



Optimization of continuous flow pulsed light system process parameters for microbial inactivation in tender coconut water, pineapple and orange juice

P. Preetha¹  | N. Varadharaju¹ | G. Jeevarathinam² | J. Deepa¹ |
A. P. Mohan Kumar³ | M. Balakrishnan¹ | P. Rajkumar¹ | R. Pandiselvam⁴ 

¹Department of Food Process Engineering, Agricultural Engineering College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, India

²Department of Food Technology, Hindusthan College of Engineering and Technology, Coimbatore, Tamil Nadu, India

³Department of Farm Machinery and Power Engineering, Agricultural Engineering College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, India

⁴Physiology, Biochemistry and Post-Harvest Technology Division, ICAR-Central Plantation Crops Research Institute, Kasaragod, Kerala, India

Correspondence

R. Pandiselvam, Scientist, ICAR-Central Plantation Crops Research Institute, Kasaragod-671 124, Kerala, India.
Email: anbupandi1989@yahoo.co.in; r.pandiselvam@icar.gov.in

Abstract

Continuous flow pulsed light (PL) system was designed and fabricated with a residence time of 3.5 s at a flow rate of 100 ml/min to study the effect on microbial quality of the fruit juice. The circuit was designed to deliver pulse energy between 12.6 and 756 J/cm² with a flash lasted 360 μs at a frequency of three pulses per second. Tender coconut water, pineapple and orange juice was treated with different input voltage (500–1000 V) to the lamp, distance of the lamp exposed to the sample (5–15 cm) and treatment time (15–45 s) to reduce the population of aerobic plate count (APC), yeast and mold. Box–Behnken design (BBD) was used to optimize the PL process parameters. The process condition was optimized based on the microbial reduction rate. The optimized condition was input voltage of 1492, 1499, and 1486 V, distance of 7.6, 10.2, and 5.3 cm, and treatment time of 43 s (12 passes), 44 s (13 passes), and 45 s (13 passes) for tender coconut water, pineapple and orange juice, respectively at the target of five log reduction for APC and four log reduction for Y&M. These results recommend that a continuous flow PL system could improve the microbial safety of the fruit juice. PL is a nonthermal method for inactivating food microbes, extending food's shelf life. For industrial purposes, the batch system is virtually impossible. As a result, continuous flow systems for liquid foods can provide a better way to handle large volumes. The optimized setup was found to achieve a microbial death rate of at least 5 log CFU/ml, consistent with FDA guidelines for fruit juice. The BBD of response surface methodology helps in optimizing the process parameters such as input voltage of the lamp, distance of the PL lamp to treatment tube and treatment time. The resulting mathematical model provides the PL process conditions for designing the equipment for different fruit beverages to obtain microbiologically safe products.

KEYWORDS

box–Behnken design, nonthermal treatment, optimization, pulsed light, tender coconut water

1 | INTRODUCTION

Consumption rate of fresh and refrigerated fruit juice is increasing for its nutritious and health benefits (Prithviraj et al., 2021; Chakraborty,

Ghag, Bhalero & Gokhale, 2020). Fruit juices are preferred by the consumers for their fresh flavor, taste and nutritional properties (Dhar et al., 2022; Kongkachuichai et al., 2015). Common method for preservation of fruit juices is thermal processing, but it has a negative impact

on sensory and nutritional properties (Jadhav et al., 2021; Kaavya et al., 2019; Petruzzi et al., 2017). Hence, there is an opportunity to switch from a thermal to nonthermal method for preservation of fruit juices.

Pulsed light (PL) is an emerging nonthermal technique that produces high power flashes of broad spectrum of light with a wavelength of 200–1100 nm for inactivating the microorganisms (Oms-Oliu et al., 2010). With a short period of time, the preferred inactivation of microbes can be achieved by PL and it has been attracted by the food industry. The destruction of microorganism in PL is mainly because of photochemical action of the UV-C (200–280 nm) and this emitted energy falls in the ultraviolet region (Pataro et al., 2011).

The PL inactivation depends on several factors viz., distance between sample and lamp, number of pulses and frequency, initial population, treatment time, thickness, composition of the sample and transparency of the sample (Dhar & Chakraborty, 2020). In continuous flow PL system, Pataro et al. (2011) attained 4 and 2.90 log reduction in apple and orange juice at a fluence of 4 J/cm²; Maftai, Ramos, Nicolau, Martín-Belloso, & Soliva-Fortuny (2014) had maximum reduction of 3.6 log cfu/ml in apple juice at the PL treatment condition of 16 J/cm²; Ferrario and Guerrero (2016) achieved only about 1.8–4.2 log cfu/ml in apple juice at PL dose of 0.73 J/cm² and Xu et al. (2019) revealed that maximum of 3.7 log cfu/ml in grape juice when treated with a flow rate of 30 ml/min, 100 pulses and at an intensity of 0.66 J/cm². Artiguez & Maranon (2015) reported that in continuous flow PL system the entire liquid surface are not treated completely since the layer of the juice which is exposed to the lamp surface alone gets treated whereas the lower layer is affected by the shadow effect. The main problem in PL is “shadowing effect” penetration of light to the samples which will limit the destruction of microbes (Ferrario & Guerrero, 2016; Wang et al., 2018). These limitations can be approached by developing a continuous flow system with a maximum fluence of the light that must reach every particle of the sample.

Fruit juices are mostly acidic in nature spoiled by bacteria, yeast and mold (Y&M). Many studies in PL treatment focussed on bacterial inactivation (Caminiti, 2011; Ferrario et al., 2013, 2014; 2015; Ferrario & Guerrero, 2018). Only few studies have been focussed on inactivation of Y&M (Voller et al., 2020; Dhar & Chakraborty, 2020). Y&M are more resistant than bacteria in fruit juices (Anderson, Rowan, Macgregor, Fouracre, & Farish, 2000). Dhar, Basak, & Chakraborty, (2022) reported that APC and Y&M are more resistant to PL than single microbial strain since group of microbes are affected by shadowing effect. This also pathed a way in investigating the effect of PL treatment on Y & M in fruit juices.

For designing the food processing equipment, process parameter optimization was considered as significant concern (Le Chau et al., 2019; Srikanth et al., 2020; Sudha et al., 2016). In approach to this issue, several researchers use Box Behnken's design of response surface methodology as a modeling tool (Chau et al., 2018; Chau et al., 2019; Srikanth et al., 2020). It reduces the experimental runs to predict statistically accepted (Nourbakhsh et al., 2014). It is also used for designing of food

processing equipments (Richa et al., 2021), process optimization (Nandhulal et al., 2021) and food preservation (Srinivas et al., 2020).

A study on continuous flow PL system can be useful for the food industry for commercial treatment of fruit juices. There is a scarcity of information on the design and development of continuous flow PL system and its effectiveness on fruit juices. Food and Drug Administration (FDA) declare that the fruit juice should be treated to ensure the minimum microbial safe limit of 5 log₁₀ reduction (FDA, 2001). Hence, to increase the microbial inactivation rate, a study was attempted to develop a continuous flow PL system and to optimize the PL process parameter which influences the microbial inactivation using Box–Behnken design (BBD).

2 | MATERIALS AND METHOD

2.1 | Extraction of fruit juices

Tender coconut water (var. WCT), Mandarin orange (var. Coorg) and pineapples (var. Queen) were procured and juices were extracted as per the procedure given in our previous study (Preetha et al., 2021).

2.2 | Design of a laboratory scale continuous flow PL treatment system

The laboratory scale PL system for juice treatment was developed at the Department of Food Process Engineering, Tamil Nadu Agricultural University, Coimbatore, India. It consists of (i) power supply (circuit) and (ii) PL reactor.

2.2.1 | Power supply (circuit)

In the flash lamp, impedance constant (K_o) is the lamp's output which is calculated as per Rashid et al. (2015). Impedance for a given lamp is constant because K_o depends only on the lamp physical dimensions, type and pressure of gas filled are given in the Table 1 (The Heraeus Lamp book, www.heraeus-nobilelight.com).

$$K_o = 1.28 \times \frac{L_A}{d} \times \left(\frac{P}{N_x} \right)^{1/6} \quad (1)$$

where K_o = impedance constant of the lamp ($\Omega A^{1/2}$), L_A = arc length of the lamp (mm), d = bore diameter of the lamp (mm), P = filled gas pressure in the lamp (Torr) and N_x = constant specific to the filled gas, for Xenon, 450 Torr (59 kPa).

The energy (2, 16, and 56 J/cm²) required per pulse with a constant pulse width (360 μ s), inductance-capacitance network (C) was calculated as given in the equation (Almabouada et al., 2011)

$$C = \left(\frac{2E_o \alpha^4 t^2}{K_o^4} \right)^{1/3} \quad (2)$$

TABLE 1 Pulsed light circuit design parameters

Properties	E1	E2	E3
Energy (Joules)	2	16	56
Arc length (mm)	300	300	300
Arc diameter (mm)	8	8	8
Gas fill pressure in the lamp (Kpa)	59	59	59
Damping coefficient	0.8	0.8	0.8
Pulse width (μ s)	360	360	360
Arc impedance ($\Omega A^{1/2}$)	48	48	48
Capacitance (μ Farads)	18	33	50
Voltage Required	500	1000	1500
Current (Ampere)	16	33	50
Frequency (Hertz)	3	3	3
Power consumption (watts)	2	16	56

Note: E1, E2, E3 – Energy dose required for the lamp.

where E_o = energy stored in capacitor (J), α = damping coefficient, t = time constant of circuit (s).

Duration of pulse (T_d) is defined as 3 t which was calculated as given in the equation (Rashid et al., 2015).

$$T_d(s) = 3t, \quad (3)$$

The voltage (V_o) required for generating the desired pulse energy was obtained from the below equation (Song et al., 2018)

$$V_o = (2E_o/C)^{0.5} \quad (4)$$

where C = capacitance of the capacitor (Farads).

Lamp current (I) required to produce pulse was determined using the equation given below as proposed by Ash et al. (2008).

$$I(A) = \left(\frac{E_o}{K_o T} \right)^{2/3} \quad (5)$$

where T = pulse width (s).

Pulse power (P_p) is the power expended over the substrate material by the lamp during the pulse was calculated as given by Ash et al. (2008)

$$P_p = K_o I^{3/2}, \quad (6)$$

The overall power consumed (P_c) by the circuit for producing the high pulse power was calculated as given by Ash et al. (2008)

$$P_c(W) = P_p \times T \times f, \quad (7)$$

where T-treatment time (s), and f = frequency of flashing (Hz).

2.3 | PL generation

The gas was ionized by passing the current through the lamp and a flash was produced. Pulse in the form of thousands of amperes can be traveled through the lamp because of the ionization which decreases the electrical resistance of the gas. When the pulse current was passed through the lamp, electrons excited with the xenon atom resulting in high energy. The higher energy electrons fall back to lower energy, resulting in the form of photons. Thus, conversion of electrical energy to light energy was achieved.

2.4 | Circuit for the PL treatment

The PL circuit was designed to flash 3 pulses per second and frequency with a 360 μ s pulse width is presented in the Table 1. The lamp energy such as 2 joules (500 V), 16 joules (1000 V), and 56 joules (1500 V) were selected based on the results of Pataro et al. (2011) and Schaefer et al. (2007). The circuit was divided in to two major parts namely (i) Flashing supply and (ii) Triggering supply.

In flashing supply, line power was connected to the transformer to vary the voltage to 500, 1000, and 1500 V. Two rectifier provided in the circuit rectify the AC voltage to DC. Through two limiting resistors, the fixed voltage charges the capacitors C. The energy stored in the capacitor was passed over the positive supply of flash lamp; simultaneously trigger voltage was supplied over the outside of the lamp and ignites the flash lamp to produce with an intense flash light. The storage capacitor recharges again and the cycle continued. The selector switch provided in the main control board was used to vary the input voltage of the lamp.

Trigger transformer was designed with an input power from the line has been stepped down using the Transformer. The rectifier provided in the circuit changes the input AC voltage to DC voltage. The corresponding DC voltage was stored in the capacitor C1 which provided an input to the trigger pulse transformer. Trigger pulse transformer was step up to 30 kV to produce a sharp pulse which has been transferred to the trigger electrode wire that surrounds the lamp (external triggering). The contractor relay was provided to give a gap of 2 s between charging and discharging of the voltage from the capacitor to build up again and continue the process.

2.5 | Construction of the PL reactor

The PL reactor (Figure 1a) consists of feeding system, treatment unit, recirculation unit and the power supply. The complete unit was fabricated with stainless steel (SS) 304. The feeding system consists of a feed tank with 2 L of capacity. The SS pipe line having an inner diameter of 6 mm was fixed with the feed tank and a ball valve was fixed in the pipe for regulating the flow of the fluid. Followed by the pipe, a quartz tube with 5 mm diameter and 300 mm length was attached

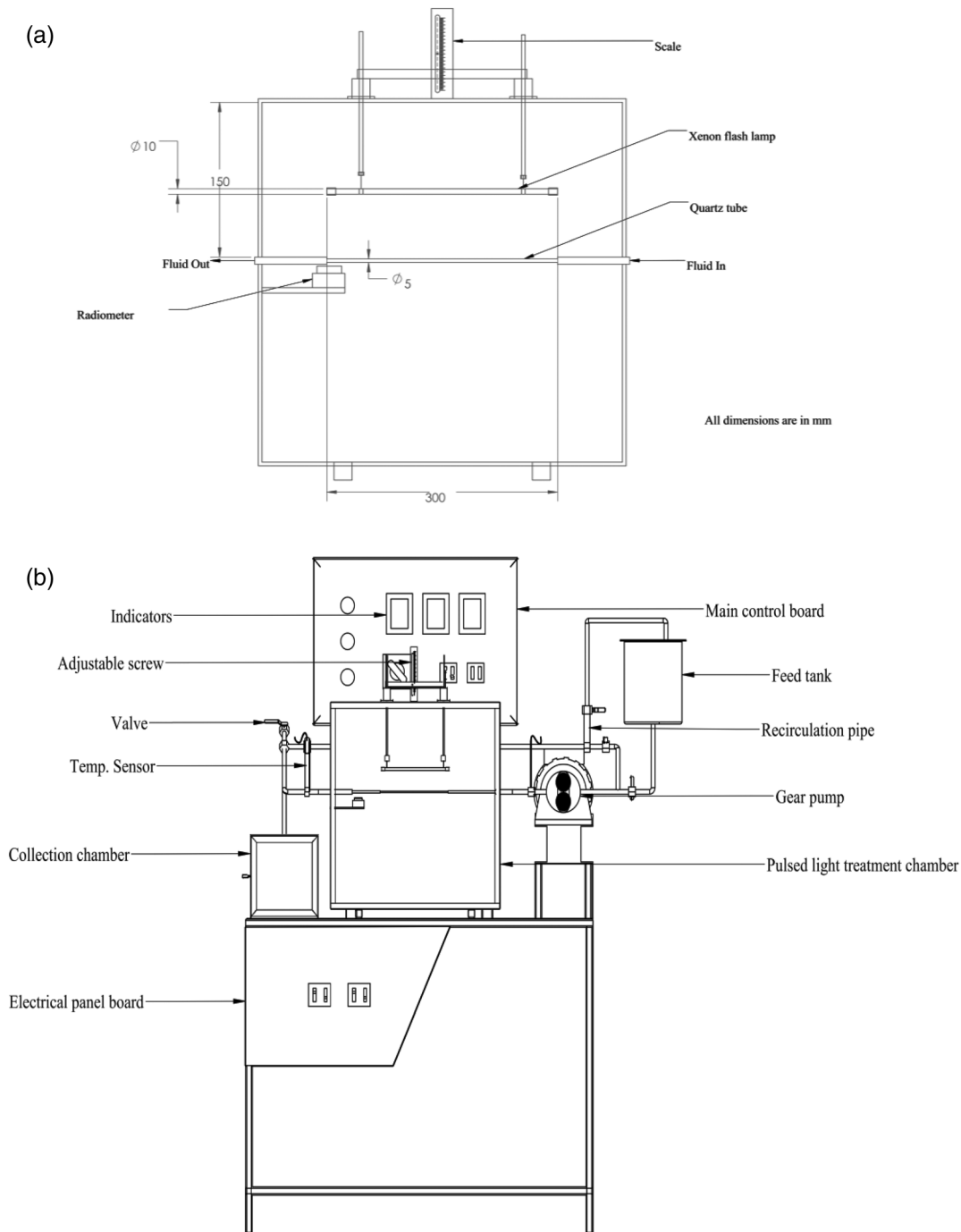


FIGURE 1 (a) Dimension of pulsed light continuous flow treatment chamber (all dimensions in mm) and (b) continuous flow pulsed light treatment system used for the treatment of fruit juices

which allows 90% of the light to pass through the treatment unit (Pataro et al., 2011).

The treatment unit (Figure 1b) consists of Xenon lamp at the top. The quartz flash lamp was filled with an inert gas that emits an irradiation spectrum of 200 to 1100 nm (Hwang et al., 2017). The lamp was provided with a provision of adjusting the height of treatment tube from 5 to 15 cm using adjusting screws. Two temperature sensors (Make: OMEGA) were attached with treatment tube (before and after treatment) to measure the difference in temperature.

The main inactivation in PL could be due to the ultra violet C spectrum. A radiometer of UV-C (Delta OHM) sensor was attached below the tube to measure the light intensity. The fruit juices circulated more than once in the treatment chamber depending upon the exposure time selected. The fruit juices were pumped using 0.25 hp gear pump (Make: Rotofluid FT 050) along the stainless steel pipe line to the quartz tube. The collection chamber made of SS sheet with 0.6 mm thickness, 140 mm diameter, and 140 mm height was fabricated to collect the treated fruit juice. Selector switch was provided

to change the intensity and pulse number. ON/OFF switch for pump and triggering circuit, two temperature indicators and radiometer indicator were fixed in the main control board.

The laboratory scale PL unit was sanitized before and after the process using standard procedures. The unit was flushed with hot water at 50°C for 10 min, then alkaline (1%) solution for 15 min and then sterile distilled water to make sure that PL unit was completely sterilized (Keyser, Muller, Cilliers, Nel, & Gouws, 2008).

2.6 | Flow profile characteristics

The capacity for the developed unit was 100 ml/min of fruit juice. The basic properties of the selected fruit juices namely density (AOAC, 2000) and viscosity using DV-III ultra-Programmable Rheometer (Brookfield's) were determined.

2.7 | Velocity

Velocity reflects the residential time of the fruit juices in the treatment chamber. To increase the residence of the liquid in the treatment chamber, diameter of the tube (quartz) was selected as 5 mm and was determined using the following formula (Ibarz & Barbosa-Canovas, 2003):

$$q = A.v, \quad (8)$$

where q = flow rate (ml/min), A = cross sectional area of the tube (cm^2), and v = velocity (cm/min).

2.8 | Reynolds number

Reynolds number indicates the flow pattern in a system and it describes the flow of the fluid is laminar or turbulent flow. Reynolds number was determined as given by Telis-Romero, Thomas, Bernardi, Telis, & Gabas (2006).

$$N_{Re} = \frac{\rho v D}{\mu}, \quad (9)$$

where N_{Re} = Reynolds number, ρ = density (g/ml), μ = viscosity (g cm/s), D = diameter of the treatment tube (mm).

2.9 | Residence time

Residence time is one of the prime factors that power the PL waves to penetrate in the fruit juices. As the liquid passes through the quartz tube, the incidence of the PL has to fall throughout the tube. The residence time of the fruit juice inside the treatment tube was calculated using Ibarz and Barbosa-Canovas (2003).

$$\theta = \frac{V}{q}, \quad (10)$$

where θ = residence time (s), V = volume of the fruit juice passing through the treatment chamber (ml), and q = flow rate (ml/s).

2.10 | Number of passes

The residence time in a single pass through the tube was much lesser than the selected exposure time. Thus, the required processing time of the liquid inside the treatment chamber was attained by recirculating the fruit juices. The number of passes that the fruit juice has to undergo was calculated as prescribed by Kaya and Unluturk (2016)

$$TP = \frac{t}{\theta}, \quad (11)$$

where TP = number of times the liquid passes through the treatment system, t = processing time (s), θ = residence time (s).

2.11 | Estimation of PL radiation dose

The intensity of the PL treatment is expressed as pulsed light dose. Radiometer with an accuracy of UV-C wavelength (254 nm) measures the irradiance which was placed below the quartz tube where the liquids flow through it. The overall effect of the radiation depends on combination of applied intensity and exposure time. The irradiation dose was calculated as explained in our previous study (Preetha et al., 2021).

2.12 | Microbiological analysis

The serial dilution spread plate method was used to compute aerobic plate count (APC) and Y&M in the sample (Dhar & Chakraborty, 2020). Plate count agar and yeast and malt agar (HIMEDIA) were used. The plates were then incubated for APC and Y&M at 37°C for 24 h and 30°C for 48 h, respectively. After the incubation period, the colonies were counted and expressed the survival fraction as the ratio of the initial number of microorganisms (N_0) to the number of microorganisms after treatment (N). The inactivation data was determined as mean \pm SD.

2.13 | Validation of design and statistical analysis

The performance of PL system was optimized based on the APC, and Y&M. BBD was used to optimize the process parameters using Software Design Expert Version 13(Stat-Ease Inc., Minneapolis, MN, USA).

The PL process parameters viz., input voltage to the lamp, distance of the PL lamp to treatment tube and treatment time need to be optimized. The minimum and maximum values of input voltage (x_1) were selected in the range from 500 to 1500 V, distances from the PL lamp (x_2) were selected in the range from 5 to 15 cm, and treatment time (x_3) was selected between 15 and 45 s. The experimental design obtained from the BBD is shown in the Table 3. Polynomial model was fitted using the response

$$Y = \beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_{11}x_1^2 + \beta_{22}x_2^2 + \beta_{33}x_3^2 + \beta_{12}x_1x_2 + \beta_{13}x_1x_3 + \beta_{23}x_2x_3, \quad (12)$$

where β_0 = intercept, β_1 , β_2 , and β_3 = linear term β_{11} , β_{22} , and β_{33} = quadratic term and β_{12} , β_{13} , β_{23} = interaction coefficient, and Y = response obtained from the model.

Mathematical modeling was done using the multiple linear regressions for all response value. Accuracy of the model can be obtained from the analysis of variance (ANOVA) which determines coefficient of variation, level of significance, R^2 and adjusted R^2 . Desirability of the levels can be attained from the BBD. The interaction between the factors can be obtained as response 3D graphs. With 3 replications, the experiments were conducted as per the design and fitted for numerical optimization of parameters.

3 | RESULTS AND DISCUSSION

3.1 | Flow characteristics of the fruit juices

The Reynolds number was calculated by allowing the fruit juices to flow at a flow rate of 100 ml/min. The Reynolds number was 715, 32, and 89 for tender coconut water, pineapple and orange juice, respectively. The Reynolds number obtained for different juice has clearly shown that, the flow of fruit juices inside the treatment was in laminar condition.

At the flow rate of 100 ml/min, the residence time was 3.5 s for all three selected fruit juices. Based on the residence time for single pass, the number of passes required for 15, 30, and 45 s were

determined as 4, 8, and 12, respectively. The fruit juices were circulated through the system to achieve the required treatment time.

3.2 | Temperature profile during the treatment

The temperature rise during the treatment was noted and presented in the Table 2. The maximum rise in temperature of 3.2°C was noted in tender coconut water and this may be due to its higher transmittance (81%) in contrast to orange and pineapple juice it was only 0.6 and 0.9°C respectively. Elmnasser et al. (2007) also reported that the rise in temperature is due to the absorbance of light by the food samples. The increase in treatment time increased the temperature of fruit juices. Ferrario et al. (2013) found that the treatment for 60 s increased the temperature up to 7.4°C for the fruit juices. In our study, the treatment lasted up to 45 s and a maximum temperature rise of 3.2°C was observed. The temperature of the fruit juices after the treatment was well below 40°C which ensured that the current study comes under nonthermal process. Guerrero-Beltran and Barbosa-Canovas (2005) pointed out that temperature rise not more than 40°C is said to be nonthermal process (cold process).

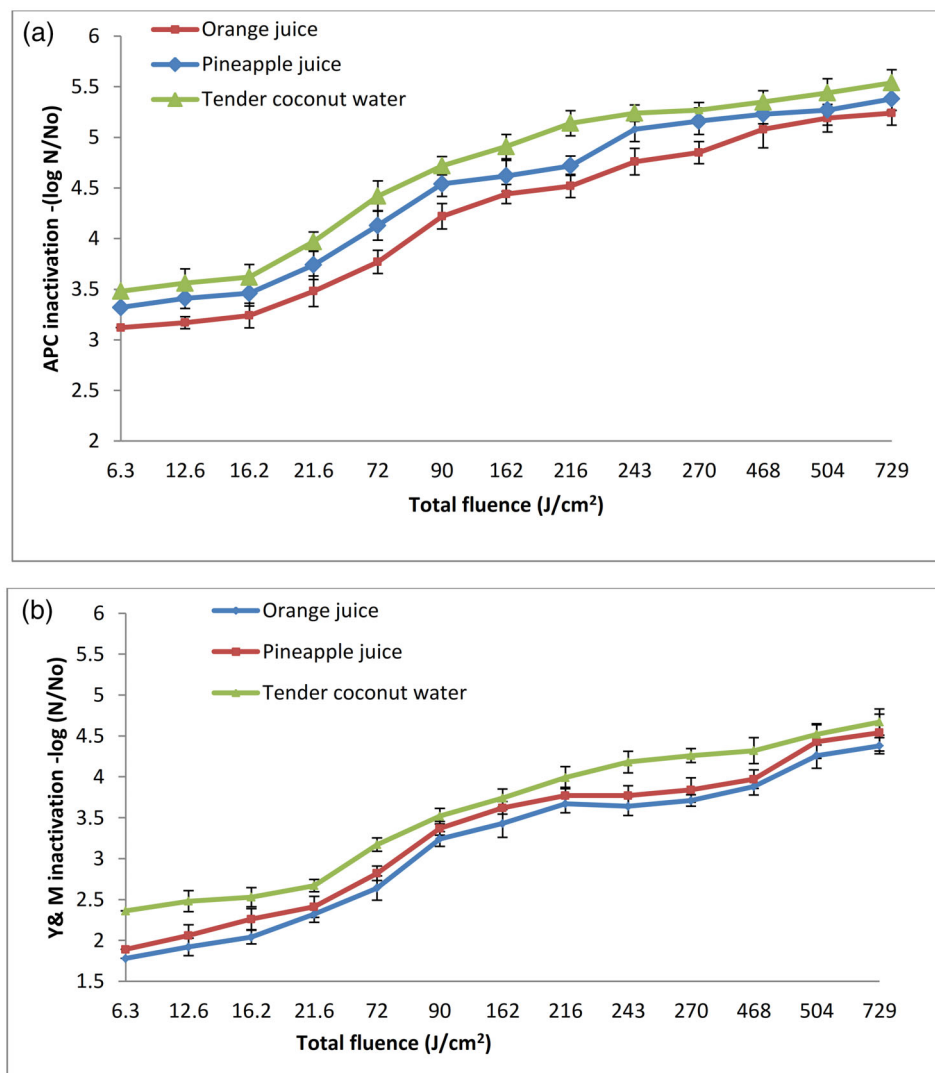
3.3 | Impact of PL process factors on the APC reduction in fruit juices

The log reduction of APC populations in fruit juices during the PL treatment at different fluence energy are shown in the Figure 2a. The maximum log reduction of 5.54 cfu/ml of APC populations were observed in tender coconut water followed by pineapple and orange juice with 5.38 and 5.24 log reductions by treating it with higher energy of 729 J/cm². Inactivation of microbes depends on the properties of light absorption by the fruit juices. Pataro et al. (2011) observed a higher reduction of *E. coli* and *L. innocua* in apple juice than orange juice because of the difference in the absorbance of the juice. Munoz et al. (2011) reported that orange juice treated at different flow rate of 20, 8 ml/min achieved 2.42 log reduction of *E.coli* K12 DSM1607.

TABLE 2 Temperature rise during the pulsed light treatment

Fruit juices	Exposure time (s)	Initial temperature (°C)	Final temperature (°C)	Rise in temperature (°C)
Orange juice	15	30.3	30.5	0.2
	30	29.8	30.1	0.3
	45	30.1	30.7	0.6
Pineapple juice	15	29.6	29.9	0.3
	30	30.9	31.3	0.4
	45	30.1	31.0	0.9
Tender Coconut water	15	28.4	28.8	0.4
	30	29.1	29.9	0.8
	45	30.1	31.3	3.2

FIGURE 2 (a) APC inactivation in the fruit juices using continuous flow PL system and (b) Y&M inactivation in the fruit juices using continuous flow PL system



The APC inactivation had a significant effect ($p < 0.001$) over the process factors for all the juices. Treating the juice at higher voltage and near to the xenon lamp increases the exposure of UV light which increases the inactivation rate (McDonald et al., 2000). Similar trend of data was found by Vollmer et al. (2020) for pineapple juice and the maximum of 5 log reduction of APC can be achieved by treating at 757 J/cm^2 . As per the earlier design suggested by Artiguez & Maranon (2015), the PL inactivation efficiency has been increased by providing the turbulence or mixing of the liquids enhances the exposure of the entire surface to light intensity. The current design of the PL system was provided with recirculation to increase the turbulence or mixing of the fruit juices. By treating at higher energy, the inactivation of APC increased. Maftai et al. (2014) stated that the input voltage supplied to the lamp, exposure time and distance from the lamp play a major role in microbial inactivation. Xu et al. (2019) achieved only 4.89 log reduction of *E.coli* in red grape juice treated with flow rate of 40 ml/min in continuous spiral flow tube.

The minimum log reduction of 3.48, 3.32, and 3.12 cfu/ml was observed for tender coconut water, pineapple and orange juice, respectively, when it was treated with lesser energy of 6.3 J/cm^2 . The

lesser energy corresponds to the input voltage of 500 V, 10 cm distance and 15 s treatment time. Inactivation rate decreases due to the lesser number of photons were produced over the sample (Wang et al., 2016). Dhar and Chakraborty et al. (2020) also experienced that increasing the distance of 9.6 cm between the sample and lamp impacts the microbial destruction.

APC inactivation is mainly due to photochemical effect (UV light), photothermal (infrared rays and visible light) and pulse effect (Dhar & Chakraborty, 2020). Xu et al. (2019) also reported that inactivation increases by applying higher fluence which resulted in striking the higher photons over the sample. The continuous flow PL system resulted in higher inactivation (Pataro et al., 2011; Xu et al., 2019).

3.4 | Impact of PL process factors on the reduction of Y&M in fruit juices

The log reduction of Y&M populations in fruit juices during the PL treatment at different fluence energy are shown in the Figure 2b. The maximum of 4.67 log reduction of Y&M population was obtained in

TABLE 3 Experimental results of box–Behnken design

Run	Input voltage, V (X ₁)	Distance from the lamp, cm (X ₂)	Treatment time, s (X ₃)	APC Inactivation ^a (log N/log No) cfu/ml, (Y)			Y&M inactivation ^b (log N/log No) cfu/ml, (Y)		
				Orange juice	Pineapple juice	Tender coconut water	Orange juice	Pineapple juice	Tender coconut water
1	1000	10	30	4.44	4.62	4.91	3.43	3.62	3.74
2	1000	15	45	4.52	4.72	5.14	3.67	3.72	3.99
3	1000	10	30	4.44	4.6	4.9	3.46	3.67	3.76
4	500	10	15	3.12	3.32	3.48	1.78	1.89	2.36
5	1000	5	15	4.22	4.54	4.72	3.24	3.37	3.52
6	500	5	30	3.24	3.46	3.62	2.04	2.26	2.53
7	1000	10	30	4.5	4.64	4.94	3.45	3.66	3.78
8	1000	15	15	3.77	4.13	4.42	2.64	2.82	3.17
9	1000	5	45	4.85	5.16	5.27	3.71	3.84	4.26
10	500	10	45	3.48	3.74	3.97	2.32	2.41	2.67
11	1000	10	30	4.48	4.6	4.88	3.48	3.68	3.72
12	1500	5	30	5.19	5.27	5.44	4.26	4.43	4.52
13	1500	10	15	4.76	5.08	5.24	3.64	3.77	4.18
14	500	15	30	3.17	3.41	3.56	1.92	2.06	2.48
15	1500	15	30	5.08	5.23	5.35	3.88	3.97	4.32
16	1500	10	45	5.24	5.38	5.54	4.38	4.54	4.67
17	1000	10	30	4.46	4.62	4.92	3.4	3.64	3.73

^aAPC and

^bY&M Initial population for tender coconut water $7.4 \pm 0.05 \times 10^5$ cfu/ml & $5.8 \pm 0.1 \times 10^4$, Pineapple juice - $6.8 \pm 0.3 \times 10^5$ cfu/ml, & $4.8 \pm 0.1 \times 10^4$ cfu/ml and orange juice - $6.3 \pm 0.2 \times 10^5$ cfu/ml & $5.2 \pm 0.1 \times 10^4$ cfu/ml.

tender coconut water followed by pineapple and orange juice with 4.54 and 4.38 log reductions. The higher inactivation was achieved at higher energy dosage of 756 J/cm^2 which corresponds to input voltage of 1500 V, treatment time of 45 s and treating the juice at 5 cm nearer to the lamp. These results were in accordance with Maftai et al. (2014) that observed a 1.2 log reduction with a lower energy dose of 2 J/cm^2 whereas higher energy resulted in 3.76 log mold reduction. Paniagua-Martinez, Ramírez-Martínez, Serment-Moreno, Rodrigues, & Ozuna (2018) also reported that PL treatment reduced 2–5 log cfu/ml of *Saccharomyces cerevisiae* in fruit juices. Dhar & Chakraborty et al. (2020) conveyed that at higher fluence rate of 17.42 W/cm^2 ($2400 \text{ V}/2.5 \text{ cm}/3 \text{ min}$) could be able to achieve more than 5log reduction of Y & M in mixed fruit beverage.

The minimum Y&M log reduction of 2.36, 1.89, and 1.78 were observed for tender coconut water, pineapple and orange juice, respectively when it was treated with 6.3 J/cm^2 . The lower energy dose of 6.3 J/cm^2 corresponds to input voltage of 500 V, 10 cm distance from the lamp and 15 s treatment times. These findings were in agreement with Vollmer et al. (2020) that lesser energy of 160 J/cm^2 resulted in 2.2 log reduction of Y&M in pineapple juice. Tran and Farid (2004) stated that Y&M are less affected by UV light than bacteria since DNA molecules of Y&M produce less pyrimidine base, especially

thymine. An opposite trend of results was reported in pineapple juice by Shamsudin et al. (2014) that highly affected by Y&M than APC. This difference may be due to varying fluence level, sample depth, process parameters and initial contamination level.

The UV light is main reason in the PL treatment for the reduction of microbes in fruit juices (Shamsudin et al., 2014). Three processes which take place in the PL treatment are photochemical, photothermal and pulse effect (Ferrario et al., 2014). The effect due to photochemical is the development of thymine dimers, which hinders the replication of DNA. Photothermal effect is due to the infrared radiation and visible light which generates heat used for destruction of microbes. The effect of pulse causes the changes in cellmembrane permeability and shrink of the cell wall (Krishnamurthy et al., 2010).

3.5 | Model fitting and statistical results for APC inactivation

The experimental results of the BBD at different PL process factors over the APC inactivation are given in the Table 3. The influence of PL process factors on the APC inactivation is described by the second order polynomial equation from the BBD is given below

TABLE 4 F value of the ANOVA results for the model predicted in log reduction of APC and Y&M in fruit juices

Sources	APC log reduction			Y&M log reduction			
	Df	Orange juice	Pineapple juice	Tender coconut water	Orange juice	Pineapple juice	Tender coconut water
		F value					
Model	9	44.89 ^a	41.53 ^a	97.69	376 ^a	1071 ^a	58.84 ^a
X ₁	1	351 ^a	324.78 ^a	722 ^a	2777 ^a	7796 ^a	460 ^a
X ₂	1	6.02 ^a	5.80 ^a	5.04	55 ^a	210 ^a	5.95 ^a
X ₃	1	33.19 ^a	24.47 ^a	63.66 ^a	327 ^a	842 ^a	43.8 ^a
X ₁ X ₂	1	0.02	0.0013	0.027	5.72 ^a	16.1 ^a	0.35
X ₁ X ₃	1	0.19	0.19	1.083	3.38	14.89 ^a	0.5
X ₂ X ₃	1	0.22	0.01	0.86	26.55 ^a	44 ^a	0.1
X ₁ ²	1	11.2 ^a	18.04 ^a	81.16 ^a	176 ^a	577 ^a	18.5 ^a
X ₂ ²	1	0.21	0.17	2.52	6.4 ^a	35 ^a	0
X ₃ ²	1	0.57	0.02	0.016	5.48	59 ^a	0.018
Residual	7						
Lack of fit	3	6.16	5.97	6.5	6.07	0.32	5.43
Pure error	4						
Corr. total	16						
R ²		0.983	0.9816	0.9921	0.9979	0.9993	0.987
R ² _{Adj}		0.9611	0.958	0.9819	0.9953	0.9983	0.9702
CV (%)		3.2	3.06	1.93	1.69	0.9602	3.5

^aSignificant at 1% level.**Orange juice**

$$\begin{aligned} \text{APC log reduction} = & 4.42 + 0.90^* x_1 - 0.11^* x_2 \\ & + 0.27^* x_3 - 0.010^* x_1^* x_2 \\ & + 0.03^* x_1^* x_3 + 0.03^* x_2^* x_3 \\ & - 0.22^* x_1^2 - 0.03^* x_2^2 - 0.050^* x_3^2 \end{aligned} \quad (13)$$

Pineapple juice

$$\begin{aligned} \text{APC log reduction} = & 4.66 + 0.87^* x_1 - 0.11^* x_2 + 0.24^* x_3 - 0.002^* x_1^* x_2 \\ & - 0.03^* x_1^* x_3 - 0.007^* x_2^* x_3 - 0.28^* x_1^2 - 0.028^* x_2^2 \\ & + 0.009^* x_3^2 \end{aligned} \quad (14)$$

Tender coconut water

$$\begin{aligned} \text{APC log reduction} = & 4.96 + 0.86^* x_1 - 0.07^* x_2 + 0.25^* x_3 - 0.007^* x_1^* x_2 \\ & - 0.0475^* x_1^* x_3 + 0.042^* x_2^* x_3 - 0.400^* x_1^2 \\ & + 0.070^* x_2^2 - 0.005^* x_3^2 \end{aligned} \quad (15)$$

The above equations can be used to predict the response of given levels of each parameter. Analysis of variance (ANOVA) for APC inactivation obtained from the BBD are presented in the Table 4. The coefficient of determination (R²) was 0.9921, 0.9816, and 0.983, for tender coconut water, pineapple and orange juice, respectively. Higher R² value indicates that model was well adapted to the experiment. The F value of the model 97.69, 41.53 and 44.89 suggests that quadratic model is significant ($p < 0.001$). The process parameters x_1 ,

x_3 and x_1^2 for tender coconut water, x_1 , x_2 , x_3 , and x_1^2 for pineapple and orange juice respectively, were significant. The input voltage supplied to the lamp implies higher inactivation of APC. Ferrario and Guerrero (2018) also developed polynomial model for the destruction of *A. acidoterrestris* in apple juice by PL. The author has developed to predict treatment time by keeping survival count as a function.

Response surface plots for the APC log inactivation in the fruit juices are shown in the Figure 3. From these responses, it is inferred that APC log inactivation for all the fruit juices increased with increase of input voltage, treatment time and a decrease in the lamp distance. Artiguez & Maranon (2014) reported that bacterial log reduction increases with increase in the input voltage applied. Continuous PL treatment over the APC inactivation in fruit juice suggest that input voltage supplied to the lamp has greater effect followed by treatment time and distance from the lamp.

3.6 | Model fitting and statistical results for Y&M inactivation

Table 3 provides the experimental results of the BBD at various PL process factors over Y&M inactivation. It was inferred that PL treatment had significant ($p < 0.001$) effect on Y&M log reduction. The second order polynomial equation from the BBD is given below, and it describes how the PL process factors affect the Y&M inactivation.

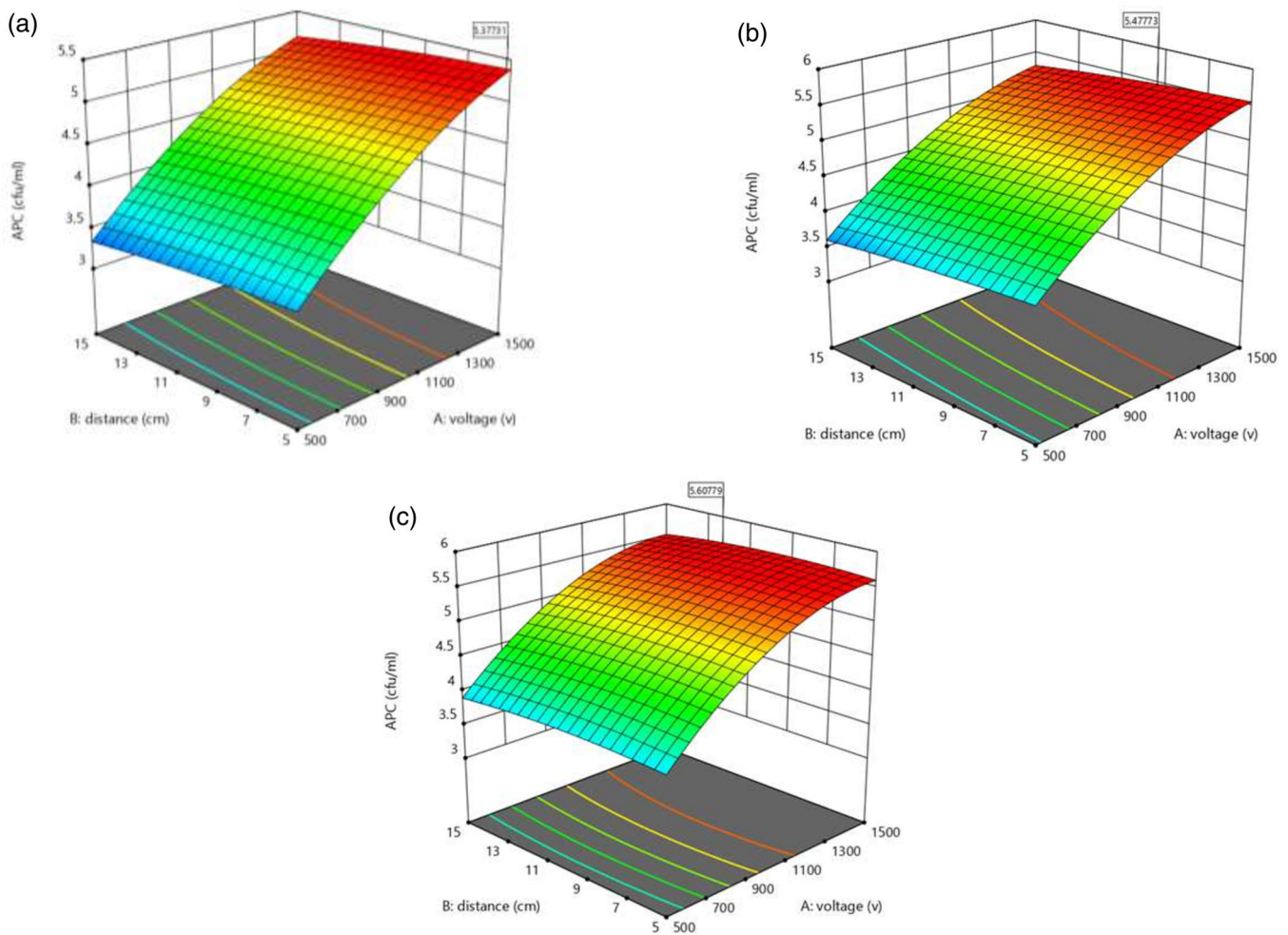


FIGURE 3 Response surface plot of process parameter on the aerobic plate count of the fruit juices (a) Orange juice, (b) pineapple juice, and (c) tender coconut water

Orange juice

$$\begin{aligned} \text{Y\&M log reduction} = & 3.44 + 1.01^* x_1 - 0.14^* x_2 \\ & + 0.347^* x_3 - 0.065^* x_1^* x_2 \\ & + 0.050^* x_1^* x_3 + 0.140^* x_2^* x_3 \\ & - 0.35^* x_1^2 - 0.067^* x_2^2 - 0.062^* x_3^2 \end{aligned} \quad (16)$$

Pineapple juice

$$\begin{aligned} \text{Y\&M log reduction} = & 3.73 + 1.01^* x_1 - 0.16^* x_2 + 0.33^* x_3 - 0.065^* x_1^* x_2 \\ & + 0.0625^* x_1^* x_3 + 0.10^* x_2^* x_3 - 0.37^* x_1^2 - 0.09^* x_2^2 \\ & - 0.12x_3^2 \end{aligned} \quad (17)$$

Tender coconut water

$$\begin{aligned} \text{Y\&M log reduction} = & 3.55 + 0.79^* x_1 - 0.088^* x_2 + 0.13^* x_3 + 0.11^* x_1^* x_2 \\ & + 0.040^* x_1^* x_3 - 0.085^* x_2^* x_3 - 0.015^* x_1^2 \\ & - 0.085^* x_2^2 - 0.11^* x_3^2 \end{aligned} \quad (18)$$

The Y&M inactivation of given values of the process parameters can be predicted using the above equation. The process parameters x_1 ,

x_2 , x_3 and x_1^2 for tender coconut water, all the terms for pineapple juice and x_1 , x_2 , x_3 , x_1x_2 , x_2x_3 , x_1^2 and x_2^2 of orange juice respectively, were significant. The input voltage (x_1) had maximum impact on the Y&M inactivation based on the higher magnitude of coefficient $\beta_1 = 0.79$, 1.01, and 1.01 for tender coconut water, pineapple and orange juice respectively. The model is well adapted to the experiment because of the higher R^2 (coefficient of determination) values of 0.987, 0.9983, and 0.9953 for tender coconut water, pineapple and orange juice respectively. Higher value indicates that model is well adapted to the experiment. The quadratic model is significant ($p < 0.001$) which is indicated by the F value of the model 58.84, 1071 and 376. A similar type of model was developed by Xu et al., (2019) by finding the survival count (Y) and function as number of pulses and flow rate. The model showed good fit of $R^2 = 0.97$ and significant.

Response surface plots for the Y & M inactivation in the fruit juices are shown in the Figure 4. From these responses, it is inferred that by increasing the input voltage and treatment time with minimum distance increases the Y&M inactivation. Input voltage supplied to the lamp has greater effect on the Y&M inactivation followed by treatment time and distance from the lamp.

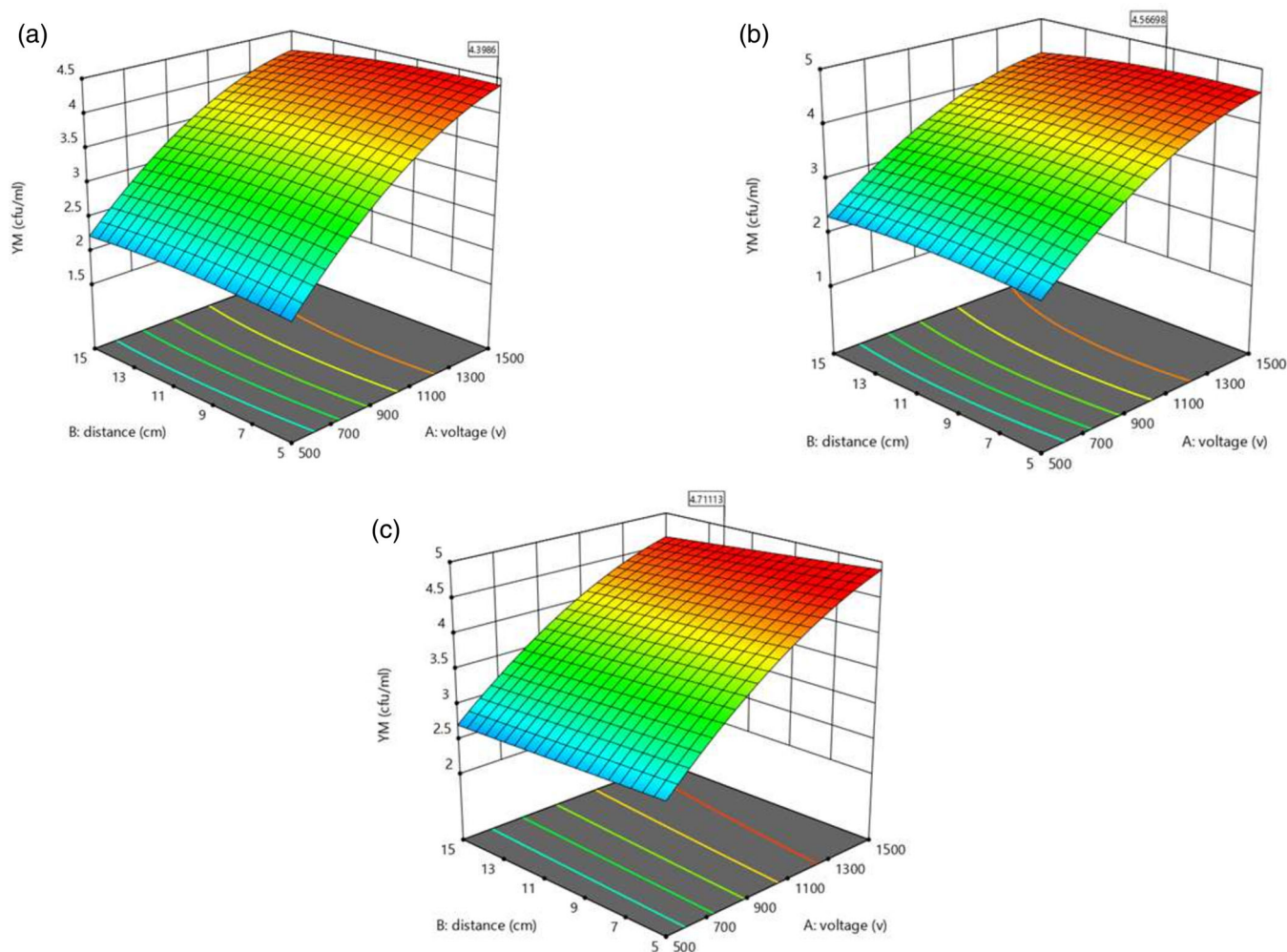


FIGURE 4 Response surface plot of process parameter on the yeast and mold of the fruit juices. (a) Orange juice, (b) Pineapple juice, and (c) Tender coconut water.

TABLE 5 Error % for predicted and actual value at the optimum conditions

Sl. No	Juices	Input voltage, V (X_1)	Distance from the lamp, cm (X_2)	Treatment time, s (X_3)	Predicted value		Experimental value		Error %	
					APC log (cfu/ml)	Y&M log (cfu/ml)	APC log (cfu/ml)	Y&M log (cfu/ml)	APC log (cfu/ml)	Y&M log (cfu/ml)
1	Tender coconut water	1492	7.6	43	5.59	4.63	5.54	4.59	0.89	0.86
2	Pineapple juice	1499	10.2	44	5.52	4.56	5.48	4.54	0.72	0.43
3	Orange juice	1486	5.3	45	5.28	4.36	5.26	4.32	0.37	0.91

Note: Error % = (Predicted value - Experimental value) / Predicted value \times 100.

3.7 | Optimization

From the second order polynomial equation, optimal values of the tested parameters for APC inactivation were determined from BBD. The optimized process parameters were input voltage of 1492,

1499, and 1486 V, distance of 7.6, 10.2, and 5.3 cm and treatment time of 43, 44, and 45 s for tender coconut water, pineapple and orange juice, respectively. Triplicate experiments were carried out and achieved a log reduction of 5.54, 5.48 and 5.26 CFU/ml of APC and 4.59, 4.44, and 4.32 CFU/ml of Y&M for tender coconut water,

pineapple and orange juice, respectively. The experimental values obtained are close to the predicted value which is shown in the Table 5. A deviation of the experimental value from the predicted value was established to be less and error was <2%. The obtained level was satisfied for achieving the minimum requirements of FDA of 5 log APC reductions and 4 log Y&M reductions for fruit juices in continuous flow PL system.

4 | CONCLUSION

The developed continuous flow PL system produces pulse energy between 12.6 and 756 J/cm² with a flash lasted 360 μs at a frequency of 3 pulses per second. It was adequate to achieve the FDA recommended level of minimum 5 log cfu/ml microbial inactivation for fruit juices. The process parameters were optimized using RSM. The results declared that by increasing the input voltage, treatment time and shorter lamp distance to the sample increased the destruction rate. The BBD study established the optimum process parameters for achieving higher inactivation of APC and Y&M significantly ($p < 0.01$) in fruit juice. The experimental values are close to predicted values and the generated model predicted the response value.

AUTHOR CONTRIBUTIONS

P. Preetha: Conceptualization; investigation; writing – original draft. **N. Varadharaju:** Conceptualization; investigation; project administration; supervision; writing – original draft. **G. Jeevarathinam:** Software; validation; writing – review and editing. **J. Deepa:** Methodology; software; validation; writing – review and editing. **A.P. Mohan Kumar:** Data curation; formal analysis; methodology; resources; software. **M. Balakrishnan:** Software; supervision; visualization; writing – review and editing. **P. Rajkumar:** Project administration; resources; visualization; writing – review and editing. **R. Pandiselvam:** Resources; software; validation; writing – original draft; writing – review and editing.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

ORCID

P. Preetha  <https://orcid.org/0000-0002-9892-7319>

R. Pandiselvam  <https://orcid.org/0000-0003-0996-8328>

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