Vietnam Journal of Science and Technology ***63*** *(6) (2025) 1083-1093*

doi:10.15625/2525-2518/18887

Bioactive of betel leaves extract grown in Vinh Long province and its application to extend shelf-life of Da Xanh pomelo

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Received: 12 September 2023; Accepted for publication: 29 August 2025

Abstract It is well known that plant extract is a safe additive for coating systems that can extend fruit shelf-life. In this study, bioactive compounds of betel leaves (*Piper betle* L.) grown in Vinh Long province were extracted, characterized, and proposed to use in the coating preparation for Da Xanh pomelo (*Citrus maxima* (Burn.) Merr.). Characteristic tests showed that betel leaves extract had high phenolic content and antioxidant activities. It could also inhibit seven different strains of mould presented in Da Xanh peel. Thus, betel leaves extract proved to be a suitable additive to coating Da Xanh pomelo. The betel leaves extract was then mixed in the coating preparation at different concentrations (150; 200; 250; 300, and 400 µg GAE/mL), and the fruits were then coated with the coating sample. After 15 days of storage, Da Xanh pomelo coated at 250 µg GAE/mL showed the least changes in ΔE value, weight loss, and total yeast and mould count. Extended storage test results also showed that Da Xanh pomelos coated with betel leaf extract-added preparation were stable after 30 days of storage at room condition.

Keywords: betel leaf extract, coating, Da Xanh pomelo, shelf-life, storage.

Classification numbers: 1.2.1, 1.3.1, 2.5.3

1. INTRODUCTION

The Mekong Delta Region (MDR) has approximately 307,000 ha of fruit cultivation, supplying roughly 4 million tons of fruit annually. MDR was designated as a key pomelo-producing region in 2003, with an area of 27,900 ha [1]. After the 2025 administrative merger, Vinh Long became one of Vietnam's largest pomelo areas, cultivating at least 21,000 ha—approximately 20 % of the national total - and its Ben Tre Da Xanh pomelo has gained official geographical indication status [2,3]. The region is expanding into specialized, high-quality production zones with strong market linkages. Despite their large output, pomelo quality varies widely because of inconsistent cultivation, fertilization, transport, and storage practices. Fungal

contamination, post-harvest disorders, and inadequate handling are the main causes of fruit deterioration. Therefore, improved post-harvest management is essential to control microbial growth and maintain quality during the storage period. Incorporating plant extracts into edible coatings is a promising solution for extending pomelo shelf life.

Betel leaf extract (PBE), rich in polyphenols, flavonoids, alkaloids, eugenol, carvacrol, and chavicol [4], is increasingly used as a natural food preservative [5]. In this study, PBE was added to an edible coating to preserve the Da Xanh pomelos during storage. These findings are expected to support the development of effective coating formulations that stabilize pomelo quality and enhance food safety.

2. MATERIALS AND METHODS

2.1. Materials

Ben Tre Da Xanh pomelo was picked in Binh Dai district, Vinh Long province. It was grown using VietGAP or GlobalGAP standards. The fruit had to meet the fresh pomelo standard (TCVN10746:2015). The best pomelos, weighing 1,400 to 1,700 grams each, were used in this study. They were picked in the morning with the stem on, wrapped in paper, and taken to the lab in boxes. This had to be done by 10:00 a.m. At the lab, the pomelos were cleaned twice: first with a 3 % NaHCO3 solution, then with a 200 ppm chlorine solution for 15 minutes, and finally rinsed with clean water.

Betel leaves were bought from Binh Minh district, Vinh Long province. Only mature and healthy leaves were chosen. Only mature and healthy leaves were chosen, and they were carefully transported to the lab to prevent any damage.

2.2. Methods

2.2.1 Preparation of betel leaves extract (PBE)

PBE was prepared as according to Dai Thi Xuan Trang *et al.* with minor modifications [6], using a modified domestic microwave oven as described in Fig. 1 [7].

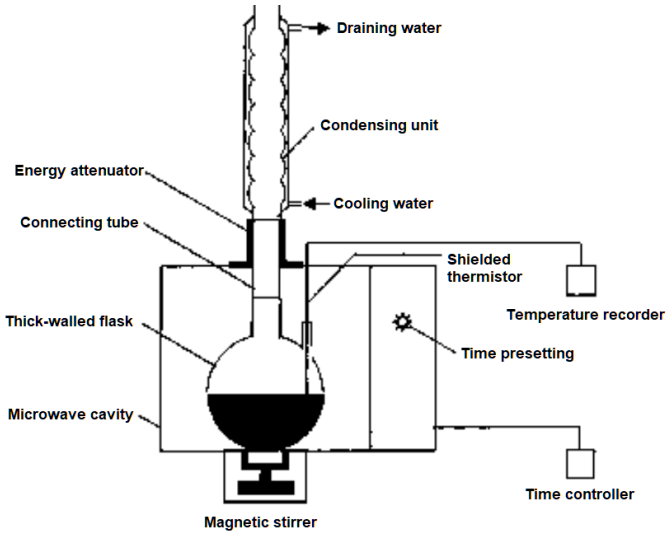

Figure 1. Scheme of a modified domestic microwave oven [7].

At the laboratory, the leaves are thoroughly washed and then dried in a convection drying at an average temperature of 50 °C until the moisture content of the sample decreases below 10 % when the leaves can be finely ground. Then, the ground sample undergoes microwave-assisted extraction by mixing with ethanol (99.5 %) at the ratio of 1:10 (w/v) under a microwave power of 480 W for 6 minutes (100 g/sample). The extract is filtered using Whatman No.1 filter paper to remove residues. The solvent was removed using rotary evaporation equipment (RV 10 digital V, IKA, Malaysia) at 60 °C to obtain a concentrated extract around 70° Brix.

2.2.2 PBE characterization method

Total phenolic content was measured using Folin-Ciocalteau (FC) with gallic acid standard [8], expressed as mg gallic acid equivalents per 1 g extract (mg GAE/g).

Trolox equivalent antioxidant capacity was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) with Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) standard [9], expressed as µg trolox equivalents per 1 g extract (µg TE/g).

The extract's specific antioxidant capacity was determined by six methods: ATBS•+ scavenging activity [10], DPPH antioxidant assay [11], Ferric reducing antioxidant power (FRAP) [12], Total reducing power (RP) [13], Total antioxidant capacity (TAC) [14], and nitric oxide (NO•) scavenging ability [15]. Results were expressed as EC_{50} values (µg/mL), calculated from correlation between extract concentration and scavenging/reduction effect (%).

Antifungal activity was determined following Gakuubi et al. [16]. The extract was added to PDA medium at concentrations of 625 - 10,000 µg/mL. Agar discs with mycelia were inoculated centrally, and fungal colony diameter was measured after 48 and 72 hours. Five plant pathogenic fungi (*Neurospora intermedia, Schizophyllum commune, Aspergillus aculeatus, Aspergillus niger*, and *Aspergillus brunneoviolaceus*), isolated from pomelo peel in the Mekong Delta by Huynh et al. [17], were tested.

2.2.3 Film coating preparation

The edible film was prepared according to Lam & Tuan with minor modifications [18]. Carnauba emulsion (65 - 85 g; SMM Group & Co., Brazil) and polyethylene emulsion (65 - 85 g; Westlake, USA) were prepared separately by melting the respective ingredient with oleic acid (10 - 14 g) at 105 °C then added ammonia 8 % (40 - 60 g) and distilled water (300 - 360 g) mixed with an antifoaming agent (0.04 g), stirred the mixture until a homogeneous colloidal system was formed. The obtained emulsion was then let to stabilize at room temperature.

The betel leaves extract film (PBEF) coating preparation was formed prior to usage by mixing two emulsion wax (carnauba: polyethylene at a ratio of 3:5) with betel leaves extract at the speed of 2.000 - 2.500 rpm for 15 - 20 min. The final product was a microemulsion (particle sizes ranged from 100 to 200 nm), light green in color, and had a dry matter concentration of about 20 %.

2.2.4 Physicochemical properties of pomelo

Weight loss (%). The fruit was weighed by a digital balance at the beginning and at the exact times of the experiment. Weight loss (%) was calculated by the Eq. 1:

$$\text{Weight loss (\%)} = [(\text{Initial weight} - \text{Current weight})/\text{Initial weight}] \times 100 \quad (1)$$

The color was measured using a colorimeter (CR-20, Konica Minolta, Japan) in the CIELAB color space. The total color difference (ΔE) was calculated using the Eq. 2:

$$\sqrt{\left(L^* - L^*_0\right)^2 + \left(a^* - a^*_0\right)^2 + \left(b^* - b^*_0\right)^2} \quad (2)$$

where: L^*, a^*, b^* are values of measured sample and L^*_0, a^*_0, b^*_0 are values of control sample.

Total soluble solids (TSS) were measured using a refractometer (Atago, 0 - 32°Brix, Japan).

Titratable acidity (TA, %) was determined by titration with 0.1 N NaOH. The results were expressed as a percent of citric acid per total sample (AOAC Official Method 942.15).

Vitamin C (mg%) or Ascorbic acid content was measured by titration with 2, 6-dichlorophenol-indophenol (AOAC Official Method 985.33).

The yeasts and moulds (colony forming unit per gram, CFU/g) were assayed according to ISO 21527-1:2008.

2.3 Experimental design

Experimental 1 is carried out to determine the appropriate concentration of PBE used in the film coating preparation for Da Xanh pomelo. The fruits were coated with PBEF at different concentrations of PBE (0, 150, 200, 250, 300, and 400 µg/mL). The amount of the PBEF used was fixed at 1.35 mL/fruit. PBEF was then coated on the fruit surface by paint brushing. This method was exclusively used for the large fruits to ensure an evenly covered film coating [18]. Fruit storage was conducted under the ambient conditions. Changes in the physicochemical properties of pomelo are evaluated over 15-day periods to determine the approximate amount of PBE. The experiment is repeated three times.

Experimental 2 is carried out to assess the changes of Da Xanh pomelo during storage. Two samples were used: a control sample without coating and a sample coated with PBEF (selected from Experiment 1). Changes in the physicochemical properties of pomelo are monitored for up to 30 days of storage. The experiment is repeated three times.

2.4 Data analysis

All data were statistically analyzed and presented as means ± standard deviations (SD). The analysis of variance and Duncan's Multiple Range Test was applied to assess the difference between means, processed by Statgraphics Centurion 16.2 (Copyright (C) PP, USA) and Excel 2016 programs. Significance was defined at $p \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1 Characteristic of betel leaf extract (PBE)

3.1.1 Quantification of total phenolic content and antioxidant capacity

The total phenolic content and antioxidant capacity of betel leaf extract are the foremost parameters that indicate the quality of the sample. Therefore, those parameters were quantified, and the results were presented in Table 1.

The results revealed that PBE had a notably high antioxidant activity equivalent to 127.28 µmol TE per gram extract. Simultaneously, phenolic content was also observed at 55.93 ± 0.40 mg GAE per gram extract. According to the previous study, the polyphenolic composition in the betel leaf extract was mainly hydroxy chavicol, 4-chromanol and eugenol, 1-phenylpropene-3,3-diol diacetate, and 4-allyl-1,2-diacetoxybenzene, all possess antioxidant, anti-inflammatory,

antithrombotic, and antimicrobial activities [19]. Recent studies also showed that phenolic compounds in PBE protect against photosensitization-induced lipid peroxidation in the rat liver while enhancing antioxidant capabilities. These same phenolic compounds in betel leaf have effectively treated gastric ulcers induced by indomethacin [20].

Table 1. Total phenolic content and antioxidant capacity of PBE.

Parameter	Value (**)
Total phenolic content (mg GAE/g)	55.93 ± 0.40
Trolox equivalent antioxidant capacity (µmol TE/g)	127.28 ± 2.78
The specific antioxidant capacity (expressed as EC_{50} - µg/mL)	
$ABTS^{\bullet+}$	7.77 ± 0.01
DPPH	63.14 ± 1.89
FRAP	8.81 ± 0.04
RP	2.37 ± 0.03
TAC	18.01 ± 0.82
$NO^{\cdot}$	45.22 ± 0.85

Values are means of triplicate assays ± SD; EC50: half maximal effective concentration

Antioxidant activities of PBE are expressed in EC_{50} value, which is the effective concentration required to scavenging/reducing 50 % of free radicals. A lower EC_{50} value implies a higher antioxidant capacity [21]. EC_{50} < 50 µg/mL indicates very strong antioxidant capability, while 50-100 µg/mL reflects a strong antioxidant potential, 101-150 µg/mL signifies moderate antioxidant ability, and EC_{50} >150 µg/mL indicates weak antioxidant capacity [22]. The results (Tab. 1) demonstrated that BPE has a remarkably strong antioxidant capacity, indicated by 5 (out of the 6) EC_{50} values lower than 50 µg/mL. The sole exception is the EC_{50} values assessed by the DPPH method, with an EC_{50} value higher than 50 µg/mL.

3.1.2 Antifungal activity of PBE

As revealed by the research of Tam *et al.*, there were seven strains of mould presented on the peel of Da Xanh pomelo, including *Trichoderma asperellum, Aspergillus brunneoviolaceus, Neurospora intermedia, Aspergillus carbonarius, Schizophyllum commune, Aspergillus niger*, and *Aspergillus aculeatus* [17]. Based on that finding, the antifungal activity of PBE was tested directly on those seven strains. The results, involving the measuring zone of inhibition diameter (ZOI) and antifungal efficacy (AFE), are presented in Tabs. 2 and 3.

Table 2. ZOI values (cm) of PBE against the seven strains of mould after 72 hours of cultivation.

Mould strains	Concentration of PBE (µg/mL)				
	0	625	1,250	2,500	5,000
Neurospora intermedia	8.14 ± 0.29^{d}	1.23 ± 0.12^{c}	0.23 ± 0.06^{b}	0^{a}	0^{a}
Schizophyllum commune	2.37 ± 0.12^{c}	0.67 ± 0.12^{b}	0.57 ± 0.12^{b}	0^{a}	0^{a}
Aspergillus aculeatus	3.93 ± 0.12^{d}	1.03 ± 0.06^{c}	0.43 ± 0.12^{b}	0^{a}	0^{a}
Aspergillus niger	4.87 ± 0.12^{d}	1.23 ± 0.12^{c}	0.13 ± 0.06^{b}	0^{a}	0^{a}
Aspergillus brunneoviolaceus	4.87 ± 0.12^{c}	0.53 ± 0.06^{b}	0.03 ± 0.06^{a}	0^{a}	0^{a}

Values are means of triplicate assays ± SD. Different letters in the same row show statistical differences ($p \leq 0.05$)

Table 3. AFE (%) of BPE against the seven strains of mould after 72 hours of cultivation.

Mould strains	Concentration of PBE (µg/mL)				
	0	625	1,250	2,500	5,000
Neurospora intermedia	-	84.84	97.13	100.00	100.00
Schizophyllum commune	-	71.83	76.06	100.00	100.00
Aspergillus aculeatus	-	73.73	88.98	100.00	100.00
Aspergillus niger	-	74.66	97.26	100.00	100.00
Aspergillus brunneoviolaceus	-	89.04	99.32	100.00	100.00

Values are means of triplicate assays ± SD. Different letters in the same row show statistical differences ($p \leq 0.05$)

In general, PBE can inhibit seven strains of mould presented on Da Xanh pomelo peel. The highest (100 %) AFE against all seven strains of mould was observed at 2,500 and 5,000 µg/mL concentrations. The antifungal and antioxidant effectiveness of PBE relies on the presence of polyphenolic compounds in the extract. For instance, hydroxychavicol found in betel leaf has been confirmed to possess antifungal capabilities against pathogenic strains such as *Aspergillus* sp., *Candida albicans*, and *Candida glabrata* [23], as well as strains within the *Mucor* fungi [24]. The compound piperlonguminine found in PBE also exhibits considerable antifungal activities [25]. Thus, the high quantified polyphenolic content also affirmed the antifungal activity of PBE extracts.

In conclusion, PBE was proven to be a suitable additive to coating Da Xanh pomelo, which can help prolong the fruit's freshness and inhibit disease-related fungal strains presented on Da Xanh pomelo peel.

3.2 Suitable concentration of PBE for coating Da Xanh pomelo

Three parameters were evaluated to determine the effect of PBEF on Da Xanh pomelo quality: total color difference, weight loss, and total yeast and mold count (Tabs. 4 - 6).

The total color difference (ΔE) reflects the peel color changes during storage, where $\Delta E > 0$ indicates a shift from green to yellow. As shown in Table 4, the ΔE increased over time. After 15 days, the 250 µg/mL PBE treatment produced the smallest color change ($\Delta E = 0.49$), which was lower than that of the 350 µg/mL treatment (0.60) and much lower than that of the control (1.24). Color changes result mainly from chlorophyll degradation, which occurs post-harvest through the oxidation of the pyrrole ring, leading to the loss of green color and greater visibility of carotenoids that create yellowing [26,27]. Because PBE itself has a strong green color, coating the peel enhances green retention and reduces ΔE, helping to maintain pomelo appearance after 15 days of storage.

Table 4. Effect of PBE on total color difference (ΔE) of Da Xanh pomelo during storage.

Storage time (days)	BE concentrations (µg/mL)					
	0	150	200	250	300	400
0	0^{aA}	0^{aA}	0^{aA}	0^{aA}	0^{aA}	0^{aA}
3	0.24 ± 0.02^{bA}	0.19 ± 0.04^{bA}	0.18 ± 0.01^{bA}	0.17 ± 0.03^{bA}	0.18 ± 0.01^{bA}	0.18 ± 0.03^{bA}
6	0.35 ± 0.02^{cD}	0.27 ± 0.02^{cBC}	0.27 ± 0.01^{cBC}	0.22 ± 0.03^{cA}	0.24 ± 0.03^{cAB}	0.28 ± 0.01^{cC}
9	0.59 ± 0.03^{dD}	0.36 ± 0.01^{dC}	0.33 ± 0.02^{dBC}	0.27 ± 0.03^{dA}	0.3 ± 0.01^{dAB}	0.36 ± 0.02^{dC}
12	0.85 ± 0.02^{eE}	0.5 ± 0.01^{eD}	0.42 ± 0.01^{eBC}	0.32 ± 0.01^{eA}	0.39 ± 0.01^{eB}	0.45 ± 0.04^{eC}
15	1.24 ± 0.03^{fE}	0.74 ± 0.03^{fD}	0.64 ± 0.03^{fBC}	0.49 ± 0.01^{fA}	0.60 ± 0.02^{fB}	0.68 ± 0.05^{fC}

Different letters in the same column (lowercase) or row (uppercase) show means significant at $p \leq 0.05$

Loss in weight is also an inevitable phenomenon in the post-harvest management of fruits and vegetables in general, including pomelos. Weight loss is the outcome of two natural biological processes of the fruit: the water evaporation process [6] and the inherent respiration process (where dry matter is converted into CO_2 and H_2O) [26]. The results in Table 5 indicated that weight loss of pomelo was increased consistently during storage. The addition of PBE did not significantly affect the weight loss rate during the first nine days of preservation. However, after 15 days, the addition of PBE noticeably influenced the weight loss rate. Among the treated samples, the addition of PBE at 250 µg/mL exhibited the lowest weight loss (3.11 %) compared to other samples. In contrast, the control sample experienced a weight loss up to 4.81 %, and when the concentration was increased to 300 µg/mL, the weight loss rate also rose to 3.68 %. This phenomenon can be explained by the varying degrees of solubility of PBE in the film preparation. At a suitable concentration, PBE dissolves and forms a semi-permeable film layer, thereby moderating gas permeability and limiting weight changes due to the fruit's respiration process [17].

Table 5. Effect of PBE on weight loss (%) of Da Xanh pomelo during storage.

Storage time (days)	BE concentrations (µg/mL)					
	0	150	200	250	300	400
0	0^{aA}	0^{aA}	0^{aA}	0^{aA}	0^{aA}	0^{aA}
3	1.19 ± 0.18^{bA}	1.13 ± 0.32^{bA}	1.01 ± 0.14^{bA}	0.91 ± 0.23^{bA}	1.01 ± 0.34^{bA}	1.11 ± 0.28^{bA}
6	2.03 ± 0.21^{cB}	1.51 ± 0.41^{cAB}	1.48 ± 0.32^{cA}	1.28 ± 0.12^{cA}	1.28 ± 0.32^{cA}	1.54 ± 0.21^{cA}
9	2.93 ± 0.16^{dC}	2.37 ± 0.14^{dB}	2.11 ± 0.44^{dAB}	1.82 ± 0.14^{dA}	2.01 ± 0.31^{dAB}	2.26 ± 0.12^{dB}
12	3.91 ± 0.21^{eD}	3.26 ± 0.32^{eC}	3.02 ± 0.22^{eBC}	2.46 ± 0.21^{eA}	2.91 ± 0.09^{eB}	3.31 ± 0.34^{eBC}
15	4.81 ± 0.10^{fD}	4.31 ± 0.31^{fC}	4.29 ± 0.11^{fC}	3.11 ± 0.14^{fA}	3.68 ± 0.28^{fB}	4.40 ± 0.11^{fC}

Different letters in the same column (lowercase) or row (uppercase) show means significant at $p \leq 0.05$.

On the pomelo surface, there are always residual traces of yeast and mould mycelium (below 10 CFU/cm^2). The results in Table 6 demonstrated that samples coated with PBE on day 0 all had the betel leaf bioactive compounds that could inhibit the growth of pathogens in food [28]. During storage, the density of yeast and mould consistently increased in all samples, including those coated with PEB at the highest concentration (400 µg/mL). After 15 days of storage, on the control sample, the total yeast and mould count reached 2.05×10^2 CFU/cm^2, whereas, in the samples treated with betel leaf extract, the yeast and mould density gradually decreased with increasing concentration. Specifically, at concentrations of 150, 200, 250, 300, and 400 µg/mL, the yeast and mould densities were 8.85×10^1, 8.18×10^1, 7.30×10^1, 6.58×10^1, and 5.39×10^1 CFU/cm^2, respectively.

Table 6. Effect of PBE on total yeast and mould count (CFU/cm^2) of Da Xanh pomelo during storage.

Storage time (days)	BE concentrations (µg/mL)					
	0	150	200	250	300	400
0	<10	ND*	ND	ND	ND	ND
3	<10	<10	<10	<10	<10	<10
6	1.10×10^2	2.45×10^1	2.00×10^1	1.82×10^1	1.55×10^1	1.12×10^1
9	2.05×10^2	8.85×10^1	8.18×10^1	7.30×10^1	6.58×10^1	5.39×10^1
12	<10	ND*	ND	ND	ND	ND
15	<10	<10	<10	<10	<10	<10

Different letters in the same column (lowercase) or row (uppercase) show means significant at $p \leq 0.05$; ND: not detected

In conclusion, based on total color difference, weight loss, and total yeast and mould count, the suitable concentration of PBE for preserving Da Xanh pomelo is determined to be 250 µg/mL film preparation.

3.3 Changes in quality of Da Xanh pomelo during storage

An extended storage test was performed to continue monitoring the changes in the quality of Da Xanh pomelo up to 30 days of preservation. In this experiment, five parameters were chosen to assess the changes in the quality of Da Xanh pomelo, including weight loss, total color difference, TSS, TA, and vitamin C content of the pulp. The results are presented in Fig. 2.

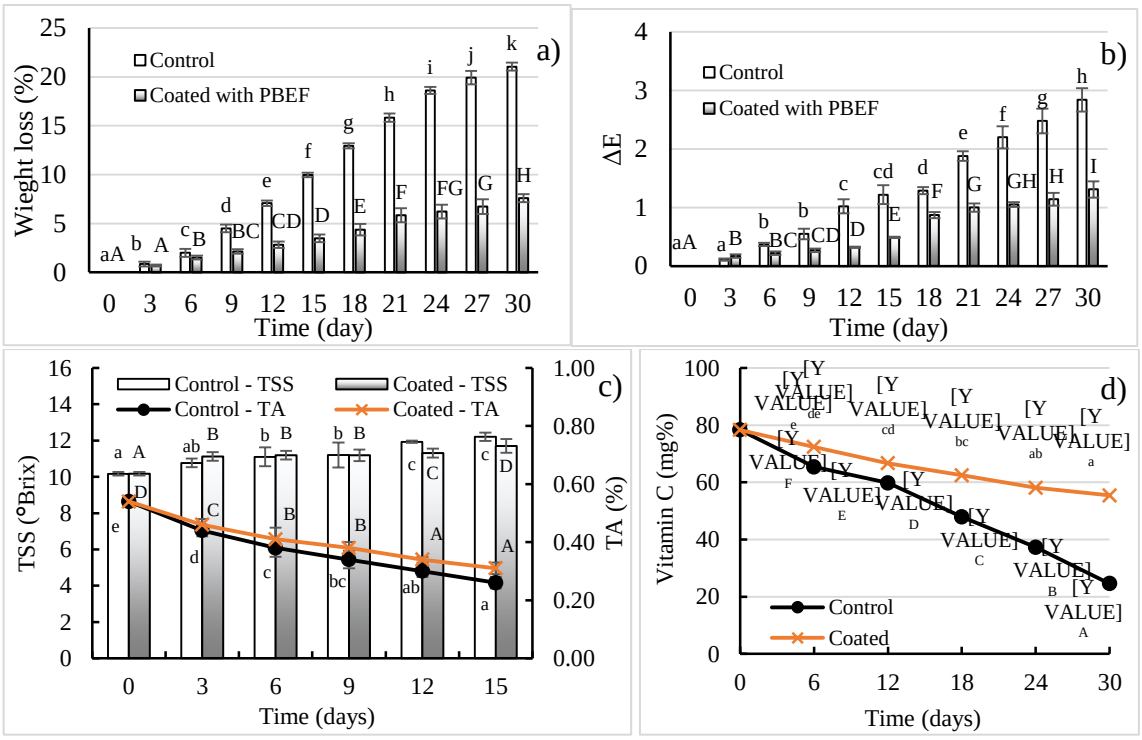

Figure 2. Changes in physicochemical properties of Da Xanh pomelo during storage.

The most noteworthy effect of PBEF coating was the reduction in weight loss. The collected data indicates that the control sample experienced a weight loss of up to 21.05 % on the 30th day, while the film-coating sample with betel leaf extract reduced only 7.60 %. Thus, the results again affirm the film's efficacy in minimizing the loss of water via evaporation. In corresponding to the weight loss, the TSS value increased over time. By the 30th day, PBEF-coated pomelo had a TSS value over 11.47°Brix, which aligns with the geographical indications for Ben Tre Da Xanh pomelo [29].

The chlorophyll degradation, expressed by the increase in ΔE value, continued during storage time in the control sample. However, the PBEF-coated pomelos exhibited a lower color difference, as ΔE value was only 1.31, in contrast to the control sample with a ΔE value of 2.84 after 30 days. The results in Fig. 2 also demonstrated that the fruit pulp's titratable acidity and vitamin C content tended to decrease during the storage period. The breakdown of organic acids during the ripening process of the fruit is related to the increased activity of enzymes such as succinate

dehydrogenase and citrate dehydrogenase, as well as the decreased activity of citrate synthase as the fruit ripens [26]. Meanwhile, the vitamin C content in the fruit tends to decrease because of oxidation and natural respiration processes [29].

The PBEF-coated samples exhibited a slower decline rate in both titratable acidities (remaining at 0.31 % after 30 days, compared to the control sample at 0.26 %) and vitamin C content in the fruit (remaining at 55.4 mg% after 30 days of preservation compared to the control sample's 24.6 mg%). According to geographical indications, Da Xanh pomelo should have titratable acidity lower than 0.46 %; both control and PBEF-coated Da Xanh pomelo meet this criterion.

In summary, the observed results have demonstrated the efficacy of film coating supplemented with betel leaf extract in stabilizing the changes in the quality of Da Xanh pomelo over 30 days of storage.

4. CONCLUSIONS

In conclusion, the study showed that PBE can be added to film coating preparation and used to stabilize the changes in the quality of Da Xanh pomelo during storage. Thus, PBEF coating preparation is recommended in the post-harvest management of Da Xanh pomelo to extend the shelf-life of the fruit.

Acknowledgements. This work was supported by key science and technology program of Ministry of Education and Training (program code: CT2020.01: Application and Development of Advanced Technologies in Preservation and Processing of Aquatics and Agricultural Products in the Mekong Delta).

CRediT authorship contribution statement. Tran Thanh Truc: Methodology, Investigation and writing drafts of the manuscript; Phan Minh Trong and To Nguyen Phuoc Mai: Experiments, Data curation; Nguyen Van Muoi: organized research and completed the manuscript.

Declaration of competing interest. The authors declare no conflict of interest.

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