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Valorization of durian seed coat polyphenol-rich extract for chitosan-based coatings to improve postharvest quality

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Abstract

This study investigated the valorization of durian (*Durio zibethinus*) seed coat as a source of bioactive polyphenol-rich extract and its application in chitosan-based edible coatings for postharvest preservation of seedless lime fruits. Polyphenol extraction was performed using a combined heat-assisted and ultrasound-assisted approach. Under the experimental conditions tested, including a solvent-to-solid ratio of 20:1, 50°C, pH 5, 2 h, and ultrasound treatment at 20 kHz and 400 W for 5 min, the extract achieved a total polyphenol yield of 51.22 mg GAE/g dry weight, representing a significant increase compared to conventional extraction. The extract also showed moderate antioxidant and antimicrobial activities. For postharvest application, seedless lime fruits were assigned to three treatments: uncoated control, 1.5% chitosan coating, and 1.5% chitosan coating containing 795 µg/mL durian seed coat extract. Fruits were stored at 25±2°C for 25 days. Compared with the uncoated control, the chitosan-extract composite coating reduced weight loss (15.06% vs. 31.64%), limited firmness reduction (34.53%), and better maintained key quality attributes, including soluble solids, pH, vitamin C, total acidity, and color ($p < 0.05$). Decay incidence also decreased from 50.0% in the uncoated control to 10.0% in the composite coating treatment after 25 days of storage. These results suggest that durian seed coat extract can serve as a functional additive in chitosan-based coatings, contributing to improved postharvest quality of seedless limes under laboratory storage conditions.

1. Introduction

Durian (*Durio zibethinus*) is a tropical fruit widely cultivated in Southeast Asia and generates substantial amounts of agricultural by-products during consumption and processing. Among these, durian seeds represent a significant fraction of the fruit, particularly in certain cultivars such as Kho Qua Xanh (KQX), where the seed proportion can reach 18–19% of the fruit mass. Durian seed consists of two distinct parts: the inner kernel, which is nutrient-rich, and the outer seed coat, which is typically rich in polyphenolic compounds. Despite their abundance, these by-products remain largely underutilized, raising both environmental and economic concerns. Recent studies have suggested that durian seeds may serve as a promising source of bioactive compounds, including polyphenols, with potential applications in food systems (Charoenphun and Klangbud, 2022; Lou *et al*., 2026).

Previous research has explored the extraction and bioactivity of polyphenols from durian seeds of various cultivars. For example, Liu *et al*. (2013) reported the extraction of oligomeric proanthocyanidins using acetone-based solvents, while Ramli *et al*. (2021) demonstrated the antibacterial activity of durian seed extracts against several foodborne pathogens. Other studies have also highlighted the antioxidant and anti-inflammatory properties of durian-derived materials, including seeds, peels, and pulp (Ang *et al*., 2018; Charoenphun and Klangbud, 2022). However, the reported bioactivities are inconsistent, with some studies indicating limited antioxidant performance depending on extraction conditions and concentrations (Ramli *et al*., 2021). These discrepancies suggest that both extraction methodology and raw material characteristics play critical roles in determining the functional properties of durian-derived extracts.

In parallel, plant-derived polyphenols have gained increasing attention as natural alternatives to synthetic

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preservatives, particularly in the development of active packaging and edible coatings. Chitosan, a biodegradable and biocompatible polysaccharide, has been widely applied in postharvest preservation due to its film-forming ability and inherent antimicrobial activity. Chitosan-based coatings can effectively reduce moisture loss, delay microbial growth, and maintain the physicochemical quality of fresh produce (Ma *et al*., 2024; Wang *et al*., 2024). Furthermore, the incorporation of polyphenol-rich plant extracts into chitosan matrices has been shown to enhance antioxidant and antimicrobial performance, leading to improved shelf life and quality retention in fruits (Ma *et al*., 2024; Dai *et al*., 2025).

Citrus fruits, including lemon, orange, mandarin, and lime (*Citrus* spp., Rutaceae), are highly susceptible to postharvest deterioration. After harvest, these fruits undergo various physiological and biochemical changes, including weight loss, softening, sensory and nutritional quality degradation, and increased vulnerability to microbial infection. Fungal decay is one of the major causes of postharvest losses in citrus fruits, with common pathogens including *Penicillium digitatum*, *Fusarium oxysporum*, and *Colletotrichum gloeosporioides* (Piccirillo *et al*., 2018; Vashpanov *et al*., 2021). Therefore, the development of natural edible coating systems with antioxidant and antimicrobial properties represents a promising strategy for improving citrus fruit preservation.

Despite these advances, the seed coat of durian, particularly from specific cultivars such as Kho Qua Xanh, has received little scientific attention. To date, no studies have systematically investigated the extraction, chemical composition, and functional properties of polyphenols derived from KQX durian seed coat, nor their integration into edible coating systems for postharvest applications. This represents a critical knowledge gap, especially considering the potential of cultivar-specific by-products as novel functional ingredients.

Therefore, the objectives of this study were: (i) to evaluate polyphenol extraction from KQX durian seed coat under selected experimental conditions using a combined heat-assisted and ultrasound-assisted approach; (ii) to assess the antioxidant, antibacterial, and antifungal activities of the obtained extract; and (iii) to investigate its application in chitosan-based edible coatings for maintaining the postharvest quality of seedless lime fruits under laboratory storage conditions.

2. Materials and methods

2.1 Raw material and chemicals

KQX durian seeds were collected from local processing facilities in Southern Vietnam. Seeds of uniform size and free from germination, decay, mold contamination, and physical damage were selected for this study.

Seedless lime fruits of uniform size, color, and maturity, and free from visible defects or mechanical damage, were procured from Chavi Garden, Tay Ninh province, Vietnam.

Chitosan, with a degree of deacetylation of approximately 90% and a molecular weight of 100–150 kDa, was used as the film-forming biopolymer. Ethanol 99.5%, gallic acid, DPPH (2,2-diphenyl-1-picrylhydrazyl), acetic acid, sodium hydroxide, and other analytical-grade reagents were purchased from standard commercial suppliers.

2.2 Preparation of durian seed coat powder

Durian seeds were washed thoroughly with distilled water to remove adhering pulp and impurities. The seed coats were manually separated from the inner kernels. Both fractions were dried in a hot-air oven at 50–60°C for 48 h until the moisture content reached approximately 6%. The dried seed coats were ground using a laboratory mill and sieved through a 0.3 mm mesh to obtain a uniform powder. The resulting powders were packed in aluminum foil bags containing silica gel desiccants and stored at 4–6°C until further analysis. The seed coat powder was used for polyphenol extraction.

2.3 Polyphenol extraction

Polyphenols were extracted from KQX durian seed coat powder using a combined heat-assisted and ultrasound-assisted method adapted from Pinela *et al*. (2019). The extraction conditions used in this study were selected based on preliminary screening experiments conducted in our previous research. Therefore, these conditions are referred to as the experimental conditions or the most effective conditions among those tested, rather than fully optimized conditions. Briefly, 1 g of durian seed coat powder was mixed with 20 mL of ethanol-water (50:50, v/v), corresponding to a solvent-to-solid ratio of 20:1 mL/g. The pH of the extraction medium was adjusted to 5. The mixture was first subjected to ultrasonic treatment at 20 kHz with a power of 400 W for 5 min. Subsequently, heat-assisted extraction was performed at 50°C for 2 h. After extraction, the resulting mixture was subjected to centrifugation at 6000 rpm for 10 min at ambient temperature. The supernatant was collected and filtered through Whatman No. 4 filter paper to remove residual particulates. The filtered extract was used for the determination of total polyphenol content and biological activity assays. All extraction experiments were

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performed in triplicate.

The extraction efficiency was evaluated based on total polyphenol yield, expressed as mg gallic acid equivalents per gram dry weight of seed coat powder (mg GAE/g DW). The extract obtained under the selected experimental conditions was subsequently used for biological activity evaluation and incorporation into the chitosan-based coating system.

2.4 Determination of chemical composition and bioactive compounds

The chemical composition and bioactive compounds of KQX durian seed coat powder were determined using standard analytical methods. Unless otherwise stated, all analyses were performed in triplicate, and results were expressed on a dry weight basis.

2.4.1 Moisture content

Moisture content was determined according to AOAC Official Method 934.06 using a vacuum oven drying method.

2.4.2 Ash content

Ash content was determined using the dry ashing method according to AOAC Official Method 923.03.

2.4.3 Crude protein

Crude protein content was determined using the Kjeldahl method according to AOAC Official Method 978.04.

2.4.4 Lipid content

Lipid content was determined using the Soxhlet extraction method. Approximately 5 g of dried sample was placed in a cellulose extraction thimble and extracted with petroleum ether, boiling range 40–60°C, for 6–8 h. The solvent was removed after extraction, and the extraction flask containing the lipid residue was dried to constant weight. Lipid content was calculated from the weight of the extracted residue and expressed as percentage of the sample dry weight.

2.4.5 Total carbohydrate

Total carbohydrate content was calculated by difference according to Food and Agriculture Organization (FAO) guidelines (FAO, 1998). The carbohydrate content was estimated using the following equation: Total carbohydrate (%) = 100 − (% moisture + % protein + % lipid + % ash + % dietary fiber). The results were expressed as percentage of sample dry weight.

2.4.6 Dietary fiber

Dietary fiber content was determined according to TCVN 9050:2012 using the enzymatic-gravimetric method. Approximately 1 g of sample was sequentially digested with heat-stable α-amylase, protease, and amyloglucosidase to remove starch and protein. Insoluble dietary fiber was separated by filtration, while soluble dietary fiber was precipitated with ethanol. The residues were dried, weighed, and corrected for residual protein and ash. Total dietary fiber was calculated as the sum of soluble and insoluble dietary fiber fractions.

2.4.7 Total phenolic content

Total phenolic content (TPC) was determined using the Folin–Ciocalteu colorimetric method according to Matić and Jakobek (2021), with slight modifications. Briefly, 0.1 mL of extract was mixed with 0.5 mL of 10% Folin–Ciocalteu reagent and allowed to react for 5 min. Then, 1.5 mL of 7.5% sodium carbonate solution was added, and the mixture was incubated at room temperature in the dark for 30 min. Absorbance was measured at 765 nm using a UV–Vis spectrophotometer (Selecta UV 2005, Spain). Gallic acid was used as the calibration standard, and results were expressed as mg gallic acid equivalents per gram dry weight (mg GAE/g DW).

2.4.8 Total flavonoid content

Total flavonoid content was determined using the aluminum chloride colorimetric method described by Zhishen *et al.* (1999), with slight modifications. Briefly, the reaction mixture was prepared by mixing distilled water, the sample extract or standard solution, 5% sodium nitrite, and 10% aluminum chloride. After incubation, 1.0 mol/L sodium hydroxide was added, and the final volume was adjusted with distilled water. Absorbance was measured at 510 nm using a UV–Vis spectrophotometer (Selecta UV 2005, Spain). Quercetin was used as the calibration standard, and the results were expressed as mg quercetin equivalents per gram dry weight (mg QE/g DW).

2.4.9 Total alkaloid content

Total alkaloid content was determined using a spectrophotometric method based on ion-pair complex formation between alkaloids and acidic dye reagents, following Le *et al.* (2018), with slight modifications. Briefly, alkaloids in the extract reacted with the acidic dye reagent to form an alkaloid–dye complex, which was subsequently extracted into an organic phase. Absorbance was measured at an appropriate wavelength, approximately 415–470 nm, depending on the reagent system used. Atropine was used as the calibration

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standard, and the results were expressed as mg atropine equivalents per gram dry weight (mg AE/g DW).

2.4.10. Saponin content

Saponin content was determined using a spectrophotometric colorimetric method. The extract was reacted with the appropriate color-forming reagent, and absorbance was measured using a UV–Vis spectrophotometer. Saponin content was calculated from a standard calibration curve and expressed as mg saponin equivalents per 100 g dry weight.

2.4.11 Tannins content

Tannin content was determined by titration with potassium permanganate using indigo carmine as an indicator, following a modified pharmacopoeial/AOAC procedure. Briefly, an aliquot of the sample infusion was mixed with indigo carmine solution and distilled water, and then titrated with 0.1 N potassium permanganate until the color changed from blue to green and finally to golden yellow (Atanassova and Christova, 2009). A blank titration was performed under the same conditions. Tannin content was calculated from the volume of potassium permanganate consumed and expressed on a dry weight basis.

2.4.12 Vitamin C content

Vitamin C content was determined according to TCVN 6427-2:1998, based on redox titration with 2,6-dichlorophenolindophenol. A known amount of sample was homogenized and extracted using an acidic extraction solution to minimize ascorbic acid oxidation. The filtrate was titrated with standardized DCPIP solution until a persistent light pink endpoint was observed. Vitamin C content was calculated from the titrant volume and expressed as mg ascorbic acid per 100 g sample.

2.4.13 Zinc content

Zinc content was determined according to TCVN 10915:2015 using atomic absorption spectrophotometry. Samples were mineralized by dry or wet ashing, followed by acid digestion and dilution. Zinc concentration was measured at 213.8 nm using external calibration with zinc standard solutions and expressed as mg/kg dry weight.

2.5 Biological activities of the extract

2.5.1 Antibacterial activity

The antibacterial activity of the durian seed coat extract was evaluated against *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus* sp., obtained from the Institute of Tropical Biology, Vietnam Academy of Science and Technology, using the agar well diffusion method, following Bhattacharya *et al*. (2016) with minor modifications. Bacterial suspensions (~10^6 CFU/mL) were uniformly spread onto potato dextrose agar plates, and wells were then filled with extract solutions at different concentrations.

The plates were incubated at 37°C for 24 h. The antibacterial activity was evaluated by measuring the inhibition zone diameter around each well (Johnson *et al*., 2024). Streptomycin, tetracycline, and azithromycin (10 mg/L) were used as positive controls, while distilled water served as the negative control.

2.5.2 Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of the extract MIC (µg/mL) was determined using a 96-well microdilution method. Bacterial suspensions (10^6 CFU/mL) were exposed to serially diluted extract solutions prepared in 10% DMSO. After incubation at 37°C for 24 h, 0.01% resazurin was added as a growth indicator. The MIC was defined as the lowest concentration at which no visible color change occurred, indicating inhibition of bacterial growth (Ngan *et al*., 2012).

2.5.3 Antifungal activity

Antifungal activity of the extract was evaluated against *Fusarium oxysporum* and *Colletotrichum gloeosporioides* using the poisoned food technique (Fogliata *et al*., 2013). The strains used in this study were isolated by our research group from diseased lime samples and subsequently identified by 28S rRNA gene sequencing with the NCBI.

The inhibition percentage was calculated according to Attia *et al*. (2020) as follows:

$$Inhibition\ (\%) = \frac{D_c - D_t}{D_c} \times 100$$

where D_c is the colony diameter in the control treatment (mm) and D_t is the colony diameter in the extract-treated samples (mm).

2.5.4 Antioxidant activity

The antioxidant activity of the extract was assessed using the DPPH radical scavenging assay according to Mensor *et al*. (2001). The decrease in absorbance was measured after reaction with DPPH solution, the radical-scavenging activity was calculated as:

$$DPPH\ (\%) = \frac{A_o - A_S}{A_o} \times 100$$

where A_o is the absorbance of the control and A_s is the absorbance of the sample. The antioxidant activity was expressed as IC_{50}, defined as the extract

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concentration required to inhibit 50% of DPPH radicals.

2.6 Preparation and characterization of chitosan-based films

2.6.1 Preparation of chitosan–extract film

The chitosan solution was prepared by dissolving chitosan powder at 1.5% (w/v) in 5% (v/v) acetic acid under continuous stirring at ambient temperature for 4–6 h until complete dissolution. The solution was filtered to remove insoluble residues, and the pH was adjusted to 5–6 using 0.1 N NaOH. No external plasticizer was added to the formulation.

The film-forming solution was prepared by dissolving 1.5% (w/v) chitosan and incorporating 795 µg/mL KQX durian seed coat extract. The solution was stirred for 1 h at ambient temperature to obtain a homogeneous mixture. A fixed volume of 100 mL of the film-forming solution was cast onto each mica sheet with a surface area of 160 cm^2 and dried at ambient temperature for 8 h. After drying, the films were carefully peeled from the mica sheet, cut into 2 × 8 cm^2 specimens, and conditioned prior to analysis.

2.6.2 Film characterization

2.6.2.1 Film thickness

The thickness of the films was measured using a digital caliper with an accuracy of ±0.001 mm. Measurements were taken at randomly selected points across the film surface. Ten film samples were analyzed, and the mean thickness was calculated.

2.6.2.2 Optical properties

The optical properties of the films were evaluated by measuring the transmittance at a wavelength of 643 nm. Ten film samples were analyzed, and the mean value was reported. Film transparency at 643 nm (T_{643}) was calculated according to Kanatt *et al*. (2012) using the following equation:

$$T_{643} = \frac{-log(\%T)}{b} = \frac{OD}{b}$$

where −log (%*T*) represents the optical density at 643 nm, and *b* is the film thickness (mm).

2.6.2.3 Surface morphology

The surface morphology of the films was examined using field-emission scanning electron microscopy (FE-SEM), following the procedure described by Vilela *et al*. (2019).

2.6.2.4 Mechanical properties

Mechanical properties were determined using a Zwicki Z1.0TH texture analyzer (ZwickRoell, Germany). Tensile strength and elongation at break were measured according to ASTM D638, using ZwickRoell testing software and following the methods described by Padrao *et al.* (2016).

2.7 Edible coating experiment for seedless lime preservation

2.7.1 Experimental design

A total of 180 seedless lime fruits were randomly divided into three treatments, with 60 fruits per treatment: (i) negative control (NC): uncoated fruits; (ii) chitosan coating (CS): fruits coated with 1.5% (w/v) chitosan solution; and (iii) chitosan–durian seed coat extract coating (CD): fruits coated with 1.5% (w/v) chitosan solution containing 795 µg/mL durian seed coat extract. The selected concentration was used as a practical baseline concentration derived from the antibacterial MIC assay and was not considered an optimized antifungal dose.

The extract concentration of 795 µg/mL was selected as a practical working concentration for incorporation into the chitosan-based coating because it corresponded to the MIC determined against *S. aureus* in the antibacterial assay. This concentration was used as an initial functional level to evaluate the performance of the composite coating, rather than as an optimized antifungal dose for citrus postharvest pathogens. Although fungal spoilage is highly relevant to lime deterioration, the antifungal assay showed a concentration-dependent response, indicating that higher extract concentrations may be required for stronger direct fungal inhibition. Therefore, the preservation effect of the composite coating was interpreted as the combined contribution of the chitosan film-forming barrier and the moderate bioactivity of the durian seed coat extract

2.7.2. Preparation of coating solutions

The chitosan coating solution was prepared as described in Section 2.6.1. For the composite coating, durian seed coat extract was added to the chitosan solution to obtain a final extract concentration of 795 µg/mL. The solution was stirred until homogeneous before use. The prepared coating solutions were stored at 4°C and used within 48 h.

2.7.3. Fruit treatment and storage conditions

Before coating, seedless lime fruits were surface-sterilized by immersion in 0.0025% (w/v) calcium hypochlorite solution for 2–3 min, rinsed with distilled water, and air-dried at room temperature. Fruits were then immersed in the respective coating solutions for 2 min, drained, and air-dried at room temperature to allow

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coating formation. The treated fruits were placed in labeled plastic containers to avoid cross-contamination and stored at 25±2°C for 25 days (Huong *et al*., 2021).

2.7.4 Sampling procedure

Sampling was conducted at 5-day intervals during storage. For each treatment, 60 fruits were used in total. Of these, 30 fruits were used for destructive analyses, including firmness, soluble solids, pH, titratable acidity, and vitamin C content. The remaining 30 fruits were used for non-destructive monitoring, including weight loss, color, and decay incidence.

At each sampling time, five fruits per treatment were collected for destructive analysis, while five fruits per treatment were monitored for non-destructive quality attributes. All measurements were performed in triplicate under a completely randomized design.

2.8 Evaluation of fruit quality

2.8.1 Color measurement

Color parameters (L*, a*, b*) were measured using a colorimeter (Konica Minolta CM-5, Japan).

2.8.2 Weight loss

Weight loss (%) was determined gravimetrically using the following equation:

$$Weight\ loss = \frac{W_1 - W_2}{W_1} \times 100$$

where: W_1 is the initial sample weight (g); W_2 is the sample weight at each sampling time (g).

2.8.3 Fruit firmness

Fruit firmness was measured using a fruit firmness tester. Measurements were taken at three different positions on each fruit using a flat cylindrical probe (8 mm diameter). Firmness was calculated as:

$$X = \frac{F}{S}$$

where: X is fruit firmness (kg/cm²); F is the applied force (kg); S is the probe tip area (cm²), calculated as: $S = \pi r^2$.

2.8.4 Titratable acidity

Titratable acidity was determined according to AOAC Official Method 942.15.

2.8.5 Vitamin C content

A 10 mL aliquot of clear filtrate was transferred into a 100 mL conical flask. Subsequently, ten drops of 0.5% starch indicator were added, and the solution was titrated with 0.01 N iodine (I_2) until a light blue endpoint appeared (Nielsen, 2010). Vitamin C content was calculated as:

$$X = \frac{a \times V \times 0.00088 \times 100 \times 1000}{v \times c}$$

where X is vitamin C content (mg/100 g), a is the volume of iodine used (mL), V is the total extract volume (mL), v is the aliquot volume (10 mL), and c is the sample mass (g). The factor 0.00088 represents grams of ascorbic acid equivalent to 1 mL of 0.01 N iodine.

2.8.6 Total soluble solids

The total soluble solids were measured using a handheld refractometer (SLI-32, Hong Kong) with a range of 0–32°Bx and a resolution of 0.2%.

2.8.7 pH measurement

A 10 mL aliquot of freshly extracted lime juice was placed in a 50 mL beaker, and pH was measured using a benchtop pH meter (PH 1200, HORIBA, Japan; resolution 0.001). The instrument was calibrated using standard buffer solutions at pH 4.01, 7.00, and 10.00 prior to analysis.

2.8.8 Decay incidence

Decay incidence was evaluated using a sensory scoring system (Table 1). Each fruit was individually assessed and assigned a decay score. Fruits with scores of 4–5 were classified as sound, fruits with a score of 3 were classified as moderately affected, and fruits with scores of 1–2 were classified as decayed. For statistical analysis, each fruit was treated as an individual observational unit, and decay status was coded as a binary outcome: sound/non-decayed or decayed. Decay incidence was calculated as follows:

$$Decay\ incidence\ (\%) = \frac{Number\ of\ decayed\ fruits}{Total\ number\ of\ fruits} \times 100$$

2.9 Statistical analysis

All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using Minitab (version 16). Differences among means, including weight loss, firmness, total soluble solids, pH, titratable acidity, vitamin C content, and color attributes, were evaluated using analysis of variance (ANOVA), followed by post hoc test at a significance level of $p < 0.05$.

Decay incidence was treated as proportion/count data and reported as the number and percentage of decayed fruits. Differences among treatments were interpreted based on proportion data rather than

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Table 1. Sensory quality scale for postharvest seedless lime fruits.

Score	Degree of deterioration	Sensory description
1	Very severe	Completely decayed; extensive mold; strong off-odor; black discoloration
2	Severe	>50% surface decay; soft, watery tissue; off-odor; black discoloration
3	Moderate	Slight softening; localized decay; partial yellowing
4	Slight	Minor black spots, slight softening; no off-odor; green coloration
5	Good	Firm; no visible defects; no off-odor; green color

continuous-variable ANOVA. Therefore, decay incidence was discussed cautiously as an indicator of visible spoilage occurrence during storage.

3. Results and discussion

3.1 Proximate composition and bioactive compounds in KQX durian seed coat

The proximate composition and bioactive compound contents of KQX durian seed coat powder are presented in Table 2.

Table 2. Proximate composition and bioactive compound contents of KQX durian seed coat powder.

No.	Analytical parameter	Unit	Result
1	Moisture	%	5.66±0.03
2	Ash content	%	3.78±0.00
3	Total carbohydrates	%	84.04±0.03
4	Starch	%	28.53±0.19
5	Protein	%	5.98±0.01
6	Soluble fiber	%	1.04±0.00
7	Insoluble fiber	%	2.09±0.01
8	Lipid	%	0.52±0.00
9	Phenolic compounds	mg GAE/g	239.87±0.87
10	Flavonoids	mg QE/g	88.95±0.51
11	Alkaloids	mg AE/g	11.39±0.06
12	Saponins	mg/100 g	47.46±0.48
13	Tannins	mg/100 g	6.80±0.06
14	Vitamin C	mg/100 g	1.09±0.00
15	Zn	mg/kg	6.87±0.08

Values are presented as mean±SD.

The results indicate that KQX durian seed coat is predominantly composed of carbohydrates (84.04%), with moderate protein content (5.98%) and low lipid content (0.52%). The relatively low moisture level (5.66%) suggests good storage stability and reduced susceptibility to microbial spoilage. The presence of both soluble (1.04%) and insoluble fiber (2.09%) further enhances its potential as a functional ingredient, particularly for applications targeting dietary fiber enrichment.

Notably, the seed coat exhibited a high concentration of total phenolic compounds (239.87 mg GAE/g), along with substantial levels of flavonoids (88.95 mg QE/g) and saponins (47.46 mg/100 g). These values are considerably higher than those typically reported for durian seed kernels, indicating that the seed coat serves as a major reservoir of phytochemicals. The elevated phenolic and flavonoid contents are particularly relevant, as these compounds are strongly associated with antioxidant capacity and antimicrobial activity.

In comparative terms, the phenolic content of the seed coat was approximately fourfold higher than that of the kernel, while flavonoid and saponin contents were approximately threefold and twofold higher, respectively. This compositional disparity underscores the importance of valorizing durian seed coat, which is often treated as an agro-industrial by-product.

From a functional perspective, the high abundance of bioactive compounds suggests that KQX durian seed coat has strong potential for incorporation into functional food systems, edible coatings, or biodegradable films. In particular, its rich phenolic profile supports its use as a natural antioxidant and antimicrobial agent, which may contribute to shelf-life extension and quality preservation in perishable food products.

3.2 Factors affecting polyphenol extraction yield

The effects of extraction conditions on polyphenol recovery from KQX durian seed coat were systematically evaluated. The highest polyphenol yield (51.22 mg GAE/g dry weight, DW) was obtained under the following conditions: solvent-to-solid ratio of 20:1 (mL/g), extraction temperature of 50°C, pH 5, heating time of 120 min, ultrasonic power of 400 W, and ultrasonication time of 5 min. These conditions are therefore considered the most effective among those tested for maximizing polyphenol extraction.

The yield obtained in this study is higher than values reported for comparable durian-derived materials, for both peel and seed samples the maximum amount of TPC with 32.80 and 0.09 mg GAE/g (Ramli *et al*., 2021); 32.81 and 0.63 mg GAE/g in seed samples and the lowest in pulps (Lou *et al*., 2026). Although direct comparisons should be interpreted cautiously due to differences in extraction protocols and reporting units, the results indicate that the combined heat-assisted and

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ultrasound-assisted approach enhances the recovery of phenolic compounds from the seed coat matrix.

A comparison between extraction approaches further demonstrated the contribution of ultrasound treatment. Conventional extraction yielded 39.81 mg GAE/g DW, whereas the inclusion of ultrasonication (20 kHz, 400 W, 5 min) increased the yield to 51.22 mg GAE/g DW, corresponding to an improvement of approximately 28.6%. This enhancement can be attributed to acoustic cavitation, which promotes cell wall disruption, increases solvent penetration, and accelerates mass transfer of intracellular phenolic compounds.

In addition to improving extraction efficiency, the selected extraction conditions likely contributed to preserving the chemical integrity of phenolic compounds. The moderate temperature (50°C) and mildly acidic pH (pH 5) are known to limit oxidative degradation and structural modification of phenolics, thereby maintaining their redox-active functional groups.

These compositional features are closely associated with the observed biological activities of the extract. Extracts obtained under the most effective conditions exhibited higher antioxidant capacity and stronger antimicrobial activity, reflecting the increased availability of phenolic and flavonoid compounds. Phenolics are well established as hydrogen- or electron-donating molecules, which explains the improved radical scavenging activity observed in the DPPH assay. Furthermore, these compounds can interact with microbial cell membranes, disrupt permeability, and inhibit essential enzymatic processes, thereby contributing to antimicrobial efficacy.

The observed relationship between extraction conditions, phenolic content, and bioactivity highlights a clear composition-function linkage, where enhanced recovery of bioactive compounds translates into improved functional performance. Similar trends have been reported in plant-based systems, where ultrasound-assisted extraction increases both phenolic yield and associated antioxidant activity (Tran *et al*., 2016).

Overall, the results demonstrate that the combined extraction approach employed in this study is effective in enhancing polyphenol recovery while preserving bioactivity, thereby supporting the potential application of durian seed coat extract as a natural antioxidant and antimicrobial agent in food systems.

3.3 Biological activities of KQX durian seed coat extract

3.3.1 Antibacterial activity

The antibacterial activity of KQX durian seed coat extract against *Bacillus* sp., *E. coli*, and *Staphylococcus aureus* is presented in Figure 1. A clear concentration-dependent response was observed, with inhibition zone diameters increasing as extract concentration increased.

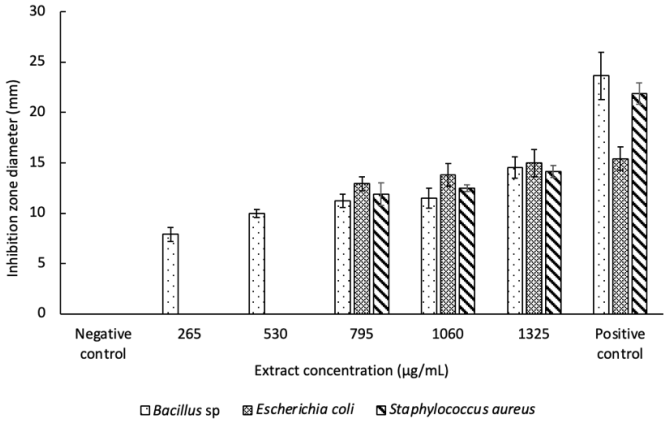


Figure 1. Relationship between extract concentration and inhibition zone diameter.

At lower concentrations (265–530 µg/mL), antibacterial activity was limited, with inhibition zones observed only for *Bacillus* sp. (7.91–9.99 mm), while no detectable inhibition was recorded for *E. coli* and *S. aureus*. At higher concentrations (795–1325 µg/mL), the extract exhibited moderate antibacterial activity against all tested strains, with inhibition zones ranging from approximately 11 to 15 mm.

The earlier onset of inhibition in *Bacillus* sp. compared to *E. coli* and *S. aureus* suggests differences in cell wall structure and susceptibility. Gram-negative bacteria such as *E. coli* possess an outer membrane that can restrict the penetration of phenolic compounds, whereas Gram-positive bacteria are generally more susceptible due to their simpler cell envelope. However, statistical analysis indicated no significant differences among the three strains at higher concentrations ($p > 0.05$), suggesting a broadly comparable antibacterial spectrum under effective dosing conditions.

The antibacterial activity observed in this study is consistent with the phytochemical composition and extraction performance of the durian seed coat extract. The relatively high phenolic and flavonoid contents obtained under the most effective extraction conditions likely contributed to the inhibitory effects against bacterial growth. Phenolic compounds can impair microbial viability through multiple mechanisms, including disruption of cell membrane integrity, denaturation of cellular proteins, alteration of membrane permeability, and interference with intracellular metabolic processes. Therefore, the moderate antibacterial activity recorded in this study is biologically plausible and aligns with the extract's phenolic-rich profile.

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3.3.2 Minimum inhibitory concentration

Based on the agar diffusion results, *S. aureus* was selected for minimum inhibitory concentration (MIC) determination due to its consistent and representative response to the extract. The MIC was assessed using a resazurin-based microdilution assay following incubation at 37°C for 24 h. Subsequently, 20 µL of 0.01% (w/v) resazurin solution was added to each well, and bacterial growth was evaluated based on the color change (Figure 2).



Figure 2. Minimum inhibitory concentration of durian seed coat extract against *S. aureus.* Column 1: medium control, Column 2: bacterial control, Column 3: antibiotic control, Columns 4–9: extract concentrations of 1060, 795, 530, 265, 132.5, and 66.25 µg/mL, respectively.

The results indicate that the lowest concentration of the extract capable of completely inhibiting visible bacterial growth was 795 µg/mL, which was therefore defined as the MIC. This finding confirms that the durian seed coat extract possesses measurable antibacterial activity against *S. aureus*, albeit at relatively high concentrations.

The comparatively high MIC value, relative to purified plant-derived antimicrobials reported in the literature, is expected given the crude nature of the extract. Crude extracts typically contain a heterogeneous mixture of active and inactive constituents, which can dilute the effective concentration of bioactive compounds. In addition, direct comparisons should be interpreted cautiously due to differences in reporting units and experimental conditions. For example, plant extracts such as *Syzygium antisepticum*, *Punica granatum*, and *Curcuma longa* have demonstrated substantially lower MIC values when expressed in mg/mL, reflecting higher apparent potency under different extraction and assay systems (Yuan and Yuk, 2018; Imran *et al*., 2021). Similarly, a wide range of antibacterial efficacy has been reported for other plant-derived extracts, with MIC values varying from highly active to relatively weak depending on composition and extraction method (Preuttiporn *et al*., 2022).

From a mechanistic perspective, the antibacterial activity observed at 795 µg/mL concentration is consistent with the phenolic-rich nature of the extract and the improved recovery of bioactive compounds under the selected extraction conditions. Phenolic compounds and flavonoids are known to disrupt bacterial cell membranes, increase permeability, and interfere with intracellular metabolic processes, thereby inhibiting microbial growth. However, the relatively high concentration required suggests that the extract exhibits moderate potency, which is typical for non-fractionated plant extracts.

Importantly, the MIC value obtained in this study provided a rational basis for selecting the extract concentration used in subsequent applications. The incorporation of 795 µg/mL extract into chitosan-based coatings ensures that the formulated system retains antimicrobial functionality at or above the inhibitory threshold. Overall, the MIC results, together with the agar diffusion assay, confirm that KQX durian seed coat extract exhibits concentration-dependent antibacterial activity and support its potential application as a natural antimicrobial agent in food preservation systems.

It should be noted that the MIC value of 795 µg/mL was obtained against *S. aureus* and was therefore used as an antibacterial reference point for subsequent coating formulation. This concentration should not be interpreted as an optimized antifungal concentration for citrus pathogens. Rather, it provided a rational and reproducible baseline for incorporating the crude extract into the chitosan matrix while maintaining a moderate level of antimicrobial functionality.

3.3.3 Antifungal activity

The antifungal activity of KQX durian seed coat extract against *Fusarium oxysporum* and *Colletotrichum gloeosporioides* is presented in Figure 3. After 7 days of incubation, the extract exhibited a clear concentration-dependent inhibitory effect on the growth of both fungal strains.

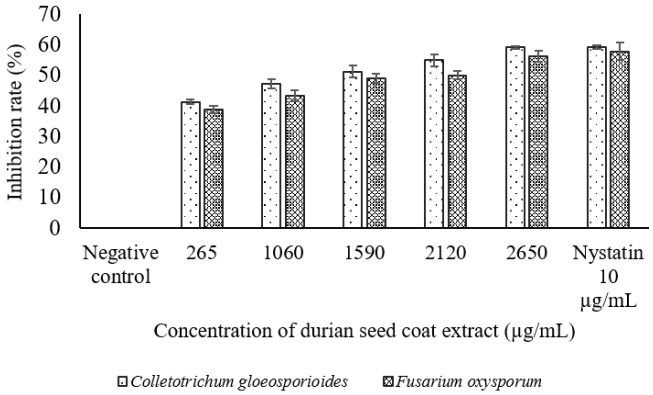


Figure 3. Inhibition rate of fungal growth at different extract concentrations.

As shown in Figure 3, increasing the extract concentration from 530 µg/mL to 2650 µg/mL resulted

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in a progressive reduction in colony diameter for both fungi, accompanied by a corresponding increase in inhibition percentage. At the highest concentration (2650 µg/mL), the inhibition rates reached 59.14±0.50% for *C. gloeosporioides* and 56.33±1.79% for *F. oxysporum*, indicating slightly higher sensitivity of *C. gloeosporioides*. Across all tested concentrations, the inhibitory effect against *C. gloeosporioides* consistently exceeded that observed for *F. oxysporum*, suggesting species-specific susceptibility differences.

Despite this notable activity, the antifungal efficacy of the extract remained lower than that of the positive control (nystatin, 10 µg/mL), which is expected given the crude nature of the extract. Statistical analysis confirmed significant differences among extract concentrations ($p < 0.05$), with the highest activity observed at 2650 µg/mL, while both the negative control and solvent control exhibited negligible inhibition, confirming that the antifungal effect was attributable to the extract.

The observed antifungal activity is consistent with the phenolic- and flavonoid-rich nature of the extract and the improved recovery of bioactive compounds under the selected extraction conditions. The compounds may contribute to fungal growth inhibition by disrupting membrane integrity, increasing cellular permeability, and interfering with enzymatic systems involved in cell wall synthesis and metabolic regulation. The moderate but concentration-dependent antifungal activity observed in this study therefore appears to be associated with the phytochemical profile of the extract, although further compound-level characterization would be needed to confirm the specific active constituents and mechanisms involved.

Comparable antifungal effects have been reported for other polyphenol-rich plant extracts. For instance, pomegranate (*Punica granatum*) peel extracts have demonstrated inhibitory activity against postharvest pathogens such as *Penicillium digitatum*, *P. expansum*, and *Botrytis cinerea* (Nawaz *et al*., 2025). Similarly, extracts from Agave species rich in flavonoids and phenolics have shown strong *in vitro* antifungal activity (Khadiri *et al*., 2024). In the context of durian-derived materials, extracts from *Durio zibethinus* have been reported to inhibit *Candida albicans* and exhibit bioactive properties comparable to standard antifungal agents (Abdul *et al.,* 2022), while durian peel-derived polyphenols have also been utilized in antifungal nanomaterial synthesis (Duong *et al*., 2023).

Mechanistically, the antifungal action of phenolic compounds involves multiple targets, including disruption of membrane integrity, interference with ergosterol biosynthesis, and inactivation of essential enzymes (Liu *et al*., 2013; Redondo-Blanco *et al*., 2020; Khanzada *et al*., 2021). The moderate yet consistent antifungal activity observed in this study is therefore in line with the extract's phytochemical profile and supports its functional potential.

Overall, KQX durian seed coat extract exhibited concentration-dependent antifungal activity against both tested fungal strains, with stronger inhibition observed at higher concentrations, particularly at ≥ 2120 µg/mL. However, its efficacy remained lower than that of the positive control, which is expected for a crude plant extract. Therefore, the concentration of 795 µg/mL used in the coating experiment should not be interpreted as the most effective antifungal dose; rather, it was selected as a practical baseline concentration derived from the antibacterial MIC assay against *S. aureus*. Further formulation studies are needed to optimize the extract concentration specifically against major citrus postharvest fungal pathogens.

3.3.4 Antioxidant activity

The antioxidant activity of KQX durian seed coat extract was evaluated using the DPPH radical-scavenging assay, with ascorbic acid as the reference standard. The IC_{50} values obtained were 72.7 µg/mL for vitamin C and 347.18 µg/mL for the extract, indicating that the extract exhibited approximately 4.8-fold lower radical-scavenging capacity.

Despite this lower activity relative to the pure standard, the observed antioxidant capacity is consistent with the high total phenolic content and flavonoid content reported previously. Phenolic compounds are known to contribute to antioxidant activity primarily through hydrogen atom transfer and single electron transfer mechanisms, which are directly assessed by the DPPH assay. However, the relationship between total phenolic content and IC_{50} is not necessarily linear, as antioxidant efficiency depends not only on concentration but also on structural characteristics such as hydroxyl group distribution, degree of conjugation, and molecular size.

The relatively high IC_{50} value observed in this study, despite substantial phenolic content, suggests that the extract may contain a higher proportion of phenolic compounds with moderate radical-scavenging efficiency, such as condensed tannins or bound phenolics with limited accessibility. In addition, the crude extract likely contains non-active constituents (e.g., carbohydrates and dietary fibers), which may dilute the effective concentration of antioxidant compounds.

Extraction efficiency is another key factor

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influencing antioxidant activity. Parameters such as solvent polarity, extraction time, temperature, and solid-to-solvent ratio significantly affect the recovery of phenolic compounds, particularly those bound to cell wall structures. Suboptimal extraction conditions may limit the release of active compounds, resulting in higher IC_{50} values. This may partly explain the differences observed when compared with previous studies. For example, reported a lower IC_{50} value (99.70 µg/mL) (Aisyah *et al*., 2024), while Tran *et al*. (2024) reported an even stronger DPPH scavenging activity for durian peel extract (IC_{50} = 38.72 µg/mL). Similarly, Ang *et al*. (2018) reported EC_{50} values of 229.29, 54.14, and 44.17 mg/L for ethanol extracts of durian leaves, peels, and seeds, respectively.

These variations can also be attributed to differences in plant matrices. Peel and leaf tissues typically contain higher levels of low-molecular-weight phenolics and flavonoids, which exhibit stronger radical-scavenging activity, whereas seed coats may be richer in structurally complex or matrix-bound phenolics with lower apparent reactivity.

From an application perspective, although the antioxidant activity of the KQX durian seed coat extract was lower than that of purified standards and some previously reported durian-derived materials, it remains within a functionally relevant range. In real food systems, antioxidant performance depends not only on intrinsic radical-scavenging capacity but also on compound stability, interactions with the food matrix, and protection provided by the coating system. Therefore, the relatively high phenolic content, together with its moderate antioxidant activity, supports the potential use of this by-product as a natural antioxidant source in food preservation applications.

Further studies should focus on optimizing extraction conditions to enhance phenolic recovery, identifying key bioactive compounds using advanced analytical techniques (e.g., LC-MS/MS), and evaluating antioxidant efficacy in real food matrices to validate its practical applicability.

3.4 Application of durian seed coat extract in edible coating for seedless lime preservation

3.4.1 Film-forming ability

The composite chitosan–durian seed coat extract (CD) solution exhibited excellent film-forming capacity when applied to spherical substrates. The resulting films were continuous, uniform, and free from visible defects such as cracks, peeling, or structural discontinuities after drying, indicating strong interfacial adhesion between the coating matrix and the substrate surface. These characteristics are essential for effective barrier formation and support the applicability of the CD system for postharvest fruit coating.

For physicochemical characterization, films were prepared via solvent casting onto mica sheets (2 × 8 cm; thickness < 0.05 mm), yielding homogeneous and handleable films suitable for subsequent analyses (Figure 4).



Figure 4. CS film (a) and CD film (b).

3.4.2 Mechanical and physicochemical properties of the films

3.4.2.1 Mechanical properties

The incorporation of durian seed coat extracts significantly modified the mechanical behavior of chitosan films (Table 3). Specifically, the tensile modulus (Et) decreased from 935.00±7.74 MPa to 881.55±6.90 MPa, while maximum tensile stress (σm) and tensile strength at break (σ_β) were reduced from 15.05±1.36 MPa to 12.47±0.15 MPa and from 13.19±1.27 MPa to 10.01±0.90 MPa, respectively ($p < 0.05$).

Table 3. Mechanical properties of chitosan films and chitosan-extract composite films.

Parameter	CS Film	CD Film
Et (MPa)	$935.000±7.74^a$	$881.545±6.9^b$
σm (MPa)	$15.054±1.36^a$	$12.470±0.15^b$
εm (%)	$2.915±0.39^a$	$2.750±0.18^b$
σ_β (MPa)	$13.192^{\pm}1.27^a$	$10.010±0.90^b$
ε_β (%)	$2.608±0.14^b$	$3.120±0.37^a$

Values are presented as mean±SD. Values with different superscripts in the same row are statistically significantly different between films ($p<0.05$).

This reduction in stiffness and strength suggests partial disruption of intermolecular hydrogen bonding within the chitosan network, likely due to the incorporation of low-molecular-weight phytochemicals acting as internal plasticizers. Conversely, elongation at break (ε_β) increased significantly from 2.61±0.14% to 3.12±0.37% ($p < 0.05$), indicating enhanced film

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flexibility. No statistically significant change was observed for elongation at maximum stress (εm) ($p > 0.05$). Overall, the results indicate a plasticization effect, where the extract improves ductility at the expense of mechanical strength, an outcome commonly reported for biopolymer and polyphenol systems.

3.4.2.2 Film thickness

The thickness of the chitosan (CS) film and the chitosan-extract (CD) composite film is presented in Table 4.

Statistical results indicated that no significant difference in thickness was observed between the two films ($p > 0.05$). The low standard deviation values indicate good uniformity and reproducibility of the film-forming process. The negligible change in thickness suggests that the incorporation of 795 µg/mL extract did not substantially alter the total solid content or the structural packing of the film matrix. This may be attributed to the homogeneous dispersion of extract components within the chitosan network, as well as the controlled casting and drying conditions, which ensured consistent film formation.

Table 4. Thickness of chitosan films incorporated with 795 µg/mL extract.

Film type	Thickness (mm)
1.5% chitosan film	0.0259±0.0020[a]
1.5% chitosan film + 795 µg/mL extract	0.0265±0.0014[a]

Values are presented as mean±SD. Values with different superscripts in the same column are statistically significantly different between films ($p<0.05$).

3.4.2.3 Surface morphology

The FE-SEM micrograph (Figure 5) shows that the chitosan-extract (CD) composite film exhibits a continuous and crack-free structure, confirming good film-forming ability. However, compared with the smooth and homogeneous surface of the pure chitosan (CS) film, the composite film displays a more heterogeneous morphology characterized by dispersed particulate features.

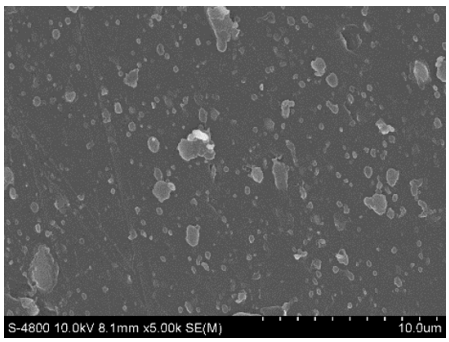


Figure 5. FE-SEM image of the 1.5% chitosan film incorporated with 795 µg/mL extract at 5,000× magnification.

These particles, ranging from submicron to several micrometers, are attributed to the incorporation of plant extract components within the chitosan matrix. The presence of such dispersed phases suggests partial phase separation or aggregation of extract constituents, leading to a less compact and less uniform polymer network. Similar morphological features have been reported in chitosan films containing plant extracts, such as betel leaf extract, where particulate structures were observed on the film surface (Bich and Thuong, 2019).

This microstructural heterogeneity provides a direct explanation for the observed changes in mechanical properties (Table 3). Specifically, the reduction in tensile modulus (Et) and tensile strength (σm and σ_β) can be attributed to the disruption of intermolecular hydrogen bonding within the chitosan matrix caused by the incorporation of extract components. The dispersed particles act as structural discontinuities or weak points, reducing stress transfer efficiency across the polymer network. Conversely, the increase in elongation at break (ε_β) indicates enhanced chain mobility, which is consistent with a plasticization effect induced by low-molecular-weight compounds present in the extract.

The observed morphology also correlates with the optical properties of the films (Table 5). The presence of particulate structures and phase heterogeneity increases light scattering within the film matrix, resulting in higher optical density and reduced transparency. In addition, phenolic compounds present in the extract contribute to light absorption in the UV-visible range, further enhancing the light barrier properties and imparting a brownish-yellow coloration to the composite film.

Table 5. Optical properties of chitosan (CS) and chitosan-extract (CD) films.

Property	Chitosan 1.5%	Chitosan 1.5% and extract 795 µg/mL
Optical density	0.224±0.0174[b]	0.337±0.022[a]
Transparency (mm^{-1})	9.34[b]	12.72[a]
Film color	White	Light brownish-yellow
Light barrier ability	Low	High

Values are presented as mean±SD. Values with different superscripts in the same row are statistically significantly different between films ($p<0.05$).

Overall, the FE-SEM observations provide clear microstructural evidence supporting the mechanical weakening, increased flexibility, and enhanced light-blocking capacity of the chitosan-extract composite film. These structure-property relationships highlight the dual role of the extract as both a plasticizing agent and a functional additive, contributing to the performance of the film in food preservation applications.

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3.4.2.4 Optical properties of the films

The optical properties of food packaging films are critical parameters influencing their protective performance. In general, higher light transmittance indicates greater transparency, whereas increased absorbance or optical density (OD) reflects enhanced turbidity and light-blocking capacity. Improved light barrier properties are particularly desirable in food preservation, as they help mitigate photooxidation and degradation of light-sensitive compounds. The optical properties of the films are presented in Table 5.

As shown in Table 5, the incorporation of 795 µg/mL extract significantly increased the OD value ($p < 0.05$), indicating reduced transparency and enhanced light attenuation. This effect can be attributed to two main factors. First, phenolic and flavonoid compounds present in the extract exhibit intrinsic UV-visible light absorption, contributing directly to increased optical density. Second, as observed in the FE-SEM analysis (Figure 5), the presence of dispersed particulate structures within the composite film enhances light scattering, further reducing transmittance. The combined effects of light absorption and scattering result in the observed light brownish-yellow coloration and improved light barrier properties of the composite film. This structural-optical relationship is consistent with previous observations in chitosan films containing plant-derived additives, where increased turbidity and reduced transparency are associated with heterogeneous microstructures and the presence of bioactive compounds.

From an application perspective, the enhanced light-blocking capacity of the chitosan-extract composite film is advantageous for food packaging, as it can reduce the rate of photoinduced oxidation reactions and extend the shelf life of light-sensitive products. Therefore, despite reduced transparency, the optical characteristics of the composite film are functionally beneficial for preservation purposes.

3.4.3 Effectiveness of chitosan-extract films in the preservation of seedless limes

3.4.3.1 Firmness

Fruit firmness decreased progressively during storage across all treatments (Figure 6), reflecting natural softening due to physiological and biochemical changes in the cell wall. However, the extent of softening differed significantly among treatments ($p < 0.01$).

The control exhibited the greatest reduction (51.66%), followed by chitosan-coated samples (41.39%), whereas the chitosan-extract treatment showed the lowest decrease (34.53%), retaining higher structural integrity after 25 days. This improved performance may reflect the combined contribution of the chitosan matrix and durian seed coat extract. The semipermeable nature of chitosan limits moisture loss and gas exchange, while phenolic compounds in the extract may inhibit oxidative and enzymatic degradation of cell wall components.

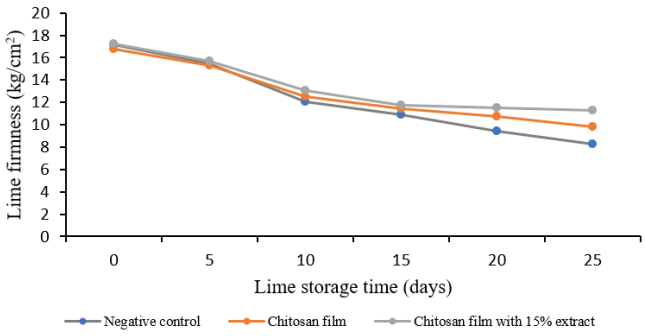


Figure 6. Changes in lime firmness during storage.

Mechanistically, firmness loss is associated with the enzymatic conversion of protopectin into soluble pectin, catalyzed by protopectinase and polygalacturonase, leading to cell wall weakening (Kays, 1991). The slower decline observed in coated samples is consistent with previous reports on fruit softening during storage (Lợi and Tùng, 2019; Hasbullah and Ismail, 2022).

3.4.3.2 Weight loss

Weight loss increased steadily during storage in all treatments (Figure 7), driven by transpiration and respiration processes. However, significant differences were observed among treatments ($p < 0.001$).

The control showed the highest weight loss (31.64%), while chitosan coating reduced this to 19.55%. The composite coating further minimized weight loss to 15.06%, demonstrating superior barrier performance. This effect may be associated with the semipermeable barrier formed by the coating, which can reduce moisture transfer and may partially modulate gas exchange at the fruit surface.

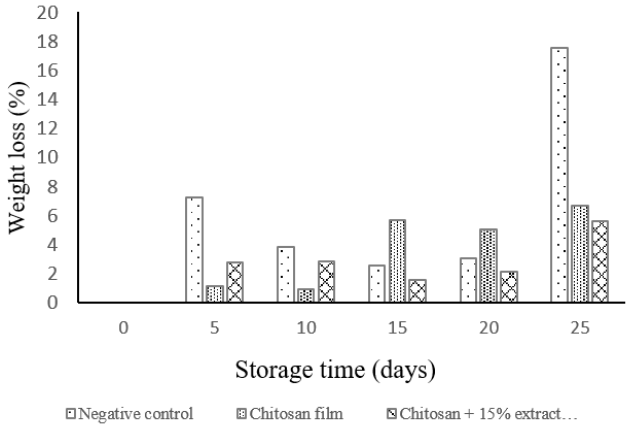


Figure 7. Weight loss of limes during the storage period.

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These findings are consistent with previous studies on chitosan-based coatings enriched with phenolic compounds, which effectively reduce mass loss and maintain fruit quality (Riaz *et al.,* 2021; Demircan and Velioglu, 2024). Weight loss during storage is primarily governed by water evaporation and metabolic activity, both of which are influenced by temperature and storage duration (Lợi and Tùng, 2019; Hasbullah and Ismail, 2022).

3.4.3.3 Total soluble solid

Changes in °Brix followed a typical pattern, with a slight initial increase followed by a gradual decline (Figure 8). The reduction was most pronounced in the control (41.82%), followed by chitosan (30.95%), and lowest in the composite coating (24%) ($p < 0.05$).

The decline in soluble solids is attributed to their utilization as substrates in respiration. The slower decline in soluble solids in coated fruits may be related to the semipermeable nature of the coating, which can partially restrict gas exchange and thereby slow metabolic changes during storage. Additionally, phenolic compounds may contribute to structural stabilization and oxidative protection (Nxumalo and Fawole, 2022; Dai *et al.,* 2025). These results align with previous findings on chitosan-polyphenol coatings in fruit preservation (Riaz *et al*., 2021).

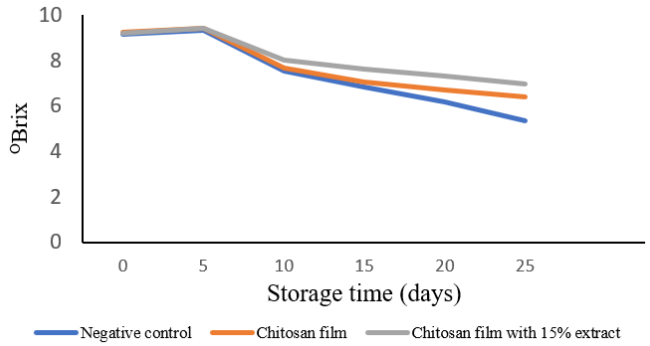


Figure 8. Changes in °Brix of lime juice during the storage period.

3.4.3.4 pH and total acidity

During storage, pH increased (Figure 9) while total acidity decreased (Figure 10), reflecting the consumption and transformation of organic acids. The composite coating most effectively stabilized both parameters ($p < 0.001$), indicating reduced metabolic activity.

The decline in acidity is associated with the decarboxylation and utilization of organic acids such as citric and malic acids in respiration pathways (Mahajan and Goswami, 2004; Mditshwa *et al*., 2017). Similar trends have been reported in citrus fruits during storage (Ma *et al*., 2019; Samad *et al.,* 2019).

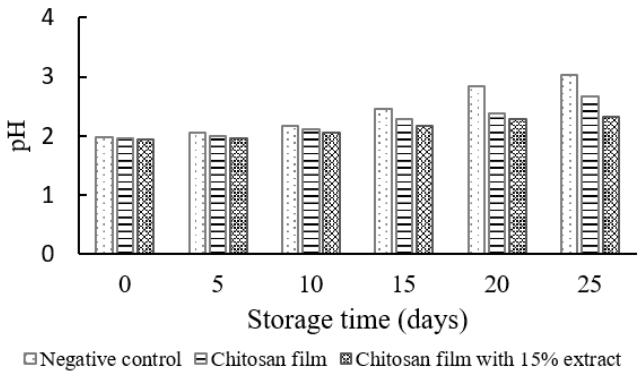


Figure 9. Changes in the pH of lime juice during storage.

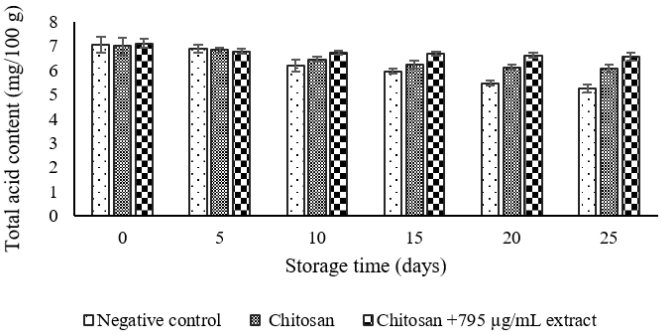


Figure 10. Changes in total acid content of lime juice during storage.

3.4.3.5 Vitamin C retention

Vitamin C content decreased in all treatments over time (Figure 11), primarily due to oxidative degradation. However, the rate of loss differed significantly among treatments ($p < 0.05$).

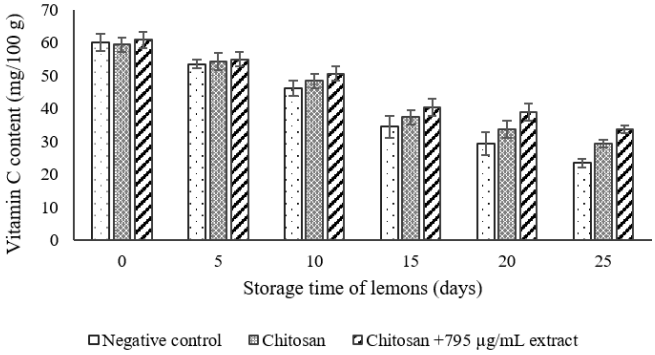


Figure 11. Changes in vitamin C content during storage.

The composite coating showed the highest retention (44.6% loss), compared with chitosan alone (50.6%) and control (61%). This protective effect may be associated with the barrier properties of the coating and the antioxidant activity of phenolic compounds in the extract, which could help reduce oxidative degradation of ascorbic acid (Thuy *et al*., 2008; Duguma *et al.,* 2014; Ngọc and Phong, 2018).

3.4.3.6 Color stability

Color changes during storage (Figure 12) reflected chlorophyll degradation and carotenoid accumulation.

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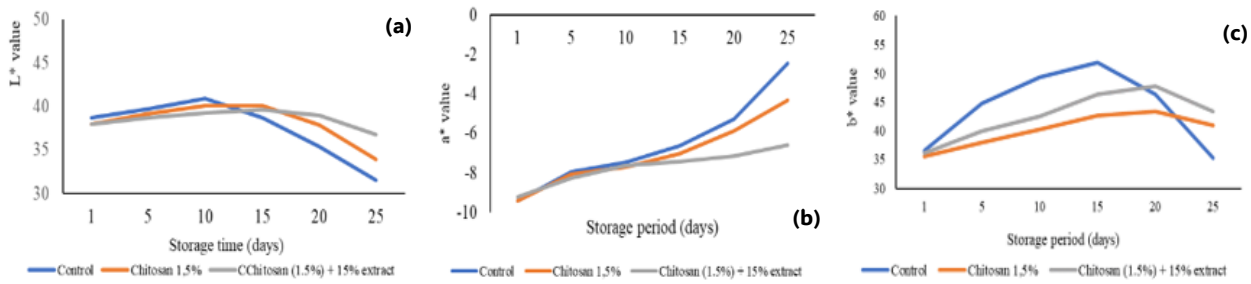


Figure 12. Changes in color value (*L**, *a**, *b**) during the storage period.

The control exhibited the most pronounced changes, indicating rapid senescence.

The composite coating most effectively preserved L*, a*, and b* values, maintaining higher lightness and greener coloration while slowing yellowing. This suggests delayed chlorophyll degradation and pigment transformation. Such effects are associated with reduced oxidative reactions and slower metabolic rates. These findings are consistent with previous studies on fruit color stability during storage (Thuy *et al*., 2008; Santamaría *et al*., 2009; Lo'ay and Dawood, 2017; Odetayo *et al*., 2022).

3.4.3.7 Decay incidence

After 25 days of storage, visible decay differed among treatments, with decay incidence reaching 50.0% in the uncoated control, 16.7% in the chitosan-coated samples, and 10.0% in the chitosan–extract composite coating (Table 6). Because decay incidence represents proportion/count data, the results were interpreted based on the number of decayed fruits relative to the total number of assessed fruits. The lower decay incidence observed in coated fruits indicates that both chitosan coating and chitosan–extract composite coating reduced the occurrence of visible spoilage compared with the uncoated control.

Table 6. Decay rate of limes after 25 days of storage.

Preservation treatment	Total fruits	Number of decayed fruits	Decay rate (%)
No coating (control)	30	15	50.0
Chitosan coating	30	5	16.7
Chitosan coating + 795 µg/mL extract	30	3	10.0

This reduction may be associated with the barrier-forming properties of chitosan, which can limit moisture transfer and reduce direct exposure of the fruit surface to spoilage microorganisms. The durian seed coat extract may further contribute through its moderate antimicrobial and antioxidant activities. However, because microbial population dynamics on fruit surfaces, respiration rate, and defense-related enzyme activity were not directly measured in this study, these mechanisms should be regarded as plausible explanations rather than confirmed causal pathways.

3.4.3.8 Integrated interpretation of preservation mechanism

Overall, the chitosan coating combined with durian seed coat extract showed better preservation performance than the uncoated control across the evaluated quality parameters. This effect may be attributed to the combined contribution of the chitosan matrix and the bioactive compounds present in the durian seed coat extract. The chitosan matrix likely acted as a semipermeable barrier that reduced moisture transfer and helped maintain fruit quality, while the polyphenol-rich extract may have contributed additional antimicrobial and antioxidant functionality. The film characterization results, including continuous film formation, modified surface morphology, increased flexibility, and reduced transparency, further support the functional role of the composite coating in postharvest preservation.

Nevertheless, the proposed mechanisms should be interpreted cautiously because respiration rate, enzymatic activity, and microbial population changes were not directly determined. Therefore, the observed preservation effect is best described as a combined protective effect of the coating system rather than a confirmed synergistic interaction. These findings are consistent with previous studies on chitosan-based composite coatings enriched with bioactive compounds for fruit preservation (Riaz *et al.*, 2021; Zhang *et al.*, 2022; Yang *et al.*, 2023; Nikhil *et al.*, 2025), and support the potential use of durian seed coat extract as a functional additive in edible coating systems.

4. Conclusion

Durian seed coat was demonstrated to be a promising source of bioactive compounds, particularly phenolics, flavonoids, and saponins. Under the experimental extraction conditions, including a solvent-to-solid ratio of 20:1, 50°C, pH 5, 2 h, and ultrasound treatment at 400 W for 5 min, total polyphenol recovery reached 51.22 mg GAE/g DW, representing an improvement of 11.41 mg GAE/g DW compared with conventional extraction. The obtained extract exhibited moderate antibacterial, antifungal, and antioxidant activities, with a DPPH IC_{50} value of 347.18 µg/mL.

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Incorporation of 795 µg/mL extract into a 1.5% chitosan matrix produced a composite edible coating that showed potential for maintaining the postharvest quality of seedless limes during 25 days of storage at 25±2°C. Compared with the uncoated control, the composite coating reduced firmness loss and weight loss, better maintained key physicochemical attributes, and lowered decay incidence from 50.0% to 10.0% under laboratory storage conditions. Overall, these findings support the potential valorization of durian seed coat as a value-added agro-industrial by-product for the development of functional edible coatings. However, further studies are needed to optimize the extract concentration against major citrus postharvest fungal pathogens, validate coating performance under commercial storage conditions, and assess process scale-up feasibility.

Conflict of interest

The authors declare no conflict of interest.

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