



Effect of Pulsed Light Treatment for the Preservation of Coconut Inflorescence Sap

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Abstract

The present study aims to investigate the impact of pulsed light (PL) treatment on the preservation of coconut inflorescence sap. In order to avoid fermentation of the sap, the freshly collected sap was exposed to various PL process parameters such as the input voltage, which is supplied to the lamp from 500 to 1500 V; the exposure time for treatment ranges from 60 to 180 s; and the distance of the lamp to the treatment chamber, which is selected from 50 to 150 mm. Using Box–Behnken design, the process parameters, such as 1000 V of input voltage, 100 mm of distance and a treatment time of 120 s were optimised based on the physico-chemical and microbial properties. Maximum log reduction of 3.57 log cfu/ml in total plate count and 2.79 log cfu/ml in yeast and mould were observed at the optimum condition. The quality parameters, such as pH and TSS do not have significant effect ($p < 0.05$) over the treatment. The colour difference value (ΔE) was 0.92, which is mildly noticeable, whereas the turbidity reduced to 42%, total sugars to 6.34% and reducing sugar reduced to 13.58%, with a significant effect ($p < 0.05$) over the PL treatment. From the results obtained, it was determined that pulsed light technology might be used as a preservation approach for coconut sap with the minimum effect on its quality parameters.

Keywords Pulsed light · Coconut inflorescence sap · Microbial · Quality parameters · Coconut sugar · Neera

Introduction

Coconut inflorescence sap is a nourishing, nutritious non-alcoholic beverage traditionally extracted from the unopened inflorescence of the Coconut palm (*Cocos nucifera* L.). It

contains sugars, minerals, vitamins, proteins, antioxidants, volatiles, etc. The sap has a high sugar content (12–15% of the total weight) and an enormous amount of nutrients, which can be easily transformed into several value-added products such as palm gur, coconut sugar, honey, syrup, palm candy, soft drinks, neera chocolate, cookies, candies, and so on (Ghosh et al. 2018). All of these products are highly valued in the market and can be derived from fresh, unfermented sap. Due to the rich sugar content of sap, it is extremely prone to natural fermentation in the presence of bacteria and yeast, which results in the production of the alcoholic beverage called toddy with an alcohol level of 5–8% (Pandiselvam et al. 2021). Under normal conditions, the freshly collected sap begins to ferment within 2–3 h (Hebbbar et al. 2018). The natural sap contains native bacteria such as *Bacillus*, *Lactobacilli*, *Micrococci*, *Enterobacter*, *Leuconostoc*, *Saccharomyces*, *Candida* and *Pichia* (Sukumaran and Radhakrishnan 2021).

Among these, yeast, LAB (lactic acid bacteria) and acetic acid bacteria are the main causes of the faster fermentation of sap (Atputharajah et al. 1986). As a result of biochemical and microbiological modifications to sap, three stages

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of fermentation, namely lactic acid fermentation, alcoholic fermentation and finally acetic acid fermentation takes place (Hebbar et al. 2020). The fermented sap is astringent, produces unfavourable volatile properties and appears hazy. Furthermore, dodecanoic acid and palmitoleic acid produced by microbial decomposition make neera sap astringent and unsuitable for human consumption (Borse et al. 2007). To prevent this fermentation, the farmers traditionally add substances like lime, *Cotylelobium lanceolatum* craib wood shavings, *Shorea roxburghii* wood shavings, water extracts of *Sacoglottis gabonensis* bark and mangosteen skins to the palm sap during neera tapping which may delay spoilage but only to a limited extent (Victor et al. 2022).

Thermal treatments are typically employed for reducing spoilage and to increase the keeping quality of neera. By heating the sap to high temperatures (pasteurisation) can lead to thermal degradation and the loss of nutrients, colour, and flavour that are distinctive to palm sap (Naknean 2013). Moreover, high-temperature pasteurisation of coconut sap (Prashanth and Patil 2020) and palm sap (Baliga and Ivy 1961) imparted a cooked flavour. According to Naknean (2013), the sugar content and pH of palm neera decreases with addition of nisin as a preservative, and the total acidity increases where nisin is the bacteriocin peptide produced by certain strains of *Lactococcus lactis* subsp. *Lactis*. Also, an excessive quantity of nisin causes an undesirable high level of sourness, despite the fact that high concentrations could only reduce the microbial load. According to Sukumaran and Radhakrishnan (2021), freezing coconut neera could lower its microbial load while increasing other chemical quality parameters like pH and protein levels. Leena et al. (2021) explored another approach, filtration of coconut nectar using electrospun nanofibrous membrane and found that it could reduce yeast load by 2 log; whereas, mineral levels and total polyphenol content are significantly decreased. Also, it was revealed that ΔE was higher in the filtered sample, which is apparent and might influence consumer preference.

To minimise the microbial load, Anbarasan et al. (2019) and Pandiselvam et al. (2020) examined whether microwave heat treatment affected the physico-chemical characteristics of neera. The quality parameters such as colour value, acidity, TSS, and pH changed at greater power levels and exposure times. The sap subjected to high temperature changes the flavour, colour and nutritional properties. Thermochemical processes such as the Maillard reaction, inversion reaction, and caramelization takes place which affects the properties of palm sap (Hebbar et al. 2018; Pandiselvam et al. 2021).

At the industrial level, commercial sterility is primarily achieved through thermal processes, which result in significant nutritional and sensory losses. Compared to thermal methods, non-thermal technologies have improved sensory qualities and nutrient retention in food items while

prolonging shelf life (Gomez-Lopez et al. 2007). The effects of plasma bubbling with varied flow rate and time on the free radical production and their influence on the characteristics of neera were examined by Aparajhitha and Mahendran (2019). The pH increased dramatically, but the colour and TSS values barely changed and the vitamin C content declined somewhat, with a reduction of 1 log (cfu/ml) for the given plasma treatment.

Among the various non-thermal technologies, pulsed light is one of the most novel, innovative, and recently developed technologies for effective microbial reduction and enzymatic inactivation. With the help of this technology, food or packaging is not only decontaminated but also has its texture and nutritional value preserved (Abid et al. 2014). Microorganisms are eliminated during pulsed light treatment by exposing them to brief, intense light pulses (Mandal et al. 2020). According to Vollmer et al. (2020), greater pulsed light treatments were preferable to heat treatments with respect to preserving colour, vitamins and the desired enzyme (bromelain). They also successfully reduce the microbial load in pineapple juice. However, there is no research on the effects of pulsed light treatment of fresh coconut inflorescence sap on microbial reduction and quality attributes. Hence, the aim of the present study is to assess the impact of pulsed light processing parameters (input voltage, distance from the lamp and treatment time) on pH, TSS, turbidity, total sugars, reducing sugars, colour difference (ΔE) and microbial reduction of coconut inflorescence sap.

Materials and Methods

Raw Material

Fresh, unfermented Neera from Coconut trees of Tall \times Dwarf variety was harvested from Thondamuthur local farm, Coimbatore, Tamil Nadu, India. The traditional mud pot was placed on the spadix to collect the dripping sap and for treatment trails the sap was collected in the early morning at 6.00 A.M. The fresh sap was immediately collected in a sterile glass storage bottle and preserved in an ice box (4 °C) to avoid fermentation. The samples were brought to the food biochemistry laboratory (Department of Food Process Engineering, Tamil Nadu Agricultural University, Coimbatore, India) for analysis within half an hour of collection.

Experimental Setup of Pulsed Light (PL) Treatment System

A pulsed light continuous treatment system which is available at the Dept. of Food Process Engineering was used for conducting the study (Preetha et al. 2022). The laboratory

model experimental setup of pulsed light (PL) comprises of a treatment chamber, a feed tank, a circulation system, a radiometer, a collection chamber, a main control board, and an electrical panel board (Fig. 1).

The Xenon Flash Lamp is made up of a rod-sealed tube constructed of clear fused quartz (CFQ) with a thickness of 3 mm that is filled with xenon gas, electrodes that transport electrical current to the gas, and a trigger electrode which is a thin nickel wire looped around the length of the lamp. CFQ transmits short-wave ultraviolet (UV) light, whereas the other types of quartz limit UV wavelength. To produce the broad spectrum from 100 to 1100 nm, a xenon flash lamp (Heraeus Noble Light Ltd., U.K.) with a bore diameter of 8 mm and a length of 300 mm was employed with a clear fused quartz envelope. A high voltage of 30 kV supplied as a pulse to the trigger electrode which ionises the xenon gas and commences conduction. A current of 16 A running through the lamp at this point causes increased ionisation and thus electron conduction inside the tube.

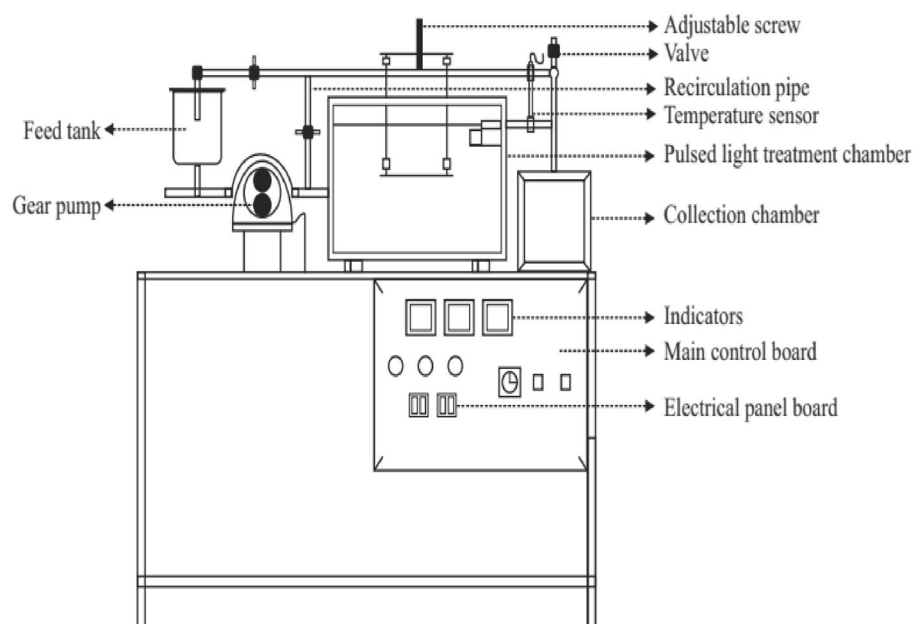
To monitor the temperature change of the liquid sample flowing during the treatment, two class B wire-type resistance temperature detector (RTD) sensors (OMEGA with an accuracy of $0 \pm 25^\circ\text{C}$) were installed at the outlet and input points of the sample flowing quartz tube. The broad spectrum of UV-C waves has a significant role in the inactivation efficiency of pulsed light treatment (PL) (Takeshita et al. 2003). Consequently, the radiometer (Delta OHM, Italy) was used to measure the UV-C light intensity at the irradiation tube's surface which is placed beneath the sample tube. A photodiode SiC (HD 2021 T4) sensor with a peak sensitivity of 254 nm makes up the detector. The flash pulses are produced with a 360 s pulse width at a frequency of 3 pulses/

second. For an input of 500, 1000, and 1500 V, the lamp receives 2, 16, and 56 J of energy, respectively.

The first step in initiating a flash is ionising the gas, followed by passing a very strong pulse of current through the ionised gas. In order for a pulse with a current of thousands of amperes to pass through the tube, the electrical resistance of the gas must be reduced through Ionisation. This current pulse stimulates the electrons around the xenon atoms as it passes through the tube, causing them to leap to excited energy levels. The instantaneous return of the electrons to a lower orbit results in the production of photons. So electrical energy has been transformed into light energy.

The pH and TSS (total soluble solids) of the coconut neera samples were measured using a digital pH meter (Eutech pH 700, accuracy: 0.01) and a handheld °Brix refractometer (Erma Inc., Japan). The turbidity of the coconut sap was determined using a digital turbidity meter (model 335; range: 0 to 1000 NTU; 0.01 NTU resolution; accuracy: $\pm 3\%$ FS, ± 1 digit). The sample to be tested was kept inside the test tube and a light source (IR LED) was focussed on it. The photodiode is the detector that detects the light reflected at a right angle to the focussed light by the suspension in the solution, amplifies it and displays the value on a 3 1/2 digit LED. The phenol–sulfuric acid method was used to measure the total sugars in the coconut neera, and the Nelson–Somogyi method was used to assess the reducing sugar before and after treatment (Ranganna 1986). The measurement was done using a UV spectrophotometer (UV-1800, SHIMADZU). The colour value of fresh and treated neera samples was assessed using a handheld imaging spectroradiometer with viewing stand (Lovibond LC 100 SV 100 Kit). The colour values are measured by the instrument

Fig. 1 Schematic diagram for the experimental setup of pulsed light treatment system



in terms of L^* (lightness to darkness), a^* (redness to greenness), and b^* (yellowness to blueness). Colour difference (ΔE), Chroma (C) and Hue (h) angles were calculated by the following formula:

$$\Delta E = \sqrt{(L^*_{untreated} - L^*_{treated})^2 + (a^*_{untreated} - a^*_{treated})^2 + (b^*_{untreated} - b^*_{treated})^2} \tag{1}$$

$$\text{Chroma}(C) = \sqrt{a^{*2} + b^{*2}} \tag{2}$$

$$\text{Hue}(h^\circ) = \tan^{-1} \frac{b^*}{a^*} \tag{3}$$

Microbiological Analysis

The microbiological analysis was carried out on treated and untreated Neera samples. The media employed for enumeration studies were plate count agar medium for total plate count and Sabouraud’s dextrose agar medium for yeast. Using the standard spread plate procedure, the PL-treated samples were serially diluted, and 10^{-5} and 10^{-6} dilutions were plated in triplicate on the necessary medium and incubated at 30 °C for 24 to 48 h. The results are represented as colony-forming units (log cfu/ml) (Preetha et al. 2022).

Statistical Analysis

The response surface methodology (RSM) was adopted to optimise the process variables of pulsed light treatment. The effect of independent variables on the responses, such as microbial load and biochemical parameters of coconut sap was analysed by Box–Behnken Design (BBD) using the statistical software Design Expert version 13.0.5.0 (Stat-Ease Inc., Minneapolis, MN, USA). The ranges of independent variables for the design are shown in Table 1. The selection of the model was based on a number of statistical factors such as R^2 value, p value, lack of fit and F-value. ANOVA was used to assess the statistical significance of each term in the polynomial functions.

Table 1 Level of Independent variable for BBD design

Factor	Name	Units	Level of independent variables	
			Minimum	Maximum
A	Voltage	V	500	1500
B	Time	S	60	180
C	Distance from the lamp	mm	50	150

Results and Discussion

Effect of Pulsed Light Treatment on pH and TSS of Coconut Inflorescence Sap

The pH of fresh coconut neera was found to be 6.48 ± 0.15 and total soluble solids (TSS) was $15.52 \pm 0.15^\circ$ brix after harvesting. At various process parameters of PL, coconut sap was treated and presented in Table 2. Which infers that the pH and TSS of the PL-treated sap did not alter significantly ($p > 0.05$) (Table 3). The pH was unchanged when the sap was treated at a low voltage of 500 V, a 100 mm distance, and an exposure time of 60 s (Table 2). Negligible pH changes were found in lime juice treated with different UV dosages (Mohd-Hanif et al. 2016). Orange juice treated to a UV–C energy level of 12.3–147.6 mJ/cm^2 by Tran and Farid (2004) showed no variation in pH. According to Gouma et al. (2015) and Noci et al. (2008), apple juice treated with UV–C did not change pH. Shamsudin et al. (2014) also reported that the pH of UV-treated ($10.76 \text{ mJ}/\text{cm}^2$) and untreated pineapple juice does not differ noticeably.

It was noticed that the TSS content of the coconut sap remained constant throughout the treatment (Table 2). The current research findings are consistent with those of Noci et al. (2008), Falguera (2011) for apple juice ($3.88 \times 10^{-7} \text{ E min}^{-1}$ incident energy) and Pala and Toklucu (2013) for orange juice (12.03 to 48.12 kJ/L). The apple juice treated with pulsed UV light treatments at various energy doses ($1850 \text{ mJ}/\text{cm}^2$ to $3354 \text{ mJ}/\text{cm}^2$) also showed no discernible variations in the TSS content (Kasahara et al. 2004).

Effect of Pulsed Light Treatment on Colour Profile of Sap

Colour is an important factor that determine consumer acceptance. Figure 2 displays photos of the sap before and after PL treatment. Colour parameters including L^* , a^* , b^* , chroma, hue angle (h°) and to assess the effects of non-thermal processing, total colour differences (ΔE) were examined. The lightness (L^*) value indicates brightness of the colour. L^* value of fresh sap was 61.5, whereas there was a modest drop in L^* value of treated sap when the voltage increased from 500 to 1500 V, the exposure time increased from 60 to 180 s, and the distance from the lamp decreased from 150 to 50 mm. A similar drop in L^* upon PL was reported in verjuice (Kaya et al. 2020), lime juice (Mohd-Hanif et al. 2016) and apple juice (Caminiti et al. 2011).

The a^* value denotes greenness ($-a^*$) and redness ($+a^*$); whereas, the b^* values denote blueness ($-b^*$) and yellowness ($+b^*$). In contrast to PL-treated sap, the a^* values decreases and b^* values slightly increases. The a^* and b^* values of fresh coconut sap was 3.3 and 11.4, respectively.

Table 2 Experimental design for optimizing the process parameters of PL-treated coconut sap and its response

Run	Factors			Responses							
	A: Voltage V	B: Distance from the lamp mm	C: Exposure Time s	pH	TSS	ΔE	Turbidity (NTU)	Total Sugars (%)	Reducing sugars (mg/100 ml)	Total plate count (log cfu/ml)	Yeast and mould (log cfu/ml)
1	1000	100	120	6.48	15	0.954	18.7	13.24	1.54	3.58	3.26
2	1000	150	60	6.45	15.2	0.981	17.7	13.32	3.13	3.69	3.37
3	500	100	60	6.48	15.2	0.944	18.5	13.29	3.15	4.16	4.05
4	1500	150	120	6.48	15.1	1.11	17.2	12.8	2.3	3.22	3.16
5	1000	100	120	6.43	14.5	0.94	24	12.95	1.6	3.46	3.31
6	1000	50	180	6.45	15	0.93	18.9	13.05	2.2	3.55	3.5
7	1000	100	120	6.46	15.1	0.955	18	13.33	1.55	3.61	3.29
8	500	50	120	6.42	14.8	0.96	22	12.96	3.26	4.11	3.81
9	1500	50	120	6.5	15.4	1.19	17.2	12.84	1.3	3.18	3.02
10	500	100	180	6.44	14.8	0.905	25.4	12.78	3.21	4.12	3.82
11	1500	100	60	6.51	15.2	1.21	17	12.5	2.28	3.25	3.13
12	1000	50	60	6.46	15.3	0.92	19	13.03	3.24	3.68	3.74
13	1000	150	180	6.47	15.2	0.948	17.9	13.35	3.09	3.55	3.47
14	1000	100	60	6.45	15	0.962	18.5	12.92	1.5	3.74	3.65
15	1500	100	180	6.49	15.3	1.22	16.8	12.66	2.29	3.39	3.31
16	1000	100	120	6.47	15.4	0.952	18.6	13.41	1.52	3.47	3.28
17	500	150	120	6.43	14.8	0.91	26	12.95	3.22	4.15	3.82

Table 3 ANOVA table indicating the significance of each factor on process parameters of PL-treated coconut sap on its responses

Factor	F-value							
	pH	TSS	Colour difference	Turbidity	Total sugars	Reducing sugars	Total plate count	Yeast and mould count
A (voltage)	1.95	1.96	16.32**	52.2**	37.9*	12.00*	542.5**	350.3**
B (time)	4.86	3.79	104.3**	14.39*	7.939*	12.4*	7.90*	76.84**
C (distance)	0.8923	0.7939	26.5*	7.40*	24.50*	9.671*	10.32*	9.08**
AB	–	–	0.183	–	0.004	8.156*	32.2*	7.35*
AC	–	–	0.490	–	2.44*	9.160*	32.2*	0.42*
BC	–	–	0.377	–	0.000	20.30**	0.221	0.167
A ²	–	–	39.71*	–	10.06*	10.06*	127.80**	28.37*
B ²	–	–	1.06	–	0.217	0.217	14.79**	5.19
C ²	–	–	0.323	–	0.089	0.089	14.79*	0.085
Lack of fit	0.9153 NS	0.2493 NS	0.249 NS	0.686 NS	0.820 NS	0.26 NS	0.8586 NS	0.927 NS
R ²	0.8917	0.8512	0.9545	0.9020	0.8398	0.8980	0.9903	0.9856
Adj. R ²	0.8626	0.8103	0.8960	0.8834	0.8189	0.8269	0.9777	0.9671

NS Non significant, ** significant at 0.001, * significant at 0.05

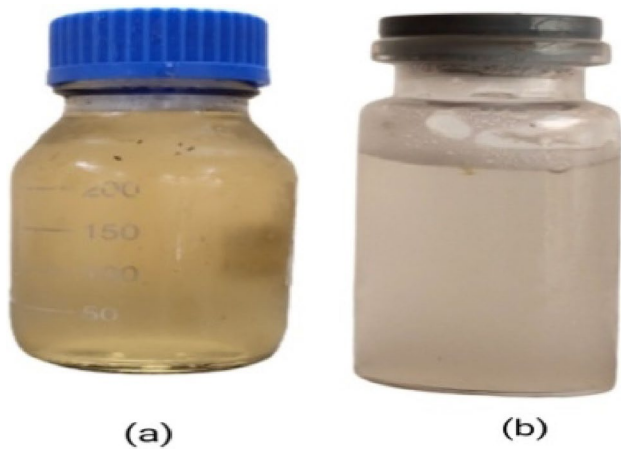


Fig. 2 Images of coconut inflorescence sap (a) before PL treatment and (b) after PL treatment at optimised process parameters (1000 V, 60 s and 100 mm)

A similar trend of decrease in a^* and increase in b^* was seen in Mohd-Hanif et al. (2016) who stated that the amino acids in lime juice underwent Maillard reactions with reducing carbohydrates like glucose and fructose, which resulted in browning and colour changes. Furthermore, carotenoids isomerization and interactions with free radicals produced by irradiation could both contribute to colour deterioration.

The chroma (c^*) is given as colour intensity. A grey tone or shade was indicated by a low Chroma value. After PL treatment, the c^* value of the sap increased. A comparable increase in c^* values was seen in PL-treated verjuice (Kaya et al. 2020). The hue angle ($^\circ$) was given in the specific red, blue, yellow or green colour. The hue angle of fresh sap was found to be 73.50° whereas PL-treated sap with voltages of

500 V, 1000 V, and 1500 V showed values of 75.60° , 760° , and 76.70° , respectively.

There was a significant difference ($p < 0.05$) in colour (ΔE) for the samples before and after PL treatment, which lies between 0.5 and 1.5 (Table 3). Total colour difference values (ΔE) were divided into five categories by Cserhalmi et al. (2006): undetectable (0–0.5), visible (1.5–3.00), mildly visible (0.5–1.5), obviously detectable (1.5–6.0), and highly detectable (6.0–12.0). The ΔE of the PL-treated coconut sap belongs to the category of “mildly noticeable” (Table 2). Consumers reported that $\Delta E \leq 3$ is not recognisable (Patras et al. 2009). Similar findings were given by Vollmer et al. (2020).

Effect of Pulsed Light Treatment on Turbidity of Coconut Inflorescence Sap

The turbidity of untreated coconut neera was observed to be 32.5 ± 0.11 NTU. When the input voltage was increased, the turbidity of the PL-treated sap was significantly ($p < 0.05$) reduced and the turbidity value found at the optimised condition was 18.7 ± 0.24 NTU (Table 2). The reduction of maximum turbidity after pulsed light treatment was 30% at 1500 V for 120 s with a 50 mm distance, while the minimum of 20.3% was recorded at 500 V for 120 s at a distance of 150 mm (Fig. 3 and Table 2). This drop in turbidity may be caused by a shift in colour pigments brought on by UV-C exposure (Kaya et al. 2016). The absorptivity value was affected by the existence of absorbing particles in the UV spectrum, which reduces the effectiveness of the disinfection procedure. Additionally, the photon makes contact with the target cells in UV-C treatment as a result (Gomez-Lopez

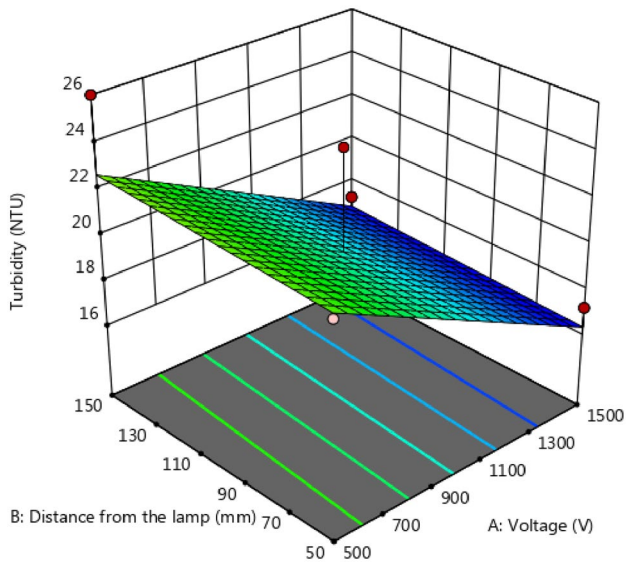


Fig. 3 Effect of pulsed light applied voltage and distance from the lamp on turbidity

et al. 2007). The results are similar to the study conducted by Mohd-Hanif et al. (2016) who found a comparable drop in turbidity in lime juice with higher UV dosages and in apple cider (Donahue et al. 2004).

Effect of Pulsed Light Treatment on the Total Sugars and Reducing Sugars of Coconut Sap

The untreated coconut sap contains $13.420 \pm 0.52\%$ of total sugar content and 1.52 ± 0.28 g/100 ml of reducing

sugar. After pulsed light treatment at 1000 V for 120 s at 100 mm distance, there was only a small decrease in total sugars (0.82%), whereas samples treated at a maximum voltage of 1500 V for 180 s at 50 mm distance showed a greater decrease in total sugar content (2.56%), as shown in Fig. 4 and Table 2. The total sugars significantly decreased with voltage and time due to sugar degradation ($p < 0.05$) when compared with the control (Table 3). Similar findings of a decline in the total sugar content of strawberry fruit following UV-C treatment were also reported by Pan et al. (2004).

In fresh coconut sap, sucrose was the non-reducing sugar that is abundant. With the action of invertase which produces lactic acid bacteria and yeast. The enzymatic hydrolysis of sucrose during fermentation results in the production of reducing sugars such as glucose and fructose (Shetty et al. 2017). It was noticed that the PL treatment parameters (voltage, time and distance) had a substantial ($p < 0.05$) rise in the reducing sugar of sap due to the breakdown of sucrose (Table 3). Both Manzoor et al. (2020) and Vidhya et al. (2018) noted that the ultrasonic treatment increased the formation of reducing sugars in tender coconut water due to hydrolyzation of the polysaccharides. It was recorded that the reducing sugar value was lowest at 1000 V for a brief exposure of 120 s at a distance of 100 mm (Fig. 4).

Effect of Pulsed Light Treatment on Microbial Load of Coconut Sap

The microbial load of coconut sap was examined in relation to input voltage, distance from the lamp and exposure period after pulsed light treatments. The key elements in microbial

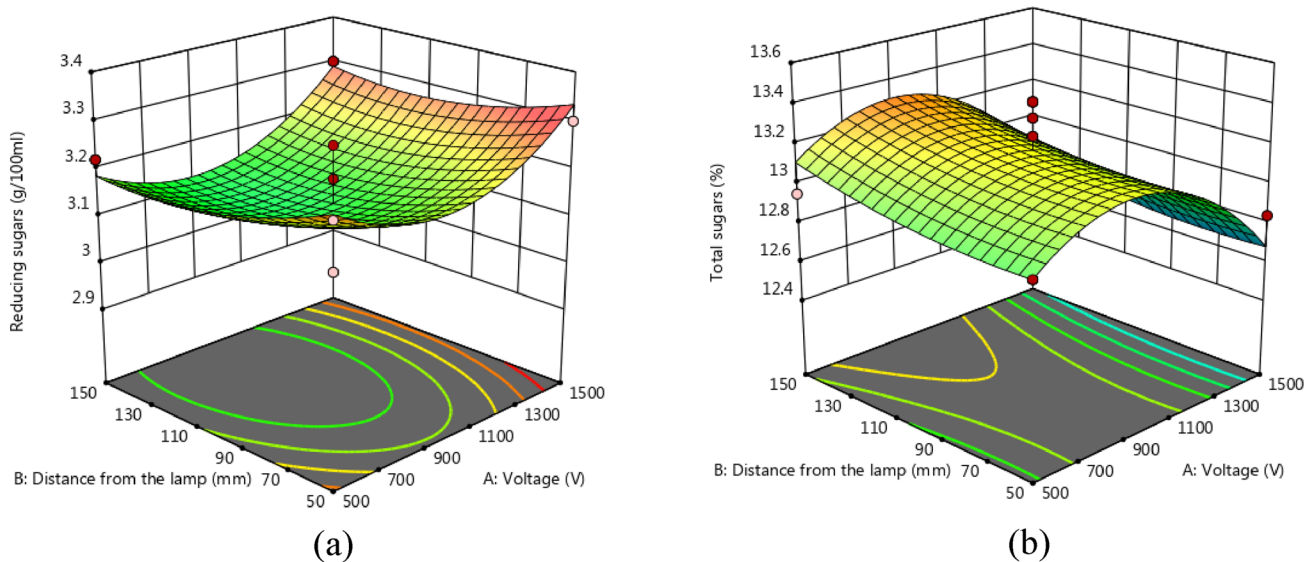


Fig. 4 Effect of pulsed light applied voltage and distance from the lamp on (a) reducing sugars and (b) total sugars of sap

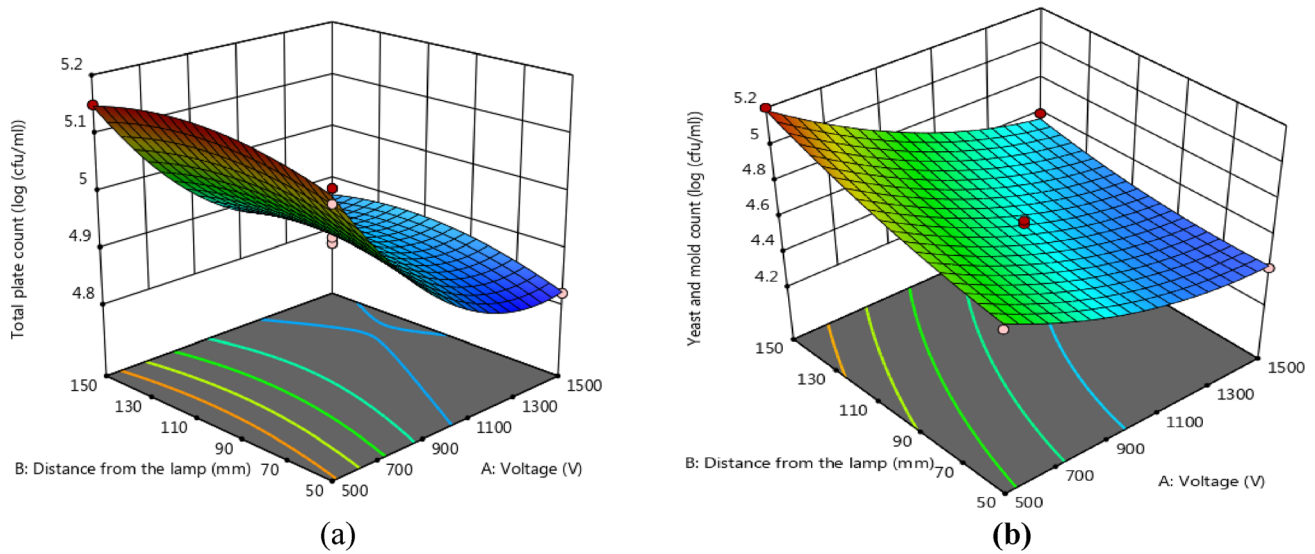


Fig. 5 Effect of pulsed light applied voltage and distance from the lamp on (a) Total plate count and (b) Yeast and mould count of sap

inactivation are the input voltage given to the lamp and the exposure period (Maftei et al. 2014). With increased voltage and treatment time, a progressive rise in microbial inactivation efficiency was seen. The control coconut sap had a total plate count of 7.18 ± 0.21 log (cfu/ml). The pulsed light treatment significantly slowed down ($p < 0.05$) the microbial populations (Table 3). The maximum reduction in total plate count of 4.0 log (cfu/ml) was achieved at the treatment combination of 1500 V, 180 s and 50 mm; whereas, the minimum reduction of 3.01 log (cfu/ml) was seen at 500 V, 60 s and 150 mm.

Figure 5a shows that the difference between the distance from the lamp and voltage was statistically influenced by microbial inactivation ($p < 0.05$). There was less log reduction at a distance of 150 mm because the light absorption of the sample decreases with distance from the lamp. At a distance of 50 mm, the rate of microbial inactivation was higher which might be because the sample was more exposed to light. Preetha et al. (2017) reported similar outcomes for tender coconut water treated with pulsed light.

The lethal activity of PL can be attributed to both photo-thermal and photochemical mechanisms. However, microscopic examination of *S. aureus* microbial cells revealed that the pulsing impact of the PL treatment had damaged the cellular structure (Krishnamurthy et al. 2008). This damage included cell wall damage, cytoplasmic membrane shrinkage, cellular content leakage and mesosome disintegration. The creation of pyrimidine dimers, particularly thymine dimers, is the principal cause of germicidal action by UV light on bacteria. The dimer prevents the creation of new DNA chains during cell replication, which causes infected microorganisms to be inactivated (unable to replicate, also known as clonogenic death) by UV light (Gomez-Lopez et al. 2007).

The yeast and mould count of fresh coconut sap was 6.10 log cfu/ml. When the pulsed light treated samples were examined for the presence of yeast and mould, it was found that the population was reduced significantly ($p < 0.05$) to 3.08 log cfu/ml at 1500 V voltage, 50 mm distance apart and time of 120 s. In the sap exposed to an input voltage of 500 V, 100 mm distance from the light, and 60 s exposure

Table 4 Final equation in terms of coded factors for the responses

Response	Regression model
pH	$+6.46 + 0.0263 A + 0.0012 B - 0.0062 C$
TSS	$+0.1750 + 15.08 A - 0.0250 B - 0.0750 C$
Turbidity	$+19.49 - 2.96 A + 0.2125 B + 0.8500 C$
Total sugars	$+13.17 - 0.1475 A + 0.0675 B - 0.0375 C - 0.0075 AB + 0.1675 AC + 0.0025 BC - 0.3313 A^2 + 0.0488 B^2 - 0.0313 C^2$
Reducing sugars	$+3.13 + 0.0412 A - 0.0325 B - 0.0012 C + 0.0100 AB - 0.0125 AC + 0.010 BC + 0.1047 A^2 + 0.0373 B^2 - 0.0003 C^2$
Colour difference	$+0.9526 + 0.1264 A - 0.0064 B - 0.0065 C - 0.0075 AB + 0.0122 AC - 0.0108 BC + 0.1074 A^2 - 0.0176 B^2 + 0.0097 C^2$
Total plate count	$+4.93 - 0.1450 A + 0.0175 B - 0.0200 C + 0.0050 AB + 0.0050 AC + 0.0050 BC + 0.0970 A^2 - 0.0330 B^2 - 0.0330 C^2$
Yeast and mould	$+4.61 - 0.2562 A + 0.1200 B - 0.0413 C + 0.0525 AB + 0.0050 AC + 0.0025 BC + 0.1005 A^2 - 0.0430 B^2 - 0.0055 C^2$

Table 5 Solution for optimization of factors and desirability analysis

S. No	Input voltage (V)	Treatment time (s)	Distance from the lamp (mm)	pH	TSS	Turbidity	ΔE	Total Sugar	Total plate count log (cfu/ml)	Yeast and mould count log (cfu/ml)	Reducing sugar	Desirability
Predicted data	1007.4	120	100	6.51	15.17	18.9	0.962	13.56	1.52	3.78	3.898	0.894
Experimental data	1000	120	100	6.48	15.14	18.7	0.954	13.48	1.54	3.61	3.31	
Error %				0.46	0.19	1.05	0.83	0.58	1.31	0.81	0.51	

period, a minimum yeast and mould count of 2.05 log cfu/ml was found in the sap. According to Tran and Earid (2004), yeast and mould were less vulnerable to UV radiation than bacteria because their DNA molecules create fewer pyrimidine bases, particularly thymine. Similar findings by Maftai et al. (2014) stated that by increasing the energy dose to 32 J/cm² and the exposure time, a mould reduction of 3.76 log cfu/ml was achieved. The response surface plots for the inactivation of yeast and mould in coconut sap with respect to process variables were depicted in Fig. 5b. These plots suggested that inactivation of yeast and mould increased significantly ($p < 0.05$) with increasing input voltage, exposure time and distance (Table 3).

A similar investigation on the pulsed light treatment of pineapple juice was studied by Vollmer et al. (2020). They noted a 5 log reduction in the yeast and mould counts as well as the aerobic mesophile count at the treatment parameters of 2.4 kV and either 94 or 187 pulses). Preetha et al. (2021) showed a higher reduction (5.33 log cfu/ml) in the microbial population of tender coconut water at a fluence of 95 J/cm². *E. coli* ATCC 25922 and *E. coli* O157:H7 were found to have decreased by 2.5–2.7 log in commercially clarified apple cider (Sauer and Moraru 2009). In apple juice, Ferrario et al. (2013) discovered a 2.9 log reduction in *E. coli*.

Optimization of Process Parameters

It is possible to predict the response for each factor using the equation given as coded factors. The coded equation can be used to assess the impact of an independent factor on response, as displayed in Table 4, by comparing the factor coefficients. The suitability of the model was evaluated with regard to the following factors: R^2 (the coefficient of determination), adjusted R^2 , p value and lack of fit. The best model to depict the relationship between the factors and response is the quadratic model, which has high R^2 (coefficient of determination) values and a p value less than 0.05, suggesting model terms are significant (Table 3). From the ANOVA, it was revealed that experimental data could be well represented with a linear and second-quadratic polynomial model, with coefficient of determination (R^2) values for total sugars, reducing sugars, colour difference (ΔE) and microbial load being 0.8159, 0.8160, 0.9585 and 0.99, respectively. The results suggested that the regression model could fit the dependent variables considerably and the error analysis indicated that the lack of fit was insignificant for these dependent variables.

The result of RSM-based numerical optimisation yielded a desirability of 0.894. The targets chosen for optimisation were turbidity, reducing sugar, colour difference, total plate count and yeast count, which were set to minimum values while pH, TSS and total sugars were set to maximum levels. Each independent variable received a score of “3” for

importance. Coconut sap was found to perform best in optimum conditions with a voltage of 1000 V, a 100 mm distance and a time of 120 s, with the least amount of change in its quality parameters. Table 5 lists these ideal solutions and their responses. The experimental value deviated from the predicted value by only < 2% (Table 5).

Conclusion

Assessment of pulsed light treatment for the preservation of coconut inflorescence sap was studied and revealed that process parameters of Pulsed light (PL) significantly reduced the microbial population and maintained the quality parameter. The use of Box–Behnken Design (BBD) in the experiment revealed the optimum processing conditions to achieve maximum quality and a reduction in microbes. The optimum condition obtained was an input voltage of 1000 V for 120 s at a distance of 100 mm from the lamp. PL treatment could achieve a 3.57 log reduction in total plate count and a 2.79 log reduction in yeast and mould while preserving the safety of the coconut inflorescence sap. This method might serve as an alternative method for the thermal preservation process. The effect of combining non-thermal process technology on microbial inactivation should be investigated in a future study in order to achieve higher log reduction, which is crucial for food preservation.

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