PLA/starch bi-layer films reinforced with rice straw cellulose nanofibers and functionalized with organosolv-lignin nanoparticles and grapefruit bioactives for shelf life extension of green grapes

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2.5.3 Moisture content (MC), film solubility (FS), and film swelling ratio (SR)

The moisture content of the films was calculated according to the AOAC method [1]. A piece of the sample (4 cm x 4 cm), weight (W_1) of 0.5 g was dried for 4 h at 105 °C in a hot air oven before being put in a petri dish. The final weight (W_2) was measured after it had cooled in a desiccator until a constant weight. The Eq. (1) was used to calculate the sample's moisture content (%).

$$\text{Moisture content (wb. \%)} = \frac{W1-W2}{W1} \quad (1)$$

Film solubility was ascertained gravimetrically following a previous method with slight modification [2]. Films (1x4 cm) were oven-dried for 24 h at 105 °C and then weighed to determine their initial dry weight (W_3). Afterward, samples were immersed in 50 mL of DL and agitated (at 100 rpm) for the entire night. The final solutions were filtered, and the final weight of the sample was determined after oven-drying (W_4). The FS (%) was calculated using Eq.2

$$\text{Film solubility (\%)} = \frac{W3-W4}{W3} \quad (2)$$

To conduct a swelling investigation, pre-weighed films (Wo) were soaked in beakers filled with distilled water while being shaken at room temperature. Until the samples achieved equilibrium, the weight of the swollen film (Ws) was given after two hours. The SR (%) was calculated using Eq. (3)

$$\text{Film swelling ratio (\%)} = \frac{Ws-W0}{W0} \quad (3)$$

2.5.4 Contact angle measurements

The water contact angle of the different bi-layer films was measured by a contact angle measurement system (Data Physics OCA 20, Germany). Briefly, the system includes a software-controlled high-precision liquid dispenser that allows for precise control of the drop size of the used liquid. A drop of 5 µl deionized water was dropped on the material surface through a micro-syringe, which was controlled by the system process, and the contact angle on the surface of the material was observed immediately using Drop Shape Analysis software (DSA 4). The changes in drop shape were recorded at different time intervals (0 and 1 min) for both plate materials [3].

2.5.6 Color parameters and opacity

The film's lightness (L*), redness/greenness (a*), and yellowness/blueness (b*) were measured using a Hunter colorimeter model D25 optical sensor (Hunter Associates Laboratory Inc., USA) concerning a background of white plate.

The total color change (ΔE) and whiteness index (WI) were calculated using the following Eq.4 and Eq.5

$$\Delta E = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2}^2 \quad (4)$$

$$WI = 100 - \sqrt{(100 - L*)^2 + (a*)^2 + (b*)^2}^2 \quad (5)$$

Film opacity was also calculated using Eq. 6

$$\text{Opacity value} = \frac{Abs600}{X} \quad (6)$$

Abs 600 is the absorbance value at 600 nm, and x is the film thickness (mm).

2.5.8 Total phenolic content and total flavonoid content

A modified procedure was employed to quantify the total phenolic content of the bi-layer films [4]. Firstly, the films were cut into small pieces and packed in a closed vial filled with methanolic solution (10 mL) and centrifuged (150 rpm, 30 min) at RT to the solution extraction. In brief, 0.25 mL of the bi-layer film extract (5 mg/mL) or gallic acid standard (0–10 mg/mL) was combined with 2.5 mL of a 10% Folin-Ciocalteu solution and incubated in the dark for 3 minutes. Subsequently, 0.75 mL of a 7.5% sodium carbonate solution was added, and the mixture was further incubated in the dark for 30 minutes. The absorbance was then measured at 765 nm, with the phenolic content expressed as mg GAE/g of the sample.

The total flavonoid content of the bi-layer films was determined using a previous method with slight changes [5]. A 0.5 mL bi-layer film extract (5 mg/mL) or quercitin standard (0–10 mg/mL) was mixed with 2.5 mL of water and 0.15 mL of 5% sodium nitrite. After 10 minutes, 0.3 mL of aluminum chloride and 1.0 mL of 1 M sodium hydroxide were added. The mixture was then stirred and left to stand for 5 minutes before measuring the absorbance at 510 nm, with the results expressed as mg QE/g.

2.5.9 Antioxidant activity (AA)

2.5.9.1 DPPH radical scavenging activity

The radical scavenging activity (RSA) of the films was investigated through a 2, 2- diphenyl-1-picrylhydrazyl (DPPH) assay, followed by a previously reported method [6]. In brief, the film extracts (200 µL) were mixed with methanolic DPPH (2 mL, 1 mM) solution, followed by vigorous agitation and the solution was left for 30 min in a dark place. The blank and sample absorbance (A) was recorded spectrophotometrically at 517 nm, and radical scavenging activity (RSA) was calculated by Eq. (7):

$$\text{RSA (\%)} = \frac{Ablank - A\ sample}{Ablank} \times 100 \quad (7)$$

2.5.9.2 ABTS assay

The antioxidant activity of the bi-layer films was assessed using the ABTS [2,2-azinobis(3-ethylbenzothiazoline-6-sulphonate)] radical cation decolorization assay with slight modifications [7]. ABTS (7 mM/L) was reacted with potassium persulfate (2.45 mM/L) in the dark for 12–16 hours to generate the blue-green ABTS*+ radical. The solution was then diluted with ethanol to an absorbance of 0.80 ± 0.05 at 734 nm. A 10 µL bi-layer film extract was mixed with 90 µL ethanol, followed by 1 mL ABTS solution, and incubated for 15 min. Absorbance was measured at 734 nm, and antioxidant activity was quantified using a Trolox calibration curve (R^2 = 0.9688) in the 0.04–1.6 mM/L range. A control was prepared with ethanol instead of extract, and results were expressed as Trolox equivalents per mL and per gram dry weight.

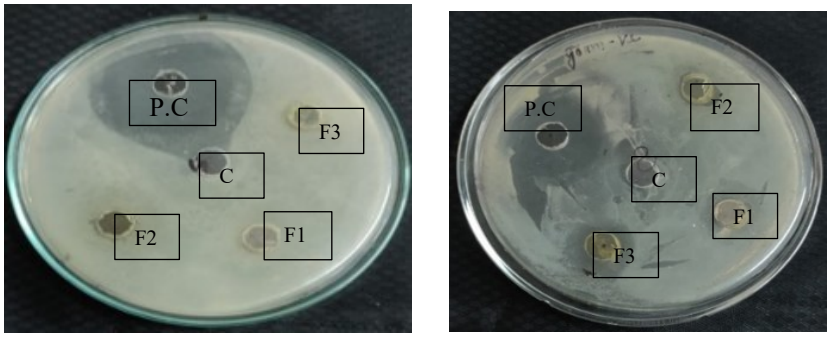
2.5.9.3 FRAP assay

The ferric-reducing antioxidant power (FRAP) of bi-layer films was evaluated following the method described by [8] with slight modifications. In summary, 0.05 mL of sample extract (10 mg/mL) or ferrous sulfate (0–10 mg/mL) was mixed with 0.1 mL of freshly prepared FRAP reagent (10:1:1, consisting of 10 mM TPTZ, 20 mM FeCl3, and 0.1 M acetate buffer). The mixture was then incubated in darkness for 30 minutes. Absorbance measurements were taken

at 593 nm, and the results were expressed as milligrams of ferrous sulfate equivalent per gram (mg FeE/g) of the samples.

3. Results and Discussion

3.10 Anti-microbial capacity



P. aeruginosa ***S. aureus***

Fig. S1 Zone of inhibition of PLA/Starch bi-layer films

P.C: Positive Control; C: PLA_SACNFs/starch; F1: PLA_SACNFs/starch_2MLNP; F2: PLA_SACNFs/ starch_5GFE; F3: PLA_SACNFs /starch_2MLNP_5GFE

Reference

(1) A.O.A.C. Official Methods of Analysis, 18th Edition, Association of Official Analytical Chemists, Washington, DC, 2005.

(2) Nordin, N. Othman, S. H. Rashid, S. A. Basha, R. K. Effects of Glycerol and Thymol on Physical, Mechanical, and Thermal Properties of Corn Starch Films. *Food Hydrocoll.,* **2020**, *106* (December 2019), 105884. https://doi.org/10.1016/j.foodhyd.2020.105884.

(3) Machado, C. M. Benelli, P. Tessaro, I. C. Study of Interactions between Cassava Starch and Peanut Skin on Biodegradable Foams. *Int. J. Biol. Macromol.,* **2020**, *147*, 1343–1353. https://doi.org/10.1016/j.ijbiomac.2019.10.098.

(4) Das, D. Panesar, P. S. Saini, C. S. Effect of Montmorillonite (MMT) on the Properties of Soybean Meal Protein Isolate-Based Nanocomposite Film Loaded with Debittered Kinnow Peel Powder. *Food Res. Int. J.,* **2024**, *185* (April).

(5) Abdel-Naeem, H. H. S. Sallam, K. I. Malak, N. M. L. Improvement of the Microbial Quality, Antioxidant Activity, Phenolic and Flavonoid Contents, and Shelf Life of Smoked Herring (Clupea Harengus) during Frozen Storage by Using Chitosan Edible Coating. *Food Control,* **2021**, *130*, 108317.

(6) Lian, H. Peng, Y. Shi, J. Wang, Q. Effect of Emulsifier Hydrophilic-Lipophilic Balance (HLB) on the Release of Thyme Essential Oil from Chitosan Films. *Food Hydrocoll.*, **2019**, *97* (61). https://doi.org/10.1016/j.foodhyd.2019.105213.

(7) Islam, M. Malakar, S. Dwivedi, U. Prabakar, P. K. Kishore, A. Kumar, A. Impact of Different Drying Techniques on Grapefruit Peels and Subsequent Optimization of Ultrasonic Extraction Conditions for Bioactive Compounds. *J. Food Process Eng.*,

2023, No. March 2022, 1–16. https://doi.org/10.1111/jfpe.14331.

(8) Eskandarabadi, S. M. Mahmoudian, M. Farah, K. R. Abdali, A. Nozad, E. Enayati, M. Active Intelligent Packaging Film Based on Ethylene Vinyl Acetate Nanocomposite Containing Extracted Anthocyanin, Rosemary Extract and ZnO/Fe-MMT Nanoparticles. *Food Packag. Shelf Life,* **2019**, *22* (September 2018), 100389. https://doi.org/10.1016/j.fpsl.2019.100389.