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Predictive model for optimal ozone condition to control C*ollectotrichum* sp. and maintain quality of Kaew Kamin mango

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Abstract The obtained optimum conditions using RSM were a 25,000 mg/hr ozone and treatment time of 30 min. At the optimum condition, the shelf-life of Kaew Kamin mangoes was longer under the 21 days at 25±2°C. Application of the optimized ozone treatment condition, when compared to the untreated control, was significantly affected in preserving postharvest quality of the fruit. Specifically, the treatment effectively delayed weight loss, maintained alteration in peel and induced adaptaion in pulp color attributes (L*, a*, b*, chroma, and hue values), and maintained fruit firmness, titratable acidity, and cellular membrane integrity as indicated by lower electrolyte leakage. Conversely, no significant differences were observed in total soluble solids, ascorbic acid, and total phenolic contents, and antioxidant capacity between treated and untreated samples.

Keywords: Kaew Kamin mango, Ozone, *Colletotrichum* sp.

Introduction

Mango (*Mangifera indica* L.) is an economically major tropical fruit, particularly the Kaew Kamin mango in Sa Kaeo Province, which has a high production volume of approximately 50,205 tons. It is highly demanded by consumers, both fresh fruit and processed into various products. However, the anthracnose disease is one of the main significant problems in mango production from the fungus *Colletotrichum* sp. This pathogen can be developed in the high thermal and moist condition commons in Thailand. Anthracnose can contaminate mango trees at every stage of growth throughout the growing season, and the symptoms are often absent during the initial stages, a phenomenon known as quiescent infection. Symptoms present in the mangoes were that they were mature or beginning to ripen and showed signs of rotting, which confused disease

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management. Anthracnose is a significant postharvest problem, significantly resisting the domestic and international mango market. Therefore, anthracnose control measures are essential to reduce postharvest losses and maintain fruit quality.

Ozone (O_3) is a powerful oxidizing agent for antimicrobial properties, which was widely recognized. It can inhibit various microorganisms, such as bacteria, yeasts, fungi, and viruses, from inactivating enzymes, degrading nucleic acids, and destroying cell membranes. Ozone applications in postharvest disease management have attracted considerable interest in horticultural crops, fruits, and vegetables. Ozone is an unstable gas that rapidly decomposes to form highly reactive free radicals. It had highly instability and a shortage, which is recognized as safe by the U.S. Food and Drug Administration (FDA). Ozone can be applied either as gaseous ozone or aqueous ozone. Although, gaseous ozone has a longer 1–4 h half-life compared to the 1–10 min of aqueous ozone, nevertheless, aqueous ozone is suitable for microbial control because it is easy to use and low in concentrations. Previous reports have shown that ozone treatment can effectively reduce microbial populations and inhibit the germination of fungal spores. For example, an experiment of Brazilian Palmer mangoes using ozonated water at a concentration of 1 mg/L/s for 10 or 20 min and storing the fruits at 14$\pm$2°C and 90$\pm$2% relative humidity for 7 and 15 days found that the growth of microorganisms (coliforms) was as inhibited by effective as chlorine (Monaco *et al*. 2016). In papayas, using ozonated water at a concentration of 3 mg/L at a temperature of 20$\pm$2°C for 5 min and storing the fruits at 10+2°C for 7 days, followed by preservation at room temperature (23$\pm$2°C) for 8 days, showed a significantly longer control of stem rot than the control for 3 days caused by four fungi (*P. caricae-papayae*, *A. alternata*, *C. gloeosporioides*, and *L. theobromae*) (Terao *et al*. 2019).

However, in Kaew Kamin mango cultivation in Sa Kaeo Province, there remains a lack of research and scientific knowledge to support the production of high-quality fruits. Especially, the anthracnose management is a main issue that causes postharvest disease. This problem gap impulsive the research team to study the factors influencing disease development and the interactions between these factors. This study aimed to develop a model for postharvest disease management. The response surface methodology (RSM) and central composite design (CCD) were used to model the optimal ozone selection for disease control.

Materials and methods

Plant materials

Kaew Kamin Mango trees (*Mangifera indica* L.) approximately 3–4 years old were collected from a population of 40 trees at Dee Tor Jai Farm (Rai Dee Tor Jai), located in Watthana Nakhon District, Sa Kaeo Province, Thailand, between November 2023 and June 2024. The mango fruits were designated by selecting inflorescences with about 70 percent of the flowers in bloom. Days after full bloom (DAFB) were tracked, and fruit set was evaluated roughly six weeks post-flowering. The tagged Kaew Kamin mangoes were then harvested and transported to the laboratory.

The mango fruits of similar size and 70-80% maturity was selected (Lueangprasert, *et al*. 2025), they were washed and sterilized on the surface. Then, they were dried in a Biological Safety Cabinet (BSC Class II) and exposed to UV light for 1 h. A 100 µl suspension of *Colletotrichum* sp. spore ($1x10^6$ spores/ml) was added to the fruit surface and pedicel of the mangoes and allowed to dry. And then, the fruits were incubated at ambient temperature for overnight before being used in the next experiment.

Effect of ozone application on disease incidence control

The prepared of Kaew Kamin mango fruits were studied for their effects on inhibiting anthracnose disease using the Response Surface Methodology (RSM) technique. The experiment was designed using a Central Composite Design (CCD) with two factors: ozone concentration in the washing water and washing time.

Aqueous ozone preparation

Aqueous ozone was prepared in water using an ozone generator (OZONER®-013-30G, Pro-Tech-Sai Co., Ltd., Thailand). Saturated ozone water was received by adding gaseous ozone to 35 liters of tap water in a plastic container at 18±2°C using an air supply head. The ozone system managed the air flow rate at 60 L/h, resulting in an ozone concentration of approximately 30 g/h. Ozone concentration was measured semi-quantitatively using an ozone test kit (HI38054, HANNA Instruments Inc., USA).

Decontamination experiment

The decontamination experiment was conducted using various conditions, including ozone concentration in the washing water and washing times, according to the experimental design. In each treatment, 15 mango sample fruits from each prepared sample were immersed in plastic containers containing 35 L of ozone water at 18±2°C, and compared to mangoes without ozone water.

Then, the obtained mangoes were air-dry in a BSC Class II cabinet and afterward stored at 25±2°C. The results were observed every 7 days. The efficiency of disease control was examined by comparing with the control treatment, that was washed with clean water (without ozone). The percentage of disease incidence was studied using the formula (1).

$$\text{Disease incidence } (\%) = [\frac{\text{Number of diseased fruits}}{\text{Total number of fruits}}] \text{ x } 100 \quad (1)$$

Note: Diseased fruits are defined as mangoes with anthracnose symptoms (black, irregularly shaped lesions) exceeding approximately 20% of the total fruit area.

Experimental design

This experiment was designed by Design Expert version 10 software (Stat-Ease, USA) using RSM's Central Composite Design (CCD). For the modeling process of *Colletotrichum* sp. disinfection on mangoes using ozone treatment, two factors were ozone concentration in the washing water (6,900–28,000 mg/h) and washing time (6–34 min). Each factor was assigned at five levels: minimum (-1.68), low (-1), medium (0), high (+1), and maximum (+1.68), as shown in Table 1. All experiments performed were calculated using equation (2) (Owolabi *et al*., 2018).

$$N = 2^n + 2n + n_c \quad (2)$$
$$N = 2^2 + 2(2) + 6 = 4 + 4 + 5 = 13$$

Notes: n is the number of factors, nc is the number of centers, and N is the sum of all experiments.

Table 1. The effect of ozone treatment on the inhibition of anthracnose disease in Kaew Kamin mango

Variables	Symbol	Unit	Level				
			-1.68	-1	0	+1	+1.68
Dosage rate of ozone	X1	mg/h	6,900	10,000	17,500	25,000	28,000
Contact time	X2	min	6	10	20	30	34

RSM recommends using a total of 13 experiments to predict the response. As shown in Table 2, in each experiment, mango samples were immersed in ozone water at various ozone concentrations (X1) and washing times (X2). The disease incidence was planned as the response variable (Y). The response

variable was diagnosed using a regression model as displayed in Equation (3) (Hazbawi and Safaeinezhad, 2023).

$$Y = a_0 + a_1X_1 + a_2X_2 + a_3X_1X_2 + a_4X_1^2 + a_5X_2^2 \quad (3)$$

Notes: Y means the regression coefficients of the model, the response variable; a0, a1, a2, a3, a4, and a5; and X1 and X2 mean the independent variables.

The adequacy of the model was assessed; the coefficient of determination (R2), adjusted R2 (Adj. R2), predicted R2 (Pred. R2), percentage coefficient of variance (%CV), and lack of fit test were used. The model was statistically significant at the $p < 0.05$ level.

Table 2. Experimental conditions of ozone treatment for the inhibition of anthracnose in Kaew Kamin mango using Central Composite Design (CCD)

Experiment conditions	Ozone concentration (X1) mg/h	Time (X2) min
1	25,000	10
2	28,000	20
3	17,500	34
4	10,000	10
5	6,900	20
6	17,500	20
7	17,500	20
8	17,500	20
9	25,000	30
10	17,500	20
11	17,500	6
12	10,000	30
13	17,500	20

Effect of ozone application on physicochemical change

Approximately 70-80% maturity mangoes were examined and washed to disinfect the surface of the fruit. The mangoes treated with ozone at the most suitable concentration and duration for disease control were investigated for physicochemical changes and compared with the control mangoes (without ozone) to estimate the effect of ozone on mango quality.

Evaluation of weight loss, peel and pulp color, and firmness values

The fresh fruit was evaluated initially for each fruit and on the day of assessment. The percentage of fresh weight loss was calculated as formular (4) follows:

$$\text{Weight loss (\%)} = \left[\frac{(\text{Initial weight} - \text{Weight on the measurement})}{(\text{Initial weight})}\right] x\ 100 \quad (4)$$

The mango fruit color of the peel and pulp was measured at the middle on both sides of the fruit with a colorimeter (NR110, 3NH Technology CO., LTD., China). The measured color values were reported as L* (lightness/darkness), a* (redness/greenness), b* (yellowness/blueness), C* (chroma), and Hº (hue angle) values.

The firmness of the mango was measured using a fruit firmness tester (DESIK, GY-4 series, Germany Desik Instruments Group Limited, Germany). An 8 mm diameter stainless steel conical probe was installed, and the probe was allowed to penetrate to a depth of 5 mm. Firmness value was expressed in Newtons (N) value.

Assessment of electrolyte leakage value

The mango pulp was determined for electrolyte leakage (EL) value as adjusted by the method of Hong and Gross (1998). The mango pulp cylinders for fifteen pieces (approximately 10 g) were drilled into 1 cm thick and 1 cm diameter pieces with a cork borer. The mango pulp samples were washed with deionized water and dried with tissue paper. Following this, the samples were immersed in 350 ml of 0.4 M mannitol solution for 1 h at ambient temperature. The EL conductivity in the mannitol solution was assessed with a conductivity meter (Mettler Toledo, S230, Switzerland). And then, the samples were autoclaved at 121°C for 30 min using a high-pressure autoclave. The final electric conductivity was evaluated after cooling to an ambient temperature. %EL value was calculated according to the formula (5).

$$\text{EL (\%)} = \frac{(\text{EL1 x 100})}{\text{EL2}} \quad (5)$$

Notes: EL1 is the electric conductivity of the sample in the mannitol solution before autoclaving.

EL2 is the electric conductivity of the sample in the mannitol solution after autoclaving at ambient temperature.

Determination of total soluble solids and titratable acidity contents

The mango pulp was measured for total soluble solids (TSS) content using a hand refractometer (HM, SCM-1000, HM Digital, Korea). Mango juice was pressed, and it was dropped onto the refractometer, and the TSS was shown as % brix.

The mango pulp was analyzed for titratable acidity (TA) content by using the AOAC method (1995). 10 g of the mango pulp was ground and combined with deionized water. The pulp was filtered, and the suspension was titrated using 0.1 N sodium hydroxide (NaOH) and 1-2 drops of 0.1% phenolphthalein as an indicator (end point at pH = 8.2). TA was calculated as % citric acid (with an equivalent weight of 0.07) using the following formula (6):

$$\text{TA (\% citric acid)} = \left[\frac{(\text{AxBx0.07})}{\text{weight of sample (g)}}\right] x\ 100 \quad (6)$$

Notes: A is a concentration of NaOH, N.
B is a volume of NaOH, ml.

Changes in ascorbic acid contents

The mango pulp was evaluated for ascorbic acid (AA) content with the AOAC method (2000). The mango pulp of a 10 g sample (M) was extracted by a 0.4% oxalic acid solution, and the volume was adjusted to 100 ml with the same solution. The sample was filtered through a Whatman filter paper No. 1. The 10 ml of suspension solution was titrated with 2,6-dichlorophenol-indophenol dye solution until a pink color appeared (V_1). 10 ml of standard AA (100 µg/ml of AA, prepared from 10 mg of AA in 100 ml of 0.4% oxalic acid solution) was titrated with 2,6-dichlorophenol-indophenol dye solution until the pink color occurred (V_2). The AA content was calculated using the formula (7):

$$\text{Ascorbic acid (mg/100g)} = \left[\frac{(\text{V1x1mgx100ml})}{(\text{V2xmlxM})}\right] x\ 100\text{g} \quad (7)$$

Preparation of mango pulp extract for antioxidant capacity and total phenolic content

For the preparation of extraction, 1 g of mango pulp was homogenized with 25 ml of 80% ethanol at 4°C for 1 min. The centrifuge (Hermle centrifuge Z326k, Germany) at 12,000 rpm for 30 min at 4°C was extracted the sample. The supernatant extracts were estimated for total antioxidant activity and total phenolic content.

Evaluation of antioxidant capacity

Antioxidant capacity of mango pulp was measured using a modified method from Muńim *et al.* (2003). Preparation of the evaluation: 400 µl of 0.3 M acetate buffer (pH 5.5) and 2.5 ml of 0.10 mM 1,1-Diphenyl-2-picrylhydrazyl

(DPPH) in 80% ethanol was mixed, and 100 µl of ethanol extract was added. The sample mixture was incubated in absolute darkness for 30 min at ambient temperature (25±2ºC). The absorbance at 517 nm was measured by the UV-Vis spectrophotometer (C-7200 Peak Instrument Co., Ltd., China). The blank, 100 µl of 80% ethanol, was used instead of the sample extract. Antioxidant capacity was compared with the Trolox standard. And using the regression equation to determine the correlation between Trolox concentration and percentage of DPPH inhibition and indicated µmol/100 g FW.

Assessment of total phenolic content

The method for determining total phenolic content was evaluated using the adjusted Folin-Ciocalteu method from Singleton and Rossi (1965). 2 ml of samples were mixed into 10 ml of 10% Folin-Ciocalteu's phenol reagent, and it was incubated at ambient temperature for 8 min. After that, 8 ml of 7.5% Na_2CO_3 solution was added and incubated at ambient temperature for 2 h. The absorbance at 765 nm was evaluated by using the UV-Vis spectrophotometer. Total phenolic content was calculated as mg/100 g FW and compared to a gallic acid standard calibration curve.

Determination of carotenoid content

The carotenoid content of mango pulp was adjusted according to the method of More and Rao (2019). Sample extract preparation: 1 g of mango pulp (W) was extracted with 14 ml of cold working solution (hexane: acetone, 3:2 v/v) and incubated in a dark room at 4ºC for 1.5 h. After that, the sample extract was centrifuged at 10,000 rpm for 10 min at 4ºC. The suspension solution was adjusted to 25 ml with the same extractant solution. The absorbance at 450 nm was assessed using the UV-Vis spectrophotometer. The carotenoid content was calculated following the formula (8) and shown as µg/g FW.

Carotenoid content (µg/g FW) = $[(A_{450} \times 4) / \text{weight of sample (g)}] \times 100$ (8)

Note: 4 is a coefficient of equations.

Statistical analysis

For this research, the statistical data of physicochemical change were analyzed using statistical difference of mean using analysis of variance (ANOVA) and Independent-Samples T-Test. The analysis of mean difference was conducted by IBM SPSS statistic 26 (Trial version) program, using a comparison of means at a 95% confidence level ($p<0.05$). The experimental results were presented as means ± standard deviations (SDs).

Results

Control of anthracnose disease in Kaew Kamin mango

Mango fruits of the Kaew Kamin variety, contaminated with *Colletotrichum* sp., were utilized to examine the impact of ozone therapy on the suppression of anthracnose disease. The experiment employed Response Surface Methodology (RSM) utilizing a Central Composite Design (CCD), focusing on two variables: ozone content in the washing water and washing duration. Each element was assessed at five levels: minimum (−1.68), low (−1), medium (0), high (+1), and maximum (+1.68). The disease control efficacy was examined by comparing treated mango fruits with a control group washed with ozone-free water, and the disease prevalence in Kaew Kamin mangoes was assessed.

Table 3. Experimental conditions for optimizing disease incidence in Kaew Kamin mango at 28 days post-treatment

Experiments	Ozone concentration (X1) mg/h	Time (X2) min	Disease incidence (%)
1	25,000	10	43.33
2	28,000	20	30.00
3	17,500	34	33.33
4	10,000	10	76.67
5	6,900	20	80.00
6	17,500	20	56.67
7	17,500	20	53.33
8	17,500	20	53.33
9	25,000	30	33.33
10	17,500	20	50.00
11	17,500	6	63.33
12	10,000	30	53.33
13	17,500	20	53.33
Control	-	-	86.67

From the analysis of the coefficient of regression equation for the percentage of disease occurrence of Kaew Kamin mango at 28 days as shown in Table 4 and 5.

From the experiment on the effect of ozone on anthracnose disease control in Kaew Kamin mangoes, it was found that ozone concentration and washing time significantly reduced disease incidence. It was shown in Table 4, ozone concentration in washing water and washing time accounted for 93.46% of the variance in disease incidence in Kaew Kamin mangoes (R^2 = 0.934). The independent variables influencing disease incidence with a p-value less than 0.05 were ozone concentration in washing water (X1) and washing time (X2).

Furthermore, linear equations were found to be the most appropriate equation for studying the factors and surface response, with statistical significance ($p<0.05$), as shown in Table 5. These equations can be used to develop the following equation to predict disease incidence in Kaew Kamin mangoes:

$$Y = 107.683 - 0.0207X_1 - 0.947X_2$$

When considering the lack of fit of the equation, the P-value of the lack-of-fit was found to be 0.1342, which is greater than 0.05. This suggests that the model is adequate for all variables in the equation. Therefore, the above equation can be used to predict the percentage of disease occurrence in Kaew Kamin mangoes. From the equation for predicting the percentage of disease occurrence in Kaew Kamin mangoes. The disease incidence response surface graph can be generated as Figure 1. It was found that decreasing ozone concentration in washing water (X1) increased the disease incidence rate in Kaew Kamin mangoes. Similarly, decreasing washing time (X2) increased the disease incidence rate in Kaew Kamin mangoes. Therefore, to influentially reduce the disease incidence rate in Kaew Kamin mangoes, the ozone concentration in washing water (X1) and washing time (X2) should be increased. Nevertheless, the most suitable factor values obtained from this experiment was 25,000 mg/h of ozone concentration in washing water (X1) and 30 minutes of washing time (X2), which can reduce the disease occurrence rate in Kaew Kamin mangoes to only 27.59%, as shown in Figure 2.

The above data demonstrate that ozone treatment was effectively controls anthracnose in Kaew Kamin mango. Response surface methodology is a suitable instrument for improving treatment conditions to reduce disease incidence.

Table 4. Analysis of variance for anthracnose disease incidence in Kaew Kamin mango

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	2640.81	2	1320.40	71.44	< 0.0001	significant
X_1- X_1	*1923.36*	*1*	*1923.36*	*104.06*	*< 0.0001*	
X_2- X_2	*717.44*	*1*	*717.44*	*38.82*	*< 0.0001*	
Residual	184.83	10	18.48			
Lack of Fit	*153.72*	*6*	*25.62*	*3.29*	*0.1342*	*Not significant*
Pure Error	*31.11*	*4*	*7.78*			
Cor Total	2825.64	12				

Std. Dev.	4.30	R-Squared	0.9346
Mean	52.56	Adj R-Squared	0.9215
C.V. %	8.18	Pred R-Squared	0.8708
PRESS	365.18	Adeq Precision	24.186
-2 Log Likelihood	71.40	BIC	79.10
		AICc	80.07

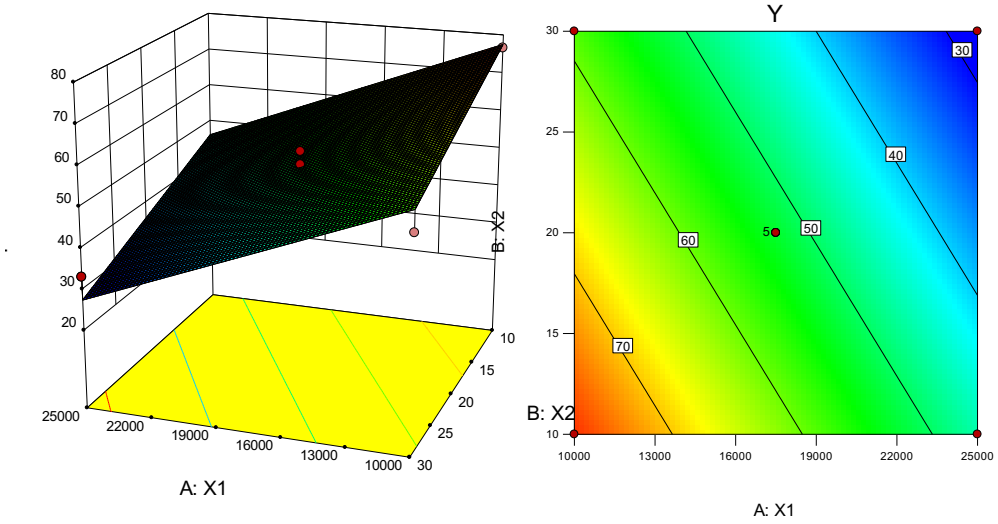

Figure 1. Effect of ozone concentration and washing duration on anthracnose incidence in Kaew Kamin mango after 28 days

Table 5. Analysis of variance (ANOVA) for regression analysis of anthracnose disease incidence in Kaew Kamin mang

Source	Sequential p-value	Lack of Fit p-value	Adjusted R-Squared	Predicted R-Squared	
Linear	< 0.0001	0.1342	0.9215	0.8708	Suggested
2FI	0.1257	0.1693	0.9338	0.8729	
Quadratic	0.1348	0.2481	0.9520	0.8618	
Cubic	0.0967	1.0000	0.9736	0.9828	

When the optimal conditions were used to evaluation the disease in Kaew Kamin mangoes, it was found that washing Kaew Kamin mangoes to destroy disease with ozone water at a level of 25,000 mg/h for 30 min was able to reduce the loss of Kaew Kamin mangoes by more than 80 percent, or the percentage of disease incidence was less than 20% for 21 days. This treatment was able to reduce the disease incidence better than the fruits that were washed with ozone-free water, which found the loss of Kaew Kamin mangoes by more than 50% for 21 days, as shown in Figure 3.

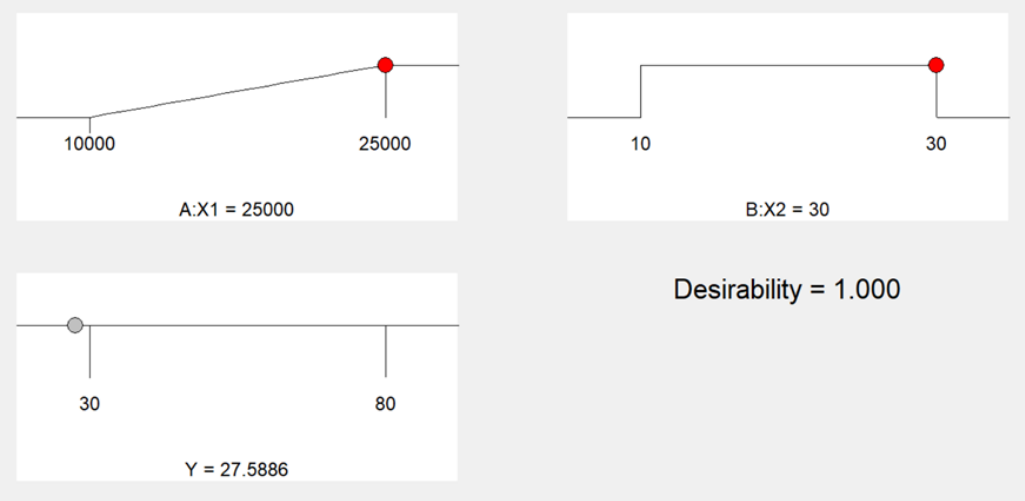

Figure 2. Prediction of optimal disease incidence in Kaew Kamin mango at 28 days

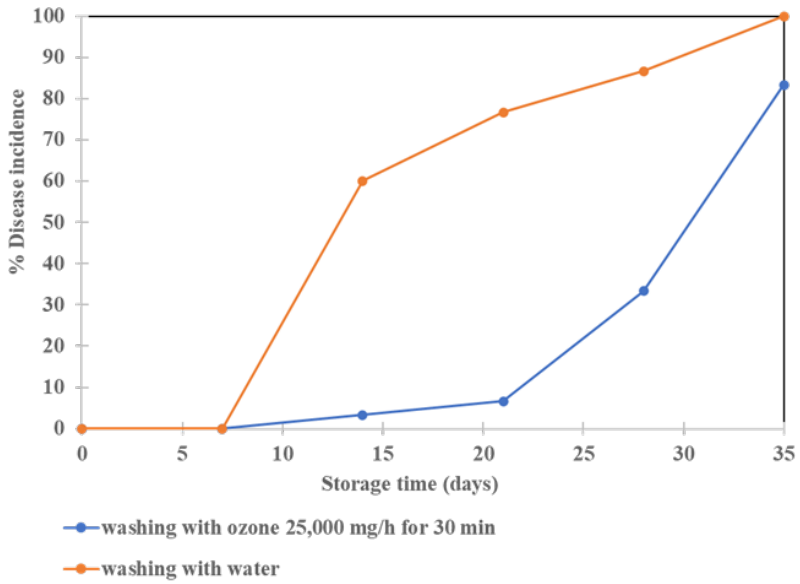

Figure 3. Effect of storage time on disease incidence in Kaew Kamin mango following ozone treatment at 25,000 mg/h for 30 min

Ozone application on physicochemical changes, Weight loss, peel and pulp color values

The experimental results of physicochemical changes indicated that ozone treatment for washing at a concentration of 25,000 mg/h for 30 min effectively maintain the quality of Kaew Kamin mangoes compared to the control without ozone treatment. It could reduce the weight loss of fruit more significantly than the control. The fruit peel color exhibited enhanced brightness (L*), yellowness (b*) and color intensity (C*) values, while the greeness (-a*) and hue values diminished, which were not significantly difference with the control. The color values of ozone treatment were statistically significantly different from the control ($p < 0.01$) (Table 6). The alteration in the fruit pulp showed elevated redness (a*), yellowness (b*) and hue values, whereas the brightness and hue values were decreased, with statistically significant differences from the control ($p < 0.01$ and $p < 0.05$) (Table 7).

Table 6. Weight loss and peel color values of fruits following ozone treatment at 25,000 mg/h for 30 min compare with control

Mean±SD	Weight loss (g)	L* value of fruit peel	a* value of fruit peel	b* value of fruit peel	C* value of fruit peel	Hue value of fruit peel
Control	287.82±43.76	64.50±3.36	-10.52±2.39	41.36±2.97	41.74±3.86	103.62±1.89
Treat-ment	287.21±43.70	66.00±3.39	-9.66±2.55	42.56±3.02	42.90±0.70	103.02±1.94
t-test	*	NS	NS	NS	NS	NS

* and NS indicate significant difference at $p < 0.01$, and non-significant differences, respectively; n=15.

Table 7. Pulp color value of fruits following ozone treatment at 25,000 mg/h for 30 min compare with control

Mean±SD	L* value of fruit pulp	a* value of fruit pulp	b* value of fruit pulp	C* value of fruit pulp	Hue value of fruit pulp
Control	76.74±0.97	4.57±0.26	43.14+0.30	42.03+0.71	84.09±0.43
treatment	75.28±0.21	6.26±0.59	43.60+0.32	43.23+0.77	82.75±0.30
t-test	**	*	**	**	*

* and ** indicate significant differences at $p < 0.01$, and $p < 0.05$, respectively; n=15.

Values of firmness, electrolyte leakage, total soluble solids, titratable acidity, and ascorbic acid contents

The change in firmness values resulting from ozone water treatment at the concentration of 25,000 mg/h for 30 min demonstrated to enhance value, while the EL values exhibited to decrease in comparison to the control, and with a statistically significant difference ($p < 0.01$). The TA content was increased value and was statistically significant ($p < 0.05$). Although, the TSS and AA values were not significantly different from the control (Table 8).

Table 8. Firmness and EL values, TSS, TA, and AA contents following ozone treatment at 25,000 mg/h for 30 min compare with control

Mean±SD	Firmness (N)	EL (%)	TSS (%brix)	TA (%)	AA (mg/100g FW)
Control	76.46±1.04	29.59±0.23	13.40±0.16	3.80±0.17	22.67±1.09
Treat-ment	77.47±1.64	26.76±0.55	13.54±0.17	4.27±0.06	23.30±0.00
t-test	*	*	NS	**	NS

*, ** and NS indicate significant differences at $p < 0.01$, $p < 0.05$, and non-significant differences, respectively; n=5.

Antioxidant capacity, total phenolic, and carotenoid contents

The antioxidant capacity and total phenolic content of mango pulp treated with ozone water at a concentration of 25,000 mg/h for 30 min were a slightly higher value; however, these differences were not statistically significant compared to the control (Table 9). The cumulative carotenoid content found that was increased significantly different as compared to the control.

Table 9. Antioxidant capacity, total phenolic content and carotenoid content following ozone treatment at 25,000 mg/h for 30 min compare with control

Mean±SD	Antioxidant capacity (µmol/100g FW)	Total phenolic content (mg/100g FW)	Carotenoid content (µg/100g FW)
Control	89.93±0.13	542.50±50.56	2,186.67±83.27
Treatment	90.26±0.47	575.00±42.06	2,533.33±61.10
t-test	NS	NS	**

** and NS indicate significant difference at $p < 0.05$, and non-significant differences, respectively; n=5.

Discussion

From the study of the effect of ozone on *Colletotrichum* sp. inhibition was investigated using Response Surface Methodology (RSM) technique, designed as Central Composite Design (CCD) with 2 factors: ozone concentration in washing water and washing time. It was found that the optimal condition for testing the disease on Kaew Kamin mango was washing with ozone water at 25,000 mg/h for 30 min, which can reduce the disease loss by more than 80 percent or the disease incidence rate was less than 20% for 21 days. This can reduce the disease incidence better than the unwashed mango, which lost more than 50% for 21 days.

Ozone is a powerful oxidizing agent that is effective in inhibiting varible microorganisms, inclusive to fungi, yeast, bacteria, and viruses. It was inhibited enzyme activity, destroied structure of cell membranes and damages DNA and RNA structure (Prabha *et al*., 2015; Xue *et al*., 2023). Ozone applications in fruits and vegetables can be done in two forms: gaseous ozone and aqueous ozone. Although the half-life of ozone in gaseous ozone (1-4 h) is longer than that in ozone in aqueous ozone (1-10 min), ozone in aqueous ozone is mostly used to inhibit microorganisms. Since ozone application in ozonated water requires a lower ozone concentration (Carletti *et al*., 2013). Consistent with the study by de Almeida Monaco *et al*. (2016) found that immersing Palmer mangoes in ozonated water at an ozone production rate of 1 mg/L/s was more effective in reducing the number of microorganisms than unozonated water. And the study of Nieto Angel *et al*. (2002) found that the application of ozone at a dose of 2.2 mg/L for 15 min was inhibited germination of *C. gloeosporioides* and *F. oxysporum.*

The results indicated that aqueous ozone is an effective method and can be used to control post-harvest diseases in mangoes and has potential applications in other horticultural crops.

The results of ozone application on physicochemical changes found that it was affected to Kaew Kamin mango fruit pulp. The mango fruit pulp b*, a* and C* values were increased; in contrast, L* and the hue were decreased. The experiment exhibited that ozone resulted in an enhanced reddish-yellow color of the fruit pulp, which was associated with the higher carotenoid content. This is related to the study of Bambalele *et al*. (2023), which reported the ozone application in Keitt mango at a concentration of 0.25 mg/L for 12, 24, 36, and 48 h and stored at 10°C for 21 days and incubated at room temperature for 7 days. It was found that the application of ozone treatment had increased the C* value from the beginning of storage and the carotenoid content after 14 days of storage when compared to the control. Especially, ozone exposure at 48 h had the most

carotenoid content at the end of storage. And consistent with the report in kiwifruit exposed to 0.3 μL/L ozone for 0, 2, 8, 24, 72, and 144 h and stored at 0°C for 4 months, the carotenoid content was found to increase by 5.7% and 8.7% in fruits exposed to ozonation for 72 and 144 h, respectively (Minas *et al*., 2010).

The results of mango pulp on ozone treatment showed firmness value to be increased, which was inversely correlated in EL value to the reduction. This result indicates that ozone supplements the strength of the cell wall, thus significantly reducing the electrolyte leakage of the cells. This evidence is consistent with the experiment of fresh-cut kiwifruit that was treated with 1 mg/L gaseous ozone for 10 min and stored at 4°C for 12 d, which delayed the softening of the fruit by decreasing firmness loss by more than 10% compared to the control. Ozone resulted in an increased calcium ion concentration, which was associated with rising calcium pectinate in the cell wall structure. Ozone suppressed the activity of cell wall-destroying enzymes, specifically polygalacturonase (PG), pectin methylesterase (PME), cellulase (Cx), and β-glucosidase (β-Glu), and maintained the levels of protopectin and cellulose, as well as preventing the leakage of electrolytes (Wang *et al*., 2023). This is consistent with a study of Keitt mangoes that were exposed to 0.25 mg/L gaseous ozone for 12, 24, 36, and 48 hours, stored at 10°C for 21 days, and then retained at room temperature for 7 days. Ozone exposure for 36 hours or more reduced the weight loss by 10.38% compared with the control. Ozone treatment preserved fruit firmness, reduced fruit deterioration, and delayed ripening more effectively than the control (Bambalele *et al*., 2023).

Furthermore, a study in Kaew Kamin mangoes reported a significant decline of TA content changes due to ozone exposure compared with the control. Consistent with the research conducted by Bambalele, *et al*. (2023), ozone treatment of Keitt mango at a concentration of 0.25 mg/L for durations of 36 and 48 hours and storage at 10°C for 21 days and curing at room temperature for 7 days was able to postpone the change of TA content to 1.4 times when the end of storage.

The results revealed the optimal ozone concentration and washing time for reducing anthracnose disease incidence and postharvest fruit loss in Kaew Kamin mangoes. These research findings allow a scientific basis for the application of ozone as a postharvest management tool, enabling accurate prediction and control of anthracnose disease in Kaew Kamin mangoes. These results could help improve the quality of mangoes and other horticultural crops, as well as reduce losses during storage and distribution for further commercial use.

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Conflicts of interest

The authors declare no conflict of interest.

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