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**RESEARCH ARTICLE** 



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# AVOGADO PEEL POLYPHENOLIC-FUNCTIONALIZED KERATIN-STARCH COMPOSITE: A NOVEL APPROACH TO EXTENDING SHELF LIFE AND ENHANCING POSTHARVEST QUALITY OF TOMATOES.

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# ABSTRACT

There is increasing research interest in the development of edible films and coatings that incorporate plant-derived antimicrobials to make perishable foods last longer and taste better. This study developed a bioactive keratin-starch composite (3:27 v/v) using varying concentrations (0.2-1.0 mL v/v) of polyphenolic extract of avocado peel. The keratin-starch composite was fabricated from 5% (w/v) keratin solution and 5% (w/v) starch solution. The functionalized keratin-starch composite was applied as a coating on light red-colored tomato fruits and stored at  $25.0 \pm 2.0 \text{ °C}$ ,  $67.0 \pm 2.0$  relative humidity, and 12 h day and night cycle for nine days. Loss in weight, as well as changes in the physicochemical attributes (pH, titratable acidity, brix value, ascorbic acid and lycopene contents, and polyphenol oxidase activity), were analyzed on days 0, 3, 6, and 9 to ascertain the effects of the coating on the quality and shelf life of tomatoes. Tomatoes treated with avocado polyphenolic-extract enriched keratin-starch composite exhibited significantly lower weight loss, lycopene content, and polyphenol oxidase activity compared with uncoated tomatoes. Additionally, the treated fruits showed improved pH stability, elevated titratable acidity, and enhanced ascorbic acid retention, compared to those coated with the base keratin-starch composite for nine days. Overall, the tomatoes coated with keratin-starch composite functionalized with 1.0 mL avocado peel polyphenolic extract exhibited the best quality attributes after the 9-day storage period. These results underscore the potential of avocado peel polyphenols as a sustainable bioactive additive for biopolymer coatings, offering an eco-friendly approach to make fresh produce stay longer on the shelf while maintaining their nutritional quality.

Keywords: Edible coatings and films, Chicken feather waste, Keratin-starch composite, Food packing, Shelf life, Tomato fruits.

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## **INTRODUCTION**

Environmental degradation has become a critical global concern, significantly exacerbated by rapid population growth and technological progress. The persistent accumulation of solid waste, especially from synthetic packaging materials, poses serious risks to human health and ecological systems (Verma *et al.*, 2021). Over eight percent of the total solid waste generated in Nigeria comes from single-use plastics (Adesokan *et al.*, 2022; Akan *et al.*, 2021; Dumbili & Henderson, 2020). Poor management and overuse have created pressing challenges for terrestrial and aquatic ecosystems, necessitating the urgent development of sustainable alternatives.

Therefore, the transition to a circular economy offers a promising path forward, prioritizing waste reduction and efficient resource use. Agricultural waste has become a significant resource, facilitating the creation of cost-effective and biodegradable packaging materials. Biocomposite films, derived from such waste, exhibit excellent potential for food packaging, serving as effective barriers to moisture, gases, and volatile compounds (Khalil *et al.*, 2019). Advances in material science have facilitated the combination of multiple biopolymers with complementary properties, significantly enhancing their mechanical and barrier functionalities (Oluba *et al.*, 2021a, Oluba *et al.*, 2021b).

Furthermore, the functional properties of biocomposites have been further improved by the addition of plant extracts or essential oils rich in bioactive components. These additives confer antibacterial and antioxidant properties, prolonging the shelf life of perishable goods such as fresh fruits and vegetables (Jafarzadeh *et al.*, 2020; Singh *et al.*, 2021). Among these, avocado peel phenolic content has shown considerable promise as an antifungal agent when incorporated into keratin-starch films (Oluba *et al.*, 2022). Despite this, the broader application of such functional composites in preserving the nutritional and structural integrity of fresh agricultural products remains underexplored. Therefore, this study investigates the effectiveness of a keratin-starch composite, enriched with avocado polyphenolic-rich extract, in enhancing postharvest shelf life and nutritional quality of tomato fruits.

# **MATERIALS AND METHODS**

## **Poultry Feather**

Keratin was extracted from poultry feathers sourced from a commercial poultry farm in Akure, Nigeria (7° 15' 2.7756" N and 5° 12' 36.9576" E).

## **Plant Materials**

Ginger rhizomes, avocado pears, and tomato fruits were sourced from the local market in Ekpoma, Edo State, Nigeria (6°45'N; 6°08'E). The plant materials received authentication from specialists in the Department of Plant Biology and Biotechnology at Adekunle Ajasin University, Akungba, Nigeria where voucher specimens (AAUAE/21/033, AAUAE/21/034, and AAUAE/21/035, respectively) were archived for future reference.

## **Starch Extraction**

Starch was extracted from peeled ginger rhizomes using a sedimentation technique with slight modifications to enhance yield and purity (Ji & Seetharaman, 2004; Oluba *et al.* 2021a). Briefly, the rhizomes were peeled, washed,

and cut into smaller pieces. These pieces were soaked in a 0.1% sodium metabisulfite solution for 1 h to inhibit enzymatic activity. The softened pieces were then ground into a slurry using a food-grade blender. The slurry was filtered sequentially through 100  $\mu$ m and 300  $\mu$ m meshes to separate fibrous material. The filtrate was centrifuged at 8,000 rpm for 5 min, and the sedimented starch was washed multiple times with distilled water. Finally, the purified starch was dried at 50°C in a hot-air oven for 10 h and stored in an airtight container for further use. When reacted with iodine solution the starch turned blue-black.

#### **Keratin Extraction**

Keratin was isolated from chicken feathers using a controlled alkaline hydrolysis method Oluba *et al.* (2021b). Cleaned and dried chicken feathers were cut into smaller pieces and treated with a sodium hydroxide solution (2% w/v) at 70 °C for 2 h. The mixture was continuously stirred to facilitate the breakdown of keratin bonds. The hydrolysate was filtered to remove insoluble residues, and the filtrate was neutralized with acetic acid until a precipitate formed. The precipitated keratin was collected by centrifugation, washed with distilled water, and lyophilized for use in composite preparation.

#### **Polyphenol Extraction**

Avocado peel polyphenolic extract was obtained using a modified solvent extraction method (Simic *et al.*, 2016). Freshly collected peels were washed, dried, and pulverized into fine particles. A weighed amount of the powder was extracted with 80% ethanol at a 1:10 (w/v) ratio under agitation at room temperature for 24 h. The extract was filtered, and the ethanol was evaporated using a rotary evaporator at 40°C to obtain a concentrated polyphenolic-rich extract.

#### **Composite Preparation**

The keratin and starch solutions (5% w/v each) were prepared by dissolving keratin and starch in distilled water. The two solutions were mixed in a 3:27 (v/v) ratio and stirred to form a uniform composite base (Oluba et al., 2025). Avocado peel polyphenolic extract was added to the base in varying concentrations (0.2–1.0 mL) to prepare the functionalized composites (K-ST-AP). Each solution was thoroughly mixed, dispensed into labeled containers, and used for tomato coating.

#### **Coating Experiment**

One hundred and forty (140) uniform, infection-free tomato fruits were selected, rinsed thoroughly with distilled water, and air-dried. The tomatoes were allocated to seven treatment groups in a randomized block design, with each group consisting of five replicates of seven fruits. Each group of fruits was immersed in its respective composite solution (Table 1) for 2 h, followed by air-drying for 3 h to allow the coating to set. The coated fruits were then placed in plastic trays within corrugated cartons and kept in the laboratory at  $25.0 \pm 2.0$  °C,  $67.0 \pm 2.0$  relative humidity and 12 h day and night cycle for nine days. The tomatoes when monitored periodically on days 0, 3, 6, and 9 for visual changes, weight, and signs of deterioration.

Table 1: Experimental grouping of tomato fruits for coating experiment

Group	Treatment
CTRL	Coated with distilled water
K-ST	Coated with K-ST
K-ST-AP <sub>0.2</sub>	Coated with K-ST functionalized with 0.2 mL of AP
K-ST-AP <sub>0.4</sub>	Coated with K-ST functionalized with 0.4 mL of AP
K-ST-AP <sub>0.6</sub>	Coated with K-ST functionalized with 0.6 mL of AP
K-ST-AP <sub>0.8</sub>	Coated with K-ST functionalized with 0.8 mL of AP
K-ST-AP <sub>1.0</sub>	Coated with K-ST functionalized with 1.0 mL of AP

Note: K-ST, keratin-starch composite; AP, avocado peel polyphenolic extract

#### Weight Loss in Tomato During Coating Experiment

Weight loss in tomatoes was assessed on days 0, 3, 6, and 9 by weighing each tomato fruit using a Sartorius BSA224S-CW (Germany) electronic weighing balance. The weights for each day were recorded as  $W_f$ . The weight loss (%)was calculated by subtracting the weight for each day from the weight of the same fruit on day 0 ( $W_0$ ) and shown in Eq. 1.

Weight loss (%) = 
$$\frac{W_0 - W_f}{W_0} x \, 100$$
 (1)

## **Physicochemical Characteristics of Coated Tomato Fruits**

Five tomatoes were chosen for analysis from each group at 3, 6, and 9 days after treatment. After homogenising the tomatoes, the filter paper was used to filter the mixture. Before undergoing additional testing, filtrates were collected in sterile bottles and kept at 4°C. To determine the pH of the treated tomatoes, the fitrate from each tomato fruit was diluted with distilled water (1:5 v/v) and the pH of the resulting solution was measured using a calibrated electronic pH meter (HANNA H12210). The titratable acidity (TA) of the tomato fitrate was determined using the Dagnew *et al.* (2021) method. Briefly, 10 mL of the fitrate was titrated against 0.05 M NaOH until a stable pink endpoint was detected to estimate the sample's titratable acidity. Equation 2 was used to determine the titratable acidity, which was expressed as grams of lactic acid per 100 g of the sample:

$$Titratable \ acidity \ (TA) = \frac{M \ NaOH \ x \ mL \ NaOH \ x \ 0.09 \ x \ 100}{mL \ of \ sample}$$
(2)

The total soluble solid (TSS) content of the filtrate obtained from each tomato fruit on days 0, 3, 6, and 9, expressed in degrees Brix, was measured using a Krüss Optronic<sup>TM</sup> handheld digital refractometer (DR 201-95). Readings were temperature-corrected to ensure accuracy. The ascorbic acid level in the tomatoes was measured using the 2,6dichlorophenol indophenol titrimetric method was used to measure the content of ascorbic acid (Hughes, 1983) of the filtrate obtained from each tomato fruit on days 0, 3, 6, and 9. To reach a stable pink endpoint, the dye solution was standardised by titrating it with a standard ascorbic acid solution; the volume was noted as V<sub>1</sub>. Titration in metaphosphoric acid was performed to the same endpoint for the tomato filtrate, with the volume  $V_2$  being recorded. Equation 3 was used to get the ascorbic acid content:

Ascorbic acid content = 
$$\frac{0.5 mg \, x \, V 2_2 \, x \, 100 \, mL \, x \, 100}{V_1 x \, 5 \, mL \, x \, weight \, of \, sample}$$
(3)

The lycopene content of the filtrate obtained from each tomato fruit on days 0, 3, 6, and 9 was measured using the method Oluba *et al.* (2021a) outlined. Polyphenol oxidase activity in the tomato filtrate was estimated using the technique described by Mayer *et al.* (1996) and further elaborated by Oluba *et al.* (2022), polyphenol oxidase activity of the filtrate obtained from each tomato fruit on days 0, 3, 6, and 9 were measured by tracking the development of a coloured benzoquinone solution from catechol. The color parameters (L\*, a\*, and b\*) of the tomato fruits were analyzed on days 0, 3, 6, and 9. After obtaining and processing the fruit samples, their images (720 × 480 pixels) were assessed using rectangular coordinates (L\*, a\*, b\*). The L\* parameter, representing luminosity, indicates the degree of light emitted by a surface, ranging from 0 (absolute black) to 100 (absolute white). The chromaticity parameters, a\* and b\*, describe color components: a\* indicates the red-green axis (positive values for green), while b\* represents the yellow-blue axis (positive values for yellow, negative values for blue) (León et al., 2006).

## **Statistical Analysis**

Every experiment was carried out in five replicates unless otherwise stated, and the mean  $\pm$  standard deviation (SD) was used to express the results. ANOVA was used to analyse the data in a randomised factorial design with treatment groups and storage time as variables. To compare group means, the Tukey multiple range test (p < 0.05) was used. GraphPad Prism 8.0 (GraphPad Software Inc., San Diego, California) was used to create the graphs and charts.

# RESULTS

## Effect of Coating on Weight Loss

Tomatoes treated with the K-ST-AP composite coatings exhibited progressively lower weight loss over the storage period compared to the uncoated control. The weight loss was most effectively reduced in tomatoes coated with the K-ST-AP<sub>0.2</sub> formulation, indicating its superior barrier properties against moisture loss (Figure 1).



Figure 1: The percentage weight loss of tomato fruits was monitored over a 9-day storage period at ambient temperature. Results are presented as mean  $\pm$  SD of five replicates.

## **Effects of Coating on Physicochemical Parameters**

The pH of the treated tomatoes remained acidic, increasing slightly with storage. Tomatoes coated with functionalized composites showed minimal pH changes, indicating slowed metabolic activity (Figure 2a). Titratable acidity was highest in K-ST-coated tomatoes and lowest in the control group (Figure 2b). The Brix value remained stable in coated fruits but declined in uncoated samples, suggesting reduced sugar utilization during storage (Figure 2c).



**Figure 2:** Effects of the chicken feather keratin-ginger starch composite coating on (a) pH, (b) titratable acidity, and (c) Brix value of tomato fruits stored at ambient temperature. Results are expressed as mean  $\pm$  SD of five replicates.

# Effects of Coating on Ascorbic Acid and Lycopene Contents

Ascorbic acid levels were significantly higher in tomatoes treated with K-ST-AP coatings, particularly K-ST-AP<sub>0.8</sub> and K-ST-AP<sub>1.0</sub> formulations, compared to other groups (Figure 3a). Lycopene content, on the other hand, was lower in coated fruits, suggesting the coatings inhibited ripening-related pigment accumulation (Figure 3b).



Figure 3: Effects of chicken feather-ginger starch composite coating on (a) ascorbic acid and (b) lycopene contents of tomato fruits stored at ambient temperature. Results are mean  $\pm$  SD of five determinations.

# Effect of Coating on Polyphenol Oxidase Activity

Polyphenol oxidase activity, which drives enzymatic browning, was significantly reduced in tomatoes treated with functionalized composites. Enzyme activity decreased proportionally with higher concentrations of avocado peel polyphenolic extract, further validating its antioxidant role (Fig. 4).



Figure 4: Effects of chicken feather-ginger starch composite coating on polyphenol oxidase activity in tomato fruits stored at ambient temperature. Results are mean  $\pm$  SD of five determinations.

## Effect of Coating Visual and Colour Attributes of Tomato Fruits

Tomatoes coated with functionalized composites maintained better color stability compared to uncoated ones. The a\* value, indicating red color development, was higher in treated fruits, reflecting slowed chlorophyll degradation and balanced lycopene synthesis during ripening (Table 2).

Color	Day	CTRL	K-ST	K-ST-AP <sub>0.2</sub>	K-ST-AP <sub>0.4</sub>	K-ST-AP <sub>0.6</sub>	K-ST-AP <sub>0.8</sub>	K-ST-AP <sub>1.0</sub>
parameter	r							
L	0	$63.61 \pm 1.1^{a}$	$62.50 \pm 0.9^{a}$	$61.41 \pm 0.7^{a}$	$63.55 \pm 0.8^{a}$	$61.07 \pm 0.5^{a}$	$60.69 \pm 0.6^{a}$	$60.12 \pm 0.3^{a}$
	3	$60.1 \pm 1.4^{b}$	$59.36 \pm 0.8^{b}$	57.12±1.6 <sup>a</sup>	$60.72 \pm 1.0^{b}$	$59.41 \pm 0.5^b$	$57.43 \pm 0.4^a$	$56.97 \pm 0.5^{a}$
	6	$57.47 \pm 0.6^{a}$	$57.79 \pm 0.4^{a}$	$56.95 \pm 0.2^{a}$	$60.16\pm0.6^{b}$	$57.56 \pm 0.4^a$	$56.78\pm0.1^a$	$56.15 \pm 0.3^{a}$
	9	$54.2 \pm 0.2^{a}$	54.15±0.4 <sup><i>a</i></sup>	52.85±1.0 <sup>a</sup>	$52.37 \pm 0.2^{a}$	$51.80\pm0.4^a$	$51.09 \pm 0.5^{a}$	$50.83 \pm 0.7^a$
a*	0	$4.57 \pm 0.9^{a}$	$4.97 \pm 0.6^{a}$	$5.95 \pm 0.9^{ab}$	$5.20 \pm 0.2^{ab}$	$6.63\pm0.5^{b}$	$6.36\pm0.4^{ab}$	$6.35 \pm 0.1^{a,b}$
	3	$9.93 \pm 0.9^{b}$	$9.08 \pm 0.5^{b}$	$7.06 \pm 1.2^{a}$	$7.89 \pm 0.8^{a}$	$9.85 \pm 0.5^b$	$10.59\pm0.6^{b}$	$10.30 \pm 0.9^{b}$
	6	10.91±0.9 <sup>a</sup>	$10.04 \pm 0.3^{a}$	11.23±0.6 <sup><i>a</i></sup>	$10.24 \pm 0.6^a$	$10.76 \pm 1.2^{a}$	$10.92 \pm 0.7^a$	$10.95 \pm 0.6^{a}$
	9	$12.98 \pm 0.5^{b}$	$11.84 \pm 0.2^{a}$	$11.36 \pm 1.2^{a,b}$	$10.98 \pm 0.2^a$	$11.05 \pm 0.3^b$	$11.63 \pm 0.5^{a,b}$	$11.33 \pm 0.2^{a,b}$
b*	0	11.97±0.7 <sup>a</sup>	12.59±0.9 <sup>a</sup>	$11.93 \pm 0.5^{a}$	$12.77 \pm 0.9^{a}$	$12.69 \pm 0.8^{a}$	$11.77 \pm 0.7^{a}$	$12.20 \pm 0.5^{a}$
	3	$8.69 \pm 1.0^{a}$	$9.04 \pm 0.6^{ab}$	$7.66 \pm 1.0^{a}$	$10.34 \pm 1.6^{b.c}$	$10.33\pm0.8^{bc}$	$11.97 \pm 0.6^{c}$	$11.95 \pm 0.3^{c}$
	6	$7.36 \pm 0.8^a$	$7.89 \pm 0.6^a$	$7.63 \pm 0.3^{a}$	$6.32 \pm 0.7^{a}$	$7.51 \pm 0.5^{a}$	$11.62\pm0.2^{b}$	$11.58 \pm 0.9^{b}$
	9	$6.16 \pm 0.9^{a,b}$	$6.22 \pm 0.5^{a,b}$	$5.05 \pm 0.7^{a}$	$5.40 \pm 0.3^{a}$	$7.80\pm0.5^{b}$	$10.91 \pm 0.9^{c}$	$10.33 \pm 0.6^{c}$

**Table 2:** Colour modification parameters of tomato fruits coated with avocado polyphenol-based keratin-starch composite film for a period of 9 days at room temperature  $(25 \pm 2 \text{ °C})$ 

Results are mean  $\pm$  SD of five determinations. L\* represents luminosity, a\* indicates the red-green axis (positive values for red, negative values for green), while b\* represents the yellow-blue axis (positive values for yellow, negative values for blue)

# DISCUSSION

Climacteric fruits like tomatoes have a naturally short shelf life due to their high respiratory rates, rapid ripening, and significant weight loss, all of which contribute to a decline in quality during storage (Javanmardi & Kubota, 2006; Alenazi *et al.*, 2020). Ripening alters key functional attributes such as texture and flavor (Chen *et al.*, 2020). The high moisture content of tomatoes makes them highly perishable, and while ripening enhances edibility, it also accelerates dehydration, thereby reducing storage potential and sensory appeal. The fruit cuticle serves as a primary defense against environmental and microbial stressors (Arya *et al.*, 2021) and plays a pivotal role in modulating ripening and postharvest behavior. Despite these natural defenses, substantial losses in fruit quality and quantity occur during storage.

This study assessed how well a keratin-starch composite enhanced with polyphenolic extract from avocado peels preserved tomato fruits when coated on the cuticle. By forming a protective layer on the fruit's cuticle and preventing stress from dehydration, the coating dramatically decreased weight loss over nine days of room-temperature storage. These results are in line with those of Ali *et al.* (2010), who found that tomatoes coated with gum Arabic comparably lost weight. The hydrophobic properties of avocado peel polyphenols and keratin improved the coating's capacity to reduce gas exchange and water loss, two essential elements in preserving fruit quality.

Uncontrolled weight loss can result in shrivelling, microbiological spoiling, and firmness loss, all of which have an adverse effect on the fruit's nutritional value, marketability, and attractiveness.

The study revealed an inverse relationship between pH and titratable acidity (TA). Lower pH levels in coated fruits were associated with higher TA, reflecting the presence of organic acids such as citric and malic acids, which are metabolized during respiration. Fruits treated with the composite coating exhibited reduced pH changes and higher TA levels, suggesting that the coating slowed ripening by limiting polyphenol oxidase activity. This enzyme, a key player in browning and ripening processes, was inhibited, which contributed to slower acid metabolism. Furthermore, the coating forms a protective layer on the outer layer of the fruit limiting the passage of gases in and out of the tomato fruits. Therefore, the rate of respiration and breakdown of organic acids in the fruit to sugars is greatly hampered due to a decrease in oxygen concentration within the fruit. The potential inhibitory effect of the polyphenol-functionalized keratin-starch composite coating on the rate of respiration (due to limited gaseous exchange) may account for the retention of acid content (low pH and higher TA) of the coated tomato fruits. The higher concentration of organic acids to sugars due to the polyphenolic extract functionalized keratin-starch composite coating fruits provides further justification for the reduced conversion of organic acids to sugars due to the polyphenolic extract functionalized keratin-starch composite coating. Decreased acidity can enhance flavor but often leads to faster ripening and reduced shelf life. Similar findings by Oluba *et al.* (2022) demonstrated that coatings containing avocado peel extract maintained acidity by stabilizing pH levels in fungal-infected tomatoes.

The reduction in Brix value due to polyphenol-functionalized keratin-starch coating in this study supports the findings by Shree *et al.* (2020) and Firdous *et al.* (2020), which emphasize the effects of fruit ripeness, species variation, and coating composition on pH and Brix values. Coated fruits showed lower Brix readings, which suggests that the coating inhibited respiration and reduced the amount of organic acid-to-sugar conversion. The inhibitory action of the composite coating to oxygen permeability is responsible for the reduced conversion of organic acids in tomato fruits to sugar through respiration hence lowering the Brix value in the coated tomato fruits.

Additionally, the composite coating slowed ascorbic acid degradation, as observed in previous studies (Oluba *et al.*, 2022; Abebe *et al.*, 2017; Kibar & Sabir, 2018). Lower lycopene content in coated tomatoes suggests that the coating impeded ripening by restricting oxygen availability necessary for respiration. Lycopene synthesis, closely tied to chlorophyll degradation during ripening, typically increases during storage (Dong *et al.*, 2004; Martinez-Hernandez *et al.*, 2016). The coating's ability to limit light penetration may have further contributed to delaying the oxidative degradation of ascorbic acid and phenolic compounds, thus enhancing shelf life.

The visual appearance of coated tomatoes was better preserved, with reduced chlorophyll loss and lower lycopene synthesis, maintaining a greener hue. The decrease in polyphenol oxidase activity also improved texture and color stability. Similar benefits have been reported in strawberries treated with chitosan-based coatings (Nguyen *et al.*, 2021).

Overall, the modulatory effect of the avocado polyphenolic extract functionalized keratin-starch composite coating on weight loss, pH, TA, Brix value, ascorbic acid, and lycopene concentration as well as polyphenol oxidase activity work in concert to prolong the shelf life of tomato fruits. The composite coating prevented water and nutrient loss by forming a protective barrier on the surface of the tomatoes. Furthermore, it reduces the rate of respiration in the coated fruits thus retaining the level of organic acids, and preventing the degradation of carotenoids due to the browning action of polyphenol oxidase. Therefore, the coated fruits showed a longer shelf life due to the barrier integrity of the coating and the consequent effect of the barrier on the nutrient composition of the coated tomato fruits.

# CONCLUSION

This study highlights the potential of an eco-friendly keratin-starch composite (Oluba et al., 2021a) functionalized with avocado polyphenols to extend the shelf life and enhance the postharvest quality of tomatoes. By reducing weight loss, slowing ripening, and preserving nutritional and aesthetic attributes, these biocomposites present a sustainable alternative to conventional coatings. Further research should explore their application to other perishable commodities and scalability for commercial use.

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