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Using the response surface methodology to establish the optimal conditions for preserving bananas (*Musa acuminata*) in a pulsed electric field and to decrease browning induced by storage at a low temperature

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ABSTRACT

In this study, a pulsed electric field (PEF) was used to establish the preservation conditions of bananas (*Musa* spp.) and explore the effect on chilling injury (CI) inhibition. The response surface methodology (RSM) demonstrated that the PEF strength of 50 kV m^{-1} has a better inhibition effect on browning; however, adverse effects are caused when the PEF strength exceeds 100 kV m^{-1} in comparison with untreated group. The optimal conditions established using RSM were PEF strength 32 kV m^{-1}, frequency 278 Hz, treatment time 32 min and width 600 μs, which can effectively inhibit the degree of banana browning by 131.6%. Further storage tests indicated that PEF could effectively improve fruit weight loss (18.91%), firmness (10.91%), browning and other changes associated with quality by maintaining the levels of total chlorophyll, carotenoids and ascorbic acid. This suggests that PEF has the potential to delay the CI of bananas stored at low temperatures and can maintain high fruit quality.

1. Introduction

Banana (*Musa* spp.) is an economic crop that is widely planted in tropical and subtropical countries in Southeast Asia. Its crop yield is the fourth largest in the world, following that of rice, wheat and corn (Maduwanthi & Marapana, 2019). Similar to most tropical fruits, banana has a short storage period owing to its high respiration that limits its shelf life (Bhande, Ravindra, & Goswami, 2008). Although there are many preservation methods that inhibit respiration for preserving bananas, storage at a low temperature is still the most widely used method. However, the critical temperature for bananas is 13 °C, below which browning, corrosion and failure to mature will normally occur (Murmu & Mishra, 2018). Therefore, the shelf life of bananas is only approximately 3–7 days at room temperature (Chen et al., 2019).

Chilling injury (CI) occurs when fruits and vegetables are harvested and stored at a specific low temperature (this temperature is also called

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the critical temperature; tropical or subtropical fruits and vegetables have a higher critical temperature for cold damage, which can occur at 10–13 °C. However, the critical temperature for cold-tolerant fruits, such as lychees and pears, may be below 2 °C). When the fruit is stored in a cold-temperature environment, the cell membranes of fruits and vegetables are composed of fluids that turn into solid gels, which further causes the cells to fail to maintain their normal metabolic physiological functions (Zhang, Jiang, Cao, & Jiang, 2021). This further induces the accumulation of reactive oxygen species in the fruit and causes lipid oxidation of the cell membrane by phenylalanine ammonia-lyase. Simultaneously, the phenolic substances in the vacuole escape the cell and accelerate the action of polyphenol oxidase (PPO) in the cytoplasm to cause browning (Aghdam & Bodbodak, 2013; Nguyen et al., 2004).

Methods of preventing CI can be divided into chemical and physical methods. Chemical methods include the use of methyl jasmonate, methyl salicylate, 1-methylcyclopropene and other biological regulators (Rehman, Singh, & Khurshid, 2018) or the coating of the fruit/vegetable surface with edible films, such as wax (Murmu & Mishra, 2018), which have gradually become unacceptable to consumers. Although coating the fruit/vegetable surface using edible films reduces this doubt, the use of edible films still has disadvantages such as uneven coating uniformity and difficulty in batch quantification. Although physical methods, including hot water and steam treatment (Wang, Zhang, Xu, Huang, & Pang, 2012) as well as the use of modified atmosphere packaging (Nguyen, Ketsa, & van Doorn, 2004) are commonly used, heat treatment can cause the fruit to lose its natural texture or browning and modified atmosphere packaging is not necessarily suitable for the presentation of the fruit for sale (e.g., most bananas are sold without packaging). Therefore, the development of a fast, harmless physical method that can preserve nutrients is an urgent issue that needs to be addressed.

A pulsed electric field (PEF) is an emerging non-thermal processing technology. Because the PEF has the characteristics of a square wave that are more likely to exhibit biological electromagnetic effects, PEFs have been used in the food industry for a variety of applications such as sterilisation, extraction, drying and frying (Arshad et al., 2020).

PEFs are mostly applied for the preservation of agricultural products and food via sterilisation and drying (Mello, Fontana, Mulet, Corrêa, & Cárcel, 2021; Pallarés, Berrada, Tolosa, & Ferrer, 2021). However, deterioration in the quality (such as browning) that affects the storage period of agricultural products is mainly induced by respiration or enzyme action, and microbial contamination is not the most important factor that affects the purchase intention of consumers. Although PEF-assisted drying can inhibit the action of most of the enzymes, it cannot maintain the fresh flavour and appearance of agricultural products.

However, related studies have shown that a PEF treatment of correct strength can enhance the antioxidant capacity of fruits and increase their phenolic compound content without destroying the tissue structure (Vallverdú-Queralt et al., 2012).

These experiments confirmed that a PEF could induce reversible/irreversible membrane perforation in plant cell membranes through a short-term high-energy impact. Such a membrane perforation phenomenon promotes the increase in plant secondary metabolites and thus enhances the ability of plants to resist adversity.

In recent years, electric field (EF) treatment has been proven to delay post-harvest oxidative browning and tissue softening in crops, including oyster mushrooms (Hsieh et al., 2020) and persimmons (Liu et al., 2017), by regulating the activity of related enzymes. In addition, the EF treatment was proven to increase the antioxidant content of kidney beans and cabbage at low temperatures, improving their ability to resist storage at low temperatures (Cakmak, Dumlupinar, & Erdal, 2010).

Based on the aforementioned literature, a PEF has the ability to inhibit the deterioration of agricultural products. It can simultaneously also protect agricultural products against stress by increasing secondary metabolite content. Therefore, PEF has the ability to inhibit the CI of bananas during storage at a low temperature.

Related research has shown that EF parameters, such as the EF strength, frequency and processing time, have complex and interactive effects on plant cells (Gürsul, Gueven, Grohmann, & Knorr, 2016; Moharrami & Hashempour, 2021; Zhu, Wang, Vanga, & Raghavan, 2021). In recent years, researchers have gradually been able to simultaneously analyse the effect of variable factors, allowing the use of response surface methodology (RSM) in the optimal condition as an experimental design to examine the interactive influence of EF parameters on biological processing and the establishment of optimal conditions (Lal et al., 2021).

Therefore, this study first used the RSM to explore the effect of PEF parameters on the inhibition of banana browning at low temperatures. A storage test was conducted to evaluate the effects of a PEF on the improvement in the quality of bananas stored at low temperature to study and establish PEF preservation modules for application to tropical fruits.

2. Materials and methods

2.1. Materials

The banana (*Musa acuminata colla* cv. Pei Chiao, AAA group) is a subspecies of Cavendish and is one of the main planted varieties in Taiwan. The bananas used in this study were purchased from Jiji Transportation and Marketing Cooperative (Nantou, Taiwan). The selection of bananas is based on the definition of commercial ripe bananas in related studies (Soltani, Alimardani, & Omid, 2011). The banana peel is turquoise instead of yellow, and those with no damage or disease in appearance are used as experimental samples.

2.2. Pulsed electric field treatment and single-factor test

The equipment used in this experiment is shown in Fig. 1. A laboratory-scale PEF device (PG403751, Youshang Technical Corp., Kaohsiung, Taiwan) with a DC supply (DSP-450-03-4HD, Chyng Hong Electronic CO., LTD, Taipei, Taiwan) was used to generate a maximum voltage of 40 kV, capacitance is 0.341 pF and current of 15 A. The generator provides a pulse width between 20 μs and 1 s and a frequency of 1–1000 Hz. The processing chamber was combined with a refrigerator

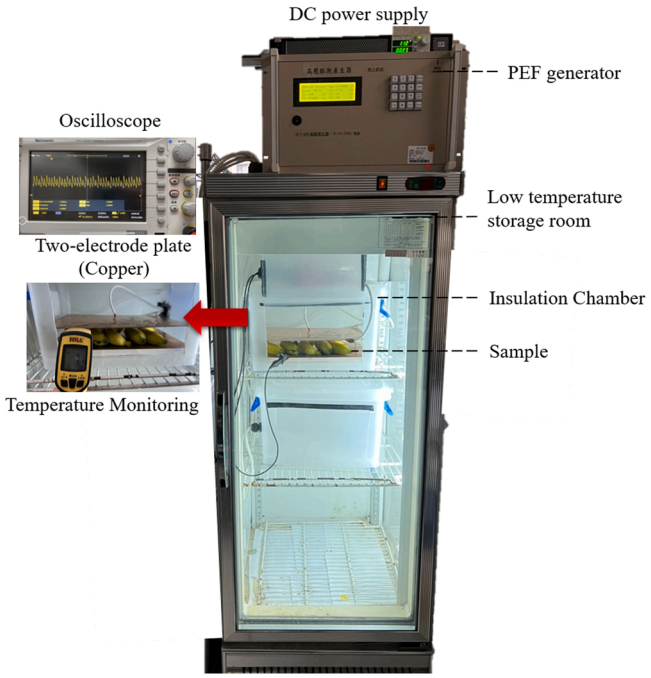

Fig. 1. Pulse electric field processing and storage device.

and consisted of parallel copper electrodes having a thickness of 1 mm, length of 350 mm and width of 250 mm. The electrode spacing was fixed at 50 mm. Each banana finger weighed 160 ± 5 g; three bananas were packed in a plastic bag and placed on the lower electrode plate for the PEF treatment.

During processing, an oscilloscope (TBS1052C, Tektronix Inc., OR, USA) was used to monitor the stability of the output voltage, frequency and bandwidth of the PEF. The temperature of the refrigerated room was maintained at 7 °C ± 2 °C during the treatment process, and no significant increase in the temperature of the processing space was found during the storage process, which was monitored using an infrared non-contact thermometer (TN498, Hila International Inc., Taipei, Taiwan).

To establish the factor boundary of RSM, we first conducted a single-factor analysis test of the inhibitory effect of PEF on the degree of banana browning. The single-factor analysis experimental design was divided into four parts: (1) different PEF strengths of 25, 50, 75, 100, 150, 200 and 250 kV m^{-1} (Eq. (1)) (fixed width, frequency and treatment time of 600 μs, 50 Hz and 30 min, respectively); (2) different pulse widths of 200, 400, 600, 800 and 1000 μs (fixed strength, frequency and treatment time of 50 kV m^{-1}, 50 Hz and 30 min, respectively); (3) different frequencies of 1, 50, 200, 350 and 500 Hz (fixed strength, width and treatment time of 50 kV m^{-1}, 600 μs and 30 min, respectively); (4) different treatment times of 1, 15, 30, 45 and 60 min (fixed strength, width and frequency of 50 kV m^{-1}, 600 μs and 50 Hz, respectively). The sample was maintained at 7 °C ± 2 °C during processing and storage, and after PEF treatment, to observe the change in the degree of browning.

$$\text{Electric field strength} = \frac{kV}{m} \tag{1}$$

where kV is the input voltage of the two electrode plates and m is the distance between the two electrode plates.

2.3. Experimental design of response surface methodology

RSM was used to optimise the PEF to the degree of browning of the banana peel. The model was constructed using Minitab version 16.0 based on the results of the single-factor analysis when three independent factors, namely, PEF strength (25–75 kV m^{-1}), frequency (50–500 Hz) and treatment time (10–60 min) were chosen, and the pulse width was fixed at 600 μs. The method used had three levels as follows: three factors, Box Behnken and a central composite design consisting of 15 experimental runs, including three replications of a central point. The degree of browning was the dependent variable, which was expressed as a function of the independent variable using quadratic polynomial Eq. (2). The optimal conditions for the PEF for browning inhibition were calculated as follows:

$$Y = b_0 + \sum_{i=1}^{3} b_{ii}X_i^2 + \sum_{i=1}^{2}\sum_{j=i+1}^{3} b_{ij}X_iX_j \tag{2}$$

where Y is the prediction of the degree of browning; b_0, b_i, b_{ii} and b_{ij} are the model constant, linear regression coefficient, interact coefficient and quadratic regression coefficient, respectively; and X_i and X_j are the coded values of independent variables.

2.4. Degree of browning

The peel (1 g) was mixed with 5 mL of 60% ethanol (dissolved in 0.1 M phosphate buffer, pH 6.8) at 4 °C for homogenisation and then centrifuged at 4 °C, 15,000 × g for 15 min. A spectrophotometer (CT-8600, E-Chrom Tech Co. Ltd., Taiwan) was used to measure the absorbance of the supernatant at 420 nm. The degree of browning was expressed as OD_{420} 100 g^{-1} fresh weight (Ali et al., 2021).

2.5. Chilling injury analysis

2.5.1. Appearance and CI index

The degree of the CI index was calculated using the following formula provided in Eq. (3) (Nguyen et al., 2004):

$$\text{CI index} = \sum \frac{CI\ \ scale\ \times\ fruit\ \ number\ \ at\ \ that\ \ scale}{Fruit\ \ number\ \ in\ \ the\ \ group} \tag{3}$$

The CI index is based on the degree of epidermal browning using a scale from 1 to 5, where 1 = no injury (no CI); 2 = mild injury (mild CI); 3 = moderate injury (moderate CI); 4 = severe injury (severe CI); and 5 = very severe CI (very severe CI).

2.5.2. Determination of fruit weight loss, total soluble solids and firmness

The analysis of fruit weight loss was calculated from the change in the weight of the bananas during different storage periods using Eq. (4). (Elbagoury, Turoop, Runo, & Sila, 2021)

$$\text{Weight loss}(\%) = \frac{(T1 - T2)}{T2} \times 100\% \tag{4}$$

where $T1$ is the fresh banana weight and $T2$ is the weight of bananas in different treatment groups during different storage periods.

The total soluble content (%) was measured using a refractometer (MASTER-53α, Atago Co., Ltd., Tokyo, Japan) after blending the pulp, filtering the mixture and measuring the total soluble solid content of the solution. The results were expressed as percentage.

The firmness of the banana pieces was measured using a texture analyser (Compac-100II, Sun Scientific Co., Ltd., Tokyo, Japan) according to related research with slight modification (Hao, Li, Xu, Huo, & Yang, 2019). The banana sample was cut in pieces to a size of 2 cm^3 for analysis. The texture analyser was equipped with a cylindrical probe with a 5-mm diameter, and Mode 1 was used. The ascending speed of the stage for the test was 120.0 mm s^{-1}, and the maximum wave peak was the fruit firmness. The results were expressed as Newton (N) units.

2.6. Determination of the quality index

Total chlorophyll and carotenoid content analyses were conducted according to related research with slight modifications (Thakur et al., 2019). Banana peel slices (1 g) were randomly selected from five samples. Pre-cooled 80% acetone (25 mL) was added to the samples, followed by blending. The mixture was extracted for 16–18 h and stored away from light. After filtering using a filter paper, a spectrophotometer was used to detect the absorbance at 663, 645 and 470 nm. The total chlorophyll content was expressed in mg 100 g^{-1}, whereas the total carotene content was expressed in μg g^{-1} and was calculated using the following Eq. (5)–(8) (Wellburn, 1994):

$$\text{Chlorophyll a(Ca)} = 12.7 \times A_{663} - 2.995 \times A_{645} \tag{5}$$

$$\text{Chlorophyll b(Cb)} = 22.95 \times A_{645} - 4.67 \times A_{663} \tag{6}$$

$$\text{Total cholorophyll}\left(\text{mg}100\ \ \text{g}^{-1}\right) = \frac{(Ca + Cb) \times V}{(W \times 1000)} \times 100 \tag{7}$$

$$\text{Total carotenoid}\left(\mu\text{g}\text{g}^{-1}\right) = \frac{[1000\ \times\ A_{470}\ -\ 3.27\ \times\ Ca\ -\ 104\ \times\ Cb]}{198} \tag{8}$$

where A_{470}, A_{645} and A_{663} are the absorbance values at 470, 645 and 663 nm, respectively. W is the sample weight (g) and V is the volume of the solvent used (mL).

The analysis of ascorbic acid in banana was performed using the 2,6-dichloroindophenol titration method (Hao et al., 2019). Separately, 10 g of the banana peel or pulp was weighed, mixed with 40 mL of 2% oxalic acid for homogenisation at 4 °C and then centrifuged at 12,000 × *g* at 4 °C for 20 min. The supernatant (5 mL) was titrated with 0.25% 2,6-indophenol to a rose-red colour until the colour did not fade within 15 s. The 2,6-indophenol value was calibrated using ascorbic acid standards. The content of ascorbic acid in the fruit was expressed in mg 100 g^{-1} FW.

2.7. Statistical analysis

RSM was conducted using SAS to conduct multiple regression analysis and ANOVA analysis. SPSS Statistics 20.0 was used to conduct statistical data analysis. The experiments were conducted in five replicates, and the values were expressed as average and standard deviation. ANOVA was employed for significance analysis and Duncan's multiple-range test for *post hoc* testing. A p value <0.05 indicated a significant difference (Granato, de Araújo Calado, & Jarvis, 2014).

3. Results and discussion

3.1. Effect of different parameters on the degree of browning of banana

Preliminary research indicates that PEF can reduce the PPO enzyme activity by altering the secondary and tertiary structures of this enzyme and that the inhibitory effect of the enzyme increases with the increase in strength and treatment time. When the PEF strength is increased to 2500 kV m^{-1} and the treatment time was 124 μs, the enzyme activity was the lowest (16.9%) (Zhong et al., 2007), indicating that PEF can delay the occurrence of enzymatic browning by reducing the PPO activity; moreover, it is speculated that it can further reduce the brown spots induced by CI. Some research has shown that irreversible/reversible electroporation increases the permeability of cell membranes and accelerates the release of polyphenols and the enzyme PPO to cause browning (Ade-Omowaye, Rastogi, Angersbach, & Knorr, 2003). In addition, it has been confirmed that when the PEF strength exceeds 120 kV m^{-1}, it causes irreversible electroporation of tomatoes, leading to cell apoptosis and acceleration of the deterioration of quality (Lafuente, Zacarias, Martínez-Téllez, Sanchez-Ballesta, & Granell,

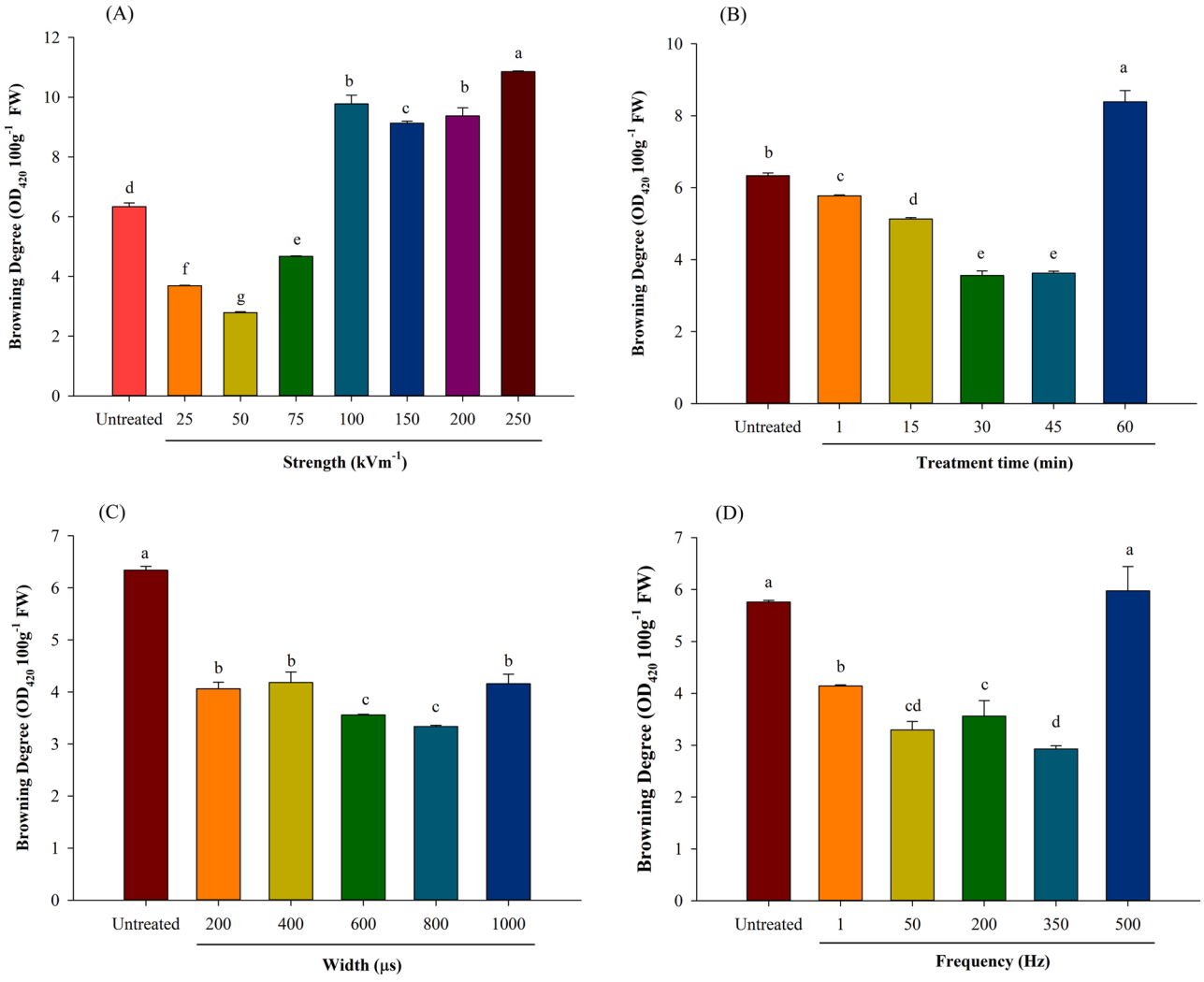

Fig. 2. Effect of different pulse electric field parameters on the degree of browning of banana during cold storage: (A) strength (fixed width, frequency and treatment time of 600 μs, 50 Hz and 30 min, respectively), (B) treatment time (fixed strength, width and frequency of 50 kV m^{-1}, 600 μs and 50 Hz, respectively), (C) width (fixed strength, frequency and treatment time of 50 kV m^{-1}, 50 Hz and 30 min, respectively) and (D) frequency (fixed strength, width and treatment time of 50 kV m^{-1}, 600 μs and 30 min, respectively). $^{a\text{-}d}$ indicate significant differences between the values of each frequency at $p < 0.05$.

2003). Thus, optimising the parameter settings is essential.

Fig. 2(A) presents the effect of different PEF strength (fixed width, frequency and treatment time of 600 μs, 50 Hz and 30 min, respectively) on the degree of browning of banana peel. The degree of browning in the untreated group was 6.33 ± 0.12 (OD_{420} 100 g^{-1}). After PEF treatments of 25, 50 and 75 kV m^{-1}, the increase in the degree of browning was effectively delayed by 3.69 ± 0.02, 2.79 ± 0.03 and 4.67 ± 0.02, of which the PEF treatment at 50 kV m^{-1} was the most effective. However, as the PEF strength was increased to 100 kV m^{-1}, the degree of browning significantly increased by 9.78 ± 0.29; furthermore, when the PEF strength was increased to 250 kV m^{-1}, the degree of browning continued to increase. This indicates that 100 kV m^{-1} is the critical value for PEF treatment, above which the destruction of the cell membrane and the flow of intra-cellular material accelerates the browning of the peel.

Fig. 2(B) presents the effect of different treatment times (fixed strength, width and frequency of 50 kV m^{-1}, 600 μs and 50 Hz, respectively) on the degree of browning of the peel. As the treatment time increased, the increase in the degree of browning reduced. When the treatment time reached 30 and 45 min, the lowest degree of browning was observed (3.56 ± 0.13 and 3.62 ± 0.05, respectively), but when the treatment time reached 60 min, the degree of browning significantly increased and was 1.4-fold higher than that of the control group.

Fig. 2(C) presents the effect of different pulse widths (fixed strength, frequency and treatment time of 50 kV m^{-1}, 50 Hz and 30 min, respectively) on the degree of browning. The degree of browning of the banana peel was significantly reduced compared with the untreated group under different pulse width treatments. The treatments at 600 and 800 μs resulted in the lowest degree of browning. The variations were 3.56 ± 0.01 and 3.34 ± 0.02 (OD_{420} 100 g^{-1}), respectively, but no significant difference was observed. Previous studies showed that the pulse width may have a crucial relationship with the decline in enzyme activity (Ho, Mittal, & Cross, 1997). Previous studies also pointed out that regulating the pulse width of PEF between 2 and 8 μs did not have a significant effect on the PPO enzyme activity (Marsellés-Fontanet & Mart ín-Belloso, 2007). However, in this study, using 200, 400 and 1000 μs to inhibit browning was not as effective as using pulse widths of 600 and 800 μs. This shows that the effect of pulse width on the inhibition of banana browning may not be linear; instead, a specific bandwidth has a more significant effect.

Fig. 2(D) presents the effect of different frequencies (fixed strength, width and treatment time of 50 kV m^{-1}, 600 μs and 30 min, respectively) on the degree of browning; the degree of browning decreases as the frequency increases. When the frequency was 350 Hz, the degree of browning of the fruit was as low as 2.93 ± 0.06. However, when the frequency increased to 500 Hz, the degree of peel browning increased with no significant difference in comparison with the untreated group. A PEF strength of 25–75 kV m^{-1}, processing time of 10–60 min, frequency of 50–500 Hz and fixed pulse width of 600 μs were subsequently chosen as the parameter ranges.

Previous studies have shown that when the frequency was between 1–60 Hz and above 1000 Hz, the enzyme activity in pectin plums considerably decreased. The possible reason is that the activity endpoint of the enzyme is affected by the disturbance of the EF. However, it has also been shown that 100–1000 Hz promotes enzyme activity. The reason may be that the reaction rate between the enzyme and substrate is increased (Samaranayake & Sastry, 2016).

This indicates that a PEF of 50 kV m^{-1}, 600 μs and 350 Hz could effectively inhibit the PPO enzyme activity in bananas. However, when the PEF frequency reached 500 Hz, the browning reaction may be accelerated owing to the increase in the frequency of PPO contact with the substrate.

3.2. Response surface analysis

Based on the experimental results, the function was obtained from Eq. (2) and Eq. (9). In the above four-factor single-factor analysis, a PEF strength (X1) of 25–75 kV/m, frequency (X2) of 50–500 Hz and processing time (X3) of 10–60 min were selected. The minimum degree of browning at #14 (50 kV m^{-1}, 275 Hz and 35 min) was 2.84 ± 0.01, and the maximum at #12 (50 kV m^{-1}, 500 Hz and 60 min) was 9.85 ± 0.02, indicating that the minimum value was close to the centre point. In addition, the good coefficient of determination of the observed vs. predicted degree of browning values $R^2 = 0.9879$ suggested that the experiment was reliable (Supplement S1).

$$Y = 2.82 + 1.53625X_1 + 0.49625X_2 + 0.8425X_3 + 1.65625X_1^2 + 1.0025X_1X_2 + 0.4X_1X_3 + 2.06625X_2^2 + 0.525X_2X_3 + 2.65875X_3^2 \quad (9)$$

Table 1 presents the variance analysis of the surface regression. The p value of the total model was 0.0003, indicating that the model is significant ($p < 0.05$). The suitability of the model was tested using the lack-of-fit test. The p value of the lack-of-fit test was 0.0858, which did not reach a level of significance ($p > 0.05$), suggesting that the experimental data fit the model.

Similarly, the linear factors X1 (strength), X2 (frequency) and X3 (time) had significant effects on the degree of browning ($p < 0.05$) (Table 1). In the quadratic factor form, $X1^2$ (strength), $X2^2$ (frequency) and $X3^2$ (time) had significant effects on the degree of browning ($p < 0.05$). From the interaction factor, only X1 × X2 (strength × frequency) had a significant effect on the degree of browning ($p < 0.05$), whereas the remaining factors X1 × X3 (strength × time) and X2 × X3 (frequency × time) were not significant ($p > 0.05$).

Fig. 3(A) presents the results at a fixed frequency of 275 Hz. The effect of the PEF strength and treatment time on the degree of browning has been discussed. When the PEF strength was 40–50 kV m^{-1} and the treatment time was 25–35 min, the lowest browning variability was observed. Fig. 3(B) presents the results at a fixed treatment time of 35 min. The effect of the PEF strength and frequency on the degree of browning is discussed. When the PEF strength was 40–50 kV m^{-1} and the frequency was 200–300 Hz, the browning variability was the lowest. Fig. 3(C) presents the results at a fixed PEF strength of 50 kV m^{-1}. The effect of the treatment time and frequency on the degree of browning is discussed. When the frequency was 250–300 Hz and the treatment time was 30–35 min, the lowest degree of browning was observed.

The literature shows that the closer the contours are to the ellipse, the stronger is the interaction between the two variables. In contrast, the closer the contours are to the circle, the weaker is the interaction between the two variables (Wang et al., 2020). It can be observed from Fig. 3 that when the treatment time is fixed, the contour graph of strength and time is close to ellipse, indicating that the mutual influence is higher. These results are in accordance with those presented in Table 1.

Table 1
Analysis of variance for the regression model.

Source	DF	Sum of squares	F Value	Prob>F[a]
Model	9	74.28	46.57	0.0003*
X1	1	18.88	106.39	0.0001*
X2	1	1.97	11.10	0.0207*
X3	1	5.67	31.99	0.0024*
$X1^2$	1	9.00	50.72	0.0008*
$X2^2$	1	14.35	80.85	0.0003*
$X3^2$	1	24.27	136.76	<0.0001*
*X1*X2*	1	4.02	22.65	0.0051*
*X1*X3*	1	0.64	3.61	0.1160
*X2*X3*	1	1.10	6.21	0.0550
Lack of fit	3	0.83	10.81	0.0858
Pure Error	2	0.05		
Total Error	5	0.89		

* significant difference ($p < 0.05$)

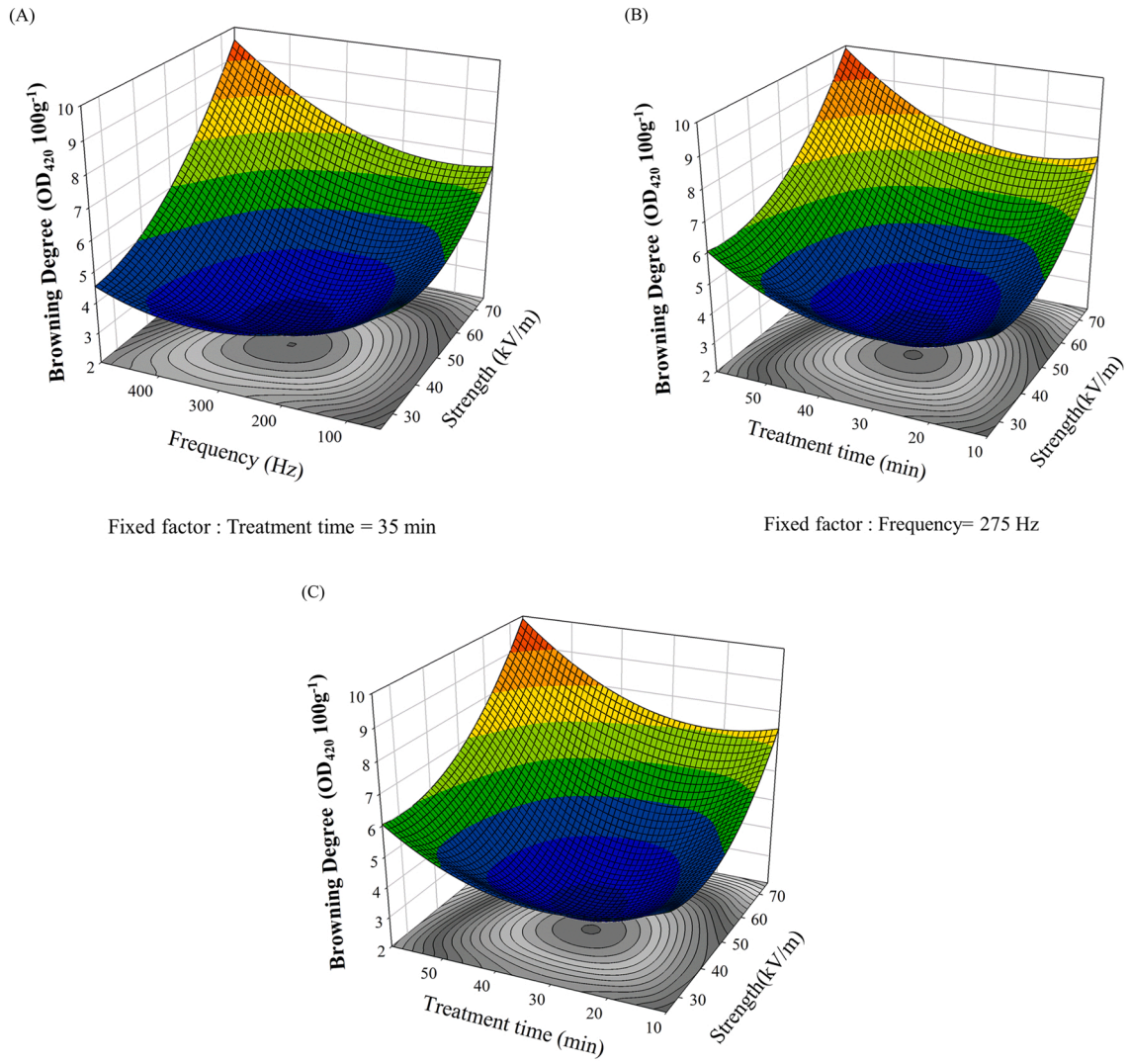

Fig. 3. The surface plots show the effects of different variables on the degree of browning at (A) a fixed treatment time at the zero level of 35 min, (B) fixed frequency at the zero level of 275 Hz and (C) fixed strength at the zero level of 50 kV m^{-1}.

The results of the ridge analysis showed that the PEF strength of 37.93 kV m^{-1}, frequency of 278.18 Hz and treatment time of 31.79 min may have the best effect on inhibiting the browning of banana when stored at 7 °C. The ridge analysis results also show that increasing the PEF strength to 40.44 kV m^{-1}, frequency to 285.42 Hz or extending the PEF treatment time has no significant effect on inhibiting banana browning. Therefore, considering future industrial practical applications, we use the following parameters: a PEF strength of 38 kV m^{-1}, frequency of 278 Hz, pulse width of 600 μs and processing time of 32 min to carry out a PEF experiment to delay the CI of bananas under low temperature storage

3.3. Change in appearance and CI during cold storage

When bananas are stored below 13 °C, CI is mainly characterised by the browning of the peel, which is accompanied by large areas of brown spots. Fig. 4(A) shows the effect of untreated and PEF treatments on banana appearance during cold storage at 7 °C for 20 days. In the untreated sample, brown spots started to show on the 5^{th} day; in the PEF-treated sample, they did not appear until the 10^{th} day. The degree of brown spots on the 20^{th} day in the PEF-treated sample was similar to that in the untreated sample on the 15^{th} day. In addition, the appearance of black spots on the 20^{th} day in the PEF-treated sample was not as severe as that in the untreated group. This shows that PEF could effectively improve the deterioration of appearance in banana CI.

Fig. 4(B) presents the change in the CI index at 7 °C for 20 days (level 0, no CI; level 1–2, slight; level 3, moderate; level 4, severe, with a browning area of >75%). Fresh bananas showed no symptoms of CI; all samples showed CI symptoms after 5 days of storage that increased with storage time; however, in the storage conditions of 7 °C and 20 days, the CI index in the PEF-treated group was lower than in the untreated group. In the first 10 days of the storage period, the CI index of the PEF-treated group was only approximately 40%–70% of that of the untreated group; during days 10–20, the CI index of the PEF-treated group was 70%–85% of that of the untreated group. Previous studies have demonstrated that the EF effect can inhibit the activity of lipoxygenase and PPO in oyster mushrooms to maintain high cell membrane integrity and delay the browning of oyster mushrooms during storage (Hsieh et al., 2020).

Previous studies have shown that using 100 kV m^{-1} and 50 Hz PEF to treat *Phaseolus vulgaris* and *Brassica oleracea* for 10 min could effectively increase the superoxide dismutase activity of *P. vulgaris* and *B. oleracea* and reduce H_2O_2 production (Cakmak et al., 2010). In this study, the use of PEF at 38 kV m^{-1} and 278 Hz could also effectively suppress the CI value of bananas. Therefore, PEF may improve the

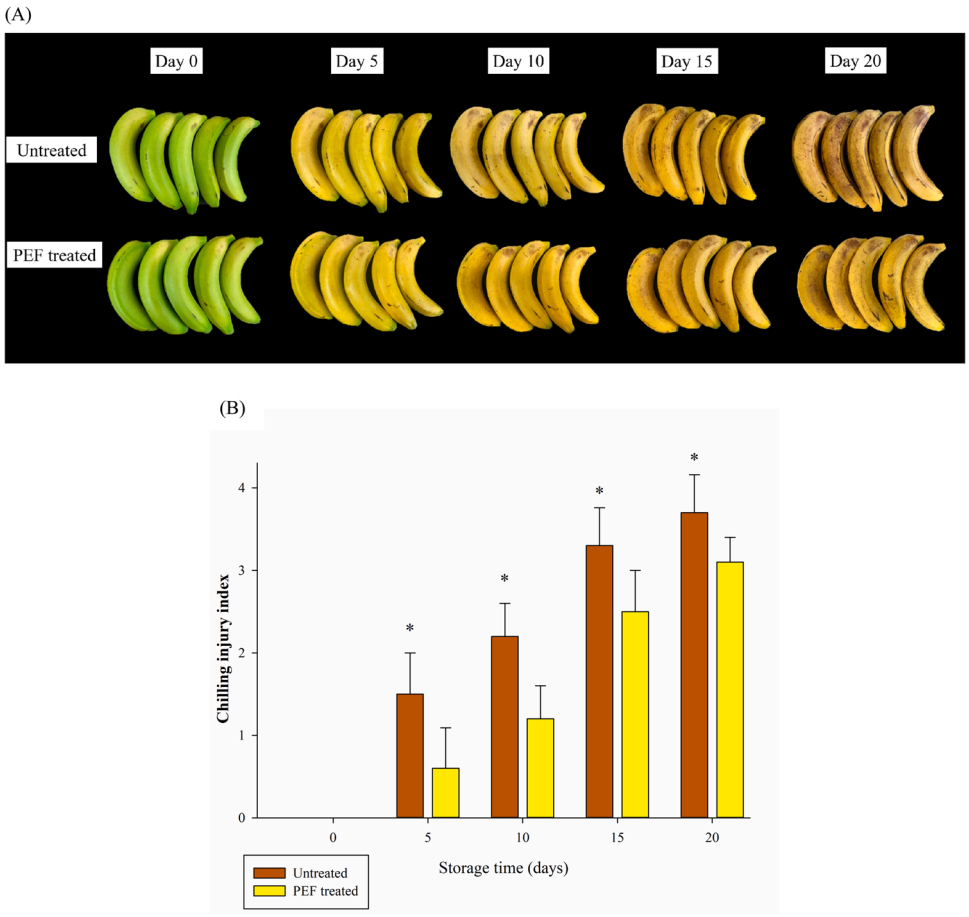

Fig. 4. Effect of the pulse electric field treatment on the (A) appearance and (B) chilling injury index in bananas during cold storage at 7 °C for 20 days. * The CI index was significantly different on the same day ($p < 0.05$).

Table 2
Effect of PEF treatment on quality changes in bananas during cold storage at 7 ℃ for 20 days.

			Storage time				
			Day0	Day5	Day10	Day15	Day20
Weight loss (%)		Untreated	-	0.47 ± 0.06^{Da}	1.10 ± 0.07^{Ca}	1.69 ± 0.27^{Ba}	2.36 ± 0.22^{Aa}
		PEF treated	-	0.40 ± 0.05^{Da}	0.79 ± 0.13^{Cb}	1.21 ± 0.07^{Bb}	1.91 ± 0.18^{Ab}
Toughness (N)		Untreated	19.69 ± 0.34^{Ca}	19.28 ± 0.64^{Ca}	23.81 ± 0.47^{Aa}	22.14 ± 0.57^{Ba}	19.24 ± 0.35^{Ca}
		PEF treated	19.69 ± 0.34^{Ba}	18.45 ± 0.43^{Cb}	20.75 ± 0.49^{Ab}	19.57 ± 0.43^{Bb}	17.13 ± 0.30^{Db}
TSS (%)		Untreated	17.26 ± 0.51^{Da}	20.20 ± 0.36^{Cb}	21.60 ± 0.13^{Bb}	21.84 ± 0.23^{Bb}	22.30 ± 0.39^{Aa}
		PEF treated	17.26 ± 0.51^{Ca}	20.98 ± 0.37^{Ba}	22.32 ± 0.27^{Aa}	22.64 ± 0.23^{Aa}	22.54 ± 0.26^{Aa}
Chlorophyll ($mg\ 100\ g^{-1}$)		Untreated	9.16 ± 0.66^{Aa}	2.80 ± 0.13^{Ba}	2.04 ± 0.04^{Cb}	1.82 ± 0.03^{CDb}	1.46 ± 0.06^{Db}
		PEF treated	9.16 ± 0.66^{Aa}	2.86 ± 0.27^{Ba}	2.38 ± 0.06^{Ca}	2.36 ± 0.22^{BCa}	2.37 ± 0.07^{BCa}
Carotenoid ($\mu g\ g^{-1}$)		Untreated	0.52 ± 0.02^{Ea}	1.06 ± 0.01^{Ab}	0.91 ± 0.01^{Bb}	0.88 ± 0.01^{Cb}	0.79 ± 0.03^{Db}
		PEF treated	0.52 ± 0.02^{Da}	1.35 ± 0.01^{Aa}	1.02 ± 0.01^{Ca}	1.07 ± 0.01^{Ba}	1.01 ± 0.01^{Ca}
Ascorbic acid ($mg\ 100\ g^{-1}$)	Peel	Untreated	4.44 ± 0.28^{Aa}	3.19 ± 0.06^{Bb}	2.81 ± 0.08^{Cb}	2.55 ± 0.11^{Db}	1.85 ± 0.09^{Eb}
		PEF treated	4.44 ± 0.28^{Aa}	3.55 ± 0.20^{Ca}	3.84 ± 0.07^{Ba}	2.74 ± 0.09^{Da}	2.40 ± 0.21^{Ea}
	Pulp	Untreated	6.48 ± 0.22^{Aa}	6.15 ± 0.26^{Bb}	4.65 ± 0.15^{Cb}	4.27 ± 0.28^{Db}	4.04 ± 0.12^{Ea}
		PEF treated	6.48 ± 0.22^{Ba}	7.00 ± 0.14^{Aa}	5.13 ± 0.08^{Ca}	4.63 ± 0.09^{Da}	3.88 ± 0.14^{Ea}

Values are expressed as mean ± standard deviation. a-b Means followed by different superscripts in the same row are significantly different at the same storage time ($p < 0.05$). A-E Means followed by different superscripts in the same column are significantly different at the same treatment. ($p < 0.05$)

antioxidant capacity of bananas against cold-induced browning by altered enzyme activity.

3.4. Change in the weight loss, total soluble solids and firmness during cold storage

Water loss is a phenomenon of CI. Cell membrane damage causes water and weight loss of the fruit (Wang et al., 2019). As shown in Table 2, in the first 5 days of storage, no significant difference was observed in the weight loss rate between the two groups, but the PEF-treated group showed a significantly delayed increase in the weight loss rate after the 10th day. The weight loss rate was delayed by 28.30% and 18.91% on the 15th and 20th days, respectively.

Previous studies using ultrasound combined with salicylic acid showed effective reduction in the weight loss of bananas during storage at a low temperature by approximately 55% (Khademi, Ashtari, & Razavi, 2019). In addition, applying a combination of methyl salicylate and calcium chloride to bananas reduced the weight loss rate of bananas

during storage at low temperature by 30% (Elbagoury et al., 2021). These studies also show that the weight loss rate of bananas during storage is positively correlated with the integrity of the fruit cell membrane. At the same time, reducing cell membrane damage can also reduce oxidative damage and improve fruit browning

Abnormal ripening is also one of the signs of CI to bananas, which causes the fruit that may fail to soften. Studies have demonstrated that at storage at a low temperature, the low activity of starch-related degrading enzymes results in high firmness and low TTS content, leading to the firmness of bananas (Song et al., 2019).

The results indicated that the toughness of the peel increased during storage and the toughness of the peel value was reach a maximum on the 10^{th} day, followed by a decrease; similar phenomena were observed in previous studies (Wang et al., 2013). The firmness in the PEF-treated group was significantly lower than that in the untreated group during storage. On the 20^{th} day of storage, the firmness of the PEF-treated and untreated groups was 17.13 ± 0.30 and 19.24 ± 0.35 N, respectively, and it decreased by approximately 10.97% in the PEF-treated group, indicating that PEF treatment can maintain the toughness of banana peels.

The same results can be observed in the content of soluble solids. Under normal ripening conditions, the starch in the fruit will be hydrolysed into soluble sugars, such as glucose, fructose and sucrose, thereby increasing the content of soluble solids. Conversely, under storage at a low temperature, the increase in the content of soluble solids is delayed (Chen, Zhao, Wu, He, & Yang, 2020).

3.5. Changes in the levels of chlorophyll, carotenoid and ascorbic acid during cold storage

The chlorophyll content of bananas is degraded during the ripening period, and the carotenoid content increases (Pongprasert, Sekozawa, Sugaya, & Gemma, 2011). Storage at low temperatures will cause damage to the chlorophyll thylakoid membrane, resulting in substantial degradation of chlorophyll and production of excessive free radicals that accelerate the CI of the fruit.

Table 2 points out that PEF treatment does not cause adverse effects on chlorophyll. Although both PEF-treated and untreated banana chlorophyll will decrease with the storage time, the PEF-treated group maintained the same chlorophyll level at 2.37 ± 0.07 mg 100 g^{-1} whereas the chlorophyll content of the untreated group continued to drop to 1.46 ± 0.06 mg 100 g^{-1} in the middle and late stages of storage.

Previous studies have pointed out that the glycine betaine in chlorophyll can resist the oxidative damage caused by CI(Aghdam & Bodbodak, 2013; Nguyen et al., 2004). The additional addition of glycine betaine with antioxidant capacity can also greatly reduce the structural damage caused by CI to cell membranes. (Rodríguez-Zapata et al., 2015). Therefore, Table 2 also shows that the PEF may reduce the structural damage caused by CI and simultaneously reduce the loss of banana chlorophyll when stored at 7°C, thereby reducing the phenomenon of banana epidermal browning.

Carotenoids are secondary metabolites of plants that scavenge free radicals to resist a harsh external environment and maintain normal metabolism (Krinsky, 1998). Table 2 shows the values increasing from 0.52 ± 0.02 to 1.06 ± 0.01 μg 100 g^{-1} (untreated l group) and 1.35 ± 0.01 μg 100 g^{-1} (PEF-treated group) in the 5 days before storage and decreased to 1.46 ± 0.06 and 1.01. ± 0.01 μg 100 g^{-1}, respectively, during storage; similar phenomena have also been found in previous studiy (Rodríguez-Zapata et al., 2015). According to the above-mentioned findings, the carotenoid content in the PEF-treated group during storage was higher than that in the untreated group, suggesting that PEF treatment alleviates the symptoms of CI by increasing the carotenoid content. Previous studies have demonstrated that processing tomato fruits for 30 pulses at 200 kV m^{-1} and storing them at 4 °C for 24 h can effectively increase the total carotenoid content by approximately 30% (González-Casado, Martín-Belloso, Elez-Martínez, & Soliva-Fortuny, 2018). In this study, the total carotenoid content increased by approximately 21% compared with the untreated group on the 5^{th} day of storage.

Ascorbic acid is a non-enzymatic water-soluble antioxidant that can effectively remove free radicals in fruits. Numerous studies have pointed out that maintaining the ascorbic acid content during storage at low temperature can effectively delay the occurrence of CI (Liu, Li, Liang, Jiang, & Chen, 2019). As shown in Table 2, the ascorbic acid content in the peel after storage decreases. The ascorbic acid content in the untreated group decreased from 4.44 ± 0.28 to 1.85 ± 0.09 mg 100 g^{-1}, whereas the ascorbic acid content in the PEF-treated group decreased from 4.44 ± 0.28 to 2.40 ± 0.21 mg 100 g^{-1}. This result showed that after the PEF treatment of bananas, a higher ascorbic acid content remains in the banana during the storage period than untreated bananas. Simultaneously, the ascorbic acid content in the pulp also decreased with the increase in the storage time and the ascorbic acid content in the PEF group increased over the first 5 days, from 6.48 ± 0.22 to 7.00 ± 0.14 mg 100 g^{-1}, and then decreased. Overall, the ascorbic acid content of the PEF treatment group was significantly higher than that of the untreated group 15 days before storage. However, no difference was observed between the two groups after the 20th day and similar phenomena have also been found in previous studies (Hao et al., 2019), where bananas treated with exogenous progesterone showed a delay in the degradation of ascorbic acid during storage and enhancement of their antioxidant capacity to resist CI.

A related study pointed out that treatment with a high-voltage electrostatic field of 0.02 kV cm^{-1} for 2 h increased the ascorbic acid content of tomatoes (Zhao, Hao, Xue, Liu, & Li, 2011). Another study indicated that treatment at 430 kV m^{-1} for 2 h did not increase the ascorbic acid content of tomatoes (Bajgai, Hashinaga, Isobe, Vijaya Raghavan, & Ngadi, 2006). In this study, a PEF strength of 38 kV m^{-1} was used and treatment for 32 min was shown to increase the ascorbic acid content of bananas. This also showed that appropriate EF treatment was helpful in improving the antioxidant ability of fresh fruit during a period of storage at low temperature after harvest, thereby improving the reduction in browning caused by CI or oxidative damage.

4. Conclusion

In this study, the PEF process (a PEF strength of 38 kV m^{-1}, frequency of 278 Hz, treatment time of 32 min and pulse width of 600 μs) established by RSM can effectively reduce the CI of bananas, thereby reducing the browning phenomenon (15 to 50%) caused by bananas when stored at low temperatures. Furthermore, the results of the quality change test showed that the PEF treatment group could avoid the decline in firmness and weight loss of bananas during storage at 7 °C and effectively maintain the carotenoids, chlorophyll and ascorbic acid content. Such results indicate that PEF treatment not only improves the resistance of tropical fruits to low temperature and CI but also does not adversely affect the nutrient content of the fruit or related post-ripening indicators. It also means that the application of PEF in food and agriculture should not only be limited to sterilisation and auxiliary extraction/drying but also can be used in the post-harvest treatment of fruits and vegetables in the future to prolong the quality of produce during the period in which they are sold.

CRediT authorship contribution statement

Bo-Kuen Chen: Conceptualization, Methodology, Software, Data curation, Writing – original draft, Visualization, Investigation, **Chao-Kai Chang:** Conceptualization, Methodology, Software, Data curation, Writing – original draft, Visualization, Investigation, Software, Validation, Writing – review & editing, **Kuan-Chen Cheng:** Data curation, Writing – original draft, Visualization, Investigation, Software, Validation, Writing – review & editing, **Chih-Yao Hou:** Visualization, Investigation, Software, Validation, Writing – review & editing, **Jer-An Lin:**

Software, Validation, Writing – review & editing, **Min-Hung Chen:** Visualization, Investigation, Supervision, **Shella Permatasari Santoso:** Visualization, Investigation, Software, Validation, **Chang-Pen Chen:** Visualization, Investigation, Supervision, Writing – review & editing, **Chang-Wei Hsieh:** Conceptualization, Methodology, Software, Data curation, Writing – original draft, Supervision, Writing – review & editing.

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Conflicts of interest

The authors declare no conflict of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fpsl.2021.100804.

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