

## COMPOSITE COATING MEDIATED IMPROVEMENT OF NUTRITIONAL QUALITY AND SAFETY OF FRESH-CUT PINEAPPLE (ANANAS COMOSUS L. MERRIL)

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### ABSTRACT

*In the present work, the efficacy of three different composite coatings in maintaining quality and storability of fresh-cut pineapple at 5°C was evaluated. The coatings consisted of sodium alginate (1.29%), carrageenan (0.5%) and xanthan gum (0.25%) as polysaccharides, enriched with olive oil (0.1%) as a lipid component and cinnamic acid (0.1%) as an antibrowning agent. During 16 days of storage, the coated fresh-cut pineapples displayed better retention in all their physicochemical characteristics like firmness, color, weight loss, ascorbic acid, total phenolic content (TPC), carotenoids, and antioxidant activity during 16 days of their storage as compared to that of uncoated samples. The coated fresh-cut pineapples displayed lower specific activity of browning-related enzymes as compared to that of untreated samples. Sensory analysis indicated that alginate coated fresh-cut pineapples have better textural and visual properties than that of uncoated samples throughout the storage period. Thus, the developed composite coating formulation can be considered as useful in fresh produce industry to enhance the shelf-life of healthy and convenient fresh-cut fruit.*

*Key words: Edible coating, Fresh-cut, Pineapple, Quality, Shelf-life, Safety.*

### INTRODUCTION

The regular consumption of fruit and vegetables has significant impact in maintaining good health as evidenced by several epidemiological studies [1]. Among many tropical and subtropical fruits, pineapple is one of the important fruit crops due to presence of several health promoting compounds such as carotenoids, vitamin C, vitamin B1 and B6, manganese and dietary fibers. However, pineapple requires preparation like removal of thorny inedible peel, crown and cutting before being consumed and therefore fresh-cut produce industries have shown interest to launch its minimally processed product. It has considerable demand in the fresh-cut market due to its exotic flavor, attractive appearance and convenient nature. But as compared to whole fruit, the faster metabolic activity of fresh-cut produce renders rapid deterioration of its overall quality due to browning, weight loss and microbial contamination which ultimately shortens their storage life.

Edible coating may prevent moisture and solute migration, respiration and oxidative reaction rates and thereby extend the shelf-life

of fresh-cut fruits and vegetables [2]. It is still challenging to develop edible coating formulations with desirable characteristics for application on fresh-cut fruits or vegetables because coatings may be dissolved and absorbed by the wet surfaces instead of drying to form a smooth and unique layer [3]. So far there is no such edible coating material which possesses all the functional properties required to slow down rapid ripening or deteriorative processes have been identified.

The development of composite coatings combining hydrocolloids (protein and polysaccharides) and lipids has emerged as one of the advanced approaches for fresh and minimally processed fruit preservation [4]. Composite coatings can be applied as a monolayer in a single step or multilayer in more than one step. In a monolayer composite edible coating, the lipid is dispersed in the hydrophilic phase (protein or polysaccharides solution) so as to obtain an emulsified coating formulation [5].

Polysaccharides such as sodium alginate, carrageenan, chitosan, pectin, gellan gum, hydroxypropylmethylcellulose (HPMC) and xanthan gum possess an excellent film forming ability against gas and solute migration

between food products and the environment [6]. In addition, these can act as the polymeric matrices for the incorporation of bioactive compounds (antimicrobial and antioxidant agents), nutraceuticals, minerals and probiotics [7]. However, most of the polysaccharides are hydrophilic in nature, limiting their moisture barrier property. In order to improve the water retention ability of edible coating formulation, the incorporation of lipid component, especially plant derived may help reduce water loss from the fresh-cut fruits due to their hydrophobic nature. Olive oil is a vegetable oil derived from fruit of olive tree (*Olea europaea* L.). It is a kind of glycerolipid with three fatty acids, mainly oleic acid and palmitic acid, attached to a glycerol backbone. It is also rich in antioxidants like vitamin E and polyphenols. Cinnamic acid, an aromatic carboxylic acid, has been reported as an effective polyphenol oxidase (PPO) inhibitor [8, 9] and approved as Generally Recognized as Safe (GRAS) substances for its use as food additive (FDA, 2013).

In the past decade, several research efforts have been attempted to design the composite coating for extending the storability of fresh and fresh-cut fruit. For instant, Dave et al. [11] observed that composite coating of soy protein, HPMC and olive oil have improved functionality as semipermeable barrier for both moisture and gaseous exchange and thereby extended shelf-life of 'Babughosha' pear fruit. Rao et al. [12] reported the effectiveness of alginate-olive oil based composite coating enriched with antioxidant in enhancing the storage life and nutritional quality of *Ziziphus mauritiana* var. Gola fruit. Similarly, Baraiya et al. [13] also demonstrated the enhanced postharvest shelf-life of carambola fruit along with improved surface appearance by using alginate-olive oil composite coating. Likewise, the incorporation of olive oil into gum arabic formulation reduces the weight loss percentage in coated citrus fruit as compared to uncoated fruit [14]. Olivas et al. [15] reported that application of methylcellulose-stearic acid based edible coating reduced the weight loss of

fresh-cut pear fruit. The application of pectin-sunflower oil edible coatings helped maintain firmness and moisture content of fresh-cut melon [16]. Hence, the application of composite edible coating on fresh and fresh-cut fruit has potentiality to overcome the drawbacks associated with only lipid or protein/polysaccharide based edible coatings [17].

In line with these reports, the present study was conducted to evaluate three different composite edible coatings [sodium alginate (1.29%), carrageenan (0.5%) and xanthan gum (0.25%) as polysaccharides, blended with olive oil (0.1%) as a lipid component and cinnamic acid (0.1%) as an antibrowning agent] for quality improvement of fresh-cut pineapple fruit.

## MATERIALS AND METHODS

### Fruit material

Pineapple (*Ananas comosus* L. Merrill cv. Smooth Cayenne) fruits were purchased from a wholesale fresh produce distributor in fruit market of Anand, Gujarat. The fruits at maturity stage 4 (40 – 80% of eyes yellow) were selected based on the uniformity in shape, size, peel color and free from any damage.

### Preparation of composite coatings formulation

Sodium alginate-based edible coating formulation was prepared according to the method described by Azarakhsh et al. [18]. Sodium alginate powder (1.29 %, w/v) was dissolved in distilled water while heating it on a stirring hot plate at 70°C until it became homogenous solution and glycerol (1.18%, v/v) was added as a plasticizer. Carrageenan solution was prepared by dissolving 0.5 g carrageenan powder in 100 mL of sterilized distilled water and glycerol was added into it at the concentration of 0.75 g/g carrageenan. Similarly, xanthan gum based edible coating solution was prepared by dissolving 0.25 g of xanthan powder into 100 mL of distilled water on magnetic stirrer at 60°C, followed by addition of glycerol (1%, v/v). Cinnamic acid (0.1 %, w/v) as antibrowning agent and olive oil

(0.1 %, v/v) as lipid component were added into each coating solution and stirring was continued on magnetic stirrer until formation of clear solution. Calcium chloride (2 %, w/v) solution was used as a cross-linker which allows the gelling of alginate and carrageenan based coatings over the cut surface of pineapple.

### **Application of composite coatings**

After removal of pineapple crown leaves, the fruits were surface sterilized with sodium hypochlorite solution (0.2 mL L<sup>-1</sup>, pH 6.5) for 10 min and then rinsed with distilled water. The fruits were manually peeled and both end parts were disposed off and the central region was sliced (0.03±0.005 m thickness) and each slice was cut in truncated cone format weigh of each cone was ~10 g. Then, pineapple pieces were dipped for 3 min into each composite coating formulation. The excess solution was drained off for 3 min on tissue paper. The control was set by dipping cut pineapple in distilled water for same duration. Both coated and uncoated samples were packed in food grade clamshell of 2.5 × 10<sup>-4</sup> m<sup>3</sup> (0.10 m × 0.05 m × 0.05 m) containing 300 g of cut chunks of pineapple in each box. For every treatment and control, three boxes were prepared and stored at 5 °C±1 °C and 95 % relative humidity (RH) to follow the changes in their quality characteristics at the regular intervals of 4 days during storage period. The details of edible coatings treatments given to the fresh-cut pineapple fruits are given in Table 1.

## **EVALUATION OF QUALITY PARAMETERS**

### **Color**

The color changes of fresh-cut pineapple fruits during storage were measured in terms of color coordinates, lightness (*L*<sup>\*</sup>) and blue to yellowness (*b*<sup>\*</sup>) values. The digital images of fresh-cut pineapple fruits were captured using digital camera (FinePix S2950, FUJIFILM, Japan) and analyzed in Adobe Photoshop CS 8.0 software (Adobe Systems,

Inc., San Jose, CA, USA) by pointing the cursor at different areas of images [19].

### **Firmness**

The firmness of fruit samples was recorded with fruit pressure tester (FT-327, FACCHINI srl, Alfonsine, Italy) applying force with an 11 mm flat-bottomed probe and penetrated up to 5 mm into the flesh. The force required to penetrate the probe into fruit tissue is expressed in terms of Newton (N).

### **Weight loss percentage (WLP)**

The change in weight loss was analyzed as per the method described by Bico et al. [20]. Fruit samples from each replicate were placed into previously tarred Petri dish and were dried at 70°C for 48 h. After drying, the Petri dish was put in a desiccator to cool to room temperature. The weight was recorded before and after drying by using an analytical balance (Shimadzu BW 380 H, Tokyo, Japan). Weight loss was calculated according to the formula:

$$\text{Weight loss (\%)} = 100 - \left[ \frac{100 \times \text{DM (\%)}_{\text{Day 0}}}{\text{DM (\%)}_{\text{Day N}}} \right]$$

DM (%)<sub>Day 0</sub> and DM (%)<sub>Day N</sub> stand for dry matter on day 0 and dry matter on day N, respectively.

### **Bioactive compounds and antioxidant activity**

Estimation of ascorbic acid was performed as per the method of Roe and Oesterling [21]. The amount of ascorbic acid was estimated as per the standard curve prepared using L-ascorbic acid and expressed as milligram per 100 gram fresh weight (mg . 100 g<sup>-1</sup>fresh weight). Total carotenoids were measured using the method described by Tomes [22] and was expressed as microgram per gram fresh weight (µg .g<sup>-1</sup>). Total phenolic content was determined by following Folin-Ciocalteu's method described by Lim et al. [22]. The different concentrations of gallic acid were used to prepare standard curve and expressed in milligrams gallic acid equivalent per gram fresh weight (mg g<sup>-1</sup>). The DPPH free

radical scavenging activity was carried out as per the method described by Brand-Williams et al. [24]. The obtained results were expressed as percentage of inhibition of the DPPH radical.

### **Browning-related enzymes**

#### **Extraction and assay of Polyphenol oxidase (PPO) and Peroxidase (POX) activity**

Two grams fruit pulp was homogenized in 25 mL of sodium phosphate buffer (0.1 mol L<sup>-1</sup>, pH 6.5). The homogenate was centrifuged for 30 min at 20,627 × g for PPO and 29,703 × g for POX at 4 °C. The supernatant was collected and used as crude enzyme extracts. Assay for PPO activity was carried out by incubating 0.1 mL enzyme extract with 2.5 mL of catechol (0.5 mol L<sup>-1</sup> in sodium phosphate buffer 0.1 mol L<sup>-1</sup>, pH 6.5) and change in absorbance at 420 nm was recorded at the interval of 30 s up to 3 min from the time the enzyme extract was added. The specific activity of PPO was expressed in Units min<sup>-1</sup> mg<sup>-1</sup> protein. One unit of PPO activity was defined as a change of 0.001 in absorbance per minute [25].

POX activity was assayed as per the procedure described by Mazumdar and Majumder [26]. The substrate ortho-dianisidine and hydrogen peroxide was reacted with enzyme extract at 30 °C, followed by addition of sulphuric acid after 5 min of incubation to stop the reaction and optical density (OD) was measured at 430 nm. The specific activity of POX was expressed in Unit min<sup>-1</sup> mg<sup>-1</sup> protein. One unit of POX was defined as a change of 1.0 in absorbance per min.

#### **Extraction and assay of Phenylalanine ammonia lyase (PAL) activity**

PAL activity was assayed according to the method described by Malik and Singh [27]. One gram fruit tissue was extracted in sodium borate buffer (0.1 mol L<sup>-1</sup>, pH 8.8) and the homogenate was centrifuged at 14000 × g for 20 min at 4°C. The supernatant was collected and used as crude enzyme extract. The reaction

mixture consisted of 0.2 mL L-phenylalanine (0.1 mol L<sup>-1</sup>) with 3.2 mL of sodium borate buffer (0.1 mol L<sup>-1</sup>, pH 8.8), and 0.2 mL enzyme extract at 37 °C for 2 h. The product released was measured at 290 nm. The PAL activity was expressed in Unit min<sup>-1</sup> mg<sup>-1</sup> protein, where one unit is defined as micromole of cinnamic acid released per hour (μM h<sup>-1</sup>).

### **Sensory evaluation**

Sensory evaluation of fresh-cut pineapple fruit was performed according to the method described by Oms-Oliu et al. [16] to determine the overall acceptability by assessing color, taste, odor and texture during 16 days of storage. Samples of fresh-cut pineapple fruits were randomly presented to the fourteen non-trained panelists consisting of students and researchers and they rated the quality attributes on the basis of a nine-point hedonic scale: 9 = excellent; 7 = good; 5 – fair; 3 - poor and 1 - unusable. A score of 6 was considered the limit of market acceptability.

### **Microbial analysis**

Samples were examined for microbial contamination by enumeration of total aerobic bacteria and yeasts and moulds colonies. Serial dilutions of samples were prepared by washing vigorously 10 g tissue with 90 mL of 1 g L<sup>-1</sup> sterile buffered peptone water in sterilized round bottom tubes at room temperature and inoculated over plate count agar (PCA) at 35 °C for 48 h and potato dextrose agar (PDA) supplemented with 0.05 g L<sup>-1</sup> chloramphenicol at 21 °C for 5 - 7 days, respectively by the spread plate method [28]. After incubation, the number of colonies were counted and expressed as log colony forming units per gram fresh weight (log CFU g<sup>-1</sup> FW).

### **Statistical analysis**

A completely randomized design was used with three replications. Statistical analysis was performed using GraphPad Prism software version 3 (GraphPad Software, Inc, San Diego, USA). Data of all analysis were expressed as mean ± standard deviation. Analysis of

Variance (ANOVA) followed by Tukey's multiple comparison post-hoc test for multiple comparisons was used to assess the statistical differences among means ( $p < 0.05$ ).

## RESULTS AND DISCUSSION

### Changes in Color

The effect of composite edible coatings on the changes in color attributes in terms of  $L^*$  and  $b^*$  values of fresh-cut pineapple fruit is presented in Table 2. The results revealed that uncoated samples had greater decline in  $L^*$  and  $b^*$  values and reached to their minimum values i.e., 42.38 and 16.75 units, respectively during 16 days of storage period. Gil et al. [29] also observed the changes in the  $b^*$  coordinate of approx. 10 units in pineapple pieces stored at 5°C after 9 days of storage. In the study, the color change in fresh-cut pineapple occurred due to decrement of  $b^*$  values through their storage period. CG and XG coated samples have higher  $L^*$  values i.e., 51.5 and 50 units than that of AG coated samples with 46.44 units, but their lower  $b^*$  values i.e., 20.38 and 19.13 units, respectively at the end of storage are responsible for their degraded color quality as compared to the AG coated samples with 26.56 units. This indicated that AG retained better color quality than XG and CG coated samples which have negative effect on color attributes during storage period of 16 days. Similarly, Benítez et al. [30] reported that alginate coatings of MD2 pineapple wedges helped retain better  $L^*$  value as compared to chitosan and antioxidants treated samples after 15 days of storage.

### Changes in Firmness

Texture is critical quality attribute that determines market acceptability of fruits and vegetables by consumer. The direct contact with the atmospheric oxygen accelerate loss of firmness in fresh-cut fruits since oxidative damage cause a reduction in membrane integrity, cellular leakage and flooding of intercellular spaces [31]. With the increase of storage period, there was a progressive decline in firmness of coated and uncoated fresh-cut

pineapple fruits (Table 2). The uncoated samples had significant loss in firmness ~60% during 8 days; whereas the application of edible coating emulsions showed only 25% - 30% decline in firmness compared to their initial values. During 16 days of storage period, the control samples showed ~71% firmness loss reaching to its least value, while the loss in firmness was reduced by 10% - 15% in coated fresh-cut pineapple, but no significant difference was observed for the change in firmness of fresh-cut pineapple among the applied treatments. Calcium ions play a critical role in keeping the alginate chains together through ionic interactions after the formation of hydrogen bonds between the chains, which produce a gel with a three-dimensional network structure. The results obtained in the present investigation are in line with that obtained by Mantilla et al. [32] who found that the fresh-cut pineapple coated with 1% and 2% alginate had better firmness than that of uncoated fruit samples during 15 days of storage at 4° C.

### Changes in Weight loss percentage (WLP)

There was a significant increment ( $p < 0.05$ ) in WLP for both coated and uncoated samples during storage (Table 2). Interestingly, the observed WLP among treated samples ranged from 5% - 7%, while it was 16% for uncoated samples during 4 days of storage. By the end of storage period, AG coated samples exhibited least WLP (~16%), followed by CG coated samples (~24%), while it was almost similar for XG and uncoated samples (37%). This indicates that the performance and physicochemical properties of the coating formulations of different polysaccharides varies according to their molecular characteristics such as molecular weight, degree of blanching, conformation, electrical charges and hydrophobicity [33]. It can be inferred that the decrease in weight loss of alginate coated fresh-cut pineapple fruits might be related to its better ability for semipermeable layer formation over the cut surface than that of carrageenan and xanthan gum based edible coatings. Furthermore, the calcium chloride

treatment followed by edible coating application might have resulted in cross linking of alginate rendering it insoluble and thus enhanced water barrier property, as reported by Olivás et al. [34] in minimally processed ‘Gala’ apples.

#### **Changes in Ascorbic acid (AA)**

Klein [35] documented that ascorbic acid oxidation increases to a much greater extent on cutting during extended storage, which could be due to enzymes such as ascorbic acid oxidase. Gil et al. [29] also documented a considerable degradation in ascorbic acid in fresh-cut pineapple chunks with respect to whole unprocessed pineapple stored for 6 days at 5 °C. The coated samples displayed slight reduction i.e., ~8%, ~4% and ~1% for AG, CG and XG, respectively in vitamin C content up to 8 days of storage period and thereafter the higher concentrations i.e., 84.02±5.42, 92.01±1.05 and 96.19±2.08 mg g<sup>-1</sup> in AG, CG and XG, respectively as compared to its initial value (67.36±1.59 mg g<sup>-1</sup>) at the end of storage (Table 3). The accumulation of vitamin C consistently increased in uncoated samples with extend of storage time, and reached to its peak value i.e., 105.21±4.77 mg g<sup>-1</sup> at the end of storage. The higher concentrations of vitamin C in coated and uncoated fresh-cut pineapple fruits could be due to increased water loss with the advance of storage period rather than the biosynthesis of vitamin C [36].

#### **Changes in Carotenoids**

In the present study, minimal processing of pineapple lead to reduction of more than 70% of carotenoids during storage period of 12 days. The amount of carotenoids noted initially was 9.97±0.13 mg g<sup>-1</sup>, which eventually declined in both coated and uncoated fresh-cut pineapple as the time of storage increased and reached to its least concentration at the end of analysis (Table 3). The decline of carotenoids was also reported by Gil et al. [29]. However, AG coated samples had insignificant change in carotenoids up to 8 days of evaluation time and thereafter exhibited

diminishing pattern with ~43% lower amount relative to its initial concentration as compared to ~70% decline in CG, XG coated and uncoated samples at the end of storage. This beneficial effect of alginate coating may be because of its better film-forming ability, thereby created a protective barrier against oxidative reactions over the fruit surface.

#### **Changes in Total phenolics (TP) content**

The amount of TP content of coated and uncoated fresh-cut pineapple fruits at 5°C initially was 3.49±0.29 mg g<sup>-1</sup> which accumulated in all the samples at certain time of storage period depending upon the metabolic activity of that particular sample (Table 3). For eg., uncoated samples had significant (p<0.05) increment of ~17% on 4<sup>th</sup> day of storage, whereas coated samples experienced maximum accumulation of ~21% on 8<sup>th</sup>, 12<sup>th</sup> and 16<sup>th</sup> days of storage in samples treated with XG, CG and AG, respectively. At the end of storage time, uncoated samples displayed highest TP content i.e., 4.62±0.16 mg g<sup>-1</sup>, whereas the least (3.30±0.07) was depicted in XG coated samples. This faster TP accumulation in control samples as compared to the treated fruits could possibly be attributed to enhanced oxidative stress in uncoated samples as a result of minimal processing. Surjadinata and Cisneros-Zevallos [37] demonstrated that increasing the wound stress intensity in tissues of three carrot cultivars lead to the enhanced accumulation of phenolic compounds. Among applied edible coatings, AG coated samples had slower extent of TP accumulation during 16 days of storage period.

#### **Changes in Antioxidant activity**

Oxidative stress due to cutting cause membrane damage and altering the composition and concentration of bioactive compounds mainly phenolics and vitamin C, resulting in changes in the total antioxidant activity of fresh-cut fruits and vegetables [38]. The results of antioxidant activity based on the DPPH assay are presented in Table 3. The initial antioxidant activity reported in fresh-cut pineapple fruits was 58.54±3.80 %. Uncoated



samples had highest antioxidant activity (~93%) on 4<sup>th</sup> day of storage but exhibited gradual decline in it till the end of storage. XG coated samples displayed the peak antioxidant activity (~78%) on 8<sup>th</sup> day, but eventually decreased to ~34% at the end of storage. However, there was significant ( $p < 0.05$ ) reduction of antioxidant activity on 4<sup>th</sup> and 8<sup>th</sup> day of storage in CG and AG coated samples respectively; however, the activity increased on 12<sup>th</sup> day and again decreased, reaching to least antioxidant activity (23% - 32%) at the end of storage period. The study on the fresh-cut pineapple fruit showed that the antioxidant activity increased during initial days of storage which subsequently declined towards the end of storage. The greater rise of antioxidant activity indicates the higher stressed condition within the tissue, as a result of cutting of fruit. As the storage time increases, the reduction may be attributed to the loss of bioactive antioxidants due to their reactions with free radicals to mitigate the consequence of minimal processing. According to Stewart et al. [39], the decrease in antioxidant capacity with prolonged storage may be due to the O<sup>2</sup>- promoted oxidation of the constitutive phenolic compounds and vitamin C.

### **Changes in Browning-related enzymes** **Polyphenol oxidase (PPO) activity**

Enzymatic browning in fresh-cut fruits and vegetables occur as a result of the oxidation of phenolic compounds, catalyze by PPO and POX enzymes [40]. At 0 day of storage, PPO activity was  $173.20 \pm 44.82$  Units  $\text{min}^{-1} \text{mg}^{-1}$  protein (Fig. 1a). With the advance of storage period, PPO activity enhanced significantly ( $p < 0.05$ ) in all the samples. In control samples, PPO reached to its peak level i.e.,  $696.76 \pm 62.48$  Units  $\text{min}^{-1} \text{mg}^{-1}$  protein on 4<sup>th</sup> day of storage and thereafter declined at the end of storage. The coated samples had 10% - 18% lower PPO activity as compared to that of control on 4<sup>th</sup> day of storage (Fig. 1a); thereafter, AG coated samples showed least fluctuation in PPO activity till the end of storage, while in CG and XG coated samples, it increased up to 12 days

of storage. At the end of storage, coated samples displayed lower PPO activity which ranged from  $361.34 \pm 24.18$ –  $399.49 \pm 115.49$  Units  $\text{min}^{-1} \text{mg}^{-1}$  protein as compared to  $521.65 \pm 27.98$  Units  $\text{min}^{-1} \text{mg}^{-1}$  protein in control samples. This significant variation regarding PPO activity pattern in coated and uncoated samples may be due to the incorporation of cinnamic acid in composite coating formulation.

### **Peroxidase (POX) activity**

The POX activity also exhibited declining behavior throughout the storage period in AG and CG coated samples while XG coated samples showed reduction up to 8 days and then increased significantly at the end of storage period (Fig. 1b). POX activity measured before processing was  $0.26 \pm 0.03$  Units  $\text{min}^{-1} \text{mg}^{-1}$  protein. Application of AG and CG coated help diminishing the activity ~65% during 16 days of storage period. In case of uncoated samples, though POX activity declined on 12<sup>th</sup> day but at the end it rose suddenly reaching to its peak level equivalent to its initial activity. This trend could be explained by the fact that incorporation of cinnamic acid as in alginate and carrageenan solution maintained its effective amount over the cut surface of pineapple, thereby prevented induction of oxidative stress condition.

### **Phenylalanine ammonia lyase (PAL) activity**

As a response to wounding, the PAL activation of the phenylpropanoid metabolism could be elicited through induced reactive oxygen species, which in turn initiates the accumulation of phenolic compounds during storage [41]. PAL activity measured at the time of processing was highest i.e.,  $4.56 \pm 0.41$  Units  $\text{min}^{-1} \text{mg}^{-1}$  protein, but it decreased subsequently in all the samples reached to its minimum level with highest reduction ranged from ~61% - 75% at 12<sup>th</sup> day of storage period (Fig. 1c). After significant reduction on 12<sup>th</sup> day of storage, PAL activity had slightly increased at the end of storage in AG, CG and control

samples, while XG coated samples did not exhibit further induction in PAL activity. In the present study, the accumulation trend of TP is not correlated with the changing behavior of PAL activity during storage period of 16 days.

### Sensory evaluation

The sensory evaluation was carried out based on the color, taste, texture, odor and overall acceptability of coated and uncoated samples. The sensory properties were evaluated after 6 and 12 days of storage period. The score for all sensory characteristics were significantly ( $p < 0.05$ ) higher in coated samples as compared to those of the control samples (Table 4). Among the coated samples, the application of alginate based edible coating on fresh-cut pineapple retained higher scores for all the sensory traits on 6<sup>th</sup> day of storage. After 12 days of storage period, the score values for color, texture and odor declined and reached close to the limit of market acceptability. Both XG and control samples were observed with significant decline in their sensory traits and these reached to the limit of market acceptability on 6<sup>th</sup> day, earlier than that of AG and CG coated samples.

### Microbial contamination

The effect of polysaccharide based composite coatings on total plate counts and yeast and mould counts of fresh-cut pineapple fruits were evaluated after 6 and 12 days of storage (Table 5). It was revealed that total plate count increased on 6<sup>th</sup> and 12<sup>th</sup> day of storage in all the samples but remained in the range from  $3.02 \pm 0.03$  –  $4.21 \pm 0.01$  log CFU/g. However, yeasts and mold growth were found below the detection level throughout the storage period of 16 days in all the coated and uncoated samples. This beneficial effect might be result of incorporation of cinnamic acid as bioactive compound with antibrowning and antimicrobial activity.

### CONCLUSION

The results obtained from this investigation indicated that the application of

composite coatings enriched with cinnamic acid on fresh-cut pineapple fruits maintained color, firmness, retained ascorbic acid content, reduced the weight loss, delayed the increase in PPO and POX of coated samples as compared to those of the uncoated samples. In addition, the results of the present study also demonstrated that the proliferation of mesophilic bacteria and yeasts and molds was significantly lesser in coated samples in comparison with that of uncoated samples. Thus, it can be concluded that alginate-based composite coating could be used for the quality improvement and shelf-life extension of fresh-cut pineapple fruits up to 16 days of storage at 5°C.

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**Table 1: Details of composite edible coatings applied on fresh-cut pineapple**

Sr. No.	Treatments	Edible coating composition
1.	AG	Sodium alginate (1.29 %) + Olive oil (0.1%) + Cinnamic acid (0.1%)
2.	CG	Carrageenan (0.5 %) + Olive oil (0.1%) + Cinnamic acid (0.1%)
3.	XG	Xanthan gum (0.25 %) + Olive oil (0.1%) + Cinnamic acid (0.1%)
4.	Control	Distilled water

**Table 2: Changes in color ( $L^*$  and  $b^*$  values), firmness and weight loss percentage (WLP) in fresh-cut pineapple treated with composite edible coatings during storage at 5°C.**

Treatments	Storage period (Days)				
	0	4	8	12	16
$L^*$					
AG	57.29±1.70 <sup>a</sup>	56.67±2.69 <sup>a</sup>	57.33±4.09 <sup>a</sup>	57.25±3.92 <sup>a</sup>	46.44±3.94 <sup>b</sup>
CG	57.29±1.70 <sup>a</sup>	58.25±2.49 <sup>a</sup>	57.14±3.63 <sup>a</sup>	53.86±4.30 <sup>b</sup>	51.50±2.33 <sup>c</sup>
XG	57.29±1.70 <sup>b</sup>	52.13±3.48 <sup>c</sup>	58.63±4.03 <sup>b</sup>	62.67±3.44 <sup>a</sup>	50.00±2.56 <sup>d</sup>
Control	57.29±1.70 <sup>a</sup>	51.57±3.05 <sup>b</sup>	42.57±5.26 <sup>c</sup>	40.89±5.60 <sup>d</sup>	42.38±3.78 <sup>c</sup>
$b^*$					
AG	48.71±2.98 <sup>a</sup>	42.67±4.85 <sup>b</sup>	42.44±5.73 <sup>b</sup>	43.63±4.81 <sup>b</sup>	26.56±4.03 <sup>c</sup>
CG	48.71±2.98 <sup>a</sup>	45.63±3.46 <sup>b</sup>	39.29±3.35 <sup>d</sup>	41.57±2.22 <sup>c</sup>	20.38±2.50 <sup>e</sup>
XG	48.71±2.98 <sup>a</sup>	40.25±3.65 <sup>c</sup>	28.25±3.85 <sup>d</sup>	42.83±3.43 <sup>b</sup>	19.13±4.82 <sup>e</sup>
Control	48.71±2.98 <sup>a</sup>	23.86±4.67 <sup>b</sup>	25.43±4.20 <sup>c</sup>	18.67±8.22 <sup>d</sup>	16.75±6.58 <sup>e</sup>
Firmness (Newton)					
AG	3.47±0.25 <sup>a</sup>	2.63±0.21 <sup>b</sup>	2.34±0.05 <sup>b</sup>	1.79±0.10 <sup>c</sup>	1.28±0.07 <sup>c</sup>
CG	3.47±0.25 <sup>a</sup>	2.70±0.20 <sup>b</sup>	2.62±0.39 <sup>b</sup>	1.50±0.10 <sup>c</sup>	1.29±0.08 <sup>c</sup>
XG	3.47±0.25 <sup>a</sup>	2.43±0.25 <sup>b</sup>	2.30±0.20 <sup>b</sup>	1.70±0.10 <sup>c</sup>	1.20±0.20 <sup>c</sup>
Control	3.47±0.25 <sup>a</sup>	2.07±0.15 <sup>b</sup>	1.38±0.14 <sup>c</sup>	1.03±0.25 <sup>c</sup>	1.00±0.10 <sup>c</sup>
Weight loss percentage (%)					
AG	0.00±0.00 <sup>d</sup>	5.07±0.14 <sup>c</sup>	4.90±0.42 <sup>c</sup>	11.97±0.09 <sup>b</sup>	15.86±0.51 <sup>a</sup>
CG	0.00±0.00 <sup>d</sup>	7.37±0.03 <sup>c</sup>	8.57±0.06 <sup>c</sup>	13.43±0.09 <sup>b</sup>	23.90±0.34 <sup>a</sup>
XG	0.00±0.00 <sup>e</sup>	6.73±0.15 <sup>d</sup>	16.30±0.04 <sup>c</sup>	18.97±0.09 <sup>b</sup>	37.42±0.03 <sup>a</sup>
Control	0.00±0.00 <sup>d</sup>	15.70±0.43 <sup>c</sup>	32.93±0.42 <sup>b</sup>	37.37±0.11 <sup>a</sup>	36.30±0.33 <sup>a</sup>

Means within the row represented by different superscript letters are significantly different at  $p < 0.05$  using Tukey's Multiple Comparison Test. The values represented (a-d) in the results indicated the range from higher to lower rank. AG – Sodium alginate, CG - Carrageenan and XG – Xanthan gum.

**Table 3: Changes in ascorbic acid, carotenoids, total phenolics and antioxidant activity in fresh-cut pineapple treated with composite edible coatings during storage at 5°C.**

Treatments	Storage period (Days)				
	0	4	8	12	16
<b>Vitamin C (mg.100g<sup>-1</sup>)</b>					
<b>AG</b>	67.36±1.59 <sup>c</sup>	63.19±9.85 <sup>d</sup>	61.80±6.33 <sup>e</sup>	73.26±2.76 <sup>b</sup>	84.03±5.41 <sup>a</sup>
<b>CG</b>	67.36±1.59 <sup>c</sup>	68.75±0.60 <sup>c</sup>	64.58±2.17 <sup>d</sup>	77.78±9.68 <sup>b</sup>	92.01±1.05 <sup>a</sup>
<b>XG</b>	67.36±1.59 <sup>c</sup>	67.71±4.17 <sup>c</sup>	66.67±10.05 <sup>d</sup>	70.83±2.08 <sup>b</sup>	96.18±10.49 <sup>a</sup>
<b>Control</b>	67.36±1.59 <sup>c</sup>	81.94±2.62 <sup>d</sup>	87.85±5.74 <sup>c</sup>	109.38±3.61 <sup>a</sup>	105.21±4.77 <sup>b</sup>
<b>Carotenoids (µg.g<sup>-1</sup>)</b>					
<b>AG</b>	9.97±0.13 <sup>b</sup>	10.23±0.96 <sup>a</sup>	9.91±0.16 <sup>b</sup>	6.69±0.08 <sup>c</sup>	5.64±0.00 <sup>d</sup>
<b>CG</b>	9.97±0.13 <sup>a</sup>	9.27±0.36 <sup>a</sup>	5.29±0.19 <sup>b</sup>	3.47±0.06 <sup>c</sup>	3.00±0.08 <sup>c</sup>
<b>XG</b>	9.97±0.13 <sup>a</sup>	6.09±0.19 <sup>b</sup>	5.56±0.11 <sup>c</sup>	3.17±0.19 <sup>d</sup>	2.87±0.02 <sup>e</sup>
<b>Control</b>	9.97±0.13 <sup>a</sup>	8.75±0.16 <sup>b</sup>	5.91±0.10 <sup>c</sup>	5.15±0.16 <sup>c</sup>	3.04±0.04 <sup>d</sup>
<b>Total phenolics content (mg.g<sup>-1</sup>)</b>					
<b>AG</b>	3.49±0.29 <sup>b</sup>	3.97±0.25 <sup>b</sup>	4.04±0.03 <sup>a</sup>	4.21±0.21 <sup>a</sup>	4.42±0.14 <sup>a</sup>
<b>CG</b>	3.49±0.29 <sup>b</sup>	3.46±0.39 <sup>b</sup>	3.97±0.01 <sup>b</sup>	4.43±0.23 <sup>a</sup>	3.96±0.11 <sup>b</sup>
<b>XG</b>	3.49±0.29 <sup>b</sup>	3.39±0.07 <sup>b</sup>	4.44±0.16 <sup>a</sup>	4.14±0.38 <sup>a</sup>	3.30±0.07 <sup>b</sup>
<b>Control</b>	3.49±0.29 <sup>b</sup>	4.24±0.26 <sup>a</sup>	3.72±0.25 <sup>b</sup>	3.55±0.12 <sup>b</sup>	4.62±0.16 <sup>a</sup>
<b>Antioxidant activity (%)</b>					
<b>AG</b>	58.54±3.80 <sup>b</sup>	49.64±1.75 <sup>c</sup>	49.15±1.62 <sup>c</sup>	85.45±8.11 <sup>a</sup>	31.96±3.88 <sup>c</sup>
<b>CG</b>	58.54±3.80 <sup>c</sup>	48.82±3.93 <sup>d</sup>	63.92±6.59 <sup>b</sup>	73.42±5.21 <sup>a</sup>	26.91±1.78 <sup>e</sup>
<b>XG</b>	58.54±3.80 <sup>c</sup>	79.79±1.68 <sup>b</sup>	83.58±3.52 <sup>a</sup>	78.34±2.52 <sup>b</sup>	22.79±3.66 <sup>d</sup>
<b>Control</b>	58.54±3.80 <sup>d</sup>	92.55±1.43 <sup>a</sup>	79.29±2.48 <sup>b</sup>	68.94±4.16 <sup>c</sup>	33.59±1.89 <sup>e</sup>

Means within the row represented by different superscript letters are significantly different at  $p < 0.05$  using Tukey's Multiple Comparison Test. The values represented (a-d) in the results indicated the range from higher to lower rank. AG – Sodium alginate, CG - Carrageenan and XG – Xanthan gum.



**Table 4: Changes in sensory attributes in fresh-cut pineapple treated with composite edible coatings after 6 and 12 days of storage at 5°C.**

Treatments	6 <sup>th</sup> day of storage				
	Color	taste	texture	odor	overall acceptability
AG	8.03±0.50 <sup>a</sup>	8.27±0.25 <sup>a</sup>	7.73±0.40 <sup>a</sup>	7.90±0.36 <sup>a</sup>	7.98±0.25 <sup>a</sup>
CG	7.27±0.25 <sup>a</sup>	8.17±0.29 <sup>a</sup>	7.80±0.26 <sup>a</sup>	7.63±0.15 <sup>a</sup>	7.72±0.17 <sup>a</sup>
XG	7.37±0.12 <sup>a</sup>	7.80±0.26 <sup>a</sup>	7.60±0.36 <sup>a</sup>	7.17±0.15 <sup>a</sup>	7.48±0.17 <sup>a</sup>
Control	2.83±0.29 <sup>b</sup>	5.50±0.50 <sup>b</sup>	4.67±0.29 <sup>b</sup>	3.77±0.68 <sup>b</sup>	4.19±0.39 <sup>b</sup>
Treatments	12 <sup>th</sup> day of storage				
	Color	taste	texture	odor	overall acceptability
AG	6.67±0.29 <sup>a</sup>	7.53±0.25 <sup>a</sup>	5.17±0.15 <sup>a</sup>	6.27±0.25 <sup>a</sup>	6.41±0.09 <sup>a</sup>
CG	5.83±0.29 <sup>b</sup>	7.67±0.32 <sup>a</sup>	4.67±0.29 <sup>b</sup>	6.07±0.12 <sup>b</sup>	6.06±0.05 <sup>b</sup>
XG	3.93±0.40 <sup>c</sup>	3.27±0.25 <sup>b</sup>	3.00±0.00 <sup>c</sup>	4.83±0.29 <sup>c</sup>	3.76±0.12 <sup>c</sup>
Control	1.00±0.20 <sup>d</sup>	0.00±0.00 <sup>c</sup>	1.07±0.12 <sup>d</sup>	0.83±0.15 <sup>d</sup>	0.73±0.04 <sup>d</sup>

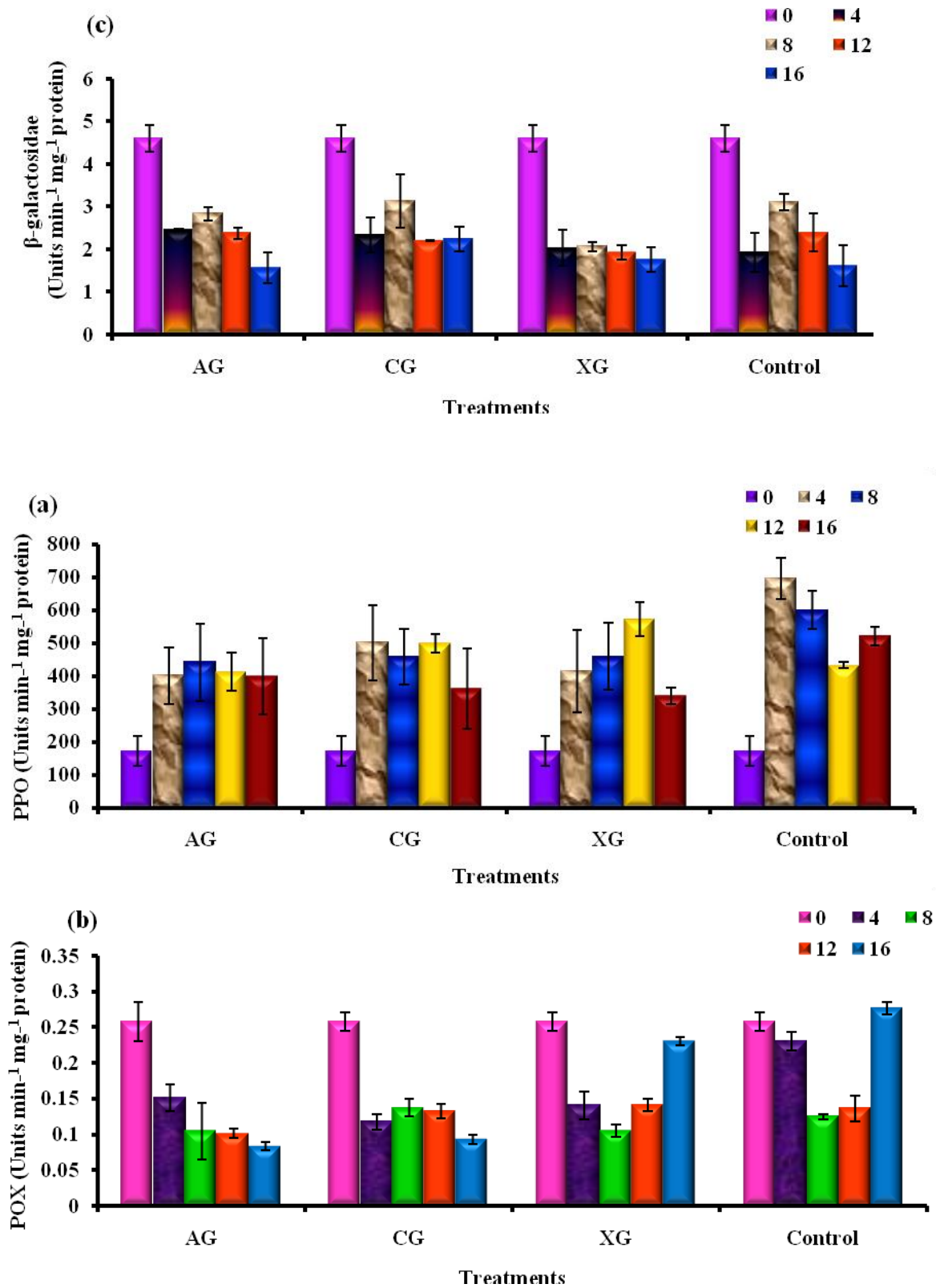
Means within the column represented by different superscript letters are significantly different at  $p < 0.05$  using Tukey's Multiple Comparison Test. The values represented (a-d) in the results indicated the range from higher to lower rank. AG – Sodium alginate, CG - Carrageenan and XG – Xanthan gum.

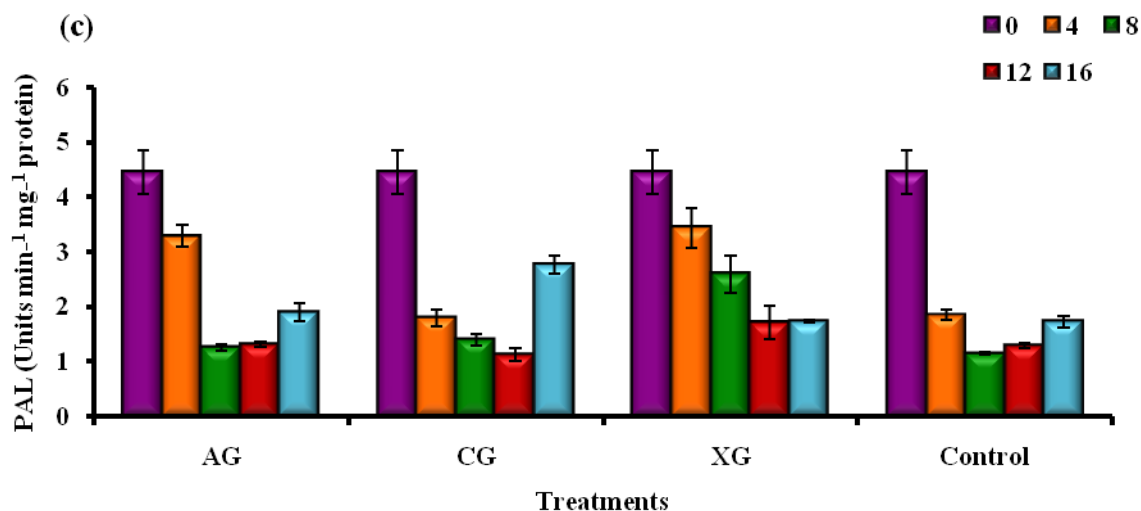
**Table 5: Microbial contamination in fresh-cut pineapple treated with composite edible coatings after 6 and 12 days of storage at 5°C.**

Treatments	Storage period (Days)		
	0	6	12
<b>Total plate count (log CFU/g)</b>			
AG	3.02±0.03 <sup>b</sup>	3.48±0.02 <sup>ab</sup>	4.07±0.03 <sup>a</sup>
CG	3.02±0.03 <sup>b</sup>	3.51±0.09 <sup>ab</sup>	3.79±0.06 <sup>a</sup>
XG	3.02±0.03 <sup>a</sup>	3.56±0.08 <sup>a</sup>	3.67±0.05 <sup>a</sup>
Control	3.02±0.03 <sup>b</sup>	3.92±0.04 <sup>ab</sup>	4.21±0.01 <sup>a</sup>

**Yeasts and molds\***

Means within the row represented by different superscript letters are significantly different at  $p < 0.05$  using Tukey's Multiple Comparison Test. The values represented (a-d) in the results indicated the range from higher to lower rank. AG – Sodium alginate, CG - Carrageenan and XG – Xanthan gum. \*Below the detection level.





**Figure 1** Changes in specific activity of (a) Polyphenol oxidase (PPO), (b) Peroxidase (POX) and (c) Phenylalanine ammonia lyase (PAL) in fresh-cut pineapple treated with composite edible coatings during storage at 5°C. Here, AG – Sodium alginate, CG - Carrageenan and XG – Xanthan gum.