



**EFFECTS OF ACIDIC ELECTROLYZED WATER AND
ULTRAVIOLET-C ON SURFACE MOULD AND
QUALITY OF 'PHULAE' PINEAPPLE**

HLWAN MOE THU
MASTER OF SCIENCE
IN
POSTHARVEST TECHNOLOGY AND INNOVATION

SCHOOL OF AGRO-INDUSTRY

MAE FAH LUANG UNIVERSITY

2023

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Hlwan Moe Thu

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ABSTRACT

Pineapple cv. Phulae (*Ananas comosus* L. Merr.) is an economically and geographically indication (GI) crop in Chiang Rai Province, Thailand. Market demand in both local and abroad is increasing due to its sweet, crispy and chewable of the core. The crown is cut as postharvest practices to economically safe for logistic process. Nonetheless, a major challenge as unmarketable condition of postharvest loss lies due to the mould growth on the de-crown area of the fruit during cold storage facilities to prolong the shelf life while transportation. Consumer concern for chemical residues also becoming a challenge to sustain the pineapple industries. In this study, acidic electrolyzed water (AEW) treatment (experiment 1), ultraviolet-C radiation (experiment 2) and the selected single treatment or combination of these selected best treatments (experiment 3) from experiment 1 and 2 tested comparing with the control fruit while storage at 13 °C for 28 days. The purpose of these operations is to explore the effects of these postharvest treatments on surface mould and also to assess the fruit quality changes during cold storage.

In the experiment 1, effect of acidic electrolyzed water (AEW) treatment on 'Phulae' pineapple fruit was analysed. Pineapple fruit at green mature stage was treated with AEW (100 ppm, 200 ppm, 300 ppm) and air dry with a fan and stored at

13 °C, 85-95% relative humidity for 28 days. Mould incidence and mould severity were investigated every 7-day interval. The results showed that until 7-days of storage, all AEW (100, 200 and 300 ppm) treatments were significantly inhibited mould incidence (20.00 %, 26.67% and 16.67% respectively) in 'Phulæ' pineapples, revealing its antifungal potential from untreated control (60.00%). However, prolonged storage led to 100% mould incidence, indicating limited sustained effectiveness. Mould severity percentage at day 14 of cold storage, AEW 300 ppm ($38.33 \pm 1.67\%$) shown significantly different from 200 ppm ($60.00 \pm 3.82\%$) and the control ($71.67 \pm 10.44\%$) but no difference from 100 ppm ($51.25 \pm 3.31\%$). There were no significantly different between the treatments and the control in days 21 and 28 of storage. While AEW treatments consistently decreased mould severity compared to the control, complete prevention was not attained. The study proposes a potential threshold in AEW's antifungal action and highlights a dose-dependent relationship, with higher concentrations correlating with reduced severity. So, AEW 300 ppm was chosen for next experiment. Further exploration across a wider concentration range is advised to optimize efficacy.

In the experiment 2, effect of UV-C irradiation on 'Phulæ' pineapple fruit was investigated. Pineapple fruit at green mature stage was treated with UV-C (13.2, 26.4 and 39.6 kJ/m^2) then stored at 13 °C and 85-95% RH for 28 days. Mould incidence and mould severity were investigated every 7-day interval. The results observed that all UV-C treatments (13.2 kJ/m^2 , 26.4 kJ/m^2 and 39.6 kJ/m^2) found lower mould incidence percentage ($63.33 \pm 8.82\%$, $53.33 \pm 8.82\%$ and $53.33 \pm 14.53\%$) than the control ($80 \pm 0\%$) but there is no significantly different until 7-day of storage. 100% of mould incidence was observed after 14-days of storage. Mould severity percentage until 7-days found that UV-C 39.6 kJ/m^2 was significantly lowest percentage ($13.33 \pm 3.63\%$) than the control ($31.37 \pm 4.64\%$) but there were no different from the other treatments 13.2 kJ/m^2 ($21.67 \pm 4.41\%$) and 26.4 kJ/m^2 ($18.33 \pm 3\%$). At 14-days of storage, mould severity treated by all treatments found significantly different than the

control but there were no different between the treatments. On 28-days of storage, UV-C dose at 13.2 kJ/m² investigated significantly lowest mould severity percentage ($80.83\pm3\%$) than UV-C dose at 26.4 kJ/m², 39.6 kJ/m² and the control ($90\pm2.5\%$, $90.83\pm0.83\%$ and $90\pm1.44\%$ respectively). UV-C treatment effectively reduced mould incidence and severity in 'Phulae' pineapples during cold storage. However, 100% mould incidence was observed by Day 14, indicating a delay rather than prevention. The absence of notable differences among UV-C doses (13.2, 26.4, 39.6 kJ/m²) suggests a potential efficacy plateau, prompting further exploration of higher doses or combining treatments for optimal mould control. While UV-C consistently lessened mould severity, a gradual increase over time implied diminishing effectiveness or limitations against established infections. The evident dose-dependent relationship supports the exploration of a broader dose range. UV-C doses of 13.2 kJ/m² and 39.6 kJ/m² were chosen for study in combination with AEW treatment (for experiment 3), aiming to enhance efficacy while mitigating potential drawbacks. In the experiment 3, the most suitable treatments from each previous experiment (1 and 2) such as in the experiment 1, AEW 300 ppm and in the experiment 2, UV-C dose at 13.2 kJ/m² and 39.6 kJ/m² were chosen. Effects of selected single treatments (experiment 1 and 2) and combination of these selected AEW and UV-C treatments on 'Phulae' pineapple fruit were investigated. Pineapple fruit at green mature stage was treated with untreated control, AEW (300 ppm), UV-C (13.2 kJ/m²), UV-C (39.6 kJ/m²), AEW (300 ppm) + UV-C (13.2 kJ/m²) and AEW (300 ppm) + UV-C (39.6 kJ/m²) then stored at 13 °C and 85-95% RH for 28 days. Mould incidence, mould severity, physico-chemicals and antioxidant activity were investigated every 7-day intervals. The results until 7-days of storage, AEW (300 ppm) ($26.67\pm16.67\%$) and combination of AEW (300 ppm) with UV-C (39.6 kJ/m²) ($26.67\pm6.67\%$) were reduced mould incidence significantly different than UV-C (13.2 kJ/m²) ($60\pm10.00\%$), UV-C (39.6 kJ/m²) ($60\pm5.77\%$) and the control ($90\pm5.77\%$) but there is no significantly different from AEW (300 ppm) + UV-C (39.6 kJ/m²) ($33.33\pm3.33\%$).

Mould incidence found 100% from 14-days of storage in all treatments. Combination of AEW (300 ppm) + UV-C (39.6 kJ/m²) treatments observed that it was significantly reduced mould severity percentage than all others treatments in 21-days of storage but no different from UV-C (13.2 kJ/m²) and AEW (300 ppm) + UV-C (13.2 kJ/m²) in 28-days of storage. The results observed that the treatments were significantly reduced (P<0.05) in mould incidence than the control until 7-day of storage and AEW (300 ppm) + UV-C (39.6 kJ/m²) found mould severity effectively until 28-days of storage than the control.

In vitro study, the treatments reduced spore survival, spore germination, germ tube elongation, and mycelium growth than the untreated control. The parameters which the treatments have no significantly reduction (P<0.05) were weight loss, moisture content, dry matter, colour, internal browning severity, total soluble solids, titratable acidity, TSS-TA ratio, pH and vitamin C content. But the treatments have significantly induced (P<0.05) in total phenolic compound, total flavonoid content, antioxidant activity (measured by DPPH and FRAP). These findings indicate that the combination of AEW (300 ppm) and UV-C (13.2 kJ/m²) exhibits potential synergistic effects. Further research is required to fine-tune the concentrations and timing of AEW and UV-C treatments. Exploring a broader spectrum of combinations will aid in determining the optimal treatment conditions to enhance both quality retention and germicidal effects.

Keywords: Ionized Water, Irradiation, Non-residue, Disinfectant, Non-thermal Sterilization

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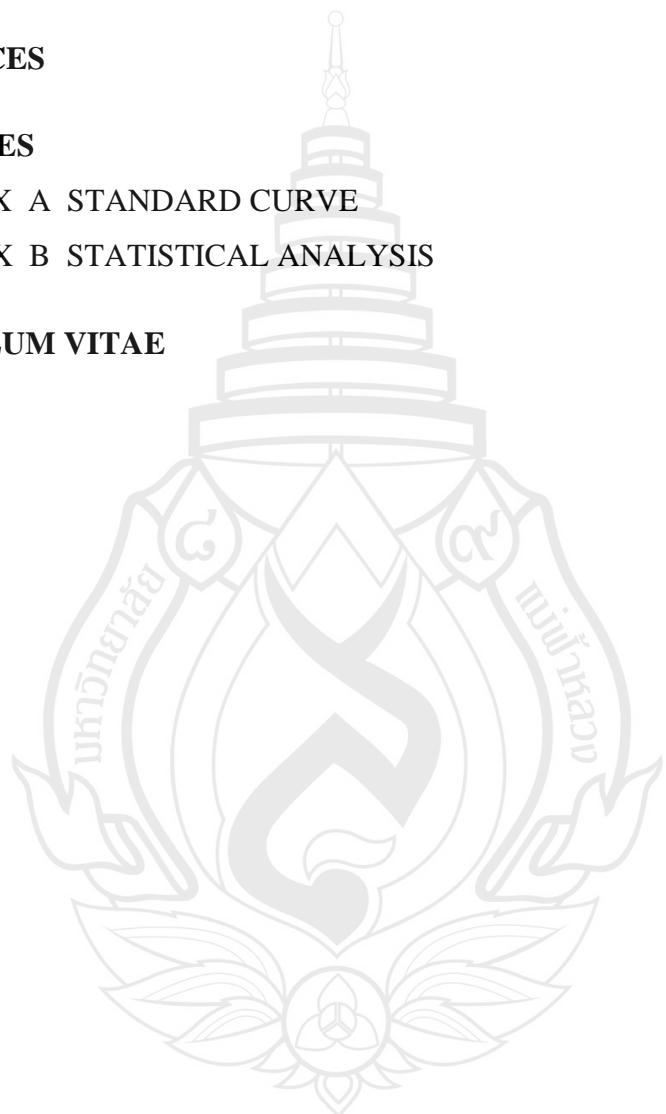
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ABBREVIATIONS AND SYMBOLS

°C	Degree Celsius
µL	Microliters
µW	Microwatt
xg	The gravitational acceleration that is standard at the Earth's surface
AA	Ascorbic Acid
ACC	Available Chlorine Content
AEW	Acidic Electrolyzed Water
AF	After Treatment
ATP	Adenosine Tri-Phosphate
BF	Before Treatment
CTAB	Cetyltrimethyl ammonium bromide
cv.	Cultivar
DNA	Deoxyribonucleic acid
DPPH	2,3-Diphenyl-1-picrylhydrazyl
DV	Daily Value
DW	Dry Weight
Eq.	Equation
FRAP	Ferric Reducing Antioxidant Power
FW	Fresh Weight
g	Gram
GAE	Gallic Acid Equivalents
GI	Geographical Indications
h	Hour
IAA	Indole acetic acid
IB	Internal Browning

ABBREVIATIONS AND SYMBOLS (continued)

in	Inch
ITS	Internal transcribed spacer
JA	Jasmonic Acid
kg	Kilogram
kJ	Kilojoule
L	Liter
M	Molar
m^2	Meter square
MA-AA	Metaphosphoric Acid-Acetic Acid
mg	Milligrams
MHz	Megahertz
mL	Millilitre
mM	Millimolar
mV	Millivolt
N	Normality
nm	Nanometres
ORP	Oxidation-Reduction Potential
PDA	Potato Dextrose Agar
ppm	Parts Per Million
RCF	Relative Centrifugal Force
rpm	revolutions per minute
sec.	Seconds
TA	Titratable Acidity
TBRC	Thailand Bioresource Research Centre
TPC	Total Phenolic Content
TSS	Total Soluble Solid

CHAPTER 1

INTRODUCTION

1.1 Background

According to Villacís-Chiriboga et al. (2020), people consume fruits for their health benefits, taste, and personal preferences. Tropical fruit consumption is growing in both domestic and international markets, owing to increase awareness of their nutritional and medical benefits (Bhat & Paliyath, 2016). However, tropical fruits are extremely sensitive to both qualitative and quantitative losses, particularly sensory, microbiological, and nutritional losses. Food and Agriculture Organization of the United Nations (FAO), (2022a) estimated that the postharvest sector accounts for 9-49% of global fruit and vegetables losses.

Pineapple (*Ananas comosus* L. Merr.) is one of the most important fruits cultivated in tropical and sub-tropical areas. It is a member of the bromeliaceae family. The six leading pineapple producing countries are Costa Rica, Indonesia, Philippines, Brazil, China (mainland) and Thailand (FAOSTAT, 2021).

Pineapple cv. Phulae is originated in Chiang Rai Province, Thailand. Because of its small size, thick skin, and crispy texture, this pineapple has become popular for both domestic and export consumption (Kongsuwan et al., 2009). However, postharvest losses of 'Phulae' pineapple have been a problem for export due to growing of mould on the fruit surface and cutting stem during storage at commercial storage temperature (10–13 °C) (Figure 1.1). Despite the fact that the surface mould on pineapple does not itself cause disease, it does make the fruit un-marketable. Fungicides have been used in conventional control measures, but consumers are more concerned about chemical residues' potential health effects. Heat treatments are among the alternative control strategies that would diminish the fruit quality, hence

non-thermal treatments have been recommended as a technique to extend shelf life. Acidic electrolyzed water (AEW) and ultraviolet-C (UV-C) have drawn a lot of attention recently for their capacity to improve bioactive compounds and to have germicidal effects without harming the environment. A report on employing AEW and UV-C to suppress surface mould on ‘Phulae’ pineapple has not yet been published.

1.2 Objectives

- 1.2.1 To determine the effect of acidic electrolyzed water, ultraviolet-C and combination of both treatments on reducing surface mould incidence on ‘Phulae’ pineapple fruit.
- 1.2.2 To investigate the effect of AEW and UV-C treatments on quality attributes of ‘Phulae’ pineapple fruit.

1.3 Scope of Research

1.3.1 Effect of AEW Treatment on Mould Incidence and Mould Severity in ‘Phulae’ Pineapple

Green mature pineapple fruit were dipped in AEW at various free chlorine concentrations (untreated control, 100 ppm, 200 ppm and 300 ppm) at ambient temperature (25 °C) for 10 min. Then, the treated fruits were put in plastic basket, air-dried with a fan, placed in a corrugated box and stored at 13 °C, 85–90% RH for 8 days. Mould incidence and mould severity were observed every 7-day intervals. Major mould grew on the de-crowned pineapple fruit was isolated and subculture to receive a pure culture before sending to Thailand Bioresource Research Centre (TBRC), Bangkok for identification.

1.3.2 Effect of UV-C Treatment on Mould Incidence and Mould Severity in ‘Phulae’ Pineapple

Green mature pineapple fruit were irradiated in UV-C chamber at various doses (untreated control, 13.2 kJ/m², 26.4 kJ/m² and 39.6 kJ/m²). The treated fruits were placed in a corrugated box and stored at 13 °C, 85–90% RH for 8 days. Mould incidence and mould severity were observed every 7-day intervals.

1.3.3 Effects of AEW and UV-C Treatments on Surface Mould and Quality of ‘Phulae’ Pineapple

1.3.3.1 *In vivo* study

The most suitable treatment results from the experiment (I) (AEW 300 ppm) and the experiment (II) (UV-C 13.2 and 39.6 kJ/m²) used in this investigation as untreated control, AEW (300 ppm), UV-C (13.2 kJ/m²), UV-C (39.6 kJ/m²), AEW (300 ppm) + UV-C (13.2 kJ/m²) and AEW (300 ppm) + UV-C (39.6 kJ/m²). And the treated fruits were stored at 13 °C for 28 days. The assessments were included mould incidence, mould severity, postharvest quality of fruit (weight loss, moisture content, dry matter, peel colour, internal browning, total soluble solids and titratable acidity), bioactive compounds and antioxidants (vitamin C, total phenolic contents, total flavonoids, DPPH, and FRAP).

1.3.3.2 *In vitro* study

The treatments were used the same as in the section 1.3.3.1. The mould of pure culture from the section 1.3.1 was used in spore survival, spore germination ratio, germ tube length and mycelium growth inhibition.

1.4 Expected Outputs

Combination of AEW and UV-C could inhibit the incidence of surface mould in ‘Phulae’ pineapple and maintain fruit quality.

1.5 Expected Outcomes

The current work is prepared to treat harvested 'Phulae' pineapple fruit with AEW and hormic doses of UV-C light. It is hypothesized that metabolism phenomenon of stressed fruits enhances active to cope with the stress accelerate to the generation of different secondary metabolites. This change is hoped for having consequences on controlling surface mould, ripening behaviour and physico-chemical quality of treated produce.

1.6 Research Location

Postharvest technology laboratory, Scientific and Technological Instruments Centre (S7-B), Mae Fah Luang University, Chiang Rai, Thailand.

CHAPTER 2

LITERATURE REVIEW

2.1 Pineapple

Pineapples (*Ananas comosus* (L.) Merr.) stand as the third most-produced tropical fruit on a global scale, surpassing 28 million tons in 2020 (FAO, 2022b). Leading the charge pineapple production in 2022 were Indonesia, Philippines, Costa Rica, Brazil and China Table 2.1. The world's most pineapple importing country was United States of America with the value of 1,241,422 tonnes in 2022. The top three exporting countries in 2022 are Costa Rica, Philippines and Netherlands (FAOSTAT, 2022). Growers navigate through challenges like fluctuating prices, unpredictable weather patterns, and the ongoing imperative to optimize yields while adhering to sustainable practices (Assumi et al., 2021).

Table 2.1 The world's top five pineapple producing countries in 2022

Ranking	Countries	Tonnes
1	Indonesia	3,203,775
2	Philippines	2,914,425
3	Costa Rica	2,909,750
4	Brazil	2,337,302
5	China, mainland	1,960,000

Source FAOSTAT (2022)

Pineapples, being a natural source of vitamin C, antioxidants, and dietary fibre, resonate with health-conscious consumers seeking wholesome alternatives (Ferreira et al., 2016).

In Thailand, pineapple is one of the major crops among durian, rambutan, mango, mangosteen, papaya, guavas, coconut, orange and bananas (Somsri, 2014). Thailand is seventh ranking in pineapple producing country in 2020 (FAOSTAT, 2021). The most grown pineapple cultivars are ‘Tradsithong’, ‘Phuket’, ‘Sawee’, ‘Thainan’, ‘Nanglae’ and ‘Phulae’. Among them, ‘Nanglae’ and ‘Phulae’ are the main growing variety in Chiang Rai province. The ‘Phulae’ is an economically important and Geographical Indication (GI) crop of Chiang Rai province. It belongs to Queen variety and has small size with 150-1000 grams. The peel is rather thick and suitable for long distance transportation than ‘Nanglae’ (Kongsuwan et al., 2009). The skin colour is yellow or greenish yellow when ripen whereas the flesh is light yellow. The core was edible with crispy texture. It was popular for both local consumption and export trading (Kongsuwan et al., 2009). The yields of ‘Phulae’ and ‘Nanglae’ pineapple from Chiang Rai province in 2017 were 31,110 and 4,443 tons respectively (Table 2.2). Most of the fresh fruit is exported to China via Loas, which takes a substantial amount of time due to insufficient infrastructure and transportation services (Meechaiyo & Guo, 2019).

Table 2.2 Pineapple plantation data in Chiang Rai Province in 2017

Items	Cultivar	
	Phulae	Nanglae
Planting Area (rai)	18,760	3,210
Harvested Area (rai)	10,370	1,481
Productivity (tons)	31,110	4,443

Source DOAE (2017)

During long transportation or storage, mould was developed on pineapple fruit. Saprophytes, such as *Ceratocystis parpdoxa*, *Penicillium* sp., grown on the broken end of peduncle or the cut end of de-crowned, causing a marketing difficulty (Figure 2.1). This characteristic is more common in exceptionally translucent fruit (Rohrbach, 1989).



Figure 2.1 Mould growth found on de-crowned of 'Phulæ' pineapple fruits after 28 days of storage at 13 °C

Maintaining a cold storage room at 7-13°C with 90-95% relative humidity is crucial during the distribution of fresh pineapples to prevent issues like chilling injury, internal browning, and flesh translucency. High humidity helps preserve pineapple quality, as fruits contain 90-95% water, and preventing a 3-10% water loss is essential to avoid rendering produce unsalable. Control of water loss, influenced by internal and external factors, is vital for prolonging shelf life and maintaining quality (El-Ramady et al., 2015). Lower humidity can lead to increased water loss, affecting appearance and causing shrinkage, while excessive humidity promotes microbial growth. Exposure to extremely high temperatures above 35°C should be avoided, as it increases weight loss and causes dryness (Hu et al., 2012).

Pineapple transportation for domestic use often utilizes road vehicles due to their convenience, availability, flexibility, and reasonable cost. Road transportation for fresh produce is a growing trend globally and may involve pick-up trucks,

enclosed trucks, open trucks, or refrigerated vehicles (ITFN, 2011). In the export market, pineapples are typically stored in refrigerated containers to maintain quality during longer sea transportation. Fruit is packed, palletized, and loaded directly into refrigerated containers for transport (Dawson et al., 2016). It is crucial for the transport system to regulate temperature and humidity to preserve pineapple quality. Refrigerated transportation is necessary for long-distance journeys to slow down the ripening process. Careful packing is essential to minimize physical damage to the produce, considering container type and size (De La et al., 2005).

Among pathogen, the black rot's (*Thielaviopsis paradoxa*) conidia, found in soil and plant debris, thrive in pineapple and sugarcane growing areas. This pathogen grows actively between 21-22 °C and is almost inactive below 10 °C. Moisture is crucial for conidia germination. Asexual conidia spread through rain splashing, causing infection before harvest via insect punctures, growth cracks, or natural crevices. As a wound pathogen, the fungus enters through cut peduncle stems or handling wounds (Agrios, 2004).

Researchers and growers have deployed a diverse arsenal of control methods for controlling the mould growth, each with its own strengths and limitations. Historically, fungicides have been the primary means of combating postharvest mould on pineapples. Benomyl, thiabendazole, and imazalil were once the go-to options, effectively preventing fungal growth and extending the fruit's shelf life (Rohrbach & Phillips, 1990). However, concerns regarding their toxicity, exceeding permissible residue limits, and the emergence of fungal resistance prompted restrictions and bans in certain regions. In the pursuit of safer and more efficient fungicides, new options have surfaced. Propiconazole and fludioxonil have shown promise as potential replacements, proving effective against a wide range of moulds while demonstrating lower toxicity to humans and the environment. Nonetheless, the continuous resistance on fungicides poses the risk of resistance development, emphasizing the need for responsible rotation and integrated pest management practices (Assumi et al., 2021).

Alternative options by using physical and chemical treatments have been applied. The vapour heat treatment at 52.5 and 53 °C for 10 min found effectively control the germination and conidia growth of *Thielaviopsis paradoxa* (Ames, 1915). The findings from research carried out in Sri Lanka revealed that subjecting the

trimmed peduncle of pineapples to a 3-minute hot water dip at 54 °C proved to be an effective method in managing the occurrence of black rot but it is important to ensure that the water in treatment tanks is replaced before the spore count of the pathogen reaches critical levels (Wijeratnam et al., 2005).

Subjecting pineapples to controlled doses of UV light can deactivate mould spores and hinder their germination, resulting in prolonged shelf life and reduced mould growth (Sari et al., 2016).

Utilizing coatings derived from natural substances such as chitosan establishes a physical barrier, preventing mould spores from reaching the pineapple's surface (Basumatary et al., 2022). These coatings can be augmented with natural antifungal compounds to enhance their effectiveness.

Subjecting pineapples to controlled amounts of ozonated water has displayed potential in impeding mould growth and prolonging shelf life, though additional research is required to optimize this approach (Nur-Aida et al., 2011).

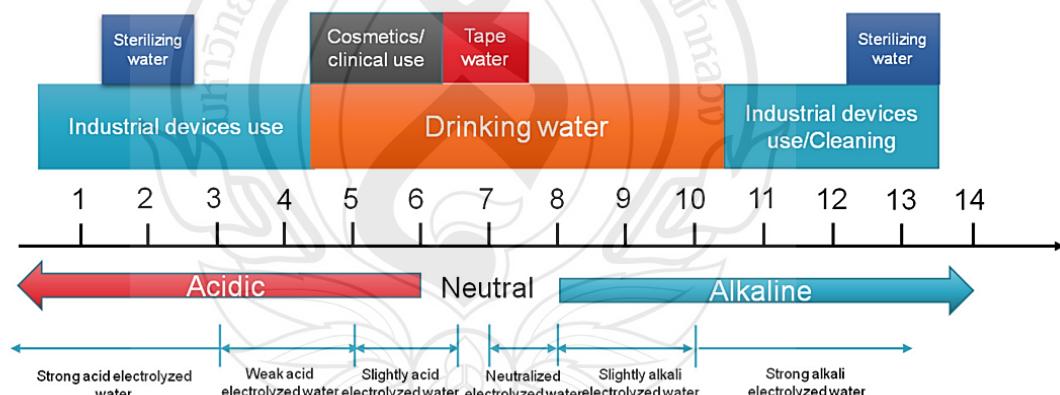
Although these treatments could control the mould incidence, the disadvantages have been observed such as chemical residues, pathogen's resistance, fruit quality losses due to physical methods, limited effectiveness in commercial scales and high cost per unit. Therefore, acidic electrolyzed water (AEW) and ultraviolet-C (UV-C) irradiation has been introduced as green sanitizers to control mould growth on produce.

2.2 Acidic Electrolyzed Water (AEW)

Electrolyzed water (EW) is created by mixing tap water with common salt solution (sodium chloride, NaCl) within an electrolysis device. Potassium chloride (KCl) or magnesium chloride (MgCl₂) can also be used instead of NaCl as its availability in the market (Al-Haq et al., 2005). Among the parts of this devices, the electrolysis chamber is the principal portion where EW is created. This chamber contains negative and positive electrodes where electric current is connected and may or may not have a separating membrane (Hricova et al., 2008; Huang et al., 2008; Rahman et al., 2016). The device settings are primarily the amount of salt present, the

amperage and voltage level, the time of electrolysis, and the water flow rate (Hsu, 2003). This phenomenon converts the salt into an activated situation and high chemical reactivity and longer shelf stability up to 3 months when kept in closed containers (Thorn et al., 2012).

Electrolyzed water is a chemical product with three major physico-chemical properties: available chlorine concentration (ACC), oxidation-reduction potential (ORP) (>1000 mV) and low pH (<2.7) (Nan et al., 2010). Their properties can be varied rely on the electrolysis instruments and the device settings. ACC will be governed by hypochlorous acid (HOCl) species, hypochlorite ion (OCl^-), and/or chlorine gas (Cl_2). EOW chlorine concentrations can also be called free chlorine, free available chlorine (FAC) or available chlorine concentration (ACC; mg/L or ppm). Different available chlorine concentrations are studied by many researchers including 2-5 ppm (Nan et al., 2010; Rahman et al., 2010; Wang et al., 2011), 300 ppm (Guentzel et al., 2008; Yang et al., 2003) and 10–100 ppm ACC (Villarreal-Barajas et al., 2022).



Source Yan, Daliri et al. (2021)

Figure 2.2 Application of electrolyzed water at different pH values in various fields

Four types of EOW are divided by its pH value: acidic electrolyzed water (AEW), slightly acid electrolyzed water (SAEW) or near NEW, neutral electrolyzed

water (NEW), and alkaline electrolyzed water (AEW). AEW is broadly explored for its physico-chemical properties; low pH <3.0, high ORP >1000 mV, and high chlorine species such as 85% HOCl, 15% Cl₂ and traces of OCl^- , hydrochloric acid (HCl), and oxygen gas (O₂) (Hsu, 2005). Figure 2.1 shows the application of electrolyzed water (EW) in different sectors at different pH values.

Investigators recommended that AEW ingredients such as pH (McPherson, 1993), chlorine compound (Liu et al., 2006; Park et al., 2004), ORP (Kim et al., 2000; Liao et al., 2007) or combinations of these factors (Huang et al., 2008) are the main reasons in the antimicrobial activity. The modification of metabolic fluxes and ATP production of the bacteria was happened by the change in electron flow in the cell as AEW with higher ORP (>1000 mV). The outer membrane of bacterial cells was sensitive to permit hypochlorous acid (HOCl) to enter into bacterial cells under the low pH (2.3–2.7) of AEW (McPherson, 1993). The most active of the chlorine compounds was HOCl. It destroys the microbial cell by suppressing glucose oxidation by chlorine-oxidizing sulfhydryl groups of certain enzymes which was the key in carbohydrate metabolism. Various types of microbes have different capacity against cleaning medium. Therefore, understanding the physicochemical of EW is the key for creating suitable EW for different types of microbes (Khalid et al., 2018).

Acidic electrolyzed water (AEW) is a novel antimicrobial agent that is remarkably effective inhibit fungal microorganisms on fresh produces (Table 2.3 and 2.4) and also *in vitro* study (Table 2.5).

Table 2.3 Application of EW against various microorganisms in fruit and vegetables

Item	Microorganisms	EW type	Exposure time(min)	ACC (ppm)	pH	ORP (mV)	References
Spinach leaves	<i>E. coli</i> , <i>Salmonella Enteritidis</i> and <i>Listeria</i>	NEW	1	50	7.0	857	(Ogunniyi et al., 2021)
Coriander	<i>E. coli</i> and <i>Salmonella</i>	SAEW	5	20, 40, 60	5.0- 6.0	-	(Jiang et al., 2020a)
Fresh-cut eggplant	Aerobic bacteria, yeast and mould	AEW/ SAEW	5	51/ 31	2.34/ 6.25	1170/ 861	(Li et al., 2020)
Lettuce	<i>Salmonella</i>	SAEW	0.75	50	6.38	872- 885	(Cap et al., 2020)
Cherry tomato/ Strawberry	Aerobic bacteria, yeast and mould	SAEW	10	34.33	6.49	853.7	(Ding et al., 2015)
Lettuce	<i>E. coli</i> and <i>I. innocua</i>	AEW	7	4	3.84	920.5	(Zhao et al., 2019)
Fresh-cut apple	Yeasts	AEW/ NEW	5	102/ 101	2.87/ 7.95	1113/ 757	(Graça et al., 2020)
Black olives	Aerobic bacteria, yeast and mould	AEW	1	80	2.7	1100	(Gök & Pazır, 2020)
Apples, cherry tomato, mandarins	<i>E. coli</i> O157:H7 and <i>Listeria monocytogenes</i>	SAEW	3	30	5.42	818	(Chen et al., 2019)
Minimally processed mangos	<i>E. coli</i> and <i>Cronobacter sakazaki</i>	AEW/ NEW	5	102/ 101	2.87/ 7.95	1113/ 757	(Santo et al., 2018)
Apple and tomato	<i>E. coli</i> O157:H7 and <i>Listeria monocytogenes</i>	SAEW	3	30	5.42	854	(Tango et al., 2017)
Fresh-cut bell peppers	<i>Listeria monocytogenes</i> sp.	SAEW	2	/	/	/	(Saravanakumar et al., 2021)
Fresh-cut pears	<i>E. coli</i> , <i>S. enterica</i> and <i>Listeria spp</i>	AEW/ NEW	3	99/ 102	2.9/ 8.2	1121/ 754	(Graça et al., 2017)
Carrot	<i>E. coli</i> and <i>Salmonella</i>	SAEW	3	4	4.02	956	(Liu et al., 2019)
Spinach, beet leaf and iceberg lettuce	<i>Bacillus cereus</i>	SAEW	15	80	5.74	832	(Hussain et al., 2019)
Avocado	<i>Collectotrichum spp</i>	NEW	0.5	/	8.2	906	(Hassan & Dann, 2019)
Strawberry	<i>Collectotrichum fructicola</i>	NEW	0.2	10	6.5- 7.5	/	(Hirayama et al., 2016a)
Tomato	<i>Fusarium oxysporum</i> , <i>Galactomyces geotrichum</i> and <i>Alternaria sp.</i>	NEW	3	60	/	/	(Vásquez-López et al., 2016)

Source Zhang et al. (2021)

Table 2.4 Application of EW against various fungal microorganisms on fresh produce

Fruit/ Veget- able	Fungi	EW properties			Expo- sure time (min)	Stor- age Condi- tion	Re- duction/ Disease control	References
		EOW type	pH	ACC (ppm)	ORP (mV)			
Tomato	<i>Alternaria sp.</i>	NEW	7.0	10	850	3,5,10	22 ° C, 8 d	30-40 % 20-40 % 50-60 %
				30				
				60				
Tomato	<i>Fusarium oxysporum</i>			10		10	20-50 % 40-60 % 60-70 %	(Vásquez- López et al., 2016)
				30				
				60				
Tomato	<i>Galactomyces geotrichum</i>			10		10	40-50 % 10-50 % 60-70 %	(Guentzel et al., 2010)
				30				
				60				
Grapes	<i>Botrytis cinerea</i>	NEW	6.3- 6.5	250	800- 900	10	25 ° C, 28 d	20 % 1.7 log CFU/g
				32	1130			
Straw- berry	<i>Fungi</i>	AEW	2.6	150	1170	10	NA	1.7 log CFU/g
Pear	<i>Botryosphaeria berengeriana</i>	AEW	2.6	180	940	1,5,10	20 ° C, 7 d	55 %
Peach	<i>Monilinia fructicola</i>	NEW AEW SAEW	6.8 3 5.8	220	1165	30 2,5,10	20 ° C, 6 d 20 ° C, 10 d	5-30 % 20-80 % 70-80 %
				270	990			
				300				
Pineapple	<i>Fusarium sp.</i>	AEW	NA	100	NA	10	13 ° C, 20 d	60 % at day 20
				200				
				300				
Pineapple	<i>Fusarium sp.</i>	AEW	NA	100	NA	10	28 ° C, 7 d	68.75 % at day 3
				200				
				300				
Apple	<i>Penicillium expansum</i>	SAEW	3 5.1	80	1154	5	25 ° C, 6 d	0 % Laborde, 2004)
				36	927			
Tangerine	<i>P. digitatum</i>	AEW	3.9	102	NA	4,8,16	5 ° C, 18 d	80 % (Whangchai et al., 2010)
				1490				
Longan	<i>Phomopsis longanase and Lasiodiplodia theobromae</i>	AEW	2.5	80	1490	10	25 ° C, 6 d	22% (Chen et al., 2020)
				230	NA			
Broccoli sprouts	<i>Yeast and moulds</i>	AEW	3.6	NA	Dip every 2 s for 60 s	NA	1 log CFU/g	(Puligundla et al., 2018)

Source Villarreal-Barajas et al. (2022)

Table 2.5 Application of EW against various fungal microorganisms in suspension

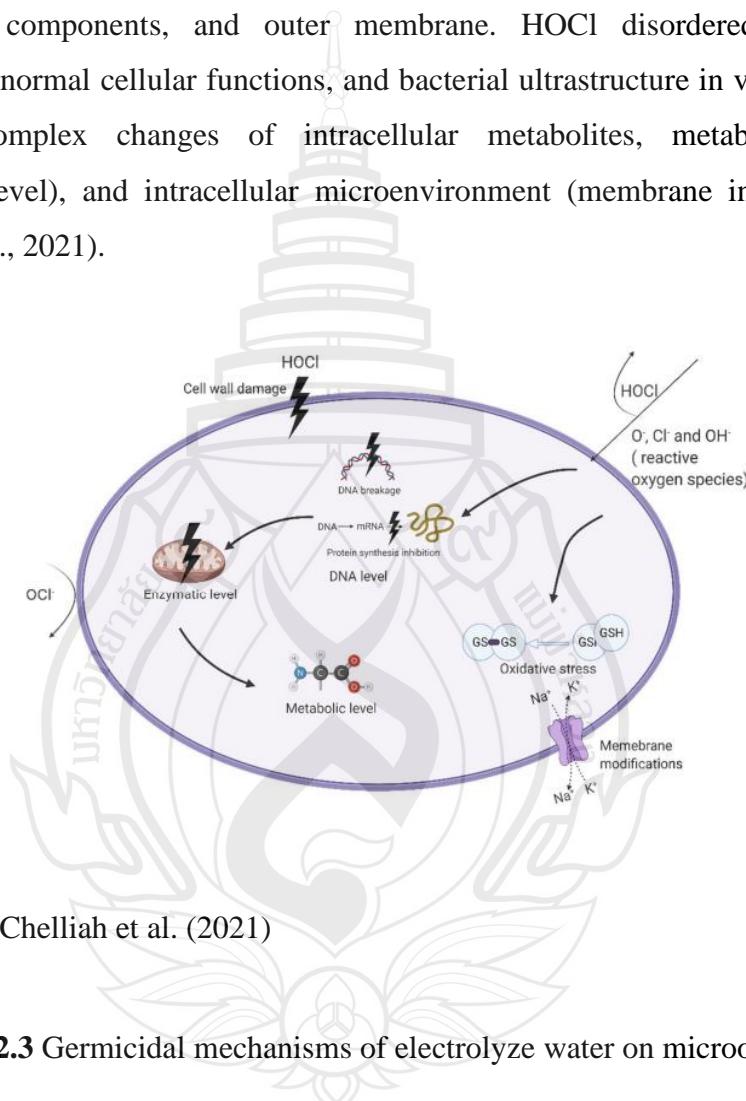
Fungi	EW properties				Exposure time (min)	Results	References
	EOW type	pH	ACC (ppm)	ORP (mV)			
<i>Alternaria sp.</i>	AEW	2.8	54-56	1071-1079	0.5-2	100% inhibition	(Buck et al., 2002)
<i>A. panax</i>	AEW	2.8	54-56	1071-1079	0.5-2	100% inhibition	
<i>Aspergillus brasiliensis</i>	AEW	2.6, 2.7	60, 85, 121	1147, 1120, 1188	15	1-1.9 log CFU/g reduction at 85 ppm	(Lemos et al., 2020)
<i>A. carbonarius</i>	AEW	11.2, 11.1, 11	NA	-869, -209, -909	15	1-1.9 log CFU/g reduction at -869 mV	
	AEW	2.6, 2.7	60, 85, 121	1147, 1120, 1188	15	1-1.9 log CFU/g reduction at 85 ppm	
<i>A. flavus</i>	AEW	2.8	54-56	1071-1079	0.5-2	100% inhibition	(Buck et al., 2002)
<i>A. flavus</i>	AEW	2.7	30	1087	1	1.48 log conidia/mL reduction	(Xiong et al., 2010)
	NEW	5.2	30	850	1	Not detectable	
	AEW	2.2	30, 60, 90	1146	0.5-2	Not detectable at 60 and 90 ppm	
<i>A. niger</i>	AEW	2.8	54-56	1071-1079	0.5-2	100% inhibition	(Buck et al., 2002)
<i>A. nomius</i>	AEW	2.6-2.7	60, 85, 121	1147, 1120, 1188	15	1-1.9 log CFU/g reduction at 60 ppm	(Lemos et al., 2020)
<i>A. ochraceus</i>	AEW	2.6-2.7	60, 85, 121	1147, 1120, 1188	15	1-1.9 log CFU/g reduction at 85 ppm	
<i>A. parasiticus</i>	AEW	2.5	20-30	1164	15	100% inhibition	(Suzuki et al., 2002)
	AEW	11.6	0.51	-878	15	0% inhibition	
<i>Botrytis allii</i>	AEW	2.8	54-56	1071-1079	0.5-2	100% inhibition	(Buck et al., 2002)
<i>B. cinerea</i>	NEW	7.0	3,5,6,8,18,24, 27,29,36,43	862	5	100% inhibition at 18 ppm	(Gómez Jaimes et al., 2017)
<i>Candida albicans</i>	AEW	2.9, 2.83, 2.77, 2.63	32.6, 42, 53.2, 65.5, 76	1050, 1076, 1090, 1100, 1125	0.5-2	100% inhibition at 65.5 ppm	(Zeng et al., 2011)
<i>Cladosporium sp.</i>	AEW	2.8	54-56	1071-1079	0.5-2	100% inhibition	(Buck et al., 2002)
<i>Colletotrichum acutatum</i>	SAEW	5-6.5	20-30	NA	10 sec, 1, 3	100% inhibition at 10 sec	(Song et al., 2013a)
<i>Colletotrichum sp.</i>	AEW	2.8	54-56	1071-1079	0.5-2	100% inhibition	(Buck et al., 2002)
<i>Colletotrichum gloeosporioides</i>	NEW	7.0	3,5,6,8,18,24, 27,29,36,43	862	5	100% inhibition at 6 ppm	(Gómez Jaimes et al., 2017)
<i>Colletotrichum fructicola</i>	NEW	6.5-7.5	1.8, 3.6, 7, 10	NA	0.16-1	100% inhibition at 10 ppm	(Hirayama et al., 2016)
<i>Fusarium sp.</i>	AEW	NA	100, 200, 300	NA	10, 30, 60	100% inhibition at 100-300 ppm	(Khayankarn et al., 2013)
<i>Fusarium sp.</i>	AEW	NA	100, 200, 300	NA	10	Mycelial growth diameter inhibited at 300 ppm	(Whangchai et al., 2017)

Table 2.5 (continued)

Fungi	EW properties				Exposure time (min)	Results	References
	EOW type	pH	ACC (ppm)	ORP (mV)			
<i>F. culmorum</i>	NEW	7.2	12.5, 25, 100, 200	889	4, 24, 48 (h)	100% inhibition at 25-200 ppm	(Audenaert et al., 2012)
<i>F. graminearum</i>						100% inhibition at 25-200 ppm	
<i>F. sporotrichoides</i>						100% inhibition at 25-200 ppm	
<i>F. moniliforme</i>	AEW	2.8	54-56	1071-1079	0.5-2	100% inhibition	(Buck et al., 2002)
<i>F. oxysporum</i>	AEW	2.3-2.6	27-35	1007-1025	0.5, 2, 5, 10	100% inhibition	(Abbas & Lazarovits, 2006)
<i>F. poae</i>	NEW	7.2	12.5, 25, 100, 200	889	4, 24, 48 (h)	100% inhibition at 25-200 ppm	(Audenaert et al., 2012)
<i>F. solani</i>	NEW	7.0	3.5,6,8,18,24, 27,29,36,43	862	5	100% inhibition at 6 ppm	(Gómez Jaimes et al., 2017)
<i>Helminthosporium sp. 1</i>	AEW	2.8	54-56	1071-1079	0.5-2	81-100% inhibition	(Buck et al., 2002)
<i>Monilinia fructicola</i>						100% inhibition	
<i>M. fructicola</i>	NEW	7.0	3.5,6,8,18,24, 27,29,36,43	862	5	100% inhibition at 8 ppm	(Gómez Jaimes et al., 2017)
<i>Phytophthora capsici</i>	SAEW	5-6.5	20-30	NA	10 sec, 1, 3	100% inactivation at 10 sec	(Song et al., 2013b)
<i>P. parasitica</i>	SAEW	6.0	30, 60, 90	922	0.5-5	>50% inhibition at 90 ppm	(Hou et al., 2012)
	AEW	2.3		1187		>50% inhibition at 90 ppm	
<i>Penicillium digitatum</i>	AEW	3.9	5, 25, 51, 102	NA	0,2,4,8, 16,32		(Whangchai et al., 2010)
<i>P. digitatum</i>	NEW	7.0	3.5,6,8,18,24, 27,29,36,43	862	5	100% inhibition at 8 ppm	(Gómez Jaimes et al., 2017)
<i>P. expansum</i>	SAEW	5,6,6.5	10.1,33.5,50	851, 895, 968	0.25-5	2.73 log CFU/mL reduction at 10.1 ppm	(Okull & Laborde, 2004)
	AEW	3.1	59.6	1133	0.25-5	4.62 log CFU/mL reduction	(Okull et al., 2006)
<i>P. expansum</i>	AEW	3.5	18	1027	0.5-5	Not detectable	
<i>P. hirsutum</i>	SAEW	5-6.5	20-30	NA	10 sec, 1, 3	100% inactivation at 10 sec	(Song et al., 2013)
<i>Penicillium sp.</i>	NEW	7.0	3.5,6,8,18,24, 27,29,36,43	862	5	100% inhibition at 8 ppm	(Gómez Jaimes et al., 2017)
<i>Thielaviopsis basicola</i>	AEW	2.8	54-56	1071-1079	0.5-2	100% inhibition	(Buck et al., 2002)

Source Villarreal-Barajas et al. (2022)

As for applying only tap water and NaCl solution, AEW can be recognized as environmental-friendly sanitizer (Wang et al., 2015). Figure 2.2 shows the germicidal mechanisms of EW on microorganisms. HOCl can penetrate the cell membrane's lipid bilayer while ClO^- , Cl^- and OH^- can attach only on the cell wall and outer membrane of the cell. EW attacks various cellular targets including cytoderm, intracellular components, and outer membrane. HOCl disordered intracellular components, normal cellular functions, and bacterial ultrastructure in various degrees including complex changes of intracellular metabolites, metabolic pathway (enzymatic level), and intracellular microenvironment (membrane integrity) (Yan, Chelliah et al., 2021).



Source Yan, Chelliah et al. (2021)

Figure 2.3 Germicidal mechanisms of electrolyzed water on microorganisms

2.3 Ultraviolet-C (UV-C)

Ultraviolet-C light is, a non-thermal germicidal treatment, surface decontamination of foods (Food and Drug Administration [FDA], 2002). Bacteria, yeasts and several types of moulds are inactivated by UV-C light within the wavelength 250 nm and 260 nm (Diesler et al., 2019). UV-C light is a non-penetrating type of

electromagnetic emission which limits its utilization just for surface disinfection of food products (Fan et al., 2017). UV-C treatments could be used to suppress mould growth on food surfaces as it was an aerobic group that live on surface of foods. Pathogenic and spoilage microorganisms have been inactivated by UV-C light application (Bintsis et al., 2000). The number of articles examining the efficacy of UV-C treatment for the suppression of mould on food has increased in recent years (Baysal et al., 2013; Caminiti et al., 2012; Green et al., 2011). UV-C inactivation features of each mould responsible for fruit rot must be known in order to minimize or eliminate mould by giving UV-C treatments. Screening and selecting acceptable target microorganisms for UV-C processing of certain food commodities is a critical step in developing new process schedules and evaluating existing ones (Estilo & Gabriel, 2017). Previous studies indicated that UV-C stress on fruits and vegetables enhanced bioactive compounds Table 2.6 and 2.7.

UV-C treatment represents a non-thermal and chemical-free approach that utilizes short-wavelength ultraviolet radiation (200-280 nm) to manage postharvest pathogens and uphold the quality of fruits. This treatment operates through two primary modes of action: direct and indirect.

In the direct mode of action, UV-C treatment deactivates microorganisms by inducing damage to their DNA and other cellular components. UV-C radiation can instigate various disruptions, including single-strand or double-strand breaks, cross-linking, and dimerization of DNA bases, impeding the replication and transcription processes. Furthermore, it affects membrane integrity, enzyme activity, and metabolic functions, leading to the demise or inhibition of microbial growth (Darré et al., 2022). Demonstrating efficacy against a range of postharvest pathogens like fungi, bacteria, and viruses on fruits and vegetables has been observed through UV-C treatment (Table 2.6 and 2.7).

The indirect mode of action involves the elicitation of plant defense responses and the improvement of fruit quality. UV-C treatment stimulates the production of secondary metabolites, such as phenolic compounds, flavonoids, anthocyanins, carotenoids, and ascorbic acid, possessing antioxidant and antimicrobial properties. Additionally, it activates the expression of defense-related genes, including those encoding pathogenesis-related proteins, chitinases, glucanases, and peroxidases,

providing resistance against biotic and abiotic stresses. UV-C treatment also influences the ripening and senescence processes, impacting ethylene production, respiration rate, firmness, colour, and aroma. Consequently, UV-C treatment enhances the nutritional and sensory attributes of fruits and vegetables while extending their shelf life (Table 2.6 and 2.7).

The effectiveness of UV-C treatment depends on various factors, such as the fruit or vegetable type, maturity, cultivar, and physiological state, UV-C dose, intensity, duration, and frequency, spectral region, and radiation exposure pattern. It is crucial to determine the optimal conditions and parameters for UV-C treatment tailored to each specific product and objective to achieve desired effects and prevent potential adverse outcomes, such as tissue damage, browning, or off-flavours (Cantwell & Suslow, 2002).

Table 2.6 Application of UV-C irradiation and effect on produce

Fruit	UV light conditions	Results	References
Mandarin	UV-C 10 kJ/m ²	- Inhibited green mould (<i>Penicillium digitatum</i>) growth. - Maintained the integrity of membrane structure reducing. - Increased jasmonic acid (JA) accumulation, bioactive compounds and antioxidant acitivity (DPPH).	(Phonyiam et al., 2021)
Pineapple cv. Phulae	UV-C 13.2, 26.4, 39.6 kJ/m ²	- Peel's antioxidant capacity and total phenolic compound was increased at 39.6 kJ/m ² but have no effect on the pulp. - The internal browning incidence was reduced at 26.4 kJ/m ² and 39.6 kJ/m ² than the control and 13.2 kJ/m ² . - This research found that disease incidence was lower as the bioactive compounds increasing.	(Sari et al., 2016)
Mango cv. Tommy Atkins	UV-C 0.5, 1, 2.5, 5, 7.5, 10 kJ/m ²	- Inducing favourable biological responses to conservation on the fruits, increasing the internal resistance of fruit tissue as well as preserving the colour and texture.	(Terao et al., 2015)

Table 2.7 Application of UV-C treatment in fruit surface

Fruit (Cultivar)	UV light conditions	Results	References
Apple (<i>Malus domestica</i> , cv. Red Delicious)	UV-C $\lambda= 254$ nm 7.5 kJ/m ²	- The earliest application of UV treatment (96 h) before inoculating with <i>Penicillium expansum</i> provided the best defense against disease.	(De Capdeville et al., 2002)
Apple (<i>Malus domestica</i> , cv. Red Delicious)	UV-C = 253.7 nm 1.5-24 mW/cm ²	- Reduction of <i>E. coli</i> O157:H7 with 3.30-log CFU/cm ²	(Yaun et al., 2004)
Blueberry (<i>Vaccinium corymbosum</i> L. cvs. Collins, Bluecrop)	UV-C = 0-4 kJ/m ² Storage 7 d at 5 °C plus 2 d at 20 °C	- Weight loss and firmness were not affected by light treatment. - Decay incidence from ripe rot (<i>Collectrichum acutatum</i> , syn. <i>C. gloeosporioides</i>) on fruit was decreased by 10%. - Antioxidants (measured by total anthocyanin) total phenolics, and ferric reducing antioxidant power (FRAP) increased by treatment.	(Perkins-Veazie et al., 2008)
Blueberry (<i>Vaccinium corymbosum</i> L. cv. Duke)	UV-C = 254 nm 0.43, 2.15, 4.30 and 6.45 kJ/m ²	- Flavonoids levels increased. - Antioxidant capacity was higher in fruit treated with 2.15, 4.30 and 6.45 kJ/m ² .	(Wang et al., 2009)
Cantaloupe melon (<i>Cucumis melo</i> L. cv. reticulatus) sliced	UV 15 and 60 min	- Aliphatic esters decreased 60 %	(Lamikanra et al., 2002)
Cantaloupe melon (<i>Cucumis melo</i> L. cv. reticulatus) fresh cut	UV-C Storage at 10 °C	- Esterase activity found the lowest during storage. - Lipase activity was higher in post-cut treated fruit than fruit processed under UV-C light and control fruit. - Effective reducing yeast, mould and <i>Pseudomonas</i> spp. density.	(Lamikanra et al., 2005)
Grape (<i>Citrus paradisi</i> , cv. Star Ruby)	UV-C $\lambda= 254$ nm 0.5-3.0 kJ/m ²	- Quality and disease resistance determined after storage at 7 °C for 4 weeks followed by 1 week at 20 °C. - Scoparone and scopoletin levels were increased at all UV doses. - Rind browning and tissue necrosis occurred at >1.5 kJ/m ² .	(D'Hallewin et al., 2000)
Grape (<i>Vitis vinifera</i> L. cv. Italia)	UV-C $\lambda= 254$ nm 0.125-4 kJ/m ²	- Grapes irradiated 24-48 h before inoculating with <i>Botrytis cinerea</i> showed a lower disease incidence than inoculated immediately before irradiation. - Doses above 1.0 kJ/m ² caused skin discolouration. - Treatment with the optimum range did not significantly reduce the numbers of epiphytic yeasts that showed antagonism towards pathogenic moulds.	(Nigro et al., 1998)

Table 2.7 (continued)

Fruit (Cultivar)	UV light conditions	Results	References
Grape (<i>Vitis vinifera</i> L.) - table grapes cvs. Thompson Seedless, Autumn Black, Emperor - green grape selection B36-55	UV-C 0.36 J/cm ² 5 min	- Reduction of gray mould incidence (<i>Botrytis cinerea</i>). - UV-C light induced catechin in cv. Autumn Black berries and trans-resveratrol in both cv. Autumn Black and selection B36-55.	(Romanazzi et al., 2006)
Kumquat (<i>Citrus japonica</i> , cv. Nagami)	UV-C = 254 nm 0.2-12 kJ/m ²	- Inactivation of <i>Penicillium digitatum</i> inoculated after UV treatment. Treated fruit found signs of damage after 2 weeks of storage at 17 °C. Damage was absent when fruits were stored at lower temperature.	(Rodov et al., 1992)
Mandarin (<i>Citrus unshiu</i> Marc.)	UV-C 10 min	- UV-light treatment reduced green mould but caused some injury to the fruit. The disease incidence was very low when stored at 30 °C, 90-95% RH for 72 h.	(Kinay et al., 2005)
Satsuma			
Mango (<i>Mangifera indica</i> cv. Tommy Atkins)	UV-C $\lambda=$ 254 nm 4.9 and 9.9 kJ/m ²	- Quality and disease resistance determined after storage at 5 °C for 14 days followed by 7 days at 20 °C. The treatment at 4.9 kJ/m ² improved fruit appearance and texture while 9.9 kJ/m ² induced senescence.	(González-Aguilar et al., 2001)
Oranges (<i>Citrus sinensis</i> cv. Bionodo Comune, Washington Navel, Tarocco, Valencia Late)	UV-C $\lambda=$ 254 nm 0.5-3.0 kJ/m ²	- Quality and disease resistance determined after storage at 7°C for 4 weeks followed by 1 week at 20 °C. - Peel quality was affected in all cultivars except Valencia L. - Percentage of damaged fruit at the higher dosages decreased as the season progressed. - 0.5 kJ/m ² was effective in reducing decay development while 1.5 kJ/m ² was more effective but only in early harvested fruit.	(D'hallewin et al., 1999)
Peach (<i>Prunus persica</i> , cv. Elberta)	UV-C $\lambda=$ 254 nm 0.4-40 kJ/m ²	- Exposure to UV delayed ripening, suppressed ethylene production and increased phenylalanine ammonia-lyase (PAL) activity. Inactivation of <i>Monilinia fructicola</i> inoculated after treatment. 40 kJ/m ² increased susceptibility to brown rot. Increased number of the antagonist yeast <i>Debaryomyces hansenii</i> on the surface of the fruit.	(Stevens et al., 1998)
Peach (<i>Prunus persica</i> L. Batsch cv. Loring)	UV-C $\lambda=$ 254 nm 7.6 kJ/m ² 10 min	- Treatment caused a rapid induction of enzymes activities: chininase, β -1,3-glucanase, and PAL starting 6 h after treatment and reaching maximum levels at 96 h after treatment.	(El Ghaouth et al., 2003)

Table 2.7 (continued)

Fruit (Cultivar)	UV light conditions	Results	References
Pear (<i>Pyrus communis</i> L.)	UV-C = 253.7 nm 0.87 kJ/m ²	- Reduction of different strains (<i>L. innocua</i> ATCC 33090, <i>L. monocytogenes</i> ATCC 19114D, <i>E. coli</i> ATCC 11299, <i>Z. bailii</i> NRRL 7256) with 2.6-3.4-log	(Schenk et al., 2008)
Fresh-cut pear-slices without peel	0-20 min		
Pear (<i>Pyrus communis</i> L.)	UV-C = 253.7 nm 0.87 kJ/m ²	- Reduction with 1.8-2.5-log of cocktail strains of: Listeria, <i>L. innocua</i> CIP 8011, <i>L. welshimeri</i> BE 313/01, <i>L. monocytogenes</i> (ATCC 19114, ATCC 33090), and yeasts: <i>Z. bailii</i> NRRL 7256, <i>Z. rouxii</i> ATCC 52519, <i>D. hansenii</i> NRRL 7268.	(Schenk et al., 2008)
Fresh-cut pear-slices with peel	0-20 min		
Persimmon fruit (<i>Diospyros kaki</i> Thunb. cv. Karaj)	UV-C 1.5, 3 kJ/m ² Storage 0-4 month at 1 °C	- Disease incidence was reduced without important effect on fruit attributes (firmness, ethylene production and skin colour)	(Khademi et al., 2013)
Pineapple (Ananas comosus L.) fresh-cut	UV-C for 15 min Kept 24 h at 4 °C	- UV produced a considerable decrease in the ester's concentration and increase in the relative amount of copaene.	(Lamikanra & Richard, 2004)
Pomegranate (<i>Punica granatum</i> , cv. Mollar of Elche)	UV-C 0.56-13.62 kJ/m ² Kept 15 d at 5 °C	- Respiration rate was not affected. Reduction of mesophilic, psychrotrophic, LAB and enterobacteriacease counts. Yeast and mould were unaffected.	(López-Rubira et al., 2005)
Fresh cut arils			
Strawberries (<i>Fragaria ananassa</i> cv. Kent)	UV-C = 254 nm 0.25 and 1 kJ/m ² Kept at 4 °C and 13 °C	- Treatment controlled the decay caused by <i>Botrytis cinerea</i> at both temperature and extended the shelf-life of the fruits by 4 to 5 d. - Lower rate of senescence found in 0.25 kJ/m ² . - UV-treated fruit found lower respiration rate, higher titratable acidity and anthocyanin content, and were firmer than control. Damage occurred at 1 kJ/m ² .	(Baka et al., 1999)
Strawberry (<i>Fragaria ananassa</i> cv. Elsanta) sepals	UV-C = 254 nm 0.05, 0.50, 1, and 1.5 J/cm ²	- <i>Botrytis cinerea</i> MUCL growth was inhibited significant starting from a dose of 0.05 J/cm ² .	(Lammertyn et al., 2003)
Strawberry (<i>Fragaria ananassa</i> <i>Duch</i>)	UV-C 0.43, 2.15, 4.3 kJ/m ² Storage at 10 °C	- Antioxidant capacity was enhanced after storage for 15 d - Best decay inhibition was found in 2.15 and 4.3 kJ/m ² .	(Erkan et al., 2008)
Strawberry (<i>Fragaria ananassa</i> <i>Duch</i> . cv. Kurdistan)	UV-C = 254 nm 0.25, 0.5 J/cm ² Kept 7 d at 1-5 °C	- Growth of yeast was deceased by all doses. - Fruits treated with 0.5 J/cm ² was firmer on day 7 and this dose improved the sensory quality of the product.	(Darvishi & Davari, 2012)
Sweet cherries (<i>Prunus avium</i>)	UV-C = 254 nm 0.5-15 J/cm ²	- Treatment had no affect either on fungal growth or fruit quality.	(Marquenie et al., 2002)

Source Turtoi (2013)

2.4 Combination of Treatments

Combination of different treatments has been found more effective to inhibit fungi growth than single treatment alone (Table 2.8).

Table 2.8 Application of combined treatments in fruit surface

Fruit	Treatments	Results	References
Pineapple cv. Phulae	Electrolyzed Water (100, 200, 300 ppm) + Ultrasound (108, 400, 700 kHz and 1 MHz)	AEW (100 ppm) + Ultrasond (1 MHz) significantly reduced decay caused by <i>Fusarium</i> sp. without effect on fruit qualities.	(Khayankarn et al., 2013)
Pineapple	Electrolyzed Water (100ppm) + Megasonic (1 MHz)	- Treatment could enhance phenylalanine ammonia (PAL) and peroxidase (POD) enzymes activity which was important for plant defense response. - Significantly reduced decay caused by <i>Fusarium</i> sp. without effecting on fruit qualities.	(Khayankarn et al., 2014)
Date palm	UV-C (2.37, 6.22, 8.29, 12.14 kJ/m ²), NEW 100ppm + 6.22 kJ/m ² , AIEW 1.83 ppm + 6.22 kJ/m ² , Ozone 0.55 ppm + 6.22 kJ/m ²	- AIEW + 6.22 kJ/m ² found the best effective for controlling microbial proliferation while keeping overall quality and prolonging shelf-life.	(Jemni et al., 2014)
Strawberry	Dipped in tape water Dipped in tape water+2 kJ/m ² Dipped AEW+ 2 kJ/m ² Dipped in 2% citric acid+ 2 kJ/m ² Dipped in 0.2% benzoic acid+ 2 kJ/m ² Dipped in 0.2% sorbic acid+ 2 kJ/m ²	- Benzoic acid + UV-C shown lowest decay % among the treatments. - UV-C suppressed water loss, maintained firmness and increased antioxidant activity. - Sorbic acid + UV-C and Citri acid + UV-C extended postharvest life and preserved health beneficial compounds than other treatment.	(Nour et al., 2021)
Cilantro fresh-cut	Tape water 5 min AIEW 5 min AEW 5 min SAEW 5 min ALEW 5 min + AEW 5 min ALEW 2.5 min + AEW 2.5 min AEW 2min+AIEW 2min+AEW 2min	- AIEW + AEW demonstrated superior bactericidal efficacy compared to individual treatments and tap water. - AIEW 5 min + AcEW 5 min resulted in the complete elimination of detectable microbial populations on the produce, surpassing the disinfection abilities of single AEW and slightly SAEW treatments.	(Hao et al., 2015)
Dry persimmon disc	Clove oil (1.56, 3.2 %) UV-C (0.6, 3.6 kJ/m ²) 1.56 % + 0.6 kJ/m ² 3.2 % + 0.6 kJ/m ² 1.56 % + 3.6 kJ/m ² 3.2 % + 3.6 kJ/m ²	- Clove oil (1.56%) and UV-C (3.6 kJ/m ²) combination was found the lowest decay incidence (caused by <i>Rhizopus oryzae</i> , <i>Alternaria tenuissima</i> and <i>Aspergillus niger</i>) and high overall acceptability percent than others during storage at 20 °C for 28 days.	(Vurmaz & Gündüz, 2020)

Table 2.8 (continued)

Fruit	Treatments	Results	References
Kailan- hybrid broccoli	Water NaClO Peroxyacetic acid (PA) Neutral Electrolyzed water (NEW) UV-C (7.5 kJ/m ²) Super-atmospheric O ₂ packaging (HO) PA+UV-C NEW+UV-C PA + HO NEW + HO UV-C + HO PA + UV-C + HO NEW + UV-C + HO	- PA+NEW proved more effective in reducing initial <i>E. coli</i> and <i>S. Enteritidis</i> populations on the produce than the conventional NaClO treatment. - UV-C showed similar effects to NaClO against <i>S. Enteritidis</i> but lower efficacy against <i>E. coli</i> . - PA + UV-C and NEW + UV-C achieved up to 3 log CFU/g reductions for both bacteria. - Throughout a 5 °C shelf life, PA-treated broccoli exhibited the highest reductions, with no increases at 10 °C. - Combinations of UV-C, PA or NEW, with hydrogen peroxide, provided better control than single treatments at both temperatures. Triple combinations of sanitizers did not yield additional benefits.	(Martínez-Hernández et al., 2015)

There has been no published research on the effects of combining of AEW and UV-C on 'Phulae' pineapple for controlling surface mould growth. Thus, the goal of this study was to look into the effects of AEW and UV-C on controlling surface mould on 'Phulae' pineapples, as well as to observe how the fruit quality attributes changed as the treatments were applied.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Materials

Pineapple cv. Phulae with green mature, similar size (300–400 g) was harvested from commercial farm in Chiang Rai Province, Thailand. Harvested fruit was transported within 3 h to Postharvest Technology Laboratory, Mae Fah Luang University. Defect and injury from pest and mechanical damage fruits were rejected. The selected fruit were kept in ambient temperature (25 °C) prior to use.

Table 3.1 Chemicals

Chemical Name	Brand/Source
2,2-Diphenyl-1-picrylhydrazyl (DPPH)	Sigma-Aldrich (St. Louis, MO 63103, USA)
2,6-Dichloroindophenol sodium salt	Ajax Finechem (Taren Point, Australia)
Acetic Acid Glacial	RCI Labscan (Bangkok, Thailand)
Aluminium Chloride Hexahydrate (AlCl ₃ .6H ₂ O)	QRëC (New Zealand)
Folin-Ciocalteu's phenol reagent	Merck KGaA (Darmstadt, Germany)
Ferric 2,4,6-tripyridyl-s-triazine (TPTZ)	Sigma-Aldrich (St. Louis, MO 63103, USA)
Ferric Chloride Hexahydrate (FeCl ₃ .6H ₂ O)	Chem-Supply (Gillman, Australia)
Gallic acid	Sigma-Aldrich (St. Louis, MO 63103, USA)

Table 3.1 (continued)

Chemical Name	Brand/Source
Hydrochloric acid 37%	RCI Labscan, (Bangkok, Thailand)
Iron (II) Sulphate 7-Hydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$)	KemAus (New South Wales, Australia)
L-Ascorbic Acid	Ajax Finechem (Taren Point, Australia)
Metaphosphoric acid (HPO_3)	AppliChem (Darmstadt, Germany)
Methanol	RCI Labscan (Bangkok, Thailand)
Potato Dextrose Agar (PDA)	KemAus (New South Wales, Australia)
Potato Dextrose Broth (PDB)	HiMedia Laboratories Pvt.Ltd. (India)
Potassium Disulfate	Hanna Instruments, Inc. (Salaj, Romania)
Potassium Iodide	Hanna Instruments, Inc. (Salaj, Romania)
Quercetin	Sigma-Aldrich (St. Louis, MO 63103, USA)
Tween-20	AppliChem (Darmstadt, Germany)
Sodium Acetate Anhydrous (CH_3COONa)	QRëC (New Zealand)
Sodium carbonate (NaHCO_3)	KemAus (New South Wales, Australia)
Sodium chloride (NaCl)	RCI Labscan (Bangkok, Thailand)
Sodium Hydroxide	RCI Labscan (Bangkok, Thailand)
Sodium Nitrite (NaNO_2)	RCI Labscan (Bangkok, Thailand)
Sodium Thiosulfate 5-Hydrate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$)	KemAus (New South Wales, Australia)

3.2 Effect of Acidic Electrolyzed Water Treatments on Surface Mould in 'Phulae' Pineapple

3.2.1 Acidic Electrolyzed Water Preparation

Acidic electrolyzed water (AEW) was produced from AQUECA-40 (Izumrud Research and Production Enterprise, Russia) device (Figure 3.1) by electrolysing a 26% sodium chloride (NaCl) solution (300 g NaCl + 0.31gal water). The pH and oxidation-reduction potential (ORP) were checked by using the benchtop pH/ORP meter (Mettler Toledo, FE20-Basic FiveEasy, Switzerland). The amount of available chlorine concentration (ACC) was determined by using the colorimeter (Hanna Instrument Inc., HI 96771 C, Romania) where using chlorine UHR reagent A (Potassium Iodide) and chlorine UHR reagent B (Potassium Disulfate). The initial AEW had pH 4.01 ± 0.1 , ORP 1084 ± 2 mV and ACC 500 ppm. The generated electrolyzed water was kept in a close container before used and diluted with reverse osmosis (RO) water to obtain the concentration for treatments.

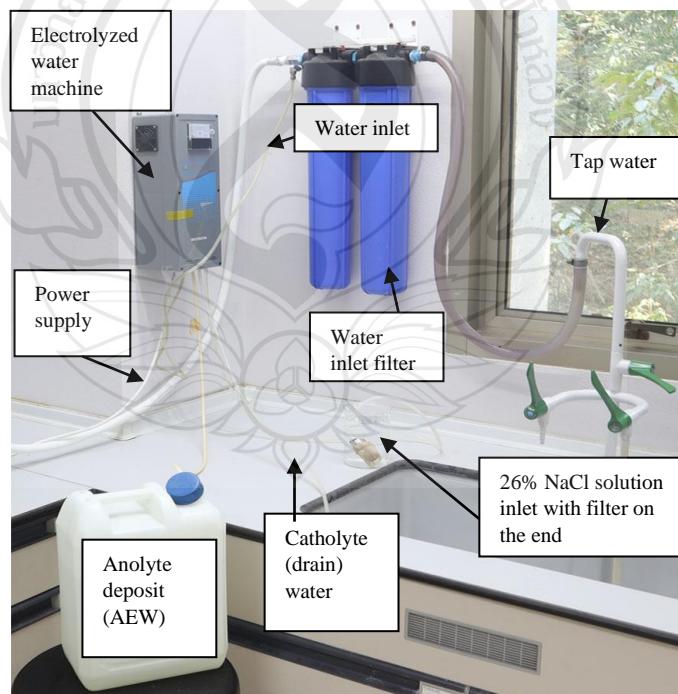


Figure 3.1 Electrolyzed water producing process

3.2.2 Acidic Electrolyzed Water Treatment

The initial electrolyzed water of available chlorine concentration (ACC) 500 ppm was diluted with RO water in a 12 L container to obtain 10 L of 100, 200 and 300 ppm. ACC was measured again after diluted. ‘Phulae’ pineapple fruit was dipped in diluted acidic electrolyzed water container for 10 min in different concentrations: Treatments concentration was followed as in Whangchai et al. (2017): untreated control, ACC 100, 200 and 300 ppm. Each treatments included three replications and each replication used ten fruits. Total number of fruits had 120 fruits. After dipping the fruit for 10 min, the fruits were air-dried in plastic basket with a fan for 10 min, and then put in a corrugated box and stored at 13 °C, 85-95% RH for 28 days. The assessment was taken in every 7-day interval.

3.2.3 Assessments

3.2.3.1 Mould incidence

Mould incidence percentage was investigated every 7-day interval for 28 days. The fruit was recorded as an infected fruit when the mould growth found on the surface of the fruit, cut-stem and de-crowned. The mould incidence percentage was calculated as the equation (1):

$$\text{Mould Incidence (\%)} = \frac{\text{Number of infected fruits}}{\text{Total number of fruits in treatment}} \times 100 \quad (\text{Eq. 1})$$

3.2.3.2 Mould severity

Mould severity (MS) (%) was evaluated as described by Safari et al. (2020) with slightly modification (Table 3.1) by using the equation (2).

$$\text{MS (\%)} = \frac{\sum (\text{Severity rating} \times \text{Number of pineapple fruit clusters in the rating})}{\text{Total number of pineapple fruit clusters assessed} \times \text{Highest MS score (4)}} \times 100\% \quad (\text{Eq. 2})$$

Table 3.2 Mould severity score assessment for pineapple fruit

Mould severity score	Description	Inference
0	No visible sign on the de-crown	No infection
1	1-25% of the de-crown area covered by the mould	Mild infection
2	26-50% of the de-crown area covered by the mould	Moderate infection
3	51-75% of the de-crown area covered by the mould	Severe infection
4	>75% of the de-crown area covered by the mould and rotten parts found	Very severe/ Devastating

3.2.3.3 Mould identification

The mould on de-crowned of 'Phulae' pineapple was isolated into a pure culture on potato dextrose agar (PDA) plate and sent to Thailand Bioresource Research Centre (TBRC), Bangkok for identification. The four steps of fungus identification methods from TBRC practices are as below-

Fungal Identification Methods

1. DNA extraction

Genomic DNA was extracted from fresh mycelia by using a modified CTAB method of Doyle and Doyle (1987). Fungal mycelia were scraped from a culture plate into a microtube containing CTAB buffer (600 µL) by using a sterile spatula. Grind mycelia using the microtube pestle. Incubate the microtube at 65 °C for 20 min. Add 600 µL of CHCl₃: IAA (24:1), and invert repeatedly. Centrifuge at 13,000 rpm for 15 min at 4 °C. Remove the upper aqueous phase to a clean microtube. Add 300 µL of cold isopropanol. Invert repeatedly and place at -20 °C for 20 min. Centrifuge at 13,000 rpm for 15 min at 4 °C to pellet the DNA. Discard supernatant. Add 50 µL of 1x TE to dissolve DNA pellet.

2. PCR: ITS

The internal transcribed spacer (ITS) region was amplified in a 50 µL reaction volume containing 10x buffer, 2.5 mM MgCl₂, 0.2 mM dNTPs, 0.2 µM of each primer (ITS5 and ITS4), and 1U *Taq* DNA polymerase. The PCR temperature

profile began with an initial denaturation at 96 °C for 2 min, followed by 35 cycles of 1 min, 53 °C for 1 min and 72 °C for 1:30 min. The final extension was carried out for 10 min at 72 °C.

3. Gel Electrophoresis and Sequencing

PCR product was checked by 1% agarose gel electrophoresis, stained with DNA-Dye NonTox, and visualized under ultraviolet (UV) transilluminator. The PCR product was sent to be sequenced for both directions on an automated DNA sequencer (Macrogen Inc., Korea).

4. Sequence analyses

The nucleotide sequences obtained from all primers were assembled using Cap contig assembly program, an accessory application in BioEdit (Biological sequence alignment editor). Program (<http://www.mbio.ncsu.edu/BioEdit/BioEdit.html>). The sequences were compared with nucleotide sequences databases on Genbank, CBS or suitable databases.

3.3 Ultraviolet-C Irradiation for Controlling Surface Mould in ‘Phulæ’ Pineapple

3.3.1 UV-C Installation

The UV-C irradiation chamber (homemade) was used according to (Safitri et al., 2015). The UV-C lamps were placed 25 inches above (4 lamps), 10 inches below (4 lamps) the radiation rack and 13 inches from centre to each side (6 lamps) (Figure 3.2). The UV-C intensity for this experiment has around 8000 $\mu\text{W}/\text{cm}^2$ that was measured by a digital UV Light Meter (Linshang, Model-LS126C, China).



Figure 3.2 UV-C chamber (a) External design and (b) Internal design

3.3.2 UV-C Treatments

The fruits were placed in a line parallel with the UV-C lamp. The doses were followed from Sari et al. (2016): untreated control, 13.2, 26.4 and 39.6 kJ/m^2 . After irradiation, the fruit was placed in a corrugated box and stored at 13 °C, 85–95% RH for 28 days. There were three replications per treatment and ten fruits per replication and assessed every 7-day interval.

3.3.3 Assessments

3.3.3.1 Mould incidence

It was conducted as described in Section 3.2.3.1.

3.3.3.2 Mould severity

It was measured as described in Section 3.2.3.2.

3.4 Combined AEW and UV-C for Controlling Surface Mould and Investigate Fruit Quality Changes

The most suitable results obtained from the Section 3.2 and 3.3 were used in this experiment. The treatments were untreated control; AEW (300 ppm); UV-C (13.2 kJ/m^2); UV-C (39.6 kJ/m^2); AEW (300 ppm) + UV-C (13.2 kJ/m^2); and AEW (300

ppm) + UV-C (39.6 kJ/m²). Due to the differences of destructive and non-destructive and storage duration nature of the assessments, this experiment had run three times.

3.4.1 Effects of AEW and UV-C Treatments on Surface Mould

The treatments were operated as mentioned in Section 3.4. Three replications were used in each treatment. There were 10 fruits per replication. Total number of fruits were 180 fruits for this experiment. AEW treated fruits were dried first with the fan for 15 min and continued for UV-C treatment. Then the fruits were moved into a corrugated box and stored in a cold room at 13 °C, 85-95% RH for 28 days. Non-destructive investigation was taken every 7-day interval.

3.4.1.1 Mould incidence

The assessment was conducted as described in Section 3.2.3.1.

3.4.1.2 Mould severity

The assessment was conducted as described in Section 3.2.3.2.

3.4.2 Effects of AEW and UV-C Treatments on Fruit Quality Attributes Changes

The same treatments were used as in the section 3.4. There were three replications in each treatment. Each replication was included 25 fruits. Total number of fruits was 450 fruits for this experiment (6 Treatment x 3 Replication x 25 fruits = 450 fruits). After given the treatments, the fruit was placed in a corrugated box and stored in a cold room at 13 °C for 28 days. Investigation was taken every 7-day interval. In each week, five fruits were randomly selected from each replication which was destructed for the following assessments (6 Treatment x 3 Replication x 5 fruits = 90 fruits per week).

3.4.2.1 Weight loss

The weight loss was taken from weighting pineapple fruit prior to storage and 7-day interval until 28 days of storage. Weight loss of each fruit was calculated as a percentage by using the equation (3).

$$\text{Weight Loss (\%)} = \frac{\text{Weight before storage} - \text{Weight after storage}}{\text{Weight before storage}} \times 100 \quad (\text{Eq. 3})$$

3.4.2.2 Moisture content

Sample two gram was weight by electronic balance (Mettler Toledo, ML204, Switzerland) and dried in an oven at 105 °C for 24 h and re-weighting. The percentage of moisture content was calculated by using the equation (4).

$$\text{Moisture Content (\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100 \quad (\text{Eq. 4})$$

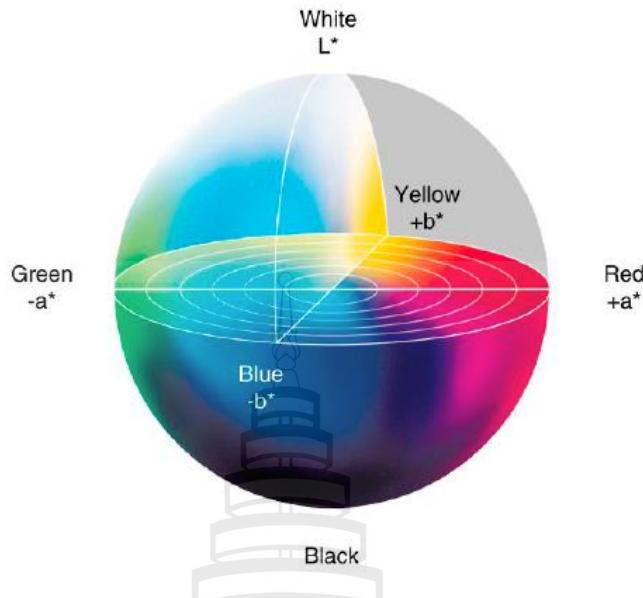
3.4.2.3 Dry matter

The percentage of dry matter was calculated by using the equation (5).

$$\% \text{ Dry matter} = 100 - \% \text{ Moisture Content} \quad (\text{Eq. 5})$$

3.4.2.4 Peel colour

The two sides of pineapple peel colour were measured on top, central and basal part of the fruit by Spectrophotometer (Konica Minolta, Model-CM 600d, Japan). The CIE L* a* b* was used. L* express the lightness from black to white on a scale from 0 to 100. Chromaticity a* represents redness (+a*) or greenness (-a*) and b* indicates yellow (+b*) or blue (-b*). Chroma (c), represents saturation, mention the vividness (more saturation toward the outside) or dullness (greyer at the centre) of the wheel. h° is the angle of the hue. Hue moves in a circle to describe the colour family – red, yellow, green, and blue – and all of the colours that fall in-between. Its value ranges from 0° to 360°, starting with red at 0°, then moving counter-clockwise through yellow, green, blue, then back to red as in the Figure 3.3.



Source Agudo et al. (2014)

Figure 3.3 Colour space- Commission International de L'Eclairage (CIE LAB)

The total colour changes of (L^* , a^* , b^*) were checked with delta E (ΔE) by using the equation (6).

$$\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \quad (\text{Eq. 6})$$

Where, L_0^* , a_0^* , b_0^* represent the initial colour of the fruit and L^* , a^* , b^* expressed for the colour of measurement taken during storage at 13 °C for 28 days.

3.4.2.5 Internal browning severity

Internal browning (IB) severity (%) was evaluated by virtual rating scaled (Figure 3.5) according to the Teisson (1979) method of rating scale with modification. The fruit was cut in a half longitudinal cross-section and severity was estimated from the extent surface of browning area in the fresh fruit near the core. The scales were rating from 0 to 4. The results were shown as a percentage of internal browning severity by using the equation (7).

$$\text{IB severity \%} = \frac{\sum (\text{Severity rating} \times \text{Number of fruit at the rating})}{\text{Total number of fruits in treatment} \times \text{Highest score (4)}} \times 100\% \text{ (Eq. 7)}$$

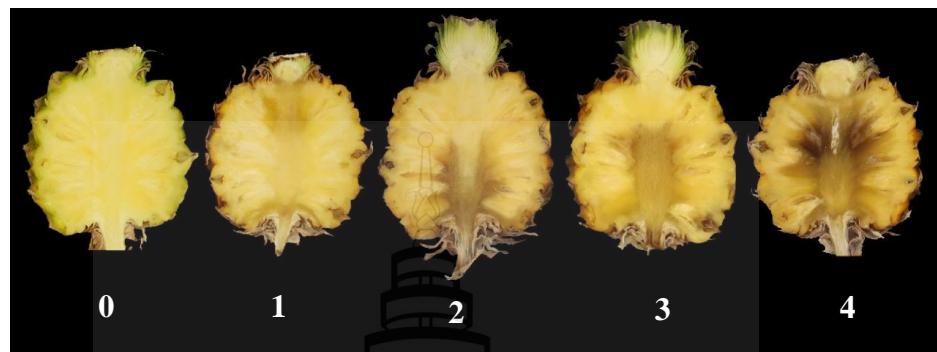


Figure 3.4 Internal browning severity rating scale in 'Phulae' pineapple

Where, Rating scale 0 = No browning surface area around the core
 Rating scale 1 = 10-25% browning surface area around the core
 Rating scale 2 = 26-50% browning surface area around the core
 Rating scale 3 = 51-75% browning surface area around the core
 Rating scale 4 = >75% browning surface area around the core

3.4.2.6 Total soluble solids (TSS)

'Phulae' pineapple juice was cut and squeezed out from longitudinal cross-section of the fruit as in the Figure 3.4. The juice was analysed by Brix-Acidity digital refractometer (Atago, Model PAL-BXIACID F5, Japan). The value was shown as °Brix.



Figure 3.5 Cutting method for taking out the pineapple juice

3.4.2.7 Titratable acidity (TA)

The pineapple juice was diluted with water in 1:50 ratio. The value was taken by using Brix-Acidity digital refractometer (Atago, Model PAL-BXIACID F5, Japan). The value was expressed as percentage of citric acid equivalent.

3.4.2.8 TSS-TA ratio

The TSS-TA ratio was calculated by TSS content which was divided by TA content.

3.4.2.9 pH of the fruit juice

The juice was collected as in the TSS and the value was taken by using a bench-top pH meter (Mettler Toledo, FE20-Basic FiveEasy, Switzerland).

3.4.2.10 Vitamin C content

The vitamin C content was measured by using indicator dye 2,6-dichloroindophenol in a titration method as described in AOAC method 967.21 (AOAC International, 2000). Two mL of pineapple juice was mixed with 5 mL of metaphosphoric acid-acetic acid solution and then titrated with 2,6-dichloroindophenol (dye solution) until the solution changed into pink colour which persisting for 5 sec (end point). Ascorbic acid was used as a standard and metaphosphoric acid-acetic acid was used as a blank. Vitamin C was calculated by using the equation (8) and (9). The results were expressed as mg of ascorbic acid per 100 g of fresh sample.

$$\text{Titer (F)} = \frac{(W_a \times V_1/V_2) \times 1}{(V_{sa} - V_b)} \quad (\text{Eq. 8})$$

Where,

Titer (F) = mg ascorbic acid equivalent to 1.0 mL indophenol standard solution

W_a = Weight of ascorbic acid (50 mg) as recorded (e.g., 50.1 mg)

V₁ = Volume of ascorbic acid standard taken to analyse (2 mL)

V₂ = Total volume of ascorbic acid standard (50 mL)

V_{sa} = Volume of dye used for ascorbic acid standard titration (mL)

V_b = Volume of dye used for blank titration (mL)

After that,

$$\text{Vitamin C content} = \frac{(V_x - V_b) \times (F/V_3) \times (V_{ss}/W_x) \times 100}{(mg \text{ AAE}/100 \text{ g FW})} \quad (\text{Eq. 9})$$

Where,

- V_x = volume of dye used for sample solution titration (mL)
- V_b = volume of dye used for blank titration (mL)
- F = Titer (F)
- V_3 = volume of sample solution taken to analyse (2 mL)
- V_{ss} = volume of extracted sample solution (mL)
- W_x = weight of edible part of samples used for extraction (g)
- 100 = conversion factor for converting 1 g to 100 g

3.4.2.11 Sample extraction

Sample (wet pulp) 3 ± 0.05 grams, taken from 5 fruits which were randomly selected from each replication, was homogenized with 15 mL of 95% methanol for 30 sec. and vortex for 1 min. The ratio of sample and methanol was 1:5. The mixture was then centrifuged (Thermo Scientific, Sorvall Legend X1R, Germany) at 5000xg, 4 °C for 5 min. The supernatant was filtered through Whatman filter paper No.4 and collected in the centrifuge tube and kept in -20 °C until used for analysis. The wet peel (sample) was also extracted the same procedure as the pulp.

3.4.2.12 Total phenolic compound (TPC) content

Total phenolic content (TPC) of the extract was measured by using the FolinCiocalteu assay according to ISO (ISO 14502-1, 2005) with some modification. Inside one well of 96-Well-Microplate, the total volume of 300 μ L was added step-by-step. Sample extract 30 μ L was added into 150 μ L of 10% Folin-Ciocalteu reagent, after 3 min, followed by addition of 120 μ L of 7.5% sodium carbonate (Na_2CO_3) The reagents were diluted and dissolved in DD water. There were four replications for each sample analysis. The mixture was shaken a little and allowed to stand for one hour at room temperature (25 °C). The absorbance of the solution was measured by using a microplate spectrophotometer (Thermo Scientific, Multiskan GO) at 765 nm. A standard curve was arranged by using a standard solution of gallic acid (0-150 μ g/mL). The blank sample consisting of water and reagents was

used as a reference. The results were expressed as mg gallic acid equivalents (GAE) per 100 g FW of pineapple by using the equation (10).

$$\text{TPC (mg GAE/100 g FW)} = \frac{c \times V \times 100}{W \times 1000} \times DF \quad (\text{Eq. 10})$$

Where,

TPC = Total phenolic content as mg of gallic acid equivalent per 100 g of sample extract in fresh weight basic (mg GAE/100 g extract)

c = Concentration of gallic acid from the calibration curve ($\mu\text{g/mL}$)

v = Volume of extract (mL)

w = Weight of the sample (g)

DF = Dilution factor

100 = Conversion factor for converting sample 1 g to 100 g

1000 = Conversion factor for converting concentration unit μg to mg

3.4.2.13 Total flavonoids content (TFC)

Total flavonoid content was determined by using the aluminium chloride colorimetric method as reported by Heimler et al. (2005) with modification. Inside one well of 96-well-microplate, the total volume for 300 μL of solution was added sequentially as 80 μL of sample extract, 119 μL of DD water, 11 μL of 5% sodium nitrite (NaNO_2), waited 3 min, 11 μL of freshly prepared 10% aluminium chloride hexahydrate ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$), waited 3 min, and added 79 μL of 1 M sodium hydroxide (NaOH). Then the mixture was shaken a little and incubated for 5 min at room temperature (25 °C). The wavelength of absorbance was measured at 415 nm by using a microplate spectrophotometer (Thermo Scientific, Multiskan GO). A calibration curve was manipulated by using a standard solution of quercetin (0-500 $\mu\text{g/mL}$). The results were expressed in mg quercetin equivalents per 100 g of fresh weight (mg QE/100 g FW) sample and calculation were the same as in the section 3.4.2.12.

3.4.2.14 Antioxidant activity measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The free radical scavenging activity was calculated by following the method of Khalaf et al. (2008) with minor modification. Inside one well of 96-microplate, the total volume had 300 μ L. Sample extract 10 μ L was mixed with 290 μ L of methanolic solution of DPPH (60 μ M or 0.06 mM or 2.36-2.4 mg/100 mL). The mixture was then shaken a little and kept in the dark or packed with aluminium foil at room temperature (25 °C) for 30 min. The wavelength of absorbance was set at 517 nm by using a microplate spectrophotometer (Thermo Scientific, Multiskan GO). Ascorbic acid was applied as a standard (0-200 μ g/mL). The result was expressed as mg ascorbic acid equivalent per 100 g fresh weight sample [DPPH (mg AAE/100 g FW)] and calculation was the same as in the section 3.4.2.12.

3.4.2.15 Antioxidant activity measured by Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay was determined according to the method of Benzie and Devaki (2017) with slightly modification. Acetate buffer (300 mM, pH 3.6) was prepared by dissolved 1.6 g of sodium acetate anhydrous (CH_3COONa) into 400 mL of DD water inside a 500 mL beaker. Then the solution was adjusted to pH 3.6 with acetic acid glacial inside fume hood by using a bench-top pH meter. When the pH was reached at pH 3.6, it was diluted with DD water to 500 mL volumetric flask. Hydrochloric acid (40 mM) solution was prepared by dissolving 331.2 μ L of 37% HCl and diluted with DD water to 100 mL inside the fume hood. The 2,2-Diphenyl-1-picrylhydrazyl (TPTZ) (10mM) solution was made by dissolving 312.3 mg of TPTZ powder in HCl (40mM) solution a little and diluted to 100 mL with HCL (40mM) solution. Iron (III) chloride (FeCl_3) (20mM) solution was prepared by dissolving 324.4 mg of ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) into DD water a little and diluted to 100 mL with DD water. The working FRAP solution was obtained by mixing the acetate buffer (300 mM, pH 3.6), TPTZ (10mM), and FeCl_3 (20mM) into a 10:1:1 ratio which should be pale yellow-orange in colour. If the blue colour observed, this shown contamination of reagents or equipment with free Fe^{2+} . Inside one well of 96-well-plate, the total volume was 310 μ L. Sample extract 10 μ L was added into 300 μ L of FRAP solution. The mixture was incubated at 37 °C for 30 min. Then, the absorbance of the mixture was calibrated at wavelength 595 nm with a microplate spectrophotometer. For the standard, the sample extract was replaced with

0-1000 μ M (0-278 μ g/ml) of ferrous sulphate 7-hydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) and the results was expressed as mg Fe(II) equivalent per 100 g of fresh weight sample [FRAP (mg Fe(II)E/100 g FW)] and calculation was the same as in the section 3.4.2.12.

3.4.3 Germicidal Effect of AEW and UV-C Treatments: *In Vitro* Study

The 7 days old mould of pure culture from the section 3.2.3.3 was used in this study.

3.4.3.1 Spore survival

The spore survival was assessed with spread plate method as in Khayankarn et al. (2013) with modification. The spore suspension was prepared by flooding the sporulating culture of 7-days-old mould (*C. paradoxa*) with 10 ml of sterile distilled water which contained 0.05% (v/v) Tween-20 and spread with glass spreader. Then the suspension was poured into Erlenmeyer flask (250 mL) and shaken for 10 min (200 rpm) on an orbital shaker at 27 °C. After that it was filter through two layers of sterile muslin cloth. The haemocytometer was used for counting conidia density under microscope and diluted with sterile distilled water until 10^6 conidia/mL was obtained.

In AEW treatment, 1 mL of spore suspension (10^6 conidia/mL) was added into test tube which was included 9 mL of AEW (300 ppm). The serial dilution was made (10^{-1} , 10^{-2} , 10^{-3} , 10^{-4}) with AEW (300 ppm). After incubated at room temperature (25 °C) for 10 min, 0.1 mL of spore suspension was added into 0.9 mL of 0.1 N sodium thiosulfate (15.811 g/100 mL of DDwater) tube. The serial dilution was become 10^{-1} into 10^{-2} , 10^{-2} into 10^{-3} , 10^{-3} into 10^{-4} and 10^{-4} into 10^{-5} . After well mixing, 0.1 mL of suspension was spread on the PDA plates with glass spreader and incubated at 27 °C for 48 h. The control treatment was treated by replacing the AEW (300 ppm) with distilled water.

As for the UV-C treatment, 5 mL of spore suspension (10^6 conidia/mL) was placed into sterilized petri dish plate and irradiated with UV-C (13.2 and 39.6 kJ/m^2) without cover lids. After that 1 mL of spore suspension was added into test tube which included 9 mL of sterilized distilled water. The following process were the same as above.

In the combination of AEW and UV-C treatments, 5 mL of spore suspension (10^6 conidia/mL) was placed into the sterilized petri dish plate and irradiated with UV-C (13.2 and 39.6 kJ/m^2) without cover lids. After that, 1 mL of spore suspension was added into test tube which included 9 mL of AEW (300 ppm). The following processes were the same as above.

All inoculated PDA plates were incubated at 27 °C for 48 h. The data was collected in every 0, 12, 24, 36, 48 h. There were 120 plates in total (6 treatments x 4 serial dilutions x 5 Rep in each dilution = 120 plates). Survival of the spore was expressed as the mean number of colony-forming-unit (CFU/mL) by using the equation (11). The process was repeated twice independently.

$$\text{Spore survival (CFU/mL)} = \text{CP} \times \text{DF} \times 10 \quad \text{Eq. (11)}$$

Where: CP = Number of colonies countable on the plate

DF = Dilution factor was number written on plate

10 = For effective dilution when spreading 0.1 mL of spore suspension on the plate

3.4.3.2 Spore germination ratio and germ tube length

The spore germination ratio and germ tube length were assessed as in Zhou et al. (2017) with modification. The spore suspension was prepared as in the section (3.4.4.1.). For AEW treatment, 1 mL of spore suspension (10^6 conidia /mL) was put in 15 mL centrifuge tube which contained 9 mL of AEW (300 ppm) and waited for 10 min. After that 1 mL of suspension was added into 100 mL size Erlenmeyer flask which contained 9 mL of sterilized potato dextrose broth (PDB) solution (24 g/L of water). The suspension flask was incubated on a rotary shaker (IKA®, KS 4000 i control, China) at 200 rpm, 25 °C. The assay was checked microscopically during incubation periods at 3, 6, 9, 12 and 15 h. Distilled water was replaced in AEW as for the control treatment.

In UV-C treatments, 5 mL of spore suspension (10^6 conidia/mL) was put inside the sterilized petri dish plate and irradiated (13.2 kJ/m^2 and 39.6 kJ/m^2). And then 1 mL of spore suspension was added into 9 mL of sterilise distilled water. After waiting for 10 min, 1 mL of suspension was added into 9 mL of PDB and checked microscopically.

In combined AEW and UV-C treatments [AEW (300 ppm) + UV-C (13.2 kJ/m²); AEW (300 ppm) + UV-C (39.6 kJ/m²)], 5 mL of spore suspension (10⁶ conidia/mL) was placed in the sterilized petri dish and treated with UV-C first. After that 1 mL of spore suspension was pipetted into the 15 mL centrifuge tube that contained 9 mL of AEW (300 ppm) and waited for 10 min. Afterward, 1 mL of spore suspension was added into 100 mL Erlenmeyer flask which contained 9 mL of PDB and checked microscopically. Spore germination (%) was microscopically counted by placing 0.1 mL of treated spore suspension on the haemacytometer and using the equation (12).

$$\text{Spore germination (\%)} = \frac{\text{Total no. of germinated spores}}{\text{Total no. of observed spores}} \times 100 \quad (\text{Eq. 12})$$

Germ tube length was measured with a digital microscope (Nikon, Model-ECLIPSE Ni-U, Japan) by using NIS-Elements D software and expressed in micrometer (μm). The assay was checked during incubation at 5, 7, 9, 11 and 13 h.

3.4.3.3 Mycelium growth inhibition

Mycelium growth inhibition (%) assessment was determined by following Whangchai et al. (2017) with modification. The 7-days-old *C. paradoxa* culture was treated the same as in the Section 3.4. In control treatment, untreated mycelium disc was put directly on the PDA plate. In AEW treatment, 3 ml of AEW solution was mixed with PDA solution before solidify to obtain AEW 300 ppm in the final volume of 15 mL. After solidified, the mycelium disc was placed in the centre. In UV-C treatments, pure culture plate was treated with UV-C (13.2 and 39.6 kJ/m²) without cover lid and then the mycelium disc was placed on the PDA plate. In combination treatments (AEW = 300 ppm + 13.2 kJ/m² and AEW 300 ppm + UV-C 13.2 kJ/m²), mould pure culture plate was treated with UV-C irradiation first without cover lid and then mycelium disc was put on the PDA plate which was already mixed with 3 ml of AEW in the concentration of 300 ppm in the total volume of 15 mL. There were 5 replications per treatment and one plates per replication (6 treatments x 5 replications = 30 plates). The plates were kept at 25 °C for 7 days and checked every 24 h. Assessment was observed by using the equation (13).

$$\text{Mycelium growth inhibition (\%)} = \frac{\text{CC} - \text{CT}}{\text{CC}} \times 100 \quad (\text{Eq. 13})$$

Where: CC = Colony diameter of control (cm)

CT = Colony diameter of treatment sets (cm)

3.5 Statistical Analysis

Analysis of variance (ANOVA) were using the Statistical Analysis System (SPSS, version 20, Inc., USA). The significant tests of mean were separated by using Duncan's multiple range test at $P<0.05$ and considered under completely randomized design (CRD). All of the data value were indicated as mean \pm standard error (SE) and analysed in triplicate at least.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Effects of AEW on Mould Incidence and Mould Severity in 'Phulæ' Pineapple

4.1.1 Mould Incidence

Mould incidence results from Figure 4.1 (A) and 4.2 shown that by Day 7, mould incidence in the control group had surged to $60.00 \pm 10.00\%$, in contrast to significantly lower rates observed in the AEW-treated groups ($20.00 \pm 0.00\%$ for 100 ppm, $26.67 \pm 3.33\%$ for 200 ppm, and $16.67 \pm 3.33\%$ for 300 ppm), suggesting an initial effectiveness of AEW in reducing mould onset compared to the control. However, by Day 14, and continuing through Days 21 and 28, mould incidence in all groups, including those treated with varying AEW concentrations, reached a consistent 100 %. These results indicate that although AEW can delay mould growth initially, it ultimately does not prevent it over the 28-day storage period at 13 °C.

4.1.2 Mould Severity

Mould severity results from Figure 4.1 (B) observed that on Day 7, the contrast in mould severity between the control group and the AEW-treated groups was pronounced. The control group recorded a mould severity of $18.33 \pm 5.83\%$, while the groups treated with AEW showed much lower severities ($5.00 \pm 0.00\%$ at 100 ppm, $7.50 \pm 1.44\%$ at 200 ppm, and $4.17 \pm 0.83\%$ at 300 ppm), with the 300 ppm treatment achieving the greatest reduction. By Day 14, mould severity had increased in all treatments, yet it remained generally lower in the AEW-treated groups compared to the control, which was as $71.67 \pm 10.44\%$. Notably, the 100 ppm and 300 ppm AEW treatments maintained significantly lower severity levels, at $51.25 \pm 3.31\%$ and $38.33 \pm 1.67\%$ respectively. By Days 21 and 28, mould severity in all

treatments converged towards higher levels, with only minor variation among them. Although the AEW treatments continued to show generally lower severities than the control, these differences became less pronounced over time, with all treatments reaching severe levels of mould ($>70\%$). The data shown that AEW can effectively reduce the initial severity of mould growth in 'Phulae' pineapples during storage at 13 °C. The most pronounced effects are seen in the early to mid-storage period (up to Day 14), particularly with higher concentrations of AEW (300 ppm). However, as storage continues towards 28 days, the efficacy of AEW diminishes, and mould severity converges across all treatment groups to high levels. Mould severity in AEW treated pineapples increased over time, but at slower rate than the control, indicating a potential decline in AEW's efficacy or limitations against established infections. This finding is consistent with the research conducted by Whangchai et al. (2017), which highlighted the antifungal activity of acidic electrolyzed water against fungal pathogens. The trend of lower mould severity with higher AEW concentrations (200 and 300 ppm) supports a dose-dependent relationship.

The findings support the idea that ingredients in AEW, such as pH (McPherson, 1993), chlorine compounds (Liu et al., 2006; Park et al., 2004), ORP (Kim et al., 2000; Liao et al., 2007), or combinations of these factors (Huang et al., 2008), play a pivotal role in its antimicrobial activity. The alteration in electron flow within microorganism cells, induced by AEW with higher ORP (>1000 mV), led to modifications in metabolic fluxes and ATP production. The outer membrane of microorganism demonstrated sensitivity, allowing hypochlorous acid (HOCL) entry under the low pH (2.3–2.7) conditions of AEW (McPherson, 1993). Among chlorine compounds, HOCL exhibited the highest activity, disrupting microbial cells by inhibiting glucose oxidation through chlorine-oxidizing sulfhydryl groups of specific enzymes crucial in carbohydrate metabolism. Microbes exhibit varied resistance to cleaning media, emphasizing the importance of understanding the physicochemical properties of EW to formulate effective EW tailored to different microbial types (Khalid et al., 2018). AEW 300 ppm was selected for study in combination with UV-C irradiation treatment.

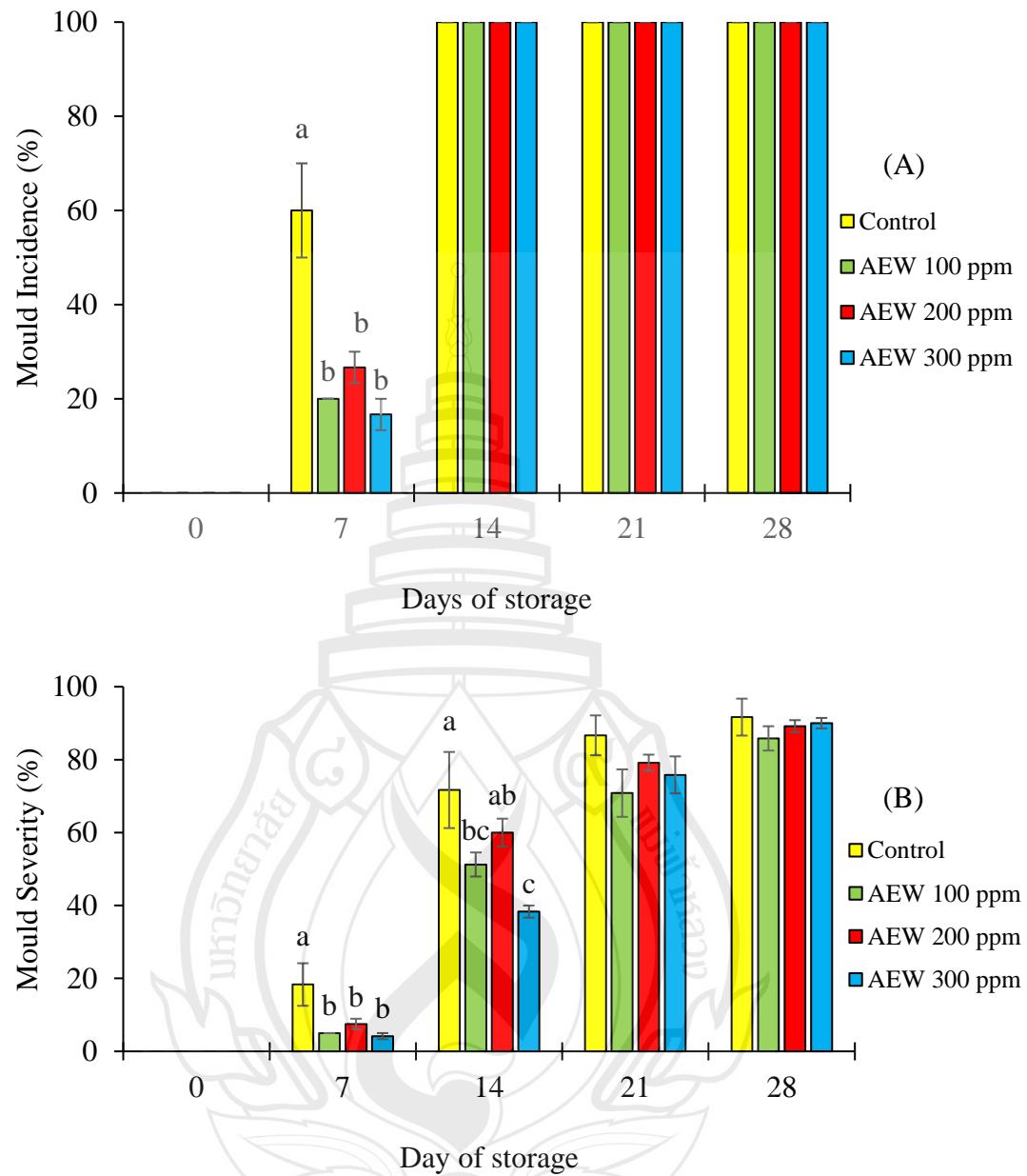


Figure 4.1 Effect of AEW treatment on (A) mould incidence and (B) mould severity in 'Phulae' pineapple during storage at 13 °C, RH 85-95% for 28 days. Each vertical bar represents standard error (SE) of the mean (n=3). Different letters represent significance difference between treatments by using Duncan's multiple range test (P<0.05)

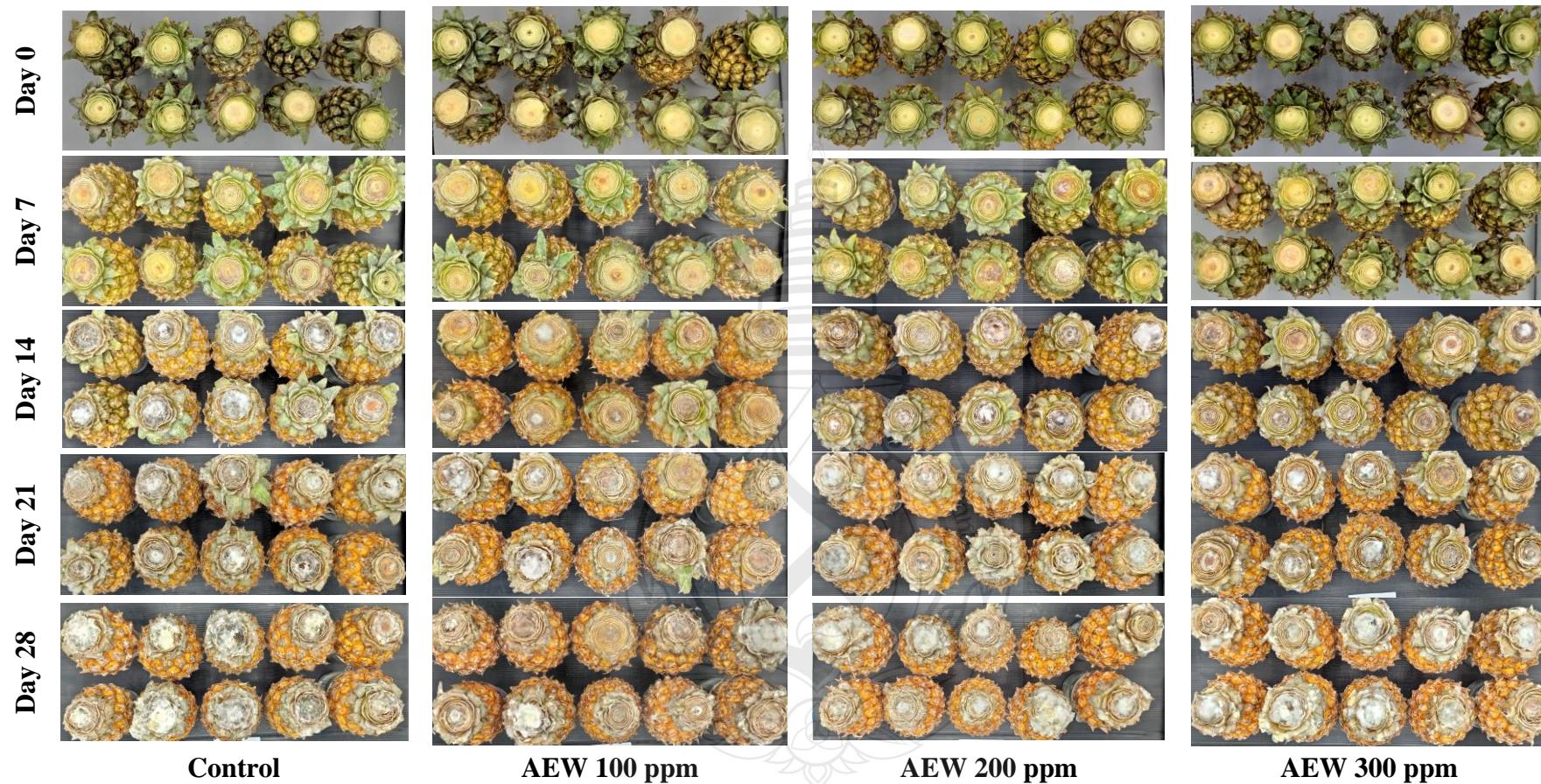


Figure 4.2 Effect of acidic electrolyzed water on mould incidence on 'Phulae' pineapple during storage at 13 °C for 28 days

4.1.3 Mould Identification

The mould identification result of pure culture from de-crowned ‘Phulae’ pineapple by TBRC was *Ceratocystis paradoxa*. It causes black rot disease in pineapple.

4.2 Effects of UV-C Irradiation on Mould Incidence and Mould Severity in ‘Phulae’ Pineapple

4.2.1 Mould Incidence

The UV-C treatment inhibited the occurrence of mould compared to the control at all monitored time points, underscoring its effectiveness in curbing mould growth on ‘Phulae’ pineapples during cold storage Figure 4.3 (A) and 4.4. In day 7, the control group experienced a significant rise in mould presence, peaking at 80%. This sharp increase in mould demonstrate that pineapples are extremely prone to mould infection within the first week of storage if left untreated. In UV-C 13.2 kJ/m² treatment, the mould incidence was reduced to 63.33%, which is lower than the control group’s incidence. This indicates that UV-C irradiation at 13.2 kJ/m² has a moderate effectiveness in slowing down mould growth when compared to untreated fruits. Both of UV-C 26.4 and 39.6 kJ/m² treatments shown the same result of incidence of 53.33% in day 7 of storage. This more notable decrease in mould presence, compared to both the control group and the lower UV-C dose, suggests a dose-response effect where increasing the intensity of UV-C irradiation leads to further delays in the development of mould. These findings align with studies conducted by (Sari et al., 2016) and (Erkan et al., 2008), which reported reduced mould incidence on pineapples and strawberries, respectively, following UV-C exposure. Despite the initial reduction, all treatments exhibited 100 % mould incidence beyond Day 14 of storage. This implies that while UV-C can postpone mould development, it may not entirely prevent it over prolonged storage durations. This observation mirrors the findings of Khademi et al. (2013) with UV-C on citrus fruits, emphasizing the necessity for supplementary strategies for sustained mould control. The absence of significant differences in mould incidence among different

UV-C doses (13.2, 26.4, 39.6 kJ/m²) within the examined range suggests a potential plateau in its efficacy against ‘Phulæ’ pineapples under this specific storage conditions.

4.2.2 Mould Severity

By Day 7, mould severity in UV-C treated groups (13.2, 26.4 and 39.6 kJ/m²) was significantly lower at 21.67%, 18.33% and 13.33% respectively, compared to 31.67% in the untreated control, demonstrating UV-C’s initial effectiveness. However, by Day 14, although mould severity remains lower in UV-C treatment fruits than in the control, the figures start converging, with treated groups showing a gradual increase in severity. This trend continues through Day 21 and by Day 28, mould severity in all UV-C treated groups approaches or matches the high levels seen in the control group, reaching up to 90.83%, indicating a substantial reduction in the efficacy of UV-C treatment over the four-week storage period. UV-C treatment consistently reduced mould severity in comparison to the control throughout the storage period Figure 4.3 (B) and 4.4. This robustly supports the capability of UV-C to mitigate the intensity of mould growth, even though complete prevention may not be entirely achievable. These findings are in line with the research conducted by (González-Aguilar et al., 2001), demonstrating reduced mould severity on mangoes following UV-C treatment. Mould severity in UV-C treated pineapples increased over time but at a slower rate than observed in the control group. This indicated a gradual decline in the effectiveness of UV-C or its limitations against established infections. Comparable observations were reported by Capdeville et al. (2002) with UV-C treatment on apples, underscoring the necessity for further exploration of the mechanisms behind this decline and the development of strategies to enhance long-term efficacy. A discernible trend of lower mould severity with higher UV-C doses is apparent, supporting the existence of a dose-dependent relationship within the investigated range. Expanding the exploration to a wider range of doses could contribute to optimizing the application rate for ‘Phulæ’ pineapples, striking a balance between efficacy and potential drawbacks associated with higher doses. UV-C 13.2 and 39.6 kJ/m² were selected for study in combination with AEW treatment.

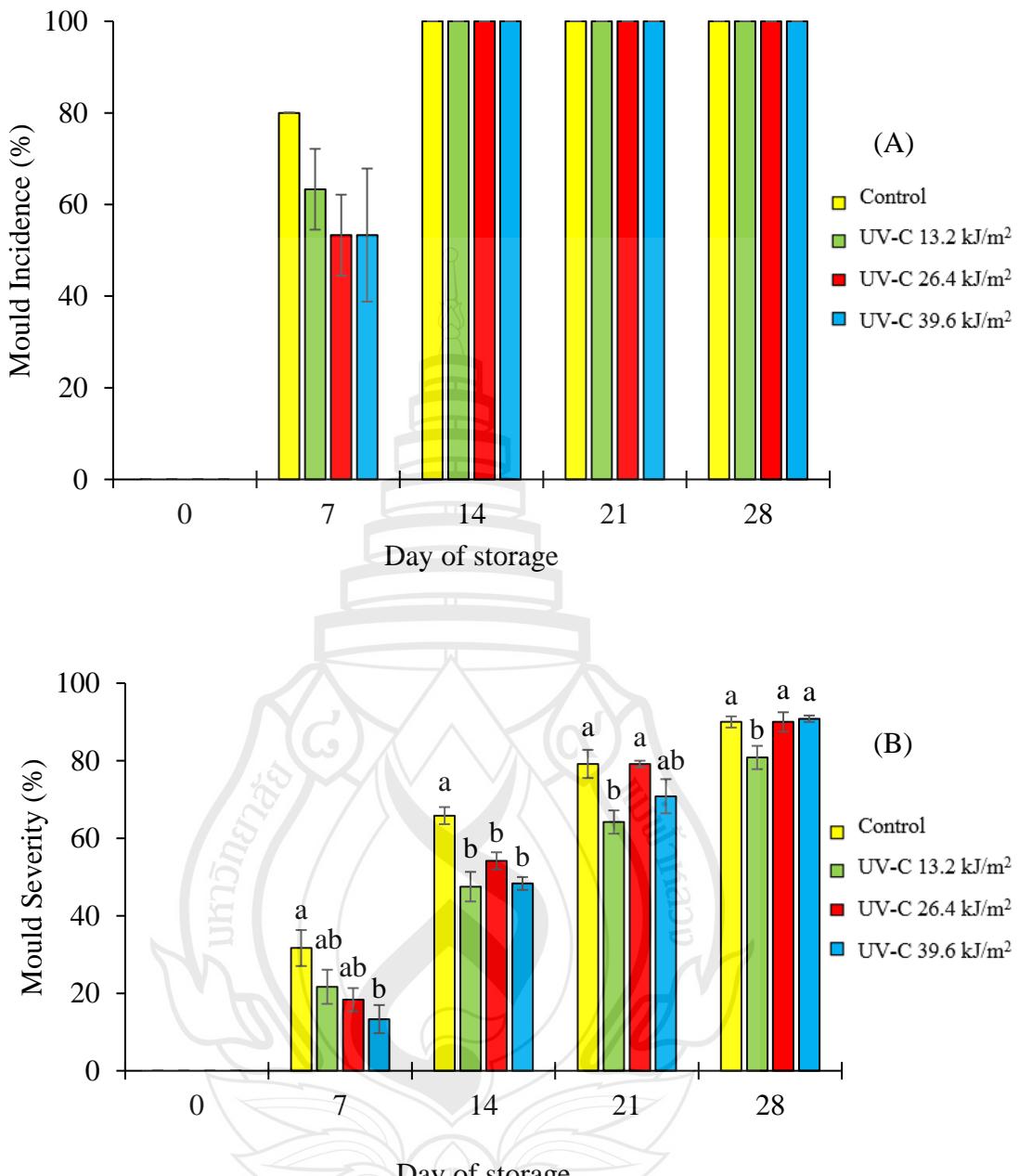


Figure 4.3 Effect of UV-C irradiation on (A) mould incidence and (B) mould severity in 'Phulae' pineapple during storage at 13 °C, RH 85-95% for 28 days. Each vertical bar represents standard error (SE) of the mean (n=3). Different letters represent significance difference between treatments by using Duncan's multiple range test (P<0.05)

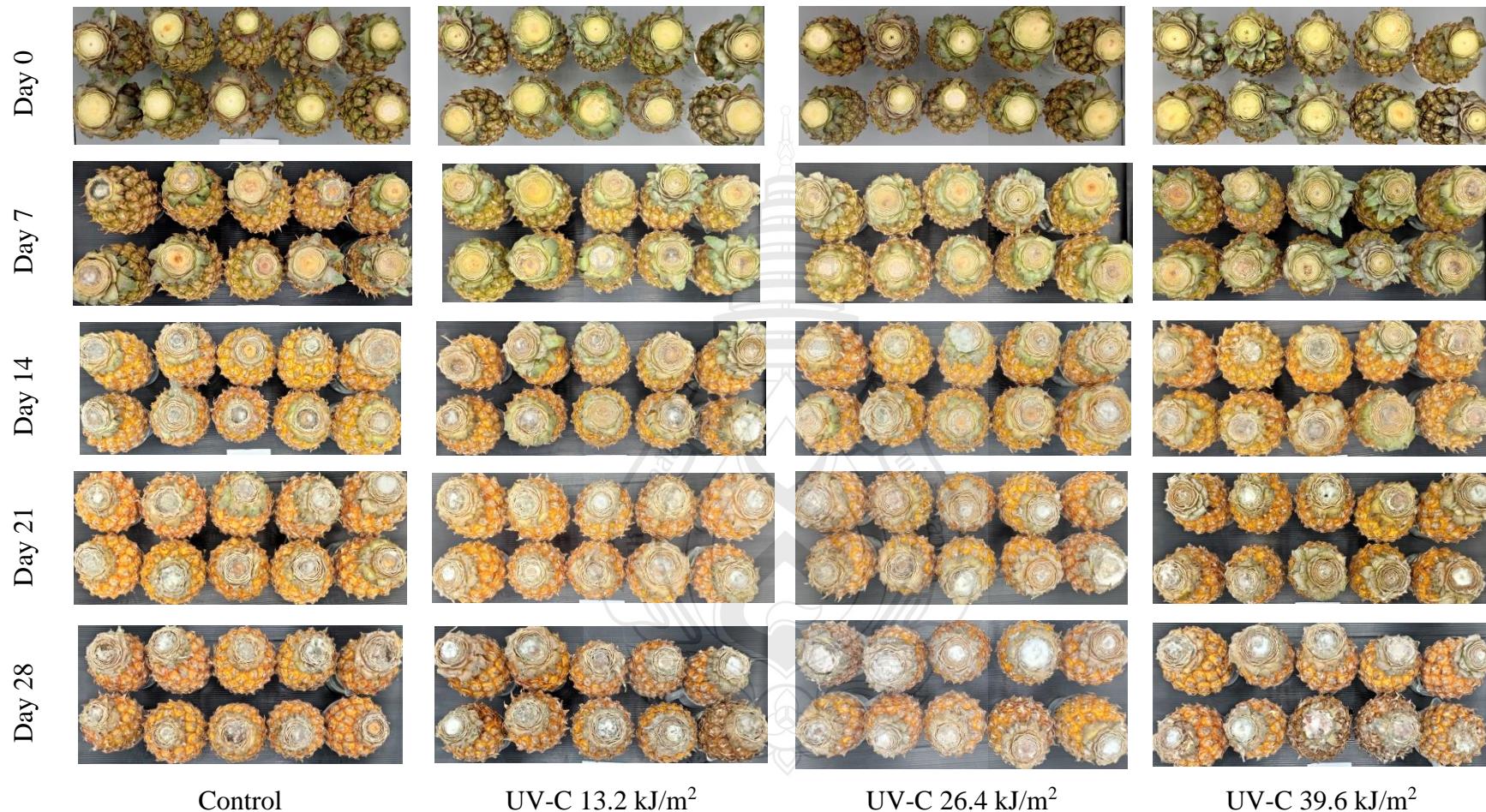


Figure 4.4 Effect of UV-C on mould incidence in 'Phulae' pineapple during storage at 13 °C for 28 days

4.3 Effects of AEW and UV-C on Mould Incidence and Mould Severity in 'Phulae' Pineapple

4.3.1 Mould Incidence

Initially, both AEW (300 ppm) and UV-C treatments (13.2 kJ/m² and 39.6 kJ/m²) effectively suppressed mould incidence compared to the control Figure 4.5 (A) and 4.6, showcasing their potential as antifungal agents against common pineapple rots. On Day 7, the control group saw mould incidence surge to 90%, highlighting the untreated pineapple's extreme vulnerability to mould. In contrast, AEW 300 ppm significantly curtailed mould growth to 26.67%, proving to be highly effective at this early stage. The UV-C treatments, both at intensities of 13.2 kJ/m² and 39.6 kJ/m², managed to lower mould incidence to 60%, demonstrating a moderate level of effectiveness. Furthermore, the combined treatments of AEW 300 ppm with either UV-C intensity further improved mould control, reducing incidence to about 33.33% and 26.67%, respectively, indicating a superior initial control over the single treatment options. However, Day 14 and through to Day 28, mould incidence in all groups, including those treated, escalated to 100%. This pattern reveals that while these treatments, individually or in combinations, can initially delay mould development, they are ultimately ineffective in preventing mould over the long term within the experimental conditions. This finding aligns with Sari et al. (2016) research, which observed reduced mould incidence on 'Phulae' pineapples with UV-C, and with studies highlighting the antimicrobial properties of acidic electrolyzed water, such as those by Sierra et al. (2019). However, all treatments eventually reached 100% mould incidence by Day 14, indicating limitations in their efficacy over extended storage. Similar observations were reported by (Khademi et al., 2013) for UV-C on citrus fruits and by (Hirayama et al., 2016a) for AEW on strawberries, emphasizing the necessity for complementary strategies for long-term mould control. Interestingly, the combination of AEW and UV-C at both dose levels did not confer any additional benefit in terms of mould incidence compared to individual treatments. This suggests potential overlap in their antifungal mechanisms or that the higher

overall efficacy reached a ceiling effect. Further research could explore synergistic effects at lower dose combinations or with different application timing.

4.3.2 Mould Severity

In the study of the effects of AEW, UV-C and their combinations on mould severity in 'Phulae' pineapples stored at 13 °C for 28 days Figure 4.5 (B) and Figure 4.6, significant variations were observed across different treatment groups and storage intervals. By Day 7, the control group's mould severity had increased to 25.83%, highlighting rapid mould progression in untreated fruits. In contrast, AEW treatment alone reduced mould severity dramatically to 6.67%, Chen et al. (2020) and Guentzel et al. (2010) on longan and grapes, respectively, support these findings, demonstrating the effectiveness of AEW in reducing mould severity on various fruits. The combination of AEW with the higher UV-C dose (39.6 kJ/m²) was similarly effective, also reducing it to 6.67%. The combination of AEW with higher doses of UV-C showed a superior ability to suppress mould initially compared to UV-C alone at lower doses or the control. As the experiment continued, all treatments saw increases in mould severity by Day 14, with AEW plus higher dose UV-C maintaining the lowest severity at 30.00%. By Day 21, while mould severity continued to climb, reaching 76.67% in the control, the most effective treatment found in AEW with 39.6 kJ/m² UV-C, which recorded a significantly lower severity at 49.17%. However, by the end of the storage period on Day 28, even the best treatments saw an increase in mould severity, with the combination of AEW and 39.6 kJ/m² UV-C finalizing at 69.17%, albeit still below the control's 85.83%. These results indicate that while initial mould suppression is notably effective with combined AEW and UV-C treatments, the escalating severity over time suggests that these methods alone might not be sufficient for long-term mould control in stored pineapples, pointing to a potential need for ongoing or additional mould management strategies.

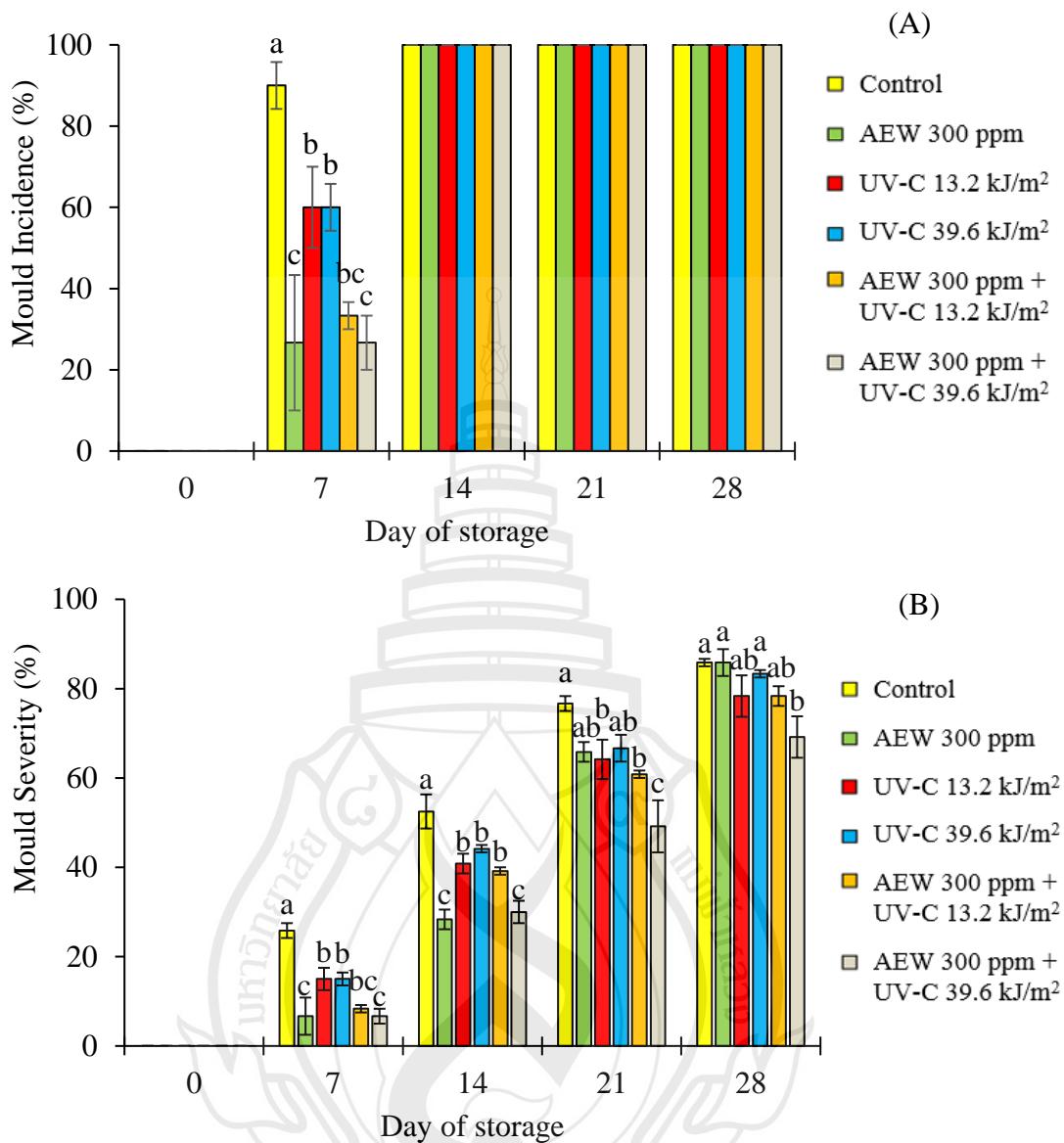


Figure 4.5 Effects of AEW and UV-C on (A) mould incidence and (B) mould severity in 'Phulae' pineapple during storage at 13 °C, RH 85-95% for 28 days. Each vertical bar represents standard error (SE) of the mean (n=3). Different letters represent significance difference between treatments by using Duncan's multiple range test (P<0.05)

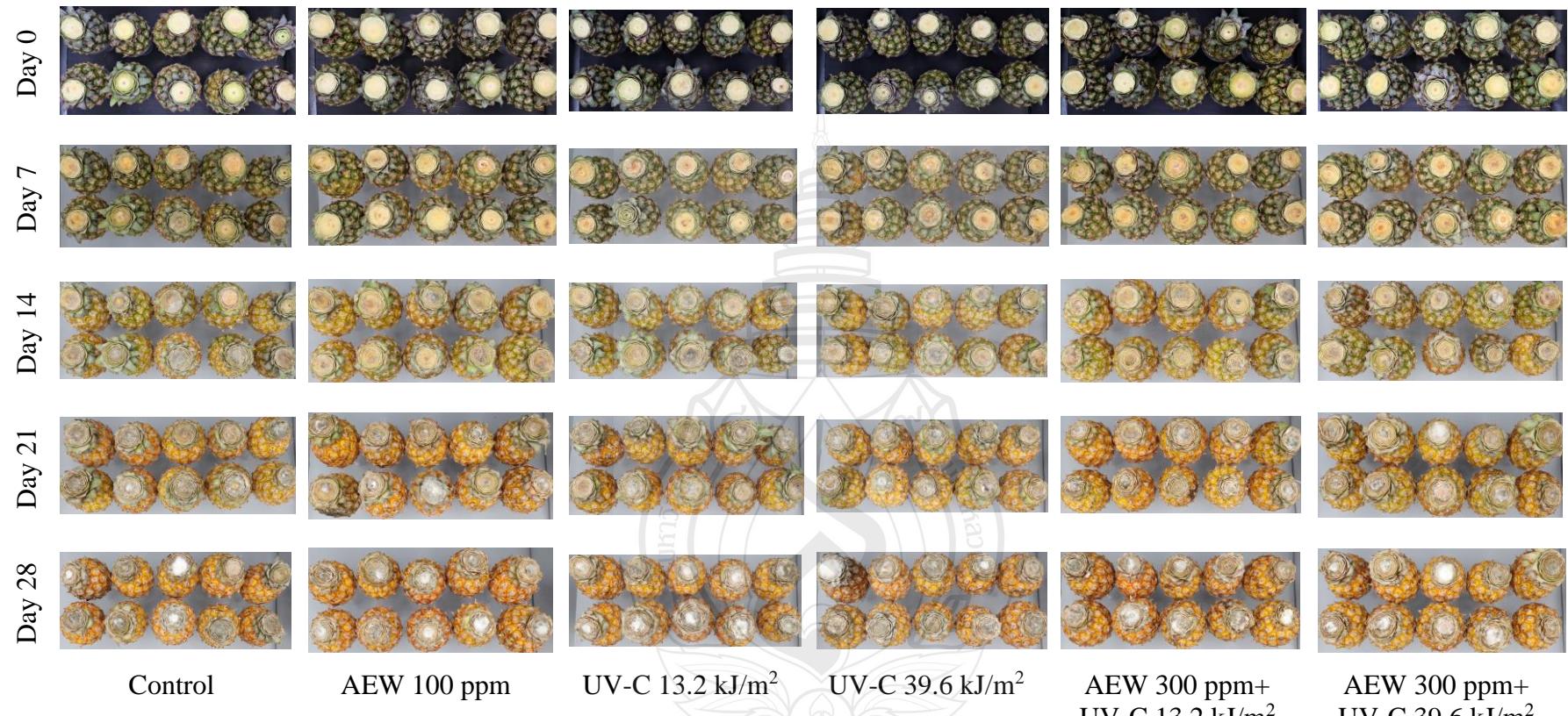


Figure 4.6 Effects of AEW and UV-C on mould incidence in 'Phulae' pineapple during storage at 13 °C for 28 days

4.4 Effects of AEW and UV-C on Fruit Quality of 'Phulæ' Pineapple

4.4.1 Weight Loss

Throughout the storage period, all applied treatments successfully mitigated weight loss in 'Phulæ' pineapples. The control group exhibited the highest weight loss at each time point, reaching 8.43% by day 28. Notably, both AEW and UV-C treatments, whether applied individually or in combination, significantly reduced weight loss compared to the control ($p < 0.05$). While UV-C at 39.6 kJ/m² displayed a slightly more pronounced effect than AEW (300 ppm) across most time points, the difference was not statistically significant by day 28. The combined AEW + UV-C treatments exhibited the highest reduction in weight loss, particularly evident at day 7 (4.59% vs. 3.60% for UV-C 13.2 kJ/m² and 3.96% for AEW 300 ppm). However, this advantage diminished by day 28.

The application of AEW and UV-C treatments effectively mitigated weight loss in 'Phulæ' pineapples during storage, indicating their potential as valuable postharvest interventions. This positive impact can be attributed to their antimicrobial properties. AEW, which contains hypochlorous acid (HClO) as the primary active ingredient, demonstrated the ability to inhibit microbial growth on the fruit surface. This inhibition, in turn, reduces respiration and ethylene production, thereby slowing down water loss (Jin-Woong et al., 2006). Simultaneously, UV-C irradiation disrupts microbial DNA and cell membranes, suppressing microbial activity and preventing fruit deterioration (Jiao et al., 2017). The synergistic action of AEW and UV-C against a broader range of microorganisms might explain the enhanced effectiveness observed in the combined treatment (Jiang et al., 2020).

This study underscores the potential of AEW and UV-C as practical postharvest strategies for reducing weight loss and prolonging the shelf life of 'Phulæ' pineapples. These findings align with previous research on various fruits, affirming the efficacy of these technologies in minimizing physiological weight loss and maintaining overall quality (Jemni et al., 2014; Kukanoor & Chavan, 2011).

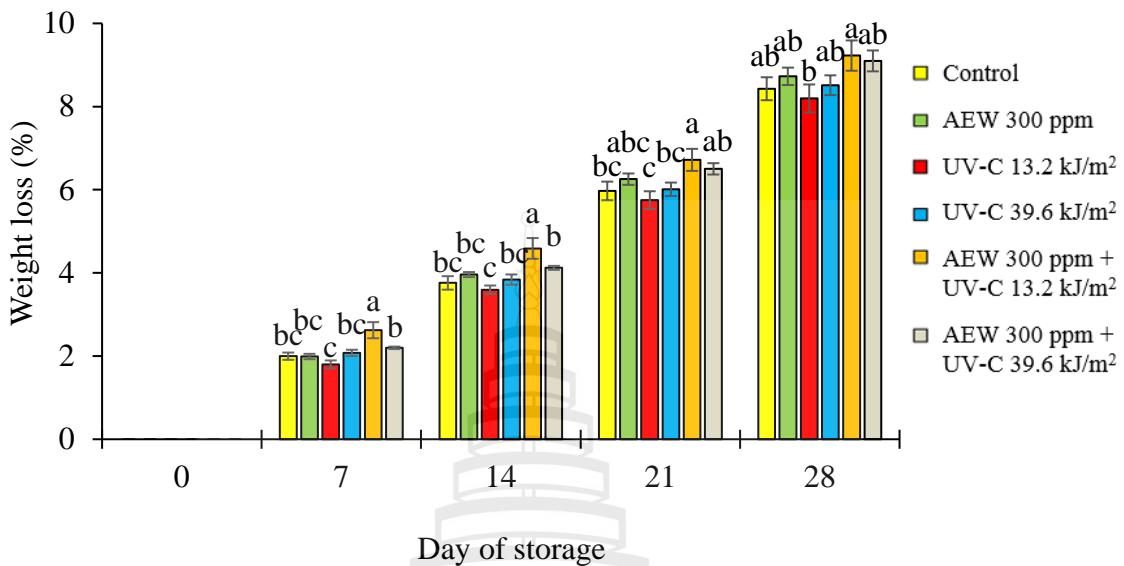


Figure 4.7 Effects of AEW and UV-C on weight loss (%) in 'Phulae' pineapple fruit during storage at 13 °C, RH 85-95% for 28 days. Each vertical bar represents standard error (SE) of the mean (n=3). Different letters represent significance difference between treatments by using Duncan's multiple range test (P<0.05)

4.4.2 Moisture Content

Pulp: In general, the moisture content of the pulp remained relatively constant throughout storage across all treatments. Control fruits consistently exhibited the highest moisture content (ranging from 84.32% to 84.85%) at most time points, although these differences did not reach statistical significance compared to other treatments. Both AEW and UV-C treatments, either applied individually or in combination, did not induce a significant impact on pulp moisture content when compared to the control.

Peel: The moisture content of the peel decreased steadily in all treatments during storage. Control fruits experienced the most substantial decline, reaching 79.61% by day 28. AEW and UV-C treatments, especially at higher dosages (39.6 kJ/m²), exhibited a slight yet statistically significant delay in peel moisture loss

compared to the control at certain time points. Interestingly, combining AEW and UV-C did not provide an additional advantage in terms of preserving peel moisture content.

AEW and UV-C treatments showcased limited influence on the moisture content of pineapple pulp. This suggests that these interventions primarily impact microbial activity and surface properties, with minimal effects on water transport within the fruit tissue. The observed reduction in peel moisture content reflects a natural physiological process associated with transpiration and respiration. AEW and UV-C, by suppressing microbial activity and potentially reducing membrane permeability, may slightly postpone this moisture loss in the peel. The absence of a significant improvement in peel moisture content with the combined AEW and UV-C treatment suggests that their individual effects may be sufficient and independent in this aspect.

These findings contribute to our understanding of how AEW and UV-C affect various aspects of 'Phulæ' pineapple quality during storage. While these treatments do not significantly alter pulp moisture content, their capacity to slow down peel moisture loss could be advantageous for maintaining fruit firmness and minimizing shrivelling.

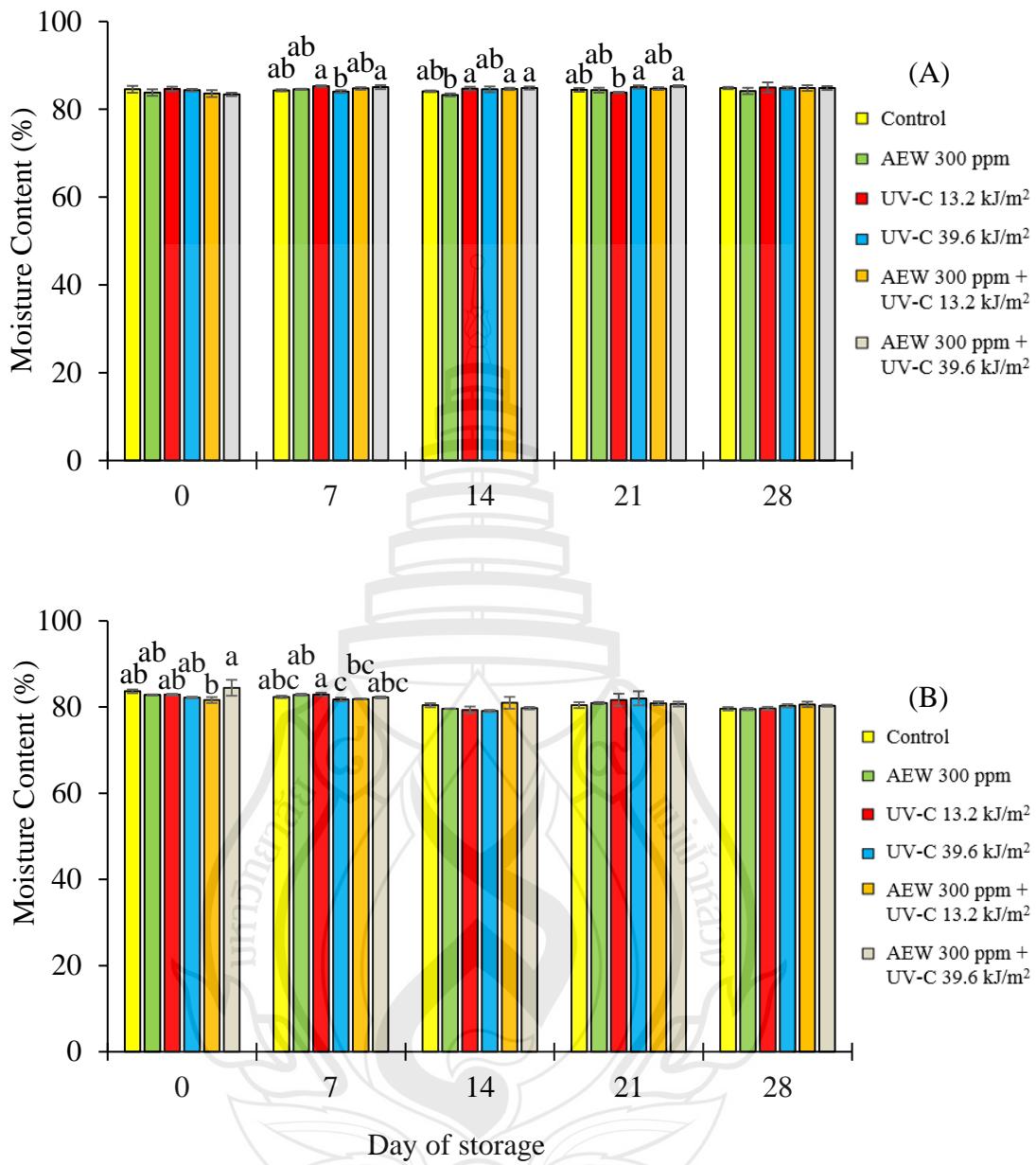


Figure 4.8 Effects of AEW and UV-C on moisture content (%) in 'Phulae' pineapple (A) pulp and (B) peel during storage at 13 °C, RH 85-95% for 28 days. Each vertical bar represents standard error (SE) of the mean (n=3). Different letters represent significance difference between treatments by using Duncan's multiple range test (P<0.05)

4.4.3 Dry Matter

Pulp: The dry matter content in the pulp generally showed an increase across all treatments during the storage period. Control fruits consistently displayed the lowest dry matter levels at most time points, although these differences compared to other treatments were not statistically significant. AEW treatments, whether applied alone or in combination with UV-C, did not significantly alter the pulp's dry matter content when compared to the control. However, UV-C alone, at both dosages (13.2 and 39.6 kJ/m²), resulted in a slight reduction in pulp dry matter at certain time points compared to the control and AEW treatments.

Peel: In contrast to the pulp, the dry matter content in the peel progressively increased in all treatments throughout storage. Control fruits again exhibited the lowest dry matter at most time points, with significant differences from other treatments observed only at day 0 and 7. AEW and UV-C treatments, particularly at the higher dosage (39.6 kJ/m²), demonstrated a slight but statistically significant increase in peel dry matter compared to the control at certain time points. However, combining AEW and UV-C exhibited inconsistent effects on peel dry matter, with no significant advantage over individual treatments.

The observed rise in dry matter content, both in the pulp and peel, is a natural outcome of water loss during storage. As water evaporates, the concentration of soluble and insoluble solids in the fruit tissue increases. AEW and UV-C treatments, while influencing other quality parameters like moisture content and microbial activity, seem to have a limited impact on the overall rate of dry matter accumulation in the pulp. The slight decrease in pulp dry matter observed with UV-C treatment may be attributed to its potential negative effects on fruit metabolism or cell wall integrity. The lack of a consistent additive effect of combined AEW and UV-C on peel dry matter indicates that their individual influences might be sufficient and independent in this context.

These findings enhance our understanding of the distinct effects of AEW and UV-C on various aspects of 'Phulae' pineapple quality during storage. While these treatments do not significantly alter pulp dry matter, their ability to slightly increase peel dry matter could be advantageous for preserving certain textural attributes.

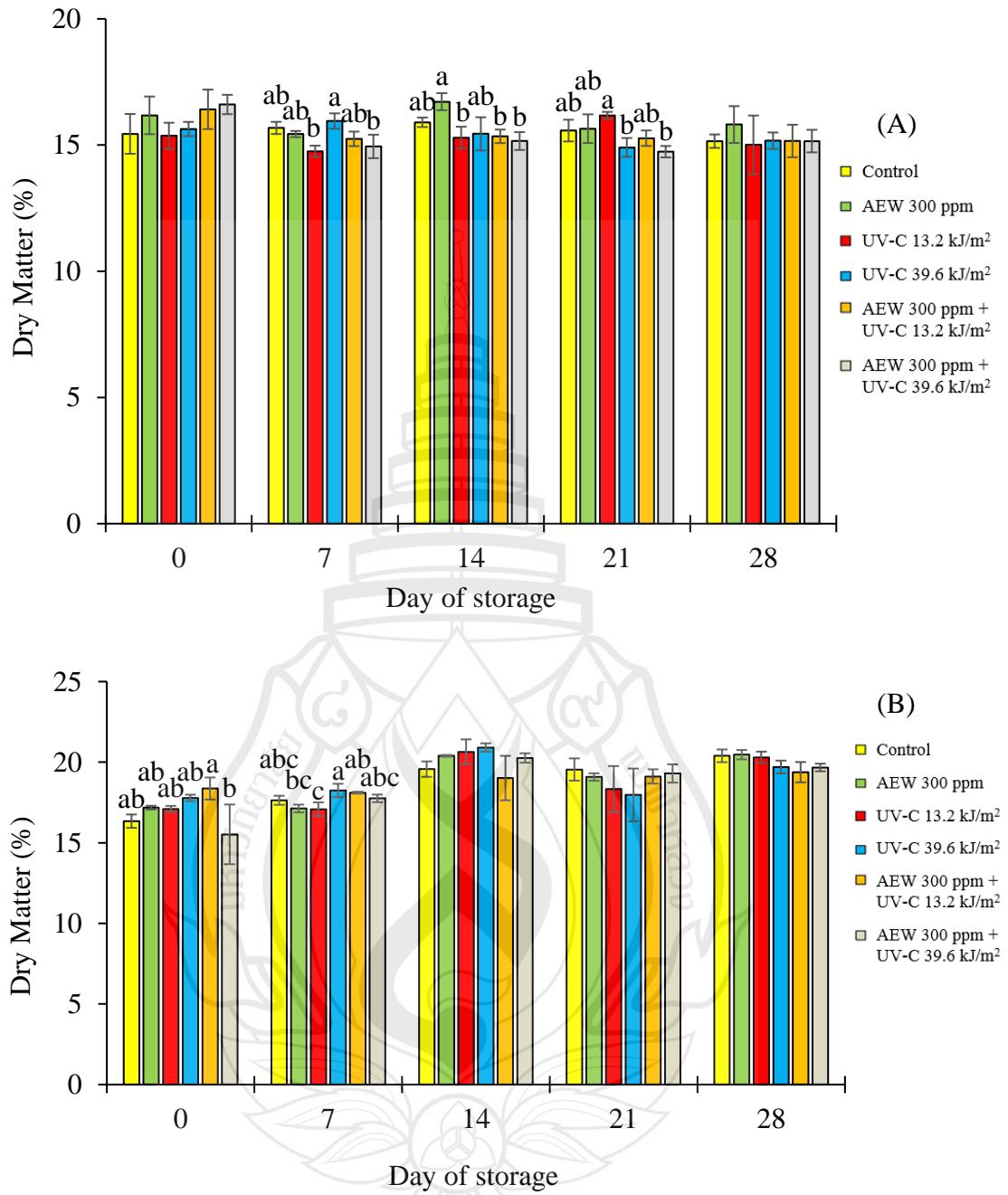


Figure 4.9 Effects of AEW and UV-C on dry matter (%) in 'Phulae' pineapple (A) pulp and (B) peel during storage at 13 °C, RH 85-95% for 28 days. Each vertical bar represents standard error (SE) of the mean (n=3). Different letters represent significance difference between treatments by using Duncan's multiple range test (P<0.05)

4.4.4 Peel Colour

L*(Lightness): All fruits experienced an increase in L* (brightening) throughout storage, signalling a reduction in chlorophyll and carotenoid pigments. AEW and UV-C treatments, whether applied individually or in combination, did not produce a significant effect on L* compared to the control.

a*(Redness/Greenness) values gradually rose in all treatments, indicating a transition from green to yellow/orange due to the accumulation of carotenoid pigments. AEW treatments slightly amplified the increase in a* compared to the control, suggesting a potential influence on carotenoid metabolism. UV-C treatment, particularly at the higher dosage, initially boosted a* but exhibited a slight decline later in storage. Combining AEW and UV-C did not yield a significant additive effect on a* compared to individual treatments.

b*(Yellowness/Blueness) values steadily increased in all treatments, reflecting the development of a yellow colour attributed to carotenoids and anthocyanins. AEW and UV-C treatments did not exert a significant impact on b* compared to the control.

ΔE (Total colour change) values increased in all treatments, indicating the overall extent of colour alteration during storage. AEW and UV-C treatments, whether applied alone or in combination, did not significantly influence ΔE compared to the control.

Chroma (Colour saturation) values generally increased in all treatments, indicating an intensification of colour as pigments accumulated during storage. AEW and UV-C treatments did not significantly alter chroma compared to the control.

Hue angle (h°) values decreased in all treatments, suggesting a shift toward yellow/orange hues as storage progressed. AEW and UV-C treatments did not significantly impact h° compared to the control.

The observed rise in L* and decrease in h° align with the natural degradation of chlorophyll during storage, resulting in a brighter and more yellow/orange appearance. The gradual increase in a* and b* indicates the formation of carotenoids and anthocyanins, contributing to the overall yellow coloration of the fruit. AEW and UV-C treatments appear to have minimal influence on the overall pace and direction of colour changes in 'Phulae' pineapple during storage. The slight augmentation of a* with AEW suggests a potential role in carotenoid metabolism, but further research is

required for validation. The absence of significant effects on other colour parameters implies that AEW and UV-C may be insufficient for modifying or preserving specific colour attributes in this pineapple variety.

The findings contribute to our understanding of the limited impact of AEW and UV-C on 'Phulae' pineapple colour during storage. While these treatments offer advantages in terms of microbial control and the preservation of other quality parameters, their influence on colour development seems minimal.



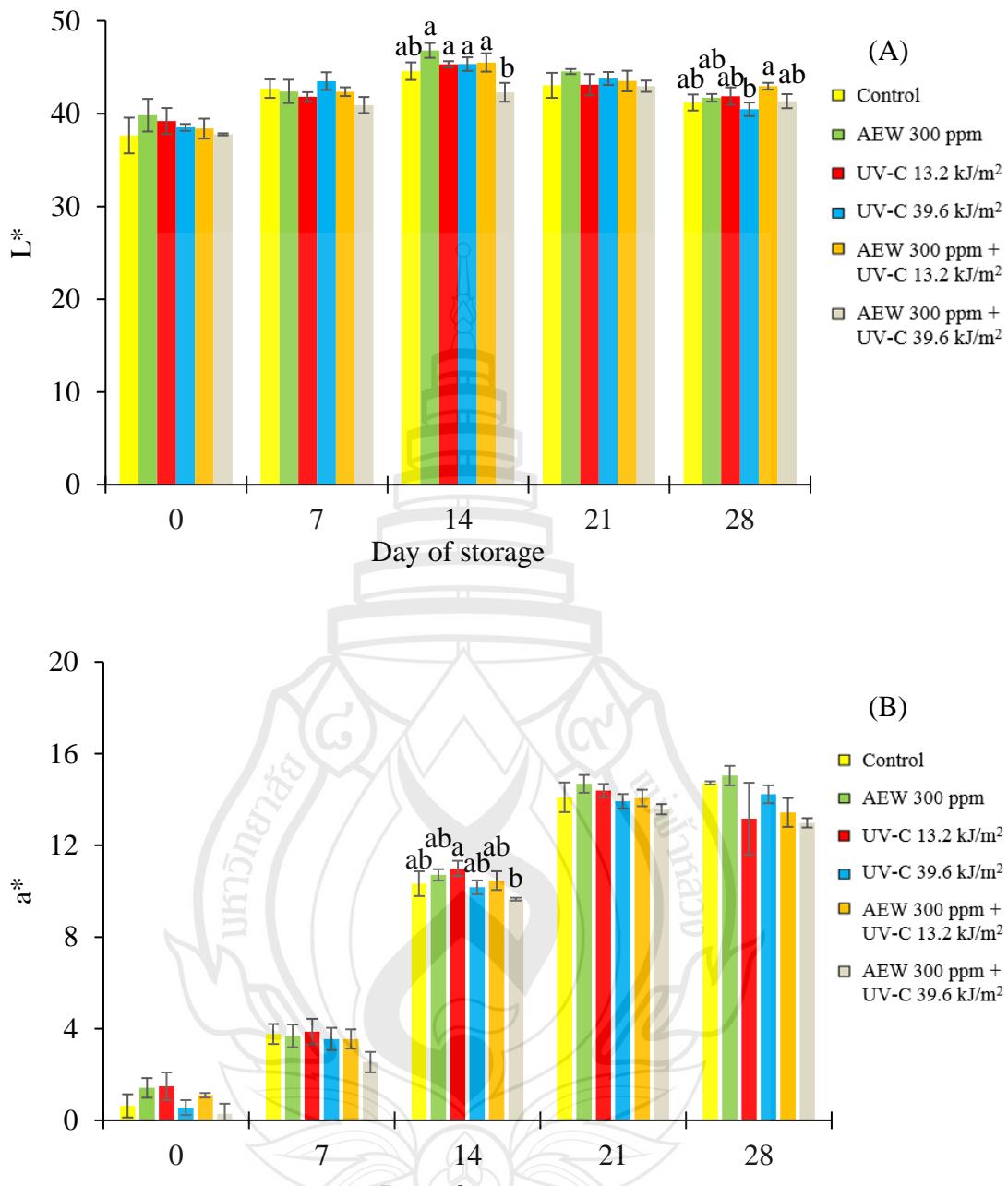


Figure 4.10 Effects of AEW and UV-C on peel colour (A) L^* , (B) a^* , (C) b^* and (D) total colour changes (ΔE) of 'Phulae' pineapple peel during storage at 13 °C, RH 85-95% for 28 days. Each vertical bar represents standard error (SE) of the mean ($n=3$). Different letters represent significance difference between treatments by using Duncan's multiple range test ($P<0.05$)

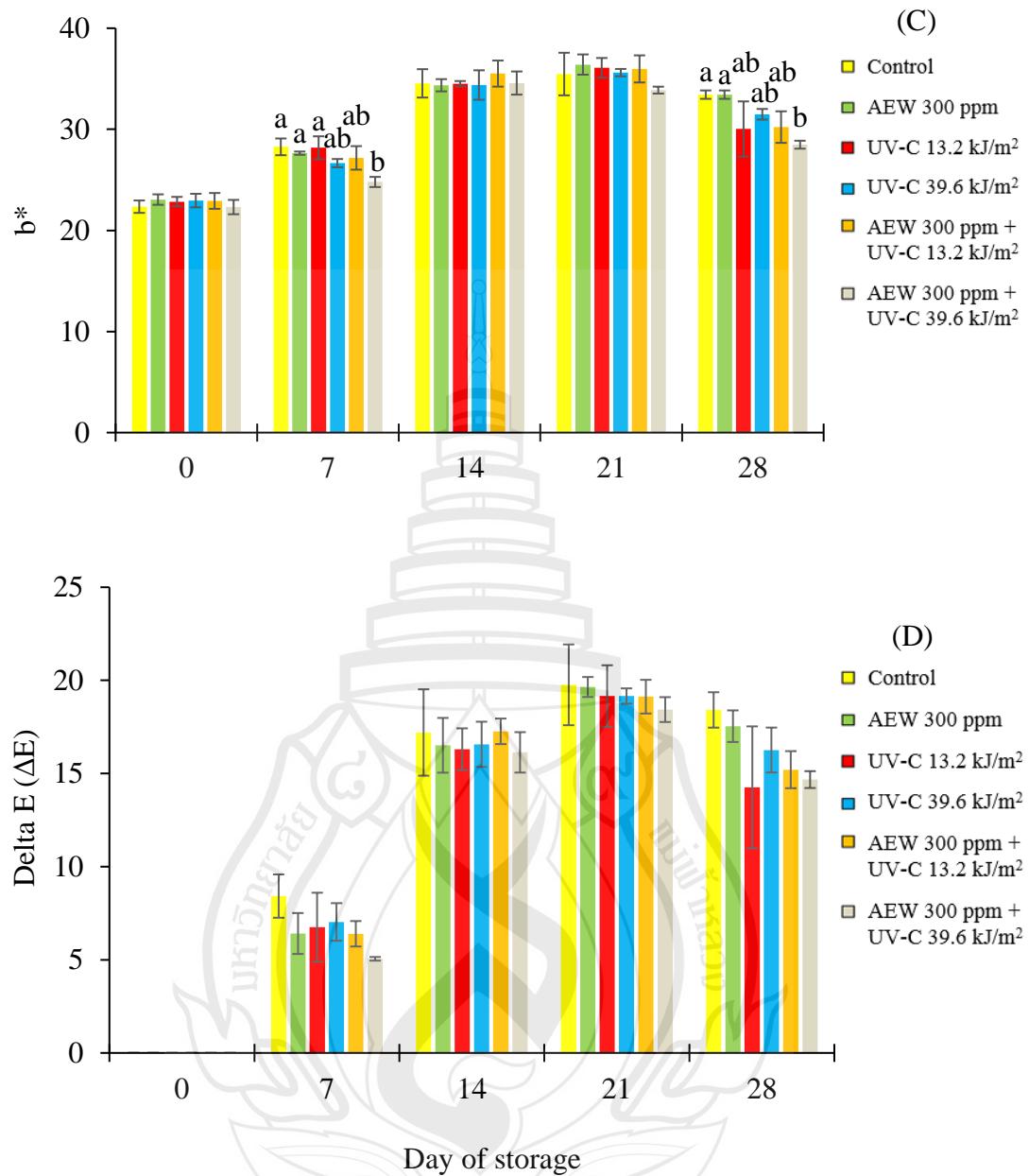


Figure 4.10 (continued)

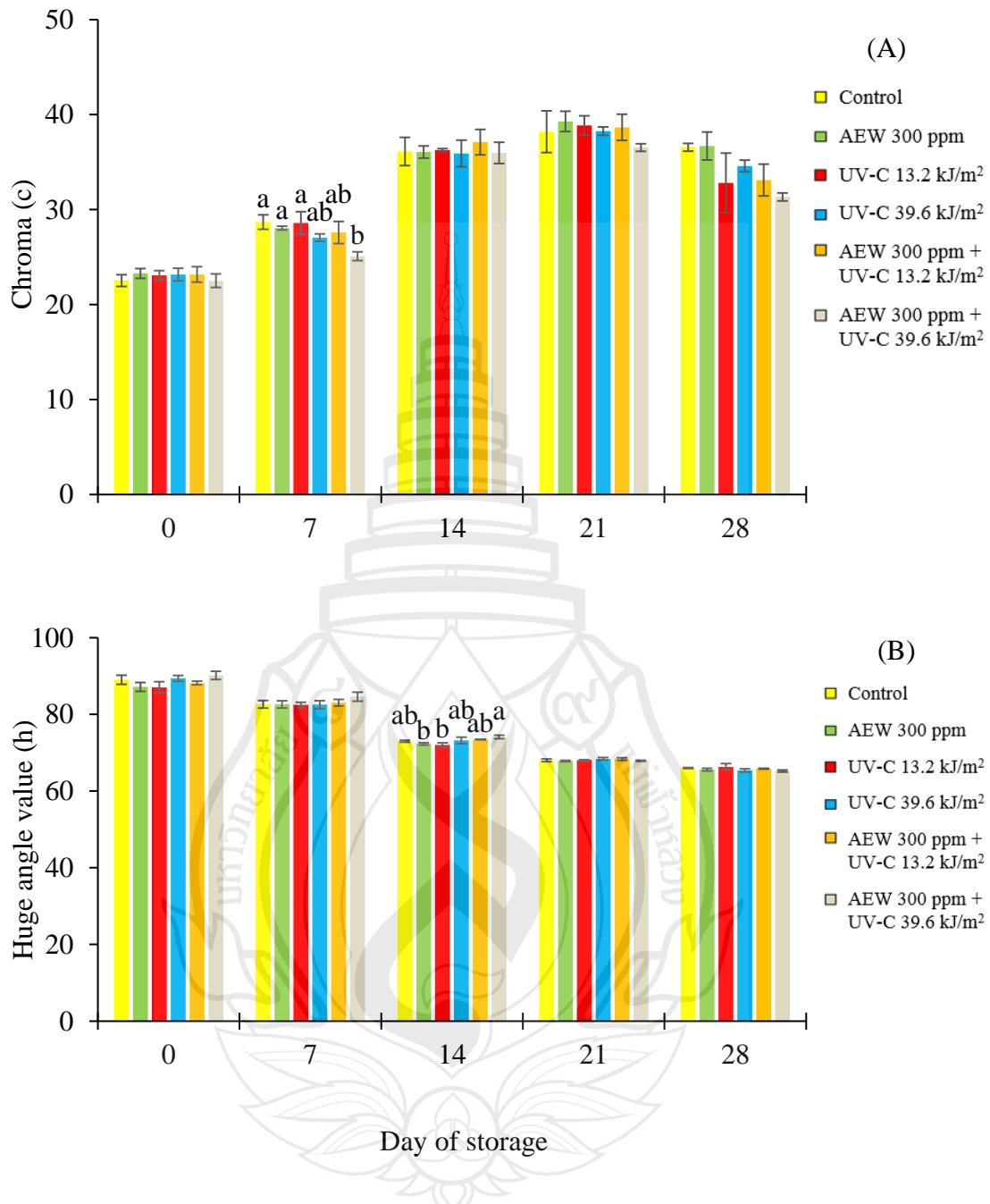


Figure 4.11 Effects of AEW and UV-C treatments on (A) chroma and (B) hue angle value of 'Phulae' pineapple peel during storage at 13 °C, RH 85-95% for 28 days. Each vertical bar represents standard error (SE) of the mean (n=3). Different letters represent significance difference between treatments by using Duncan's multiple range test (P<0.05)

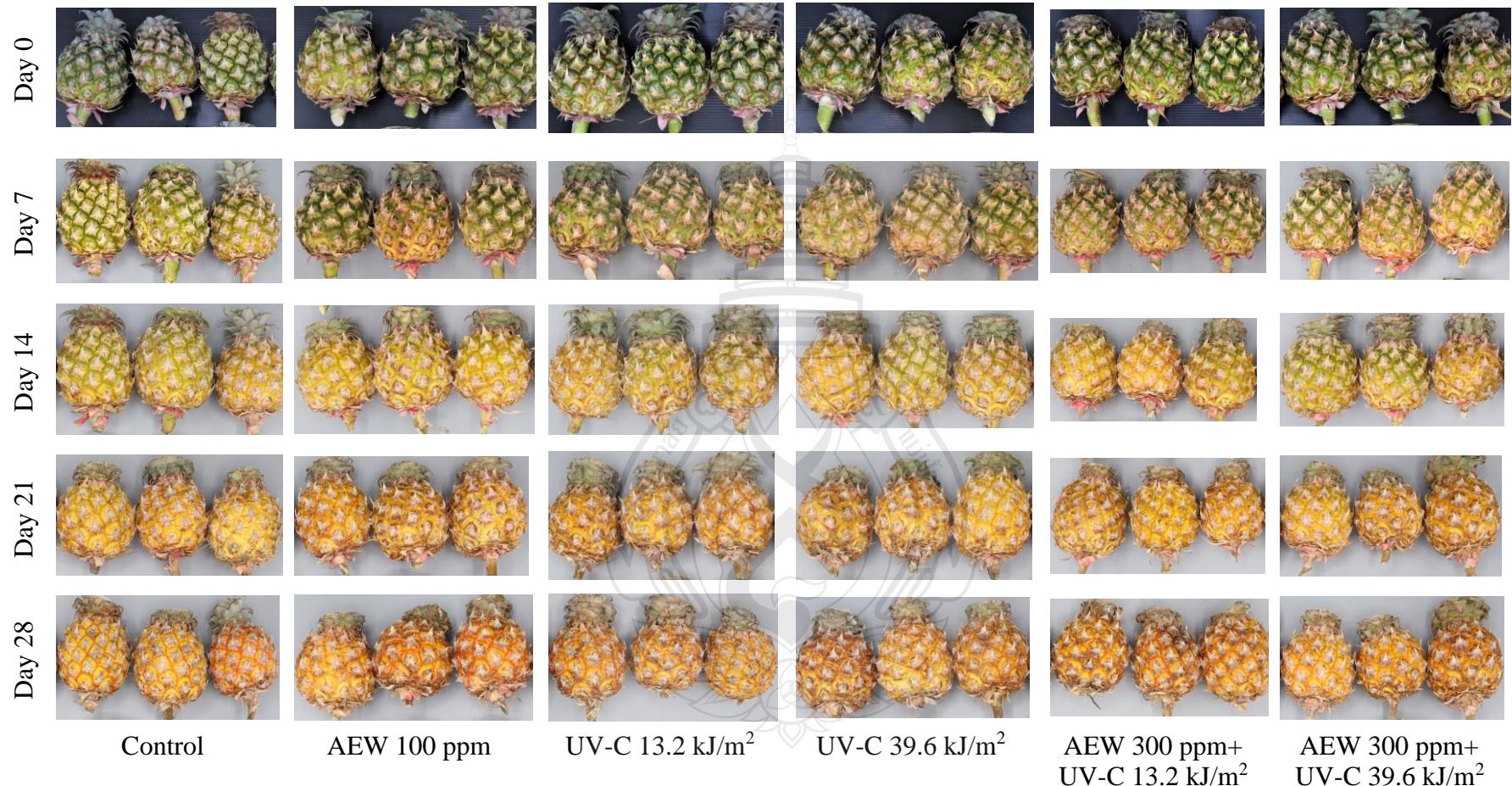


Figure 4.12 Effects of AEW and UV-C treatment on peel colour of 'Phulæ' pineapple fruit during storage at 13 °C for 28 days

4.4.5 Internal Browning Severity

The control group exhibited a continuous rise in the occurrence of internal browning severity (IBS) throughout the storage period, peaking at 61.67% by day 28. This aligns with prior research indicating the susceptibility of 'Phulae' pineapples to IBI during storage (Luengwilai et al., 2016).

The application of AEW (300 ppm) alone did not significantly impact IBS compared to the control group, although it did show a minor delay in browning onset. This implies that while AEW may have some effectiveness against IBS. But it was not entirely adequate for comprehensive control. Studies have suggested AEW's efficacy in reducing postharvest diseases in certain fruits, but its effectiveness against IBS in pineapples is not well-established (Cheng et al., 2023).

Both UV-C doses (13.2 and 39.6 kJ/m^2) had a marginal, though statistically insignificant, effect in decreasing IBI compared to the control group. This indicates a potential for UV-C in IBS control, but the doses utilized in this study were insufficient to yield significant reductions. Previous research has shown UV-C's effectiveness in reducing postharvest diseases in some fruits, emphasizing the importance of determining optimal doses and treatment times based on specific fruits and pathogens (Sari et al., 2016).

The combination of AEW 300 ppm and UV-C 13.2 kJ/m^2 demonstrated IBS levels similar to those observed with individual AEW and UV-C treatments. However, the combination of AEW 300 ppm and UV-C 39.6 kJ/m^2 resulted in the lowest IBS at day 28 (53.33%), suggesting a potential synergistic effect at this higher dose. This may be attributed to AEW enhancing the penetration of UV-C into fruit tissue, leading to a more effective reduction of internal pathogens. Further research is necessary to validate this synergistic effect and determine the optimal combination of AEW and UV-C for IBS control in 'Phulae' pineapples.

These findings imply that AEW and UV-C treatments have the potential to control IBS in 'Phulae' pineapples during a 28-day storage period at 13°C. However, further research is essential to fine-tune treatment combinations and dosages for optimal IBS reduction while preserving fruit quality and marketability.

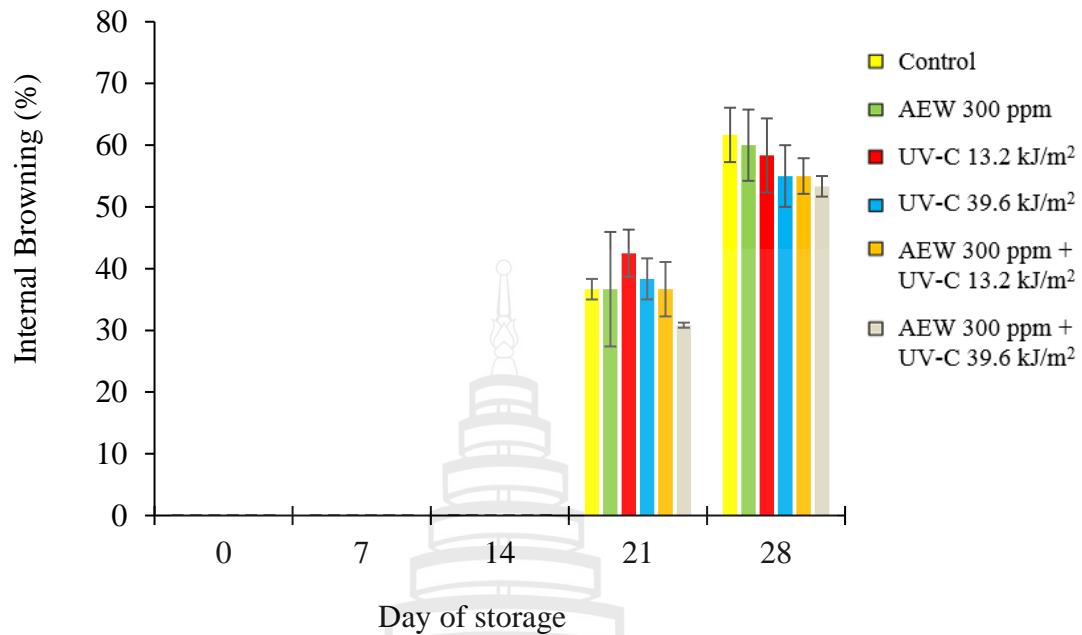


Figure 4.13 Effects of AEW and UV-C treatments on internal browning severity of ‘Phulae’ pineapple fruit during storage at 13 °C, RH 85-95% for 28 days. Each vertical bar represents standard error (SE) of the mean (n=3). Different letters represent significance difference between treatments by using Duncan’s multiple range test (P<0.05)

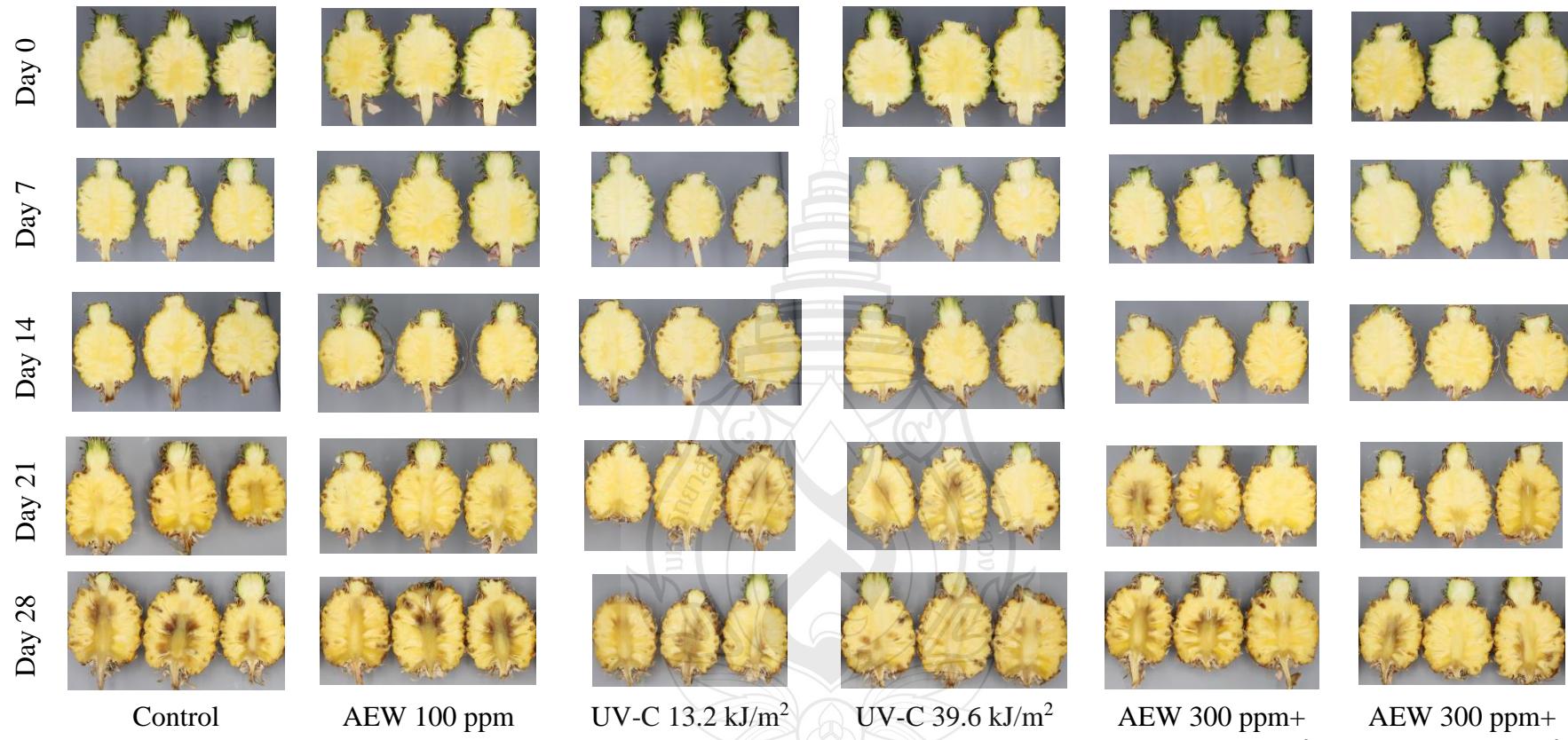


Figure 4.14 Effects of AEW and UV-C on internal browning severity of 'Phulae' pineapple during storage at 13 °C for 28 days

4.4.6 Total Soluble Solids (TSS), Titratable Acidity (TA), TSS/TA, pH

Total Soluble Solids (TSS): In the control group, there was a gradual decline in TSS from 16.27 °Brix on day 0 to 15.29 °Brix on day 28, likely attributable to the natural processes of respiration and sugar conversion during storage. AEW (300 ppm) and both UV-C doses (13.2 and 39.6 kJ/m²) generally maintained TSS levels similar to the control group, indicating minimal impact on sugar metabolism. The combination of AEW 300 ppm and UV-C 13.2 kJ/m² exhibited a slight, non-significant increase in TSS on days 14 and 21 compared to the control group, suggesting a potential synergistic effect. However, further research is necessary for confirmation.

Titratable Acidity (TA): The control group showed a modest increase in TA from 1.19 g/L on day 0 to 1.30 g/L on day 28, a common trend in pineapples as organic acids accumulate during storage. AEW 300 ppm and both UV-C doses displayed TA trends similar to the control group, suggesting minimal impact on acid metabolism. Combination treatments did not significantly influence TA compared to the control group, indicating no interaction between AEW and UV-C on this parameter.

TSS/TA Ratio: The TSS/TA ratio, indicative of the balance between sweetness and tartness, generally decreased in all treatments during storage, reflecting the rise in TA and decline in TSS. The control group exhibited the most substantial decline in TSS/TA ratio, reaching 11.84 on day 28. AEW (300 ppm) and UV-C treatments maintained slightly higher TSS/TA ratios compared to the control group, suggesting potential preservation of the sweetness-to-tartness balance. The combination of AEW (300 ppm) and UV-C (13.2 kJ/m²) maintained the highest TSS/TA ratio on days 14 and 21, potentially indicating a synergistic effect in retaining sweetness.

pH: All treatments showed a minor decrease in pH over storage, with the control group decreasing from 4.06 to 3.69 by day 28, reflecting increasing acidity due to organic acid accumulation. AEW (300 ppm) and both UV-C doses exhibited similar pH changes to the control group, suggesting no significant impact on acidification. Combination treatments did not significantly differ from the control

group in terms of pH, indicating no interaction between AEW and UV-C on this parameter.

The findings imply that AEW (300 ppm) and UV-C treatments alone had minimal impact on the key quality attributes of 'Phulae' pineapple pulp during the 28-day storage period at 13°C. However, the combination of AEW (300 ppm) and UV-C (13.2 kJ/m²) demonstrated a potential synergistic effect in maintaining a higher TSS/TA ratio, suggesting improved preservation of the sweetness-to-tartness balance. Further research involving diverse AEW and UV-C combinations and varying storage durations is crucial to optimizing treatment conditions for maximal quality retention in 'Phulae' pineapples.



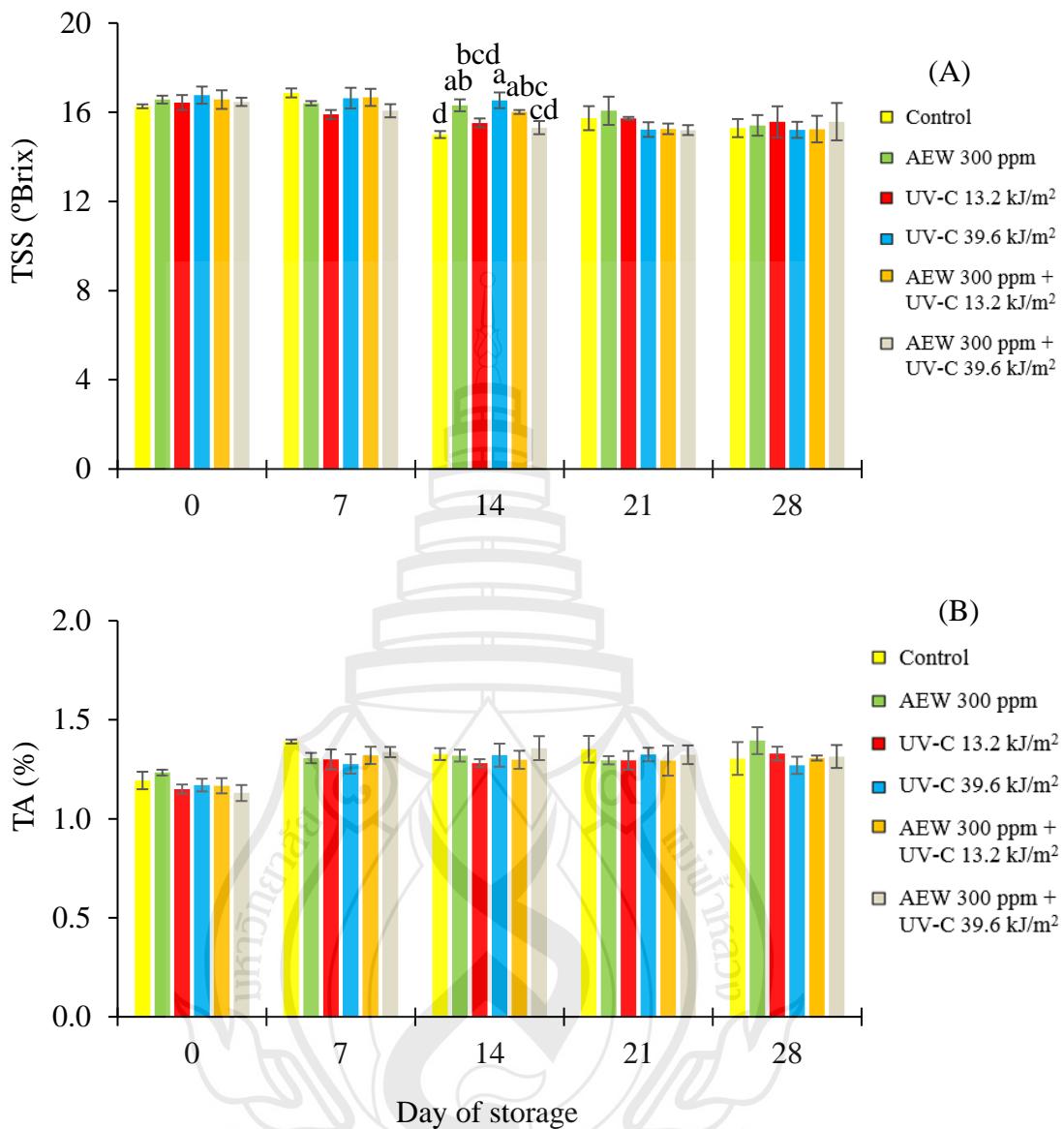


Figure 4.15 Effect of AEW and UV-C treatments on (A) TSS, (B) TA, (C) TSS/TA and (D) pH of eatable 'Phulae' pineapple parts during storage at 13 °C, RH 85-95% for 28 days. Each vertical bar represents standard error (SE) of the mean (n=3). Different letters represent significance difference between treatments by using Duncan's multiple range test (P<0.05)

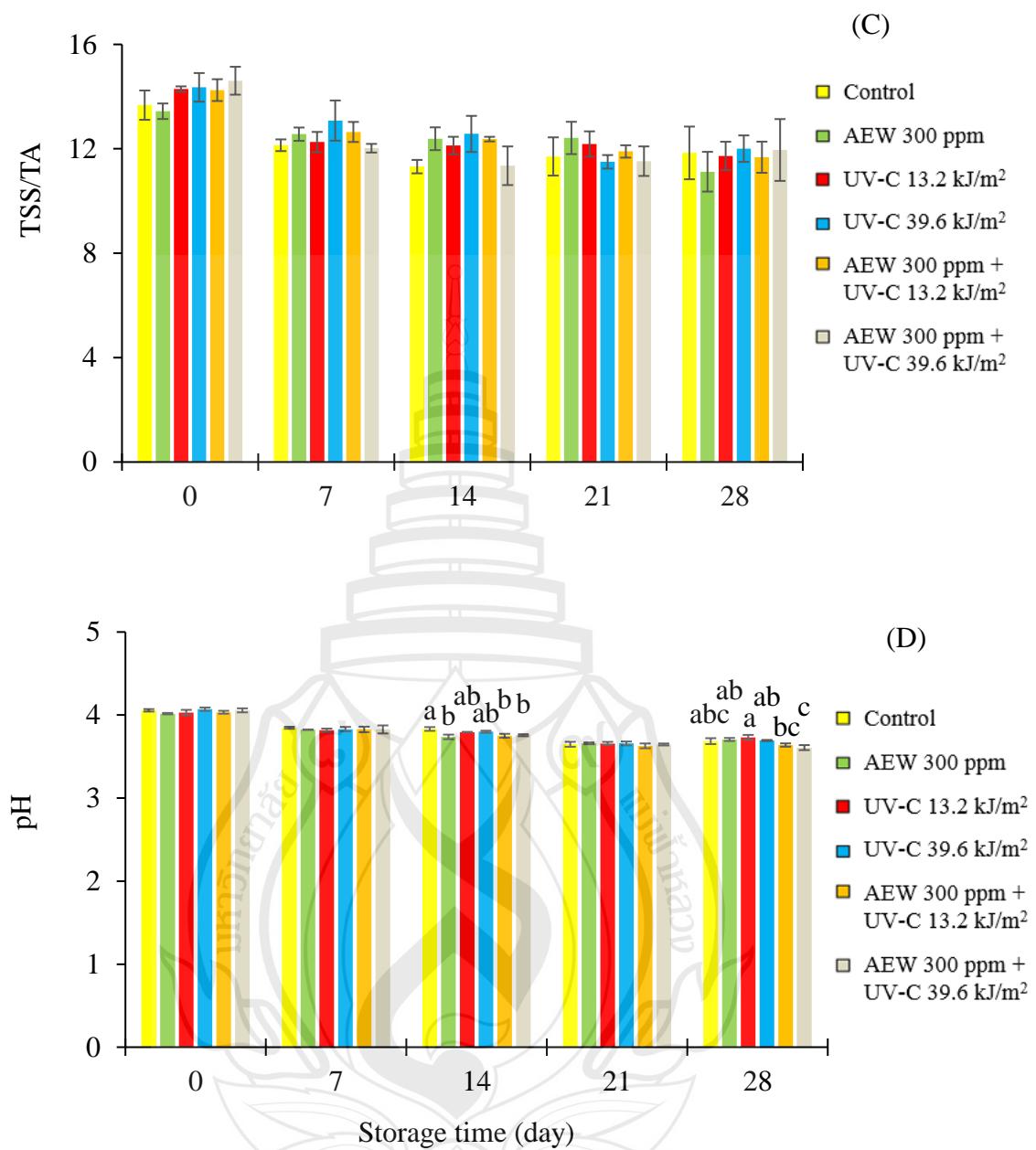


Figure 4.15 (continued)

4.4.7 Vitamin C Content

The control group initially experienced a slight decline in vitamin C content on day 7, followed by a notable increase until day 14, and subsequently, a decrease until day 28. This observed pattern may reflect initial utilization, followed by accumulation during ripening, and eventual degradation during storage.

The AEW treatments-maintained vitamin C levels comparable to the control throughout storage, indicating a minimal impact on its synthesis or degradation.

Both UV-C doses initially elicited significant increases in vitamin C content compared to the control, particularly at day 14. This suggests a potential stress response or an upregulation of biosynthesis pathways induced by UV-C treatment. However, by day 28, vitamin C levels in the UV-C groups had declined to values similar to the control and AEW treatments.

Combining AEW and UV-C resulted in similar vitamin C patterns to individual treatments, displaying an initial increase followed by a decrease by day 28.

The inconsistent impact of AEW and UV-C on vitamin C content throughout storage implies complex interactions between these treatments and pineapple metabolism. The initial increase in vitamin C observed with UV-C might represent a transient stress response or indicate upregulation of biosynthesis pathways. However, further research is required to comprehend the underlying mechanisms and determine the long-term consequences of this response. The decline in vitamin C observed in all treatments, including the control, during later storage aligns with typical postharvest degradation in fruits. Optimizing storage conditions, such as temperature and atmosphere, could potentially alleviate this loss.

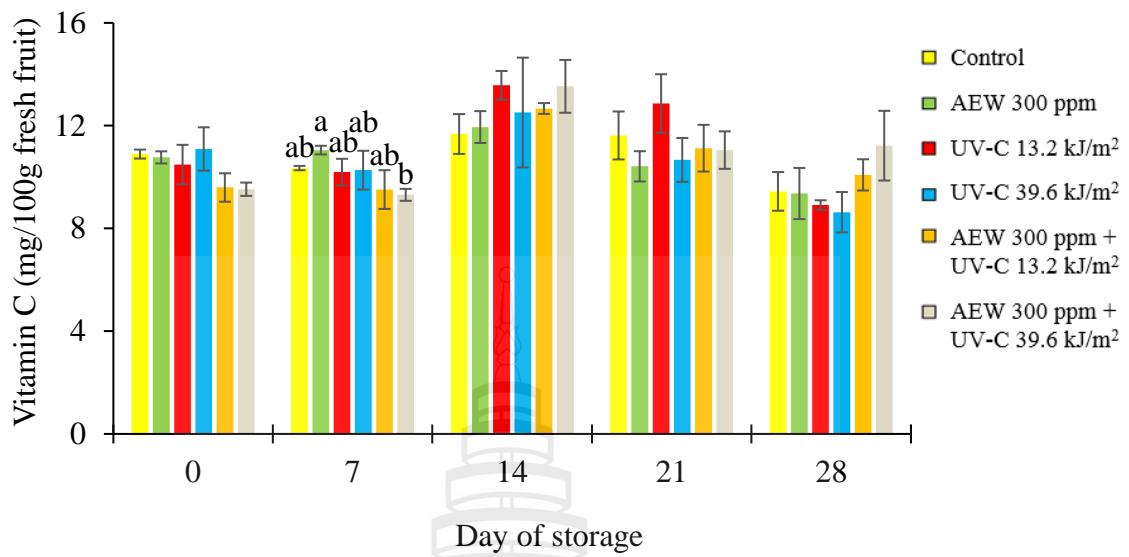


Figure 4.16 Effects of AEW and UV-C treatments on vitamin C content of 'Phulae' pineapple pulp during storage at 13 °C, RH 85-95% for 28 days. Each vertical bar represents standard error (SE) of the mean (n=3). Different letters represent significance difference between treatments by using Duncan's multiple range test (P<0.05)

4.4.8 Total Phenolic Compound (TPC) Content

Pulp: The control group exhibited an initial rise in TPC on day 7, likely attributed to stress-induced synthesis or increased extractability during early storage. This was followed by a gradual decline until day 28, indicative of natural degradation processes. AEW (300 ppm) treatments maintained TPC levels similar to the control throughout storage, indicating minimal impact on phenolic compound metabolism. Both UV-C doses initially induced slight increases in TPC compared to the control, but these effects were transient and did not significantly differ from the control by day 28. The combination of AEW and UV-C did not consistently alter TPC content compared to individual treatments. However, the AEW 300 ppm + UV-C 13.2 kJ/m² combination exhibited a noticeable peak on day 21, suggesting a potential synergistic interaction at this specific dose and timing.

Peel: The control group displayed a substantial increase in TPC throughout storage, possibly due to a stress response, ripening-related synthesis, or improved extractability from the peel matrix. AEW 300 ppm significantly raised TPC in the peel compared to the control, with the highest levels observed at day 28. This implies that AEW potentially stimulated phenolic compound biosynthesis or enhanced their accumulation in the peel. UV-C treatments also significantly increased TPC in the peel, with both doses demonstrating similar effectiveness. This indicates that UV-C exposure, irrespective of dose within the tested range, effectively induced or activated phenolic compound synthesis pathways in the peel. Combining AEW and UV-C further heightened TPC in the peel compared to individual treatments, with the highest levels observed for both combination groups at day 28. This suggests a synergistic effect between AEW and UV-C in promoting phenolic compound accumulation in the peel.

The findings underscore the distinct responses of pineapple pulp and peel to AEW and UV-C concerning TPC content. While pulp TPC remained relatively stable with these treatments, the peel exhibited significant increases, particularly with AEW application and UV-C exposure. These observations imply that AEW and UV-C primarily influence phenolic metabolism in the peel tissue, potentially through stress-induced biosynthesis or enhanced synthesis pathway activity. Optimizing AEW application (concentration and timing) and UV-C dosage could offer promising

strategies for enriching the phenolic content of 'Phulae' pineapple peel, enhancing its potential for functional food and nutraceutical applications. Further research is required to elucidate the specific mechanisms underlying the divergent effects of AEW and UV-C on pulp and peel TPC, as well as to explore the impact of these treatments on individual phenolic compound profiles and their associated bioactivities.



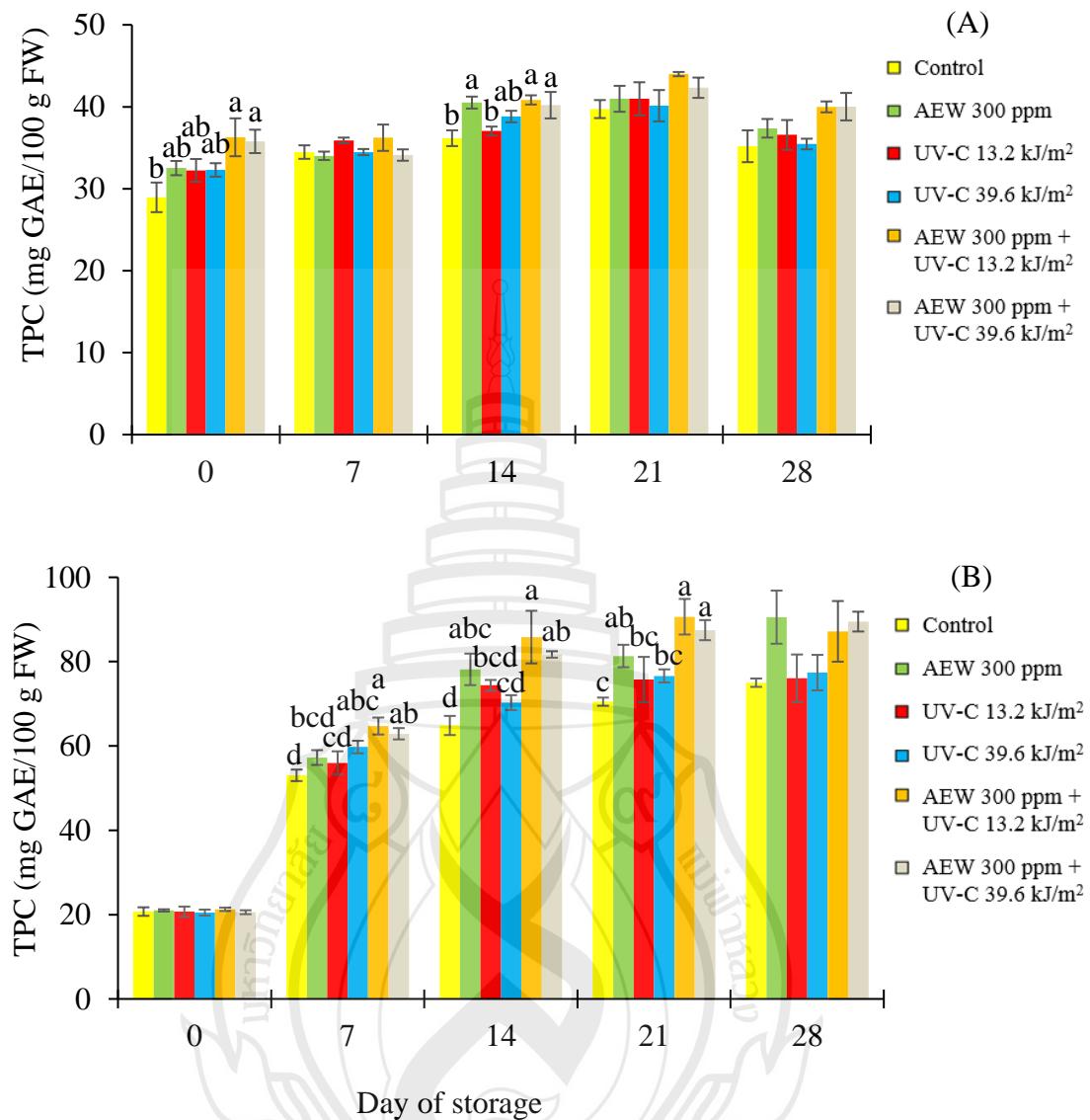


Figure 4.17 Effects of AEW and UV-C on total phenolic compound content of 'Phulae' pineapple (A) pulp and (B) peel during storage at 13 °C, RH 85-95% for 28 days. Each vertical bar represents standard error (SE) of the mean (n=3). Different letters represent significance difference between treatments by using Duncan's multiple range test (P<0.05)

4.4.9 Total Flavonoid Content (TFC)

Pulp: The control group exhibited a consistent increase in TFC throughout storage, possibly indicating natural biosynthesis or increased extractability over time. AEW 300 ppm treatments generally maintained TFC levels similar to the control, suggesting minimal impact on flavonoid metabolism in the pulp. Notably, UV-C treatments at both doses initially led to lower TFC compared to the control on day 7, but by day 28, their levels reached and even exceeded the control. This temporary decline followed by an increase implies a potential stress response or delayed stimulation of flavonoid biosynthesis pathways in the pulp triggered by UV-C exposure. Combining AEW and UV-C did not consistently alter TFC content compared to individual treatments, indicating no clear synergistic or antagonistic effects on flavonoid metabolism in the pulp.

Peel: The control group demonstrated a significant progressive increase in TFC throughout storage, mirroring the pattern observed for TPC. This suggests stress-related synthesis, ripening-associated accumulation, or improved extractability of flavonoids from the peel matrix. AEW 300 ppm significantly raised TFC in the peel compared to the control, with the highest levels observed at day 28. This implies that AEW effectively stimulated or enhanced the accumulation of flavonoids in the peel tissue. UV-C treatments also significantly increased TFC in the peel, with both doses showing similar effectiveness. This indicates that UV-C exposure, irrespective of the dose within the tested range, effectively activated or elevated flavonoid biosynthesis pathways in the peel. Combining AEW and UV-C further boosted TFC in the peel compared to individual treatments, exhibiting the highest levels at day 28 for both combinations. This reinforces the synergistic effect between AEW and UV-C in promoting flavonoid accumulation in the peel.

The findings unveil distinct responses of pineapple pulp and peel to AEW and UV-C concerning TFC. While pulp TFC remained relatively stable with these treatments, the peel exhibited significant increases, particularly with AEW application and UV-C exposure. These observations suggest that AEW and UV-C primarily influence flavonoid metabolism in the peel tissue, potentially through stress-induced biosynthesis or enhanced synthesis pathway activity. Optimizing AEW application (concentration and timing) and UV-C dosage could offer promising strategies for

enriching the flavonoid content of 'Phulae' pineapple peel, enhancing its potential for functional food and nutraceutical applications. Further research is required to elucidate the specific mechanisms underlying the differential effects of AEW and UV-C on pulp and peel TFC, as well as to explore the impact of these treatments on individual flavonoid compound profiles and their associated bioactivities.

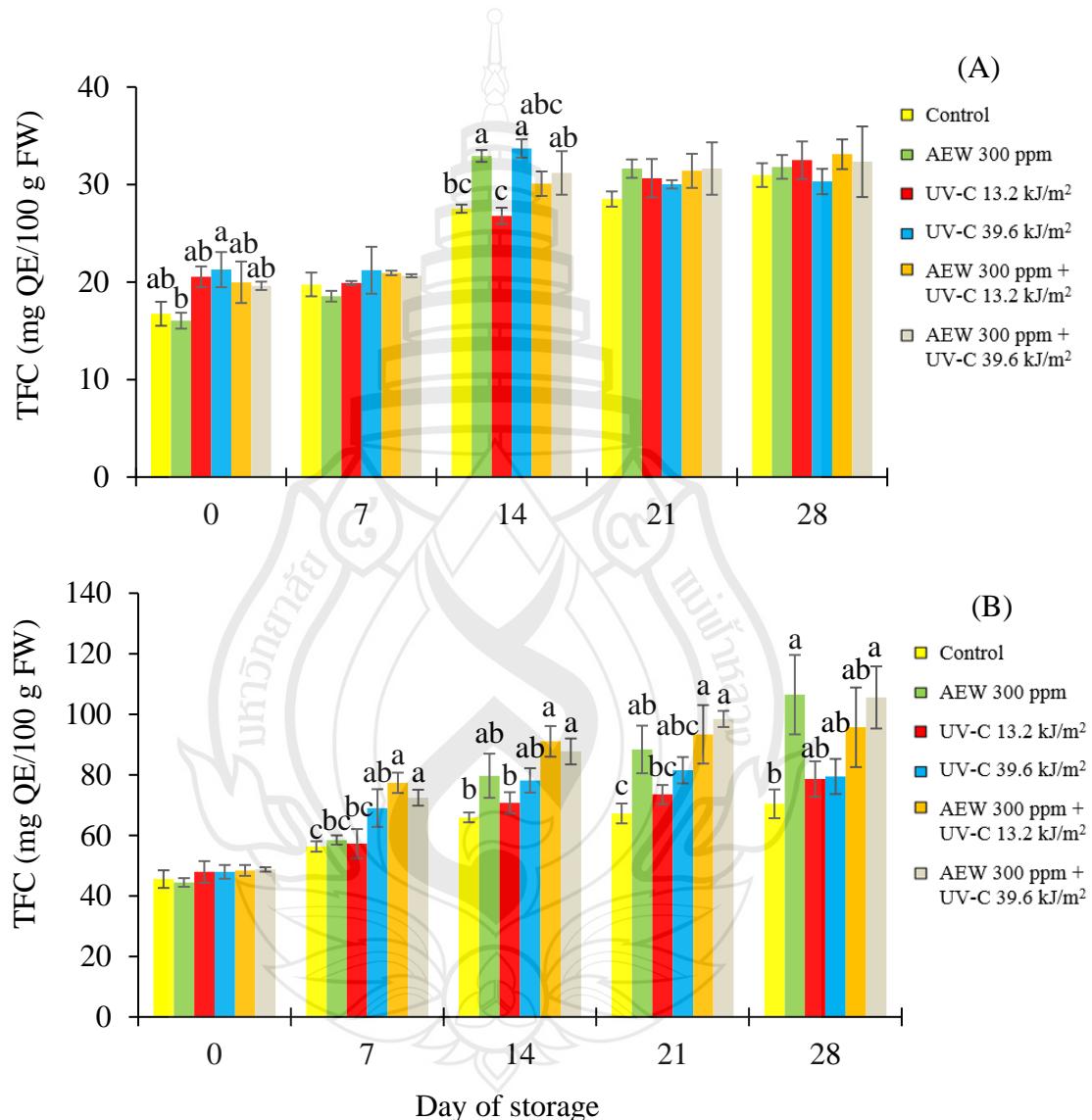


Figure 4.18 Effect of AEW and UV-C treatments on total flavonoid content of 'Phulae' pineapple (A) pulp and (B) peel during storage at 13 °C, RH 85-95% for 28 days. Each vertical bar represents standard error (SE) of the mean (n=3). Different letters represent significance difference between treatments by using Duncan's multiple range test (P<0.05)

4.4.10 Antioxidant Activity (DPPH)

Pulp: The control group displayed a notable increase in antioxidant activity (AA) on day 7, likely stemming from stress-induced synthesis of antioxidant compounds. This was followed by a decline towards day 28, indicative of natural degradation processes. AEW 300 ppm treatments generally maintained AA levels akin to the control, suggesting minimal impact on overall antioxidant production in the pulp. UV-C treatments at both doses initially induced minor increases in AA compared to the control on day 7, but these effects were short-lived and did not significantly differ from the control by day 28. This suggests a brief stress response without sustained enhancement of antioxidant machinery in the pulp. Combining AEW and UV-C exhibited moderate and inconsistent effects on AA compared to individual treatments. Notably, the AEW 300 ppm + UV-C 13.2 kJ/m² combination showed heightened AA compared to the control on day 14 and 21, suggesting a potential synergistic interaction at this specific dose and timing. However, further investigations are needed to validate this observation.

Peel: The control group demonstrated a significant progressive increase in AA throughout storage, mirroring the observed patterns for TPC and TFC. This implies stress-related synthesis, ripening-associated accumulation, or improved extractability of antioxidant compounds from the peel matrix. AEW 300 ppm significantly elevated AA in the peel compared to the control, with the highest levels observed at day 28. This indicates that AEW potentially stimulated biosynthesis or enhanced the accumulation of antioxidants in the peel tissue. UV-C treatments also significantly increased AA in the peel, with both doses showing similar effectiveness. This suggests that UV-C exposure, regardless of the dose within the tested range, effectively activated or elevated antioxidant synthesis pathways in the peel. Combining AEW and UV-C further boosted AA in the peel compared to individual treatments, reaching the highest levels at day 28 for both combinations. This reinforces the synergistic effect between AEW and UV-C in promoting antioxidant accumulation in the peel.

The findings unveil a distinct response of pineapple pulp and peel to AEW and UV-C concerning AA. While pulp AA remained relatively stable with these treatments, the peel exhibited significant increases, particularly with AEW application

and UV-C exposure. These observations underscore the differential impact of these treatments on antioxidant metabolism across different pineapple tissues. AEW and UV-C primarily influence antioxidant production in the peel tissue, potentially through stress-induced biosynthesis or enhanced synthesis pathway activity. Optimizing AEW application (concentration and timing) and UV-C dosage could offer promising strategies for enriching the antioxidant content of 'Phulae' pineapple peels, increasing their potential applications as functional food ingredients and nutraceuticals. Further research is needed to elucidate the specific mechanisms underlying the divergent effects of AEW and UV-C on pulp and peel AA, as well as to explore the impact of these treatments on individual antioxidant compound profiles and their associated bioactivities.

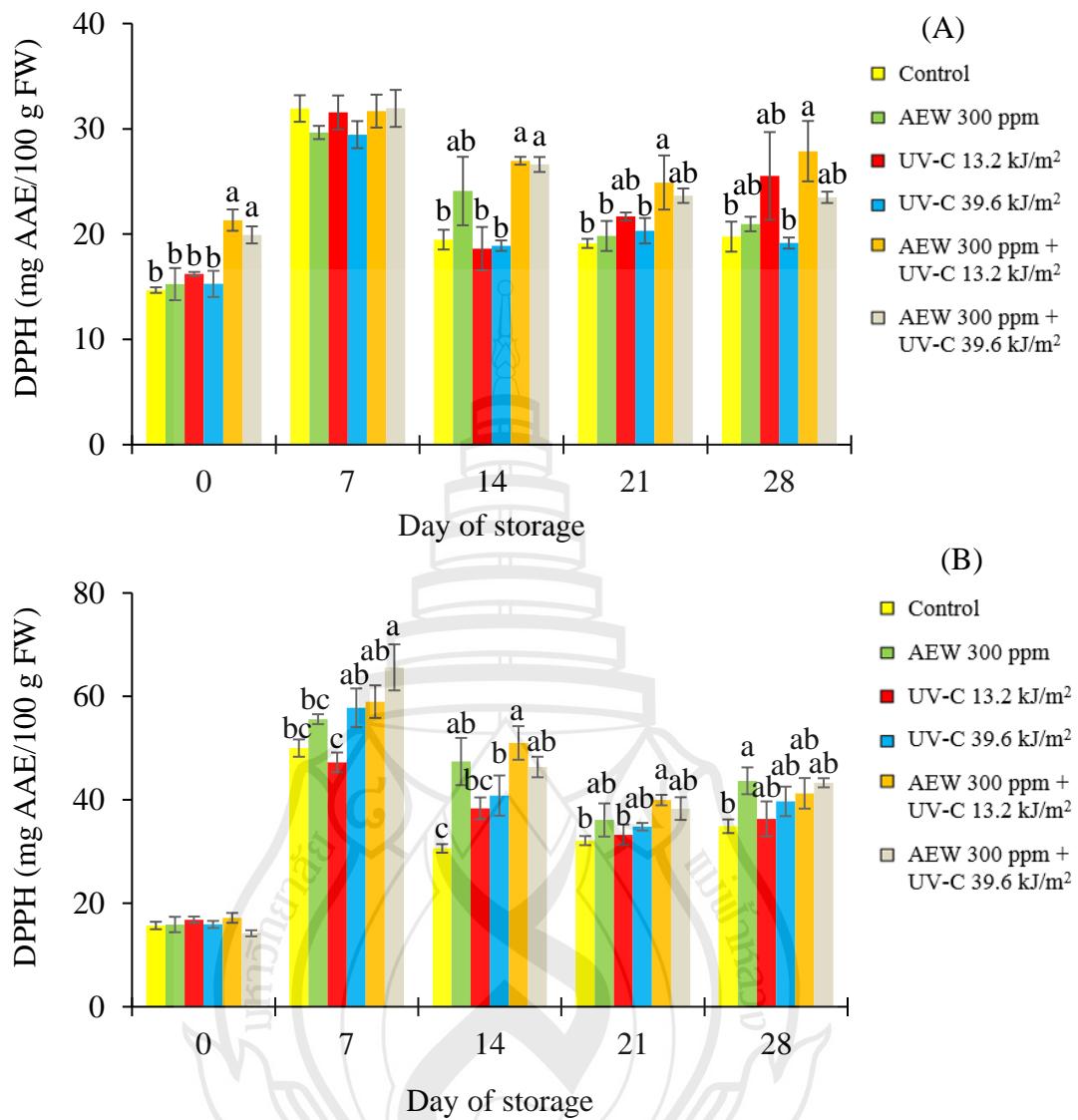


Figure 4.19 Effects of AEW and UV-C on antioxidant activity (DPPH) of 'Phulae' pineapple (A) pulp and (B) peel during storage at 13 °C, RH 85-95% for 28 days. Each vertical bar represents standard error (SE) of the mean (n=3). Different letters represent significance difference between treatments by using Duncan's multiple range test (P<0.05)

4.4.11 Antioxidant Activity (FRAP)

Pulp: The control group displayed a gradual rise in FRAP values during storage, indicating a slow and steady accumulation of ferric reducing antioxidant power (FRAP) compounds. AEW 300 ppm treatments consistently maintained FRAP levels similar to the control, suggesting minimal impact on overall FRAP activity in the pulp. UV-C treatments at both doses initially led to transient increases in FRAP on day 7 compared to the control, but these effects did not significantly differ from the control by day 28. This suggests a brief stress response without sustained enhancement of FRAP-active compounds in the pulp. Notably, the AEW 300 ppm + UV-C 13.2 kJ/m² combination significantly increased FRAP compared to the control from day 14 onwards, particularly at day 28. This implies a potential synergistic interaction between AEW and UV-C at this specific dose and timing, promoting higher FRAP activity in the pulp. However, further investigations are needed to confirm and elucidate the underlying mechanisms.

Peel: The control group exhibited a dramatic surge in FRAP throughout storage, surpassing pulp values at later stages. This underscores the significant contribution of the peel to the overall FRAP activity of pineapple fruit and suggests stress-related synthesis, ripening-associated accumulation, or improved extractability of FRAP-active compounds during storage. AEW 300 ppm significantly increased FRAP in the peel compared to the control, with the highest levels observed at day 28. This indicates that AEW effectively stimulated the biosynthesis or enhanced the accumulation of FRAP-active compounds in the peel tissue. UV-C treatments also significantly increased FRAP in the peel, with both doses showing similar effectiveness. This suggests that UV-C exposure, irrespective of the dose within the tested range, effectively activated or elevated FRAP synthesis pathways in the peel. Combining AEW and UV-C further boosted FRAP in the peel compared to individual treatments, exhibiting the highest levels at day 28 for both combinations. This reinforces the synergistic effect between AEW and UV-C in promoting FRAP activity in the peel.

The findings highlight a distinct response of pineapple pulp and peel to AEW and UV-C in terms of FRAP activity. While pulp FRAP remained relatively stable with these treatments, the peel exhibited significant increases, particularly with AEW

application and UV-C exposure. These observations underscore the differential impact of these treatments on antioxidant metabolism across different pineapple tissues. AEW and UV-C primarily influence FRAP activity in the peel tissue, potentially through stress-induced biosynthesis or enhanced synthesis pathway activity. Optimizing AEW application (concentration and timing) and UV-C dosage could offer promising strategies for enriching the FRAP activity of 'Phulae' pineapple peels, expanding their potential applications as functional food ingredients and nutraceuticals. Further research is needed to elucidate the specific mechanisms underlying the differential effects of AEW and UV-C on pulp and peel FRAP activity, as well as explore the impact of these treatments on individual FRAP-active compound profiles and their associated bioactivities.

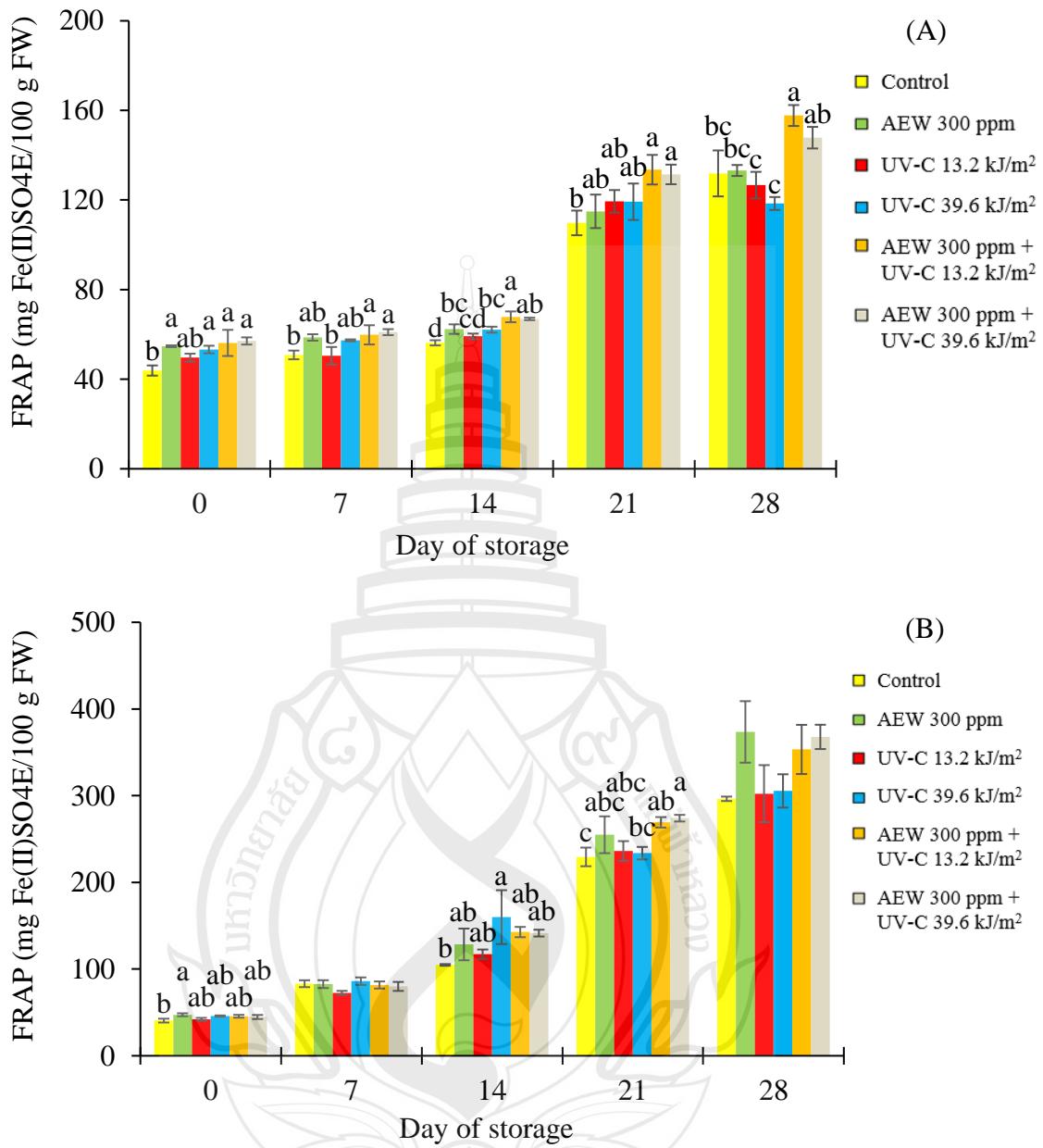


Figure 4.20 Effects of AEW and UV-C on antioxidant activity (FRAP) of 'Phulae' pineapple (A) pulp and (B) peel during storage at 13 °C, RH 85-95% for 28 days. Each vertical bar represents standard error (SE) of the mean (n=3). Different letters represent significance difference between treatments by using Duncan's multiple range test (P<0.05)

4.5 Germicidal Effects of AEW and UV-C: *In vitro* Study

4.5.1 Spore Survival

Control: The control group showed a consistent increase in spore survival over time, reaching 1.56 million CFU/mL at 48 hours. This indicates that the storage conditions provided a conducive environment for the germination and proliferation of *C. paradoxa* spores. AEW 300 ppm: AEW exhibited a remarkable fungicidal effect against *C. paradoxa*, completely inhibiting spore survival at all time points. This suggests that AEW disrupts essential cellular processes or damages the spore wall, preventing germination and growth. UV-C treatments: Both UV-C doses (13.2 and 39.6 kJ/m²) significantly reduced spore survival compared to the control, but their effectiveness varied over time. At 12 and 24 hours, UV-C exposure led to comparable reductions in spore count compared to AEW. However, by 36 and 48 hours, spore survival in UV-C-treated samples surpassed those treated with AEW alone. This could indicate initial stress-induced dormancy or repair mechanisms in *C. paradoxa* spores following UV-C exposure, leading to delayed germination and higher spore counts at later time points. Combined treatments: Combining AEW with UV-C did not confer any additional advantage compared to AEW alone. Spore survival remained undetectable throughout the storage period. This suggests that AEW's fungicidal effect was predominant, potentially masking any further reduction from UV-C at this concentration and timing.

The findings underscore the potential of both AEW and UV-C as antifungal treatments against *C. paradoxa*. AEW demonstrated exceptional fungicidal activity, completely eliminating spore survival, possibly through the disruption of cellular processes or membrane damage. UV-C treatment, while initially effective, showed delayed germination and higher spore counts at later stages, indicating potential stress-induced dormancy or repair mechanisms in *C. paradoxa* spores. The combination of AEW and UV-C did not exhibit a synergistic effect in this study, possibly due to the overwhelming fungicidal action of AEW.

Table 4.1 Effects of AEW and UV-C treatments on spore survival of *C. paradoxa* during storage at 27 °C for 48 h

Treatments	Spore Survival (CFU/mL)			
	12 h	24 h	36 h	48 h
Control	0	130000 ± 2236 b	1380000 ± 89554 b	1560000 ± 93434 b
AEW 300 ppm	0	800 ± 84 a	1200 ± 84 a	1400 ± 114 a
UV-C 13.2 kJ/m ²	0	9000 ± 474 a	16600 ± 483 a	62000 ± 1789 a
UV-C 39.6 kJ/m ²	0	4400 ± 270 a	11000 ± 212 a	30000 ± 2000 a
AEW 300 ppm + UV-C 13.2 kJ/m ²	0	1600 ± 89 a	2000 ± 122 a	2000 ± 122 a
AEW 300 ppm + UV-C 39.6 kJ/m ²	0	2800 ± 303 a	16000 ± 894 a	16000 ± 894 a

Note Values are mean ± standard error from n = 5. Different letters within the same column differ significantly within treatments by using Duncan's multiple range test (P<0.05)

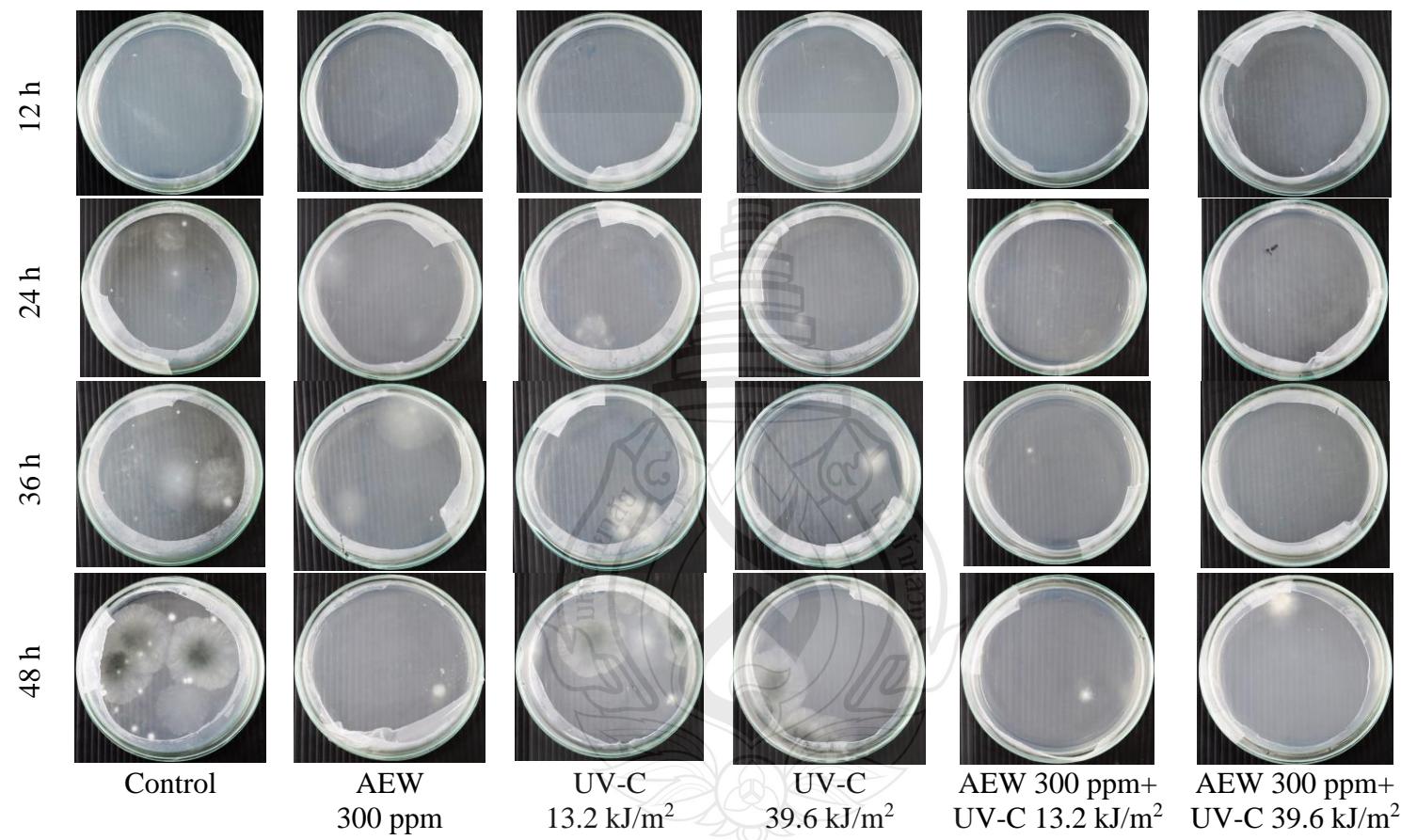


Figure 4.21 Effects of AEW and UV-C treatments on spore survival of *C. paradoxa* during storage at 27 °C for 48 h

4.5.2 Spore germination ratio

The data indicates that both AEW and UV-C treatments effectively hindered the spore germination of *C. paradoxa* compared to the control during the 15-hour of incubation period. The following breakdown outlines the observations and potential explanations:

Control: The control group displayed a typical sigmoidal spore germination pattern, with the germination rate gradually increasing over time, reaching 55.81 % by 15 hours. This suggests that favourable incubation conditions allowed *C. paradoxa* spores to germinate and initiate mycelial growth.

AEW 300 ppm: AEW significantly suppressed spore germination at all time points compared to the control, with the germination rate remaining under 20.3% even after 15 hours of incubation. This implies that AEW disrupts crucial metabolic processes or damages the spore wall, preventing the emergence of germ tubes and subsequent hyphal growth.

UV-C treatments: Both UV-C doses (13.2 and 39.6 kJ/m^2) significantly reduced spore germination compared to the control, although their effectiveness varied over time. At 3 and 6 hours, the lower UV-C dose showed less inhibition compared to AEW, but by 9 hours, both doses achieved similar levels of germination reduction. Interestingly, by 15 hours, the higher UV-C dose exhibited slightly higher germination compared to the lower dose and AEW. This could be attributed to the biphasic effects of UV-C, where low doses primarily inhibit germination, while higher doses can induce stress responses or DNA damage repair mechanisms, leading to delayed germination in some spores.

Combined treatments: Combining AEW with UV-C did not confer any additional advantage compared to AEW alone at most time points. However, the AEW + UV-C 13.2 kJ/m^2 combination showed the lowest germination rate (14.3%) at 15 hours, even slightly lower than AEW alone. This suggests a possible synergistic interaction at this specific dose and timing, warranting further investigation.

The findings underscore the potential of both AEW and UV-C as antifungal treatments against *C. paradoxa* by inhibiting spore germination. AEW demonstrated remarkable efficacy, consistently keeping germination below 20 %, possibly due to the disruption of essential cellular functions or membrane damage. UV-C treatment,

while effective, exhibited a biphasic response with delayed germination at higher doses, indicating potential stress-induced dormancy or repair mechanisms in *C. paradoxa* spores. The combination of AEW and UV-C showed limited but potential synergistic effects at the lower dose, necessitating further exploration to optimize the combined treatment for enhanced antifungal activity.

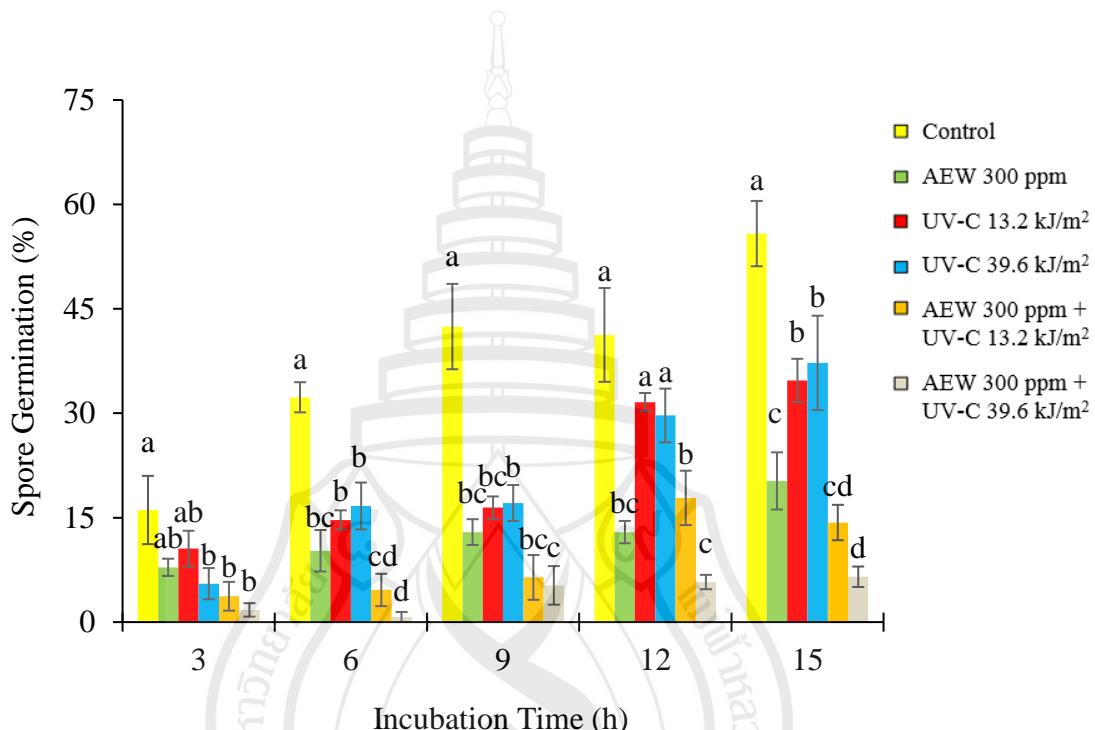


Figure 4.22 Effects of AEW and UV-C on spore germination (%) of *C. paradoxa* during incubated for 15 h at 25 °C. Each vertical bar represents standard error of the mean (n=3). Different letters represent significance difference between treatments by using Duncan's multiple range test (P<0.05)

4.5.3 Germ Tube Length

Figure 4.23 and 4.24 shown that germ tube length in the control group displayed a typical sigmoidal growth pattern, with the length of germ tubes steadily increasing over time, reaching 843.37 µm by 13 hours. This suggests that favourable incubation conditions facilitated the rapid germination and extension of germ tubes in *C. paradoxa* spores.

Effects of AEW: AEW exerted a complete inhibition of germ tube elongation at all time points, consistently registering zero μm values throughout the 13-hour incubation. This exceptional antifungal efficacy implies that AEW disrupts critical developmental processes or damages the spore wall, preventing the emergence of germ tubes and subsequent hyphal growth.

Effects of UV-C: Both UV-C doses (13.2 and 39.6 kJ/m^2) significantly suppressed germ tube length compared to the control, but their effectiveness varied over time. At 5 and 7 hours, both doses markedly hindered germ tube elongation compared to the control, while AEW remained highly effective at complete inhibition. By 9 and 11 hours, although still significantly lower than the control, both UV-C doses showed some recovery in germ tube growth, highlighting the biphasic response of UV-C exposure. By 13 hours, both UV-C doses achieved germ tube lengths similar to the control group, indicating potential repair mechanisms or delayed germination in some UV-C-treated spores.

Effects of combined treatments: Combining AEW with UV-C did not confer any additional advantage compared to AEW alone. Notably, all combined treatments also exhibited a germ tube length of 0 μm at all time points. This suggests that AEW's potent antifungal activity dominated the combined treatment, overshadowing any potential synergistic effect at these specific doses and timing.

The findings underscore the remarkable fungicidal activity of AEW against *C. paradoxa* completely inhibiting germ tube elongation and subsequent mycelial growth throughout the incubation period. UV-C exposure, while initially effective in suppressing germ tube length, exhibited a biphasic response with delayed growth or potential repair mechanisms in some spores, ultimately reaching similar levels as the control by 13 hours. The combination of AEW and UV-C did not show any synergistic effect in this study, possibly due to AEW's overwhelming efficacy masking any further reduction from UV-C at these given parameters.

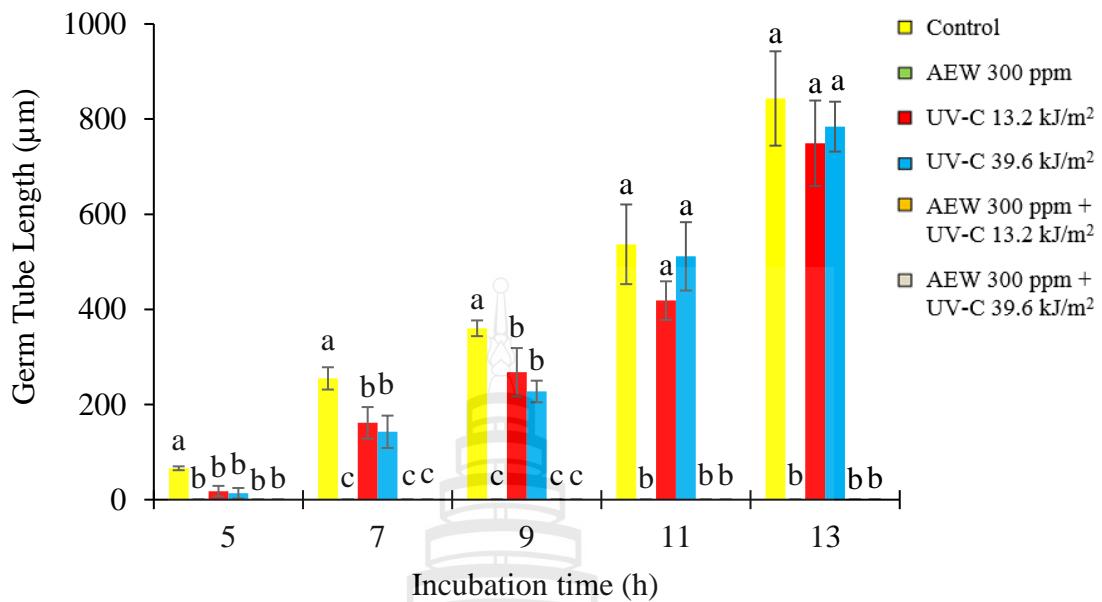


Figure 4.23 Effects of AEW and UV-C on germ tube length (μm) of *C. paradoxa* during incubated for 13 h at 25 °C. Each vertical bar represents standard error (SE) of the mean (n=3). Different letters represent significance difference between treatments by using Duncan's multiple range test (P<0.05)

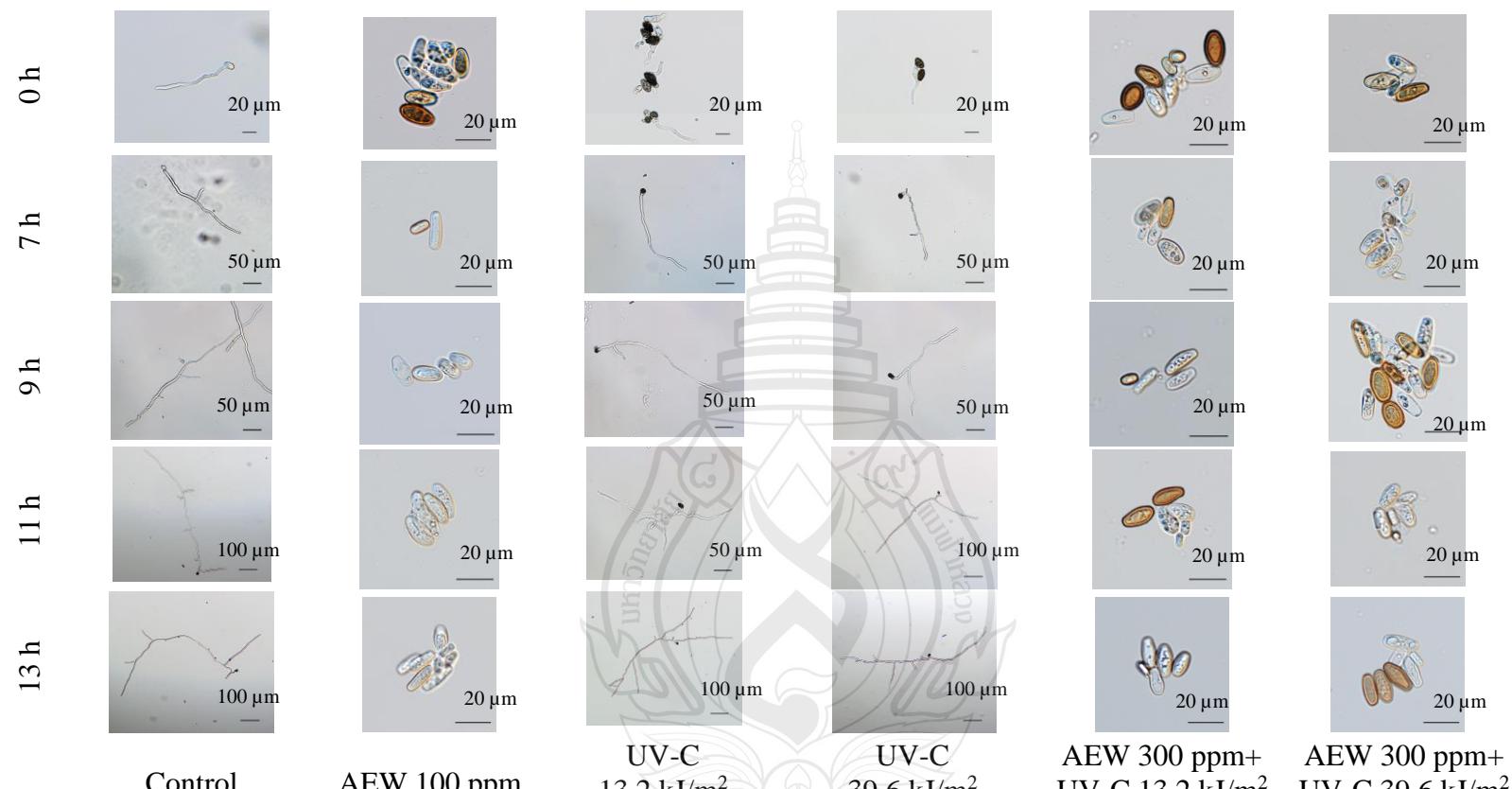


Figure 4.24 Effects of AEW and UV-C on germ tube length (μm) of *C. paradoxa* during incubation for 13 h at 25 °C

4.5.4 Mycelium Disc Growth Inhibition

The control group exhibited unhindered mycelium growth throughout the 7-day storage period, indicating conducive conditions for the expansion of *C. paradoxa* mycelia and underscoring the necessity for antifungal interventions to regulate its proliferation.

AEW displayed remarkable antifungal activity, resulting in complete inhibition of mycelium disc growth (100 % inhibition) on all observation days. This suggests that AEW disrupts essential fungal growth processes or inflicts damage to hyphae, effectively preventing the expansion of mycelia.

Both UV-C doses (13.2 and 39.6 kJ/m²) initially restrained mycelium growth, but their efficacy significantly diminished over time. On day 1, both doses exhibited moderate inhibition compared to the control but significantly lower than AEW. By day 2, the higher UV-C dose maintained some inhibitory effect, while the lower dose displayed minimal impact. Intriguingly, from day 3 onwards, both UV-C treatments completely lost their inhibitory activity, allowing mycelial growth to resume freely, similar to the control. This biphasic response suggests initial damage or stress-induced dormancy in UV-C-treated mycelia, followed by potential repair mechanisms or adaptation, leading to restored growth later in storage.

Combining AEW with UV-C achieved sustained and complete inhibition of mycelium growth (100 %) throughout the 7-day storage period, akin to the impact of AEW alone. This indicates no additional advantage or synergistic effect in this context. AEW's potent antifungal activity likely prevailed in the combined treatment, overshadowing any potential contribution from UV-C at these specific doses and timing.

The findings underscore the exceptional fungicidal efficacy of AEW against *C. paradoxa*, completely halting mycelial growth for an extended duration. UV-C exposure initially curtailed mycelial expansion, possibly due to cellular damage or a stress response, but its effectiveness was transient, with complete recovery and uninhibited growth observed later in storage. The combination of AEW and UV-C did not reveal any synergistic effect in this study, potentially because AEW's overwhelming activity obscured any further contribution from UV-C.

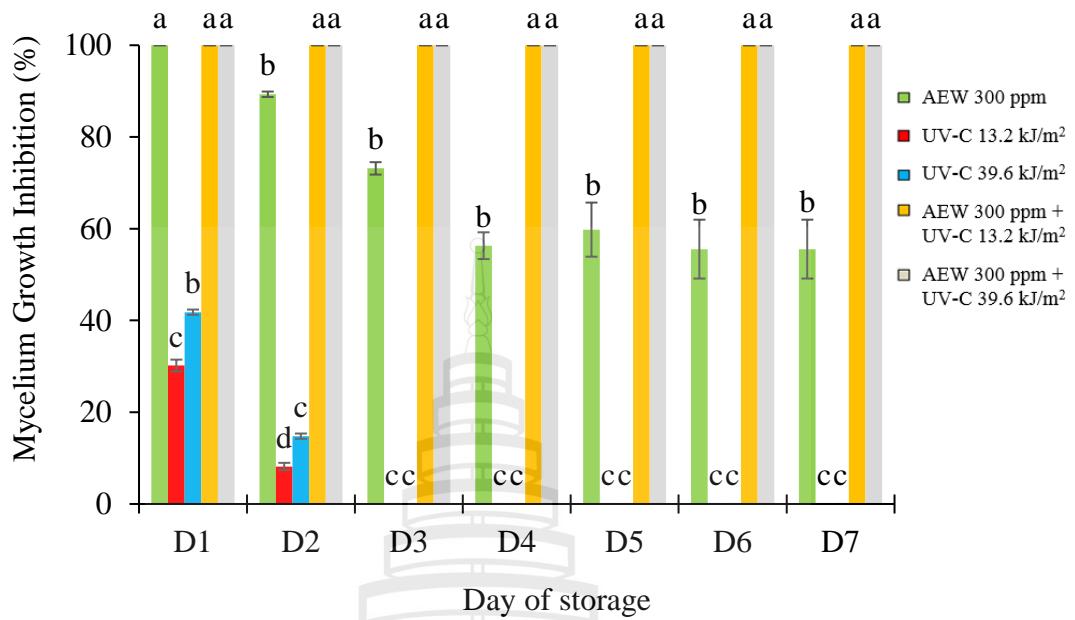


Figure 4.25 Effects of AEW and UV-C on mycelium disc growth inhibition during storage for 7 days at 27 °C. Each vertical bar represents standard error (SE) of the mean (n=3). Different letters represent significance difference between treatments by using Duncan's multiple range test (P<0.05)

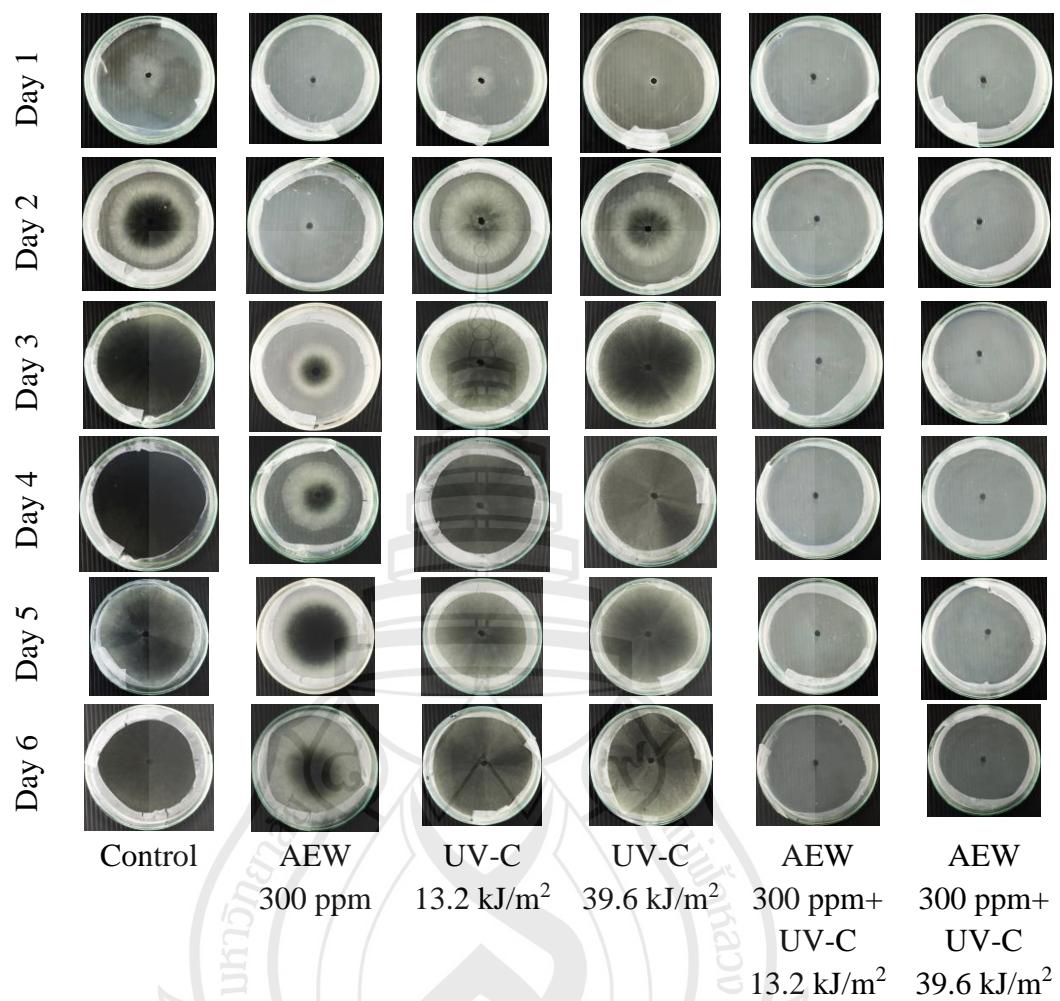


Figure 4.26 Effects of AEW and UV-C treatments on mycelium disc growth inhibition of *C. paradoxa* during storage at 27 °C for 7 days

CHAPTER 5

CONCLUSION

In conclusion, the extensive examination of Acidic Electrolyzed Water (AEW) and Ultraviolet-C (UV-C) irradiation as postharvest treatments for 'Phulæ' pineapples provides significant insights into their impact on quality parameters and antifungal properties. Key fruit quality indicators such as total soluble solids (TSS), titratable acidity (TA), TSS/TA ratio, and pH were minimally affected by AEW and UV-C treatments, indicating compatibility in preserving these attributes. Particularly, the combination of AEW and UV-C at 13.2 kJ/m² demonstrated a potential synergistic effect in maintaining a higher TSS/TA ratio, suggesting improved preservation of the sweetness-to-tartness balance.

Vitamin C content exhibited inconsistent impacts with AEW and UV-C treatments. AEW maintained levels comparable to the control, while UV-C initially increased content, followed by a decline, revealing a complex interaction with pineapple metabolism. Further research is needed to understand mechanisms and optimize storage conditions for vitamin C retention.

AEW and UV-C influenced total phenolic compound (TPC) content and total flavonoid content (TFC) in both pulp and peel, with peel showing more significant increases. This indicates that AEW and UV-C primarily influence phenolic and flavonoid metabolism in the peel, potentially through stress-induced biosynthesis or enhanced synthesis pathways.

Antioxidant activity, assessed through DPPH and FRAP assays, displayed a distinct response in pulp and peel. AEW and UV-C had minimal impact on pulp antioxidant activity, while both treatments significantly increased antioxidant activity in the peel. Combined treatments showed a potential synergistic effect, highlighting tissue-specific responses to AEW and UV-C, with the peel exhibiting higher sensitivity.

The germicidal effects (*in vitro*) on of AEW and UV-C against *Ceratocytis paradoxa* revealed their potential as antifungal agents. AEW exhibited exceptional fungicidal activity, inhibiting spore survival, germination, and germ tube elongation. UV-C treatments were initially effective, but a biphasic response indicated potential stress-induced dormancy or repair mechanisms. The combination of AEW and UV-C did not show a synergistic effect, possibly due to the overwhelming fungicidal action of AEW.

In summary, AEW and UV-C treatments offer promising strategies for enhancing postharvest quality and controlling fungal pathogens in 'Phulae' pineapples. The observed effects on IBS, TSS/TA ratio, phenolic compounds, flavonoids, antioxidant activity, and antifungal properties underscore the need for further research to optimize treatment conditions and understand underlying mechanisms. These findings contribute valuable insights to postharvest technology, offering potential applications to improve marketability and nutritional value while addressing storage-related challenges.

CHAPTER 6

SUGGESTION

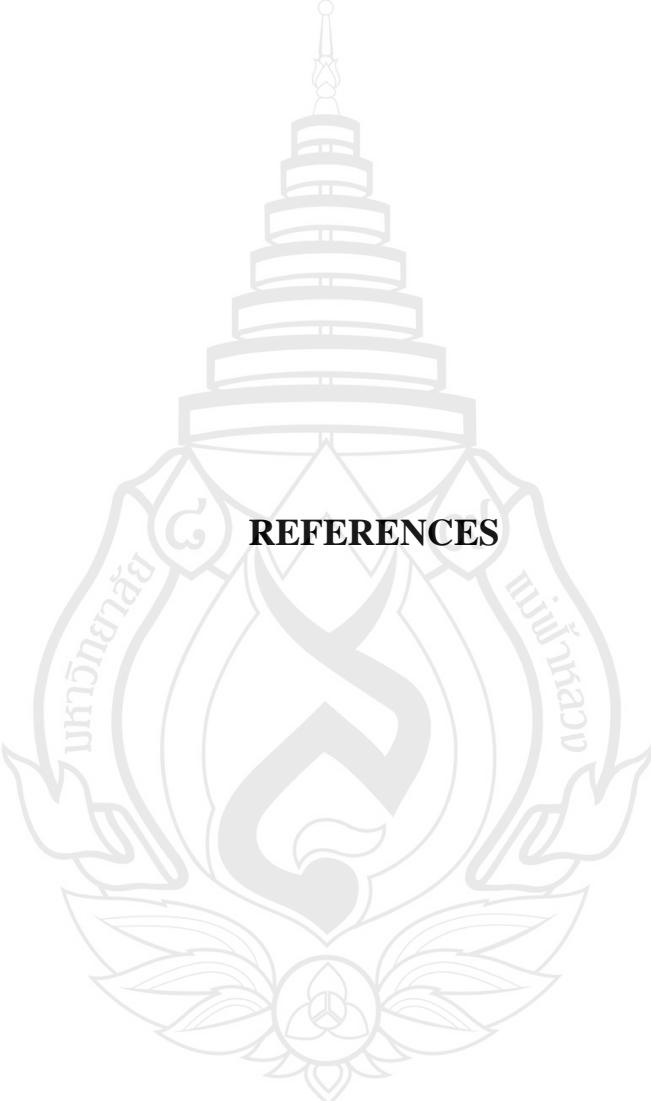
Based on the outcomes of this investigation, several recommendations can be put forth to guide further research and practical applications in the postharvest management of 'Phulæ' pineapples:

1. Optimizing AEW and UV-C Combinations: While the pairing of AEW 300 ppm and UV-C at 13.2 kJ/m² demonstrated potential synergies, further exploration is necessary to optimize the concentrations and timings of AEW and UV-C treatments. Investigating a wider range of combinations will help identify the most effective treatment conditions for maximizing quality retention and germicidal effects.

2. Extended Storage Studies: The study covered a 28-day storage period, and extending the investigation to longer durations is recommended to assess the sustainability of observed effects. This will provide insights into potential benefits or challenges associated with AEW and UV-C treatments over extended periods.

3. In-Depth Mechanistic Investigation: To unravel the underlying mechanisms of AEW and UV-C effects on pineapple physiology, conducting in-depth mechanistic studies is advised. This includes exploring specific pathways involved in the synthesis and degradation of key compounds, understanding stress responses, and elucidating genetic and biochemical changes induced by these treatments.

Addressing these recommendations will contribute to refining the use of AEW and UV-C treatments in postharvest management, providing sustainable solutions for enhancing the quality and safety of 'Phulæ' pineapples in the supply chain.



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APPENDICES

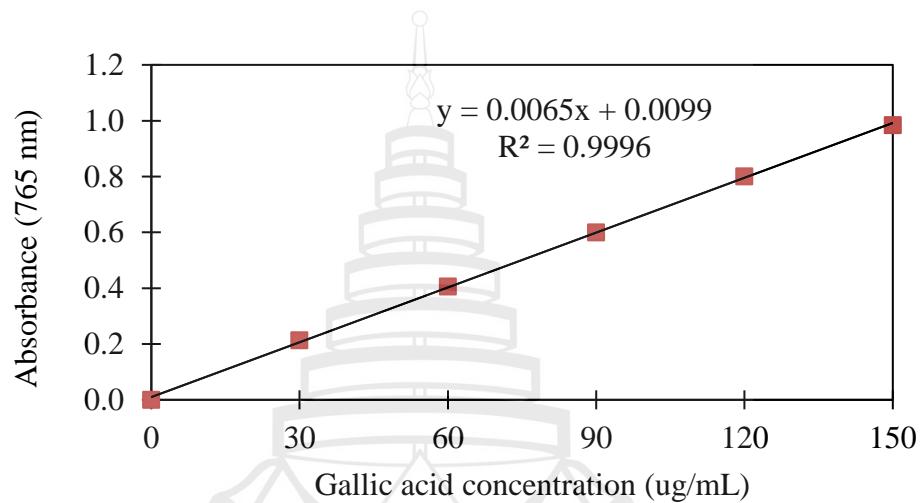
APPENDIX A**STANDARD CURVE**

Figure A1 Linearity between absorbance at 765 nm of gallic acid concentration for total phenolic compound content assay

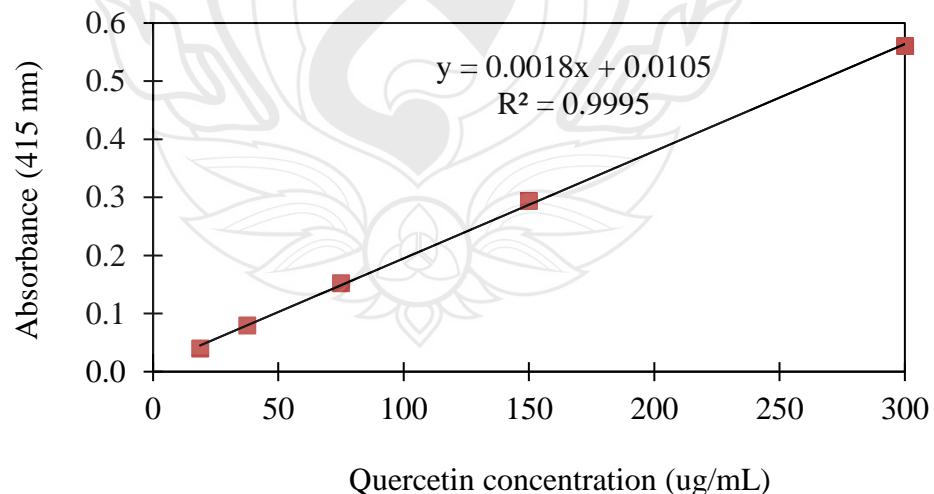


Figure A2 Linearity between absorbance at 415 nm of quercetin concentration for total flavonoid content assay

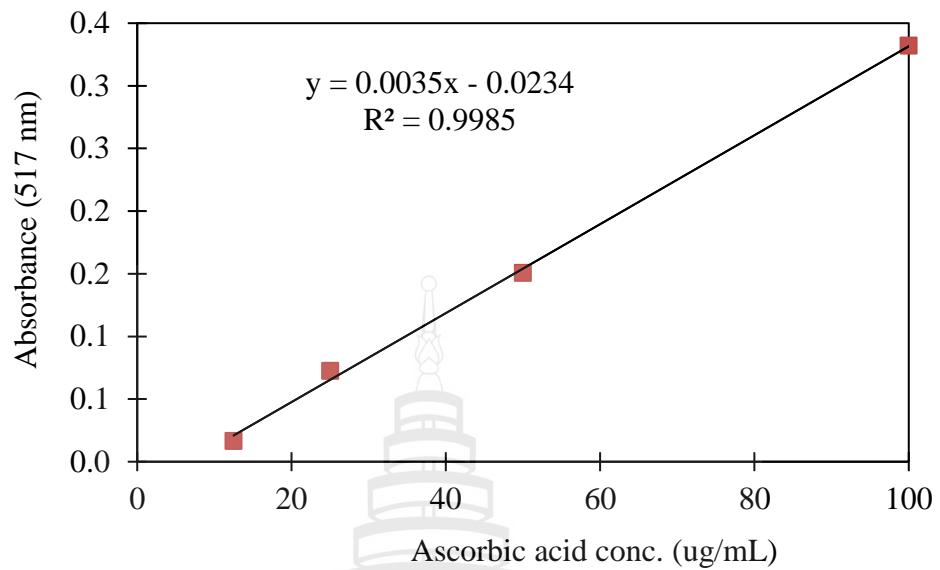


Figure A3 Linearity between absorbance at 595 nm of ascorbic acid concentration for antioxidant assay (measured by DPPH assay)

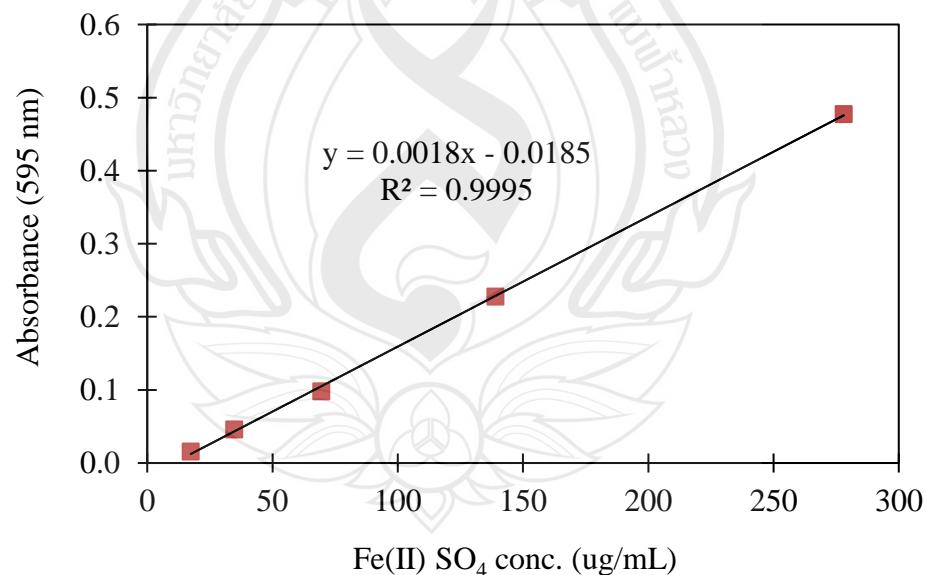


Figure A4 Linearity between absorbance at 595 nm of iron (II) sulphate (Fe(II) SO₄) concentration for antioxidant activity assay (measured by FRAP assay)

APPENDIX B

STATISTICAL ANALYSIS

Table B1 Effect of AEW on mould incidence and mould severity of 'Phulae' pineapple during storage at 13 °C for 28 days

Treatments	Day of storage				
	Day 0	Day 7	Day 14	Day 21	Day 28
Mould Incidence (%)					
Control	0	60.00 ± 10.00 a	100.00 ± 0 a	100.00 ± 0 a	100.00 ± 0 a
AEW 100 ppm	0	20.00 ± 0.00 b	100.00 ± 0 a	100.00 ± 0 a	100.00 ± 0 a
AEW 200 ppm	0	26.67 ± 3.33 b	100.00 ± 0 a	100.00 ± 0 a	100.00 ± 0 a
AEW 300 ppm	0	16.67 ± 3.33 b	100.00 ± 0 a	100.00 ± 0 a	100.00 ± 0 a
Mould Severity (%)					
Control	0	18.33 ± 5.83 a	71.67 ± 10.44 a	86.67 ± 5.46 a	91.67 ± 5.07 a
AEW 100 ppm	0	5.00 ± 0.00 b	51.25 ± 3.31 bc	70.83 ± 6.51 a	85.83 ± 3.33 a
AEW 200 ppm	0	7.50 ± 1.44 b	60.00 ± 3.82 ab	79.17 ± 2.20 a	89.17 ± 1.67 a
AEW 300 ppm	0	4.17 ± 0.83 b	38.33 ± 1.67 c	75.83 ± 5.07 a	90.00 ± 1.44 a

Note Values are mean ± standard error (SE) from n = 3. Different letters within the same column differ significantly between the treatments by using Duncan's multiple range test (P<0.05)

Table B2 Effect of UV-C irradiation on mould incidence and mould severity of 'Phulae' pineapple fruit during storage at 13 °C for 28 days

Treatments	Day of storage				
	Day 0	Day 7	Day 14	Day 21	Day 28
Mould Incidence (%)					
Control	0	80.00 ± 0.00 a	100.00 ± 0 a	100.00 ± 0 a	100.00 ± 0 a
UV-C 13.2 kJ/m ²	0	63.33 ± 8.82 a	100.00 ± 0 a	100.00 ± 0 a	100.00 ± 0 a
UV-C 26.4 kJ/m ²	0	53.33 ± 8.82 a	100.00 ± 0 a	100.00 ± 0 a	100.00 ± 0 a
UV-C 39.6 kJ/m ²	0	53.33 ± 14.53 a	100.00 ± 0 a	100.00 ± 0 a	100.00 ± 0 a
Mould Severity (%)					
Control	0	31.67 ± 4.64 a	65.83 ± 2.20 a	79.17 ± 3.63 a	90.00 ± 1.44 a
UV-C 13.2 kJ/m ²	0	21.67 ± 4.41 ab	47.50 ± 3.82 b	64.17 ± 3.00 b	80.83 ± 3.00 b
UV-C 26.4 kJ/m ²	0	18.33 ± 3.00 ab	54.17 ± 2.20 b	79.83 ± 0.83 a	90.00 ± 2.50 a
UV-C 39.6 kJ/m ²	0	13.33 ± 3.63 b	48.33 ± 1.67 b	70.83 ± 4.41 ab	90.83 ± 0.83 a

Note Values are mean ± standard error from n = 3. Different letters within the same column differ significantly between the treatments by using Duncan's multiple range test (P<0.05)

Table B3 Effects of acidic electrolyzed water and UV-C treatments on mould incidence and mould severity in ‘Phulae’ pineapple fruit during storage at 13 °C for 28 days

Treatments	Day of storage				
	Day 0	Day 7	Day 14	Day 21	Day 28
Mould Incidence (%)					
Control	0	90.00 ± 5.77 a	100.00 ± 0.00 a	100.00 ± 0.00 a	100.00 ± 0.00 a
AEW 300 ppm	0	26.67 ± 16.67 c	100.00 ± 0.00 a	100.00 ± 0.00 a	100.00 ± 0.00 a
UV-C 13.2 kJ/m ²	0	60.00 ± 10.00 b	100.00 ± 0.00 a	100.00 ± 0.00 a	100.00 ± 0.00 a
UV-C 39.6 kJ/m ²	0	60.00 ± 5.77 b	100.00 ± 0.00 a	100.00 ± 0.00 a	100.00 ± 0.00 a
AEW 300 ppm + UV-C 13.2 kJ/m ²	0	33.33 ± 3.33 bc	100.00 ± 0.00 a	100.00 ± 0.00 a	100.00 ± 0.00 a
AEW 300 ppm + UV-C 39.6 kJ/m ²	0	26.67 ± 6.67 c	100.00 ± 0.00 a	100.00 ± 0.00 a	100.00 ± 0.00 a
Mould Severity (%)					
Control	0	25.83 ± 1.67 a	52.50 ± 3.82 a	76.67 ± 1.67 a	85.83 ± 0.83 a
AEW 300 ppm	0	6.67 ± 4.17 c	28.33 ± 2.20 c	65.83 ± 2.20 ab	85.83 ± 3.00 a
UV-C 13.2 kJ/m ²	0	15.00 ± 2.50 b	40.83 ± 2.20 b	64.17 ± 4.41 b	78.33 ± 4.64 ab
UV-C 39.6 kJ/m ²	0	15.00 ± 1.44 b	44.17 ± 0.83 b	66.67 ± 3.00 ab	83.33 ± 0.83 a
AEW 300 ppm + UV-C 13.2 kJ/m ²	0	8.33 ± 0.83 bc	39.17 ± 0.83 b	60.83 ± 0.83 b	78.33 ± 2.20 ab
AEW 300 ppm + UV-C 39.6 kJ/m ²	0	6.67 ± 1.67 c	30.00 ± 2.50 c	49.17 ± 5.83 c	69.17 ± 4.64 b

Note Values are mean ± standard error (SE) from n = 3. Different letters within the same column differ significantly between the treatments by using Duncan’s multiple range test (P<0.05)

Table B4 Effects of acidic electrolyzed water and UV-C on weight loss of ‘Phulae’ pineapple fruit during storage at 13 °C for 28 days

Treatments	Weight Loss (%)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Control	0	2.00 ± 0.09 bc	3.76 ± 0.16 bc	5.97 ± 0.22 bc	8.43 ± 0.28 ab
AEW 300 ppm	0	1.99 ± 0.06 bc	3.96 ± 0.06 bc	6.25 ± 0.14 abc	8.73 ± 0.21 ab
UV-C 13.2 kJ/m ²	0	1.80 ± 0.10 c	3.60 ± 0.10 c	5.75 ± 0.21 c	8.19 ± 0.34 b
UV-C 39.6 kJ/m ²	0	2.08 ± 0.12 bc	3.84 ± 0.12 bc	6.01 ± 0.16 bc	8.51 ± 0.24 ab
AEW 300 ppm + UV-C 13.2 kJ/m ²	0	2.62 ± 0.19 a	4.59 ± 0.25 a	6.72 ± 0.27 a	9.23 ± 0.36 a
AEW 300 ppm + UV-C 39.6 kJ/m ²	0	2.20 ± 0.03 b	4.12 ± 0.05 b	6.50 ± 0.14 ab	9.10 ± 0.25 ab

Note Values are mean ± standard error (SE) from n = 3. Different letters within the same column differ significantly between the treatments by using Duncan’s multiple range test (P<0.05)

Table B5 Effects of acidic electrolyzed water and UV-C on moisture content of 'Phulae' pineapple during storage at 13 °C for 28 days

Treatments	Moisture Content (%)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Pulp					
Control	84.56 ± 0.79 a	84.32 ± 0.24 ab	84.10 ± 0.19 ab	84.43 ± 0.43 ab	84.85 ± 0.27 a
AEW 300 ppm	83.82 ± 0.75 a	84.56 ± 0.12 ab	83.28 ± 0.34 b	84.35 ± 0.57 ab	84.19 ± 0.73 a
UV-C 13.2 kJ/m ²	84.63 ± 0.52 a	85.25 ± 0.22 a	84.71 ± 0.43 a	83.83 ± 0.14 b	84.99 ± 1.16 a
UV-C 39.6 kJ/m ²	84.36 ± 0.28 a	84.05 ± 0.30 b	84.56 ± 0.66 ab	85.09 ± 0.37 a	84.83 ± 0.33 a
AEW 300 ppm + UV-C 13.2 kJ/m ²	83.58 ± 0.78 a	84.75 ± 0.29 ab	84.65 ± 0.27 a	84.73 ± 0.31 ab	84.84 ± 0.64 a
AEW 300 ppm + UV-C 39.6 kJ/m ²	83.39 ± 0.38 a	85.06 ± 0.46 a	84.84 ± 0.23 a	85.26 ± 0.23 a	84.84 ± 0.45 a
Peel					
Control	83.66 ± 0.41 ab	82.36 ± 0.28 abc	80.43 ± 0.48 a	80.45 ± 0.69 a	79.61 ± 0.39 a
AEW 300 ppm	82.82 ± 0.12 ab	82.87 ± 0.24 ab	79.61 ± 0.06 a	80.92 ± 0.23 a	79.53 ± 0.29 a
UV-C 13.2 kJ/m ²	82.90 ± 0.19 ab	82.92 ± 0.42 a	79.36 ± 0.78 a	81.67 ± 1.43 a	79.70 ± 0.36 a
UV-C 39.6 kJ/m ²	82.22 ± 0.20 ab	81.75 ± 0.42 c	79.09 ± 0.26 a	82.03 ± 1.64 a	80.30 ± 0.40 a
AEW 300 ppm + UV-C 13.2 kJ/m ²	81.63 ± 0.68 b	81.89 ± 0.06 bc	80.99 ± 1.38 a	80.88 ± 0.44 a	80.62 ± 0.63 a
AEW 300 ppm + UV-C 39.6 kJ/m ²	84.48 ± 1.85 a	82.25 ± 0.24 abc	79.74 ± 0.29 a	80.70 ± 0.57 a	80.33 ± 0.24 a

Note Values are mean ± standard error (SE) from n = 3. Different letters within the same column differ significantly between the treatments by using Duncan's multiple range test (P<0.05)

Table B6 Effects of acidic electrolyzed water and UV-C on dry matter of 'Phulae' pineapple fruit during storage at 13 °C for 28 days

Treatments	Dry Matter (%)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Pulp					
Control	15.44 ± 0.79 a	15.68 ± 0.24 ab	15.90 ± 0.19 ab	15.57 ± 0.43 ab	15.15 ± 0.27 a
AEW 300 ppm	16.18 ± 0.75 a	15.44 ± 0.12 ab	16.72 ± 0.34 a	15.65 ± 0.57 ab	15.81 ± 0.73 a
UV-C 13.2 kJ/m ²	15.37 ± 0.52 a	14.75 ± 0.22 b	15.29 ± 0.43 b	16.17 ± 0.14 a	15.01 ± 1.16 a
UV-C 39.6 kJ/m ²	15.64 ± 0.28 a	15.95 ± 0.30 a	15.44 ± 0.66 ab	14.91 ± 0.37 b	15.17 ± 0.33 a
AEW 300 ppm + UV-C 13.2 kJ/m ²	16.42 ± 0.78 a	15.25 ± 0.29 ab	15.35 ± 0.27 b	15.27 ± 0.31 ab	15.16 ± 0.64 a
AEW 300 ppm + UV-C 39.6 kJ/m ²	16.61 ± 0.38 a	14.94 ± 0.46 b	15.16 ± 0.35 b	14.74 ± 0.23 b	15.16 ± 0.45 a
Peel					
Control	16.34 ± 0.41 ab	17.64 ± 0.28 abc	19.57 ± 0.48 a	19.55 ± 0.69 a	20.39 ± 0.39 a
AEW 300 ppm	17.18 ± 0.12 ab	17.13 ± 0.24 bc	20.39 ± 0.06 a	19.08 ± 0.23 a	20.47 ± 0.29 a
UV-C 13.2 kJ/m ²	17.10 ± 0.19 ab	17.08 ± 0.42 c	20.64 ± 0.78 a	18.33 ± 1.43 a	20.30 ± 0.36 a
UV-C 3.96 kJ/m ²	17.78 ± 0.20 ab	18.25 ± 0.42 a	20.91 ± 0.26 a	17.97 ± 1.64 a	19.70 ± 0.40 a
AEW 300 ppm + UV-C 13.2 kJ/m ²	18.37 ± 0.68 a	18.11 ± 0.06 ab	19.01 ± 1.38 a	19.12 ± 0.44 a	19.38 ± 0.63 a
AEW 300 ppm + UV-C 39.6 kJ/m ²	15.52 ± 1.85 b	17.75 ± 0.24 abc	20.26 ± 0.29 a	19.30 ± 0.57 a	19.67 ± 0.24 a

Note Values are mean ± standard error (SE) from n = 3. Different letters within the same column differ significantly between the treatments by using Duncan's multiple range test (P<0.05)

Table B7 Effects of acidic electrolyzed water and UV-C on colour of ‘Phulae’ pineapple fruit during storage at 13 °C for 28 days

Treatments	Day of storage				
	Day 0	Day 7	Day 14	Day 21	Day 28
L*					
Control	37.63 ± 1.93 a	42.69 ± 1.00 a	44.57 ± 0.95 ab	43.05 ± 1.35 a	41.19 ± 0.86 ab
AEW 300 ppm	39.83 ± 1.76 a	42.39 ± 1.26 a	46.81 ± 0.80 a	44.53 ± 0.27 a	41.70 ± 0.41 ab
UV-C 13.2 kJ/m ²	39.19 ± 1.40 a	41.80 ± 0.51 a	45.31 ± 0.34 a	43.11 ± 1.15 a	41.88 ± 0.95 ab
UV-C 3.96 kJ/m ²	38.51 ± 0.37 a	43.50 ± 0.96 a	45.33 ± 0.73 a	43.78 ± 0.70 a	40.46 ± 0.73 b
AEW 300 ppm + UV-C 13.2 kJ/m ²	38.38 ± 1.07 a	42.36 ± 0.47 a	45.51 ± 0.99 a	43.51 ± 1.12 a	42.95 ± 0.37 a
AEW 300 ppm + UV-C 39.6 kJ/m ²	37.76 ± 0.11 a	40.92 ± 0.87 a	42.30 ± 1.02 b	42.95 ± 0.61 a	41.34 ± 0.76 ab
a*					
Control	0.63 ± 0.51 a	3.77 ± 0.44 a	10.33 ± 0.54 ab	14.10 ± 0.65 a	14.72 ± 0.07 a
AEW 300 ppm	1.41 ± 0.43 a	3.69 ± 0.50 a	10.71 ± 0.25 ab	14.68 ± 0.39 a	15.04 ± 0.43 a
UV-C 13.2 kJ/m ²	1.49 ± 0.60 a	3.87 ± 0.56 a	10.99 ± 0.34 a	14.39 ± 0.29 a	13.16 ± 1.57 a
UV-C 39.6 kJ/m ²	0.56 ± 0.33 a	3.55 ± 0.49 a	10.17 ± 0.30 ab	13.92 ± 0.32 a	14.23 ± 0.36 a
AEW 300 ppm + UV-C 13.2 kJ/m ²	1.09 ± 0.10 a	3.55 ± 0.42 a	10.46 ± 0.41 ab	14.07 ± 0.36 a	13.43 ± 0.63 a
AEW 300 ppm + UV-C 39.6 kJ/m ²	0.29 ± 0.44 a	2.54 ± 0.45 a	9.65 ± 0.06 b	13.58 ± 0.23 a	12.98 ± 0.20 a

Table B7 (continued)

Treatments	Day of storage				
	Day 0	Day 7	Day 14	Day 21	Day 28
b*					
Control	22.35 ± 0.61 a	28.27 ± 0.83 a	34.55 ± 1.40 a	35.47 ± 2.11 a	33.43 ± 0.42 a
AEW 300 ppm	23.05 ± 0.52 a	27.64 ± 0.16 a	34.36 ± 0.60 a	36.40 ± 1.00 a	33.43 ± 0.42 a
UV-C 13.2 kJ/m ²	22.84 ± 0.47 a	28.17 ± 1.14 a	34.50 ± 0.27 a	36.08 ± 0.97 a	30.03 ± 2.73 ab
UV-C 39.6 kJ/m ²	22.95 ± 0.67 a	26.65 ± 0.41 ab	34.38 ± 1.46 a	35.61 ± 0.36 a	31.49 ± 0.53 ab
AEW 300 ppm + UV-C 13.2 kJ/m ²	22.92 ± 0.78 a	27.17 ± 1.16 ab	35.51 ± 1.29 a	35.97 ± 1.34 a	30.22 ± 1.56 ab
AEW 300 ppm + UV-C 39.6 kJ/m ²	22.31 ± 0.71 a	24.79 ± 0.50 b	34.58 ± 1.14 a	33.88 ± 0.34 a	28.48 ± 0.39 b
ΔE					
Control	0	8.42 ± 1.16 a	17.20 ± 2.32 a	19.76 ± 2.17 a	18.41 ± 0.95 a
AEW 300 ppm	0	6.41 ± 1.10 a	16.52 ± 1.47 a	19.65 ± 0.54 a	17.54 ± 0.85 a
UV-C 13.2 kJ/m ²	0	6.74 ± 1.86 a	16.31 ± 1.12 a	19.16 ± 1.65 a	14.26 ± 3.27 a
UV-C 39.6 kJ/m ²	0	7.03 ± 1.01 a	16.57 ± 1.21 a	19.16 ± 0.41 a	16.26 ± 1.21 a
AEW 300 ppm + UV-C 13.2 kJ/m ²	0	6.40 ± 0.68 a	17.27 ± 0.69 a	19.13 ± 0.90 a	15.21 ± 1.00 a
AEW 300 ppm + UV-C 39.6 kJ/m ²	0	5.05 ± 0.10 a	16.14 ± 1.09 a	18.43 ± 0.67 a	14.67 ± 0.45 a

Table B7 (continued)

Treatments	Day of storage				
	Day 0	Day 7	Day 14	Day 21	Day 28
Chroma (c)					
Control	22.53 ± 0.62 a	28.69 ± 0.76 a	36.12 ± 1.49 a	38.20 ± 2.20 a	36.56 ± 0.41 a
AEW 300 ppm	23.28 ± 0.52 a	28.06 ± 0.20 a	36.06 ± 0.64 a	39.29 ± 1.07 a	36.70 ± 1.47 a
UV-C 13.2 kJ/m ²	23.08 ± 0.50 a	28.59 ± 1.20 a	36.26 ± 0.16 a	38.87 ± 1.01 a	32.81 ± 3.14 a
UV-C 39.6 kJ/m ²	23.16 ± 0.66 a	27.05 ± 0.39 a	35.91 ± 1.40 a	38.27 ± 0.44 a	34.60 ± 0.62 a
AEW 300 ppm + UV-C 13.2 kJ/m ²	23.18 ± 0.81 a	27.59 ± 1.16 a	37.10 ± 1.34 a	38.67 ± 1.38 a	33.11 ± 1.67 a
AEW 300 ppm + UV-C 39.6 kJ/m ²	22.52 ± 0.71 a	25.09 ± 0.46 a	35.97 ± 1.12 a	36.53 ± 0.40 a	31.33 ± 0.42 a
Huge Angle Value (h)					
Control	89.03 ± 1.18 a	82.64 ± 0.98 a	73.01 ± 0.26 ab	68.03 ± 0.35 a	66.01 ± 0.13 a
AEW 300 ppm	87.16 ± 1.16 a	82.62 ± 0.94 a	72.32 ± 0.30 b	67.84 ± 0.16 a	65.59 ± 0.35 a
UV-C 13.2 kJ/m ²	87.08 ± 1.45 a	82.47 ± 0.69 a	72.06 ± 0.53 b	68.06 ± 0.12 a	66.33 ± 0.86 a
UV-C 39.6 kJ/m ²	89.40 ± 0.76 a	82.53 ± 1.05 a	73.23 ± 0.83 ab	68.41 ± 0.35 a	65.38 ± 0.42 a
AEW 300 ppm + UV-C 13.2 kJ/m ²	88.19 ± 0.48 a	83.07 ± 0.85 a	73.45 ± 0.08 ab	68.36 ± 0.33 a	65.83 ± 0.11 a
AEW 300 ppm + UV-C 39.6 kJ/m ²	90.18 ± 1.06 a	84.62 ± 1.18 a	74.13 ± 0.45 a	67.91 ± 0.13 a	65.23 ± 0.26 a

Note Values are mean ± standard error (SE) from n = 3. Different letters within the same column differ significantly between the treatments by using Duncan's multiple range test (P<0.05)

Table B8 Effects of AEW and UV-C treatments on internal browning incidence of 'Phulae' pineapple during storage at 13 °C for 28 days

Treatments	Internal Browning Incidence (%)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Control	0	0	0	36.67 ± 1.67 a	61.67 ± 4.41 a
AEW 300 ppm	0	0	0	36.67 ± 9.28 a	60.00 ± 5.77 a
UV-C 13.2 kJ/m ²	0	0	0	42.50 ± 3.82 a	58.33 ± 6.01 a
UV-C 39.6 kJ/m ²	0	0	0	38.33 ± 3.33 a	55.00 ± 5.00 a
AEW 300 ppm + UV-C 13.2 kJ/m ²	0	0	0	36.67 ± 4.41 a	55.00 ± 2.89 a
AEW 300 ppm + UV-C 39.6 kJ/m ²	0	0	0	30.83 ± 0.42 a	53.33 ± 1.67 a

Note Values are mean ± standard error (SE) from n = 3. Different letters within the same column differ significantly between the treatments by using Duncan's multiple range test (P<0.05)

Table B9 Effects of AEW and UV-C on TSS, TA, TSS/TA and pH of ‘Phulae’ pineapple pulp during storage at 13 °C for 28 days

Treatments	Day of storage				
	Day 0	Day 7	Day 14	Day 21	Day 28
TSS					
Control	16.27 ± 0.09 a	16.87 ± 0.20 a	15.00 ± 0.16 d	15.73 ± 0.54 a	15.29 ± 0.41 a
AEW 300 ppm	16.57 ± 0.18 a	16.40 ± 0.10 a	16.31 ± 0.27 ab	16.07 ± 0.63 a	15.41 ± 0.46 a
UV-C 13.2 kJ/m ²	16.43 ± 0.34 a	15.90 ± 0.20 a	15.52 ± 0.20 bcd	15.72 ± 0.07 a	15.57 ± 0.70 a
UV-C 39.6 kJ/m ²	16.77 ± 0.38 a	16.63 ± 0.46 a	16.53 ± 0.35 a	15.22 ± 0.33 a	15.21 ± 0.36 a
AEW 300 ppm + UV-C 13.2 kJ/m ²	16.57 ± 0.42 a	16.67 ± 0.38 a	16.01 ± 0.09 abc	15.26 ± 0.24 a	15.24 ± 0.60 a
AEW 300 ppm + UV-C 39.6 kJ/m ²	16.47 ± 0.19 a	16.07 ± 0.30 a	15.31 ± 0.30 cd	15.20 ± 0.22 a	15.58 ± 0.84 a
TA					
Control	1.19 ± 0.04 a	1.39 ± 0.01 a	1.33 ± 0.03 a	1.35 ± 0.07 a	1.30 ± 0.08 a
AEW 300 ppm	1.23 ± 0.01 a	1.31 ± 0.03 a	1.32 ± 0.03 a	1.30 ± 0.02 a	1.39 ± 0.07 a
UV-C 13.2 kJ/m ²	1.15 ± 0.02 a	1.30 ± 0.05 a	1.28 ± 0.02 a	1.29 ± 0.05 a	1.33 ± 0.04 a
UV-C 39.6 kJ/m ²	1.17 ± 0.03 a	1.28 ± 0.05 a	1.32 ± 0.06 a	1.32 ± 0.03 a	1.27 ± 0.04 a
AEW 300 ppm + UV-C 13.2 kJ/m ²	1.17 ± 0.04 a	1.32 ± 0.04 a	1.30 ± 0.05 a	1.29 ± 0.08 a	1.31 ± 0.01 a
AEW 300 ppm + UV-C 39.6 kJ/m ²	1.13 ± 0.04 a	1.34 ± 0.03 a	1.36 ± 0.06 a	1.32 ± 0.05 a	1.31 ± 0.06 a

Table B9 (continued)

Treatments	Day of storage				
	Day 0	Day 7	Day 14	Day 21	Day 28
TSS/TA					
Control	13.67 ± 0.56 a	12.14 ± 0.22 a	11.32 ± 0.26 a	11.71 ± 0.73 a	11.84 ± 1.01 a
AEW 300 ppm	13.44 ± 0.30 a	12.56 ± 0.26 a	12.39 ± 0.44 a	12.42 ± 0.62 a	11.12 ± 0.76 a
UV-C 13.2 kJ/m ²	14.29 ± 0.10 a	12.26 ± 0.39 a	12.13 ± 0.34 a	12.18 ± 0.49 a	11.72 ± 0.55 a
UV-C 39.6 kJ/m ²	14.35 ± 0.55 a	13.08 ± 0.77 a	12.57 ± 0.69 a	11.50 ± 0.26 a	12.01 ± 0.51 a
AEW 300 ppm + UV-C 13.2 kJ/m ²	14.25 ± 0.78 a	12.65 ± 0.39 a	12.37 ± 0.49 a	11.90 ± 0.89 a	11.68 ± 0.57 a
AEW 300 ppm + UV-C 39.6 kJ/m ²	14.61 ± 0.53 a	12.02 ± 0.17 a	11.35 ± 0.74 a	11.53 ± 0.57 a	11.95 ± 1.18 a
pH					
Control	4.06 ± 0.01 a	3.85 ± 0.01 a	3.83 ± 0.02 a	3.65 ± 0.03 a	3.69 ± 0.04 abc
AEW 300 ppm	4.02 ± 0.01 a	3.82 ± 0.00 a	3.74 ± 0.03 b	3.66 ± 0.01 a	3.71 ± 0.02 ab
UV-C 13.2 kJ/m ²	4.03 ± 0.03 a	3.82 ± 0.02 a	3.79 ± 0.00 ab	3.66 ± 0.02 a	3.73 ± 0.03 a
UV-C 39.6 kJ/m ²	4.07 ± 0.02 a	3.83 ± 0.03 a	3.80 ± 0.01 ab	3.66 ± 0.02 a	3.69 ± 0.01 ab
AEW 300 ppm + UV-C 13.2 kJ/m ²	4.03 ± 0.02 a	3.83 ± 0.03 a	3.75 ± 0.02 b	3.63 ± 0.03 a	3.64 ± 0.02 bc
AEW 300 ppm + UV-C 39.6 kJ/m ²	4.06 ± 0.02 a	3.83 ± 0.05 a	3.76 ± 0.01 b	3.65 ± 0.01 a	3.61 ± 0.03 c

Note Values are mean ± standard error (SE) from n = 3. Different letters within the same column differ significantly between the treatments by using Duncan's multiple range test (P<0.05)

Table B10 Effects of AEW and UV-C on vitamin c content of ‘Phulae’ pineapple pulp during storage at 13 °C for 28 days

Treatments	Vitamin C (mg Ascorbic Acid equivalent/100 g fresh fruit)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Control	10.89 ± 0.17 a	10.35 ± 0.08 ab	11.67 ± 0.78 a	11.62 ± 0.93 a	9.44 ± 0.75 a
AEW 300 ppm	10.76 ± 0.24 a	11.05 ± 0.17 a	11.94 ± 0.62 a	10.42 ± 0.59 a	9.36 ± 1.00 a
UV-C 13.2 kJ/m ²	10.48 ± 0.77 a	10.19 ± 0.52 ab	13.57 ± 0.56 a	12.86 ± 1.14 a	8.91 ± 0.19 a
UV-C 39.6 kJ/m ²	11.09 ± 0.84 a	10.27 ± 0.75 ab	12.51 ± 2.14 a	10.67 ± 0.85 a	8.63 ± 0.79 a
AEW 300 ppm + UV-C 13.2 kJ/m ²	9.59 ± 0.55 a	9.51 ± 0.75 ab	12.67 ± 0.21 a	11.12 ± 0.91 a	10.08 ± 0.61 a
AEW 300 ppm + UV-C 39.6 kJ/m ²	9.52 ± 0.26 a	9.31 ± 0.23 b	13.53 ± 1.03 a	11.05 ± 0.73 a	11.22 ± 1.36 a

Note Values are mean ± standard error (SE) from n = 3. Different letters within the same column differ significantly between the treatments by using Duncan’s multiple range test (P<0.05)

Table B11 Effects of AEW and UV-C on total phenolic compound content of ‘Phulae’ pineapple during storage at 13 °C for 28 days

Treatments	TPC (mg gallic acid equivalent/100 g of sample wet weight basic)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Pulp					
Control	28.95 ± 1.81 b	34.47 ± 0.82 a	36.17 ± 0.96 b	39.72 ± 1.10 a	35.19 ± 1.94 a
AEW 300 ppm	32.52 ± 0.87 ab	34.02 ± 0.51 a	40.51 ± 0.73 a	40.98 ± 1.58 a	37.38 ± 1.14 a
UV-C 13.2 kJ/m ²	32.25 ± 1.38 ab	35.90 ± 0.34 a	37.04 ± 0.55 b	40.98 ± 2.02 a	36.57 ± 1.82 a
UV-C 39.6 kJ/m ²	32.30 ± 0.82 ab	34.47 ± 0.37 a	38.81 ± 0.70 ab	40.13 ± 1.91 a	35.46 ± 0.65 a
AEW 300 ppm + UV-C 13.2 kJ/m ²	36.28 ± 2.31 a	36.23 ± 1.61 a	40.84 ± 0.56 a	43.99 ± 0.26 a	39.99 ± 0.67 a
AEW 300 ppm + UV-C 39.6 kJ/m ²	35.78 ± 1.43 a	34.11 ± 0.69 a	40.20 ± 1.62 a	42.33 ± 1.24 a	40.02 ± 1.68 a
Peel					
Control	20.72 ± 1.01 a	53.03 ± 1.37 d	64.86 ± 2.27 d	70.50 ± 0.97 c	75.00 ± 1.00 a
AEW 300 ppm	20.99 ± 0.27 a	57.25 ± 1.75 bcd	78.17 ± 3.74 abc	81.34 ± 2.67 ab	90.56 ± 6.31 a
UV-C 13.2 kJ/m ²	20.69 ± 1.22 a	55.98 ± 2.75 cd	74.37 ± 1.29 bcd	75.75 ± 5.38 bc	76.07 ± 5.63 a
UV-C 39.6 kJ/m ²	20.48 ± 0.71 a	59.75 ± 1.48 abc	70.28 ± 1.77 cd	76.61 ± 1.56 bc	77.39 ± 4.22 a
AEW 300 ppm + UV-C 13.2 kJ/m ²	21.26 ± 0.41 a	64.73 ± 2.01 a	85.83 ± 6.24 a	90.64 ± 4.23 a	87.18 ± 7.19 a
AEW 300 ppm + UV-C 39.6 kJ/m ²	20.57 ± 0.46 a	62.90 ± 1.34 ab	81.70 ± 0.77 ab	87.47 ± 2.39 a	89.50 ± 2.35 a

Note Values are mean ± standard error (SE) from n = 3. Different letters within the same column differ significantly between the treatments by using Duncan’s multiple range test (P<0.05)

Table B12 Effects of AEW and UV-C on total flavonoid content of 'Phulæ' pineapple during storage at 13 °C for 28 days

Treatments	TFC (mg Quercetin Equivalent/100 g of sample wet weight basic)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Pulp					
Control	16.74 ± 1.23 ab	19.75 ± 1.22 a	27.52 ± 0.42 bc	28.51 ± 0.78 a	30.97 ± 1.22 a
AEW 300 ppm	16.03 ± 0.82 b	18.54 ± 0.55 a	32.93 ± 0.61 a	31.63 ± 0.94 a	31.81 ± 1.22 a
UV-C 13.2 kJ/m ²	20.54 ± 1.06 ab	19.90 ± 0.20 a	26.79 ± 0.83 c	30.65 ± 1.97 a	32.49 ± 1.93 a
UV-C 39.6 kJ/m ²	21.27 ± 1.81 a	21.20 ± 2.41 a	33.70 ± 0.94 a	30.02 ± 0.42 a	30.31 ± 1.30 a
AEW 300 ppm + UV-C 13.2 kJ/m ²	19.97 ± 2.13 ab	20.93 ± 0.23 a	30.08 ± 1.26 abc	31.40 ± 1.75 a	33.10 ± 1.53 a
AEW 300 ppm + UV-C 39.6 kJ/m ²	19.61 ± 0.43 ab	20.64 ± 0.16 a	31.19 ± 2.23 ab	31.64 ± 2.69 a	32.34 ± 3.62 a
Peel					
Control	45.54 ± 2.92 a	56.32 ± 1.72 c	65.92 ± 1.63 b	67.24 ± 3.28 c	70.40 ± 4.74 b
AEW 300 ppm	44.43 ± 1.43 a	58.46 ± 1.53 bc	79.69 ± 7.30 ab	88.37 ± 7.86 ab	106.47 ± 13.12 a
UV-C 13.2 kJ/m ²	47.92 ± 3.57 a	57.24 ± 4.84 bc	70.71 ± 3.53 b	73.50 ± 3.12 bc	78.58 ± 5.89 ab
UV-C 39.6 kJ/m ²	47.93 ± 2.28 a	69.03 ± 6.22 ab	78.15 ± 4.03 ab	81.50 ± 4.37 abc	79.44 ± 5.80 ab
AEW 300 ppm + UV-C 13.2 kJ/m ²	48.40 ± 1.79 a	77.36 ± 3.36 a	91.04 ± 5.07 a	93.36 ± 9.66 a	95.69 ± 13.15 ab
AEW 300 ppm + UV-C 39.6 kJ/m ²	48.73 ± 0.73 a	72.44 ± 2.64 a	87.71 ± 4.28 a	98.45 ± 2.67 a	105.56 ± 10.26 a

Note Values are mean ± standard error (SE) from n = 3. Different letters within the same column differ significantly between the treatments by using Duncan's multiple range test (P<0.05)

Table B13 Effects of AEW and UV-C on antioxidant activity (DPPH assay) of 'Phulae' pineapple during storage at 13 °C for 28 days

Treatments	DPPH (mg Ascorbic Acid Equivalent/100 g of sample wet weight basic)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Pulp					
Control	14.68 ± 0.27 b	31.93 ± 1.26 a	19.47 ± 0.94 b	19.12 ± 0.43 b	19.76 ± 1.43 b
AEW 300 ppm	15.25 ± 1.53 b	29.65 ± 0.63 a	24.09 ± 3.25 ab	19.82 ± 1.43 b	20.96 ± 0.69 ab
UV-C 13.2 kJ/m ²	16.22 ± 0.19 b	31.55 ± 1.62 a	18.62 ± 2.05 b	21.66 ± 0.39 ab	25.52 ± 4.16 ab
UV-C 39.6 kJ/m ²	15.27 ± 1.24 b	29.45 ± 1.28 a	18.90 ± 0.50 b	20.32 ± 1.21 b	19.15 ± 0.53 b
AEW 300ppm+ UV-C 13.2 kJ/m ²	21.33 ± 1.02 a	31.68 ± 1.56 a	26.97 ± 0.37 a	24.90 ± 2.57 a	27.88 ± 2.87 a
AEW 300ppm+ UV-C 39.6 kJ/m ²	19.92 ± 0.81 a	31.94 ± 1.76 a	26.61 ± 0.71 a	23.65 ± 0.69 ab	23.49 ± 0.54 ab
Peel					
Control	15.71 ± 0.73 a	50.01 ± 1.68 bc	30.62 ± 0.83 c	32.12 ± 0.88 b	34.90 ± 1.32 b
AEW 300 ppm	15.90 ± 1.50 a	55.60 ± 0.96 bc	47.41 ± 4.56 ab	36.11 ± 3.02 ab	43.69 ± 2.60 a
UV-C 13.2 kJ/m ²	16.84 ± 0.61 a	47.25 ± 0.91 c	38.36 ± 2.11 bc	33.25 ± 1.99 b	36.33 ± 3.37 ab
UV-C 39.6 kJ/m ²	15.92 ± 0.68 a	57.83 ± 3.71 ab	40.83 ± 3.89 b	34.81 ± 0.73 ab	39.71 ± 2.86 ab
AEW 300ppm+ UV-C 13.2 kJ/m ²	17.19 ± 0.96 a	58.99 ± 3.15 ab	51.01 ± 3.25 a	39.96 ± 1.01 a	41.27 ± 2.96 ab
AEW 300ppm+ UV-C 39.6 kJ/m ²	14.20 ± 0.59 a	65.61 ± 4.46 a	46.36 ± 1.99 ab	38.32 ± 2.19 ab	43.31 ± 0.89 ab

Note Values are mean ± standard error (SE) from n = 3. Different letters within the same column differ significantly between the treatments by using Duncan's multiple range test (P<0.05)

Table B14 Effects of AEW and UV-C on antioxidant activity (FRAP assay) of ‘Phulae’ pineapple during storage at 13 °C for 28 days

Treatments	FRAP (mg Fe (II) SO ₄ Equivalent/100 g of sample wet weight basic)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Pulp					
Control	43.87 ± 2.28 b	50.83 ± 1.95 b	56.23 ± 1.15 d	109.77 ± 5.51 b	131.88 ± 10.26 bc
AEW 300 ppm	54.71 ± 0.38 a	58.64 ± 1.45 ab	62.35 ± 2.14 bc	114.95 ± 7.48 ab	133.18 ± 2.46 bc
UV-C 13.2 kJ/m ²	49.62 ± 1.76 ab	50.43 ± 3.94 b	59.15 ± 1.22 cd	119.37 ± 5.07 ab	126.64 ± 5.93 c
UV-C 39.6 kJ/m ²	53.25 ± 1.68 a	57.36 ± 0.43 ab	62.11 ± 1.34 bc	119.22 ± 8.14 ab	118.45 ± 2.88 c
AEW 300 ppm + UV-C 13.2 kJ/m ²	56.19 ± 5.84 a	59.80 ± 4.29 a	67.85 ± 2.41 a	133.54 ± 6.61 a	157.72 ± 4.68 a
AEW 300 ppm + UV-C 39.6 kJ/m ²	57.07 ± 1.60 a	61.00 ± 1.34 a	66.91 ± 0.54 ab	131.42 ± 4.37 a	147.83 ± 4.82 ab
Peel					
Control	40.56 ± 2.23 b	83.13 ± 3.97 a	104.79 ± 0.96 b	229.38 ± 10.76 c	296.37 ± 2.58 a
AEW 300 ppm	47.30 ± 1.68 a	82.65 ± 4.51 a	128.43 ± 18.29 ab	254.92 ± 21.26 abc	373.52 ± 35.51 a
UV-C 13.2 kJ/m ²	42.10 ± 1.61 ab	72.57 ± 2.28 a	117.10 ± 5.46 ab	236.29 ± 11.30 abc	302.32 ± 32.93 a
UV-C 39.6 kJ/m ²	45.80 ± 0.73 ab	86.04 ± 4.33 a	159.92 ± 31.06 a	233.70 ± 7.20 bc	305.49 ± 19.21 a
AEW 300 ppm + UV-C 13.2 kJ/m ²	45.57 ± 1.62 ab	81.65 ± 4.09 a	142.71 ± 5.93 ab	269.27 ± 6.03 ab	353.35 ± 28.36 a
AEW 300 ppm + UV-C 39.6 kJ/m ²	44.72 ± 2.36 ab	80.01 ± 5.20 a	141.48 ± 3.94 ab	274.05 ± 3.82 a	367.80 ± 14.01 a

Note Values are mean ± standard error (SE) from n = 3. Different letters within the same column differ significantly between the treatments by using Duncan’s multiple range test (P<0.05)

Table B15 Effects of AEW and UV-C treatments on spore germination (%) of *C. paradoxa* during incubate at 27 °C for 15 hours

Treatments	Spore germination (%) in different incubation time				
	3 h	6 h	9 h	12 h	15 h
Control	16.10 ± 4.91 a	32.29 ± 2.17 a	42.45 ± 6.13 a	41.25 ± 6.74 a	55.81 ± 4.69 a
AEW 300 ppm	7.87 ± 1.24 ab	10.25 ± 2.98 bc	12.93 ± 1.85 bc	12.93 ± 1.61 bc	20.28 ± 4.11 c
UV-C 13.2 kJ/m ²	10.55 ± 2.57 ab	14.68 ± 1.38 b	16.44 ± 1.61 bc	31.60 ± 1.31 a	34.71 ± 3.12 b
UV-C 39.6 kJ/m ²	5.52 ± 2.24 b	16.68 ± 3.36 b	17.11 ± 2.58 b	29.68 ± 3.86 a	37.24 ± 6.78 b
AEW 300 ppm + UV-C 13.2 kJ/m ²	3.71 ± 2.07 b	4.62 ± 2.33 cd	6.42 ± 3.22 bc	17.84 ± 3.91 b	14.30 ± 2.54 cd
AEW 300 ppm + UV-C 39.6 kJ/m ²	1.75 ± 0.97 b	0.72 ± 0.72 d	5.28 ± 2.77 c	5.75 ± 1.03 c	6.51 ± 1.47 d

Note Values are mean ± standard error (SE) from n = 3. Different letters within the same column differ significantly between the treatments by using Duncan's multiple range test (P<0.05)

Table B16 Effects of AEW and UV-C treatments on germ tube length of *C. paradoxa* during incubate at 27 °C for 13 hours

Treatments	Germ Tube Length (μm) in different incubation time				
	5 h	7 h	9 h	11 h	13 h
Control	65.94 ± 4.04 a	254.89 ± 23.42 a	360.28 ± 16.58 a	536.82 ± 83.74 a	843.37 ± 99.25 a
AEW 300 ppm	0 b	0 c	0 c	0 b	0 b
UV-C 13.2 kJ/m ²	17.95 ± 11.04 b	161.38 ± 33.26 b	267.81 ± 50.87 b	418.48 ± 40.54 a	749.18 ± 89.89 a
UV-C 39.6 kJ/m ²	13.49 ± 11.07 b	142.65 ± 34.04 b	227.43 ± 22.76 b	511.49 ± 71.85 a	784.12 ± 52.46 a
AEW 300 ppm + UV-C 13.2 kJ/m ²	0 b	0 c	0 c	0 b	0 b
AEW 300 ppm + UV-C 39.6 kJ/m ²	0 b	0 c	0 c	0 b	0 b

Note Values are mean ± standard error (SE) from n = 3. Different letters within the same column differ significantly between the treatments by using Duncan's multiple range test (P<0.05)

Table B17 Effects of AEW and UV-C Treatments on Mycelium Disc Growth Inhibition during Storage at 27 °C for 7 days

Treatments	Mycelium Disc Growth Inhibition (%)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	0 d	0 e	0 c	0 c	0 c	0 c	0 c
AEW 300 ppm	100 a	89.31 ± 0.60 b	73.16 ± 1.36 b	56.30 ± 2.92 b	59.80 ± 5.90 b	55.56 ± 6.42 b	55.56 ± 6.42 b
UV-C 13.2 kJ/m ²	30.17 ± 1.29 c	8.18 ± 0.79 d	0 c	0 c	0 c	0 c	0 c
UV-C 39.6 kJ/m ²	41.81 ± 0.57 b	14.78 ± 0.58 c	0 c	0 c	0 c	0 c	0 c
AEW 300ppm + UV-C 13.2 kJ/m ²	100 a	100 a	100 a	100 a	100 a	100 a	100 a
AEW 300ppm + UV-C 39.6 kJ/m ²	100 a	100 a	100 a	100 a	100 a	100 a	100 a

Note Values are mean ± standard error (SE) from n = 3. Different letters within the same column differ significantly between the treatments by using Duncan's multiple range test (P<0.05)

Example for UV-C dose calculation

(targeted dose: 13.2 kJ/m²)

The UV meter is a machine measuring the intensity power of UV lamp. In this case, the UV meter (Linshang, Model-LS126C, China) has $\mu\text{W}/\text{cm}^2$ as a displayed unit. The doses could be checked from online at

<https://www.waveformlighting.com/uv-c-led/uv-unit-calculator>.

Where; 1 W/cm² = 10 000 J/m². 1 sec (or) 1 $\mu\text{W}/\text{cm}^2$ = 0.01 J/m². 1 sec

Equation for UV-C dose:

$$\begin{aligned} \text{Dose (kJ/m}^2\text{)} &= \text{Intensity } (\mu\text{W}/\text{cm}^2) \times \text{Time (sec)} \\ 13.2 \text{ kJ/m}^2 &= 8000 \mu\text{W}/\text{cm}^2 \times \text{Time (sec)} \\ 13200 \text{ J/m}^2 &= 80 \text{ J/m}^2 \times \text{Time (sec)} \\ 165 \text{ (sec)} &= \text{Time} \end{aligned}$$



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รายงานผลการจำแนกจุลินทรีย์ / IDENTIFICATION'S REPORT

ชื่อผู้ขอรับบริการ / Customer's name:	Mr. Hlwan Moe Thu	เลขที่ / No.:	2022ID1130-166
หน่วยงานและที่อยู่ / Institute and address:	Mae Fah Luang University 333 M. 1 T.Tasud Muang Chiang Rai Thailand 57100	วันที่ได้รับตัวอย่าง / Sample receive date :	7.Dec.2022
		วันที่รายงานผล/ Report date:	14.Dec.2022

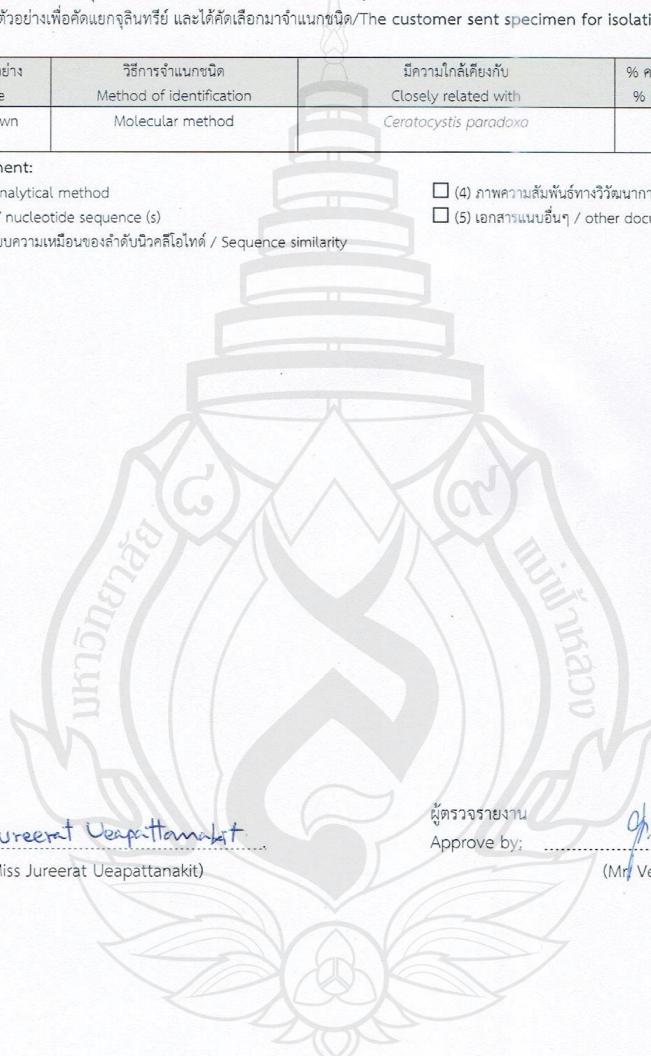
ตัวอย่างบริการส่งตัวอย่างเป็นเชื้อบริสุทธิ์เพื่อจำแนกชนิด/The customer sent pure isolate for identification
 ผู้ขอรับบริการส่งเป็นตัวอย่างเพื่อคัดแยกจุลินทรีย์ และได้คัดเลือกมาจำแนกชนิด/The customer sent specimen for isolation and chose isolate(s) identification

ลำดับที่ No.	รหัสตัวอย่าง Code	วิธีการจำแนกชนิด Method of identification	มีความใกล้เคียงกับ ¹ Closely related with	% ความเหมือน % similarity	หมายเหตุ Note
1	Unknown	Molecular method	<i>Ceratocystis paradoxa</i>	100%	

เอกสารแนบ / Attachment:

(1) วิธีการวิเคราะห์ / Analytical method
 (2) ลักษณะนิวเคลียติก / nucleotide sequence (s)
 (3) ข้อมูลการเปรียบเทียบความเหมือนของลักษณะนิวเคลียติก / Sequence similarity

(4) ภาพความสัมพันธ์ทางวิวัฒนาการ / Phylogenetic relationship
 (5) เอกสารแนบเนื่ງ / other document



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Figure B1 Mould identification report from TBRC

เอกสารแนบ / Attached document 1

วิธีการจำแนกชนิดรา

Fungal Identification Methods

1. DNA extraction

Genomic DNA was extracted from fresh mycelia using a modified CTAB method of Doyle and Doyle (1987).

1. Use a sterile spatula to scrape fungal mycelia from a culture plate into a microtube containing CTAB buffer (600 μ l).
2. Grind mycelia using the microtube pestle.
3. Incubate the microtube at 65°C for 20 min.
4. Add 600 μ l of CHCl₃; IAA (24:1), and invert repeatedly.
5. Centrifuge at 13,000 rpm for 15 min at 4°C.
6. Remove the upper aqueous phase to a clean microtube.
7. Add 300 μ l of cold isopropanol. Invert repeatedly and place at -20°C for 20 min.
8. Centrifuge at 13,000 rpm for 15 min at 4°C to pellet the DNA.
9. Discard supernatant. Add 50 μ l of 1x TE to dissolve DNA pellet.

2. PCR: ITS

The internal transcribed spacer (ITS) region was amplified in a 50 μ l reaction volume containing 10X buffer, 2.5 mM MgCl₂, 0.2 mM dNTPs, 0.2 μ M of each primer (ITS5 and ITS4), and 1U Taq DNA polymerase. The PCR temperature profile began with an initial denaturation at 96°C for 2 min, followed by 35 cycles of 96°C for 1 min, 53°C for 1 min and 72°C for 1:30 min. The final extension was carried out for 10 min at 72°C.

3. Gel Electrophoresis and Sequencing

PCR product was checked by 1% agarose gel electrophoresis, stained with DNA-Dye NonTox, and visualized under ultraviolet (UV) transilluminator. The PCR product was sent to be sequenced for both directions on an automated DNA sequencer (Macrogen Inc., Korea).

4. Sequence analyses

The nucleotide sequences obtained from all primers were assembled using Cap contig assembly program, an accessory application in BioEdit (Biological sequence alignment editor) Program (<http://www.mbio.ncsu.edu/BioEdit/BioEdit.html>). The sequences were compared with nucleotide sequences databases on Genbank, CBS or suitable databases.

References

- Doyle JJ and Doyle JL (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytoch. Bull.* 19: 11-15.
- Hall TA (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids Symp. Ser.* 41: 95-98. Available at [<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>]. Accessed July 25, 2008.
- Omega Bio-Tek, Inc. (2013). E.Z.N.A. Forensic DNA Kit: Standard protocol: 6-9.
- Techaprasarn J. (2010). Genetic variation of *Kaempferia* (Zingiberaceae) in Thailand based on chloroplast DNA (psbA-trnH and petA-psbJ) sequences. *Genetics and Molecular Research* 9 (4): 1957-1973.

Disclaimer:

Figure B2 Mould identification methods from TBRC



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