, skin pitting, uneven ripening, reduction in the levels of carotenoids and aroma, and the presence of undesirable flavors [2]. Therefore, there is a need to find alternative adequate postharvest technologies for reducing postharvest losses, thus extending the shelf life and maintaining the quality at low costs. These techniques should be simple, easily available, environmentally friendly and reasonable, and have no known harmful effects on human health, hence the current interest in the formulation of edible coatings [7].

Edible films and coatings can be potentially used as an elective preservation technique that can provide an additional protective coating that can not only keep fruit plumpness, fresh appearance and hardness but also improve the luster of the fruit surface, thereby increasing the commercial value of fruits [8]. Currently the consumer interest in biodegradable edible films or coatings made from natural bio-based products could be recognized as safe. Different biological materials such as polysaccharides, proteins and lipids have been used to prepare the coating material with the purpose of modifying the internal atmosphere of fruits and vegetables [9]. The use of edible coatings affords numerous advantages by avoiding the need for capital-intensive storage techniques. Protein-based coatings have been made from some proteins which include corn zein, wheat gluten, soy protein, rice protein, egg albumin, milk proteins and gelatin [10].

Zein is a natural storage protein found in corn kernels. Zein coatings have been used to coat nuts and candy for increased gloss, prevention of oxidation and development of off-odors because of their good barrier properties against oxygen and lipids [11]. Zein coatings offer a reasonable alternative to shellac and carnauba wax and that has received considerable attention in maintaining the overall fruit quality [11, 12]. Gelatin, an important biopolymer obtained by hydrolysis from collagen, is widely used with a broad range of functional properties and applications such as in the food, pharmaceutical and photographic industries, including its film-forming ability. Gelatin films generally have effective barrier properties against oxygen and carbon dioxide [13]. Aguilar-Mendez *et al.* found that the application of gelatin-starch coatings extended the postharvest shelf life of avocado [14]. There is little information available about the effect of protein-based edible coatings on extending the shelf life of fruits and vegetables. Therefore, the objective of our study was to assess the potential of zein and gelatin coatings on the extension of shelf life and postharvest quality retention of mango fruit.

## 2. Materials and methods

### 2.1. Fruit material

Fresh mango (*Mangifera indica* L., cv. Kesar) fruits were harvested at their mature stage from an orchard located in the vicinity of Karamsad village in Anand district, Gujarat, India. The fruits were transported to the research laboratory and they were graded for their uniformity in size, shape and color, and fruit without any blemishes were selected for the present study. The fruits were sanitized in 2% sodium hypochlorite solution for 5 min, and then rinsed with water and air-dried at room temperature.

### 2.2. Chemicals

Zein and gelatin were purchased from Himedia (Mumbai, India). All other chemicals and solvents were of analytical grade and obtained from Himedia, Merck and SRL (Mumbai, India).

### 2.3. Preparation of coating formulations

Zein [5% and 10% (w/v)] coating solutions were prepared by dissolving 5 g and 10 g of zein powder in 36.8 mL 95% alcohol, 35 mL of 100% isopropanol and 28.2 mL of distilled water, and the solution was heated for 10–15 min at 75 °C on a magnetic stirrer hotplate [11]. Glycerol monostearate (0.75%) was added as a plasticizer and the solution was stirred for 10 min under the same magnetic stirring conditions (model: GENEL, SLM- HP- MS- 150). Gelatin [5% and 10% (w/v)] coatings were prepared by solubilizing 5 g and 10 g of gelatin powder in 100 mL of distilled water and constantly stirring on a magnetic stirrer hotplate at 70 °C for 30 min [15]. Glycerol monostearate (0.75%) was added as a plasticizer and the solution was stirred for 10 min under the same magnetic stirring conditions and subsequently cooled at 40 °C.

### 2.4. Coating application

The fruit were randomly categorized into five groups with seventeen fruits in each

treatment and each treatment was conducted with three replicates. Four groups were categorized into one of the four coating treatments (T): zein 5% (T1), zein 10% (T2), gelatin 5% (T3) and gelatin 10% (T4), while the fifth group contained untreated fruit dipped in distilled water, designated as control or uncoated (T5). The mango fruit were subjected to the above-mentioned coating solutions for 1 min. Residual solutions of fruit were allowed to drip off and the fruit was dried at room temperature and stored at  $(32 \pm 1)$  °C and 70–75% relative humidity (RH). The coated and control fruit were evaluated for quality attributes and the activities of enzymes at the beginning of the experiment (*i.e.*, 0 days) and after (6, 12 and 18) days of their storage period. For control fruit the data were recorded only up to 12 days of the storage period, as thereafter they decayed completely.

### 2.5. Determination of weight loss

The mango samples (seven fruits per replication) were weighed at the beginning of the experiment (*i.e.*, 0 days) and at the end of each storage interval. The difference between the initial and final weight of the fruit was considered as a total weight loss and the results were expressed as the percentage loss of the initial weight as per the standard method of the AOAC [16].

### 2.6. Decay percentage

The decay percentage of coated and uncoated fruit was calculated as the number of decayed fruit divided by the initial number of all fruit multiplied by 100.

### 2.7. Soluble solids, titratable acidity and pH

The soluble solids content of the fruit was determined by using a hand refractometer (Atago Co., Tokyo, Japan). A sample was prepared by mixing the fruit in a blender. The sample was thoroughly mixed, a few drops of the filtrate were placed on the prism glass of the refractometer and a direct reading was taken by reading the

scale in the meter as described in the AOAC method [16]. The titratable acidity was determined according to the method of Mazumdar and Majumder by titration of 5 mL of juice with 0.1 N sodium hydroxide using phenolphthalein as an indicator, and the results were expressed as the percent of citric acid [17]. The pH of the fruit samples was assessed using a digital pH meter (model: ELICO, LI 120) as per the standard method described by the AOAC [16].

### 2.8. Reducing sugars, non-reducing sugars and total sugars

Fruit pulp (0.5 g) was extracted with 5 mL of 80% ethanol for 5 min and refluxed for 30 min. The sample was centrifuged at  $1000 \times g$  for 30 min. The residue was again subjected to ethanol extraction. The extract was combined and the alcohol removed by evaporation. The remaining residue was dissolved in 10 mL of distilled water. The aliquots (*i.e.*, 0.2 mL to 0.5 mL) of the supernatant were taken and the contents of reducing sugars (RS) and non-reducing sugars (NRS) were measured by following the dinitrosalicylic acid (DNS) method, employing glucose as a standard [18], and the total sugars were calculated as RS plus NRS.

### 2.9. Total phenols, ascorbic acid and total carotenoids

A fruit sample of (0.5 to 1) g was extracted with 10 mL of 80% ethanol and the extract was centrifuged at 10,000 rpm for 20 min. The clear supernatant was collected and allowed to evaporate at room temperature, and the remaining residue was dissolved in a known volume of distilled water. The aliquots (*i.e.*, 0.2 mL to 2 mL) of the supernatant were taken and total phenols were determined as per the method of Bray and Thorpe using Folin-Ciocalteu reagent [19]. The standard calibration curve was prepared by using catechol at a concentration of  $0.1 \text{ mg} \cdot \text{mL}^{-1}$  in distilled water, while ascorbic acid was determined according to the method of Roe [20], which is based on the reduction of 2, 4-dichlorophenol indophenols by ascorbic acid. Levels of ascorbic

acid were estimated based on the standard curve prepared from pure ascorbic acid. The total carotenoid content was carried out as per the methods described by Wang *et al.* [21].

### 2.10. Extraction and assay of polygalacturonase (EC 3.2.1.15) and cellulase (EC 3.2.1.4)

Fruit tissue (2 g) was homogenized in 15 mL of sodium phosphate buffer (20 mM, pH 7.0) containing cysteine-HCl (20 mM), EDTA (20 mM) and Triton X-100 (0.05%). The homogenate was filtered and centrifuged at  $15,000 \times g$  for 30 min at 4 °C in a refrigerated centrifuge (model: Eppendorf, 5430R). The clear supernatant was used as enzyme extract for assaying polygalacturonase (PG) and cellulase enzyme activities [22]. For PG activity, the reaction mixture comprised 0.2 mL sodium acetate (200 mM, pH 4.5), 0.1 mL NaCl (200 mM), 0.3 mL polygalacturonic acid (PGA), 1% aqueous solution adjusted to pH 4.5 and 0.1 mL of enzyme extract in a total volume of 1.0 mL. The reaction mixture was held at 37 °C for 1 h followed by addition of dinitrosalicylic acid. D-galacturonic acid was used as the standard and one unit of enzyme activity was defined as the amount of enzyme required to liberate 1 nmol of galacturonic acid per min under the conditions of the enzyme assay [22]. For cellulase, the reaction mixture contained sodium acetate buffer (100 mM, pH 5.0), carboxymethyl cellulose (CMC) (1.5%) and enzyme in a final volume of 1.0 mL. The cellulase activity was assayed by measuring the reducing groups released from CMC. One unit of enzyme activity was the amount of enzyme required to form 1  $\mu\text{mol}$  of reducing groups per hour, per gram of the original fresh weight sample [22].

### 2.11. Extraction and assay of pectin methyl esterase (EC 3.1.1.11)

The procedure for extraction and assay of pectin methyl esterase (PME) was adapted from Lohani *et al.* with some modifications [23]. The reaction mixture contained 1 mL

pectin solution (0.01%, pH 7.5), 0.2 mL NaCl (0.15 M), 0.1 mL bromothymol blue solution (0.01%), 0.2 mL water and 0.1 mL of enzyme extract. Absorbance was measured immediately at 620 nm and again measured after 3 min. The difference in the initial absorbance and absorbance after 3 min was the measurement of PME activity. One unit of enzyme activity was defined as the amount of enzyme required for liberating 1  $\mu\text{mol}$  of methyl ester- $\text{min}^{-1}$ .

### 2.12. Extraction and assay of $\beta$ -galactosidase (EC 3.2.1.23)

The procedure for extraction and assay of  $\beta$ -galactosidase ( $\beta$ -gal) was followed in accordance with Biswas [24]. The reaction mixture contained 0.25 mL of sodium acetate buffer (0.1 M, pH 5) and 0.01 mL of p-nitrophenyl  $\beta$ -D-galactopyranoside (PNPG) (10 mM) at 55 °C. The reaction was initiated by adding 0.74 mL of enzyme extract and incubated for 10 min; similarly, the blank was prepared without the enzyme extract, which is replaced by the buffer. The reaction was terminated by adding 4 mL of NaOH (0.1 M) and the enzyme activity was expressed as  $\mu\text{mol}$  of p-nitrophenol (PNP) formed per minute per mg protein.

### 2.13. Enzyme extraction and assay of peroxidase (EC 1.11.1.7)

Peroxidase (POD) activity was assessed by following the method described by Guilbault [25]. The reaction mixture contained 10  $\mu\text{L}$  of enzyme supernatant, 2.99 mL  $\text{H}_2\text{O}_2$  (0.03% in 0.01 M potassium phosphate buffer, pH 6.0) and 0.05 mL orthodiansidine dye-6  $\text{mL}^{-1}$  of 0.03% of  $\text{H}_2\text{O}_2$  substrate solution (1% in methanol). The reaction mixture without substrate served as a blank. The changes in absorbance were recorded at 460 nm for 1 min at the interval of 15 s. Enzyme activity was expressed as the 1 unit change in optical density per min per gram of fresh tissue.

### 2.14. Shelf life or marketable period

The shelf life of the coated and uncoated mango fruit was calculated by counting the

days required for them to attain the last stage of ripening, but up to the stage when they still remained acceptable for marketing [26].

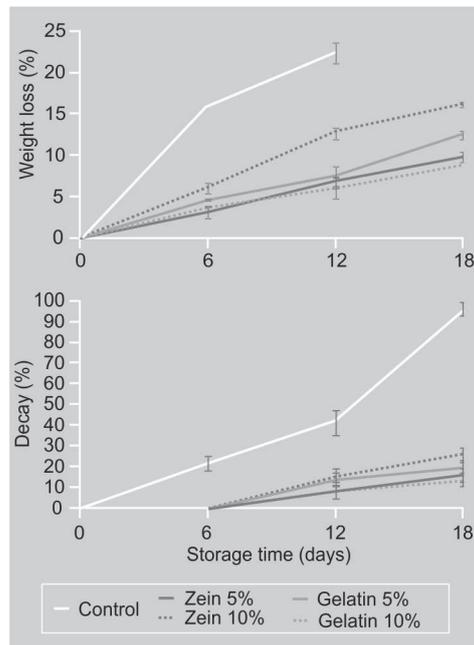
### 2.15. Statistical analysis

The experiment was conducted in a completely randomized design with three replications. All the analyses performed were carried out in triplicate and the standard deviation was calculated. Data analyses were performed by analysis of variance (ANOVA) using IRRISTAT statistical software (v. 3.1, IRRI, Manila, Philippines). Multiple comparisons among the treatments with significant differences tested with ANOVA were conducted by using the least significant difference (LSD) at the  $p < 0.05$  level. Duncan's multiple range test was used to compare the mean values at different storage intervals [27].

## 3. Results and discussion

### 3.1. Weight loss

Water loss or transpiration is considered to be the major factor that affects the storage life and postharvest quality of the majority of fruits. During our experiments, weight loss increased during the storage for all mango fruits, although a significantly ( $p < 0.05$ ) higher weight loss percentage was noted in the control fruit (*figure 1*). The weight loss of the coated mango samples was lower as compared with the control mango samples (*figure 1*). The fruits treated with zein and gelatin coatings presented a lower weight loss throughout the entire storage period as compared with the uncoated samples. At the 12th day of the storage period, the weight loss in control mango fruit was highest (*i.e.*, 22.34%); the lowest weight loss was noted in the gelatin 10%- and zein 5%-coated mango fruit, *i.e.*, 6.03% and 6.72%, respectively. Among the currently tested coatings, zein 5% and gelatin 10% coatings showed a better effect on delaying the weight loss of mango fruit at the 18th day of storage. The postharvest water loss can lead to wilting and shriveling,



**Figure 1.** Effect of zein and gelatin coatings on weight loss and decay of coated and uncoated (control) mango during its storage (vertical bar represents mean  $\pm$  standard deviation of means for three replicates).

both of which reduce the marketability of the product [28]. Further, Hassani *et al.* stated that the weight loss reduction was probably due to the effect of a semi-permeable barrier to gases and water vapor, therefore reducing respiration, enzymatic browning and water loss [28]. Our results are in agreement with those of Lim *et al.* [15], wherein gelatin-coated sweet cherry had a lower level of water loss over the storage period. According to Cipolatti *et al.*, the weight loss reduction can be due to the effect of protein-based edible coatings, which prevented the dissection of the fruits that tend to have the walls degraded and the water released, causing tissue wilting [12]. A significantly ( $p < 0.05$ ) higher weight loss was observed in zein 10%-coated mango fruit as compared with that of zein 5% coatings, that could be due to the thickness of the coatings. The coating of zein 10% was so thick that it covered the surface of the fruit; similar results were reported by Park *et al.*, who reported that tomato fruit coated with a thick coating of corn zein resulted in  $O_2$  suppression at a very low level and  $CO_2$  concentration at an excessive level, resulting in the production of ethanol [29].

### 3.2. Decay percentage

Fruits and vegetables contain a wide range of organic substrates and high water activity, and thus are good substrates for microbial infection [30]. The decay percentage was significantly ( $p < 0.05$ ) higher in the uncoated fruit, and fruit subjected to zein 10% and gelatin 10% coating treatments showed a moderate decay percentage (figure 1). Moreover, our results show that the fruit coated with zein and gelatin coatings did not show any decay percentage up to 6 days of their storage period (figure 1). The initial decay percentage in uncoated mango was 21.57%, and the incidence of decaying was increased and reached 96.08% at the 18th day of the storage period. The coatings of zein 5% and gelatin 10% significantly reduced the decay percentage during the entire storage period in comparison with control and other coating treatments. The deterioration of fruits and vegetables during storage is mainly due to moisture loss and wilting. The application of coating solution reduced the decay percentage by providing a barrier which protects fruits and vegetables against excessive transpiration [31], and our results are supported by the findings of Bai *et al.*, who found that 'Gala' apples coated with zein coatings maintained their quality and reduced the decay percentage as compared with the uncoated samples [11].

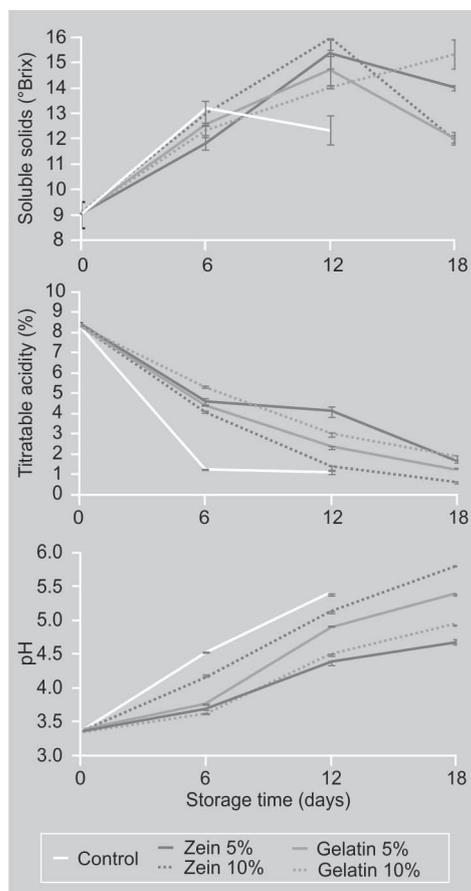
### 3.3. Concentration of soluble solids

The results of our study revealed that the concentration of soluble solids (SS) in mango fruit increased during the storage period (figure 2). Treatment with the zein and gelatin coatings reduced the increase in the concentrations of SS. The increase in SS concentration was extremely pronounced in the control samples compared with those of coated samples. In control samples, the highest SS concentration, *i.e.*, 13.2 °Brix, was noted at the 6th day of the storage period; after that, it declined and reached 12.3 °Brix at the 12th day of the storage period. All the coated samples showed a

gradual increment in SS concentration at the end of the storage period, except the zein 10%-coated samples. The treatments of zein 5% and gelatin 10% coating showed a beneficial effect on maintaining the SS accumulation at a slower level as compared with the uncoated (control) and other coating treatments. During ripening the increase in the SS content is due to starch hydrolysis and pectin degradation [12]. Sugars and organic acids of the fruits are the main substrates consumed by respiration during storage. Edible coatings have a strong effect on decreasing the metabolic processes, and finally reducing the respiration rates [32]. Our results are in accordance with those of Cipolatti *et al.*, who found that protein-phenolic-based edible coatings retarded pectin degradation and fruit ripening in tomato fruit [12].

### 3.4. Changes in titratable acidity and pH

Titratable acidity (TA) of coated and uncoated mango fruit decreased markedly during the entire storage period (*figure 2*). The results of the present study revealed that pH increased as TA decreased during the storage time. The TA of the coated mangoes decreased with the storage time but to a lesser extent than that of uncoated fruit. TA was significantly higher and pH was significantly lower in the zein- and gelatin-coated mango samples as compared with those of uncoated samples (*figure 2*). Among the applied coatings, zein 5%, gelatin 5% and gelatin 10% were the most effective coatings in maintaining the TA at a higher level and pH at a lower level as compared with control, while the zein 10% coating also had a higher TA and lower pH but with less evident results. At the 12th day of storage, the loss of TA in uncoated fruit was around 87% whilst TA in zein 5%-coated mangoes decreased by 50%. With regard to the coating treatments, gelatin 10% was more effective in retaining the TA at a higher level. As Echeverria and Valich reported that TA is directly related to the concentration of organic acids present in the fruits, the level of TA declining during storage might be due to the metabolic changes or to the use of

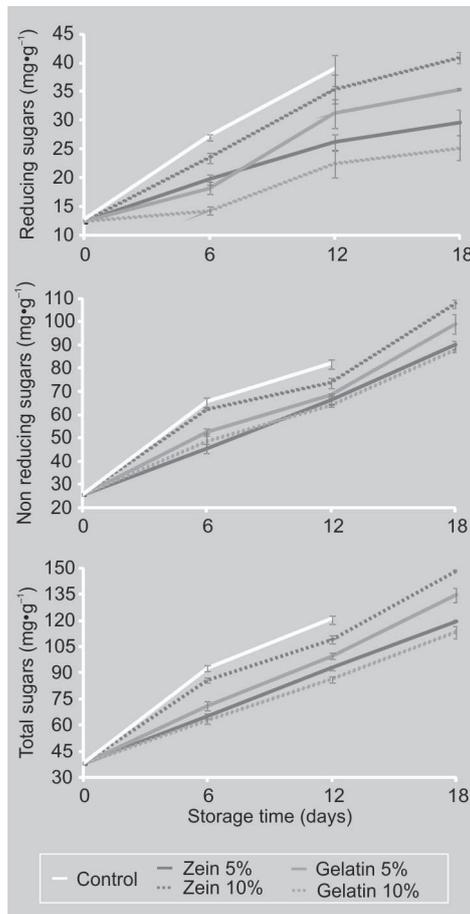


**Figure 2.** Effect of zein and gelatin coatings on soluble solids, titratable acidity and pH of coated and uncoated (control) mango during its storage (vertical bar represents mean  $\pm$  standard deviation of means for three replicates).

organic acid in the respiratory process [33]. In this regard it is also considered that the rate of respiration reduced by edible coatings may delay the utilization of organic acids present in the fruit [34]. Coating slowed the changes in pH and TA in banana fruit by effectively delaying fruit senescence, as reported by Gol and Rao [35]. Similar findings were obtained by Aguiar *et al.*, who reported that mango fruit treated with galactomannan coating retained TA at a higher level as compared with uncoated samples [36].

### 3.5. Changes in reducing sugars, non-reducing sugars and total sugars

Our results revealed that there was a significant ( $p < 0.05$ ) increase in the reducing sugar (RS), non-reducing sugar (NRS) and



**Figure 3.** Effect of zein and gelatin coatings on reducing sugars, non-reducing sugars and total sugars of coated and uncoated (control) mango during its storage (vertical bar represents mean  $\pm$  standard deviation of means for three replicates).

total sugar (TS) contents of coated as well as uncoated mango fruits during the storage period (figure 3). However, the rate of increase in RS, NRS and TS contents was significantly ( $p < 0.05$ ) lower in coated samples as compared with control samples. The maximum amount of RS ( $38.66 \text{ mg}\cdot\text{g}^{-1}$ ), NRS ( $81.72 \text{ mg}\cdot\text{g}^{-1}$ ) and TS ( $120.39 \text{ mg}\cdot\text{g}^{-1}$ ) contents was noted in the control samples at their 12th day of storage. The reducing sugar content was found to be higher in uncoated mango fruit, which might be due to a decrease in the acidity as a result of physiological changes and rapid conversion of starch to sugars as a result of moisture loss, as previously reported by Wills and Rigney [37]. In the case of coated samples, a significantly ( $p < 0.05$ ) slower increase in RS, NRS and TS contents were noted with respect to that of control, that might be due to their slower ripening rate. This view concurs with the results of Abbasi *et al.*, who

studied the effect of chitosan coatings on postharvest quality of mango fruit and observed that the RS, NRS and TS contents were less in the coated samples [38]. Among the tested coating treatments, zein 5% and gelatin 10% were the most effective in delaying RS, NRS and TS accumulation in mango fruit. These results are in agreement with those of Li and Yu, who concluded that RS content was significantly affected by coatings and/or films [39]. At the 18th day of the storage period, mango fruit coated with gelatin 10% showed a lower amount of RS, *i.e.*,  $25.21 \text{ mg}\cdot\text{g}^{-1}$ , NRS, *i.e.*,  $88.04 \text{ mg}\cdot\text{g}^{-1}$  and TS, *i.e.*,  $113.25 \text{ mg}\cdot\text{g}^{-1}$  with respect to the other tested coatings. Maqbool *et al.* cited that, during ripening, the fruit texture is changed due to alteration in the cell wall structure and the degradation of starch and resultantly bound carbohydrate fractions, especially pectic substances and hemicelluloses [40]. The present results were similar to the results on 'cat Hoa loc' mangoes where the authors studied the effect of different coatings and observed that the contents of RS and TS were lower in the coated fruit with respect to those of control fruits [41].

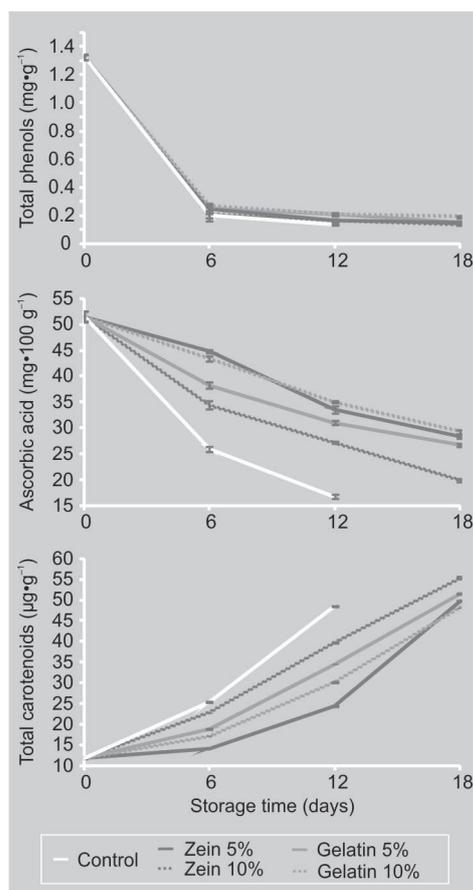
### 3.6. Changes in total phenols and ascorbic acid

Total phenols and ascorbic acid content decreased with increasing storage time in both coated and control samples (figure 4). The fruits coated with zein and gelatin coatings showed significant ( $p < 0.05$ ) improvement in the retention of total phenols and ascorbic acid with respect to that of control fruit. Mango fruit treated with zein 5% and gelatin 10% had a higher content of total phenols than either of the other coating treatments. All the coated fruit retained their phenolic content up to the 18th day of their storage period but, among them, gelatin 10% was superior in maintaining the total phenol content at a higher level, *i.e.*,  $0.19 \text{ mg}\cdot\text{g}^{-1}$ . Phenolics are considered as biologically active components and widely distributed in fruits and vegetables; they have the ability to scavenge free radicals, superoxide and hydroxyl radical by a single electron transfer [42]. Phenolic compounds

have an important role in fruit quality maintenance in terms of color, taste, aroma and flavor; in this regard, those coated fruit with a higher phenolic content would have a higher quality than control fruit [43]. The ascorbic acid content of zein- and gelatin-coated mango samples was noted to decrease with time but to a lesser extent than that of uncoated fruit. The maximum reduction in ascorbic acid values was observed in the control fruit ( $16.77 \text{ mg} \cdot 100 \text{ g}^{-1}$ ) at the 12th day of the storage period, while the least was noted in the zein- and gelatin-coated fruit. Ascorbic acid retention was significantly affected by the zein and gelatin coatings. Zein 5% and gelatin 10% coating treatments showed a more pronounced effect in maintaining the ascorbic acid content at a higher level, *i.e.*,  $28.43 \text{ mg} \cdot 100 \text{ g}^{-1}$  and  $29.26 \text{ mg} \cdot 100 \text{ g}^{-1}$ , respectively, in comparison with other coatings. The loss of ascorbic acid can be greatly favored by the presence of  $\text{O}_2$ . The coating formulations may reduce  $\text{O}_2$  diffusion and that can be attributed to a slow ripening rate, and consequently better preservation of ascorbic acid contents and delayed senescence [44]. Our results are consistent with those of Abbasi *et al.* obtained in chitosan-coated mango fruit [38].

### 3.7. Changes in total carotenoids

The carotenoid content gradually increased during storage in both coated and uncoated mango fruit (figure 4). The highest increase in carotenoids was observed in uncoated mangoes followed by zein 10%-coated fruit, while the mango fruit coated with zein 5%, gelatin 5% and gelatin 10% delayed their carotenoid accumulation throughout the storage. A significant ( $p < 0.05$ ) increase in carotenoid content was observed at regular intervals in both coated as well as uncoated mangoes. As the concentration of gelatin increased, the level of carotenoids decreased. Uncoated mango fruit showed their maximum amount of carotenoids at the 12th day of storage, while the coated mango samples maintained their carotenoid accumulation until the 18th day of storage, with a subsequent increase in their accumulation. It is possible that the coated fruits retained their

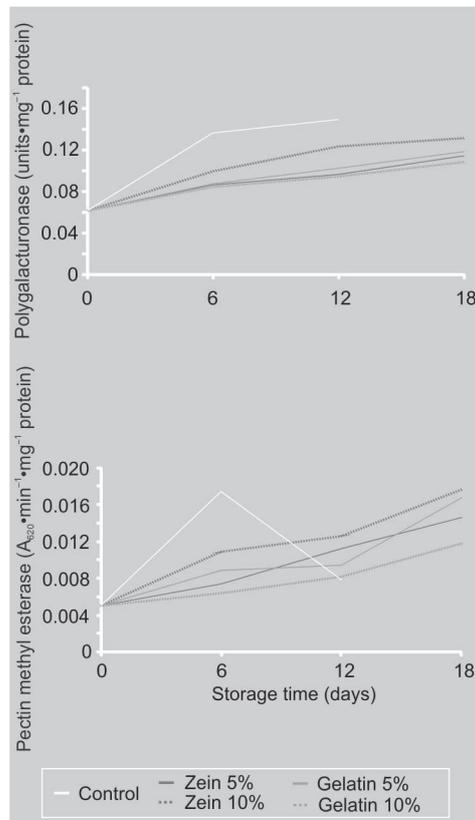


**Figure 4.** Effect of zein and gelatin coatings on total phenols, ascorbic acid and total carotenoids of coated and uncoated (control) mango during its storage (vertical bar represents mean  $\pm$  standard deviation of means for three replicates).

chlorophylls for a longer period, thereby reducing the carotenoid synthesis. In agreement with these findings, Ali *et al.* reported that coating application provided a barrier against gas exchange and production of ethylene, and therefore delayed the ripening process [45]. Similar results were observed in the tomato fruit when treated with corn zein coating and delayed the color change [46].

### 3.8. Activities of polygalacturonase and pectin methyl esterase

Usually, tissue softening and the loss of firmness of fruit are mainly due to the degradation of cell wall components, *e.g.*, pectins, and destruction of the middle lamella structure [47]. Enzymes such as polygalacturonase (PG), pectin methyl esterase (PME), cellulase and  $\beta$ -galactosidase ( $\beta$ -gal) have been known to have active



**Figure 5.** Effect of zein and gelatin coatings on polygalacturonase and pectin methyl esterase of coated and uncoated (control) mango during its storage (vertical bar represents mean  $\pm$  standard deviation of means for three replicates).

roles in the softening of cell walls [47, 48]. The results of our study showed that in both coated as well as control fruit, the activity of PG and PME enzymes increased significantly ( $p < 0.05$ ) during their storage period, but the activity of these enzymes was lower in the coated samples (figure 5). Control mango fruit showed the greatest increase in PG activity during the entire storage period and maximum activity, *i.e.*,  $0.15 \text{ units}\cdot\text{mg}^{-1}$  protein, was seen at the 12th day of the storage period, while the fruits treated with gelatin 10% and zein 5% coatings exhibited lower activities, *i.e.*,  $0.095 \text{ units}\cdot\text{mg}^{-1}$  protein and  $0.096 \text{ units}\cdot\text{mg}^{-1}$  protein, respectively. In this regard, Ruoyi *et al.* stated that partial inactivation of PG could result in reduced pectin solubilization and slower softening [49].

Pectin methyl esterase (PME) is also another important enzyme involved in textural changes [50]. In our experiments, the PME activity in control samples increased and peaked at the 6th day of storage and

after that a gradual declining trend was seen in the activity (figure 5). At the 6th day of storage, the highest and lowest PME activities were noted in the control and coated samples, respectively. Among the tested coatings, the samples coated with zein 5% and gelatin 10% maintained their PME activity at a lower level throughout the storage as compared with the zein 10% and gelatin 5% coatings. Pectins, the main constituents of the middle lamella and primary cell wall of the fruit, are hydrolyzed by PME to generate demethylated pectins that can be more easily hydrolyzed by PG, thus causing the depolymerization of pectins [51, 52]. The cell wall modifications result in loss of water, which is also considered as the important criterion for texture changes in fruits and vegetables [14]. Edible coatings create a semipermeable barrier around the fruit, modifying the internal atmosphere by reducing  $\text{O}_2$  and/or elevating  $\text{CO}_2$  concentrations. In view of this, Aguilar-Mendez *et al.* stated that low  $\text{O}_2$  and high  $\text{CO}_2$  concentrations reduce the fruit enzymatic activity, resulting in better firmness retention of fruit [14]. Similar to our results, lower activities of polygalacturonase and pectinesterase are reported to contribute to the enhanced retention of brittleness and firmness during storage of shellac-coated pears [51].

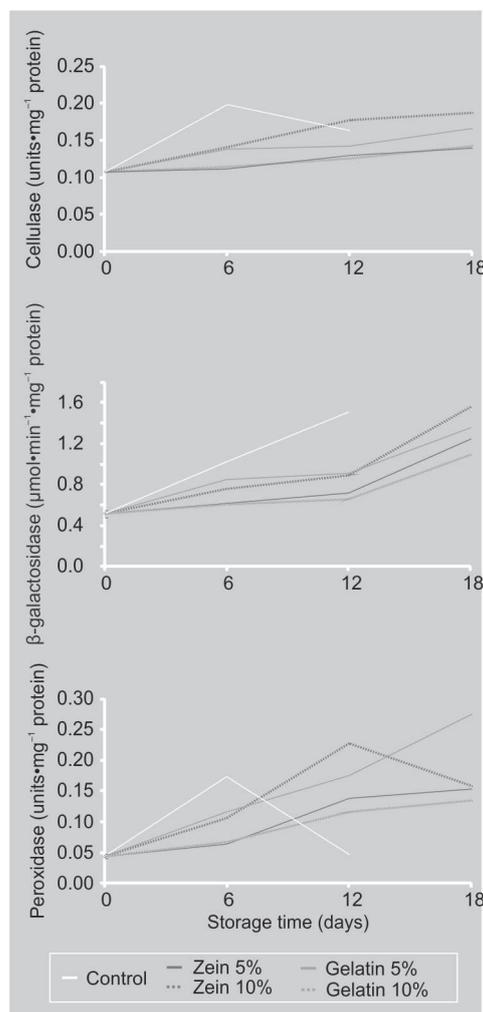
### 3.9. Activities of cellulase and $\beta$ -galactosidase

During ripening, cellulase plays an important role in fruit softening and it has a different effect on softening changes in different fruits [51]. The cellulase and  $\beta$ -galactosidase ( $\beta$ -gal) activity in the zein- and gelatin-coated mango fruits was significantly ( $p < 0.05$ ) lower than that in the control fruit during their storage period (figure 6). Maximum cellulase activity (*i.e.*,  $0.20 \text{ units}\cdot\text{mg}^{-1}$  protein) was noted at the 6th day of the storage period and thereafter a decreasing trend was seen at the 12th day of storage. Mango fruit treated with zein and gelatin coatings maintained a constant increasing behavior in the cellulase activity. Among the selected coatings, gelatin 10% and zein 5% were superior in inhibiting the

cellulase activity at a greater level. The results from our study are in agreement with those of Zhou *et al.*, where pear fruit treated with edible coating had lower cellulase activity as compared with that of control fruit [51]. Our results showed the overall response of  $\beta$ -gal activity in coated as well as control mango fruit (figure 6). They indicated that  $\beta$ -gal activity was higher in the control samples throughout the storage period. Control fruit showed a 73% increase in  $\beta$ -gal activity up to 12 days of storage; however, a significant ( $p < 0.05$ ) difference was observed among the coating treatments. Treatment with gelatin 10% and zein 5% coatings dramatically inhibited this activity at the end of the storage period and was found to be effective in maintaining the  $\beta$ -gal activity at a lower level. These results were similar to those obtained in papaya, in which the coating treatments inhibited the  $\beta$ -gal activity and maintained it at a lower level [50].

### 3.10. Changes in peroxidase activity

Peroxidase (POD) is an important oxyradical detoxification enzyme in plant tissue; it is considered to be associated with the defensive system and may be related to the multiple changes affecting the texture, flavor and color of fruits and vegetables [53]. In our experiments, the POD activity in all the samples increased gradually, and the activity was higher in the control samples with respect to that of coated samples (figure 6). The POD activity in the control fruit increased from 0 days to 6 days of storage and thereafter started to decline, while all the coated samples showed a continuous increment throughout the storage period, except the zein 10% coating. During the 12 days of storage, the fruit coated with zein and gelatin showed higher induction of POD activity and maintained it at the end of the storage period. Normally in plants the antioxidant enzyme activity is increased in response to stress, and a decrease in enzymatic potential may be associated with a reduction in the capacity to prevent damage [44]. In our study, coating treatment delayed the senescence process of mango fruit by inducing higher POD activity, and these results were in correlation with those



**Figure 6.** Effect of zein and gelatin coatings on cellulase,  $\beta$ -galactosidase and peroxidase of coated and uncoated (control) mango during its storage (vertical bar represents mean  $\pm$  standard deviation of means for three replicates).

obtained in pear fruit treated with shellac coating which showed better membrane integrity due to higher POD activity [51].

### 3.11. Shelf life or marketable period

The shelf life of mango fruit was extended significantly ( $p < 0.05$ ) with all the currently tested edible coatings. During the storage period, the fruits treated with zein 5% and gelatin 10% coatings had a longer shelf life, *i.e.*, 23 days, followed by gelatin 5% and zein 10% coatings, *i.e.*, 21 days and 19 days, respectively. Uncoated mango fruit maintained their shelf life up to only 14 days. Among the tested coating treatments, zein 5% and gelatin 10% were effective and superior in extending the mango shelf life for a

longer period. Similar results were observed in tomato fruit treated with zein coating and avocado fruit treated with gelatin-starch coating [11, 14]. The positive effect of coating on shelf life could be due to modifying the atmosphere (MA), and reducing moisture loss and surface wounding, as well as reducing a variety of diseases [11]. The MA created can delay the ripening process by delaying ethylene production and reducing the internal oxygen level, consequently extending the shelf life of fruits [54].

#### 4. Conclusions

In conclusion, our study showed that the zein and gelatin coatings could delay the ripening process of mango fruit. Changes in weight loss, soluble solids, titratable acidity, pH, total sugar content and total carotenoids were positively affected by zein and gelatin coatings. In addition, the edible coatings showed a positive effect on keeping the total phenols and ascorbic acid at a higher level as compared with the control. Samples treated with zein 5% and gelatin 10% coatings showed a lower percentage of the decay rate. The application of zein 5% and gelatin 10% coatings also maintained the higher induction of defense-related peroxidase enzyme and lower activities of softening enzymes such as polygalacturonase, pectin methyl esterase, cellulase and  $\beta$ -galactosidase. In light of these results, the use of zein 5% and gelatin 10% could be a good alternative in maintaining the quality attributes and extending the shelf life of mango fruit during storage.

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### **Influencia de un revestimiento de zeína y gelatina en la calidad postcosecha y en el aumento de la duración de vida del mango (*Mangifera indica* L.).**

**Resumen – Introducción.** El mango es el fruto tropical más importante en el plano económico y nutricional; posee un gran valor comercial, pero es muy perecedero. Su sensibilidad frente a las enfermedades postcosecha y a los daños físicos limita su comercialización. Las pérdidas postcosecha de los frutos son un grave problema, dado su rápido deterioro durante la manutención, el transporte y el almacenamiento. Las películas de envasado y los revestimientos comestibles pueden emplearse potencialmente como una técnica de conservación para prolongar la duración de vida de los frutos. **Material y métodos.** Se evaluó, en intervalos regulares, durante el periodo de su almacenamiento, la influencia de revestimientos a base de zeína y gelatina en las características físico-químicas, en el reblandecimiento y en la actividad de las enzimas antioxidantes de mangos almacenados a  $(32 \pm 1)$  °C, en relación con los mangos no revestidos. **Resultados y discusión.** Los revestimientos a base de zeína y gelatina parecieron tener un impacto benéfico en el retraso de la evolución de la pérdida de peso, de los azúcares solubles, de la acidez valorable, del pH, de los azúcares y carotenoides totales. Los revestimientos de zeína y gelatina dieron lugar a una mejor retención de contenidos de ácido ascórbico y compuestos fenólicos, en relación con la del testigo. Los revestimientos a base de zeína y gelatina atrasaron la maduración de los mangos mediante supresión de la actividad de las enzimas de reblandecimiento, tales como la poligalacturonasa, la pectina metil esterasa, la celulasa y la  $\beta$ -galactosidasa. Los revestimientos del 5 % de zeína y del 10 % de gelatina mantuvieron la mayor inducción de la enzima peroxidasa ligada a la defensa; les siguieron los revestimientos del 5 % de gelatina y del 10 % de zeína. **Conclusión.** Los revestimientos del 5 % de zeína y del 10 % de gelatina pueden aplicarse para atrasar la maduración, mantener las características de calidad y prolongar la duración de conservación del mango durante su almacenamiento.

**India / *Mangifera indica* / frutas / aptitud para la conservación / film comestible / zeína / gelatina**

