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Effect of Pulsed Electric Field on Lycopene Extraction

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ABSTRACT

The effect of application of pulsed electric field on the cell wall disruption of tomato pulp was investigated. PEF treatment (40 pulses, 1.5 kV/cm, 500μs) expeditiously performed disruption of tomato pulp cell membranes within 10s. In general, field strength (1.5–2 kV/cm) and pulse number (30–50) had major influence on the better cell wall disruption.As the cell wall disruption occurs, the amount of lycopene extracted gets increased. The PEF pre-treated tomato pulp contained 68.63% higher amount of lycopene than by the conventional method.

1. INTRODUCTION

Lycopene is a bright red carotenoid pigment found in tomatoes, red carrots, watermelons, papayas, pink guava etc. Lycopene is a polyunsaturated hydrocarbon that are composed entirely of carbon and hydrogen. It is insoluble in water and soluble in organic solvents. Lycopene's eleven conjugated double bond gives deep red colour and antioxidant activity [1]. Lycopene provides protection against aging and Cardio-vascular diseases. Lycopene intake regulates the cholesterol metabolism [2]. Lycopene is added to cosmetics and skin care products. Some cosmetic products that contain lycopene include anti-aging treatments, facial moisturizers, eye creams, lip gloss and lipstick. Lycopene is used as a colorant and appears to be a safe cosmetic ingredient [3].

23.8 % of India's population suffering from cancer. Lycopene levels have been found to be inversely related to the incidence of several types of cancer, including breast cancer and prostate cancer [4]. India is one of the country which leads in the production of Tomatoes.Hence the availability of tomatoes is abundant in India, high amount of lycopene can be extracted from PEF pre-treated tomato and can be used for the treatment of cancer.

Extraction of the lycopene can be done by various extraction methods such as Supercritical fluid CO_2 extraction technology, Membrane separation technology, Microwave assisted extraction and Ultrasonic extraction [5], [6]. Pulsed electric field treatment is used as a pre-treatment of lycopene extraction followed by low volume hexane extraction. The pulsed electric field is one of the growing and attractive applications of High Voltage Engineering. It extends its applications in the fields of cancer treatment, inactivation of microbes in food, extension of shelf life for longer period of time and enhanced juice extraction [7].

In this work, PEF treatment is used to enhance the lycopene extraction from tomato. Lycopene is present in the intra-cellular membrane of the tomato.During the extraction of the intracellular products from the cells, the natural barriers such as cell wall and cell membrane preserve the cell content. The problem with the conventional extraction methods is the poor cell wall disruption of the tomato. The cell wall disruption is not achieved because of the strong nature of the cell wall. Due to the incomplete cell wall disruption, the extraction efficiency is also low. Hence, PEF treatment is used to electroporate the cell membrane. The

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formation of the pores across the membrane allows the solvent to penetrate through the pores more efficiently and leads to an enhanced extraction.

2. EXPERIMENTAL PROTOCOL

Sample preparation, procedure for extracting lycopene from pulsed electric field pre-treated sample are highlighted here.

2.1. Sample preparation

Mature ripe red organic tomatoes were bought at Koyambedu market and stored at refrigerator. Damaged and rotten tomatoes were discarded. Before use, tomatoes were maintained at room temperature for 6-8 hours. Tomatoes were sliced, seeds were removed, cut into small pieces and then homogenized them in a mixer grinder for 30s to obtain a homogenous pulp. Tomato pulp was kept on ice and out of light after preparation until assayed. All the analysis was done at room temperature.

2.2. Pulsed Electric Field treatment

Tomato pulp was subjected pulsed electric field for better cell wall disruption and the lycopene levels of PEF treated samples was estimated. The PEF was applied to the sample by varying the parameters in two ways.

i) Keeping voltage constant and varying number of pulses.

ii) Keeping Pulse length constant and varying number of pulses.

The tomato sample was subjected to pulsed electric field by Electoporator ECM 830 (High voltage laboratory, College of Engineering, Guindy, Chennai) which is shown in Figure 1.Electroporator has the ability to select the voltage magnitude, pulse length, number of pulses, pulse interval. The voltage magnitude was varied between 550-650V, pulse duration was varied between 100-500µs and the number of pulses was varied between 10-50. Pulse interval was set to be 100ms.

To treat the samples, the tomato pulp was placed in the cuvette chamber which holds approximately 1ml of the sample. The well of the chamber was made with stainless steel electrodes while the walls were made of electrical insulation material Teflon. The electrode gap was 0.4 cm and it was filled with the tomato pulp. The parameters were set and the tomato pulp was subjected to pulsed electric field using electroporator.

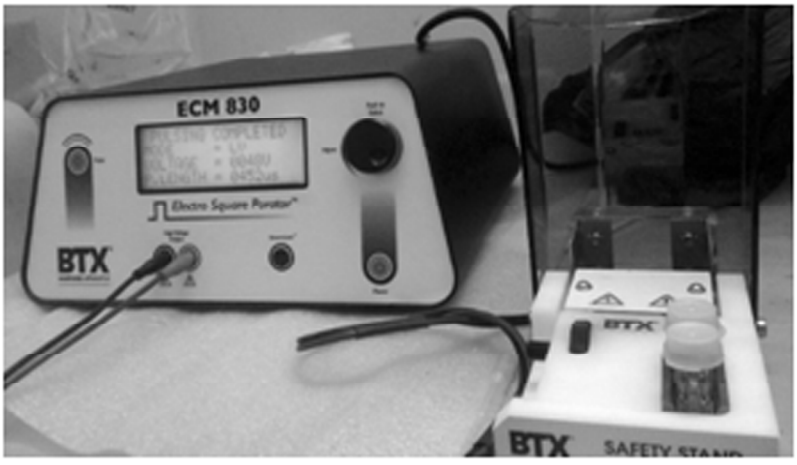

Figure 1: Electoporator ECM 830 with cuvette placed in safety stand

The samples were treated at electric field strengths of 1.125, 1.5 and 1.625 kV/cm. The pulse lengths were 100, 350 and 500μs. Numbers of pulses were 10, 20, 30, 40, and 50.After the application of each pulse, the voltage magnitude and pulse length had been displayed on the screen. The samples were collected then analysed to obtain further results.

2.3. Extraction Protocol

Lycopene content was estimated using Fish et. al. method [8] which is a rapid spectrophotometric method. The samples were centrifuged for 10min at 20,000rpm, 4°C in order to separate the tomato biomass from the water medium. The upper water layer was discarded because water doesn't have lycopene content and the bottom tomato biomasslayer was taken for further analysis.

The following solvents were added to the collected tomato sample: 2.5 ml of 0.05% (w/v) BHT in acetone, 2.5 ml of 95% ethyl alcohol and 5 ml of hexane and then macerated by using pestle.After that, 3.0 ml of deionised water was added to each vial and the samples were kept for 5 min on ice. The vials were then kept at room temperature for 15 min to allow for phase separation. Samples were analysed in triplicates.

2.4. Estimation of lycopene levels

The absorbance of the hexane was measured in a 1 cm path length quartz cuvette at 503 nm versus a blank hexane solvent using a spectrophotometer Hitachi U2900 (Centre for Advanced Studies in Botany, University of Madras, Guindy, Chennai). The lycopene content of the pulp was calculated from the absorbance value. Lycopene levels in the hexane extracts were calculated by the formula [8]:

$$\text{Lycopene}\left(\text{mg/kg}\right) = \frac{A_{503} \times 536.9\text{g} \times 10\text{ml}}{17.2 \times 10^4/\text{Mcm} \times \text{kg tissue}} \quad (1)$$

$$\text{Lycopene (mg/kg)} = A_{503} * 31.2/\text{ g tissue} \quad (2)$$

where the molar extinction coefficient of 17.2×10^4/Mcm is that reported by Zechmeister et al.[9] for lycopene in hexane. A_{503} is the absorbance of hexane at 503nm.

3. RESULTS AND DISCUSSION

The absorbance readings were taken from spectrophotometer. The amount of lycopene was estimated by the formula (2). The variation in amount of lycopene extract with respect to varying electric fields were

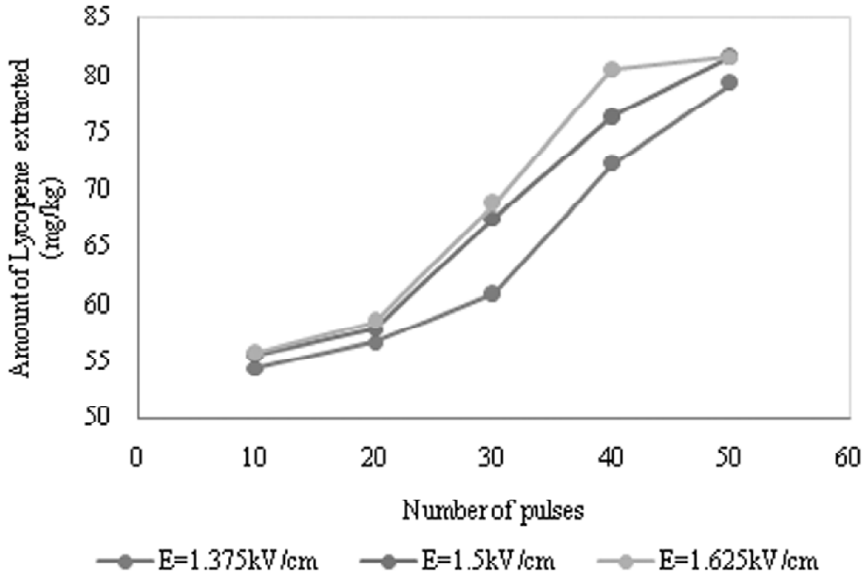

Figure 2: Amount of lycopene extracted from treated tomato for pulse duration of 100μs

analysed. For constant pulse duration of 100µs, the amount of lycopene extracted from the treated tomato pulp increased gradually as the number of pulses and the applied electric field increased which is depicted in Figure 2.The maximum percentage increase in lycopene content was 57.56% using the treatment parameters of 1.625 kV/cm, 100µs and 50 pulses.

For constant pulse duration of 350µs, the amount of lycopene extracted from the treated tomato pulp increased gradually as the number of pulses and the applied electric field increased which is depicted in Figure 3. However, when 1.625kV was applied, lycopene extraction reached the maximum amount at 40 pulses after which there was a decrease in the lycopene extraction. The maximum percentage increase in lycopene content was 65.88% using the treatment parameters of 1.625 kV/cm, 350µs and 40 pulses. Apparently, increasing energy input beyond a threshold could decrease lycopene extraction.

The larger amount of lycopene was extracted when the pulse duration was about 500µs and electric field strength of 1.5kV/cm for 40 pulses which is depicted in Figure 4. However, when energy input was increased by increasing the number of pulses to 50, the amount of lycopene extracted considerably reduced. The maximum percentage increase in lycopene content was 68.63% using the treatment parameters of 1.625 kV/cm, 350µs and 40 pulses.

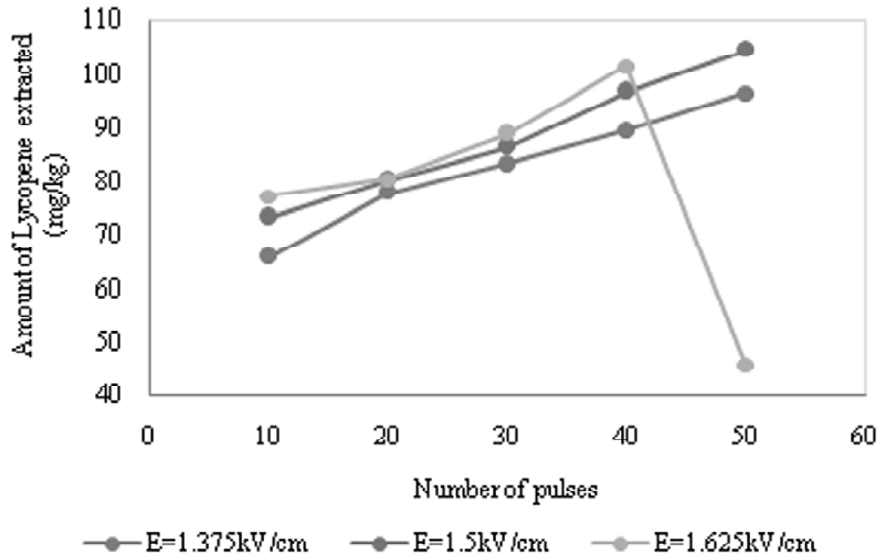

Figure 3: Amount of lycopene extracted from treated tomato for pulse duration of 350µs

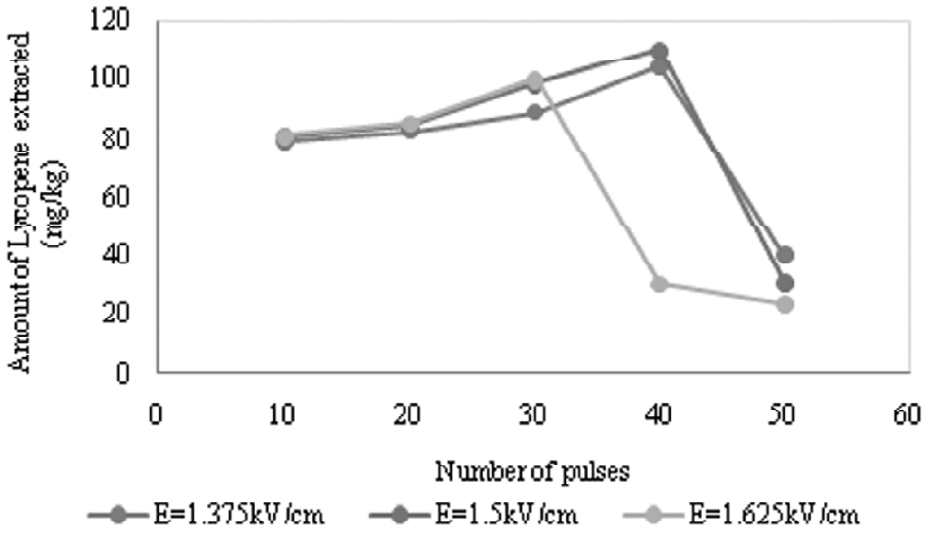

Figure 4: Amount of lycopene extracted from treated tomato for pulse duration of 500µs

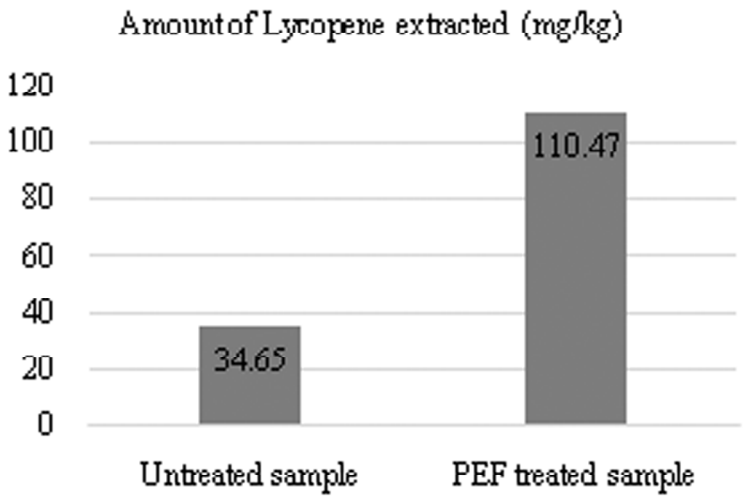

Figure 5: Comparison of results between treated and untreated sample

34.65 mg/kg of lycopene was present in untreated sample whereas PEF treated sample had 110.47 mg/ kg lycopene. PEF treated sample had 68.63% increased extract amount of lycopene than the untreated sample when the electric field was about 1.5kV/cm, pulse duration of 500μs and 40 number of pulses. Maximum energy was found to be 2.1kJ/Pulse. When the energy exceeds the maximum threshold limit, the amount of lycopene extracted drastically reduced.

4. CONCLUSION

Cell wall disruption had been achieved expeditiously when subjected to electric field. Hence, PEF treated samples have high concentration of lycopene than the untreated samples. In particular, field strength of 1.5kV/cm, pulse number 40 and pulse duration of 500μs had a key influence on the cell wall disruption. The PEF pre-treated tomato pulp contained 68.63% higher amount of lycopene than the untreated sample. The results indicate that low electric field applications result in reversible breakdown while higher electric field applications were irreversible. The application of voltage should be optimum for enhanced lycopene extraction.

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