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Pulsed Electric Field for Enhanced Extraction of Intracellular Bioactive Compounds from Plant Products: An Overview

Jeya Shree Thulasidas*, Gowri Sree Varadarajan and Raji Sundararajan

[1]*Division of High Voltage Engineering, Department of Electrical and Electronics Engineering, CEG Campus, Anna University, Chennai, India*

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

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***Corresponding author:** Jeya Shree Thulasidas, Division of High Voltage Engineering, Department of Electrical and Electronics Engineering, CEG Campus, Anna University, Chennai, India

Abstract

Bioactive compounds are additional nutritional constituents that are present in plants. Bioactive compounds have numerous health effects. They exhibit anti-diabetic, anti-mutagenic, anti-oxidant, and anti-cancer properties. Bioactive compounds help in the treatment of cancer, increasing bone strength, strengthening the immune system and lowering cholesterol. Currently, the demand for natural bioactive compounds is increasing due to their use in the functional food industries as well as in pharmaceutical industries. Extraction of bioactive compounds from the plant sources efficiently and harmlessly is the biggest task for food and pharmaceutical industry.

Many researchers have been focusing on finding cost effective and harmless method for extraction of bioactive compounds from naturally available plant sources. Although there are several conventional extraction methods, pulsed electric field extraction technique is found to be more efficient method for industrial application. Pulsed electric field-assisted extraction overcomes the drawbacks of the existing extraction methods. Pulsed electric field extraction provides higher yield of bioactive active compounds within shorter time. This review is a study of application of pulsed electric field in the extraction of bioactive compounds.

Keywords: Lycopene; Polyphenol; Pulsed electric field; Electroporation; Extraction; Alkaloids; Anthocyanins; Betanine; Betulin; Bioactive compounds; Carotenoids

Introduction

The Pulsed Electric Field (PEF) is one of the growing and attractive applications of High Voltage Engineering. Pulsed electric field induces transmembrane potential in the cell membrane which causes increase in the conductivity and permeability of the cell membrane [1]. This increase in the permeability causes reversible breakdown or irreversible breakdown on the cell membrane. Both breakdowns find its applications in biomedicine, environment, food processing and food preservation [2].

In the field of biomedicine, reversible electroporation finds its applications in cancer treatment by introducing cytotoxic drugs into tumour cells by the process called Electrochemotherapy [3], improving fusion yields with cells of different sizes by the process of electrofusion [4], transferring genes/DNA into eukaryotic cells and bacteria [5,6], enhancing transdermal drug delivery [7], inserting proteins into cell membrane and delivering foreign DNA into cells [8].

In the field of food processing and preservation, reversible electroporation pulsed electric field is widely used in enhancing juice extraction from various plant tissues and microorganisms [9], producing stable dehydrated products by osmotic dehydration [10], preserving milk by reducing the activity of bacteria and other microorganisms by the method of pasteurization [11], to killing microorganisms by non-thermal sterilization [12], preserving food by non-thermal microbial inactivation [13] and enhancing extraction of valuable or bioactive compounds from plant tissue and microorganisms [14]. Environmental applications of reversible electroporation include production of biofuel from microalgae with low energy use [15], disinfection of waste water by inactivating bacterial population [16] and treating organic waste by inactivating microorganisms and destroying sludge structure [17].

Irreversible electroporation causes cell death and hence it is used in tissue ablation for cancer treatment [18,19]. Irreversible electroporation is used in cryopreservation of biological tissues, plant metabolism stimulation, plant and animal raw material disintegration [20]. Extraction of bioactive compounds from plant sources can be done by various conventional extraction methods such as, soaking, maceration, boiling, grinding, magnetic stirring, water percolation, heat refluxing, soxhlet extraction, membrane separation technology, hydro-distillation, etc. These extraction methods have many drawbacks such as, low extract yields, poor extraction efficiency, long processing time, more solvent consumption [21].

Industries need an innovative technique to extract bioactive compounds and manufacture the products efficiently, reliably, cheaply, and eco-friendly. To meet the industry's requirements, many innovative extraction techniques were proposed and the potential of application of them was evaluated by researchers. Innovative techniques include supercritical fluid CO_2 extraction, pressurized liquid extraction, enzyme-assisted extraction, accelerated solvent extraction, micro-wave-assisted extraction, and ultrasonic-assisted extraction [22]. These innovative methods overcome the drawbacks of conventional method which has been proven by comparing the results of novel method and conventional method. The pulsed electric field-assisted extraction is the recent advancement in the field of extraction.

Pulsed electric field is the application of high voltage pulses for a very short span of time in the range of nanoseconds to micro seconds through the sample placed between two conducting electrodes [23]. Pulsed electric field-assisted extraction has been proved to be a cost-effective technique which is energy efficient, time saving and harmless to environment [24].

Pulsed electric field on enhanced extraction of bioactive compounds

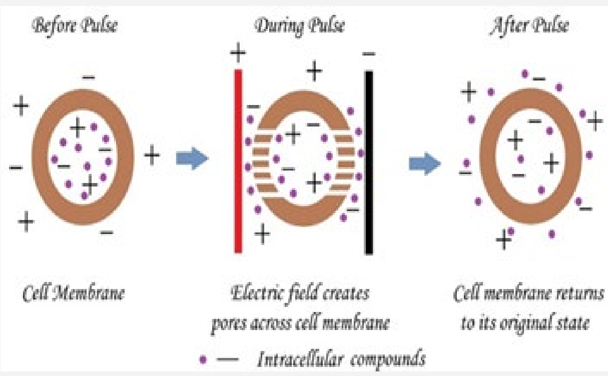

Figure 1: Process of enhanced extraction. Schema redrawn based on Figure 1 in [1]

When high voltage electrical pulses are applied to the electrodes, the sample experiences a force per unit charge called electric field. The electric field can be applied in the form of exponentially decaying, square-wave, triangular, unipolar, bipolar or oscillatory pulses. According to the electrical field, electroporation can be either reversible or irreversible, but this effect can be regulated depending on the application [25]. During the exposure of living cells to electric field, electric potential which passes through the cell membrane increases as a result of charging at membrane interfaces [21] (Figure 1).

Exposing cells to electric field separates molecules according to their charge under the dipole nature of cell membrane molecules. Pulsed electric field temporarily undermines the lipid bilayer and the proteins of cell membranes [18]. It is believed that Pulsed Electric Field creates pores on the cell membranes which accounts for transfer of large molecules across the cell membrane. The optimal pulsed electric field does not affect the intracellular membranes because the outer membrane shields the intracellular membrane [21]. For enhanced extraction, optimal pulsed electric field is applied to the sample which leads to reversible electroporation on the cell membranes.

It increases the membrane permeability and thus facilitates the mass transfer of intracellular compounds to the surrounding solution through the temporarily created pores [26]. Food contains several ions that provide a definite level of electrical conductivity to the product. This technique is usually preferred for liquid foods because electrical current flows into the liquid food more efficiently and the transfer of pulses from one point to other in liquids is quite easy due to the presence of charged molecules [27].

Antioxidants as a group help protect the cells in your body from free radical damage. If the body does not get adequate protection, free radicals can become rampant, causing the cells to perform poorly. This can lead to tissue degradation and put you at risk of diseases such as, heart disease, cancer and atherosclerosis [28]. Bioactive compounds possess antioxidant properties and are naturally present in intracellular matrix of food and animals. It can also be synthetically produced. Pulsed electric field has proven to be a promising method for enhancing the extraction of valuable bioactive compounds.

Table 1 shows the optimum values for the application of PEF to achieve enhanced intracellular bioactive compounds, such as polyphenol, anthocyanin, carotenoids, lycopene, lutein, betanine, alkaloids, betulin, lactase, protein, polysaccharides, lipid, oil, etc. from various plant products. Polyphenols are phytochemicals found richly in natural plant food sources that have antioxidant properties. There are over 8,000 identified polyphenols found in foods such as tea, wine, chocolates, fruits, vegetables, and extra virgin olive oil, etc. Anthocyanins, Flavonoids such as isoflavones and flavanols, Tannins and Catechins are the major polyphenols found in many food sources [25-54]. Polyphenols has been extracted from the red grape pomace [55], orange juices [56], orange peel [31] and tea leaves [32] by pulsed electric field. The authors discussed the effects of electric field strength, treatment time, pulse number, temperature and pH on polyphenol extraction.

Varying the pulse parameters alters the extraction of polyphenols. Polyphenols have been extracted from the red

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grape pomace by pulsed electric field. The authors discussed the effects of electric field strength on polyphenols extraction. The highest extraction yields have been obtained when the field strength is increased. At optimum field strength, the polyphenol content was 36% more than untreated samples [29]. From this, it is evident that electric field strength plays a major role in the extraction of bioactive compounds.

Table 1: Pulsed Electric Field protocol for various food products

S. No	Extract	Products	Optimal Values for PEF Application	References
1	Polyphenol	Red grape pomace	E-400 V/cm, n-2, t_p-2000±1 µs, Δt- 20 ms	[29]
		Orange juice	E-35 kV/cm, f-800 Hz, t_p-4 ms, t_{PEF}-750 ms	[30]
		Orange peel	E-5 kV/cm, n-20, t_p-3 µs, t_{PEF}-60 µs	[31]
		Tea leaves	E-0.9 kV/cm, t_{PEF}-0.5 s µs	[32]
		Green grapes	E-10 kV/cm, t_{PEF}-100 µs, n-15	[33]
2	Anthocyanin	Merlot grapes	U-7 kV, f-178 Hz, t_{PEF}-150 s	[34]
		Raspberry	E-3 kV/cm, t_{PEF}-15 µs, n-420	[35]
		Potato	E-3.4 kV/cm, t-3 µs, n-35 t_{PEF}-105 µs, W-8.92 kJ/kg	[36]
		Red cabbage	E-2.5 kV/cm, t_{PEF}-15 µs, n-50, W-15.63 J/g	[37]
		Blueberry	E-3 kV/cm, W-10kJ/kg, f-10Hz, t_p-20 µs	[38]
3	Carotenoids	Carrot	E-0.6 kV/cm, t_{PEF}-3 ms, t_p-20 µs, f-5 Hz	[39]
		Tomato	E-25 kV/cm, t_{PEF}-110 µs	[40]
		Orange juice	E-35 kV/cm, f-800 Hz, t_p-4 ms, t_{PEF}-750 ms	[41]
4	Lycopene	Tomato	E-1.5 kV/cm, n- 40, t_p- 500 µs	[42]
		Watermelon	E-35 kV/cm, f- 200 Hz, t_p- 50 µs	[43]
5	Lutein	Microalgae	E-25 kV/cm, t_{PEF}-150µs	[44]
6	Betanine	Beetroot	E-7 kV/cm, W-2.5 kJ/kg, f-1 Hz, n-5, t_p-2 µs	[45]
7	Alkaloids	Korean Monkshood	E-20 kV/cm, n-8, t_{PEF}-60 s	[46]
		Potato peel	E-0.75 kV/cm, t_{PEF}-600 µs	[47]
8	Betulin	Mushroom	E- 40kV/cm, t_p- 2 µs	[48]
9	Lactase	Kluyveromyces lactis	E-30 kV/cm, n- 100	[49]
10	Protein	Microalgae	E-38 kV/cm, f- 158 Hz, t_p-232 µs	[50]
11	Polysaccharides	Corn silk	E-30 kV/cm, t_p- 6 µs	[51]
12	Lipid	Microalgae	U-45 kV, E- 45kV/cm, t_p-10 s, t_{PEF}-30 s, n-3	[52]
13	Oil	Sunflower seeds	E-7 kV/cm, f- 15 Hz, t_p- 20 µs, t_{PEF}- 30 s	[53]
		Olive paste	E-2 kV/cm, f- 125 Hz, W-5.22 kJ/kg	[54]

E: Electric Field Strength; U: Voltage; W: Energy Input; C: Capacity; F: Frequency; t_p: Time of One Pulse; n: Number of Pulses; t_{PEF}: Total Time of PEF Treatment; Δt: Time Between Pulses

Anthocyanins are natural colorants belonging to the flavonoid family. They are widely distributed among flowers, fruits, and vegetables. Anthocyanin pigment content has a critical role in the colour quality of many fresh and processed fruits and vegetables [57]. Anthocyanin has been extracted from the Merlot grapes [34] and its by-products [58], red raspberry [35], potato [36], cabbage [37] and blueberries [38] by pulsed electric field. The authors discussed the effects of electric field strength, treatment time, pulse number, temperature and pH on anthocyanin extraction. The amount of anthocyanin extraction increases by altering the pulse parameters.

Carotenoids are also called tetraterpenoids. They are organic pigments that are produced by plants and algae, as well as several bacteria and fungi. There are over 600 known carotenoids. α-Carotene, β-Carotene, Lycopene, Lutein and Zeaxanthin are some of the main carotenoids commonly found in many food sources [59]. Carotenoid has been extracted from the carrot [39], tomato [40] and orange juice [41]. Carrot is one of the major sources of carotenoids. Carrot puree has been placed between the electrodes with the gap of 80 mm. Pulsed electric field has been applied to carrot puree with electric field strengths up to 1 kV/cm at 5 Hz. The content of carotenoids has been significantly higher compared to the untreated samples [39].

Betanine is a red carotenoid pigment which is found in beetroots. Red beetroot discs have been treated in a batch parallel plate electrode treatment chamber having an electrode gap of 5 mm and 4.9 cm^2 surface area. The exponential decay pulses at different electric field strengths, specific energies per pulse, and frequencies have been applied to the beetroot slices.

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The application of pulsed electric field with a field strength of 7 kV/cm (2.5 kJ/kg) has enabled 90% of betanine extraction in 35 minutes. The application of pulsed electric field treatment with a field strength 7 kV/cm has increased the betanine yield by 4.2 times when compared to non-treated samples [45].

Alkaloids have been extracted from potato peels by pulsed electric field. At a field strength of 0.75 kV/cm and 600 µs pulse duration, the amount of alkaloid is 1856.2 µg/g which is 99.9% higher recovery rate than the conventional method Hossain et al. [47]. Five methods (maceration extraction, percolation extraction, heat reflux extraction, ultrasonic-assisted extraction and pulsed electric field-assisted extraction) for the extraction of alkaloids from Korean monkshood (Aconitum coreanum) have been investigated. The amount of alkaloid of the four methods has been compared with the pulsed electric field method. The highest yield of alkaloid was 3.94 mg/g by PEF with conditions of 20 kV/cm electric field intensity, 8 pulses with the total treatment time less than 1 min. The results indicated that the higher extraction yield is achieved for ultrasonic-assisted extraction and pulsed electric field-assisted extraction. When compared between these two methods, PEF method provides the higher extraction yield, lower energy consumption, and at a shorter time, without adverse effects to human health [46].

Betulin is a bioactive compound which has been extracted from chaga mushroom (Inonotus Obliquus) by applying high intensity pulsed electric field. Exponentially decaying bipolar triangle pulse with a pulse duration of 2 µs and a field strength of 40 kV/cm has been applied to the sample placed between two stainless steel electrodes with a gap of 1 mm. PEF-assisted extraction has 1.3 times higher amount of betulin than the conventional extraction [48].

High voltage pulsed electric field with the electric field intensity of 30 kV/cm, electrode gap of 2 mm, 100 number of pulses has been applied to Kluyveromyces lactis. Pulsed electric field treatment enhanced the extraction of intracellular lactase from Kluyveromyces lactis to the culture medium. The lactase enzyme collection rate was 6.2% higher than the untreated sample [49]. Table 2 shows the extraction amount of PEF treated and untreated samples of various plant products with its enhancement factor. From various studies, it has been proved that, PEF helps in enhancing the extraction amount of intracellular bioactive compounds.

Table 2: Comparison of extraction amount of PEF treated and untreated samples of various plant products

S. No.	Extract	Products	Amount of Extract		Enhancement Factor of PEF Treated Sample	References
			Untreated	PEF Treated		
1	Polyphenol	Tea leaves	2.50%	27.50%	11	[32]
		Green grapes	0.782 mgGAE/ml	1.126 mgGAE/ml	1.03	[33]
2	Anthocyanin	Merlot grapes	880 mg/L	1100 mg/L	1.25	[34]
		Red raspberry	12.95%	54.98%	4.25	[35]
		Potato	8.1 mg/100g	63.9 mg/100g	7.8	[36]
		Red cabbage	142%	379%	2.67	[14]
		Blueberry	28.3 mg/100 mL	50.2325 mg/100mL	3.12	[38]
3	Carotenoids	Orange	1789.69±108.36 µg/100g	1893.49±112.23 µg/100g	1.06	[41]
4	Lycopene	Tomato	34.65 mg/kg	110.47 mg/kg	3.18	[42]
		Watermelon	87.60%	121.20%	1.38	[43]
5	Lutein	Microalgae	163.10±6.91 µg/g	753.09±36.61 µg/g	4.62	[44]
6	Alkaloids	Korean Monkshood	11.70%	36.25%	3.098	[46]
7	Betulin	Mushroom	5.41±0.08 g/kg	6.83±0.10 g/kg	1.26	[48]
8	Polysaccharides	Corn silk	6.18±0.43%	7.31±0.15%	1.18	[51]
9	Betanine	Beetroot	23.60%	99%	4.2	[45]
10	Lipid	Microalgae	2600±800 µg/L	6100±1600 µg/L	2.34	[52]
11	Oil	Olive	4.75%	13.77%	2.89	[54]

Conclusion

When compared with the other extraction techniques, pulsed electric field-assisted extraction is a promising technology for enhancing extraction of bioactive compounds from the various plant sources. The results indicate that PEF-assisted extraction have higher extract yield of bioactive compounds, consumes lower energy and less treatment time, providing the optimum process parameters. The effects of process parameters on the extraction of bioactive compounds and optimal parameter for extraction of several kinds of plant products had been discussed. To save energy cost, square wave pulses with larger pulse width can be used. From the practical point of view, development of pulsed electric field technology is very important for improvement on industrial extraction processes.

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