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Enhancing fresh-cut pineapple shelf-life: exploring the impact of ultrasonic-homogenized alginate coatings

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ABSTRACT

Purpose: This study investigates the influence of ultrasonic-homogenized alginate-based coatings on the quality and shelf-life of fresh-cut pineapple. As preservation of fresh-cut produces is still a challenging task for both producers and distributors. **Research method:** Alginate emulsion was prepared using sodium alginate, distilled water and glycerol. The primary emulsion was formulated by adding olive oil, citral and cinnamic acid into sodium alginate solution and subjected to ultrasonic homogenization for varying durations (0, 20, 40, and 60 minutes) at a fixed amplitude of 20 kHz. Changes in physicochemical properties, microbial activity, browning enzymes and sensory evaluation were studied after application of emulsions followed by storage under refrigeration. **Findings:** The viscosity of the resulting solutions decreased significantly from 110.84±25.67 mPa·s to 17.75±1.59, 10.41±0.54, and 7.74±0.39 mPa·s, respectively, while the average droplet diameter decreased to approximately 300 nm, indicating a shift from heterogeneous to homogeneous particle size distribution. Application of alginate-based coating effectively preserved freshness of pineapple, maintaining color and firmness, minimizing hydrogen peroxide and malondialdehyde accumulation and reducing weight loss percentage. Furthermore, it retained levels of ascorbic acid and total phenolics, delayed enzymatic activities such as polyphenol oxidase, peroxidase and phenylalanine ammonia-lyase and inhibited growth of mesophilic bacteria, yeasts and molds compared to control and primary emulsion coated samples. **Research limitations:** There were no limitations. **Originality/Value:** The study extended shelf-life of fresh-cut pineapple up to 12 days when stored at 5°C±1°C. The findings underscore the efficacy of ultrasonic-homogenized alginate coatings in improving coating solution homogeneity, thereby enhancing functionality as an edible coating for fresh-cut pineapple preservation.

INTRODUCTION

Pineapple (*Ananas comosus* L.) is a rich source of vitamins, dietary fibers, fructose, flavonoids, calcium and potassium (Haqbeen et al., 2019). Many supermarkets, restaurants and even street vendors have started the marketing of fresh-cut pineapple alone or in mixed fruit salad. Generally, small scale vendors and restaurants prepare these as fresh-cut products on demand basis because pre-processing and subsequent storage may induce biochemical alteration like browning, loss of texture and reduce the shelf-life (Namin et al., 2021) and may cause significant changes in their nutritional and sensory quality. Therefore, the preservation of fresh-cut produce is still a challenging task for both producers and distributors. Factors such as variety, cutting techniques, packaging materials, storage etc. are the main causes affecting the commercialization of nutritious and convenient fresh-cut pineapple (Xing et al., 2022).

Emulsion-based edible coatings technology is considered as a valuable alternative to improve fresh-cut fruit and vegetable quality. Antimicrobial edible coatings are emerging approaches to improve safety and quality of fresh-cut fruit and vegetables. Polysaccharide based edible coatings such as sodium alginate, carboxymethyl cellulose; carrageenan, starch, etc. have been found effective in reducing the rate of respiration, weight loss, texture along with acting as carrier for additives like antioxidants and antimicrobials. Due to the hydrophilic nature, alginate adheres well to the cut surface of fruit or vegetables through cross-linking of its molecules with calcium ions (Metha et al., 2024). Most recently, the enrichment of edible coatings with plant-derived food additives including vegetable oils and essential oils, or their compounds is emerging as an advanced strategy to enhance the safety against microbial growth of fresh and fresh-cut produces (Yousuf et al., 2021). The plant-derived compounds possess the antimicrobial and antioxidant activity due to their high content of secondary metabolites, mainly phenolic compounds, iso-flavonoids, terpenes, ketones, aliphatic alcohols, acids, and aldehydes (Uslu & Ozcan, 2024). Citral, a mixture of geranial and neral is an active component of lemongrass (*Cymbopogon citrates*) essential oil. The edible coating enriched with citral has demonstrated its ability in quality retention of raspberry (Guerreiro et al., 2015) and citrus fruit (Fan et al., 2014). Cinnamic acid is an aromatic carboxylic acid and well known for its antimicrobial activity. Moreover, it has also been reported as an effective anti-browning agent on fresh-cut pears (Sharma & Rao, 2015). Currently, the nanoemulsions have emerged as promising tool for the entrapment of lipophilic functional ingredients in aqueous media to improve their mixing which may facilitate the controlled release of bioactive agents during storage of food products (Akhter et al., 2024). Among various approaches, the homogenization using probe sonicator can potentially disrupt the droplets up to nanoscale by high frequency sound waves up to 20 kHz. The size reduction of emulsion droplets enhances their functionality, improves optical transparency and physical stability (Thirukumaran et al., 2023). Several studies have demonstrated the utility of nanoemulsions in enhancing the shelf-life and quality of fruits and vegetables. Prakash et al. (2019) documented that the alginate incorporated with 0.5% citral nanoemulsion coated fresh-cut pineapple maintained color, gaseous exchange and microbial growth during storage. Edible composite coatings of pectin, polyphenylene alcohol, and salicylic acid improved the shelf life of grapes (Loay et al., 2021). Similarly, Javanmardi et al. (2023) prolonged the shelf life of strawberries by application of nanoemulsion of essential oils from plants.

From the aforesaid accounts, it can be inferred that emulsification of edible coating solution incorporated with antimicrobials to prepare micro or nanoemulsion would enhance its functional attributes like transparency, adherence and antimicrobial activity which in turn protect fresh-cut fruit and vegetables from oxidative and microbial decay. Therefore, the

present investigation will examine the influences of ultrasonication of alginate solution incorporated with citral and cinnamic acid on its physical properties and efficacy for quality improvement of fresh-cut pineapple during their storage under refrigeration.

MATERIALS AND METHODS

Fruit Material

The fruit of pineapple (*Ananas comosus* L. Merril cv. Smooth Cayenne) were purchased from a wholesale fresh produce distributor in fruit market. The fruit at maturity stage 4 (40 – 80% of eyes yellow) were selected with uniform shape, size, peel color and free from any physical injuries.

Chemicals and reagents

Sodium alginate, cinnamic acid, calcium proprionate were procured from Himedia Laboratories Private Limited, India. Tween 80 and citral were purchased from Sisco Research Laboratories Private Limited, India and Sigma-Aldrich Corporation, India, respectively through local chemical dealers. Olive oil (Figaro brand) was purchased from local market of Gujarat, India.

Preparation of alginate-based edible coating

The optimized sodium alginate solution was prepared by dissolving sodium alginate powder (1.29%) in distilled water at 70 °C under magnetic stirring until the formation of clear solution, followed by the addition of glycerol (1.16%) as a plasticizer (Azarakhsh et al., 2012). The primary emulsion (E0) was formulated by adding olive oil (0.1%), citral (0.05%) and cinnamic acid (0.05%) into sodium alginate solution under magnetic stirrer at room temperature. Tween 80 (0.1%) was used as non-ionic surfactant. Thereafter, the primary emulsion was sonicated using 20 kHz Sonicator (Ultrasonics, USA) at 25 °C. Sonicator probe was symmetrically dipped into primary emulsion and at fixed amplitude of 30 µm for three different durations i.e. 20 min (E20), 40 min (E40) and 60 min (E60), in which each cycle consisted of 30 s pulses on and 30 s pulses off. Energy input was given through sonotrode equipped with a piezoelectric crystal having 13 mm in diameter. Calcium propionate (2% w/v) solution was used to carry out cross-linking reaction of sodium alginate molecules over the cut surface of pineapple.

Minimal processing and application of treatments

All the utensils, cutting boards, knives and containers used for fruit processing were sanitized with 95% ethanol to reduce microbial contamination. The crown leaves were removed and washed with tap water. After washing, fruits were disinfected with sodium hypochlorite solution (0.2 mL L^{-1}, pH 6.5) for 10 min, followed by rinsing in distilled water and air dried. After air drying at room temperature, fruit were peeled and extreme ends were disposed of. The central region was sliced (2.0 ± 0.5 cm thickness) and each slice was cut in truncated cone format weight of a single cone was ~10 g. Edible coating treatment was given by dipping of pineapple wedges for 3 min into coating forming emulsions (Table 1) and the excess solution was drained off for 3 min on tissue paper. The control was set by dipping pineapple wedges in distilled water for same duration. After air drying for 3 min, the cut pineapple was packed in food grade sterilized clamshell with 300 g wedges in each box. For every treatment and control, equal amount of pineapple was distributed in three boxes and stored at 5 ± 1 °C and 95% relative humidity (RH) to follow the changes in their quality characteristics at regular intervals of 4 days during their storage.

Table 1. Effect of ultrasonication on droplet size (*nanometer*) and viscosity (*milli Pascal second*) of alginate-based emulsions.

Treatments	Droplet size (Diameter in nanometer)	Viscosity (milliPascal second)
E0	$295.80^{b} \pm 54.02$	$110.84^{a} \pm 25.67$
E20	$687.90^{a} \pm 83.01$	$17.75^{b} \pm 1.59$
E40	$288.40^{b} \pm 1.69$	$10.41^{c} \pm 0.54$
E60	$313.40^{c} \pm 6.51$	$7.74^{d} \pm 0.39$

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. E0 – Primary emulsion [Alginate (1.29%) + Citral (0.05%)], E20 – E0 sonicated for 20 min, E40 – E0 sonicated for 40 min, E60 – E0 sonicated for 60 min.

Characterization of alginate-based emulsion

Particle size, size distribution and viscosity

The droplet size was measured by dynamic light scattering (DLS) technique using Particle size analyzer (NanoPartica SZ-100 series, Horiba Scientific Instruments, Kyoto, Japan) set at 633 nm and 25 °C and equipped with a backscatter detector at an angle of 173 °. Viscosity of emulsions was measured using a Modular Compact Rheometer (MCR 102, Anton Paar Germany GmbH, Ostfildern, Germany) and the data was compiled using Rheoplus V3.62 software.

Determination of quality attributes

Color

The changes in color of fresh-cut Pineapple during storage were measured according to the procedure of Papadakis et al. (2000) using digital camera (FinePix S2950, FUJIFILM, Japan) and Adobe Photoshop CS 8.0 software (Adobe System, Inc. SanJose, CA, USA). The color coordinates, lightness (L*), green to red (a*) and blue to yellowness (b*) values were recorded by pointing the cursor at different areas on the digital image of each fresh-cut pineapple samples.

Firmness

The fruit firmness was determined by puncture with fruit pressure tester (FT-327, FACCHINI SRL, Alfonsine, Italy) using flat-bottomed probe of 11 mm diameter and penetrated up to 5 mm into the flesh and the force required to penetrate the probe into fruit tissue is expressed in terms of Newton (N).

Weight loss percentage (WLP)

Pineapple wedges (50 g) from each replicate was placed into previously tarred petri-dish and dried at 70 °C for 48 h. After drying, the petri-dish was put in a desiccator to cool to room temperature. The dry 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 (1):

$$\text{Weight loss (\%)} = \{(\text{Initial Weight} - \text{Final Weight})/\ \text{Initial Weight}\} \times 100 \qquad (1)$$

Ascorbic acid (AA)

Ascorbic acid was determined according to the procedure of Roe and Oesterling (1944). The fruit tissue was extracted in 5% metaphosphoric acid-glacial acetic acid mixture. After centrifugation for 10 min at 5000 rpm, the known volume of supernatant was incubated in the mixture of 0.2 g L^{-1} 2, 4-dinitrophenyl hydrazine and 10 µL of 100 g L^{-1} thiourea for 3 h at 37 °C. The orange-red osazone crystals were dissolved by adding 5 mL of 85% sulphuric acid

after incubation period. The concentration was calculated based on the absorbance measured at 540 nm by comparing with the standard graph of ascorbic acid and expressed in gram of ascorbic acid per kilogram of fresh weight (g kg^{-1}).

Total phenolic content (TPC) and antioxidant activity

Five gram of fruit tissue was extracted with 50 mL of methanol and the resultant homogenate was centrifuged at 10621 × g for 15 min at 4 °C. TPC was determined by Lim et al. (2006). A volume of 0.2 mL of supernatant of each sample was mixed with 1.5 mL of Folin-Ciocalteu's reagent (which was diluted 10 × with distilled water) and 1.2 mL of sodium carbonate (7.5%, w/v) in a test tube. The tube content was vortexed and incubated in dark for 30 min at room temperature. The optical density (OD) was read at 765 nm and expressed as grams of gallic acid equivalents (GAE) per kilogram of fresh weight. Similarly, free radical scavenging activity was carried out as per the method described by Brand-Williams et al. (1995). An aliquot (100 µL) of methanolic extract was mixed with 3.9 mL of methanolic solution of DPPH (0.025 g L^{-1}). The mixture was thoroughly vortexed and kept in the dark for 30 min. The absorbance was measured at 515 nm against a blank of methanol without DPPH. Reference solution was also prepared containing DPPH and methanol instead of extract. The result of antioxidant capacity was expressed as mmoles of ascorbic acid equivalents antioxidant capacity (AEAC) per kilogram of fresh weight.

Malondialdehyde (MDA) and Hydrogen peroxide content (H_2O_2)

Fresh-cut pineapple tissue (1 g) was homogenized in a chilled 0.1% TCA (10 mL) for determination of MDA and H_2O_2 content, according to the method described by Velikova et al. (2000). The homogenate was centrifuged at 12,000 × g for 15 min at 4 °C. For MDA, an aliquot of 0.5 mL of the supernatant was added to 1 mL of 0.5% TBA prepared in 20% TCA, incubated in boiling water for 30 min, and reaction stopped at 0 °C for 10 min. The optical density was measured at wavelength of 532 nm using a UV–visible spectrophotometer (Shimadzu, UV-160A). The amount of MDA-TBA complex (red pigment) was calculated from the extinction coefficient 1.55 mM L^{-1} m^{-1}.

For H_2O_2 assay, an aliquot of 0.5 mL of supernatant was added to 0.5 mL of 10 mM L^{-1} potassium phosphate buffer (pH 7.0) and 1.0 mL of 1.0 mol L^{-1} KI. After incubation for 30 min at room temperature, the absorbance was taken at 390 nm using a UV–visible spectrophotometer (Shimadzu, UV-160A) and concentration was measured using calibration curve obtained with H_2O_2 standard solutions and the results was expressed in terms of micrograms per kilograms of fresh weight.

Browning-related enzymes

Polyphenol oxidase (PPO) and Peroxidase (POX) activity

Two grams of the pineapple tissue were homogenized in 25 mL of 0.1 mol L^{-1} sodium phosphate buffer (pH 6.5). The extract was centrifuged for 30 min at 20627 × g for PPO and 29703 × g for POX at 4 °C. PPO activity was assayed according to the method of Zhu and Zhan (2010), by incubating 0.1 mL enzyme extract with 2.5 mL of catechol (0.5 mol L^{-1}) and change in OD was recorded at 420 nm at the interval of 30 s up to 3 min. PPO activity was expressed in katals per kilogram of protein.

POX activity was assayed by the method described by Mazumdar and Majumder (2003). 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 katals per kilogram of protein.

Phenylalanine ammonia lyase (PAL) activity

The PAL activity was determined as per the methodology described by Malik and Singh (1980). Briefly, one gram of pineapple tissue was extracted in 0.1 mol L^{-1} sodium borate buffers (pH 8.8) and the homogenate was centrifuged at 19250 × g for 20 min at 4 °C. The reaction was initiated by incubating 0.2 mL L-phenylalanine (0.1 mol L^{-1}) was incubated with 3.2 mL of sodium borate buffer (0.1 mol L^{-1}, pH 8.8), and 0.2 mL enzyme extract at 37 °C for 2 h. After incubation period, the PAL activity was measured at 290 nm and the result was expressed in katals per kilogram of protein.

Microbiological analysis

The microbial contamination on the coated and uncoated fresh-cut pineapple was evaluated by enumeration of total mesophilic bacteria and yeasts and molds colonies. Ten gram of pineapple tissue was vigorously washed with 90 mL of 1 g L^{-1} sterile buffered peptone water in sterilized round bottom tubes at room temperature and serial dilutions were prepared and inoculated over plate count agar (PCA) at 35 °C for 48 h and potato dextrose agar (PDA) supplemented with 0.05 g L^{-1} chloramphenicol at 21 °C for 12 days for total mesophilic bacterial count and yeasts and molds, respectively by the spread plate method (ICMSF, 1978). The result was expressed as log colony forming units per gram of fresh weight (log CFU g^{-1} FW).

Sensory evaluation

The Nine-point Hedonic scale method as given by Amerine et al. (2013) was considered for conducting the sensory analysis of fresh-cut pineapple was performed based on color, taste, texture and odor at the beginning and at the end of storage period. The samples were randomly presented to fourteen non-trained panelists consisting of students and researchers. The panelists have judged the quality and ranked each sample by following hedonic scale of 9 points: 9 = excellent, 7 = very good; 5 = good, 3 = fair and 1 = poor.

Statistical analysis

A completely randomized design was used with three replications. At each interval of storage period, analytical determination was carried out by taking sample from three clamshells. Statistical analysis was performed using GraphPad Prism software version 3 (GraphPad Software, Inc, San Diego, USA). Data from all analyses were expressed as mean ± standard deviation. Analysis of Variance (ANOVA) followed by Turkey's multiple comparison post-hoc tests for multiple comparisons was used to assess the statistical differences among means ($p < 0.05$).

RESULTS AND DISCUSSION

Effect of ultrasonic emulsification on particle size, size distribution and viscosity of alginate-based antimicrobial emulsions

As shown in Table 1, the average droplet size of primary emulsion (E0) was 295.8±54.02 nm and the droplet size distribution curve showed two main intensity peaks of around 120 nm and 450 nm having bimodal behavior (Fig. 1). After 20 min sonication, the average droplet diameter of emulsion (E20) significantly ($p < 0.05$) increased to 687.9 ± 83.01 nm and the size distribution curve shifted from bimodal to multimodal behavior with three main intensity peaks. Among the three intensity peaks, the presence of minor intensity peaks of around 50 nm and 200 nm suggests that the 20 min sonication might have ruptured the larger oil droplets into smaller one to certain extent but the presence of major intensity peak of around

1000 nm indicated that either aggregation of smaller droplets and/or formation of thick inter-facial layer over lipophilic - surfactant complex by adsorption of alginate molecules have resulted in formation of larger size droplets (Dickinson, 2009). After 40 min sonication, ~58% reduction in the average droplet size (288.40 ± 1.69 nm) of E40 was achieved and the size distribution was changed from multimodal to bimodal behavior. However, sonication performed for 60 min (E60) did not cause the significant change in the droplet size, but bimodal changed into monomodal size distribution. This result suggests that emulsion behavior changed from heterogeneous to a homogeneous system with increasing of sonication time, which is desirable because homogenous emulsion system would form uniform edible coating layer than that derived from the heterogeneous system. Moreover, the size distribution curve shows only an intensity peak of around 300 nm after 60 min of ultrasound processing which may be corresponding to the droplets with a multilayered complex interface formed by the adsorption of alginate molecules and surfactant onto hydrophobic compounds (Fig. 1). Salvia-Trujillo et al. (2015) developed alginate-based nan emulsion enriched with different concentrations of lemongrass essential oil having droplet size ranging from 64 nm and 364 nm after microfluidization. Though, in the present study the emulsification was carried out by ultrasonicator, the droplet size reduction was achieved by two-fold. However, in the present study, the emulsion droplet size was comparatively larger than that previously reported by Salvia-Trujillo et al. (2013), which might be due to the use of low concentration of emulsifier. The viscosity of primary emulsion of alginate-olive oil enriched with citral and cinnamic acid was 110.84 ± 25.67 mPa·s (Table 1). On subjecting to sonication at 30 µm amplitude for 20, 40 and 60 min, the viscosity declined significantly to 17.75 ± 1.59, 10.41 ± 0.54 and 7.74 ± 0.39 mPa·s, respectively. This reduction in viscosity might have attributed to the breaking of chemical bonds in polymeric chains of alginate molecules and/or aggregates, which in turn facilitate the formation of emulsion with properly dispersed food additives. Similar observations were reported by Salvia-Trujillo et al. (2013), who noted the lowering in viscosity of lemongrass essential oil-alginate Nan emulsions due to the effect of ultrasound processing. According to Jadhav et al. (2024), the reduction in viscosity with simultaneous decline in the droplet size after ultrasound processing of hydrocolloid-lipid emulsion indicate enhanced stability. Therefore, in the present study, the alginate-based antimicrobial emulsion sonicated for 40 min would have greater stability as compared to that of E20 and E60.

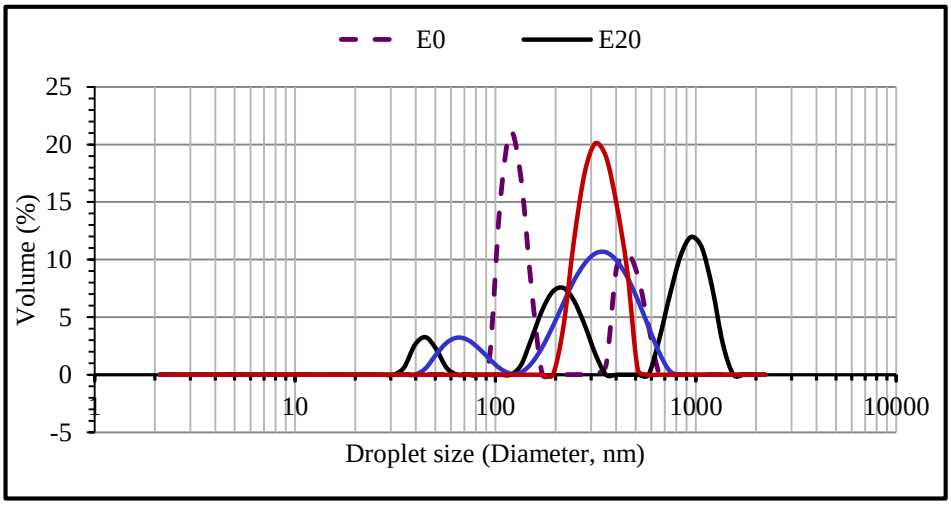


Fig. 1. Effect of ultrasonic homogenization on alginate based antimicrobial emulsions on droplet size distribution. E0 – Primary emulsion [Alginate (1.29%) + Citral (0.05%)], E20 – E0 sonicated for 20 min, E40 – E0 sonicated for 40 min, E60 – E0 sonicated for 60 min.

Changes in physicochemical quality of fresh-cut pineapple

Color

In the present study, the alginate-based edible coating of fresh-cut pineapple maintained higher lightness (L*) values throughout the storage time as compared to uncoated samples (Fig. 2a). It was found that the reduction of L* value in uncoated samples was ~23 units whereas for coated samples it decreased in the range between 2.5 to 6.1 units at the end of storage period. Regarding the changing trend of a* and b* values, there were significant ($p < 0.05$) difference among coated and uncoated samples. At 0 d of storage, a* and b* were -2.1 and 38.2 units, respectively. During the entire evaluation period, there was a gradual rise in a* values in both uncoated and coated fresh-cut pineapple (Fig. 2b). In control samples, it increased significantly ($p < 0.05$) and reached to 3.6 units on 12th d of storage, whereas the coated samples exhibited comparatively lesser change of a* values and therefore it ranged from -1.7 to 0.3 units at the end of storage. Regarding the changing pattern of b* values, there was significant ($p < 0.05$) decline in uncoated, E40 and E60 coated samples but E0 and E20 coated samples showed increment over entire storage time (Fig. 2c). This significant ($p<0.05$) decrease in L* and b* values with concomitant increase in a* value in control sample through the storage period indicated that the color quality was degraded due to tissue browning, in agreement with that reported by Aiamlaor et al. (2025) in cucumbers. Among the coated fresh-cut pineapple, E0 and E20 treated samples showed consistent rise in a* and b* values with extend of storage period might be the reason associated with the increase of flesh yellowness along with slight occurrence of browning. Our results are in line with findings of González-Aguilar et al. (2004) reported the change in L* and b* values in fresh-cut 'Smooth Cayenne' pineapple affected its color quality during 14 days of storage at 10 °C and further viewed that this may be related to conversion of phenolics compounds into melanin catalyze by polyphenol oxidase. On the contrary, E40 and E60 coated samples showed insignificant change in color components, L* and a* over the entire storage period, while b* value remained unchanged up to 8 d but thereafter it displayed the significant ($p < 0.05$) decline at the end of storage indicating that the reduction in flesh yellowness. The overall results showed that the coating created by alginate-based emulsions (E60 and E40) with ~300 nm droplet size on fresh-cut pineapple helped in maintaining the better color parameters up to 8 days of storage than that for E20 with ~600 nm droplet size.

Table 2. Changes in firmness (*Newton*) and weight loss percentage (WLP) of coated and uncoated fresh-cut pineapple during 12 days of storage at 5 °C.

Treatments	Storage period (Days)			
	0	4	8	12
Firmness (Newton)				
E0	$4.55^{a} \pm 0.34$	$3.63^{b} \pm 0.25$	$3.26^{b} \pm 0.16$	$3.33^{b} \pm 0.10$
E20	$4.55^{a} \pm 0.34$	$3.73^{b} \pm 0.19$	$3.63^{b} \pm 0.21$	$3.46^{b} \pm 0.18$
E40	$4.55^{a} \pm 0.34$	$3.55^{b} \pm 0.03$	$3.39^{b} \pm 0.06$	$3.42^{b} \pm 0.12$
E60	$4.55^{a} \pm 0.34$	$4.17^{a} \pm 0.05$	$3.48^{b} \pm 0.04$	$3.50^{b} \pm 0.26$
Control	$4.55^{a} \pm 0.34$	$3.31^{b} \pm 0.12$	$2.41^{c} \pm 0.13$	$2.19^{c} \pm 0.23$
Weight loss (%)				
E0	$0.00^{c} \pm 0.00$	$0.91^{b} \pm 0.02$	$0.90^{b} \pm 0.06$	$10.37^{a} \pm 0.04$
E20	$0.00^{d} \pm 0.00$	$0.57^{c} \pm 0.05$	$0.87^{b} \pm 0.07$	$10.77^{a} \pm 0.07$
E40	$0.00^{d} \pm 0.00$	$1.22^{c} \pm 0.02$	$1.37^{b} \pm 0.03$	$1.89^{a} \pm 0.02$
E60	$0.00^{d} \pm 0.00$	$0.36^{c} \pm 0.03$	$0.57^{b} \pm 0.01$	$5.87^{a} \pm 0.09$
Control	$0.00^{d} \pm 0.00$	$15.17^{c} \pm 0.18$	$21.10^{b} \pm 0.69$	$32.37^{a} \pm 0.55$

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. E0 – Primary emulsion [Alginate (1.29%) + Citral (0.05%)], E20 – E0 sonicated for 20 min, E40 – E0 sonicated for 40 min, E60 – E0 sonicated for 60 min.

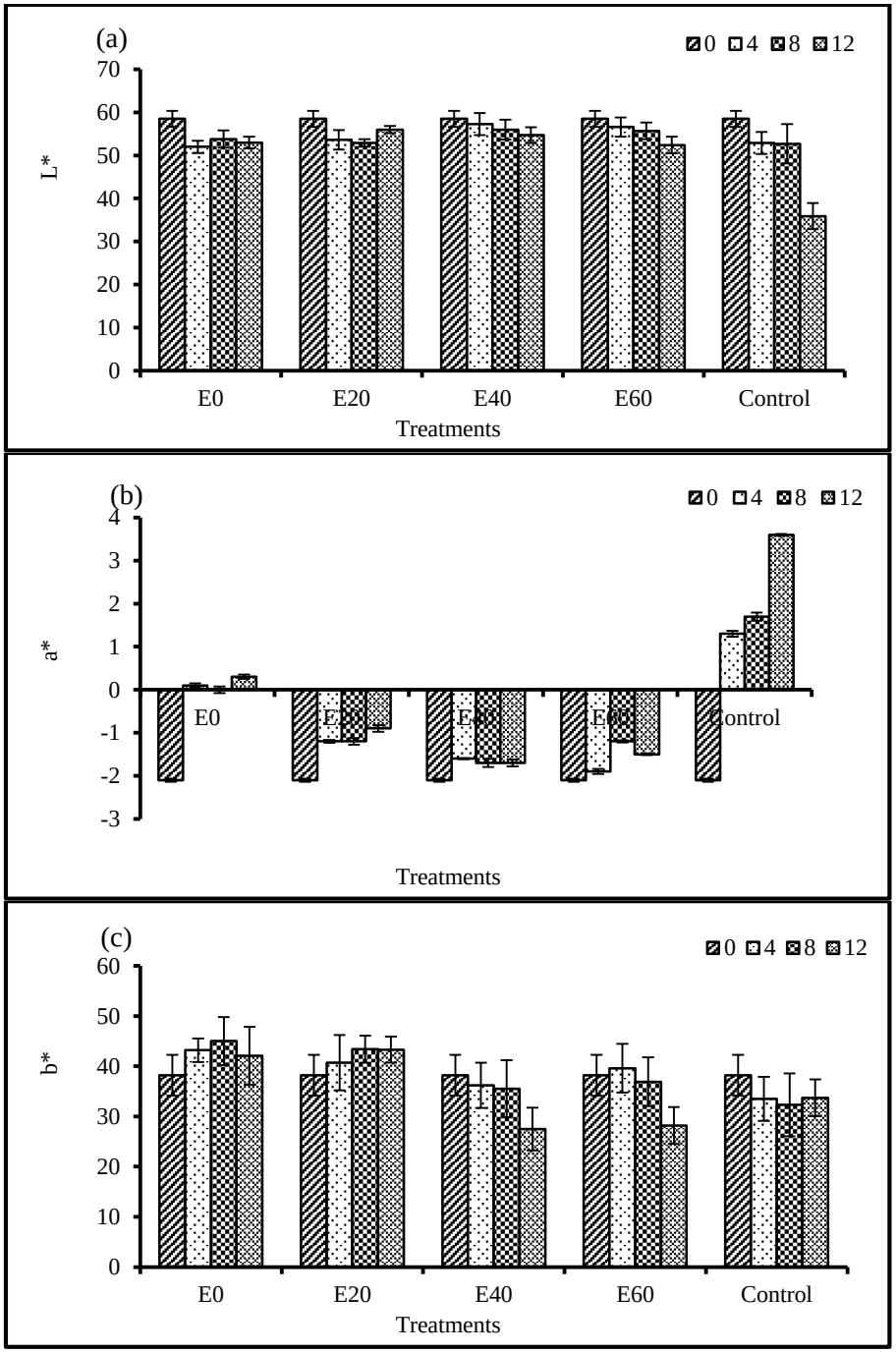


Fig. 2. Changes in color parameters (a) *L**, (b) *a**, and (c) *b* of coated and uncoated fresh-cut pineapple during 12 days of storage at 5 °C. Vertical bars represent ± standard deviation of means of three replicates. E0 – Primary emulsion [Alginate (1.29%) + Citral (0.05%)], E20 – E0 sonicated for 20 min, E40 – E0 sonicated for 40 min, E60 – E0 sonicated for 60 min.

Firmness

The loss of texture is considered one of most important factors that limit the shelf-life of fresh-cut fruit (Toivonen & Brummell, 2008). The uncoated samples exhibited consistent decline in firmness and reached to ~53% at the end of storage, while it was only ~25% loss in firmness observed in coated fresh-cut pineapple during 12 days of storage at 5 °C (Table 2). Hence, the application of alginate-based emulsions exerted better retention of firmness of fresh-cut pineapple as compared to the uncoated samples over the storage time. As

demonstrated by Quiles et al. (2007), the treatment of fresh-cut 'Fuji' apple with calcium propionate-maintained cell wall integrity through the generation of calcium pectates up to 2 weeks of storage. In the present study, this greater retention in firmness of coated samples is attributed to the dipping of fresh-cut pineapple into calcium propionate solution, followed by edible coating treatments.

Weight loss percentage (WLP)

The fresh-cut products can lose water even more rapidly than whole fruit or vegetable because of their increased surface to volume ratio. WLP was consistently increasing with the storage period in uncoated pineapple and showed 32.37 ± 0.55% decline at the end of storage (Table 2). Alginate-based coating significantly ($p < 0.05$) helped lowering WLP in fresh-cut pineapple throughout the storage period of 12 days at 5 °C. Alginate based coating are reported by Bal, (2021) for reducing weight loss and delaying the tomato-ripening process during postharvest storage. There were significant ($p < 0.05$) variations in changing trend of WLP among the coated samples which might be related to the difference in the viscosity, average droplet size and size distribution of emulsions. According to Huck-Iriart et al. (2014), high viscosity or large droplet size interferes with mobility of droplets and also suppresses the migration rate of emulsion system. E0 and E20 coated pineapple exhibited highest WLP i.e., ~10% among coated samples at the end of storage period (Table 2). E40 coated samples showed least change in WLP (1.89 ± 0.02%) at the end of storage, while E60 coated samples exhibited approx. two fold higher WLP than that of E40 coated wedges. Therefore, the better efficiency of E40 and E60 coatings in delaying weight loss as compared to E0 and E20 coatings may be correlated to the lower viscosity with smaller droplet size of E40 and E60 emulsions than those of E0 and E20 emulsions. Moreover, the proper dispersion of citral and cinnamic acid in alginate solution after 40 and 60 min sonication might have enhanced the water barrier properties of coated fresh-cut pineapple. However, E60 coated samples demonstrated comparatively higher WLP than that of E40 coated samples, suggests that the further reduction in viscosity of alginate-based emulsion during 60 min sonication might be the consequences of the extensive breaking of the polymeric chains of alginate molecules and thereby slightly compromised its water barrier property (Salvia-Trujillo et al., 2013).

Ascorbic acid (AA)

Ascorbic acid is frequently used as an indicator of the nutritional quality of fresh-cut fruit due to its high sensitivity to wounding stress (Deutsch, 2000). In the present investigation, AA significantly ($p < 0.05$) declined from 35.59 $g.kg^{-1} \times 10^{-2}$ at 0 day to 15.10 $g.kg^{-1} \times 10^{-2}$ at 12 day of storage in uncoated fresh-cut pineapple (Fig. 3a). Under stress conditions such as minimal processing, the activity of enzymes like ascorbate oxidase and ascorbate peroxidase might have enhanced which lead to the degradation of AA content (Cice et al., 2024). However, AA content decreased in a moderate manner in fresh-cut pineapple coated with alginate-based coatings. Among applied treatments, E40 and E60 coatings helped retain approx. 9 $g.kg^{-1} \times 10^{-2}$ higher AA content, whereas it was only 3 to 4 $g.kg^{-1} \times 10^{-2}$ higher in E0 and E20 coated samples as compared to uncoated samples. Mantilla et al. (2013) also reported the reduction in AA content in fresh-cut pineapple coated with multilayer coating enriched with cinnamaldehyde. However, AA content of fresh-cut pineapple measured in the present study was quite different from that accounted by Mantilla et al. (2013) which may be related to the factors such as cultivar, maturity stage, environment and agronomic conditions. This fact is supported by Lu et al. (2014), who demonstrated that pineapple genotypes vary in their carbohydrate and bioactive compounds including ascorbic acid. It is believed that the E40 and E60 coatings prevented the direct contact of atmospheric oxygen contributed by its

enhanced semipermeable behavior as well as the antioxidant properties of citral and cinnamic acid slow down the oxidative decline of AA content from fresh-cut pineapple and therefore, the loss of AA content was lesser in the present study as compared to that documented by Mantilla et al. (2013).

Change in Total phenolic content (TPC)

Wounding activates defense mechanism leading to synthesis and/or oxidation of phenolic compounds (Reyes et al., 2007). According to Lu et al. (2014), the phenolics content may range from 31.48 to 77.5 mg/100 g FW in pineapple fruit. In the present study, the amount of TPC noticed in fresh-cut pineapple at 0 day was 21.09 ± 1.42 g $kg^{-1} \times 10^{-2}$ (Fig. 3b). It was found that the TPC accumulated throughout the storage period in both the coated and uncoated samples. During initial 4 days of storage, the uncoated pineapple showed ~2.6-fold increment in TPC, while its enhancement was comparatively lesser (~0.9 times) in coated samples. In uncoated pineapple wedges, the biosynthesis of total phenolics was continued as indicated by its maximum concentration i.e., 78.72 ± 1.81 g $kg^{-1} \times 10^{-2}$ during 8 days of storage and thereafter declined to ~69.42 ± 1.42 g $kg^{-1} \times 10^{-2}$ at the end of storage period. Similarly, the treated samples were also observed with increasing trend of TPC with the advance of storage time. However, its accumulation was comparatively slower in coated pineapple than that observed in uncoated wedges. The faster TPC accumulation in control samples as compared to the treated fruit could possibly be attributed to enhanced wound stress in uncoated samples as a result of minimal processing (Reyes et al., 2007). Besides, the increase is often attributed to wounding stress and the activation of the phenylpropanoid pathway, which is involved in the biosynthesis of phenolic compounds (Amodio et al., 2014). It can also be influenced by storage conditions, such as temperature and gas composition. Thus, the coating of fresh-cut pineapple with the ultrasonically homogenized alginate-based edible coating helped in reducing the wound-induced stress by acting itself as a protective layer for the damaged surface of cut pineapple. A similar pattern of phenolics accumulation was also reported in minimally processed apple (Magri et al., 2024).

Change in Antioxidant activity

The antioxidant capacity of a substance is defined as its ability to scavenge reactive oxygen species and electrophiles. After wounding, the antioxidant capacity of tissues was enhanced significantly in all the samples including treated and non-treated one during initial 4 days of storage (Fig. 3c). This increment in antioxidant activity was approx. 57% in uncoated pineapples, followed by 49%, 40%, 36% and 38% in E0, E20, E40 and E60, respectively, greater than its initial level. The tremendous rise in control samples is likely in response to the higher oxidative stress levels as compared the treated samples. From 4 days onwards, the changing trend of antioxidant activity varied among the tested treatments and control samples. The antioxidant activity in control samples underwent progressive decline, whereas in E0 samples it consistently diminishing with the advance of storage time. E20 and E40 were obtained with slight change, while E60 shows abrupt decline at the end of storage time, similar decline of antioxidant capacity over storage period was reported by Tapia-Rodriguez et al. (2021). The overall result obtained by DPPH assay revealed that the antioxidant activity of fresh-cut pineapple was enhanced to some extent irrespective of the given treatment at the end of storage time.

Changes in Hydrogen peroxide (H_2O_2) and Malondialdehyde (MDA)

Reactive oxygen species (ROS) are produced due to various metabolic activities and their production increases under various stress conditions (Hodges and Forney, 2000). Oxidative

stress occurs when the generation of ROS exceeds the capacity of the cell to maintain cellular redox homeostasis. The generated ROS affect metabolism of the cells by oxidative damage to various cellular compartments including membrane lipids, proteins and nucleic acids (Jena et al, 2023). At the 0 d of storage, H_2O_2 level was measured highest which thereafter decreased gradually from 0.15 µmol kg^{-1} to 0.10 µmol kg^{-1}, 0.08 µmol kg^{-1} and 0.07 µmol kg^{-1} in control, E0 and E20 samples, respectively on 8 d of storage as shown in (Fig. 3d). However, the significant generation of H_2O_2 was observed in these samples at the end of storage time. In contrast to this, E40 and E60 samples showed progressive decline of H_2O_2 level and reached to the least value at the end of storage time. Malondialdehyde, the end product of lipid peroxidation, catalyzed by lipoxygenase (LOX) enzyme, is a good indicator of the structural integrity of plant cell membrane. MDA accumulation was less fluctuated throughout the storage period and showed little difference in its accumulation pattern among the treated and control fresh-cut pineapple during entire storage time as shown in (Fig. 3e).

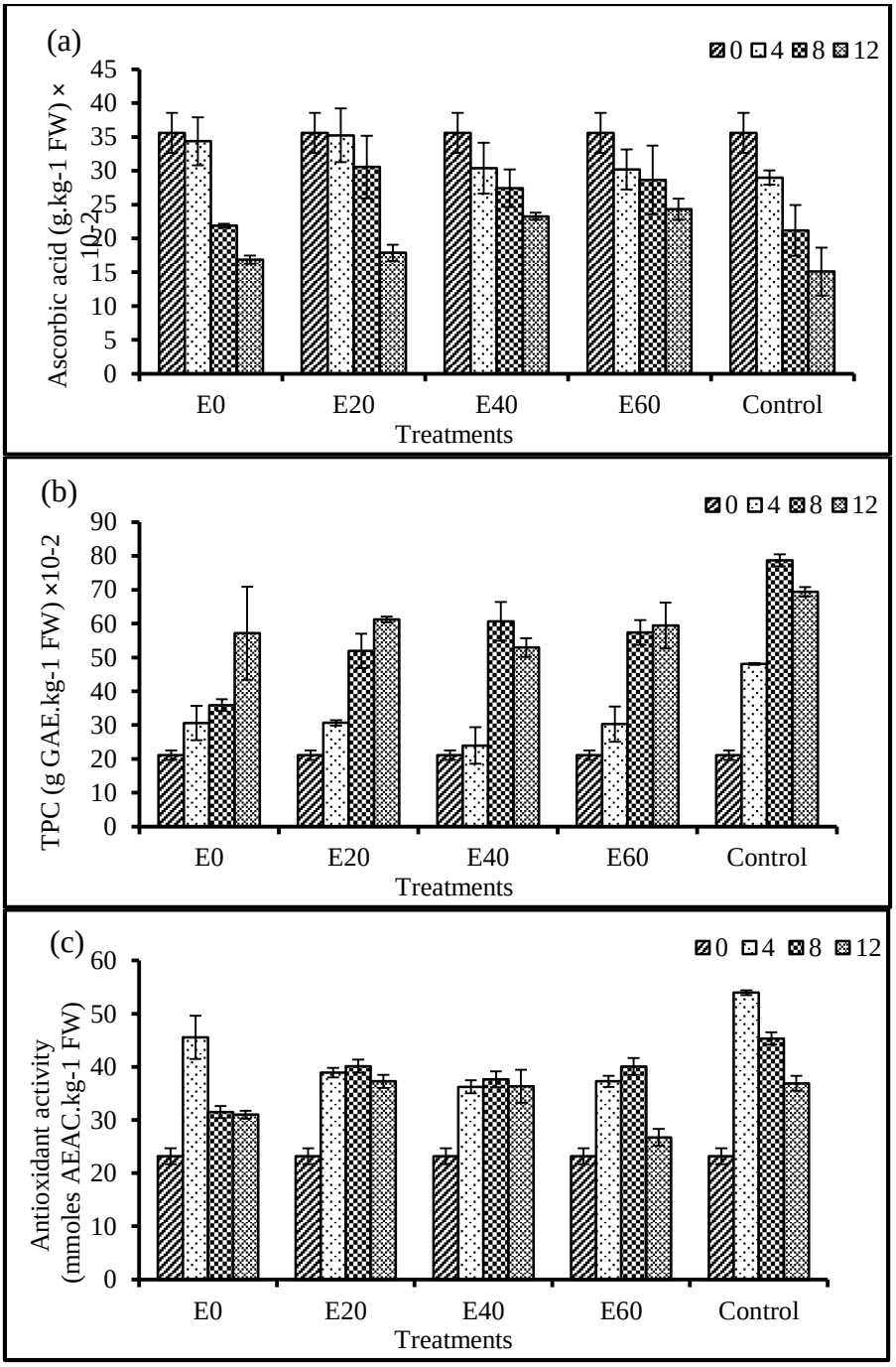
Browning-related Enzymes

Polyphenol oxidase (PPO) and Peroxidase (POX) activity

Polyphenol oxidase (PPO) and peroxidase (POX) are the main enzymes responsible for the browning reaction. Results revealed a significant ($p < 0.05$) decline in PPO activity during initial 4 days of storage in all coated as well as uncoated samples (Fig. 4a). Among tested treatments, maximal inhibition of PPO activity was noticed in E60 (~67%), followed by E40 (~52%), E20 (~43%) and E0 (~38%). The uncoated samples were found with least reduction of PPO activity (~28%) at 4^{th} day of storage period. From 4^{th} day onwards, PPO activity in coated and uncoated samples exhibited consistently declining pattern with the advance of storage time. By 12 day of the storage period, PPO activity inhibited to the greater extent in E20, E40 and E60 coated fruit as compared to that noticed in E0 and uncoated samples. The significant ($p<0.05$) reduced PPO activity may be the effect of alginate-based coatings which help the better migration of micro-range droplets with citral and cinnamic acid into the fruit tissue, thereby prevented the browning of fresh-cut pineapple over the storage time. In our previous study, the incorporation of cinnamic acid into xanthan gum-based edible coating prevented the cut surface browning of fresh-cut pear through inhibition of polyphenol oxidase (PPO) activity (Sharma and Rao, 2015). Similarly, Gumghatti based composite coatings significantly reduced the PPO activity as coatings act as barriers and led to a reduction in the oxygen supply for enzymatic oxidation of phenolics (Rao et al., 2025).

POX could enhance PPO-mediated browning reactions (Toivonen & Brummell, 2008). In the present study, POX activity rapidly increased during the first 4 d of storage period in both treated and non-treated fresh-cut pineapple and thereafter underwent abrupt decline till the end of storage period (Fig. 4b). Similar finding was reported by Mirshekari and Madani (2021) in fresh cut Papaya fruit. It was found that the increment in POX activity was ~16% higher for control samples to its initial value as compared to that of coated samples. However, the activity of POX was observed to be significantly reduced in coated samples. The fresh-cut pineapple coated with alginate-based emulsion exhibited significantly ($p < 0.05$) greater reduction in their POX activity as compared to uncoated samples. Decrease in POX after 4 days of storage may be due to prolonged storage which leads to a decline in POX activity. Also, acidification of peroxidase causes a pronounced change in the protein from the native state to the reversible denatured state (Burnette, 1977). Furthermore, this inhibition of POX activity was highest in E60 and E40 approx. by 10%, followed by E20 (~8%) and E0 (~5%). This trend could be explained by the fact that increasing the emulsification time from 20 to 40 min facilitated the proper distribution of cinnamic acid and citral within the alginate solution, thereby prevented induction of oxidative stress condition. However, the changing

trend of POX activity in E60 samples indicated that increasing the emulsification time from 40 to 60 min have no further effect on the activity pattern of POX.



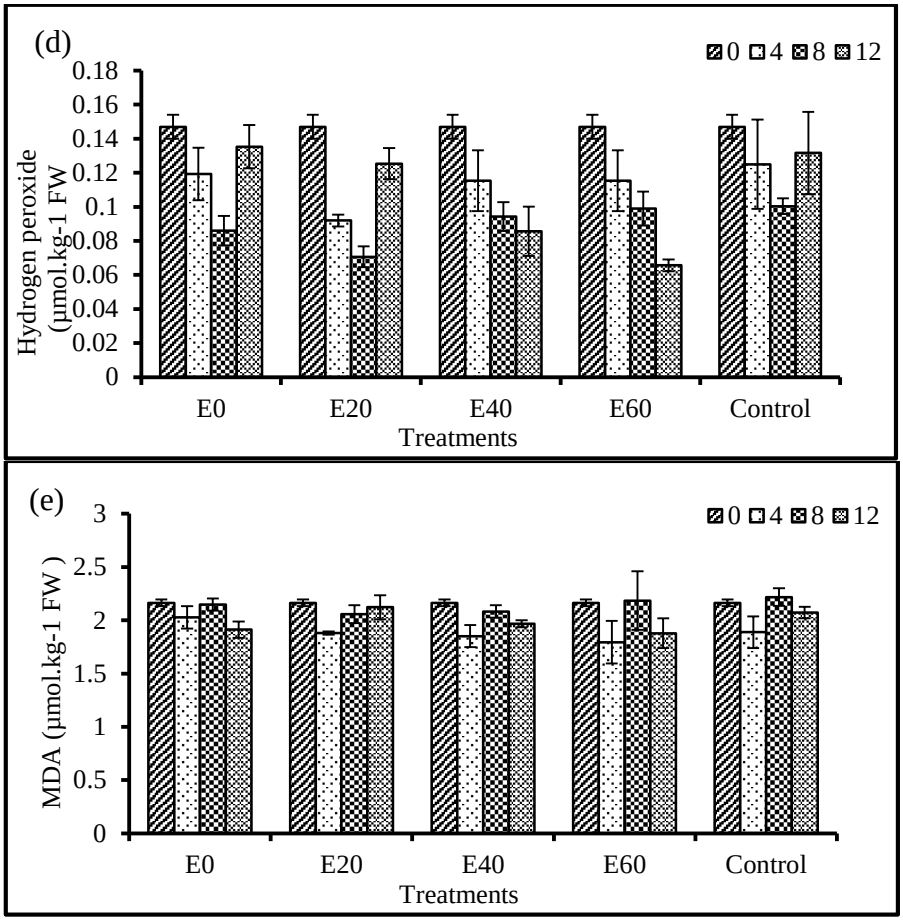


Fig. 3. Changes in (a) Ascorbic acid (b) Total phenolics content, (c) Antioxidant activity, (d) Hydrogen peroxide (H_2O_2), and (e) Malondialdehyde (MDA) of coated and uncoated fresh-cut pineapple during 12 days of storage at 5 °C. Vertical bars represent ± standard deviation of means of three replicates. E0 – Primary emulsion [Alginate (1.29%) + Citral (0.05%)], E20 – E0 sonicated for 20 min, E40 – E0 sonicated for 40 min, E60 – E0 sonicated for 60 min.

Phenylalanine ammonialyase (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 (Becerra-Moreno et al., 2015). In the present study, the PAL activity was initially increased in both coated and uncoated fresh-cut pineapple reaching to its maximum level at 4th day and thereafter significant ($p < 0.05$) declined at the end of storage period (Fig. 4c). However, biosynthesis of TPC was continued till the end of storage time. This suggested that though PAL activity declined with the storage time, its complete inactivation did not occur and therefore involved in synthesizing phenolic compounds throughout the storage time (Reyes et al., 2007). A previous study conducted by Li et al. (2022) also reported decrease in PAL activity in plum fruits treated with edible coating during storage period.

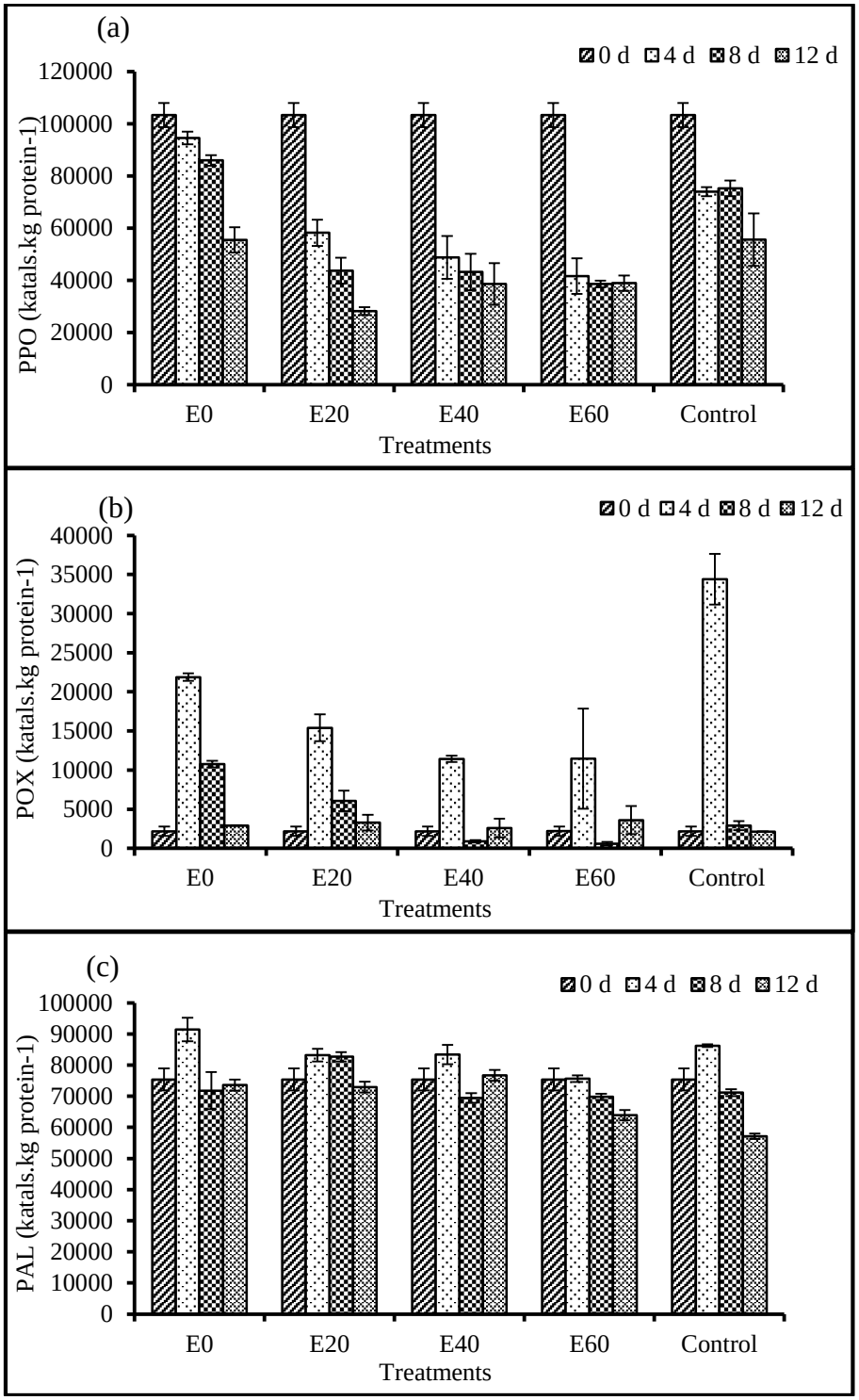


Fig. 4. Changes in specific activity of (A) Polyphenol oxidase (PPO), (B) Peroxidase (POX) and (C) Phenylalanine ammonia lyase (PAL) of coated and uncoated fresh-cut pineapple during storage for 12 d at 5 °C. Vertical bars represent ± standard deviation of means of three replicates. E0 – Primary emulsion [Alginate (1.29%) + Citral (0.05%)], E20 – E0 sonicated for 20 min, E40 – E0 sonicated for 40 min, E60 – E0 sonicated for 60 min.

Table 3. Changes in total aerobic bacterial (*log CFU g*$^{-1}$) and yeasts and molds count (*log CFU g*$^{-1}$) present on coated and uncoated fresh-cut pineapple during 12 days of storage at 5 °C.

Storage time (Days)			
Treatments	0	6	12
Total aerobic bacteria (log CFU g^{-1})			
E0	$0.00^{a} \pm 0.00$	$3.30^{c} \pm 0.03$	$3.42^{b} \pm 0.01$
E20	$0.00^{a} \pm 0.00$	$2.00^{b} \pm 0.00$	$3.13^{b} \pm 0.07$
E40	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$2.65^{c} \pm 0.07$
E60	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$2.39^{c} \pm 0.12$
Control	$0.00^{a} \pm 0.00$	$3.65^{d} \pm 0.38$	$4.29^{a} \pm 0.16$
Yeast and molds (log CFU g^{-1})			
E0	$0.00^{a} \pm 0.00$	$3.36^{c} \pm 0.11$	$4.21^{a} \pm 0.05$
E20	$0.00^{a} \pm 0.00$	$2.39^{b} \pm 0.12$	$2.59^{b} \pm 0.16$
E40	$0.00^{a} \pm 0.00$	$2.15^{b} \pm 0.21$	$2.39^{b} \pm 0.12$
E60	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$2.00^{b} \pm 0.00$
Control	$0.00^{a} \pm 0.00$	$3.55^{c} \pm 0.08$	$4.34^{a} \pm 0.06$

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. E0 – Primary emulsion [Alginate (1.29%) + Citral (0.05%)], E20 – E0 sonicated for 20 min, E40 – E0 sonicated for 40 min, E60 – E0 sonicated for 60 min.

Microbial analysis

In the present investigation, the effect of ultrasonicated alginate-based emulsion on aerobic bacterial total plate counts and yeast and mold counts of fresh-cut pineapple were evaluated at the beginning and on 6th and 12th days of storage at 5 °C (Table 3, Fig 5 & 6). After 12 days of storage period, the fresh-cut pineapple coated with primary emulsion (E0) showed that the total plate counts and yeast and mold counts similar to that of uncoated samples. However, ultrasonic emulsification has greatly enhanced the antimicrobial property and therefore E20, E40, and E60 samples had substantially lowered total plate counts and yeast and mold counts with reduction of ~2.0 log CFU g^{-1} at the end of storage period as compared to E0 and uncoated samples. As demonstrated by Salvia-Trujillo et al. (2015), the incorporation of 0.1% lemongrass essential oil into sodium alginate-based nanoemulsion significantly slowed down the growth of natural microflora of fresh-cut '*Fuji'* apples during 2 weeks of storage. From the findings of present study, it is suggested that the antimicrobial activity of citral and cinnamic acid incorporated into edible coating could be improved by ultrasonic homogenization. Smaller emulsion droplets can enhance the antimicrobial activity as smaller droplets increase the surface area for interaction between the active compound and the microbial cell membrane, potentially leading to increased effectiveness.

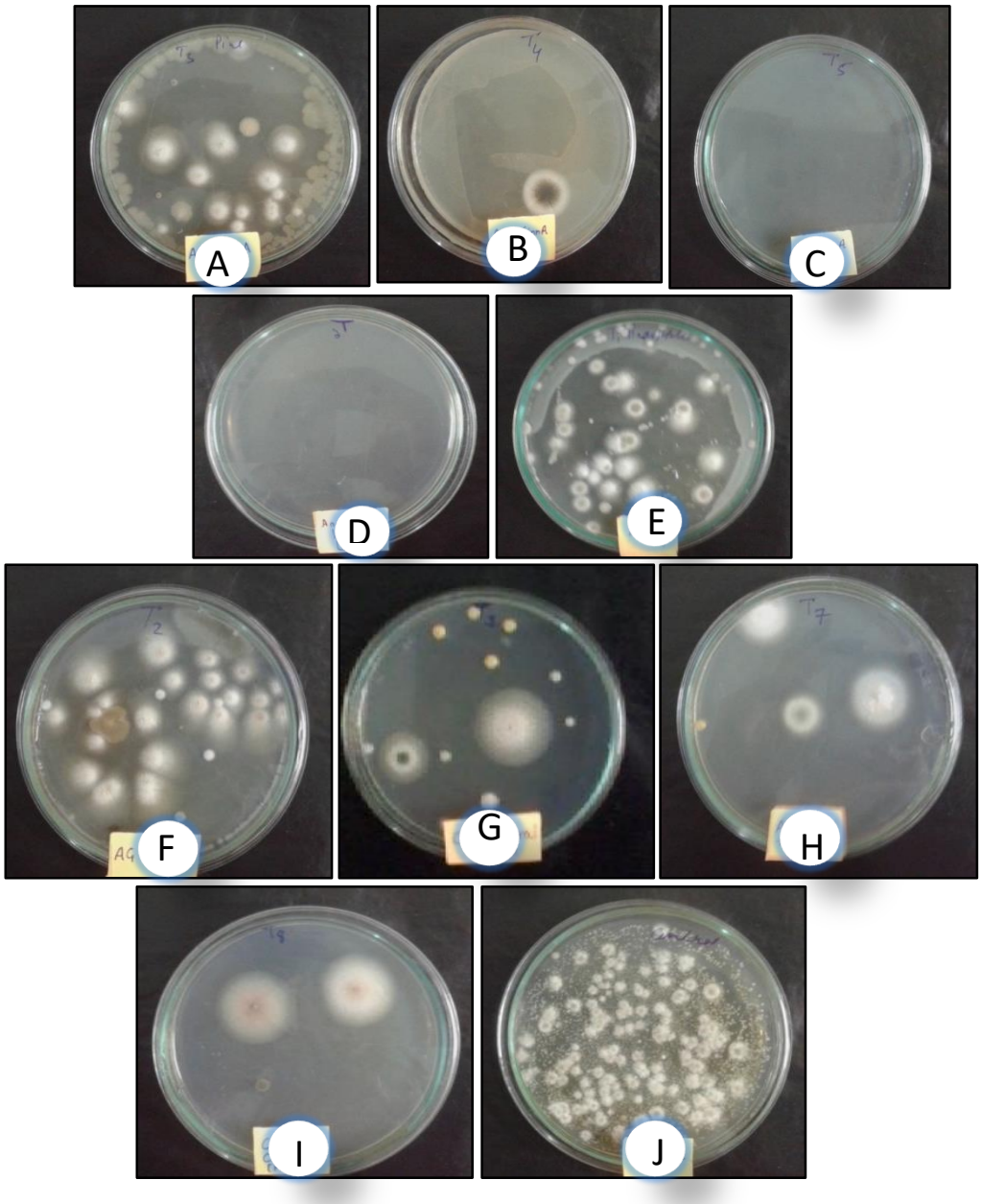


Fig. 5. [A-E] Aerobic bacterial contamination in fresh-cut pineapple during their 6 days of storage at 5°C {(A) E0 – Primary emulsion [Alginate (1.29%) + Citral (0.05%)], (B) E20 – E0 sonicated for 20 min, (C) E40 – E0 sonicated for 40 min, (D) E60 – E0 sonicated for 60 min, (E) Control} and [F-J] Aerobic bacterial contamination in fresh-cut pineapple during their 12 days of storage at 5°C {(F) E0 – Primary emulsion [Alginate (1.29%) + Citral (0.05%)], (G) E20 – E0 sonicated for 20 min, (H) E40 – E0 sonicated for 40 min, (I) E60 – E0 sonicated for 60 min and (J) Control}.

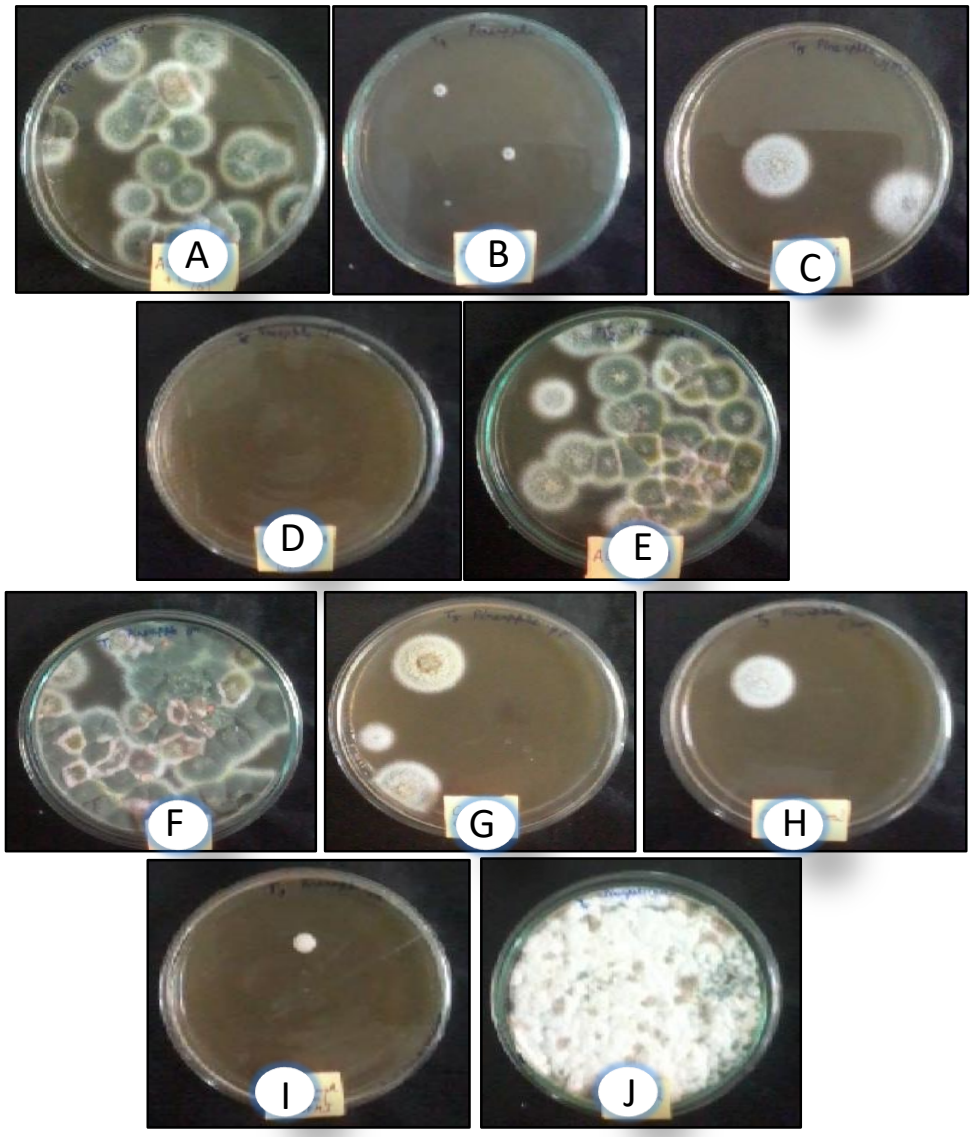


Fig. 6. [A-E] Yeasts and molds contamination in fresh-cut pineapple during their 6 days of storage at 5°C {(A) E0 – Primary emulsion [Alginate (1.29%) + Citral (0.05%)], (B) E20 – E0 sonicated for 20 min, (C) E40 – E0 sonicated for 40 min, (D) E60 – E0 sonicated for 60 min, (E) Control} and [F-J] Yeasts and molds contamination in fresh-cut pineapple during their 12 days of storage at 5°C {(F) E0 – Primary emulsion [Alginate (1.29%) + Citral (0.05%)], (G) E20 – E0 sonicated for 20 min, (H) E40 – E0 sonicated for 40 min, (I) E60 – E0 sonicated for 60 min and (J) Control}.

Sensory quality

The evaluation of sensory properties of fresh-cut pineapple coated with alginate-based emulsion enriched with citral and cinnamic acid in the present study is necessary for the acceptance of fresh-cut pineapple. The result depicted in Figure 7, showed the change in the sensory attributes of coated and uncoated pineapple. At 6 day of storage time, uncoated samples exhibited significantly ($p < 0.05$) lower color and odor scores as compared to the coated samples (Fig. 8). In the present study, the fresh-cut pineapple coated with alginate-based emulsions maintained its quality in terms of taste, odor, color and texture as these attributes had higher scores than that of uncoated samples after 12 days of storage at 5 °C (Fig. 8). In the previous study, Iacovino et al. (2024) reported that the sensory attributes of banana coated with alginate emulsion and alginate cross linked with $CaCl_2$ stored at 20 °C were maintained up to 11 days. Thus, the present study also demonstrated the positive

influence of alginate-based emulsions added with citral and cinnamic acid on the sensory traits of fresh-cut pineapple.

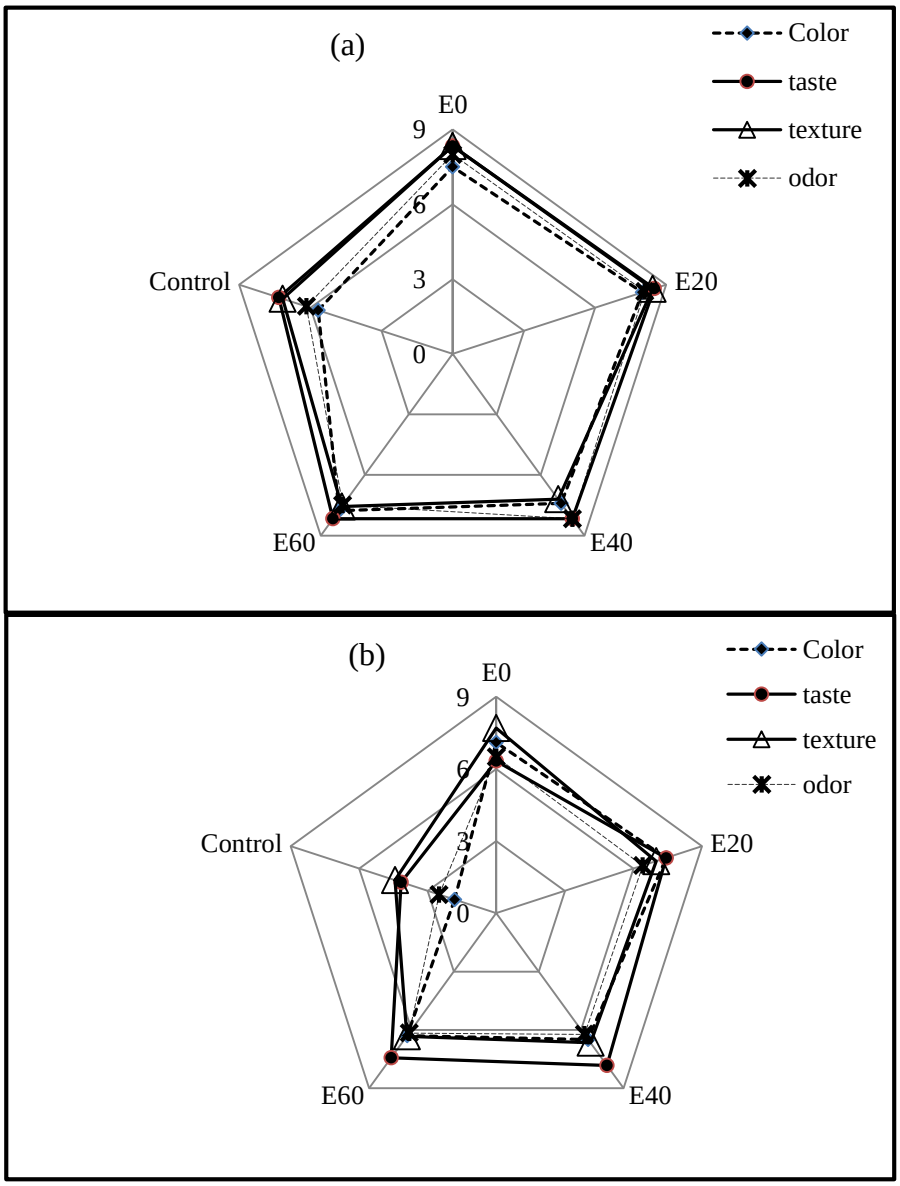


Fig. 7. Changes in sensory quality on (A) 6 d and (B) 12 d of coated and uncoated fresh-cut pineapple during storage for 12 d at 5 °C. Vertical bars represent ± standard deviation of means of three replicates. E0 – Primary emulsion [Alginate (1.29%) + Citral (0.05%)], E20 – E0 sonicated for 20 min, E40 – E0 sonicated for 40 min, E60 – E0 sonicated for 60 min.

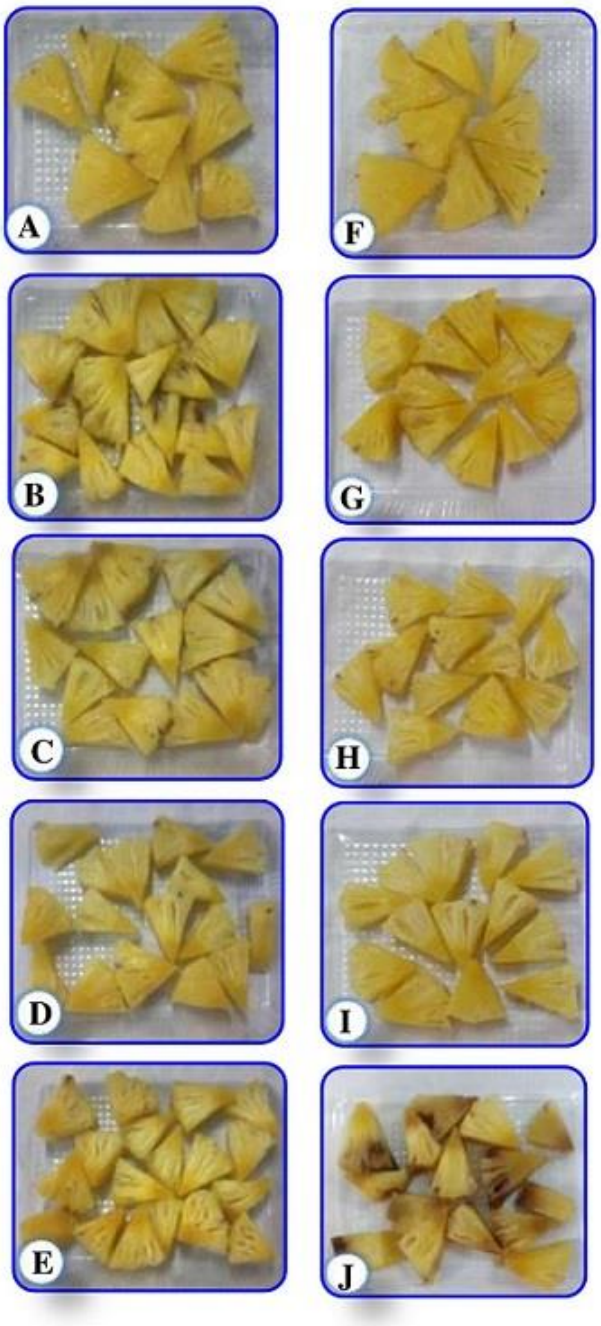


Fig. 8. [A-E] Visual changes in coated and uncoated fresh-cut pineapple during storage at 6 days at 5 °C. {(A) E0 – Primary emulsion [Alginate (1.29%) + Citral (0.05%)], (B) E20 – E0 sonicated for 20 min, (C) E40 – E0 sonicated for 40 min, (D) E60 – E0 sonicated for 60 min, (E) Control} and [F-J] Visual changes in coated and uncoated fresh-cut pineapple during storage at 12 days at 5 °C. {(F) E0 – Primary emulsion [Alginate (1.29%) + Citral (0.05%)], (G) E20 – E0 sonicated for 20 min, (H) E40 – E0 sonicated for 40 min, (I) E60 – E0 sonicated for 60 min and (J) Control}.

CONCLUSION

To conclude, the application of ultrasonicator was found efficient in developing the alginate-based emulsion incorporated with citral and cinnamic acid with micro-range droplet size of ~300 nm (E40 and E60) which further can be utilize as functional edible coating on fresh-cut pineapple. Quality analysis showed the lesser change in color and firmness, maintained ascorbic acid, reduced the weight loss and delayed the increase in PPO and POX of coated

samples as compared to those of the uncoated and primary emulsion coated samples. In addition, the proliferation of mesophilic bacteria and yeasts and molds was significantly lowered in coated samples when compared with that of uncoated samples. The present study revealed that the ultrasonic-assisted homogenization of alginate solution enriched with citral and cinnamic acid enhanced its efficacy by reducing the viscosity and improving the droplet size distribution and thereby it has potentiality to be employ as antimicrobial edible coating for the quality improvement and shelf-life extension of fresh-cut pineapple up to 12 days of storage at 5 °C.

The current work has some practical limitation as it was carried out for only 12 days storage period and needs to be evaluated for longer durations. Besides the alginate based edible coatings used were not compared with the available commercial coating technologies. Future work also needs to be carried out on investigating bioactive release kinetics, enhancing scalability for industrial applications, and testing the suggested coating on various fruit types. These efforts will help optimize performance, extend shelf life across diverse produce, and demonstrate the broader applicability and commercial potential of this innovative edible coating system.

Conflict of interest

There is no potential conflict of interest between the authors as well as any financial, personal or relationship with other people or organizations.

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