Journal of Cancer Prevention & Current Research

Research Article

Open Access

CrossMark

Enhancement of Bioactive Compounds from Green Grapes Extract using Pulsed Electric Field treatment

Abstract

Grapes, one of the world's largest fruit crops and most commonly consumed fruits in the world, are rich in antioxidants and phytochemicals. Grape extract comprises several bioactive compounds, mostly represented by polyphenols. In this study, the amount of polyphenol and antioxidant activity of Pulsed Electric Field (PEF)-treated and untreated grape extract is investigated. The results indicate PEF treated extract have higher antioxidant activity and high amount of total phenolics compared to untreated one, indicating the potency of PEF-treated extract to be more effective.

Volume 9 Issue 5 - 2018

Jeya Shree T,[1] Gowri Sree V,[1] Raji Sundararajan[2]

[1]Division of High Voltage Engineering, Department of Electrical and Electronics Engineering, College of Engineering Guindy, Anna University, Chennai

[2]School of Engineering Technology, Purdue University, USA

Correspondence: Raji Sundararajan, 187, Knoy Building, Purdue University, West Lafayette, IN 47906, USA, Tel (1) 765 494 6912, Email raji@purdue.edu, rajnish.sharma@chitkarauniversity.edu.in

Received: July 30, 2018 | **Published:** September 14, 2018

Introduction

An ounce of prevention is better than a pound of treatment. Natural (fresh and raw) fruits, like grapes are fully loaded with phenolic compounds that are anti-oxidant, anti-inflammatory, anti-platelet aggregation, anti-carcinogenic, cartilage-protective, and anti-aging properties. Resveratrol, a type of natural phenol, found in grapes, modulates the blood glucose response by effecting how the body secretes and uses insulin. This compound also improves the endothelial function.[1] Some studies have revealed that polyphenol administration improves insulin sensitivity in diabetic rats and patients with T2D. Dietary polyphenols have been suggested to lower the risk of T2D.[2] Hence grapes that are rich in polyphenols is a good choice for diabetes patients keeping its nutritional profile in mind. It can be very well used for cancer prevention.

Grapes are also a great choice for people with diabetes. Diabetes patient often avoid fruits because of the sugar content in it (Figure 1). Grapes don't tend to highly increase blood sugar, but they highly uplift insulin levels. Since high insulin levels cause insulin resistance, choosing foods with a lower insulin response rate, like grapes, can thwart the insulin resistance characteristic development in Type 2 Diabetes (T2D).[3]

Figure 1 Beneficial health effects of dietary polyphenols. Cellular, molecular, and biochemical actions of dietary polyphenols. Dietary polyphenols play an important role in inflammation, apoptosis, angiogenesis and auto-immune diseases.

Since a large amount of fruits have to be consumed, it is of practical interest to take the extracts. We hypothesize that using pulsed electric fields we can extract more amount than conventional methods. Towards this, in this research, we explored the potential of grape extract as a great source of antioxidant using PEF treatment. PEF treatment has been evidenced as a promising technology for enhancing the extraction of bioactive compounds and antioxidants.[4] PEF treatment is the application of high-frequency, electric pulses with high intensity field strengths, which can modulate the activity of biological membranes, which in turn enhances the bioactive compounds present in the food.[5] In this study, the amount of polyphenol and antioxidant activity of PEF treated and untreated grape extract is investigated.

Materials and methods

Sample preparation

500g of the green grapes were taken and then washed with tap water and then dried in shade to remove the moisture content and then it was de-clustered. They were crushed in a juicer grinder for 2min and 350ml of grape juice was obtained. The Soxhlet extraction was used to extract the bioactive compounds and the antioxidants present in the grapes. In this extraction method, the extraction solvent (Ethanol) was placed in the boiling flask. The chamber containing the extract was connected above the boiling flask. On heating boiling flask at a temperature of 70°C to 80°C, the solvent was evaporated and re-condensed in the distillation column above and drained back to the chamber containing extract. The Pythagorean cup allowed the chamber to fill the solvent to a point, above which it emptied its contents in to the boiling flask by a syphoning mechanism. The recycling of the evaporated solvent was allowed to continue until the extraction was complete. The extracted compounds were accumulated in the boiling flask.

Electrical pulse application

Electrical pulses were generated with a BTX ECM 830 electroporator. Electroporation is achieved by varying voltage from 5-3000 V, pulse duration from 10µs to 10s, pulse interval from 100ms to 10s and number of pulses from 1 to 99. To apply electrical pulses, the extract was placed in the cuvette chamber (electrode gap=1mm) which holds approximately 100µl of the sample. All experiments were performed in triplicates. After applying electrical pulses to the grape extract, it was transferred from cuvette to a vial and it was used for further analysis.Estimation of polyphenols for both untreated and PEF treated grape extract by Gallic acid standard.

Estimation of polyphenols

Solution preparation: Gallic Acid (GA) was diluted in Distilled Water (DW) in the ratio of 1:10w/v (10mg GA in 100ml DW). Folin–Ciocalteu Reagent (FCR) was diluted in distilled water in the ratio of 1:10w/v (10ml FCR in 100ml DW). Sodium carbonate (Na_2CO_3) was diluted in distilled water in the ratio of 7.5w/v (7.5g Na_2CO_3 in 100ml DW).

Gallic acid standardisation**:** The Gallic acid was pipetted out in the test tubes at a rate of 20µl, 40µl, 60µl, 80µl, 100µl. In each test tube, 2.5ml FCR solution and 2ml Na_2CO_3 was added. The reaction is to be allowed to continue for further 45min at 45˚C in water bath. Finally, the absorbance at 765nm was measured using a Shimadzu UV-1650PC spectrophotometer.

Estimation of Polyphenols: The total phenol content in the extract was determined by Folin Ciocalteu method. In brief, 200µl of grape extract was mixed with 2.5ml of FCR (diluted 1:10v/v) followed by 2ml of sodium carbonate (7.5w/v) solution. The tubes were vortexed and total polyphenols were determined after 1h of incubation at room temperature. The absorbance of sample was measured at 765nm using a UV-1650PC spectrophotometer.[6]

Antioxidant analysis

The antioxidant capacity of the grape extract 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.

Results and discussion

Total phenol determination

After applying pulsed electric field to the grape extract, we determined the total polyphenol content by Folin Ciocalteu method. Extraction profile of PEF treated and untreated green grape extracts for different field strength and number of pulses is shown in Figure 2. When the electric field is increased from 2.5kV/cm to 10kV/cm, there is a gradual increase in the amount of polyphenol. When the field is increased to 12.5kV/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 12.5kV/cm, for 5 pulses and 10 pulses, the amount of polyphenol is higher than the untreated sample. At 15kV/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=10kV/cm, t_{on}=100µs, t_{off}=100ms.

When treating the green grape extract by pulsed electric field, we noticed 41.28% increases in the total phenolic content than the untreated green grape extract.

Antioxidant activity

Since the amount of polyphenol extracted by pulsed electric field is the highest at E=10kV/cm, t_{on}=100µs, t_{off}=100 ms, n=15, these PEF parameters have been chosen for anti-oxidant 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.

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 10µl to 50µl, apparently the antioxidant activity is also increased. Figure 3 shows the results regarding the antioxidant activity of PEF treated and untreated grape extracts.

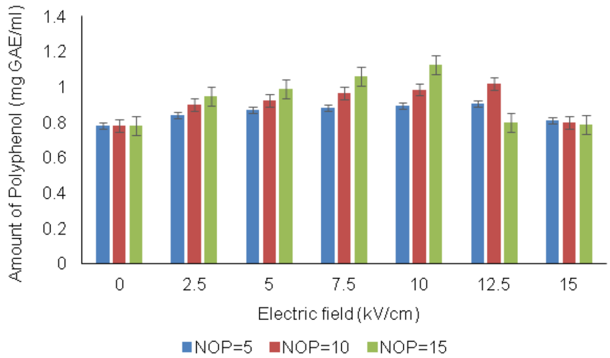

Figure 2 Amount of polyphenol extracted from green grapes.

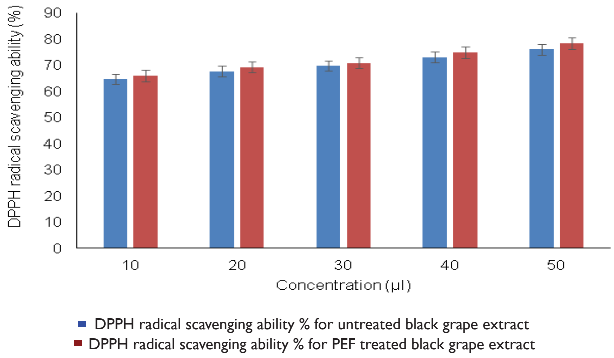

Figure 3 Antioxidant activities of green grapes.

Conclusion

PEF treatment enhanced the extraction of polyphenols and also in increasing the antioxidant activity. PEF treatment enhanced extraction of polyphenols from green grape extract by 1.44times than the conventional extraction. PEF treatment enhanced antioxidant activity of green grape extract by 1.03times than the untreated extract.

Pulsed electric field assisted extraction is cost effective, saves time, energy efficient and harmless to environment. Hence PEF treated grape extracts that are rich in polyphenols and antioxidant is a good choice for diabetes patients.

Acknowledgements

Authors thank Prof. M. Sivanantham, Secretary, Sri Venkateswara College of Engineering for providing us the necessary facilities to carry out this project.

Conflict of interest

Author declares that there is no conflict of interest.

References

1. Zhu X, Wu C, Qiu S, et al. Effects of resveratrol on glucose control and insulin sensitivity in subjects with type 2 diabetes: systematic review and meta-analysis. *Nutrition & Metabolism*. 2017:14(1).
2. Lall R, Syed D, Adhami V, et al. Dietary Polyphenols in Prevention and Treatment of Prostate Cancer. *International Journal of Molecular Sciences*. 2015;16(2):3350–3376.
3. https://www.sun-world.com/grapes-nutrition/grapes-and-diabetes.
4. Shree TJ, Sree VG, Priyanka A, et al. Antioxidant and Anticancer Activity of Pulsed Electric Field Treated Grape Extract. *Journal of Nanomedicine & Biotherapeutic Discovery*. 2018:8(1).
5. Ricci A, Parpinello G, Versari A. Recent Advances and Applications of Pulsed Electric Fields (PEF) to Improve Polyphenol Extraction and Color Release during Red Winemaking. *Beverages*. 2018;4(1):18.
6. ISO 14502-1: Determination of substances characteristic of green and black tea--Part 1: Content of total polyphenols in tea-Colorimetric method using Folin-Ciocalteu reagent. *International Organization for Standardization (ISO)*. 2005. 10p.