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Evaluation the bioactivity and applicability of flavedo extract in preserving *Citrus maxima* (Burm.) Merr. pomelo

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Abstract: Pomelo peels, often overlooked in food processing, generate significant byproducts, especially in minimal processing setups. Rich in bioactive compounds, particularly in the green peel or flavedo, these peels offer versatile applications. This study focuses on two main aspects. Firstly, the characterisation of the *Citrus maxima* (Burm.) Merr. pomelo flavedo extract was carried out, with its phenolic composition and activities such as antioxidant, and antifungal properties assessed. Secondly, the impact of applying a pectin film enriched with this extract on pomelo storage at 8 °C was evaluated. The extract has a diverse phenolic composition, including catechin, chlorogenic acid, rutin, ellagic acid, quercitrin, quercetin, apigenin, and gallic acid, with gallic acid being the most concentrated at 54 mg·g^{-1}. While the extract showed free radical-scavenging activity, it was less effective than vitamin C; the extract also demonstrated antifungal effects on 7 mold and 1 yeast strains. The extract-infused pectin coating significantly reduced colour changes, respiration intensity, and weight loss in *Citrus maxima* (Burm.) Merr. pomelo. Additionally, it preserved ascorbic acid, total soluble solids, and titratable acidity content. Sensory evaluations favoured pomelo preserved with the supplemented coating over both pre-preserved and untreated samples.

Keywords: antifungal ability; biological activity; chemical composition; half maximal effective concentration (EC_{50}); preservation

The *Citrus maxima* (Burm.) Merr. pomelo, characterised by its spherical shape and varying green to yellowish-green hues, typically weighs between 1.2 to 2.5 kg. Commonly consumed directly or as juice, the pomelo often results in the discard of its peel, which accounts for approximately 30% of the fruit's weight (Zarina and Tan 2013). Despite constituting a significant proportion of the fruit, the nutritional benefits of the pomelo peel are often underutilised. Pomelo peels, as by-products of fruit processing, represent a valuable reservoir of flavonoids known for their anti-inflammatory, and antitumor properties, and their role in preventing cardiovascular diseases, diabetes, and other ailments (Van Hung et al. 2020). Rich in water, cellulose, hemicellulose, soluble sugars, essential oils (predominantly D-limonene), and polyphenols (primarily flavonoids), the pomelo peel also contains notable quantities of pectin, vitamin A, and vitamin C [130–170 mg·(100 g)$^{-1}$ fresh

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substance] (Tocmo et al. 2020). Flavonoids, particularly polymerised flavonoids, are abundant in citrus fruits, notably pomelo, with concentrations reaching nearly 24 mg·(100 g)$^{-1}$ (Peterson et al. 2006). The lipid profile of pomelo peel includes a diverse array of compounds such as triglycerides, phosphorus glycolipids, phospholipids, and fatty acids. The essential oil pockets within the flavedo consist of various components including α-Pinene, β-Pinene, α-Phellandrene, α-Terpinene, Limonene, trans-β-ocimene, γ-Terpinene, trans-Linalool oxide, cis-Linalool oxide, Linalool, trans-p-Mentha-2,8-dien-1-ol, β-Citronellol, Neral, Geraniol, Geranial, Geranyl acetate, β-Copaene, Germacrene D, Bicyclogermacrene, α-Eudesmol, (E,E)-cis-Farnesol (Tocmo et al. 2020).

In addition to their chemical composition and antioxidant properties, pomelo peel extracts also exhibit antimicrobial effects. Notably, they demonstrate inhibitory effects against *Staphylococcus aureus*, a common source of food contamination (Aichayawanich and Ngaowthong 2012). Furthermore, antimicrobial properties against *Pseudomonas aeruginosa*, *Escherichia coli* (Das et al. 2013), and antifungal effects against *Fusarium moniliforme*, *Aspergillus niger*, and *Mucor plumbeus* have been reported using the agar well diffusion method by Hemalatha.

The *Citrus maxima* (Burm.) Merr. pomelo, while inherently possessing traits that facilitate preservation and grant it a prolonged shelf life, is susceptible to deterioration during storage due to microbial spoilage and inadequate preservation techniques. Elevated concentrations of sugars, minerals, and vitamins within the fruit create conducive conditions for the proliferation and survival of various detrimental microorganisms. Fruits affected by microbial spoilage often exhibit compromised quality, emitting unpleasant odours (Yalcin and Çapar 2017). An edible film represents a distinct material derived from natural sources, typically comprising organic compounds like polysaccharides, proteins, lipids, and various biological constituents. A defining attribute of biofilm lies in its inherent biological nature, enabling self-decomposition through biological or chemical mechanisms. This property serves to reduce adverse environmental impacts by facilitating the material's degradation and assimilation into the environment. Edible coatings have emerged as a promising strategy in post-harvest fruit preservation, forming a semi-permeable membrane that shields fruits from mechanical damage, mitigates respiration and transpiration rates, and fortifies defences against fungal infections (Falguera et al. 2011).

This study aims to assess the properties of pomelo peel extract using quantitative, antioxidant, and antifungal assays; and to investigate the efficacy of a pectin film supplemented with pomelo peel extract in preserving pomelo.

MATERIAL AND METHODS

Material. The experimental material was *Citrus maxima* (Burn.) Merr. pomelo grown in Ben Tre, Vietnam. It is called Da Xanh pomelo in Vietnam. The pomelos were harvested from trees aged between 5 and 8 years, and it took about 8 months from flowering to harvest.

The mold and yeast strains isolated from *Citrus maxima* (Burn.) Merr. and *Citrus grandis* (L.) Osbeck pomelo peels were *Aspergillus fijiensis*, *Syncephalastrum monosporum*, *Cladosporium tenuissimum*, *Aspergillus flavus*, *Schizophyllum commune*, *Aspergillus fumigatus*, *Talaromyces atroroseus*, and *Rhodosporidiobolus fluvialis*. They were kept at the Institute of Applied Technology and Sustainable Development – Nguyen Tat Thanh University.

Equipment. The equipment utilised in the investigation was the Vevor 2 500 g Electric Grain Mill Grinder, the UV spectrophotometer – Vis Evolution 60S (Thermo Fisher Scientific, USA), the Heidolph vacuum rotary evaporator (Heidolph Instruments, Germany), EClassical 3200 (U)HPLC System (EChrom, China), the Hanna HI2211-02 pH meter (Hanna Instruments, Thailand), the Class II Polypropylene Vertical PRO Laminar Clean Bench (Cleatech, USA), and the Milotech heat pump dryer (Milotech, Vietnam) and O_2 & CO_2 Headspace Analyser GS6000 (Illinois Instruments, USA).

Chemicals. High methoxyl pectin and Dichloran Glycerol Medium Base M1129 (Himedia, India); NaCl 99.5%, $AlCl_3{\cdot}6H_2O$ 97%, NaOH, CH_3COOH 99.5%, $FeCl_3$, Na_2CO_3 99.6%, $CH_3COONa{\cdot}3H_2O$ 99%, glycerol 99%, dimethyl sulfoxide, and sorbitol (China); Folin & Ciocalteu reagent, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) 98%, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), and vitamin C (USA); gelatin with bloom 125 (Germany), absolute ethanol (China), $Ca(OCl)_2$ (China), itraconazole (Korea).

Flavedo extract preparation. The study was conducted on the green peel (flavedo) of Da Xanh pomelo [*Citrus maxima* (Burm.) Merr.] grown in Ben Tre, Vietnam. Mature pomelos were harvested and stored for 7 days; after that, the white peel (albedo) and flesh of the pomelos were separated and flavedos were col-

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lected for research. Flavedo peels were dried with a heat pump at a drying temperature of 35 °C until reaching a moisture content of less than 10%, flavedo peels were crushed into small particles by Vevor 2 500 g Electric Grain Mill Grinder that passed through a 0.5 mm sieve; flavedo peels were extracted with ethanol solvent in which the ratio of absolute alcohol to water is 90:10 (*v*/*v*), the ratio of raw material/solvent is 10:146 (g·mL^{-1}) at room temperature for 48 h. Vacuum evaporation to expel the solvent and continue convection drying until the extract reaches a moisture content of less than 5%.

***Citrus maxima* (Burm.) Merr. preparation.** Pomelos were obtained from a local commercial pomelo grower at commercial maturity and transported to the Institute of Applied Technology and Sustainable Development, Nguyen Tat Thanh University. The selected pomelos weighed 950–1 050 g and had the typical shape of Da Xanh pomelos. The skin was dark green and the diameter was 13–15 cm. The fruit had the firmness of fresh pomelos; there was no mechanical or deep damage caused by insects. Selected pomelos were dipped in a solution of chlorine 200 mg·L^{-1} for five min and and rinsed with clean water, then drained and air-dried at 25 °C before coating application.

Coating preparation. The combination of pectin and gelatin facilitates molecular interactions leading to a more perfect film structure and the addition of the extract helps increase the content of phenolic compounds and antioxidant activity in the film composition (Liu et al. 2007; Siripatrawan and Harte 2010). Therefore, in this study, pectin, gelatin and pomelo peel extract were selected to conduct the research. Pectin and gelatin were dissolved in water (45 °C) at concentrations such as 0.05 g·mL^{-1} and 0.02 g·mL^{-1}. The coating mixture consists of 85% pectin solution, 5% gelatin solution, 5% sorbitol and 5% flavedo extract from pomelo and stirred until homogeneous.

Fruit coating. The coating solution is sprayed evenly on the entire fruit surface using a paint sprayer with a pressure nozzle with a pressure of 6 bar and a pump flow of 2.5 L·min^{-1} (Oshima 24L; Oshima, China). The coating forms a very thin film on the surface of the pomelo, only upon close inspection can one notice the difference between the coated and uncoated fruits. 100 g of the coating solution can be sprayed on 40 ± 2 pomelos. Then, all coated pomelo were air-dried for 1 h at 25 °C, weighed and then randomly packed into experimental units. Microclimate cabinet RGX – 250B (XingChen, China) was used to carry out preservation in 120 days at either 8 °C and humidity 85% (8DXC – coated Da Xanh pomelo; 8DXUC – uncoated Da Xanh pomelo).

Total polyphenol content (TPC) quantification. TPC (absorbance at 765 nm) was measured according to Folin-Ciocalteu (ISO 14502-1: 2005). Briefly, 1.5 mL of diluted Folin-Ciocalteu was added to 0.6 mL of flavedo extract (0.008 g·mL^{-1}) and left to stand in low light (5 min); Then 1.2 mL of Na_2CO_3 solution (7.5% *w*/*v*) was added and allowed to stand in low light for 60 min.

Total flavonoid content (TFC) quantification. TFC (absorbance at 510 nm) was measured by the $AlCl_3$ colourimetric method of (Bag et al. 2015) with minor adjustments. The mixture comprised 1 mL of flavedo extract (0.008 g·mL^{-1}) with 0.06 mL of $NaNO_2$ (5% *w*/*v*) for 5 min, followed by 0.06 mL of $AlCl_3$ (10% *w*/*v*) for 6 min. Subsequently, 0.4 mL of 1M NaOH and 0.48 mL of distilled water were added and allowed to stand for 30 min.

High-performance liquid chromatography-ultraviolet (HPLC-UV) method. HPLC method was used to determine phenolic compounds extracted from pomelo peel. One mg of extract was dissolved in 1 mL of 80% methanol and filtered through a 0.22 µm polytetrafluoroethylene (PTFE) filter membrane.

Separation of phenolic compounds was performed on an Agilent 1200 Series LC HPLC instrument equipped with a Zorbax C18 column [length (*L*) × inner diameter (*I.D.*) is 250 mm × 4.6 mm, 5 µm] at 25 °C (± 1 °C). The mobile phase consisted of 0.1% acetic acid (A) and acetonitrile (B). The flow rate was maintained at 0.2 mL·min^{-1}. Gradient elution was performed as follows: 0–3 min 95% mobile phase A and 5% mobile phase B; 3–15 min 25% A, 75% B; 15–23 min 0% A, 100% B. Chromatograms were recorded with a UV detector at 265 nm. Polyphenols were identified according to their retention times compared to commercial standards. Standard gallic acid, catechin, chlorogenic acid, rutin, ellagic acid, quercitrin, quercetin and apigenin, were prepared at a stock concentration of 500 µg·mL^{-1}.

Investigate the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging effect. The extract's antioxidant capacity was assessed using the modified DPPH method (Thaipong et al. 2006). DPPH, dissolved in methanol, forms a solution with stable radicals. Extract antioxidants neutralise these, causing a colour change. DPPH stock solutions (24 mg in 100 mL methanol) were prepared and mixed for testing. 0.15 mL extract was combined with 2.85 mL test solution, left for 30 min in the dark, and measured at 515 nm. Vitamin C was the positive control and the extraction

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solvent was the negative control based on the half maximal effective concentration (EC_{50}) comparison.

Investigate the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radical scavenging effect. $ABTS^+$ free radical scavenging activity was assessed following (Nenadis et al. 2007). $K_2S_2O_8$ (2.6 mM) and ABTS (7.4 mM) stock solutions were mixed equally. 0.15 mL sample solution was added to a test ABTS solution (2.85 mL), prepared by diluting the stock solution (1:6) with methanol, adjusting absorbance to 1.1 ± 0.02 at 734 nm. Absorbance values after 30 min were measured using a UV-Vis spectrophotometer. Antioxidant activity expressed as EC_{50}, had vitamin C as the positive control and the extraction solvent as the negative control.

Antifungal capacity, minimum inhibitory concentration (*MIC*) and minimum fungicide concentration (*MFC*). *MIC* is the lowest concentration at which mold does not grow. To determine the *MIC* concentration, the extract concentrations used in the study were 20, 40, 80, and 160 mg·mL^{-1}. *MFC* is the lowest concentration at which mold is destroyed. After determining the *MIC* concentration, the extract concentration was successively doubled to a value that could determine the minimum fungicide concentration. The disc containing no extract was used as a control. Then the fungal suspension with the number of cells (10^6 CFU·mL^{-1}; CFU – colony forming unit) was spread on the surface and incubated at 28 °C and the appearance of mold/yeast was observed after 5 days (Hu et al. 2019).

Skin colour. Colour was assessed using the CIE L^* (lightness), a^* (redness-greenness), b^* (yellowness-blueness) scale and a Minolta colourimeter (CR-400; Minolta, Japan). The results are expressed as delta E (Velickova et al. 2013):

$$\text{Delta } E = [(L_i - L_0)^2 + (a_i - a_0)^2 + (b_i - b_0)^2]^{1/2} \quad (1)$$

where: L_i, a_i, and b_i – colour measurement results at the ith analysis; L_o, a_o, and b_o – colour measurement results of input materials.

Respiration rate. Respiration rate was measured by the method described by (Velickova et al. 2013), in which 1 pomelo from each replicate were placed in an airtight jar 3 000 mL, with a metal probe in the headspace, for 2 h at 8 °C prior to gas sampling. The CO_2 concentration was recorded by a GS6600 CO_2, O_2 Headspace Analyser. Respiration rate was expressed as mL CO_2·kg^{-1}·h^{-1}, m: pomelo weight in grams and $\Delta CO_2/100$ was the difference between the initial concentration of CO_2 in the air and after 2 h of storage. Respiration rate was calculated by Equation 2:

$$Respiration\,rate = \frac{\left(3000 - m\right) \times \Delta CO_2 \times 2 \times 1\,000}{100 \times m} \quad (2)$$

where: 3 000 – volume of airtight jar (mL); m – weight of fruit (g); $\Delta CO_2/100$ – difference in measured CO_2 concentration compared to the original; 2 – time for sample to be kept in sealed vial (h); 1 000 – conversion factor for g to kg.

Weight loss. Pomelo weight loss was determined by weighing the same marked fruit at the beginning of the experiment and at the end of each storage period. The results were presented as the percentage loss of initial weight (Rojas-Argudo et al. 2009).

Ascorbic acid content. The method for determining vitamin C content is based on the Association of Official Analytical Chemists official method AOAC 967.21 (2,6-Dichlorophenolindophenol Titrimetric Method) based on the use of 2,6-dichlorophenolindophenol (DCPIP) as a titrant.

The vitamin C content (mg) in 100 g of starting ingredients is calculated according to Equation 3:

$$Vitamin\,C = \frac{\left(V_m - v_{\text{blank}}\right) E \times V_0 \times 100}{m \times V_{\text{sample}}} \quad (3)$$

where: V_m – volume of DCPIP solution used to titrate the sample to be tested (mL); v_{blank} – volume of DCPIP solution used to titrate the blank sample (mL); E = 0.088065 mg – equivalent of ascorbic acid corresponding to 1 mL of DCPIP solution (0.001 N); V_0 – initial solution volume (mL); V_{sample} – volume of solution to be titrated (mL); m – mass of solid sample (g).

Total soluble solid. The total soluble solid (TSS) content of pulp juice was assayed by a HI96800 Digital Refractometer (Hanna Instruments, USA) and expressed as °Brix.

Sensory evaluation. Flesh pomelo underwent sensory evaluation before (day 0) and after 120 days at 8 °C by a panel of thirty staff from Nguyen Tat Thanh University, Vietnam (15 females and 15 males), aged 25 to 60 years, experienced in citrus sensory evaluation. Fruit, brought to room temperature, was hand-peeled and cut in half crosswise. One half was used for

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sensory analysis, and the other for additional quality measurements. Panelists, using coded 100 mg plastic dishes, evaluated pomelo samples for colour, smell, taste, texture, and overall liking on a 7-point scale (1 = 'dislike extremely', 7 = 'like extremely'). Four samples at each tasting time were presented randomly to avoid bias. Palates were cleansed between samples with low-salt saltine crackers, room-temperature mineral water, and a short time lag. Panelists' average responses were considered for each attribute (Tietel et al. 2011).

Data processing methods. In this study, pomelo peel extract was taken only once to be used for the entire experiment. The extract was quantified for the content of phenolic compounds once. The extract was analysed for TPC and TFC content, antifungal activity, and antioxidant activity based on the ability to scavenge DPPH and ABTS free radicals. Each experiment was done three times. Next, the pomelo preservation process was repeated three times, with 10 fruits used to record images and 10 fruits used to analyse survey criteria each time. One-way analysis of variance (ANOVA) was performed to determine the statistically significant difference at the 5% level of significance between the samples and a post-hoc test (Tukey's test) was applied to determine the difference between the samples. Determine the significant difference between the mean values using SPSS software (version 20).

RESULTS AND DISCUSSION

For the green peel of Da Xanh pomelo [*Citrus maxima* (Burm.) Merr.] cultivated in Ben Tre, Vietnam, the basic components have been determined to include moisture (67.76 ± 0.17%), protein (0.021 ± 0.00%), lipid (1.71 ± 0.23%), ash (1.29 ± 0.07%), and carbohydrate (29.22 ± 0.29%).

Quantitative results of total polyphenol content (TPC) and total flavonoid content (TFC) content in flavedo extract. The analysis revealed a total polyphenol content of 3.44 ± 0.09 mg GAE (gallic acid equivalent) per g DW (dry weight) and a total flavonoid content of 9.85 ± 0.06 mg QE (quercetin equivalent) per g DW in the flavedo extract of pomelo. According to research by Hoang et al. (2021) when extracting pomelo peel at a temperature of 25 °C, the total polyphenol content is 4.83 ± 0.04 mg GAE per g DM (dry matter). However, with *Citrus grandis* (L.) Osbeck pomelo, the author said that for microwave-dried pomelo peels at a temperature of 50–60 °C, the polyphenol content is 892–1 336 mg GAE per 100 g DM (Abd Rahman et al. 2018).

Some phenolic components in flavedo extract by high-performance liquid chromatography-ultraviolet (HPLC-UV) method. HPLC-UV analysis of *Citrus maxima* (Burm.) Merr. pomelo peel revealed a diverse array of phenolic compounds, including gallic acid, catechin, chlorogenic acid, rutin, ellagic acid, quercitrin, quercetin, and apigenin. Gallic acid emerged as the most abundant compound, with a concentration of 54 $mg \cdot g^{-1}$, showcasing potent antioxidant properties. The peel also contained significant amounts of quercitrin (1.01 $mg \cdot g^{-1}$) and quercetin (0.50 $mg \cdot g^{-1}$), highlighting its potential as a functional food ingredient for promoting health. Additionally, rutin (3.66 $mg \cdot g^{-1}$) and catechin (0.31 $mg \cdot g^{-1}$) contributed to the antioxidant profile, emphasising the peel's overall bioactive composition. The presence of ellagic acid (0.12 $mg \cdot g^{-1}$), chlorogenic acid (0.24 $mg \cdot g^{-1}$), and apigenin (0.06 $mg \cdot g^{-1}$) further added to the diversity of phenolic compounds. In conclusion, Da Xanh pomelo peel is a rich source of antioxidants, making it a promising candidate for the development of functional foods and nutraceutical products. Most of the studies evaluating the compound composition in pomelo peel essential oil, research by Tocmo et al. (2020) shows that Most of these volatile compounds are terpenoids, which constitute the largest class. These include acyclic, monocyclic, and bicyclic monoterpenoids, as well as diterpenoids, and various forms of sesquiterpenoids such as acyclic, monocyclic, bicyclic, and tricyclic types.

Antioxidant activity of flavedo extract. Experimental results (Table 1) showed that pomelo peel extract had antioxidant properties, although it was less than the antioxidant activity of vitamin C. In addition, the EC_{50} value of the extract was also very different when using different determination methods or different statistical software (Sridhar and Charles 2019).

Antifungal activity of flavedo extract of pomelo. Figure 1 and Figure 2 illustrate variations in the diameter of antifungal inhibition rings with increasing extract concentrations across multiple strains, including *A. fijiensis*, *S. monosporum*, *C. tenuissimum*, *A. flavus*, *S. commune*, *A. fugamitus*, and *R. fluvialis*. The antifungal efficacy showed significant differences, notably increasing inhibition zone diameters. However, *T. atroroseus* exhibited non-significant differences in inhibition ring diameter. Notably, at 160 $mg \cdot mL^{-1}$ and 80 $mg \cdot mL^{-1}$, *T. atroroseus* demonstrated the least effectiveness, while *R. fluvialis* exhibited the highest efficacy. At 40 $mg \cdot mL^{-1}$, the best antifungal effect was observed against *C. tenuissimum*, and at 20 $mg \cdot mL^{-1}$, *A. fijiensis*,

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Table 1. The antioxidant capacity of flavedo extract compared to the antioxidant capacity of vitamin C

Method		Linear equations	R^2	EC_{50} (mg·mL^{-1})
ABTS	flavedo extract	$y = 166.99x + 7.5572$	0.9996	0.25 ± 0.0016
		$y = 163.28x + 7.9873$	0.9994	
		$y = 164.82x + 8.8368$	0.9994	
	vitamin C	$y = 1\,208.9x + 1.2879$	0.9996	0.04 ± 0.0006
		$y = 1\,184.6x + 0.8723$	0.9997	
		$y = 1\,210.7x + 1.2921$	0.9993	
DPPH	flavedo extract	$y = 3.9944x + 3.2895$	0.9997	11.73 ± 0.0290
		$y = 3.9810x + 3.2218$	0.9998	
		$y = 3.9882x + 3.2003$	0.9997	
	vitamin C	$y = 146.50x - 0.8648$	0.9999	0.34 ± 0.0015
		$y = 146.79x - 0.7712$	0.9998	
		$y = 146.35x - 0.3644$	0.9998	

ABTS – 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); DPPH – 2,2-diphenyl-1-picrylhydrazyl; EC_{50} – half maximal effective concentration

S. monosporum, and *R. fluvialis* were the least affected, with *C. tenuissimum* showing optimal inhibition. Overall, *C. tenuissimum* proved the most resilient strain across all tested extract concentrations. Flavedo extract exhibited strong inhibition against fungal strains, attributed to its composition of antifungal compounds such as alkaloids, saponins, flavonoids, phenols, carbohydrates (Okwu et al. 2007). These compounds disrupt

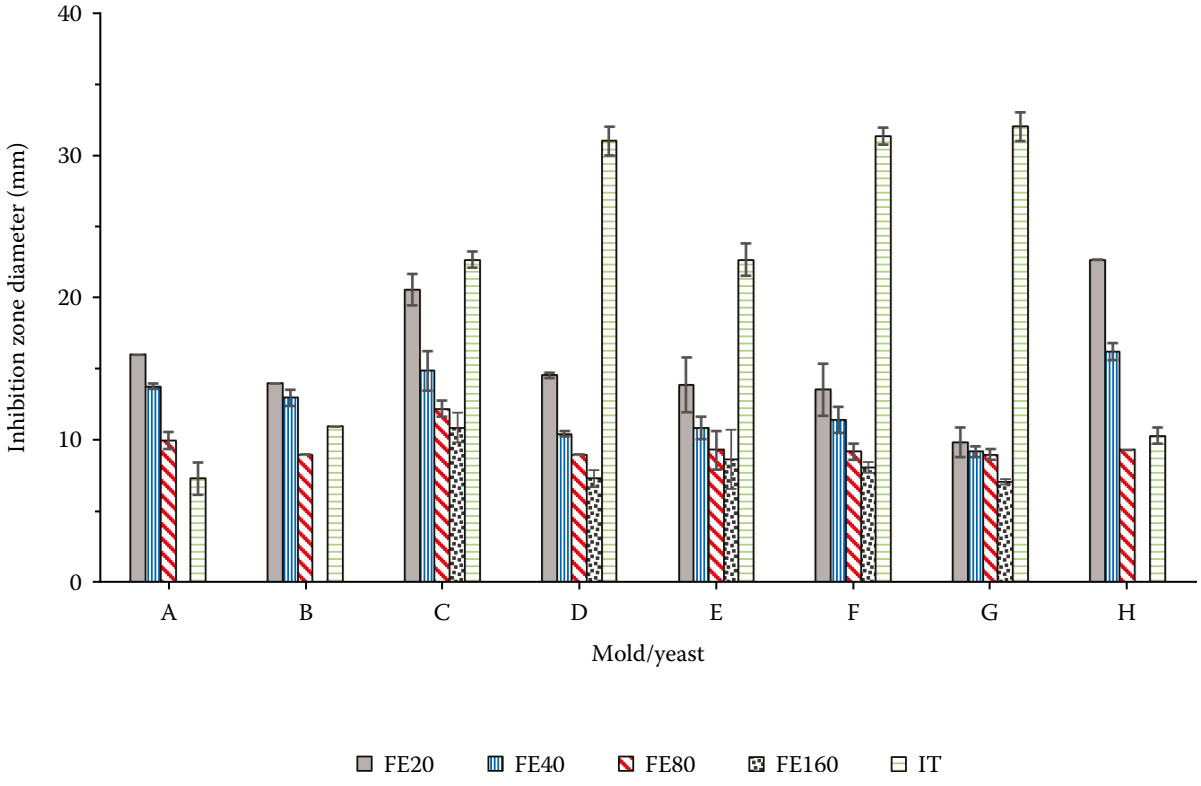

Figure 1. Mold/yeast inhibition zone diameter of itraconazole and flavedo extract

FE – flavedo extract; 20, 40, 80, 160 mg·mL^{-1} – flavedo extract concentration; IT – itraconazole 0.01 mg·mL^{-1}; A – *Aspergillus fijiensis*; B – *Syncephalastrum monosporum*; C – *Cladosporium tenuissimum*; D – *A. flavus*; E – *Schizophyllum commune*; F – *A. fumigatus*; G – *Talaromyces atroroseus*; H – *Rhodosporidiobolus fluvialis*

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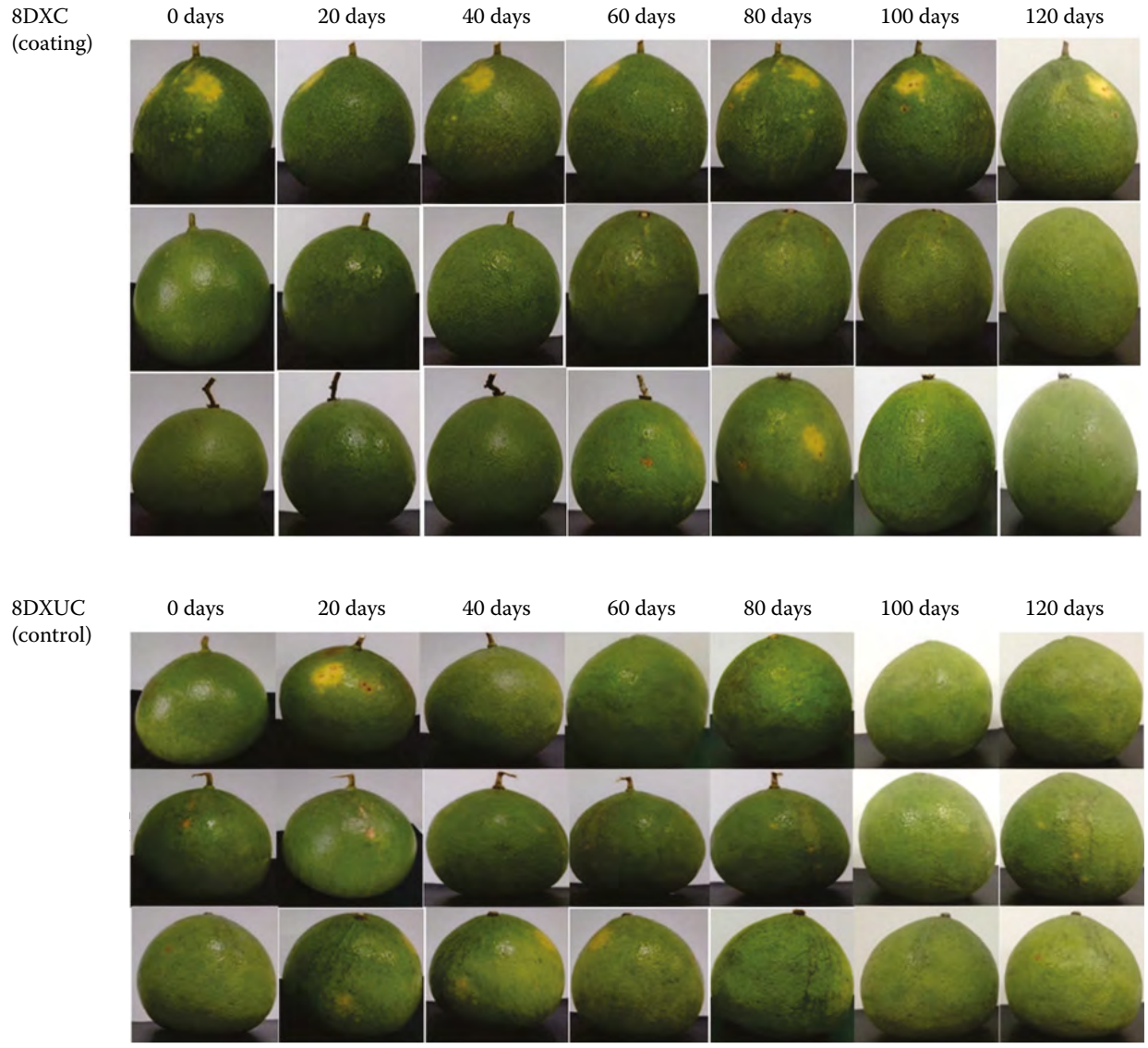

Figure 2. Images of coating and control Da Xanh pomelo during storage at 8 °C

8DXC (coating) – coated Da Xanh pomelo; 8DXUC (control) – uncoated Da Xanh pomelo

fungal cell membranes, leading to cytoplasm leakage and hindering hyphal growth. Consistent with prior studies, authors like (Liu et al. 2021) reported inhibition of *A. flavus* by citrus peel extracts rich in phenolic components. Additionally, itraconazole demonstrated antifungal activity against *Aspergillus fumigatus* (Denning et al. 1997; Figure 3).

The *MIC*/*MFC* results of the mold strains showed that the *S. monosporum* strain is the most difficult mold to kill, and the yeast *T. atroroseus* yeast was the less resistant strain. Molds often have a more complex cell structure than bacteria, which may include more cell layers or thicker cell walls. This makes them more tolerant to biocides than bacteria (Table 2).

Changes in fruit quality parameters of pomelo. The outcomes of image analysis for pomelos subjected to coating and those left uncoated over a 120-day storage period are illustrated in Figure 2, while the variations in nutritional components are presented in Figure 4. Figures 4A–F depict alterations in colour, respiration intensity, weight loss, vitamin C content, total soluble solids, and sensory evaluation results of pomelos throughout the preservation process.

During storage, prolonged storage duration reveals that the colour of pomelo gradually lightens, with the green hue diminishing and the yellow hue increasing. Concurrently, the colour change, indicated by delta *E*, progressively increases over the storage period (Fig-

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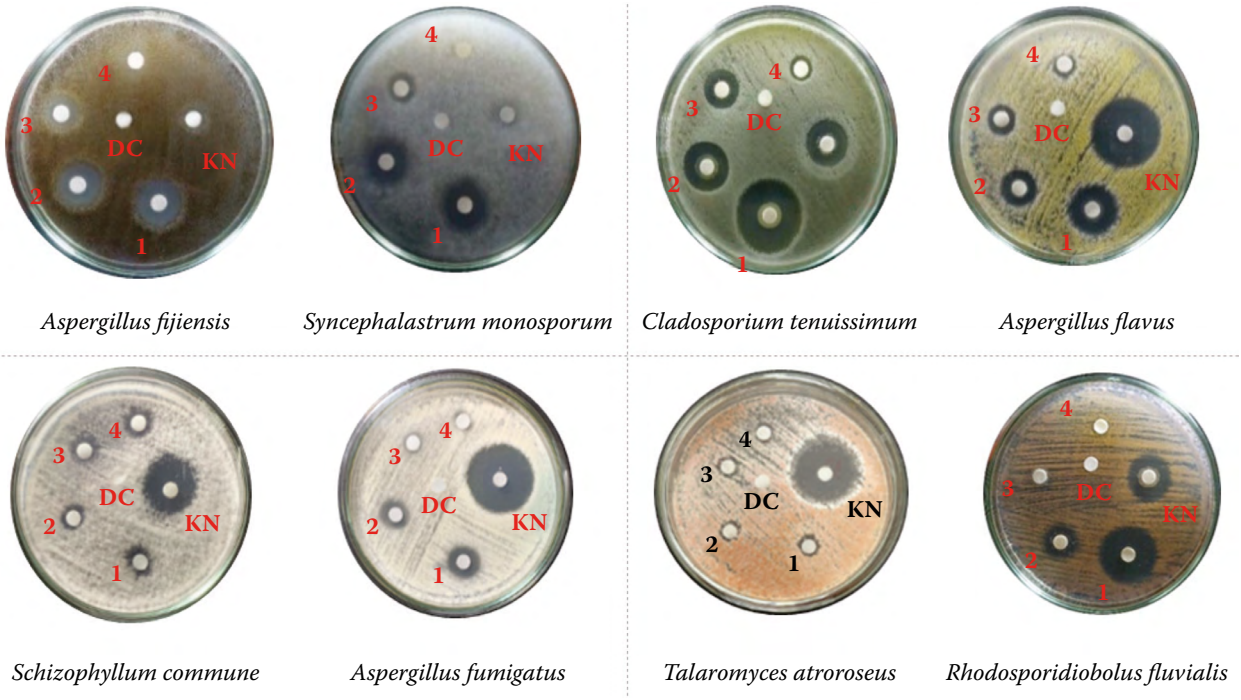

Figure 3. Image of antifungal activity of flavedo extract

1, 2, 3, 4 – flavedo extract with 160, 80, 40, 20 mg·mL^{-1} concentration; DC – dimethyl sulfoxide (DMSO) 5% (negative control); KN – itraconazole 0.01 mg·mL^{-1} (positive control)

ures 2 and 4A). Although pomelo is a fruit that does not have sudden respiration, during storage the respiration intensity of pomelo fluctuates but tends to decrease over time (Figure 4B). This is explained by the fact that the nutrient content in the fruit gradually decreases over time of storage.

Concomitantly, fruit weight loss (Figure 4C) exhibited a gradual increase during the storage period, primarily due to water loss from fruit cells resulting from respiration or variations in humidity between the fruit and its storage environment. Vitamin C content (Figure 4D) experienced a gradual decline over the storage duration, while total soluble solids (Figure 4E) increased. Additionally, the sensory evaluation scores (Figure 4F) for smell, taste, texture, and overall liking were lower for the original pomelo compared to those stored for 120 days.

Collectively, colour change, respiration intensity, weight loss, vitamin C content, total soluble solids, and sensory evaluation highlight that coated pomelos maintain superior quality compared to their uncoated counterparts. Applying a thin coating on the fruit surface mitigates aerobic respiration without inducing anaerobic respiration, thereby contributing to preserving pomelo quality during storage.

Colour changes, respiration intensity, and weight loss are important indicators of metabolic activity and provide preliminary information about the shelf life of post-harvest fruit. The results of this study showed a significant reduction of colour change, respiration intensity, and weight loss of pomelo. Gelatin is a protein capable of forming thin films, and when combined with pectin, a polysaccharide that also has film-forming ability, it enhances these properties. When mixed with pomelo peel extract, the combination exhibits antioxidant and anti-mold properties, improving the mechanical properties of the film such as increased

Table 2. The minimum inhibitory concentration (*MIC*) and minimum fungicide concentration (*MFC*) results of the fungal strains

Fungal strains	Concentration (mg·mL^{-1})	
	MIC	*MFC*
Aspergillus fijiensis	160	640
Syncephalastrum monosporum	320	1 280
Cladosporium tenuissimum	160	640
Aspergillus flavus	160	640
Schizophyllum commune	160	640
Aspergillus fumigatus	160	640
Talaromyces atroroseus	80	320

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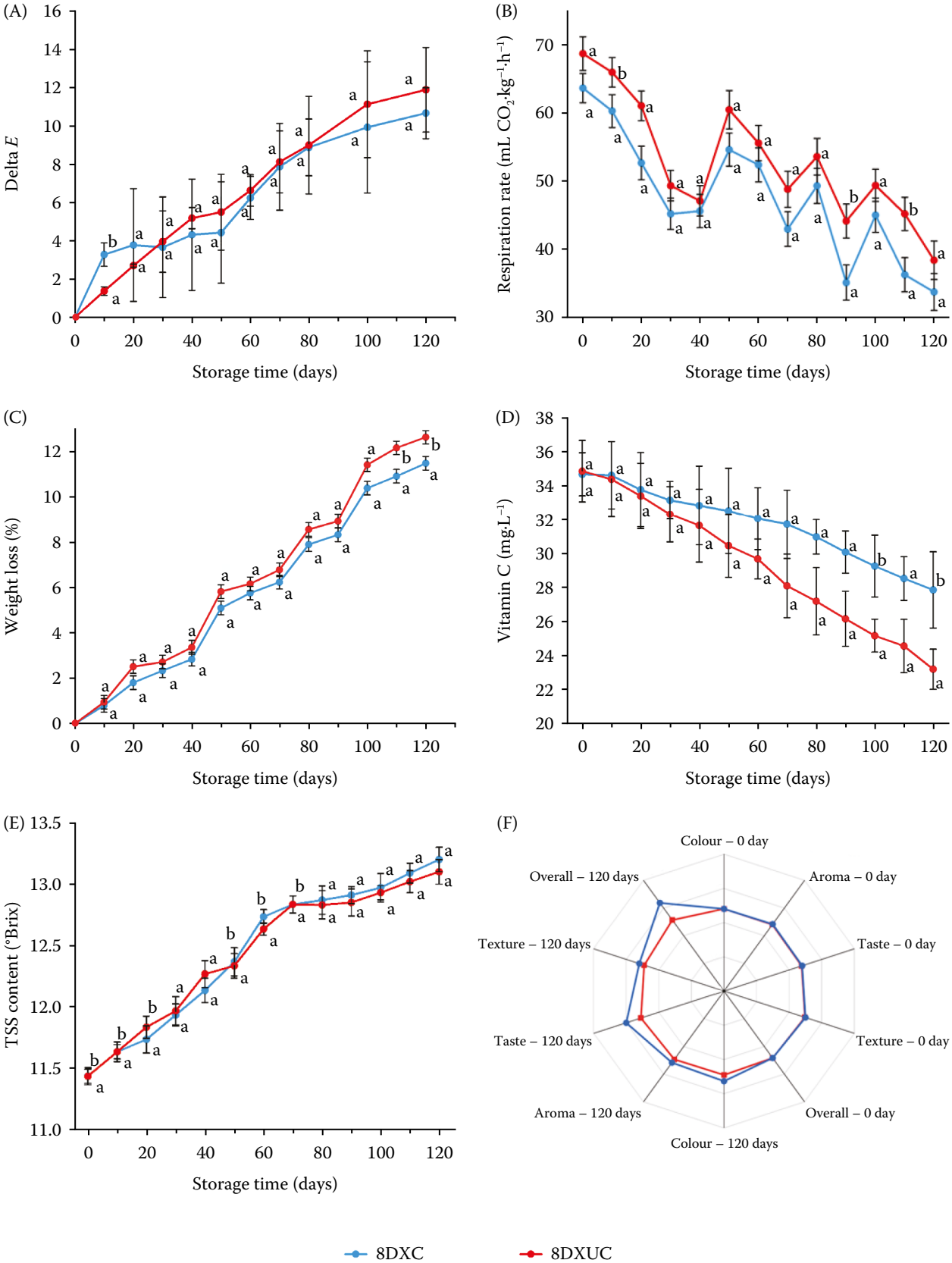

Figure 4. Changes in fruit quality parameters of Da Xanh pomelo

8DXC – coated Da Xanh pomelo; 8DXUC – uncoated Da Xanh pomelo; TSS – total soluble solid

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durability, enhanced flexibility, and better antioxidant performance. The film sprayed onto the surface of the pomelo peel acts as a beneficial semi-permeable membrane around the surface of the fruit peel, limiting exposure to atmospheric oxygen and helping to delay respiratory, absorption activities and metabolism in fruit (Chen et al. 2016). These results are consistent with the results of some authors. The feasibility of preserving citrus fruits with pectin-beeswax coating was studied by (Maftoonazad and Ramaswamy 2019). The results showed that fruits without film coating had peels that gradually turned yellow. faster than fruits coated with pectin-beeswax film at a temperature of 10–25 °C for 32 days. In a study by (Cháfer et al. 2012) on preserving oranges with chitosan film supplemented with bergamot, musk, and tea tree extracts, the results showed that all orange samples were coated with film had a lower respiration rate than the uncoated control sample during 8 weeks of storage.

Fruit quality parameters, such as vitamin C and TSS, reflect the preservative properties of the fruit. The results showed that pomelo with a coating applied had a higher vitamin C and TSS content. Similar trends have also been demonstrated by (Chen et al. 2016), the research reported that Nanfeng tangerines preserved with sodium alginate (SA) film combined with *Ficus hirta* (FH) fruit extract had a maintained vitamin C content higher than the sample control after the storage period. Research by (Zeng et al. 2013) has demonstrated that preserving oranges with a carboxymethyl cellulose (CMC) coating supplemented with *Impatiens balsamina* L. stem extract helps maintain a higher total soluble solids content compared to the control sample after the end of the preservation process.

CONCLUSION

In summary, research showed that the flavedo extract from Da Xanh pomelo contains several phenolic compounds such as gallic acid, catechin, chlorogenic acid, rutin, ellagic acid, quercitrin, quercitin, and apigenin of which gallic acid has the highest concentration; besides, the extract also exhibited antioxidant activity, antifungal capacity; thereby showing the application potential of flavedo extract. After being stored at 8 °C for 120 days, Da Xanh pomelo treated with the coating showed colour change, respiration intensity, and lower weight loss than pomelo without coating treatment. Besides, the coating also helps maintain vitamin C and TSS content and helps improve the sensory value of pomelo. Therefore, the results of this study have shown that flavedo extract from *Citrus maxima* (Burm.) Merr. pomelo added to the coating can prolong the storage time and maintain the quality of pomelo after storage.

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