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Effect of nonthermal techniques (UV, LED and atmospheric plasma) on proximate composition, vitamin C and functional group of dried red chilli (*Capsicum annuum*)

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Abstract
The proximate composition and functional groups of dried red chillies were affected by a number of light-based techniques, including Ultraviolet C (UV C) radiation, blue light emitting diode (LED), and atmospheric plasma. The proximate composition, Vitamin C content, and functional group analysis of the control and treated samples were performed using Fourier transform infrared (FTIR) spectroscopy. The results demonstrated that these techniques had a significant impact on the proximate composition and vitamin C content when compared to the control sample. With the exception of plasma, the vitamin C content of chilli increased significantly and functional groups were relatively maintained across all treatments when compared to the control sample. These light-based techniques can be preferred over other nonthermal techniques to preserve the nutrient compositions and functional properties of chilli.

Keywords: Red chilli, UV-C, LED, atmospheric plasma, FTIR

1. Introduction
Food safety and quality continues to be a major issue as they directly impact the health and social welfare of consumers. Today the consumers are more conscious of making the best food choices because they want to know the food's quality before it even gets to their plates. Spices and herbs have gained popularity recently due to their antimicrobial properties and bioactive compounds (Kaavya *et al*., 2020) [10]. The red chilli (*Capsicum annuum*, L.), which is native of South and Central America, is familiar all over the world and used in a wide variety of dishes. Due to its distinct flavour and pungency, this spice can also change the flavour of food. Chillies come in a variety of hues, from pale yellow to deep red, and can differ greatly in size, shape, and colour between and within species (Krzykowski *et al*., 2018) [11]. India is the world leader in the production, consumption and export of chillies. Indian chillies are famous for their flavour and colour and they are especially grown in the Guntur district of the state of Andhra Pradesh. For both the domestic and international markets, some buyers and exporters favour stemless chilli (Jalgaonkar K, 2017) [7].
Chilli is a well-known excellent source of vitamin C and has a high nutritional value. Numerous variables, such as cultivar, maturity, growing conditions, method of processing, and climate, affect the levels of these compounds in chillies. Chilli has been the subject of numerous studies, mostly to assess the close composition and/or functional groups of various thermal and nonthermal techniques. There is a widespread misconception that thermal processing reduces the nutritional value of foods like chilli, making them appear to be less healthy than their fresh counterparts (Hwang *et al*., 2012) [6]. The modification of surface characteristics, nutritional properties and surface decontamination through light-based technologies like Ultraviolet C (UV-C), blue light emitting diode (LED) and atmospheric plasma treatments has recently come to a potential practical method. It is known that the proximate and chemical composition of agricultural products can be changed by these techniques by generation of reactive oxygen species, free radicals, excited atoms, and molecules that penetrate up to several millimeters. Contrarily, photochemical reactions in food material can be induced at greater depths by UV-C emitted as a result of electronic transitions during the plasma discharge (promote water desorption, formation of free radicals and breaking bonds) (Molina *et al*., 2018) [16].

FTIR is a nondestructive method that offers details on the sample's molecular fingerprinting. Infra-red (IR) spectra is used to obtains interferogram signal from the interferometer by applying the Fourier transform to the interferogram. The vibrational spectroscopy method of FTIR can be used to obtain high quality structural data of a molecule. The annotated spectrum makes it possible to see the various light frequencies that the bond has absorbed. The majority of molecules have the ability to capture photons in the IR region of the electromagnetic spectrum, which is the basis for FTIR (Kaavya *et al*., 2020) [10].

Many studies have been investigated about traditional practicing processing methods such as solar drying, mechanical drying, roasting, smoking, etc. and these are significantly affect its proximate composition and heat sensitive nutrients of the chilli. However, very little information is available how light based nonthermal techniques like UV, LED and atmospheric plasma affect the proximate composition of chilli. This study focuses on the effect of these emerging techniques affect the proximate composition as well functional groups of the chilli.

2. Materials and methods

A representative sample of dried chillies (*Capsicum annuum*) was purchased from local market of Thanjavur, Tamil Nadu. The sample selected was free from broken, discoloration and foreign matter. Chilli was evenly spreaded and randomly collected and grinded in hammer mill and sieved through one mm IS sieve. Powdered samples were collected and stored in impermeable container at room temperature until analysis. All analytical chemicals and reagents were purchased from Sigma Aldrich Chemicals Co, USA.

2.1 Experimental setup

A continuous UV-C system includes a treatment chamber, conveying system, holding frame, and control system was used for the experiment that was developed in NIFTEM Thanjavur (Shwetha *et al*., 2018) [28]. A batch type LED arrangement consist of 470 nm intensity LED light strips were bought and installed in a 4 × 7 matrix network. The 470 nm LED light required 5V and 20 mA of current to operate, producing 25000 mW of light (Sharma *et al*., 2022) [27]. 5 cm distance was maintained between the LED light source and sample retained in glass petriplates during treatment. For plasma treatment, a plasma reactor operated at atmospheric pressure as atmospheric air as discharge. The gap between the electrodes were maintained at 5 cm. High AC voltages between 0 and 100 kV and currents between 0 and 50 mA were used to charge the electrodes (Madathila *et al*., 2021) [14].

For the current study, red chilli samples were exposed to UV for 45 minutes at 8×15W power level, LED for 25 minutes and plasma for 7 minutes at 45 kV. Each treatment was performed in triplicate.

2.2 Proximate analysis

The AOAC official method was followed in order to conduct the proximate analysis (fat, crude protein, ash, and moisture) for the study. The ash content is determined by gravimetric method. The crude protein content by semi-micro-Kjeldahl method, and the protein factor of 6.25 was used in the calculation. Using a Soxhlet extractor as n-hexane as the solvent, the fat was examined (Nofiani *et al*., 2021) [18]. Using a water activity meter (AQUALAB 4TE, Meter Food, Pullman, WA, USA) at 24 °C, the water activity of chilli powder was measured (Theagarajan *et al*., 2019) [29].

2.3 pH

18 mL of distilled water were used to dilute 2 g of homogenate. A digital pH-meter (Eutech Instruments Pvt. Ltd., Singapore) was used to measure the pH of mixture (Nofiani *et al*., 2021) [18].

2.4 Vitamin C

The endpoint of titration was evaluated by reduction of the blue dye 2,6-dichlorophenolindophenol by ascorbic acid (AOAC, 1999). The dye's transition into pale pink form marks the titration's end point. To eliminate any potential protein interference, 10 ml of 3% Meta phosphoric acid were added to 5 ml of each sample. Following titration against freshly standardized 2,6-Dichloroindophenol, the filtrate was analysed (DCP, 0.0012%). 10ml of standard ascorbic acid was used for the standardization. By comparison between standard ascorbic acid solution and sample, vitamin C determined and expressed as mg/100g of fresh weight (Shaha *et al*., 2013) [26].

2.5 FTIR Spectroscopic analysis

Fourier transform infrared spectrophotometer (FTIR) is one of the most effective tools for determining the chemical bonds (functional groups) present in compounds. For the FTIR analysis, dried powders of various treatments were used. The translucent sample disc was created by encapsulating 10 mg of the dried extract powder on Potassium bromide (KBr) pellet and loaded into a Japanese-made FTIR Spectrometer (Shimadzu, IR Affinity1) with 400 to 4000 cm^{-1} scan range and resolution of 4 cm^{-1} (Pakkirisamy *et al*., 2017) [19]. DLATGS Detector was used and data were analysed by lab solution IR software.

2.6 Statistical analysis

The each analysis was carried out in triplicate, and the results are presented as mean ± standard deviation for proximate composition. One-way ANOVA (Post-hoc Tukey test) was carried out using Minitab statistical software for the statistical analysis, with a significance level of 0.05.

3. Results and discussion

3.1 Moisture content and water activity

Table 1 depicts the moisture contents of control and treated chilli sample. The results are expressed in % wet basis. Moisture content decreased steadily in each treatment. The average initial moisture content of the control sample was 7.01±0.12. The reduction of moisture in each treatment might be due to evaporation of moisture from inside the chilli as there is exposed surface area. When agricultural commodities are dried, moisture is removed via diffusion from the surface and inter tissue of the sample (Buvaneswaran, 2021) [3].

Significant changes in water activity have been examined in samples after each treatment (Table 1). Untreated raw materials had an average water activity of 0.65±0.01. Variations in water activity are linked to increased surface water evaporation. The lowest water activity levels were found for plasma followed by UV and LED treated sample. A similar result also found when UV-C radiations applied on black pepper powder (Park *et al*., 2019) [21], cabbage treated with LED for Quality study (Lee *et al*., 2014) [12] and when chilli pepper (*Capsicum annuum* L.) exposed to cold plasma

as pre-treatment (Zhang *et al*., 2019) [32].

3.2 pH
The average pH of control sample is 6.03±0.08 and it reduced in UV and LED treatment and increased in plasma treated sample. The decline in pH during UV and LED treatment might be due to increase in the amount of organic acids released during the radiation treatment (Waje *et al*., 2008) [30] and electrolyte release into the solution and the leaching of cell constituents during LED treatment (LeLAS, 2007) [13]. A slight increase in pH of plasma treated sample was observed as compared to control (Table 1) might be due to decomposition of chilli protein into alkaline molecules such as ammonia and accumulated continuously. The reactive nitrogen species (RNS), free radicals, singlet oxygen, ionic radiations, etc. might lead to decomposition of protein (Qian *et al*., 2021) [23].

3.3 Ash and acid insoluble ash
As per Indian standards total ash and acid insoluble ash on dry basis in chilli should be not more than 8% and 1.3% respectively. As a consequence of the stalk being present in whole chillies, the ash content is higher than recommended. All treatments experienced a rise in ash and acid insoluble ash content as a result of dewatering, that raised the dry matter and ash values in all treatments (Romauli *et al*., 2021) [25].

3.4 Crude fiber
According to Joshi & Varma, (2016) [9], the increase in crude fibre was primarily caused by structural changes in the polysaccharides such as cellulose, glucose, and mannose present in the cell wall, indicating that the changes were brought on by an expansion of the plant's cellular structure during light treatment. Hence crude fiber content was significantly increased in all treatments compared to control sample (Table 1).

3.5 Crude fat
A slight increase in crude fat was observed after all the treatments compare to the control sample (Table 1). This might be due the penetration of light radiations into the oil glands of the chilli and leads to the elution of lipid components into the cell surface (Destandau *et al*., 2013) [4].

3.6 Crude protein
Using the Kjeldhal method, the protein content of the control and sample was determined. After exposure to UV-C radiation, the average protein content has slightly decreased. This may be due to damage to the peptide bonds between amino group and carboxyl group of two adjacent amino acids, which prevented the formation of corrosive dipeptides and then polypeptides. As a result, the polypeptide chain unfolds from the protein's various conformations, producing unique basic structures. A similar study was reported by Rajashekara *et al*., (2021) [24] when UV C radiation are used to study biological composition of *Capsicum* plants. Exposure of LED light for 30 minutes and plasma for 60 minutes increased the protein content of chilli powder. These findings are similar to Akasapu *et al*., (2020) [1].

3.7 Vitamin C
The vitamin C content of control sample was slightly lower than that of the UV-C illuminated chilli (Table 1). According to studies of Promyou & Supapvanich, (2012) [22], UV-C exposure increased the ascorbic acid accumulation. Additionally, it has been noted that the quantity and quality of light used during the exposure has a significant impact on how much ascorbic acid is produced (Marín *et al*., 2004) [15]. A similar result was observed for LED treated sample. After plasma treatment, compared to the control sample, a slight drop in the vitamin C content was observed. The slight decline in vitamin C content is most likely the result of the cold plasma's oxidation of the vitamin. Additionally, because vitamin C is light-sensitive, UV produced by plasma may also be a significant factor in the deterioration of the vitamin (Wang *et al*., 2012) [31]. Our result, a reduction in vitamin C of less than 4%, is regarded as acceptable.

Table 1: Proximate composition of control and treated chilli sample (mean ± SD)

Treatment	Water activity	Moisture content (%)	pH	Ash (%)	Acid insoluble ash (%)	Crude fiber (%)	Crude fat (%)	Crude protein (%)	Vitamin c (mg/100g)
Control	0.65±0.01	7.01±0.12	6.03±0.08	10.08±0.21	1.32±0.029	2.54±0.10	23.65±0.41	21.29±0.28	93.0 ± 1.2
UV	0.62±0.02	6.75±0.25	5.61±0.03	12.32±0.24	4.88±0.24	6.96±0.08	23.85±0.23	21.11±0.05	97±1.01
LED	0.631±0.2	6.43±0.06	5.80±0.02	12.67±0.08	1.35±0.07	8.23±0.15	23.73±0.11	21.57±0.1	100±0.5
Plasma	0.59±0.01	6.32±0.08	6.38±0.02	10.52±0.38	1.05±0.06	6.47±0.09	23.98±0.38	21.40±0.02	89.65±0.21

Note: mean within rows with different superscripts are significantly different ($p<0.05$).

3.8 FTIR analysis
The red chilli after UV, LED and plasma treatment was exposed to FTIR analysis and compared with control sample to determine chemical properties of chilli surface. The coherence and total compounds in individual sample are determined by FTIR analysis. Peaks of the graph support the existence of various functional groups and corresponding bonds in the sample. The red chilli FTIR stretch of each treatment is shown in Figure 1.

The peaks at 3535.51 cm^{-1}, 2922.16 cm^{-1}, and 1742.65 cm^{-1} represent O-H stretch (Aromatic compounds) O-H stretch (Carboxylic acid), and C=O (ester) respectively. Peaks at 1633.61 cm^{-1}, 1452.41 cm^{-1}, and 1243.16 cm^{-1} are due to N-H (amines), C-H bend (alkanes) and C-N (aliphatic amines) respectively. All these peaks authenticate the functional groups of capsaicin and dihydrocapsaicin.

Compared to control sample, treated sample contain new peaks at 1050 cm^{-1}, 1029.99 cm^{-1}, and 1028.06-1157.29 cm^{-1} respectively for UV, LED and plasma. The prominent broad peaks at 1050 cm^{-1} and 1029.99 cm^{-1} due to due to strong aromatic bending and stretching of CO-O-CO functional group (Jeong *et al*., 2018) [8]. While the peak at 1028.06 cm^{-1} represent sulphoxide group indicating strong stretching vibration of S=O functional group. Peak around 1157.29 cm^{-1} contributed by primary, secondary & tertiary alcohol, aliphatic ether groups due to C-O stretching. Similar observations were reported by Hilares *et al*., (2018) [5] when LED treated to sugar cane bagasse.

The peaks at 1410 cm^{-1} and 1415 cm^{-1} represent strong S=O stretching due to sulfonyl chlorides and sulfate groups

respectively (Figure 1). At the same time peaks at 1500 cm^{-1}, 1450-1465 cm^{-1}, 1440 cm^{-1} and 1420 cm^{-1} represent nitro compound, alkane group, carboxylic acid and alcohol group respectively. These might be due to N-O stretching, C-H and O-H bending. These results are congruent to Jeong *et al*., (2018) [8], Pankaj *et al*., (2017) [20] and Basaran, (2009) [2] who studied effect of LED on blue berry fermentation, effect of atmospheric plasma on chitosan film and application of UV C radiation on hazelnut surface.

Variations in the shape and area of peaks in the 1700-1800 cm^{-1} region were observed, according to experimental data plotted in Figure 1, due to various effects of light treatment on functional groups of chilli. Elevated peak has been observed for plasma as compared to control and other two sample at 1635.64 cm^{-1} and 1743.65 cm^{-1} due to strong C=C stretching contributed by alkene and aldehyde functional groups (Mošovská *et al*., 2018) [17]. Similar peaks has been observed in UV with respect to control sample at 1716.65 cm^{-1} due to strong C=O stretching by α, β-unsaturated esters (Basaran, 2009) [2]. Similar to control sample (2360.87 cm^{-1}), a peak was observed in UV (2358.94 cm^{-1} and 2918.30 cm^{-1}), LED (2358.94 cm^{-1}, 2852.72 cm^{-1} and 2922.16 cm^{-1}) and plasma (2360.87 cm^{-1}, 2852.72 cm^{-1} and 2922.16 cm^{-1}) treated sample. 2358-2360 cm^{-1} peaks are due to strong O=C=O stretching provided by trapped carbon dioxide in sample and 3000-2800 cm^{-1} peaks are due to strong broad N-H stretching by amine salt (Jeong *et al*., 2018) [8]. Peak between 3150-3200 cm^{-1} and 3200-3250 cm^{-1} indicate weak broad O-H stretching and strong broad O-H stretching by alcohol and carboxylic acid respectively, there is no significant difference between control and sample in this region, which is clearly visible from the graph.

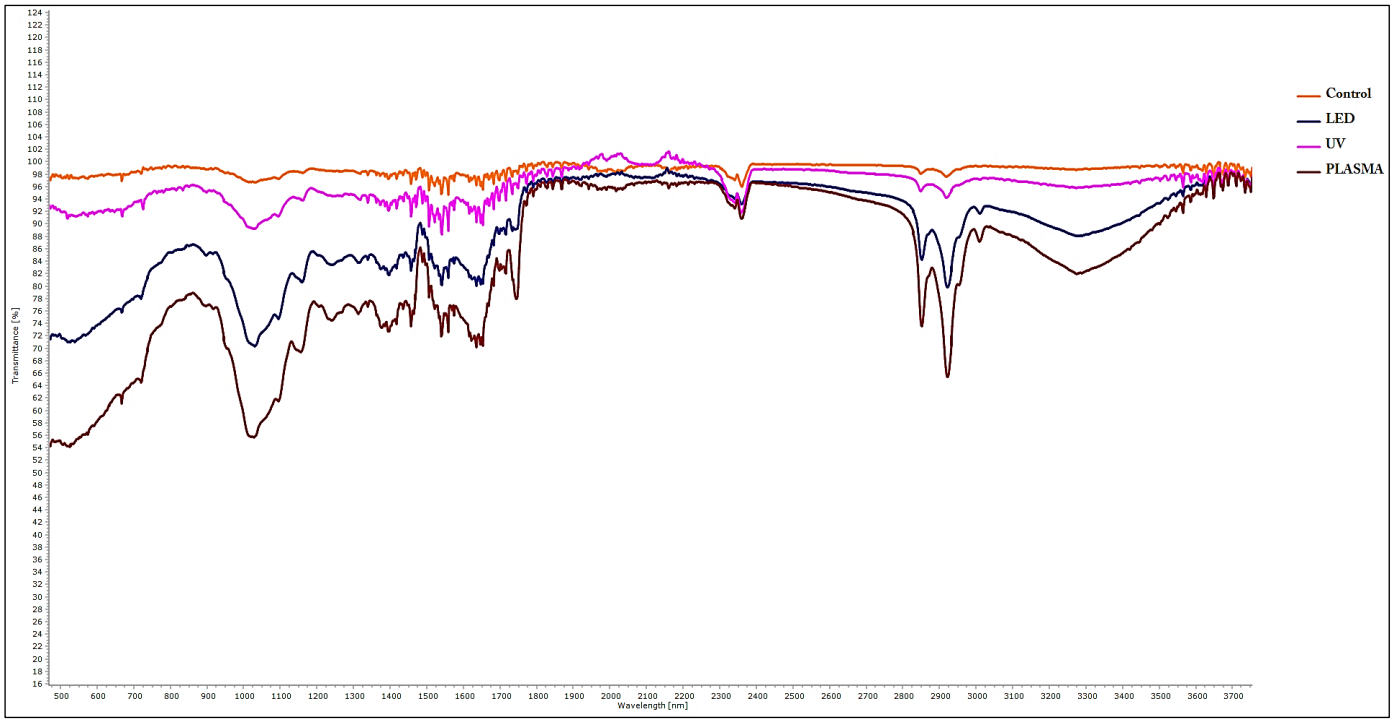

Fig 1: Fourier transform infrared spectra of control and treated dried red chilli samples

4. Conclusion

The macronutrients were well preserved for UV C and LED treatment; it can be inferred from the current study. After the plasma treatment, a negligible decrease in vitamin C and crude fibre was observed. Particularly heat sensitive compounds like vitamin C, time of exposure and type of treatment are very important factors in maintaining and preserving the essential nutrients. In all treatments, the characteristic bonds and functional group of the pungency factor, capsaicin and dihydrocapsaicin, were well preserved by surface diagnostic methods like FTIR. The results obtained point to the potential of these techniques for processing and surface sterilization of heat-sensitive food products. The low-temperature approach, open-air operation at atmospheric pressure and small treatment times (tens of seconds) are major benefits for practical use. These methods are affordable, environmentally friendly, and can be used on a large scale without the use of harmful chemicals for disinfection.

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