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RESEARCH PAPER

Evaluation of Non-thermal Process for Decontamination of Orange Juice Using a Pulsed Light System

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Abstract

Non-thermal pulsed light treatments were given to orange juice for inactivating the *Escherichia coli* while retaining the quality characteristics. A laboratory model Pulsed Light Treatment chamber was designed. To evaluate, the different process parameters were selected viz., number of flashes (120,180 and 240 flashes), depth of the juice layer (5, 10, 15 and 20 mm) shelf height (5, 10 and 15 cm) and an inoculation level (10^7cfu/ml). *E.coli* inactivation of the cells increased when the number of pulses were increased. Higher reduction of bacteria (5.385 log reduction) and fungi (4.14 log reduction) were achieved with a thin layer of juice (5mm) and minimum shelf-height from the lamp (5cm). Quality Characteristics such as pH, TSS and colour value were not affected significantly after the Pulsed treatment however, colour of the orange juice had minor change (20 per cent) when applied with higher number of 240 flashes. The results obtained suggest that pulsed light treatment technology could become an alternative preservation method for orange juice.

Keywords: *Pulsed light, orange juice, non-thermal method, microbial inactivation, quality, process parameter*

Fresh fruit juices provide antioxidant, minerals and vitamins with an important role in the prevention of cardiovascular diseases, cancer, diabetes etc. (Mattews, 2006). Seasonality of fruit production however, restricts the availability of fresh fruit juices throughout the year thus, making it necessary to develop a technology making the products available to the consumers throughout the year.

Elimination of pathogenic micro-organisms is one of the fundamental aspects of fruit juice processing and is governed by CFR (Code of Federal Regulations) Title 21. The current revision of CFR - Title 21 (FDA, 2013) states in part that: "processors of juice products shall include in their Hazard Analysis and Critical Control Point (HACCP) plans control measures that will consistently produce, at a minimum, a 5 log (i.e., 10^5) reduction, for a period at least as long as the shelf-life of the product when stored under normal and moderate abuse conditions, in the pertinent microorganism. For the purposes of this regulation, the "pertinent microorganism" is the most resistant microorganism of public health significance that is likely to occur in the juice".

Citrus fruit such as orange have high nutritional value including bioactive compounds such as ascorbic acid, flavanons and carotenoids (Lopez-Gomez *et al.*, 2010). It is one of the preferred beverages among the consumers (Plaza *et al.*, 2006). Oranges are mainly processed into fruit juices and concentrate. Fresh orange juice must be processed to inactivate the micro-organism to extend its shelf-life. Food preservation is mainly done by the industry to inhibit the microbial growth (Manas and Pagan, 2005) and thermal technologies such as pasteurization and sterilization are widely employed for this purpose. However, these treatments have a negative effect

on certain components of the food viz., reducing its vitamin content and volatile flavour compounds, induce undesirable denaturation of protein and cause sensory changes making them less accepted in terms of color and textural properties most importantly. (Elmnaser *et al.*, 2008).

Non-thermal technologies as alternatives to thermal treatment are being developed to obtain a food product with better final sensory quality, without neglecting microbial safety (FDA/CFSAN report, 2000; Woodling and Moraru, 2005). Some of the non-thermal techniques developed so far to preserve food include Pulsed Electric Field Technology, High Pressure Processing Technology, Ultrasound Technology, Oscillating Magnetic fields and Ultra Violet light technology. High pressure processing requires long treatment time while pulsed electric field is a rapid process of inactivating vegetative cells such as *E.coli,* though it is unable to inactivate bacterial spores. The use of pulsed ultra violet (UV) light is well established for air, water treatment and surface decontamination but, its use for treating liquid foods is still limited. Compared to water, liquid foods have a range of optical and physical properties and diverse chemical composition affecting UV light transmittance (UVT), dose delivery and consequently, microbial inactivation.

Pulsed light (PL) is a technique used to decontaminate surfaces by killing microorganisms using short time pulses of an intense broad spectrum, rich in UVC light. The emitted light flash has a high peak power and consists of wavelengths ranging from 200 to 1100 nm (Dunn *et al*., 1997) which includes Ultraviolet light, broad spectrum white light and near infrared light (Green *et al*., 2005). The technique used to produce flashes with high peak power generates a greater relative production of light with shorter bactericidal wavelengths (MacGregor *et al*., 1998). The aim of the research was to design a system with potential applications by the food processing industry to satisfy the consumer demand for 'wholesome, fresh like foods'. The effectiveness of the technology was assessed by determining microbial load reduction and change in pH, TSS, colour of the PL treated juices and the results are reported in this paper.

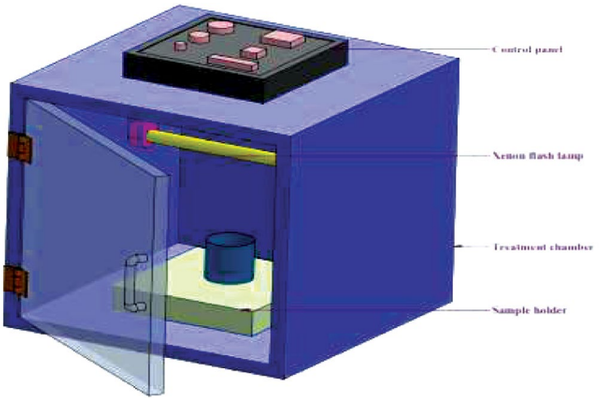

Fig. 1: Experimental Apparatus - Laboratory model of Pulsed light treatment apparatus

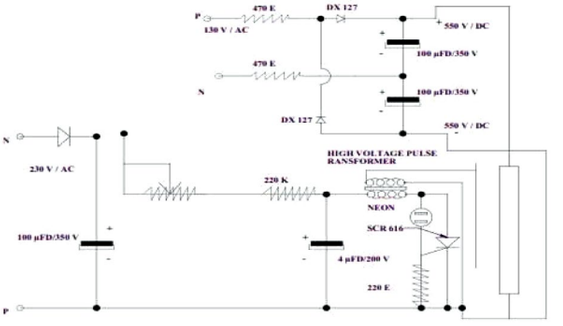

Fig. 2: Circuit Diagram for Pulsed Light

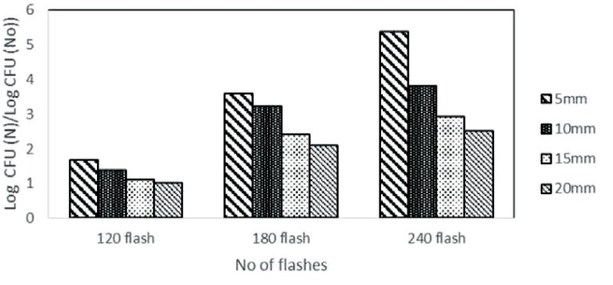

Fig. 3: Effect of layer depth(mm) on bacterial inactivation at a distance of 5cm from the lamp

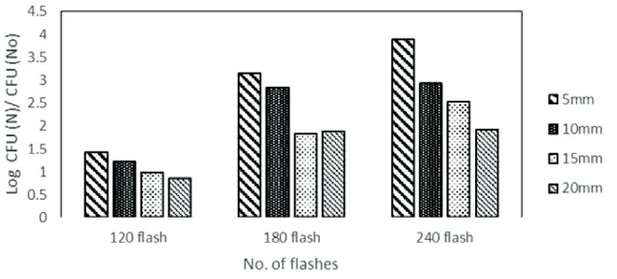

Fig. 4: Effect of layer depth (mm) on bacterial inactivation at a distance of 10 cm from the lamp

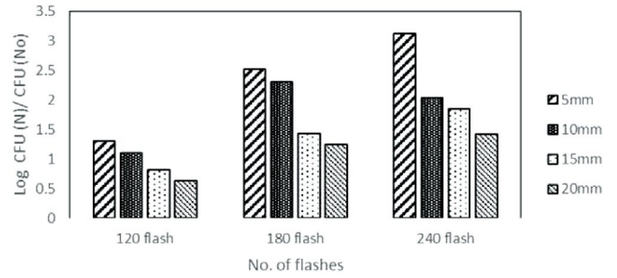

Fig. 5: Effect of layer depth (mm) on bacterial inactivation at a distance of 15 cm from the lamp

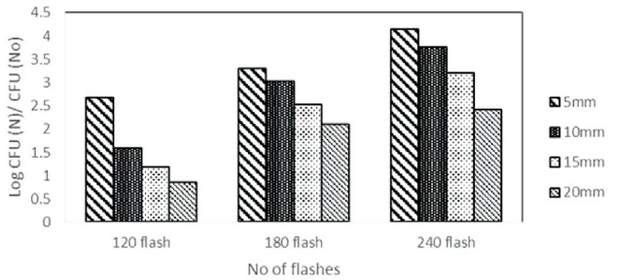

Fig. 6: Effect of layer depth (mm) on Yeast and mold inactivation at a distance of 5 cm from the lamp

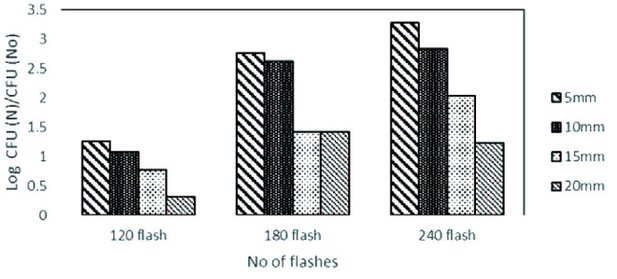

Fig. 7: Effect of layer depth (mm) on Yeast and mold inactivation at a distance of 10 cm from the lamp

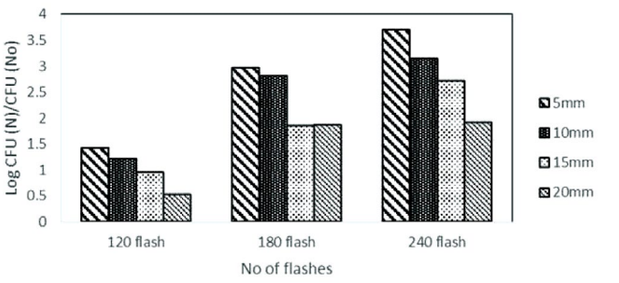

Fig. 8: Effect of layer depth (mm) on Yeast and mold inactivation at a distance of 15 cm from the lamp

Materials and Methods

Laboratory model of Pulsed light treatment apparatus

The treatment chamber was made of 12 mm thick plywood having a dimension of 30×30×30cm. Since the circuit involved high DC voltage for triggering and flashing, plywood was preferred as it is an insulating material. The inner surface of the box were covered with aluminium foil to ensure maximum incidence of light on the juice by reflection. The experimental setup included a xenon flash lamp (Make: Heraeus Noblelight Ltd., U.K) fixed to the bottom of the top face on a PC board through clamps (Figure 1). The Xenon flash lamp which had a bore diameter of 3mm and arc length of 75mm, with a clear flushed quartz envelope was used to produce the broad spectrum flash.

Pulsed light Circuit

The circuit for pulsed light treatment (Fig. 2) was designed with a lamp flash frequency of 20 flashes per minute and a flash duration of 40 microseconds. The circuit is divided into two major parts namely, the triggering supply and the flashing supply. The flashing supply is switched on first so that the capacitor got charged and discharged the voltage through resistor and the voltage reached the electrodes of the flash lamp. Then, the trigger supply was switched on so that the high trigger voltage was discharged to the trigger electrode, resulting in subsequent flashing of the lamp. The trigger supply frequency

was adjustable by changing the potentiometer, but it was maintained at the maximum level of (20 Hz), to maintain the flash frequency constant at 20 flashes per minute.

Orange Juice

The oranges were cut in to two halves with a sharp stainless steel sterile knife and were hand pressed against a revolving burr (M/S. Kenstar food processor, Kitchen appliances India Ltd., India.). Seed and Pulp were collected in the mesh and juice with coarse tissues was collected in the bottom vessel. The juice was then, filtered using a muslin cloth to remove coarse tissues. The filtered juice was collected in a sterile reagent bottle (250ml).

Inoculation of *Escherichia coli* culture

Escherichia coli culture (obtained from MTCC) was grown on nutrient agar (NA) broth at 37°C for 24 h reaching a stationery phase count of 10^9 cfu/ml. Cells collected by centrifuging (Rotor no. A4-44 producing a relative centrifugal force 10,375) at room temperature and rinsing with 0.1% peptone prior to inoculation in to juices at an initial level count of approximately at 10^6 to 10^7 cfu/ml. A working culture was prepared by aseptically transferring a colony from nutrient agar plates in to fresh nutrient agar broth that was again incubated at 37°C for 24 h. For inoculation into orange juice at final concentration of $\leq 10^7$cfu/ml culture was added directly (Ngadi *et al.*, 2003). The microbial count was expressed as colony forming unit (CFU/ml).

Enumeration of Yeast and mold by Plate count Method

The yeast and mold counts was observed for the quality of juice. 1 ml of juice sample was taken and pipetted into a test tube containing 9 ml of sterile water. The test tubes were shaken well for 10-15 minutes for uniform distribution of microbial cell in the water blank. This will give a dilution of 1:10 (10^{-1}). One ml from (10^{-1}) dilution was transferred to 9 ml of sterile water with a sterile one ml pipette, which gave a dilution of 10^{-2}. The process was repeated up to 10^{-3} dilutions with the serial transfer of the diluents. One ml aliquots from 10^{-1} and 10^{-3} dilutions were transferred to the sterile petri dishes for the enumeration of yeast. Three replications were maintained for calculating the population as a mean of three replications.

Approximately, 15-20 ml of molten and cooled (45ºC) Rose Bengal agar medium was added to each petri dish and the plates were rotated in clockwise and anticlockwise direction for though mixing of the diluents and the medium. The plates were then incubated at room temperature for three days for yeast and mold, respectively. After the incubation period, the colonies were counted and the number of organisms (total yeast and mold) per gram of sample was calculated by using the formula given below.

Number of colony forming units (cfu) per gram of the sample:

$$= \frac{\text{Mean number of cfu} \times \text{Dilution factor}}{\text{Quantity of sample on weight basis}} \qquad \ldots(1)$$

Pulsed light treatment of orange juice

The fresh orange juice was autoclaved at 121ºC at 1.5 bar for 15 minutes. The sterilized juice sample was inoculated with *E.coli* cells of 10^6 to 10^7 cfu/ml and placed inside the treatment chamber in a petri dish (100 mm diameter) and the door was closed. The samples were exposed to receive 0.08 J/cm^2 per pulse. The treatment of 60, 120, 180, 240 flashes thus resulting in an overall fluence ranging from 4.8, 9.6, 14.4 and 19.2J/cm^2. The treated samples were covered carefully and transferred to aseptic laminar air flow chamber where they were bottled in glass bottles (100ml) for shelf-life study. The treated samples were stored at 8±2°C to determine the shelf-life.

Estimation of Quality characteristics

The total soluble solids was determined using a digital hand held refractometer (Atago co., Ltd., Tokyo, Japan) and the total soluble solid content was expressed as °Brix at 20°C (AOAC, 1980). The pH of juice was measured using digital pH meter (Cyber scan, India) at 25°C. The Colour of juice was

Table 1: Influence of process parameter (Shelf-height, No of flashes, Depth of the juice) on pH, TSS and Colour Value

Shelf-height	Flashes	Depth of the juice	pH	TSS	Colour
5cm	120	5mm	3.74±0.02	10.2±0.1	0.44±0.02
		10mm	3.74±0.02	10.2±0.1	0.52±0.03
		15mm	3.75±0.01	10.2±0.1	0.58±0.02
		20mm	3.76±0.01	10.3±0.1	0.62±0.02
	180	5mm	3.73±0.03	10.2±0.1	0.46±0.01
		10mm	3.73±0.02	10.3±0.1	0.54±0.02
		15mm	3.74±0.01	10.3±0.1	0.6±0.03
		20mm	3.75±0.01	10.3±0.1	0.64±0.01
	240	5mm	3.72±0.02	10.3±0.1	0.48±0.02
		10mm	3.72±0.02	10.3±0.1	0.56±0.01
		15mm	3.73±0.02	10.3±0.1	0.64±0.02
		20mm	3.74±0.01	10.3±0.1	0.7±0.01
10cm	120	5mm	3.74±0.02	10.3±0.1	0.13±0.02
		10mm	3.74±0.02	10.3±0.1	0.24±0.02
		15mm	3.74±0.01	10.3±0.1	0.36±0.01
		20mm	3.75±0.01	10.4±0.1	0.4±0.02
	180	5mm	3.74±0.02	10.3±0.1	0.16±0.01
		10mm	3.72±0.03	10.2±0.1	0.2±0.02
		15mm	3.73±0.01	10.2±0.1	0.32±0.01
		20mm	3.75±0.01	10.3±0.1	0.43±0.02
	240	5mm	3.73±0.01	10.2±0.1	0.36±0.01
		10mm	3.73±0.01	10.3±0.1	0.44±0.01
		15mm	3.73±0.01	10.3±0.1	0.48±0.01
		20mm	3.74±0.01	10.2±0.1	0.52±0.02
15cm	120	5mm	3.75±0.01	10.2±0.1	0.4±0.02
		10mm	3.74±0.01	10.2±0.1	0.46±0.02
		15mm	3.74±0.02	10.2±0.1	0.54±0.01
		20mm	3.74±0.02	10.3±0.1	0.62±0.01
	180	5mm	3.74±0.01	10.3±0.1	0.52±0.01
		10mm	3.73±0.01	10.3±0.1	0.56±0.02
		15mm	3.73±0.01	10.3±0.1	0.68±0.01
		20mm	3.74±0.01	10.3±0.1	0.74±0.01
	240	5mm	3.74±0.01	10.3±0.1	0.48±0.02
		10mm	3.73±0.01	10.3±0.1	0.52±0.01
		15mm	3.74±0.01	10.3±0.1	0.56±0.01
		20mm	3.75±0.01	10.3±0.1	0.6±0.02

	pH			TSS			Colour		
	SED	**CD(0.05)**	**CD(0.01)**	**SED**	**CD(0.05)**	**CD(0.01)**	**SED**	**CD(0.05)**	**CD(0.01)**
S	0.00313	0.00625	0.00829	0.02222	0.0443	0.0588	0.0338	0.00674	0.00894
F	0.00313	0.00625	0.00829	0.02222	0.0443	0.0588	0.0338	0.00674	0.00894
D	0.00362	0.00722	0.00958	0.02566	0.05115	0.6789	0.0039	0.00778	0.01032
SF	0.00543	0.01082	0.01436	0.03849	0.07673	0.10184	0.0058	0.00167	0.01549
FD	0.00627	0.0125	0.01659	0.04444	0.0886	0.11759	0.00676	0.01347	0.01788
SD	0.00627	0.0125	0.01659	0.04444	0.0886	0.11759	0.00676	0.01347	0.01788
SFD	0.01086	0.02165	0.02873	0.07698	0.15346	0.20368	0.01171	0.02334	0.03097

measured using a Hunter lab colour flux meter (Hunter Associate Laboratory, Inc., Reston) which provides colour values in the terms of L, a, and b values, where L indicates whiteness to darkness, a (+) redness, a (-) greenness, b(+) yellowness and b (-) blueness.

$$\Delta E = (\Delta a^2 + \Delta b^2 + \Delta L^2)^{1/2} \quad (1)$$

$\Delta L = L_{Sample} - L_{Standard}$ +ΔL - sample is lighter than standard
-ΔL - sample is darker than standard

$\Delta a = a_{Sample} - a_{Standard}$ +Δa - sample is redder than standard
-Δa - sample is greener than standard

$\Delta b = b_{Sample} - b_{Standard}$ +Δb - sample is yellower than standard
-Δb - sample is bluer than standard

Statistical Analysis

All the analysis were carried out in triplicate. The results are expressed as mean values and standard deviation (SD). The optimisation of number of flashes to the sample 120, 180 and 240 flashes, shelf-height from the lamp- 5, 10 and 15cm from the lamp, juice depth of the juice - 5, 10, 15 and 20mm for the Pulsed light treatment process was done using the Factorial Completely Randomized Design (FCRD) and were analysed using analysis of variance (ANOVA), followed by Least Significant Difference (LSD) Test with p=0.05. This treatment was carried out using AGRESS software version 7.01.

Results and Discussion

Effect of juice depth on *E.coli* reduction

The effect of the depth of the juice showed a significant differences ($p \leq 0.01$) on microbial inactivation (Figure 3, 4&5) between the treatments (no of flashes× depth of the juice× shelf height). As expected, the microbial reduction decreased when juice depth was increased. A 5mm juice depth resulted in a higher microbial reduction when compared to 10 and 15mm for the shelf-height and number of flashes included in the study. This may be due to the absorption of ultraviolet energy (which is a major part of the pulsed light energy) in the top layer of the liquid when higher thickness of the liquid are treated. However, the highest inactivation level, 5.4 log reduction was observed for a 5mm juice depth treated with a 240 flash, the minimum distance 5cm distance from the lamp. Similar trend of results were obtained by Hillegas and Demirci (2003) when treated clover honey at different light pulse treatment.

Effect of shelf-height on *E.Coli* reduction

Shelf-height to expose juice samples at 5, 10 and 15 cm (distance from the lamp) showed significant ($p \leq 0.01$) effect on microbial inactivation for the juice depth and number of flashes included in this study (Figure 3, 4&5). Increasing the distance of the sample from the lamp decreased the microbial inactivation. At a shelf-height of 15cm, juice thickness of 20 mm exposure to

240 flashes caused only a 1.4 log reduction in bacterial counts. Lower microbial population reductions at greater shelf heights reflected an increased energy dissipation as the light pulsed travelled from its source to the juice samples (Sharma and Demirci, 2003).

Effect of number of flashes on *E.coli* reduction

The number of flashes also had significant ($p \leq 0.01$) effect on microbial count reduction. Higher number of flashes resulted in more product exposure to light and hence increased the microbial reduction as observed earlier, also (Hillegas and Demirci, 2003). At the same sample depth (5 mm) and shelf height (5 cm), 240 flashes resulted in a maximum reduction followed by 180 flashes (3.6 log) while a lower (1.4 log) bacteria reduction was observed after 120 flashes, at the highest juice depth (20mm) and the highest distance (15cm) from the lamp (Figure 3,4&5).

Yeast and Mold Reduction

Yeast and Mold Counts were observed for the quality of orange juice. Reduction of yeast and mold population in PL treated juice was smaller than for bacteria but significant differences ($p \leq 0.01$) were observed between the treatments (Figure 6,7&8). The reduction of yeast and mold population for 240 flashes, 5mm juice depth and 5cm shelf-height was 4.14 log. Hansen (1976) observed that molds are more resistant to UV radiation because of their large sizes than bacteria. The different inactivation is probably due to the absorption of UV light by the DNA molecule, causing the cross linking between neighbouring pyrimidine nucleosides in the same DNA strand. Montgomery (1985) has demonstrated that the chemical composition of the cell wall and its thickness ultimately determines the relative UV resistance of an organism.

Effect of PL treatment on quality characteristics

Determinations of juice pH and TSS (Table 1) showed no major difference between, treatments ($p \leq 0.05$). Orange juice had a pH of 3.76 and TSS of about 10.3. PL treatments with different number of flashes, shelf-height and orange juice depth did not cause any significant ($p \leq 0.05$) changes in pH and TSS values in which is agreement with the results reported by Mafeti *et al.,* (2014) for apple juice. In a accordance with our results, Noci *et al.,* (2008) have also observed no effect on pH and TSS values of apple juice treated by UV light.

Samples exposed to different treatments did not have significant colour changes (Table 1) in agreement with the results reported by Maftei, *et al.,* 2014 for apple juice and by Choi *et al.*, 2002 and Francis and Clydesdale (1975) for orange juice. These authors considered DE greater than 2 would result into a noticeable visual difference when compared with untreated samples. Treating juice at the highest number of flashes 240 did not cause a major colour modification. As indicated by the colour change, DE values ranged from 0.2 to 1.2. The lesser ΔE values implies that the more likeliness of the sample towards the standard (fresh juice).

Conclusion

Lethality of the *E.coli* in orange juice depends on the number of flashes, shelf-height and depth of the sample. All factors play a major role in *E.coli* inactivation. Microbial decimal reduction increased when number of flashes are increased. Juice quality parameters, including pH, TSS and colour did not however, change after exposure to the PL treatments included in this study. It can thus, be concluded that pulsed light could be an alternative to a thermal treatment for controlling *E.coli* in orange juice if conditions achieving five decimal reductions could be identified. Orange juice was found to be safe for human consumption. This study has been done only with *E.coli* population, other organisms also to be the considered and further research should be taken up.

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