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Effect of non-thermal plasma treatment on carrot slices

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Abstract
Non-thermal plasma can effectively facilitate microbial inactivation while maintaining the product quality due to its low temperature operation. This study investigates the effect of non-thermal plasma on physical quality parameters (weight loss and firmness) and microbial safety (total plate count) of carrot slices. A low-pressure DC glow discharge device operated at different power levels of 10, 20, and 30 W was used for non-thermal plasma treatment of carrot slices with exposure times of 5, 10, 15, and 20 min. After plasma treatment, the total plate count of carrot slices significantly reduced from an initial population of 6.3 log CFU/g. The inactivation efficiency was found to increase with both treatment power and exposure time. The measurements on physical quality parameters showed significant differences between the treatments. The results implicate the potential of non-thermal plasma treatment to decontaminate carrot slices but quality changes must be considered to guarantee consumer's acceptance.

Keywords: Decontamination, microbial quality, non-thermal, carrot, safety

Introduction
In recent years, the increasing consumer requirements for high quality convenience foods with high nutritive value and fresh sensory attributes has led to a greater demand for minimally processed, prepacked, ready-to-eat fruit and salads (Rico *et al.*, 2007) [11]. Among fruits, Carrots (*Daucus carota L.*) are widely used to make salads, home-cooked meals, and ready-to-eat products. They are the rich sources of bioactive compounds like carotenoids, ascorbic acid, dietary fiber and are known as vitaminized food with several other functional components having significant health-promoting properties (Sharma *et al.*, 2012) [12]. It is also a significant source of phytonutrients including phenolics, polyacetylenes and carotenoids which play a major role in protecting biological systems from the effects of oxidative stress. Minimally processed carrots constitute one of the major minimally processed vegetables and are prepared by peeling off the outer layer of the carrots root and cutting to slices and keeping them refrigerated in some form of containers/packaging (Yu *et al.*, 2018) [15]. The major problems that limit their shelf-life and quality are white blush discolouration caused by tissue dehydration, tissue softening due to destruction of cell membrane and loss of soluble pectin along the cell liquor, and microbial spoilage (Gomez-Lopez *et al.*, 2009) [2]. As these products are consumed raw and does not receive any lethal treatment prior to consumption, a number of food poisoning outbreaks are associated with it. Traditional methods to control food spoilage and microbial hazard are not compatible with consumer demands for fresh-like convenience food. Therefore, research efforts are directed towards developing a suitable alternative to ensure safety of minimally processed foods (Fernandez *et al*., 2013) [1]. An emerging antimicrobial technology for decontaminating infected surfaces is the use of non-thermal plasma. Their application aimed at the removal of microbial contamination from fresh and minimally processed food and it has received increased attention. Non-thermal plasmas offer distinct advantages for decontamination of heat sensitive foods as it can be produced at room temperature and near/low atmospheric pressure (Zhang *et al*., 2013) [17]. Plasma, fourth state of matter is constituted of photons, electrons, positive and negative ions, atoms, free radicals and excited or non-excited molecules that, in combination, have the ability to inactivate microorganisms more effectively. The reactive species diffuse through the cell membrane and cause surface erosion and localized lesions by reacting with membrane lipids, proteins and nucleic acids. The UV photons can also cause DNA modifications and consequent improper cell replication and these result in inhibition of microorganisms (Kim *et al*., 2014) [3]. Furthermore, a number of studies indicating the potential of non-thermal plasma treatment for decontamination of fruit and vegetable and products thereof have been reviewed by various

researchers (Niemira, 2012; Tappi *et al*., 2014; Misra *et al*., 2017) [9, 13, 6]. Therefore, the objective of this study was to investigate the effect of non-thermal plasma on inactivation of total plate count on carrot slices and to evaluate the resulting product quality.

Materials and Methods

Sample preparation

The Nantes variety of carrot (Ooty) purchased from the local market (Coimbatore, Tamil Nadu, India) were visually inspected for damage and good ones were selected.

The outer layer of the selected carrots was peeled off and washed using cold water. Then it is prepared by cutting into thin slices with a help of a sterilized stainless-steel knife.

Plasma source and plasma treatment

A direct current (DC) glow discharge plasma device was used in the present study for low-pressure non-thermal plasma treatment of carrot slices. The plasma treatment system, illustrated in [Fig-1], consists of a cylindrical treatment chamber (1), a high voltage DC power supply unit (2) and a vacuum pump (3). The cylindrical treatment chamber is of electro polished mild steel and has dimensions of 40 cm (height), and 40 cm (diameter) with an observation window. Two pure copper electrodes (4) of 20 cm diameter were used as cathode and anode, and fixed with their surfaces parallel to each other. A glass plate (5) was placed in between two electrodes for holding the sample. The DC source powered at 220 V; 50 Hz delivers a high voltage output in the range of 0 – 1000 V was connected to the electrodes for plasma generation. The carrot slices were transferred into the chamber and placed in between the electrodes so as to expose the samples to plasma discharge. The lid of the chamber was tightly closed and made leak proof. Initially, the chamber was evacuated to a base pressure of 0.05 mbar using the vacuum pump to remove adsorbed gases or water vapors from the surface of the food material. Atmospheric air was used as a process gas for plasma generation, and the working pressure was adjusted to 0.2 mbar by a vacuum valve. A constant potential difference was applied across the electrodes and adjusted to obtain stable glow discharge plasma. The carrot slices were then exposed to non-thermal plasma at different power levels – 10, 20, and 30W; and for different treatment durations – 5, 10, 15, and 20 min. After plasma treatment, the samples were taken out and analyzed for microbial reductions. All treatments and further evaluations were performed in triplicate to ensure reproducibility of the experimental data.

Microbiological analysis

The samples subjected to plasma treatment were incubated under room temperature (30 ± 2 ºC) for a period of 3 h. For microbiological analyses, 10 g of the samples were taken aseptically and serially diluted in a sterile peptone water. Total plate count was determined by surface plating of 100 µl of 10^{-5} and 10^{-6} aliquots in duplicate on plate count agar. PCA plates were then incubated at 37 °C for 24-48 h. To know the efficacy of each treatment, results were expressed based on log reduction and calculated using the following equation (Ulbin-Figlewicz *et al*., 2015) [14]:

$$Log\ reduction = logN_0 - \log N \quad \ldots.[1]$$

Where N_0 is the number of viable cells before treatment, (CFU/g) and N is the number of viable cells after treatment, (CFU/g)

Physical quality analysis

Weight loss was expressed as percentage of initial weight of sample and calculated from the formula (Misra *et al*., 2014) [7, 8]:

$$Weight\ loss, \% = \frac{w_1 - w_2}{w_1}\ x\ 100 \quad \ldots.[2]$$

Where, w_1 is the initial weight of the sample before treatment, (g) and w_2 is the final weight of the sample after treatment, (g)

Firmness as the peak force (N) necessary to cause a deformation represents the limit of the flesh elasticity (F). The surface texture of carrot slices measured from penetration tests were performed using a Texture Analyzer (Stable Micro Systems, Surrey, UK). The instrument was mounted with a 50 N load cell and equipped with a 4 mm diameter stainless steel cylindrical probe which punctures the sample at a download speed of 0.5 mm/s and a distance of 3 mm. Penetration tests were carried out at two different points on each slice and firmness value was determined (Misra *et al*., 2014) [7, 8].

Statistical analysis

Each plasma treatment was performed three times and plate count analyses were made in duplicate of each sample. Statistical analyses to evaluate significant difference between the data were performed using SPSS 19.0 (SPSS Inc., Chicago, USA). Means were compared using Duncan's multiple range test with a confidence level at $P < 0.05$. The non-thermal plasma treatment needs to be optimized with respect to two variables such as input power and exposure time. The effect of individual linear, quadratic, and interaction terms was determined using regression analyses and modeled by the following polynomial model (Lee *et al*., 2015) [5]:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 \quad \ldots.[3]$$

Where, b_n are constant regression coefficients, Y is the log reduction (log CFU/g), X_1 and X_2 are the treatment power (W) and exposure time (min.), respectively.

Results and Discussion

The non-thermal plasma treatment effectively reduced the microbial populations on the surface of carrot slices.

Microbial inactivation

The effect of plasma treatment on reduction of total plate count on the product surface are presented in Table 1, respectively. The fresh carrot slices had an initial total plate count population of 6.30 log CFU/g. This result is in agreement with Zagory (1999) [16] who reported that the total counts of microbiological populations on fresh-cut fruits and vegetables after pre-processing range from 3 to 6 log CFU/g. Time dependent inactivation of total bacterial count has been observed using non-thermal plasma treatment. Viable total plate counts of bacterial population were reduced by more than 5 log units after 20 min of plasma treatment at 30 W power. Usage of 10 and 20 W plasma power resulted in lower reduction of total plate count and was 2.91 and 4.70 log CFU/g, respectively for the same exposure time. The effect of treatment time on the inactivation of total plate count on carrot slices is shown in Fig 2. The number of bacterial counts decreased as the treatment time increased, and the relationship between the level of microbial reduction (log reduction) and exposure time appeared to be linear. It was also observed that total plate count from carrot slices were significantly reduced by about 2.74, 3.65, 4.55 after treatment at 30 W for 5, 10 and 15 min, respectively (P<0.01). These results indicate that

increasing exposure time from 5 to 20 min significantly enhance the decontamination effect of plasma treatment.

In the present study, it is observed that the inactivation of total plate count increased with both plasma power and exposure time as reported by Kim *et al.*, (2014) [3]. The higher inactivation was achieved by increasing the input power to 30 W with an exposure time of 20 min, which resulted in the higher power density of 0.10 W/cm^2. The higher electron and ion density caused by rising power at 30W, along with ultraviolet photons, led to better destruction of bacterial cells. The mechanism of inactivation by plasma treatment may be due to the oxidative damage produced by the plasma reactive species. These species are able to induce damage to biomolecules, including DNA and proteins. They can cause alterations in the functions of biological membranes by interacting with lipids (Kim *et al.*, 2015) [4]. Also, the inactivation of DNA and RNA or etching of cell wall/ membrane is affected by UV radiation, especially in low pressure plasma because the content of UV photons can be considerable (Ulbin-Figlewicz *et al.*, 2015) [14]. These factors can potentially lead to cell death. Thus, the treatment power and exposure time were found to have a significant effect (P<0.01) on microbial inactivation of carrot slices.

The polynomial model describing the effect of treatment process variables on bacterial inactivation of carrot slices is given as Y, Log reduction = -2.49083 + 0.21475*X_1 + 0.32817*X_2 + 0.0015*X_1*X_2 – 0.0035*X_1^2 – 0.00783*X_2^2 (R^2 = 0.9737). The coefficient of regression (R^2) value of 0.97 revealed that the model had a good fit with the experimental results. The positive coefficients for linear terms (plasma power and exposure time) and interaction term (plasma power x exposure time) indicates that the inactivation of bacterial population increased significantly with increase of these process variables ($p < 0.05$) and other terms had a negative effect on the variables. Therefore, the polynomial model explaining the degree of bacterial inactivation using a plasma treatment was highly dependent on the plasma power used and exposure time.

Effect of plasma treatment on weight loss

The degree of weight loss of carrot slices treated using non-thermal plasma is shown in Table 2. The minimum loss on weight of 0.71 percent was found in carrot slices treated at 10 W for 5 min and the maximum loss of 4.92 percent was found in plasma treatment of 30 W for 20 min. The result clearly indicates that the weight loss in plasma treated carrot slices increase significantly with an increase of plasma power and exposure time (P<0.001). This may be due to the escape of CO_2 and diffusion of gases from the surface of fruit slices either through aqueous/waxy layers of the epidermis or through gaseous pores (Misra *et al.*, 2014) [7, 8]. The weight loss might also be due to evaporation of moisture from the carrot slices, facilitated by the low-pressure inside the treatment chamber during processing (Kim *et al.*, 2014) [3]. It is generally considered that fruits and vegetables remain in their characteristic freshness when they do not lose more than 3 – 5 percent of their weight. From the table, it can be observed that the total loss in weight did not exceed 5 percent for all the treatments which confirms the fresh-like quality attributes of the plasma treated carrot slices.

Effect of plasma treatment on firmness

Firmness is another important attribute of fresh-cut products which is used as a useful indicator for quality assessment of fruit decay. The initial firmness of carrot slices was determined as 4.466 N. The mean values of peak force (N) required for puncturing (break) the carrot slices after plasma treatment are presented in Table 2. An increase in firmness value of 4.824 and 4.854 N was observed in plasma treated carrot slices at 20 and 30 W for an increased exposure time (20 min). It was observed that the firmness of carrot slices increased significantly upon plasma treatment and this result is in agreement with Ramazzina *et al.*, (2015) who reported a similar increase in firmness of the plasma treated fresh-cut kiwifruits. The firmness values of control and treated carrot slices implies that the tissue structure of the produce remained intact which allowed the retention of firmness.

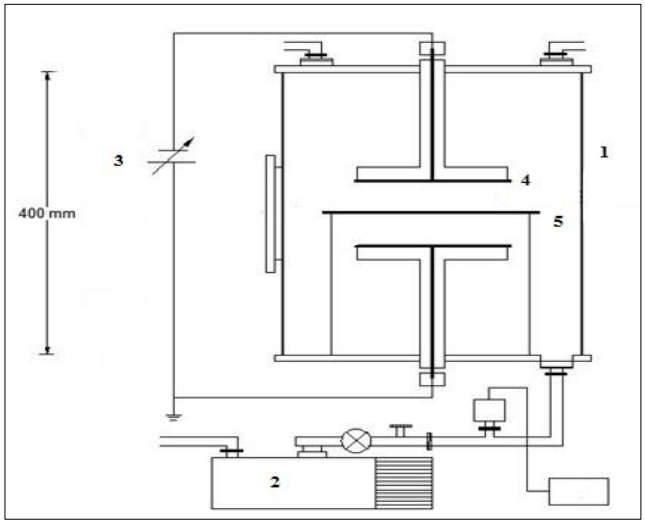

Fig 1: Schematic diagram of DC glow discharge plasma device

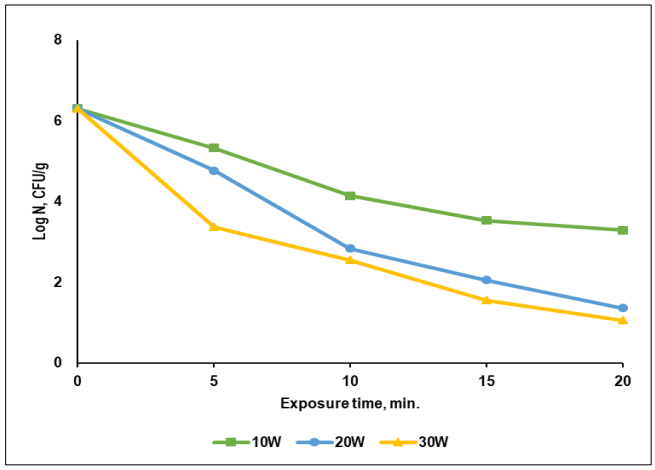

Fig 2: Inactivation efficiency of non-thermal plasma against total plate count on carrot slices

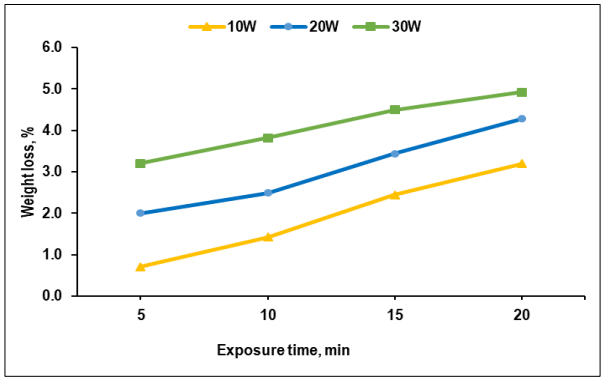

Fig 3: Effect of non-thermal plasma treatment on weight loss of carrot slices

Table 1: Effect of plasma treatment on reduction of total plate count in carrot slices

Log reduction of TPC (log N / log N_0)			
Exposure time, min.	**Plasma power, W**		
	10	**20**	**30**
05	0.97 ± 0.12^{j}	1.53 ± 0.11^{i}	2.74 ± 0.01^{f}
10	2.06 ± 0.05^{h}	3.37 ± 0.04^{e}	3.65 ± 0.09^{d}
15	2.57 ± 0.07^{g}	4.05 ± 0.02^{c}	4.55 ± 0.02^{b}
20	2.91 ± 0.08^{f}	4.70 ± 0.06^{b}	5.05 ± 0.04^{a}

The values are expressed in Mean ± S.D;
Values with different letters differ significantly according to Duncan's multiple range tests ($P<0.05$).

Table 2: Effect of plasma treatment on physical quality of carrot slices

Plasma power, W	Exposure time, min.	Weight loss, %	Firmness, N
10	05	0.71 ± 0.11^{h}	4.523 ± 0.015^{de}
	10	1.42 ± 0.15^{f}	4.612 ± 0.032^{c}
	15	2.44 ± 0.10^{e}	4.701 ± 0.051^{b}
	20	3.19 ± 0.12^{cd}	4.814 ± 0.035^{a}
20	05	2.00 ± 0.11^{gh}	4.556 ± 0.045^{cd}
	10	2.49 ± 0.16^{e}	4.654 ± 0.057^{b}
	15	3.44 ± 0.07^{cd}	4.731 ± 0.055^{b}
	20	4.28 ± 0.09^{bc}	4.824 ± 0.041^{a}
30	05	3.20 ± 0.11^{g}	4.581 ± 0.030^{cd}
	10	3.82 ± 0.10^{de}	4.682 ± 0.036^{b}
	15	4.49 ± 0.09^{ab}	4.763 ± 0.043^{a}
	20	4.92 ± 0.15^{a}	4.854 ± 0.041^{a}

The values are expressed in Mean ± S.D;
Values with different letters differ significantly according to Duncan's multiple range tests ($P<0.05$).

Abbreviations: DC – Direct current, UV – Ultra violet, DNA – Deoxyribonucleic acid**,** PCA – plate count agar, CFU – colony forming units

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Conclusion

The low-pressure non-thermal plasma treatment showed promising results for the inactivation of total plate count on the surface of carrot slices. The effectiveness of nonthermal plasma varies with the experimental parameters, such as treatment power and exposure time. It was found that the plasma treatment significantly altered the physical-quality parameters but the carrot slices remained in their characteristic freshness even after treatment at higher power level and increased exposure time. The results of this study demonstrate the potential of low-pressure non-thermal plasma for inactivation of surface microflora in minimally processed fruits and vegetables. However, more research is needed to fully understand and further improve the application of plasma and the product – plasma interactions.

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