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Effect of blue led light on Physico-Chemical properties of pomegranate juice

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Abstract
Pomegranate fruit and juice product has been known for its incredible wellbeing and wholesome perspective. It has been broadly utilised by various industry for the creation of juice, jellies and beautifiers. The investigation aimed decide the Physico-chemical changes happening in pomegranate organic product juice when exposed to LED light. LED's can protect the nutritive estimations of natural products, helps in the development of a new method for future and lessens the microbial check. Handling boundaries of 25 °C with a differing distance of 2cm, 5cm and 8cm was contemplated. The outcomes demonstrated a huge expansion in the total phenol activity for a 2cm distance test with an increment of 132%. Flavonoid content demonstrated a spike in action by 130%. Colour, pH and Brix indicated a negligible impact on the nature of juice. Minor changes in cell reinforcement movement were noted. Ascorbic acid increment with a test set at 5cm demonstrating the most noteworthy movement of 30.65 g/100ml of tartaric acid value. The results recommend that LED light's openness on food product juices can be utilised as a preparing boundary for handling and keeping up the quality.

Keywords: LED light, Pomegranate juice, Colour, Quality, Total phenol

Introduction
Pomegranate originates from the middle east or Himalayan region and is known as the oldest edible fruit. Pomegranate is rich in phenol and flavonoid content. These bioactive compounds present in pomegranate fruit and juice contribute to its antioxidant activity, which helps fight cardiovascular and cancer disease. Antioxidant activities present in pomegranate fruit and juice help maintain health and have grown interested in its large consumption as fruit and as freshly extracted fruit juice (Anahita, Asmah, and Fauziah 2015) [3] (Opara and Al-Ani 2010) [21].
The current consumer is moving in the direction of consuming fresh food or food that looks fresh such as minimally processed product. Minimally processed food allows consuming food which doesn't contain any traces of chemicals, pesticides and any other preservatives. Fruit and fruit juices are a great source of polyphenols, flavonoids, vitamin C and antioxidant compounds that play a major part in preventing heart and cancer disease (Aneja *et al.* 2014) [4].
Food industry generally provides maximum food safety by destroying microbes and extending shelf life. This is achieved by using heat as a medium to destroy microbes. Heat when applied to food to destroy microbes deteriorates the fruit quality also. To maintain the natural food quality, several new, novel and innovative techniques were developed such as High-Pressure Processing, Ultrasound Processing, Pulsed Electric field, Pulsed Light processing, Infrared heating, high Intensity LED Processing and addition of chemical preservatives to prolong the shelf life (Pushparaj 2019) (Hinds *et al.* 2019) [12] (Naik *et al.* 2020) [18]. With the trend in the current population, preference for fresh alike food products has remarkably increased without any addition of chemical preservative, and thus novel food preservation techniques (Ghate *et al.* 2013) [8] provide the solution for such market trend. Most of the techniques mentioned above are already well established and studied properly. Amongst them, LED processing remains still an unexplored area. Various properties that are beneficial to both users and industry, LED provides vast majorities of opportunities to explore in this field ranging from Food Safety, horticulture, post-harvest processing (Nájera *et al.* 2018) [19]. This makes LED one of the promising fields to explore and use it to our advantage at a safe level. LED doesn't produce heating effect over and within the product and provides a durable approach for developing new Non-Thermal technology for the microbial reduction (Keyser *et al.* 2008) [15].

LED can be termed as a non-thermal technology based on temperature as there is no increase in temperature within the system resulting in deterioration to the quality of food. LED emits lights ranging from 210nm to 750nm with 210nm to 400nm is regarded as UV-LED (Ultraviolet-LED) and further from 400nm (violet colour) to 750nm (red colour) comes under visible LED light range.

Therefore, the main aim of this study was to examine the physicochemical effect of Visible LED of 470nm on pomegranate juice. The research was done to assess product quality changes after exposure to blue LED light to blue LED light of wavelength 470nm (Akgün 2019) [1].

Materials and Methods

Procurement and juice extraction of the pomegranate fruit

The full ripened pomegranate fruits (Punica granatum L.) Were obtained from the local market of Thanjavur, Tamil Nadu in 2020 and used throughout this study. The freshly acquired pomegranate was thoroughly washed and was cut into pieces.

The edible aril seed containing juice was manually separated and crushed with adequate pressure by the hydraulic press to squeeze out the juice without crushing the seeds. By using cheesecloth to separate it from the seed, fresh juice was filtered. Immediately, fresh juice was stored for LED exposure and physicochemical study.

LED light Setup

The 4x6 matrix array style arrangement was built with a batch type LED setup. The 470 nm wavelength was selected based on literature studies, as it had the highest bactericidal effect.

The 470nm light was acquired with an arrangement of the 4x6 matrix array type. The light was 5 W at 470 nm and the current was 20 ma (Srimagal, Ramesh, and Sahu 2016) [29].

Analysis of Pomegranate Juice quality

Quality characteristics of extracted pomegranate juice were analysed before and after LED treatment. pH, colour, Brix value, titrable acidity was measured using digital pH meter, Hunter Lab colourimeter. Colour value (L, a, b) were measured using Hunter Lab colorimeter. Titrable acidity (% citric acid) was determined by the colorimetric method (Sadler and Murphy 2010) [25].

Total soluble solid (^{0}brix) value was measured using a pocket refractometer. Sample A, B, and C were named for distance of 2cm, 5cm, and 8cm respectively.

pH: A digital microprocessor pH meter was used to analyse the pH of the extracted juice.

Refractometer: A pocket refractometer was used to analyse the total soluble solids (TSS) present in extracted juice and expressed as % sucrose.

Titrable Acidity Titration (TA): Fruit juice sample titrable acidity was estimated using the method defined by (Singh and Pal 2008) [28]. Titrable acidity was found by titrating sample against 0.1 N NaOH solution by adding phenolphthalein as an indicator with the appearance of the pink colour of the sample as the end-point (Zaouay *et al.* 2012) [31].

$$\text{Total Titrable Acidity} = \frac{\text{titrate value} \times \text{acid factor}}{\text{ml of juice}} \times 100$$

Hunter Lab Colorimeter: The hunter lab colorimeter was used to measure the colour of fresh and LED treated pomegranate juice. Reading were noted in L*, a*, b*. The hunter colour lab was used to find the overall colour change of pomegranate juice after treatment (Nikitha and Natarajan 2020) [20].

Total soluble solids (TSS): TSS is an index of soluble sugar content in fruit including sugar content, carbohydrates, organic acids, proteins, fats and minerals and any kind of liquids. TSS shows a high positive correlation with sugar content and is generally accepted as an important quality trail. TSS (^{0}Brix) in juice samples was determined with a lab-scale refractometer at room temperature. Few drops of the sample were added to the sample holder and the lid was closed and the results were reported in degree Brix (Zaouay *et al.* 2012) [31].

Ascorbic acid: Ascorbic acid was evaluated by titration using 2,6-Dichlorophenolindophenol (DCPIP) method. The stock solution was prepared by adding 4% oxalic acid in 100ml distilled water and 100mg of ascorbic acid. Dye solution of sodium bicarbonate and DCPIP mix was prepared. Working standard was prepared by diluting 10 ml stock solution to 100ml with 4% oxalic acid to have the concentration of working solution as 100 µg/ml. 5g sample was taken into 100ml of 4% oxalic acid. 5ml of supernatant was taken from the above-made mix and was further diluted by adding 10ml oxalic acid (4%) and was titrated against the dye (V_2) (Rekha *et al.* 2012) [23, 24].

$$\text{Amount of Ascorbic acid (mg/100ml sample)} = \frac{0.5mg}{V1\ ml} \times \frac{V2}{5\ ml} \times \frac{100ml}{sample\ volume} \times 100$$

V_1 = amount of dye for working standard titration, ml

V_2 = amount of dye used for sample titration, ml

Flavonoid content: Flavonoid content is measured as mg quercetin equivalent (QE)/g of dried plant material. Quercetin was used to make the stock standard calibration curve. Aluminium chloride method was used for analyse the total flavonoid content. 1 ml sample was taken to which 150 µl $nano_3$ was added.

After 6 min 300 µl $alcl_3$ was added with another waiting time of 5 min. 1 ml of 0.1 N NaOH was added.

The mixture obtained was made up to 10ml with distilled water and the sample was read at 510nm (Hajimahmoodi *et al.* 2013) [11].

Total phenol Content: Total phenol content was calculated as gallic acid equivalent (GAE).

Folin-ciocalteu reagent was used in the process for the determination of total phenol content. 1ml of juice was diluted to 1000ml quantity with distilled water. 0.5 ml of folin-ciocalteu regent was added along with 1ml sodium carbonate solution (20%).

The sample obtained was incubated for 30min in dark and sample reading by taken at 660nm in UV-spectrophotometer

against blank. (Rekha *et al.* 2012) [23, 24] (Yashaswini and Venkatachalapathy 2021) [13].

Antioxidant activity

The antioxidant activity was expressed as a percentage decrease in absorption relative to power, equal to the percentage of scavenged DPPH (% DPPH) (Shekhar and Anju 2014) [26]. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging was tested with minor modifications according to (Yen and Chen 1995) [30] process. By adding 4mg of DPPH to 100ml of methanol, the DPPH solution was developed. The juice was extracted from the fruit and filtered with Whatman filter paper.

From the extracted filter, 0.1 ml was taken into the test tube and up to 2 ml of the volume was produced using methanol. The final volume was developed using DPPH solution up to 5 ml. The sample amount of makeup was then incubated. (Gil *et al.* 2000) [9, 10] (Amri and Hossain, 2018) [2].

$$\text{Antioxidant Activity} = \frac{(Absorbance\ of\ control - absorbance\ of\ sample)}{absorbance\ of\ control} \times 100$$

Result and Discussion

Fresh fruit juice was illuminated at 470nm LED light and temperature increase was monitored during illumination. Fruit juice surface temperature increased by 2.7, 2.1, 1.4 - degree Celcius for 2cm, 5cm and 8cm distance between light and fruit juice respectively. A similar trend was shown by (Kim, Bang, and Yuk 2017) [16]. Increase in temperature can be because of LED intensity and distance between LED light and fruit juices. (Ghate *et al.* 2016) [7] showed the same trend for illumination temperature and distance combination.

Changes in Brix value, pH and Titrable acidity: Brix value or total soluble solids (TSS) were found similar in all the treated sample with control sample showing Brix value of 16.189% with sample C with a minimal increase of 0.278 value. Sample A and B showed a Brix value of 16.267 and 16.433. pH value didn't change significantly with the control sample at pH of 3.92 with sample A showing pH of 4.01. Change in pH value was insignificant in terms of taste and flavour.

Total titrable acidity (grams of tartaric acid per 100 ml) was observed with minimal change, ranging from 4.89 to 5.05. Highest was noticed in sample A and C followed by control and then sample B. Sample A and C showed titrable acidity of 5.05. Control sample had the acidity of 4.92 and sample B with the acidity of 4.89.

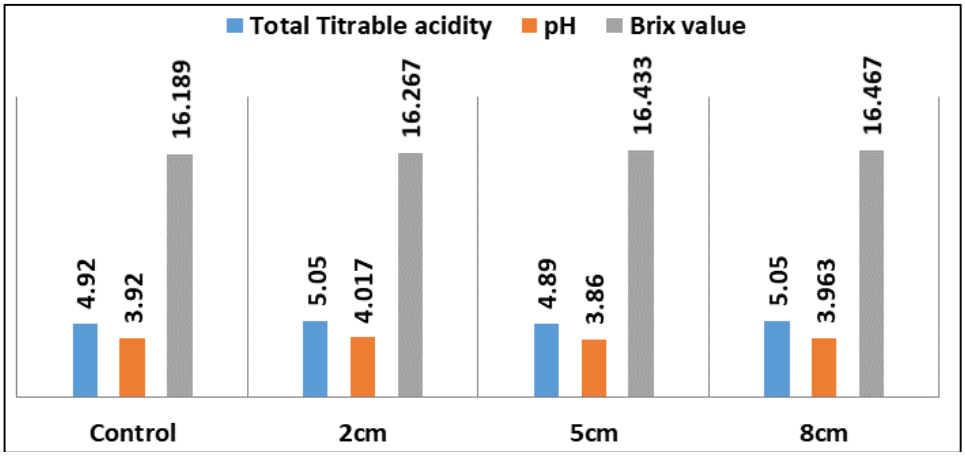
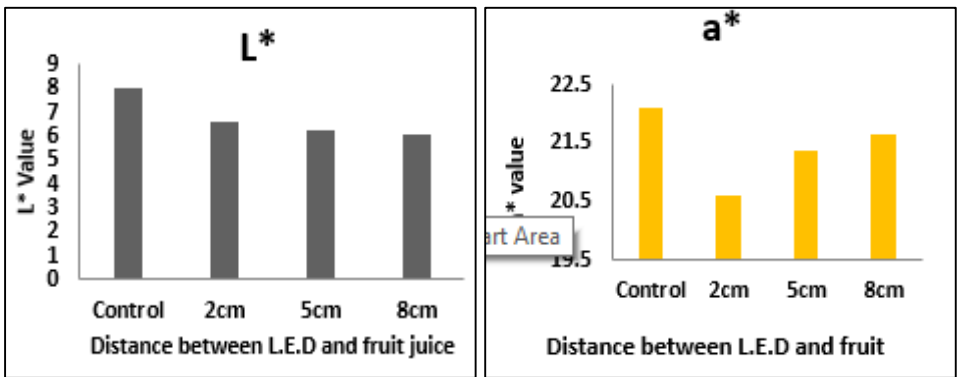


Fig 1: Comparison of Total titratable acidity, pH and Brix value between control and LED treated samples.

Changes in Colour

Illumination of LED light on pomegranate fruit juice showed a reduction in colour values (L*, a*, b*). The control sample showed L* value of 8.07 whereas sample placed at a distance of 2cm, 5cm, 8cm showed the value of 6.62, 6.3, 6.1 respectively. A decrease in a* value was seen maximum at the exposure of sample at 2cm. This phenomenon happens because of the absorption of LED light by β-carotene present in pomegranate, which has an absorption spectrum lies between 400nm to 500nm. Absorption of LED led to degradation of colour by initiating oxidative changes (Ghate *et al.* 2016) [7] (Boon *et al.* 2010) [5] (Kaya, Yıldız, and Ünlütürk, 2015) [14].



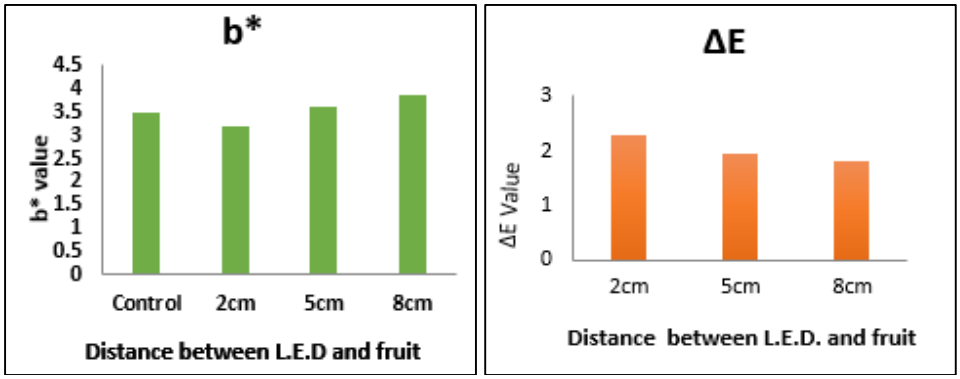


Fig 2: Change in Colour value After LED Treatment

Effect of LED illumination on antioxidant activity, flavonoid content, ascorbic acid and total phenol activity of pomegranate fruit

Table 1 shows the changes in antioxidant activity, flavonoids, total phenol and ascorbic acid content in pomegranate juice after illumination of 10h along with the control sample.

Antioxidant activity - There was a slight change in antioxidant activity among the three samples concerning control with a decrease in 1.27% antioxidant activity for sample A. Increase in % antioxidant activity was observed by 1.27% and 2.42% for sample B and C while comparing with the control sample (Gil *et al.* 2000) [9, 10].

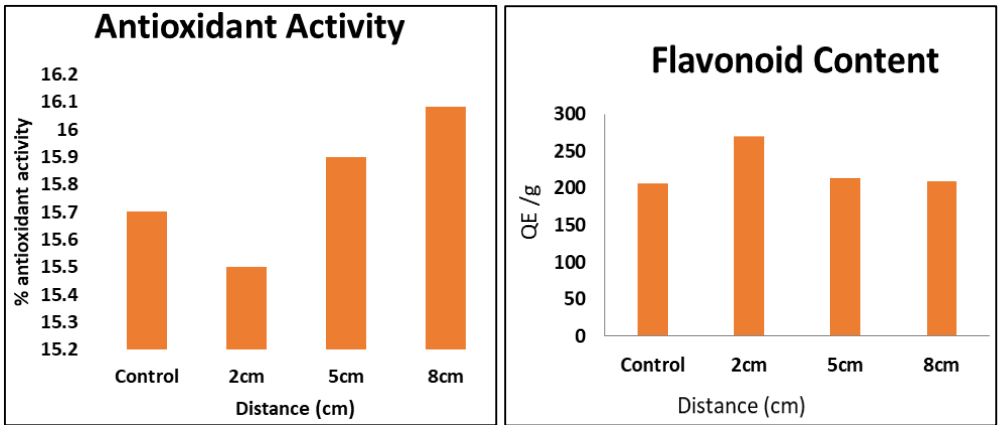


Fig 3: Change in Antioxidant activity and Flavonoid Content Value After LED Treatment

Total flavonoid content showed a significant change in the activity concerning the control sample for sample A. Increase in 1.3 times higher than that of the control sample was observed after illumination of Blue LED for 10h. Increase in flavonoid activity was observed for sample B and C also. LED light illumination showed a positive effect on total flavonoid content for 25 ºC and 2cm distance (sample A). Similar results were found for illuminating fruits and other plant materials at 440nm in the dark. (Hajimahmoodi *et al.* 2013) [11]. (Shi *et al.* 2014) [27] also showed a positive effect of Blue LED on anthocyanin accumulation.

The ascorbic acid Value of control sample was estimated at 7.9 mg/100g with 7.9%, 23.88%, 10.26% increase observed after illumination of the sample (Majidi and Al-Gubury 2016) [17] (Rekha *et al.* 2012) [23, 24].

Total phenol content was found highest in Sample A having a distance of 2cm from the LED light source with a phenolic activity of 2574.6±3.268 followed by sample C and B. Increase in phenolic activity in all three samples were observed. The control sample showed 1941.4±5.54 GAE–mg/g.

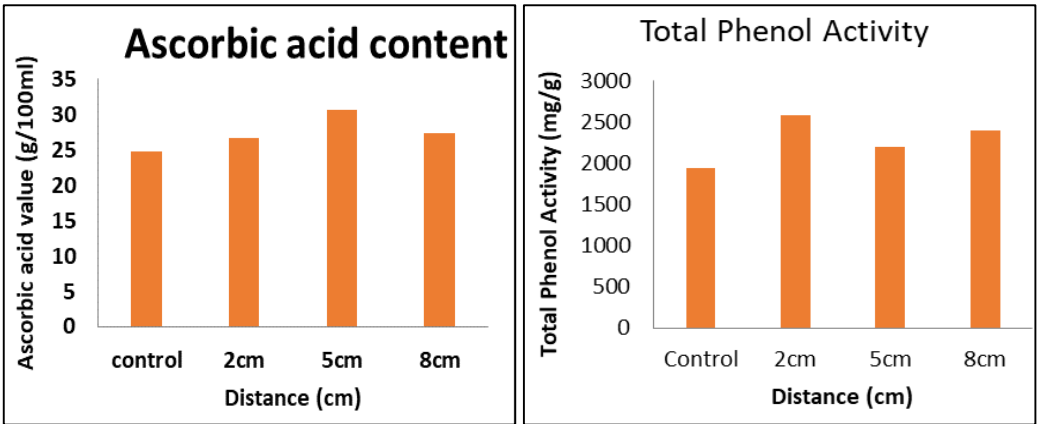


Fig 4: Change in Ascorbic acid content and total phenol activity value After LED Treatment

Table 1: Effects of LED light illumination on antioxidant capacity, flavonoid content, ascorbic acid and total phenol capacity at varying illumination distance

LED exposure temperature	Time of exposure (h)	Distance	sample	Antioxidant activity (% DPPH)	Flavonoids (mg QE/100 g FW)	Ascorbic acid (mg/100 g FW)	Total phenol capacity (gallic acid) equivalent (GAE) – (mg/g)
-	-	-	Control	15.7±0.082	206.6±2.254	7.9±1.07	1941.4±5.54
25	10	2 cm	Sample A	15.5±0.082	269.8±1.241	8.52±0.89	2574.6±3.268
25	10	5cm	Sample B	15.9±0.074	213.6±1.246	9.78±1.41	2189.6±4.689
25	10	8cm	Sample C	16.08±0.048	208.6±3.725	27.28±0.86	2390.5±1.754

Conclusion

Illumination of 470nm light was evaluated in this study to analyse the Physico-chemical effect such as colour value (L*, a*, b*), flavonoid content, antioxidant activity (%DPPH), and ascorbic acid content. Parameters such as ascorbic acid value and the colour value are light-sensitive but were preserved well without any side effects. Flavonoid content in fruit juice increased significantly. Total phenol activity was also preserved and showed a spike in the activity. Colour value didn't deteriorate and showed a negligible colour difference. These positive effects of LEDs on the quality of the fruit may be due to the stimulation effect of light on the production of primary and secondary metabolites that are involved in the protection against ROS produced during the illumination of LEDs (Darko *et al.* 2014) [6] (Kim, Bang, and Yuk 2017) [16]. Together, these data indicate that the impact of LEDs on fruit quality differs according to fruit and ripening conditions. However, the detailed mechanism for controlling the nutritional quality of fruits through LEDs, has yet to be developed.

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