Polyphenol grape extract using Pulsed Electric Field

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Abstract— **Polyphenols are found naturally in plants and they provide much of the flavor, color and taste to fruits and vegetables, seeds and other parts of plants. They also act as antioxidants which provide numerous health benefits by protecting cells against damage caused by free radicals. Polyphenols are anticancer agents which provides protection against prostate cancer and breast cancer. The influence of Pulsed Electric Field (PEF) on the extraction of polyphenols from two types of grapes (black and green) was investigated. Antioxidant level of PEF treated grape samples and untreated grape samples were compared. PEF with electric field strength E= 2.5 to 15 kV/cm, pulse duration= 100 µs, pulse interval= 100 ms and number of pulses 5 to 15 was applied to the sample. The results indicate that the black grapes have higher polyphenol content and exhibits higher antioxidant activity than the green grapes. Applying PEF to grapes enhances the amount of polyphenol and increases antioxidant activity. The optimum values for the enhanced extraction of polyphenols by PEF is found to be 12.75 kV/cm, 10 pulses, 100 µs pulse duration and 100 ms pulse interval.**

I. Introduction

Polyphenols is a bioactive substance which possess many biological activities, such as antioxidant, cardioprotective, anticancer, anti-inflammation, antiaging and antimicrobial properties. Polyphenols are present in grapes, tea leaves, hibiscus leaves, borage leaves, pomegranate, apple, strawberries, raspberries, blueberries, cranberries, onions and certain nuts. Grape is one of the major source of polyphenols which is abundantly cultivated in India. Polyphenol can be extracted from grapes by various conventional extraction methods. In these methods, the time for extraction is higher and the extraction efficiency is not effectively achieved because of the strong nature of the cell wall.

Many researchers are trying to find the optimum technology so that the polyphenols from the grapes can be extracted more efficiently. In the last years, unconventional extraction methods, known as green technologies were applied to increase the extraction of polyphenols [1]. The Pulsed Electric Field (PEF) is an innovative method which enhances the extraction efficiency of bioactive compounds by achieving effective cell wall disruption. Some of the advantages of the PEF treatment compared to conventional treatments are shorter processing time, energy efficient, harmless to environment, etc. [2]. PEF is the application of high voltage pulses to plant foods placed between two conducting electrodes.

The pulsed electric field may be applied in the form of exponentially decaying, square wave, bipolar or oscillatory pulses and at ambient, sub-ambient or slightly above ambient temperature [3]. When applying the pulsed electric field, a critical electrical potential is introduced along the cell membrane, which leads to mechanical changes. These changes help in increasing cell membrane permeability and the formation of pores in membranes enables the enhanced extraction of cell content. Due to this property, PEF treatment is applied in food industry for improving the extraction of bioactive substances [4]. PEF treatment helps in processing grapes to obtain a product that is rich in polyphenols. Our objective was to investigate extraction efficiency of polyphenols with antioxidant capacity from two varieties of grapes (green and black grapes) using pulsed electric field.

II. Materials and Methods

A. Sample Preparation

Fresh black grapes and green grapes are purchased from Pallavaram market. 500 g of green grapes are taken and then washed with tap water and then left to dry in open air away from direct sunlight to remove moisture content and then it is de-clustered. They are crushed in a juicer grinder for 2 min to obtain grape juice. The crushed green grape juice sample of 350 ml is taken and placed in the thimble of soxhlet apparatus. Solvent (300 ml ethanol) is discharged in the round bottom flask. On heating at a temperature of 70 to 80 °C, the solvent evaporates, rises to the condenser, where it condenses and drains back to the extractor. When the extractor becomes full with the hot solvent, the solvent siphons down to the flask along with the extracted constituents, the recycling of the evaporated solvent is allowed to continue until the extraction is complete. The same process is repeated for black grapes.

B. Pulsed Electric Field

Pulsed electric field is applied to the sample using electroporator (ECM 830) in High Voltage laboratory, College of Engineering, Anna University, Guindy, Chennai. Electroporator contains low voltage mode and high voltage mode. The electric field induced on the sample depends upon the applied voltage and distance between the electrode in the cuvette. To treat the samples, the extract is placed in the cuvette chamber which holds approximately 0.5 ml of the sample. The electrode gap of the cuvette chamber is 1 mm. The electrodes are made up of stainless steel and the walls are made of electrical insulation material Teflon. Pulsed electric field is applied to the sample by varying the voltage between 250 V and 1500 V, number of pulses varied between 5 and 15, pulse interval and pulse length is set to be constant as 100 ms and 100 μs respectively. After applying pulsed electric field to the sample, it is transferred from cuvette to a vial and it is used for further analysis.

C. Determination of Polyphenols

The amount of polyphenol of the extract is determined by Folin Ciocalteu method using gallic acid as standard as per ISO 14502-1 [5]. 10 mg of Gallic acid solution is mixed with 100 ml of distilled water. The Gallic acid is pipetted out in the test tube at a rate of 20 μl, 40 µl.....100 µl. The 1/10 ratio of the Folin–Ciocalteu reagent is mixed with distilled water. The prepared solution is pipetted out in the order of 2.5 ml per test tube. The mixture was kept at room temperature for 3 min. 2 ml of sodium bi-carbonate solution (7.5 g of sodium bi-carbonate is added to 100 ml of distilled water) is pipetted out in the test tube. The reaction is allowed to continue for 45 min at 45℃ in water bath. Finally, the absorbance was measured at 765 nm using a Shimadzu UV-1650PC spectrophotometer calibrated against gallic acid, and the results are reported in nm.

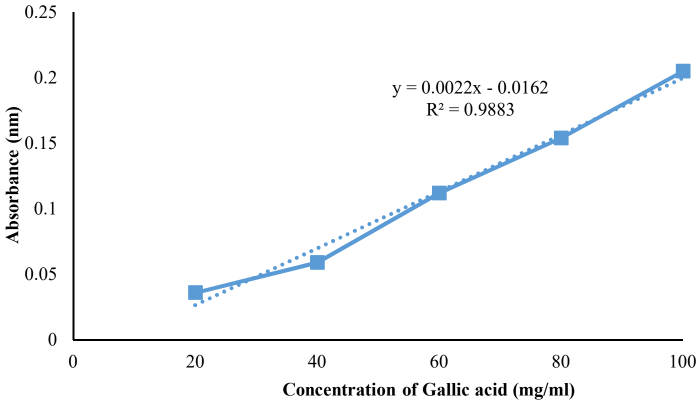

Fig. 1. Gallic acid standard graph

To estimate the amount of polyphenol in grape extracts, 0.5 ml of grape extract is mixed with 2.5 ml of Folin-Ciocalteu reagent (diluted 1:10 v/v) followed by 2 ml of sodium bi-carbonate (7.5% w/v) solution. The tubes are vortexed and total polyphenol is determined after 30 minutes of incubation at 40 ˚C temperature. The absorbance of sample is measured against a blank sample at 765 nm using the same spectrophotometer [6]. The phenol content is extrapolated from the gallic acid standard/calibration graph equation: $y = 0.0022x - 0.0162$, $R^2 = 0.9883$.

D. Antioxidant analysis

The antioxidant capacity of the samples is determined by DPPH (2,2-diphenyl-1-picryl-hydrazyl) free radical method. This method is based on the ability of the antioxidant to scavenge the DPPH cation radical. DPPH radical is stable at room temperature and produces a violet solution in ethanol. DPPH free radical is reduced in the presence of an antioxidant molecule, giving rise to colorless ethanol solution [7]. The measurement of DPPH radical scavenging activity was performed according to methodology described by Brand Williams et al. [8]. 0.5 mL of sample was added to 3 mL of absolute methanol and 0.3 mL of DPPH radical solution (0.5 mM in methanol). It was incubated in dark room for 30 min at room temperature and the discoloration of DPPH was measured against the control. The color changes (from deep violet to light yellow) were read at 517 nm using spectrophotometer. The mixture of methanol and DPPH radical solution serves as blank. Free radical scavenging activity was calculated by the following formula [9].

$$DPPH\ radical\ scavenging\ ability\ (\%) = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100 \qquad (2)$$

Where A_{blank} is absorbance of blank solution and A_{sample} is absorbance of sample.

III. Results and Discussion

A. Effect of PEF treatment on total polyphenol of two varieties of grapes

After applying pulsed electric field to the grape extract, we determined the total polyphenol content through the spectrophotometric method. When treating the green grape extract by pulsed electric field, we noticed 41.28% increase in the total phenolic content than the untreated green grape extract.

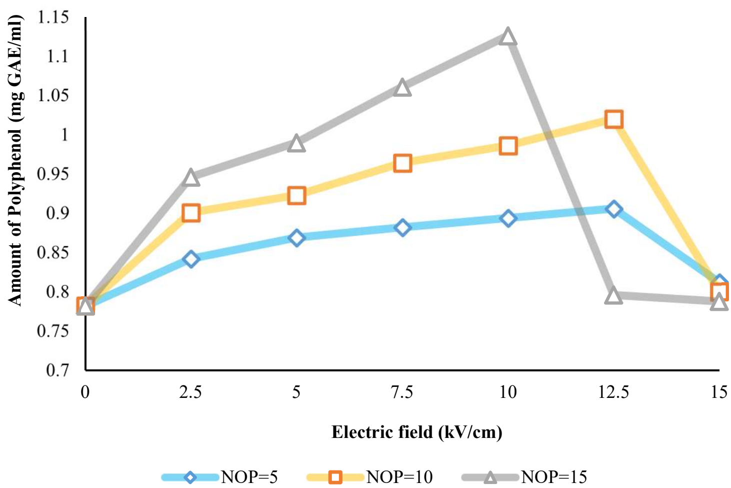

Fig. 2. Amount of polyphenol extracted from green grapes

Extraction profile of PEF treated and untreated green grape extracts for different field strength and number of pulses is shown in Fig. 2. When the electric field is increased from 2.5 kV/cm to 10 kV/cm, there is a gradual increase in the amount of polyphenol. When the field is increased to 12.5 kV/cm for 15 pulses, a spark is bridged between two electrodes which in turn decreased the amount of polyphenol. The amount of polyphenol is almost equal to that of untreated green grape extract. At 12.5 kV/cm, for 5 pulses and 10 pulses, the amount of polyphenol is higher than the untreated sample. At 15 kV/cm for all the pulse numbers, breakdown occurred which in turn decreases the amount of polyphenol. The maximum % increase in polyphenol content from green grape extract was obtained using the PEF treatment parameters: E=10 kV/cm, t_{on}=100 μs, t_{off}=100 ms, n=15.

Extraction profile of PEF treated and untreated black grape extracts for different field strength and number of pulses is shown in Fig. 3. Treating grape extract with 5 and 10 pulses resulted in a steady increase in polyphenol content for all the field levels up to 12.5 kV/cm. However, when 15 pulses were applied, polyphenol content was maximum at 10 kV/cm after which there was a decrease in polyphenol extraction. Apparently increasing energy input beyond a threshold could decrease polyphenol content. An increase of 43.99% in polyphenol content from PEF black grape extract was obtained using the PEF treatment parameters: E=10 kV/cm, t_{on}=100 μs, t_{off}=100 ms, n=15.

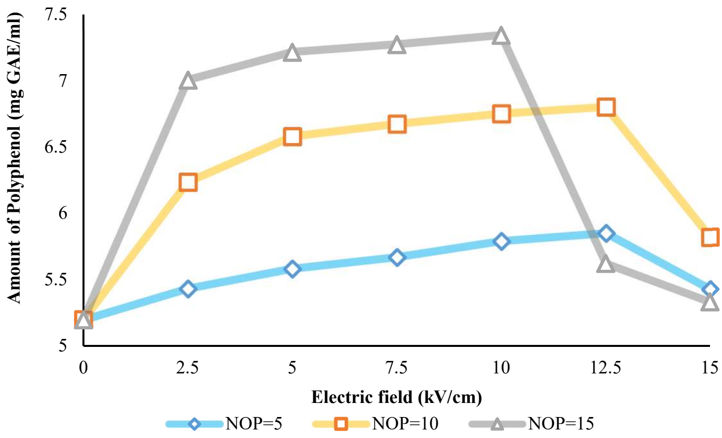

Fig. 3. Amount of polyphenol extracted from black grapes

From this study, it could be concluded that optimum PEF parameters enhances the amount of polyphenol from both the types of grape extracts. The largest amount of polyphenol was obtained from PEF treated black grape extract.

B. Effect of PEF treatment on antioxidant activity of two varieties of grapes

Since the amount of polyphenol extracted by pulsed electric field is higher at E=10 kV/cm, t_{on}=100 µs, t_{off}=100 ms, n=15, these PEF parameters have been chosen for antioxidant analysis. The antioxidant capacity of the grape extract is determined by DPPH radical scavenging assay. Antioxidant capacity depends on the ability of the sample extract to trap the DPPH radical. Higher DPPH radical scavenging ability shows that the antioxidant activity is higher in the grape extract. Both varieties of grape extract shown DPPH radical scavenging ability which in turn proves the presence of antioxidants.

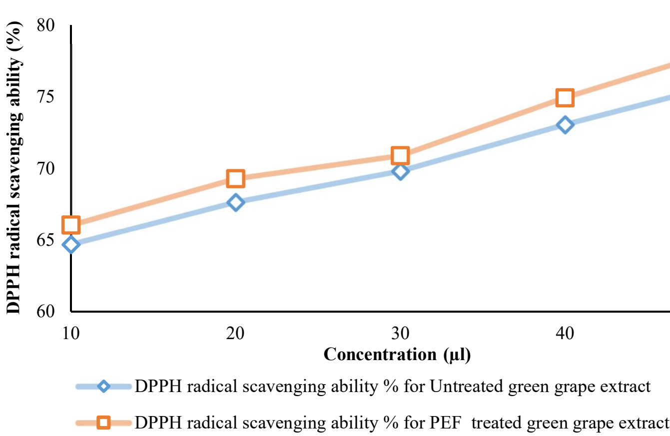

Fig. 4. Antioxidant activity of green grapes

The antioxidant analysis of PEF treated and untreated grape extracts has been done by varying the initial concentration of the grape extract. As the concentration of the grape extract increase from 10 µl to 50 µl, apparently the antioxidant activity is also increased. The results regarding the antioxidant activity of PEF treated and untreated grape extracts are presented in Fig. 4 and Fig. 5.

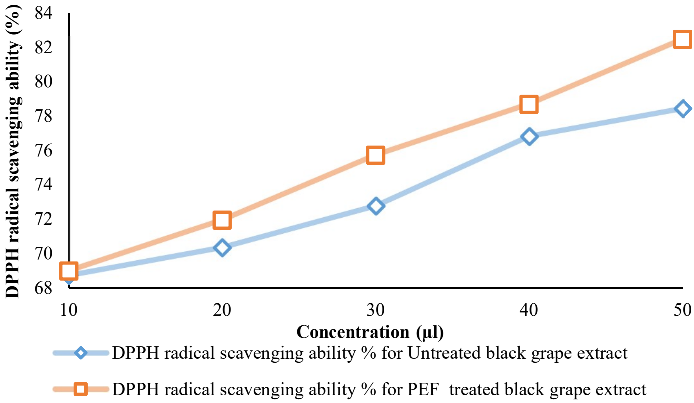

Fig. 5. Antioxidant activity of black grapes

From 10 µl to 40 µl of initial concentration of grape extracts, the difference between antioxidant activity of PEF treated and untreated grape extract is less. But for 50 µl concentration of green grape extract, the antioxidant activity of PEF treated green grape extract is 1.03 times higher than the untreated green grape extract. And for the same concentration (50 µl) of black grape extract, the antioxidant activity of PEF treated black grape extract is 1.05 times higher than the untreated black grape extract. For 50 µl concentration of grape extract, we noticed that the antioxidant activity of PEF treated black grape extract is 82.47% which is 1.05 times higher than the antioxidant activity of PEF treated green grape extract.

IV. Conclusion

PEF treatment helps in enhancing the extraction of polyphenols and also in increasing the antioxidant activity. PEF treatment enhanced extraction of polyphenols from green grape extract by 1.44 times than the conventional extraction. PEF treatment enhanced antioxidant activity of green grape extract by 1.03 times than the untreated extract. PEF treatment enhanced extraction of polyphenols from black grape extract by 1.41 times than the conventional extraction. PEF treatment enhanced antioxidant activity of black grape extract by 1.05 times than the untreated extract. Pulsed electric field assisted extraction is cost effective, saves time, energy efficient and harmless to environment. For industries that require alcoholic drinks which contain higher amount of polyphenol and higher antioxidant activity, the proposed method can be used.

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