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Resistance of *Eupenicillium javanicum* mold spores to the light-emitting diode (LED), LED-assisted thermal and thermal processing in strawberry and apple juices

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ABSTRACT

The use of light-emitting diode (LED) technology for the non-thermal processing of foods is a major topic of interest among various research groups. This study is aimed at inactivating the *Eupenicillium javanicum* ascospores present in strawberry and apple juices using a combination of a visible LED (vis-LED, 430–630 nm, 216–420 J/cm^2) and 90 °C thermal treatment, as well as to compare the findings with the inactivation done using thermal-processes alone. The results showed that violet-blue LEDs within the range of 430 and 460 nm with an energy between 300 and 420 J/cm^2 were better for the inactivation of *E. javanicum* ascospores than the green and red LEDs which were within the 550–630 nm region with an energy range from 216 to 264 J/cm^2. Furthermore, the inactivation process conducted using vis-LED was affected by the juice's soluble solid contents and the calculated D_{LED}-values were within the range of 116.3 J/cm^2 to 277.8 J/cm^2 in juices with a Brix scale value of 10–20°. Finally, the inactivation rate obtained from combining a violet-blue LED with a 90 °C thermal treatment was similar to the rate of using the thermal treatment alone.

1. Introduction

Various bacterial and fungal species have contaminating and heat-resistant spores and can survive various environmental stresses including nutrient deprivation. However, these spores can cause food spoilage, which is usually indicated by changes in the visual nature, smell, and texture of the food, thus making it inconsumable. In addition, foodborne illnesses and outbreaks can occur if the contaminating microbe is a pathogen. These illnesses and outbreaks are caused by the consumption of foods containing toxins (or poisons) or the ingestion of a large number of toxins producing cells. Some of the food-contaminating bacterial and fungal spore-producers include *Bacillus cereus*, *Clostridium perfringens*, *Alicyclobacillus acidoterrestris, Byssochlamys Nivea*, *Byssochlamys fulva*, *Neosartorya fischeri*, *Talaromyces flavus,* and *Eupenicillium javanicum* (Evelyn and Silva, 2019, 2020; Silva et al., 2014).

The process of associating and isolating the genus *Eupenicillium* with concentrated apple juice, and from the strawberry pulp after pasteurization is documented in the study conducted by Salomão et al. (2014, 2018) and Aragão (1989) respectively. Due to the facts outlined in the aforementioned studies, the *Eupenicillium* sp. including *E. javanicum* has been categorized as heat-resistant filamentous molds and identified as a potential cause of spoilage in fruit juices (Salomão, 2018). Furthermore, it is very important to control this mold in fruit juices because the *E. javanicum* has been reported to produce mycotoxins such as xanthomegnin, patulin, and some palitantin (Frisvad et al., 1990; Okeke et al., 1993).

Thermal processing is a common sterilization/pasteurization method used by stable foods production industries. Accordingly, sterilization (heating at high temperature) ensures the inactivation of all forms of microorganisms, whereas pasteurization (mild heat treatment, usually with a temperature between 85 and 95 °C) reduces the number of key pathogenic or spoilage microorganisms usually by 5 or 6 logs (Sant'Ana et al., 2014; Evelyn and Silva, 2016). Because most mold spores present in food products usually survive the pasteurization process, several studies have been conducted to investigate the kinetics involved in completely deactivating the spores. Furthermore, various studies have been conducted to determine the required decimal reduction time and temperature for thermally inactivating *Eupenicillium* sp spores in fruit juices. Spuy et al. (1975) reported that the decimal reduction time (D) and temperature required to inactivate *Penicillium brefeldianum* ascospores in apple juice were 1.0 min and 90 °C ($D_{90°C}$) respectively. Williams et al. (1941) also reported that 81 °C temperature and a 10-min

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reduction time were required for inactivating *Eupenicillium lapidosum* ascospores in blueberry juice. Moreover, Salomão et al. (2009) reported a *D*-value of 0.61 min at 60 °C for *Penicillium expansum* CCT 4680 spores in apple juice. They also reported that in order to inactivate the CCT 4680 spores, the temperature must be up to 80 °C. Regarding the *E. javanicum* ascospores present in strawberry pulp (15 °Brix), the parameter values: 90 °C and 0.8 min were reported by Aragão (1989). Also, the thermal inactivation parameters of *E. javanicum* ascospores in pineapple juice (11 °Brix) reported were *D*-values of 19.8, 5.0, and 1.45 min at 80 °C, 85 °C, and 90 °C, respectively (Evelyn et al., 2020a). In addition, this study demonstrated that older *E. javanicum* mold ascospores are more resistant than others (Evelyn et al., 2020a).

Considering the fact that pasteurization could diminish food quality due to the amount of heat used, other innovative technologies have emerged as non-thermal food pasteurization methods. Admittedly, the use of non-thermal methods alone is often not enough in reducing spores to a certain desired level (Evelyn and Silva, 2019, 2020). Therefore, several research have conducted studies regarding the combination of non-thermal technologies with heat for microbial spore inactivation in foods, as well as the minimization of heat effects on the food's sensory properties. This combination (whether sequentially or simultaneously) has also been recognized for its benefits in reducing the treatment temperatures and/or processing times for bacterial/mold/yeast spores, and for enzyme inactivation (Evelyn and Silva, 2019, 2020; Michael and Yokota, 2007). Accordingly, artificial light treatment or the use of electromagnetic radiations from light-emitting diodes (LED) with wavelengths ranging from 200 to 780 nm is one of the emerging non-thermal technologies for the inactivation of microorganisms in foods (Prasad et al., 2020). LEDs are made up of semiconductors, and they are considered to have more advantages over UV technology including the fact that they are mercury-free, compact, robust, and have a long life span (Muthukumar, 2019; Prasad et al., 2020). The process of inactivating microbial cells/spores with LEDs is generally referred to as photodynamic inactivation. This method was suggested because it activates the reactive oxygen species (ROSs) which react with the microbial cellular photosensitizers such as porphyrin, flavins, cytochromes, and NADH, thus leading to the eradication of the spores (Luksiene and Brovko, 2013; Muthukumar, 2019; Prasad et al., 2020). In addition, the antibacterial effect of LED in visible range wavelength (vis-LED) has attracted attention in the past decade. In this study, the kinetics and processes of the vis-LED and vis LED-assisted thermal processing (vis LED-thermal) were investigated.

LED technology has been considered an attractive non-thermal technology for the preservation of fruit and vegetable products (Muthukumar, 2019). However, there have been limited studies on the topic of photodynamic inactivation as well as the kinetics of using vis-LED on fungal spores and in real food such as fruit juices. Therefore in this study, *E. javanicum* ascospores in strawberry and apple juices were inactivated using both vis-LED and vis-LED thermal. The objectives were: (i) to investigate the effect of vis-LED wavelength and soluble solids (SS) content of juice on the log reductions of ascospores, (ii) to study the vis-LED non-thermal inactivation of ascospores, and (iii) to estimate the kinetic parameters of vis-LED, vis-LED thermal, and thermal processing alone.

2. Materials and methods

2.1. Eupenicillium javanicum microbiology

2.1.1. Strain

Agar slants containing *E. javanicum* InaCC F154 were obtained from the Indonesian Culture Collection (InaCC) LIPI. The strain was grown for 5 day at 27 °C on potato dextrose agar (PDA, Himedia, India) as stock cultures for spore production.

2.1.2. Spore production

In this study, the sporulation process described in the article published by Evelyn et al., (2020a) was followed. After growth for 4 weeks at 30 °C on PDA, the *E. javanicum* ascospores were obtained by flooding the surface of the culture plates with 5 mL sterile distilled water (SDW) and gently rubbing from the agar surface with a sterile bent glass rod. Furthermore, the ascospore suspension was subsequently filtered through layers of gauze to remove any remaining hyphal fragments. Spore pellets were obtained after centrifugation in sterile SDW at 4000×*g*, 15 min, and 4 °C. This procedure was repeated three times, after which the ascospore formation was monitored and confirmed using an optical microscope, and the final spore suspension was then stored at 2 °C in SDW before being used.

2.1.3. Juice inoculation

The commercial pasteurized strawberry (pH 3.9; 10.1 ± 0.1 °Brix) and apple (pH 4.1; 10.3 ± 0.1 °Brix) juices used in this study were purchased from a local supermarket and used as the suspending medium for *E. javanicum* ascospores. As aforementioned, in previous studies regarding the inactivation of genus *Eupenicillium*, the fruits utilized were strawberries and apples and thus were selected as targeted fruits in this study. The strawberry juice used had the following contents: 40% fruit juice, fructose syrup, sugar, acidity regulator (citric acid), stabilizer (Gum Xanthan E415), strawberry synthetic flavors, preservatives, and dyes. While the apple juice used contained 36% cider and additional content such as sucrose, acidity regulator (citric acid and sodium citrate), synthetic flavoring of apples, salt, natural coloring grade IV caramel, and vitamin A made up the remaining 64%. These commercial juices were used as a model to better reflect the spoilage possibility of the increasingly popular consumer juices available in the market. This spoilage can occur as a result of storage or improper packaging. Depending on the experiment, the juices' Brix values were adjusted to 15° or 20° with sucrose. Furthermore, A small portion of the ascospore solution was inoculated into the strawberry and apple juice to achieve an initial spores population of $\sim 10^6$ CFU/mL for LED experiments. Concerning LED-assisted thermal and thermal experiments, an initial spore concentration of inoculum $\sim 10^{10}$ CFU/mL of juice was used to investigate the effect of combined treatments on the total inactivation.

2.1.4. Spore enumeration

The ascospore concentration of *E. javanicum* in juices before and after processing was determined by spread plating onto PDA followed by aerobic incubation at 27 °C for 4–5 days (Evelyn et al., 2020a). Appropriate decimal dilutions were performed in 0.1% peptone water before plating, and each tube dilution was mixed repeatedly using a high-speed vortex mixer to produce a uniform spore suspension and plated twice. Average colonies were CFU/mL of juice sample.

2.2. Light-emitting diode (LED) processing of juice

A batch-type LED unit was specifically developed and equipped with a fan cooling system according to Ghate et al. (2013, 2015). LED that emits three wavelengths, ie. 430 nm (purple), 460 nm (blue), 550 nm (green), and 630 nm (red) were selected based on a previous antimicrobial efficacy study (Muthukumar 2019; Prasad et al., 2020). For each experiment, 12 LED lights were mounted on a circuit board (illumination time of 40 min equal to a dose or energy density of 420 J/cm^2 for blue light, 300 J/cm^2, 264 J/cm^2 for the green light, and 216 J/cm^2 for red light) were used to illuminate a 9 cm-diameter sterilized Petri dish containing 3 mL inoculated juice sample. The Petri dish was placed over a magnetic stirring unit to ensure an even suspension and the distance between the sample and the light source was set at approximately 5 cm. All experiments were carried out in a temperature-controlled incubator set at 20 °C allowing a maximum temperature increase of 2–3 °C at the highest energy. The power of the LED in W was taken from the manufacturer and the irradiance was calculated from the area of the Petri dish

(which is then used to obtain the dose by multiplying with the exposure time). The time (40 min) was selected such that a reduction of at least 3 logs was achieved after treatment with the highest dose, and the combination of different wavelengths was not tested.

2.3. LED-assisted thermal and thermal processing alone

For vis-LED thermal inactivation processes, LEDs having the same energy density or dose (216–420 J/cm^2) were first used to treat the inoculated (10 °Brix) juice samples, after which thermal processing was carried out at 90 °C for up to 9 min. Juices having a Brix value of 10° were selected because they are usually labeled as direct or not concentrated, have increased popularity, and are typically thought to be of higher quality i.e. consumer acceptance of their flavor, convenience, and image as a healthy food product (Berk, 2016). Additionally, the obtained results from the vis-LED thermal processing were then compared to that of the thermal processing alone. The temperature and time used for this process (90 °C and 9 min) were selected because they have been commonly used in previous studies regarding fungal ascospores, thus making it easier to compare results. (Evelyn and Silva, 2015; Evelyn et al., 2016, 2020a). Furthermore, according to earlier experiments by Evelyn et al. (2020a), the thermal processes were conducted using thermal death tubes submerged in a thermostatic water bath. The approximate time required to heat the sample to the desired temperature was 2 min, after which the samples were immediately immersed in an ice bath and subsequently enumerated as described in Section 2.1.4.

2.4. Modeling the LED, LED-thermal, and thermal inactivation of E. javanicum spore in juice

A log-linear model was used to fit the vis-LED inactivation kinetics of *E. javanicum* spores in fruit juices according to previous literature (De Souza et al., 2020) (Eq. (1)):

$$log\frac{N}{N_0} = -\frac{K\,x\,H}{ln10} \quad (1)$$

where N_0 and N (cfu/mL) is the initial spore concentration and spore survivors after being exposed to the LED treatment in the strawberry or apple juice, respectively. H is the radiation transmitted by the LED (J/cm^2) and k is the first-order rate constant in cm^2/J from which the decimal reduction LED radiation quantity can be calculated (Eq. (2)):

$$D = \frac{2.303}{K} \quad (2)$$

Equation (3) was used to estimate the D-value after thermal treatments (D_T, the time in min at a certain temperature necessary to reduce microbial population by 90%) (Bigelow, 1921) and the results were compared to the non-linear Weibull model (Eq. (4)):

$$log\frac{N}{N_0} = -\frac{t}{D} \quad (3)$$

$$log\frac{N}{N_0} = -bt^n \quad (4)$$

where b is the scale factor related to the microorganism's velocity of inactivation and n is the shape factor that describes the degree of curvilinearity. $n < 1$ and $n > 1$ correspond to the survival curves with concave-upwards (tailings) and convex or concave-downwards (shoulders), respectively (Evelyn and Silva 2019, 2020).

Furthermore, The linear and non-linear models were fitted to the spore survival lines using TableCurve 2D version 5.01 (SYSTAT Software Inc., USA), and the model's error minimizing parameters were estimated. Also, the coefficient of determination (R^2) and root mean square error (RMSE) was used to check the model's suitability. The R^2 value, which was close to 1, and a relatively small RMSE indicated the adequacy of the model to describe the data. At least two experiments and two duplicates were conducted for each technique, after which the model's parameters (D_{LED}, D_T, b, n) were estimated using the regression of a logarithmic number of survivors (log N/N$_0$) versus LED dose/time.

2.5. Statistical analysis

To examine significant differences in the microbial reductions among the treatments a one-way analysis of variance (ANOVA) was used, after which the Tukey's test with a confidence level of 95% ($p < 0.05$) was conducted (4 doses x 3 SS-soluble solids x 2 juices). This test was used to compare the D_{LED}-, D_T-, b-, and n values after the vis-LED thermal processes. The mean and standard deviation for each set of data was presented.

3. Results and discussion

3.1. LED treatment of E. javanicum InaCC F154 ascospores in juices

Fig. 1 shows the inactivation of *E. javanicum* ascospores by a visible light-emitting diode (vis-LED, 430–630 nm, 216–420 J/cm^2) in strawberry and apple juices with various soluble solids. The figure shows how 40 min of exposure to the vis-LEDs affected the inactivation of *E. javanicum* spores in strawberry and apple juices. Generally, the use of LEDs in the violet-blue (430–460 nm) region with an energy between 300 and 420 J/cm^2 for reducing *E. javanicum* ascospores in strawberry

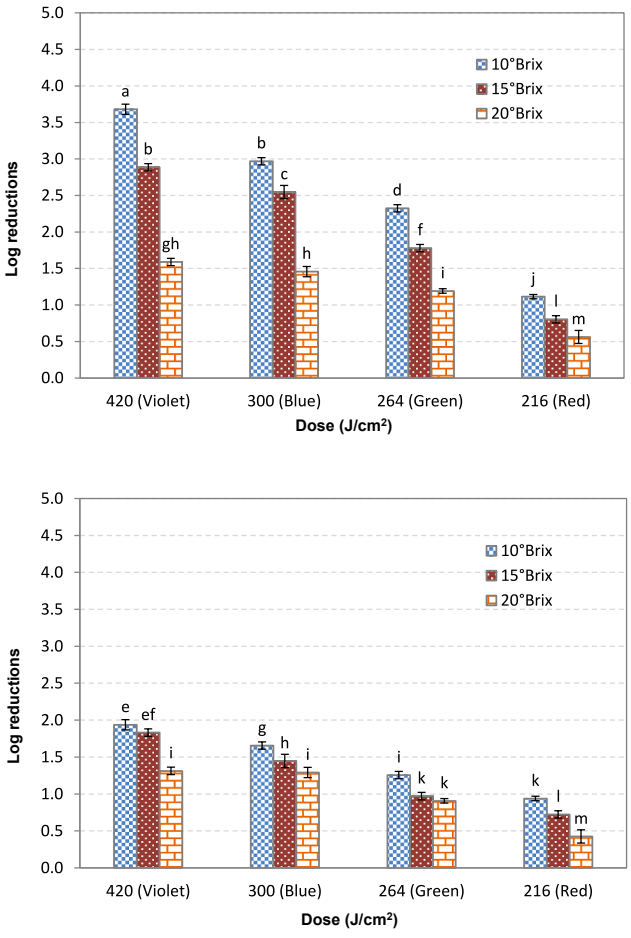

Fig. 1. Log reductions of *E. javanicum* ascospores after LED (216–420 J/cm^2) treatment in (a) strawberry and (b) apple juices. Error bars are standard deviations. Columns with different letters are statistically different ($p < 0.05$). Error bars represent ± standard deviation of the means.

and apple juices was better than the green (550 nm, 264 J/cm^2) and red (630 nm, 216 J/cm^2) regions. Also, the violet-blue LEDs were more susceptible in the 10 °Brix juices. For example, the log value obtained after violet-blue light treatments was 3.0–3.7 log compared to the value obtained using the green and red light treatments (<2.3) in strawberry juices ($p < 0.05$). Similarly, a log value of 1.7–1.9 was observed for LEDs having greater energy than those having lesser energy (<1.3 logs) in apple juices ($p < 0.05$). Previous research has also found that the antibacterial effect of vis-LEDs with wavelengths between 405 and 460 nm is greater than that between 520 nm and 642 nm for microbial inactivation (Ghate et al., 2013; Kumar et al., 2015). Furthermore, Murdoch et al. (2013) obtained compatible log reductions (3.5 logs) by 405 nm light exposure with a much higher dose of 1080 J/cm^2 for *A. niger* conidiospores in malt extract broth compared to the 3.7 log value which was obtained in this study after 430 nm (420 J/cm^2) light treatments for *E. javanicum* ascospores in strawberry juices.

Following this, Temba et al. (2016) found that *Aspergillus flavus* spores in buffer solution and maize kernels did not respond to 420 nm light treatment (84 J/cm^2), likely as a result of the lower light energy utilized as well as the effect of the suspending media. Only a few studies were found for photodynamic inactivation of fungal spores thus far. According to some theories, the excitation of reactive oxygen species (ROS) by photosensitizing light-absorbing molecules like porphyrins in the microbes leads to cellular damage and ultimately cell death during visible light inactivation of fungus spores (Donnelly et al., 2008; Fraikin et al., 1996; Murdoch et al., 2013). Other researchers reported a lower log value (~2 logs) for bacterial (*B. cereus*, *Bacillus subtilis*, *Bacillus megaterium*, and *Clostridium difficile*) spores in phosphate-buffered saline after 405 nm LED (1150 J/cm^2) treatment (Maclean et al., 2009).

In comparison to apple juice, strawberry juice showed higher log reductions of yeast spores. It is well known that berry fruits contain anthocyanin pigments, which give the fruits their red color. According to Cisowska et al. (2011), anthocyanins are potent antimicrobials having a variety of mechanisms and combinations with other substances, including weak organic and phenolic acids. This antimicrobial has an additional role to play in the inactivation process of strawberry juices. Furthermore, a potential synergism between 400 and 470 nm visible light spectrum and the juices' high acidic pH conditions could result in greater spores inactivation in these juices (Ghate et al., 2015).

According to the results, generally vis-LED with higher soluble solid contents (SS, °Brix) tended to display less spore inactivation (Fig. 1). For example at a dosage of 420 J/cm^2, 3.7 logs were obtained for 10 °Brix compared to 1.2 logs for 20 °Brix in strawberry juice, while 1.9 logs were observed for 10 °Brix compared to 0.9 logs for 20 °Brix in apple juices ($p < 0.05$). The lower inactivation in juices having higher °Brix values could be due to the higher radiation absorption by the dissolved solids. The effects of SS on the inactivation by vis-LED have not been reported by any previous research. However, a similar observation has been reported with the inactivation process using UV-C light (Menezes et al., 2019).

All of these results point to bacteria being more resistant to vis-LED treatments than fungi. The inactivation was influenced by the interaction between dose or energy, SS, the presence of a photosensitizer, the content of the suspending medium, and microbial species.

3.2. LED-assisted thermal and thermal treatment of E. javanicum F154 ascospores in juices

Fig. 2 shows the thermal log survivors of *E. javanicum* InaCC F154 ascospores and juices treated with LED (430–630 nm, 216–420 J/cm^2) at 90 °C for up to 9 min. The thermal spore inactivation of LEDs was found to be significantly influenced by LED wavelength, for example, the lower the wavelength (higher energy), the greater the spore inactivation. Furthermore, a 9-min thermal process in strawberry juice resulted in 5.9–6.8 logs for violet-blue (300–420 J/cm^2) treated juices compared to 3.1–5.3 logs for green (264 J/cm^2) and red (216 J/cm^2)

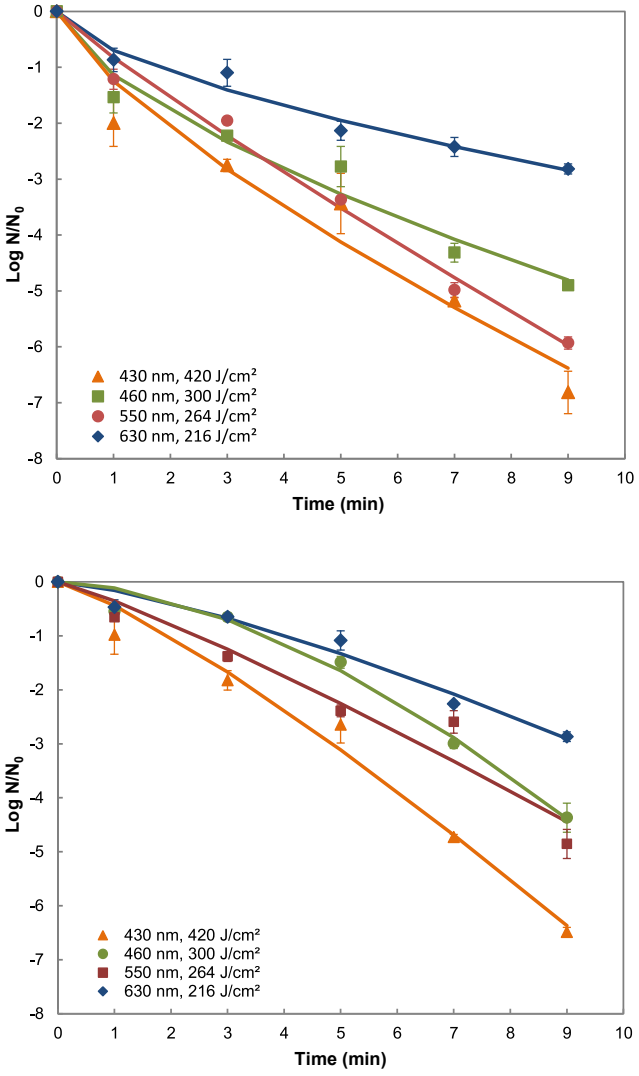

Fig. 2. Weibull model fitting of LED (216–420 J/cm^2) assisted thermal inactivation of *Eupenicillium javanicum* ascospores in (a) strawberry (10.1 °Brix) and; (b) apple (10.3 °Brix) juices for time up to 9 min.

treated juices ($p < 0.05$). Apple juices also experienced log reductions between 4.9 logs and 6.5 logs after violet-blue treatments, demonstrating the potential for 5-log reductions in fruit juices with these treatments.

Additionally, *E. javanicum* spores showed higher resistance in apple juice than in strawberry juice by 0.1–1.0 log under the same processing conditions ($p < 0.05$). The results imply that the inactivation is medium and wavelength-dependent. These results were challenging to compare because previous studies did not examine the impact of inactivating bacterial and fungal spores using a combination of vis-LEDs and thermal treatments.

Following this, the outcomes of thermal treatments alone, and that of the 90 °C thermal spore inactivation after LED illumination were contrasted (Figs. 2 and 3).). Similar spore inactivation (6.4–6.5 log) was attained in strawberry juices using both 90 °C thermal procedures and violet-LED therapy. The spores in apple juice, however, appeared to be more resistant to these thermal treatments than those in strawberry juice ($p < 0.05$).

The 40-min LED treatment's additional spore reductions, as aforementioned, is the only benefit of the sequential treatment. Furthermore, the recommended 5–6 log reductions in fruit juices contaminated by *E. javanicum* spores can be obtained by heating alone at 90 °C for 9 min.

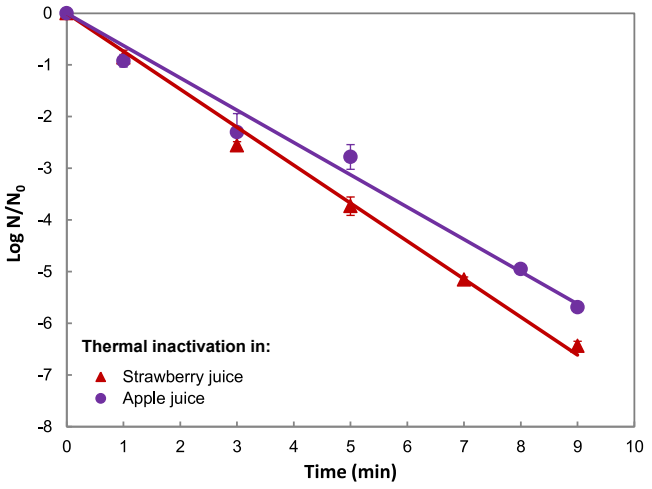

Fig. 3. First-order model fitting of 90 °C thermal inactivations of *Eupenicillium javanicum* ascospores in strawberry (10.1 °Brix) and apple (10.3 °Brix) juices for time up to 9 min.

Further studies were conducted to explain the kinetics of LED-thermal and thermal-only processes in the following section.

3.3. Kinetics of LED, LED assisted thermal and thermal only inactivation of E. javanicum ascospores in juices

Equations (1) and (2) were used to calculate the kinetic parameters of *E. javanicum* spore inactivation in 10–20 °Brix juices after vis-LED treatment. In strawberry juices, the inactivation rate constants (k) at 10–30 °Brix ranged from 0.009 to 0.020′cm^2/J (D-values = 116.3–263.2 J/cm^2) (Table 1) while the apple juices' k-values ranged from 0.008 to 0.011′cm^2/J (D-values = 208.4–277.8 J/cm^2), demonstrating that this juice had stronger resistance at the same SS with respect to the D-values ($p < 0.05$). Prior to the time of this study, no previous studies have reported the inactivation kinetics of *Eupenicillium,* especially *E. javanicum* spores in fruit juices after vis-LED and thermal treatments, thus the results obtained can not be compared.

Furthermore, the first-order and Weibull models (Eqs. (3) and (4)) were attempted to describe the log survival curves of *E. javanicum* spores in 10°Brix juices after LED-thermal treatments. The Weibull model explained spore inactivation significantly better ($0.04 \leq$ MSE ≤ 0.30, $0.96 \leq R^2 \leq 0.98$) than the first-order model ($0.07 \leq$ MSE ≤ 0.31, $0.96 \leq R^2 \leq 0.98$) (Table 2). The D parameter for the first-order and b parameter for the Weibull model is the factor that represents the spore inactivation rates, which generally showed the best performance for violet-blue LED

Table 1
k and D-values for the LED inactivation of *E. javanicum* ascospores in strawberry and apple juices*.

Food matrix	k-value (cm^2/J)	D_{LED}-value (J/cm^2)	SSE	RMSE	R^2
Strawberry juice:					
10.1 ± 0.1°Brix	0.020 ± 0.001	116.3 ± 3.20^a	0.18	0.42	0.92
15.0 ± 0.1°Brix	0.016 ± 0.004	147.1 ± 3.81^b	0.16	0.43	0.88
20.1 ± 0.1°Brix	0.009 ± 0.006	263.2 ± 6.52^d	0.04	0.23	0.86
Apple juice:					
10.3 ± 0.1°Brix	0.011 ± 0.001	208.4 ± 11.36^c	0.004	0.13	0.97
15.1 ± 0.1°Brix	0.010 ± 0.001	238.1 ± 11.22^e	0.08	0.16	0.86
20.1 ± 0.1°Brix	0.008 ± 0.0004	277.8 ± 5.64^f	0.10	0.26	0.85

*Vis-LED processes were carried out at 216–420 J/cm^2 k- and D-values are the LED first-order kinetic parameters (Eqs. (1) and (2)); SSE, RMSE, and R^2 were the sum of squares for error (SSE), root mean squared error, and regression coefficient, respectively. Different letters (a-f) within a column indicate significant differences ($p < 0.05$).

Table 2
Kinetic parameters for the LED-thermal and thermal inactivation of *E. javanicum* ascospores in strawberry and apple juices*.

Food matrix	LED light and dose (J/cm^2)	LED assisted thermal			Thermal only
		First-order	Weibull		
		D_T-value ± SD (min)	b-value ± SD	n-value ± SD	D-value ± SD (min)
Strawberry juice (10.1°Brix)	Violet (420)	1.32 ± 0.33^a	1.25 ± 0.34^d	0.74 ± 0.14^{ab}	1.36 ± 0.32^a
	Blue (300)	1.54 ± 0.11^a	1.14 ± 0.22^d	0.66 ± 0.10^a	
	Green (264)	1.97 ± 0.35^{ab}	0.82 ± 0.14^{cd}	0.90 ± 0.09^b	
	Red (216)	2.89 ± 0.22^b	0.70 ± 0.13^c	0.64 ± 0.10^a	
Apple juice (10.3°Brix)	Violet (420)	1.48 ± 0.22^a	0.44 ± 0.12^b	1.21 ± 0.15^c	1.59 ± 0.50^{ab}
	Blue (300)	2.09 ± 0.11^{ab}	0.35 ± 0.17^b	1.16 ± 0.25^{bc}	
	Green (264)	2.35 ± 0.39^b	0.11 ± 0.05^a	1.67 ± 0.19^d	
	Red (216)	3.33 ± 0.15^c	0.16 ± 0.07^{ab}	1.33 ± 0.21^{cd}	

*Thermal processes were carried out at 90 °C. D_T-values are the thermal first-order kinetic parameter (Eq. (3)); b and n are the Weibull kinetic parameters (Eq. (4)); The Weibull model worked better than a first-order model for LED-assisted thermal, presenting lower MSE values (0.04–0.30) and higher R^2 (0.96–0.98). Different letters (a-d) within a column indicate significant differences ($p < 0.05$).

treated juices ($p < 0.05$). The b-values obtained for the Weibull model decreased from 1.25 ± 0.3 to 0.7 ± 0.1 for strawberry juices and from 0.45 ± 0.1 to 0.16 ± 0.1 for apple juices as the LED wavelength was changed from 430 nm (420 J/cm^2) to 630 nm (216 J/cm^2). Furthermore, the n values obtained varied from 0.64 to 1.33 (Table 1), indicating both concave-upward (n less than 1) and concave-downward (n more than 1) trends in the spore survivor curves for LED-assisted thermal processes (Fig. 2). The D-values estimated for the first-order model increased from 1.32 ± 0.2 min to 2.9 ± 0.2 min for strawberry juice and from 1.48 ± 0.2 min to 3.3 ± 0.1 min for apple juice under the same conditions. The findings further demonstrated that increasing the dose or energy of the violet-blue LEDs might enhance the thermal inactivation rate of fungal spores in the juices or shorter the time required for the thermal treatment. Also, increased SS can potentially decrease the rates of the spores' inactivation in these juices.

Equation (3) was also used to estimate the thermal-only processes for the inactivation of *E. javanicum* spores. The obtained $D_{90°C}$-values for these processes were 1.36 ± 0.3 min for strawberry juice and 1.59 ± 0.5 min for apple juice, thus, demonstrating a comparable spore inactivation rate between the thermal-only treatments and typically blue-violet region-assisted thermal treatments (1.32 ± 0.2 min to 2.09 ± 0.1 min) ($p > 0.05$). As previously noted, vis-LED treatments before thermal operations led to greater log reductions with sequential treatments. However, the mechanisms for the inactivation are still unknown and need to be investigated before they can be implemented.

4. Conclusions

This study showed that the inactivation of *E. javanicum* ascospores in strawberry and apple juices was affected by the wavelength/energy of visible light-emitting diodes (LED). Furthermore, the violet-blue region (430–460 nm, 300–420 J/cm^2) performed best in inactivating the spores, resulting in 3.0–3.7 log reductions for strawberry juices. However, these treatments were not effective for apple juices. The kinetic

results showed that the inactivation conducted using vis-LED was also affected by the soluble solids content of the juices. Also, it may be possible to use the vis-LED processes to achieve a 5-log inactivation of *E. javanicum* spores in strawberry juices at a higher dose (> 420 J/cm^2).

The combined violet-blue LED treatment with thermal processes resulted in the same spore inactivation rate as the 90 °C-thermal processes alone but gained more advantage due to additional spore reductions by the LED before the thermal treatments. Nonetheless, factors such as practical and economical implications of this sequential technology should be made before its application. Furthermore, studies should be conducted to understand the inactivation mechanism under these processes as well as the quality perspective.

CRediT authorship contribution statement

Evelyn: Conceptualization, Funding acquisition, Formal analysis, Methodology, Writing - original draft, Writing - review & editing. **Chairul:** Conceptualization, Supervision, Methodology, Writing - review & editing. **Syaktia Aryuda:** Methodology, Investigation, Project administration. **Intan Ainunnisa:** Methodology, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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