Food Bioscience

Testing of a new high voltage electrical discharge generator prototype at high frequencies to assist anthocyanin extraction from blueberries

--Manuscript Draft--

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Abstract:	Traditional extraction methods are based on high-temperature maceration with organic solvents, which are dangerous for human health. A viable alternative to overcome the issues associated with conventional extraction is to increase cell tissue permeability by applying high voltage electrical discharge (HVED) treatments. The objective of this work was to validate the electroporation of blueberry plant cells using a new HVED generator prototype at a high frequency, investigate the effect, intensity, and duration of the applied voltage, and recover anthocyanins from its electroporated cells. The electroporation level of the HVED-treated blueberries was measured qualitatively by transmission electron microscopy (TEM) analysis. Meanwhile, it was quantitatively measured by the cell permeabilization index (Zp) and anthocyanin extraction level. Results of the micrographs (TEM) showed electroporation in all treatments in which Zp was 0.24 when applying a 2 kV treatment for 2 s, whereas a 3-fold increase in tissue damage was revealed with the most powerful treatment (10 kV voltage, 30 s). In addition, anthocyanin values ranged from 83.09 ± 1.20 (control) to 136.82 ± 0.84 (HVED), which was 64.66% higher. The HVED treatment can increase mass transfer rates during conventional extraction processes. It should be noted that the validated prototype required a low specific energy requirement (31 to 204 kJ/kg) for proper tissue electropermeabilization. In conclusion, we demonstrated the capability of the developed HVED prototype to boost mass transfer phenomena and thus potentially increase its adaptability to assist dissimilar industrial processes or waste (e.g., peels and seeds) such as freeze-drying operations.
Suggested Reviewers:	Jaime Ortíz, PhD Professor, University of Chile jaortiz@uchile.cl Extraction of bioactive compounds from food Functional properties of food
	Erick S. Scheuermann, PhD Professor, University of the Frontier ericks@ufrontera.cl Experience in bioactive compound extraction Food processing
	Alejandro Reyes, PhD Professor, University of Santiago Chile alejandro.reyes@usach.cl Expert in Food Processing Extraction of bioactive compounds

Opposed Reviewers:	
Response to Reviewers:	

Dear Editor in Chief
Giuseppe Spano, PhD

Attn
Guest editor
Dr. Carlos L. Cespedes

This paper leads to an alternative to recover compounds from fruits or waste by permeabilizing the cell membrane using a prototype of high voltage electrical discharge (HVED) generator. This equipment was developed with the aim of investigating the effect of the main processing parameters, intensity and duration of the applied voltage, on the degree of electroporation of fresh blueberries and the recovery of anthocyanins from their skins after methanolic extraction. The level of electroporation produced by the HVED system was assessed quantitatively (cell disintegration index, ZP) or qualitatively by transmission electron microscopy (TEM) analysis.
We demonstrated the ability of the developed HVED prototype to drive mass transfer phenomena and thus potentially increase its adaptability to assist different industrial processes or waste (eg husks and seeds)
We believe that the present work is of interest to food scientists and engineers involved in compound extraction or industrial waste recovery, especially for those working on an industrial scale.
We confirm that this work is original; it has not been published elsewhere nor is it currently under consideration for publication elsewhere.

Thank you for your consideration of this manuscript.

Sincerely

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The authors would like to thank the editor and all the reviewers for the time and effort spent reviewing our manuscript and for their useful and effective inputs that helped us to further improve it. The changes made by the authors, in response to the reviewers' comments, are reported below.

Reviewer #2

- Line 64 remove space before "In Conclusion)

Answer: This recommendation has been considered in the new version of the manuscript, therefore the space was removed as gently required in page 2, line 31.

- Line 44 and 53 Provide space before starting every new paragraph. Also, correct it throughout the manuscript.

Answer: This recommendation has been considered in the new version of the manuscript; spaces were added before each paragraph.

- Line 111 Provide equipment details for oven dryer.

Answer: This recommendation has been considered in the new version of the manuscript, hence the information was properly incorporated in page 05 lines 111.

- Line 110 Include the moisture content data. On dry or wet basis?

Answer: This recommendation has been considered in the new version of the manuscript and the missing information was added in page 05 lines 111.

- Line 116 -118 clear the formatting and color for % values for chemical reagent and purity correction required for previous comments.

Answer: This recommendation has been considered in the new version of the manuscript. We deleted the format and color of the % values for the chemical reagent, and the purity percentages were also reported in page 06 lines 118 to 121.

Authors have failed to respond the following comments, without suitable justification it is difficult to comment on this manuscript.

Conclusion - Conclusion section is too lengthy; authors should reduce it and include the outcome of research with future scope related to this study.

Answer: This recommendation has been considered in the new version of the manuscript. Conclusion were reduced (page 17, lines 373-384) and future scopes (lines 382-384)

Section 2.1 - Authors should clearly respond to each comment, read properly and respond them. The utilized blueberries samples were fresh or not? Why did authors purchase blueberries from fruit vendor rather than fresh farm? Authors should include the detail of harvesting of purchased blueberries samples? How long it was stored in the market at what storage conditions? What were the storage conditions during the transportation of blueberries samples from the market to the research area? After reaching the research area, it was immediately processed or stored?

Answer: This recommendation has been considered in the new version of the manuscript and incorporated in page 05 lines 105 to 110.

Line 113-115 - The authors should justify, how they homogenized blueberry anthocyanin content prior to the HVED assisted extraction step? Authors should include the detailed description of the homogenization process.

Answer: This recommendation has been considered in the new version of the manuscript and incorporated on it.

The anthocyanins are found mainly in the fruit skin, therefore the homogenizing criteria was based mainly in the color parameter. In order to clarify this aspect, we performed a correction in the new version of the manuscript in page 06 lines 114 to 117.

Section 3.1 - Authors should highlight the changes made in this section and provide line and page number on the answer sheet.

Answer: In the new manuscript version, particularly in Section 3.1, all changes that we made in the first revision are highlighted in light blue (pages 11 to 12, lines 236 to 252.

Figure 4: Authors should include the changes as mentioned in the comment. I do not observe any changes made by authors in TEM images. Authors should read the reviewers' comment again and again and respond them.

Each figure provided by the lab should include the magnification and applied voltage values. It was not necessary for authors to include them in their typewriting, else it shows the manipulating figures. For representing TEM images, authors should refer to some recently published standard articles.

Answer:

It is not possible to include the magnification and voltage values directly on TEM images according to following reasons:

- TEM images in opposite to SEM images don't include magnification or any other information of images because of the way to obtain TEM images are completely different from the SEM ones. In SEM. the images are captured from the electrons that

impact the sample surface and return to the equipment, and magnification is captured immediately from the equipment and printed in the image. On the other and in TEM an image is formed from the interaction of the electrons with the sample as the beam is transmitted through the specimen. The image is then magnified and focused onto an imaging device, such as a fluorescent screen, a layer of photographic film, or a sensor such as a scintillator attached to a charge-coupled device. In this study a camera Gatan, model 782 installed in the TEM equipment was used. This camera originally displays values different from the real magnification, therefore them it is necessary to calculate the real magnification by a formula giving by the equipment provider. Finally, the equipment operator gave us the real magnification, we can't manipulate the images.

- Second, the values written at the bottom of each image correspond to the HVED treatment voltage applied to blueberries, not the TEM voltage.
- Third, as you suggested, current papers were reviewed for the year 2022.

In the list of images obtained from papers recently publishes it is possible to observe that TEM images don’t contain any information of magnification neither the voltage.

Zhang, G., Fang, S., Regenstein, J. M., & Wang, F. (**2022**). Preparation, characterization and stability of nanoliposomes loaded with peptides from defatted walnut (Juglans regia L.) meal. ***Journal of Food Science and Technology***, 1-12.

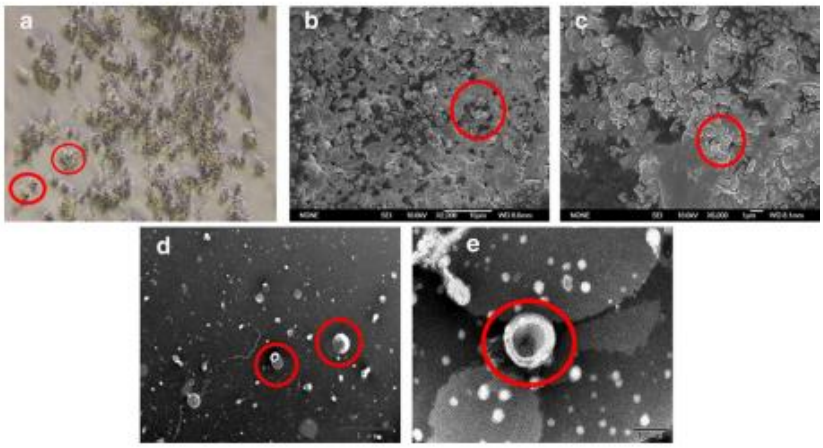
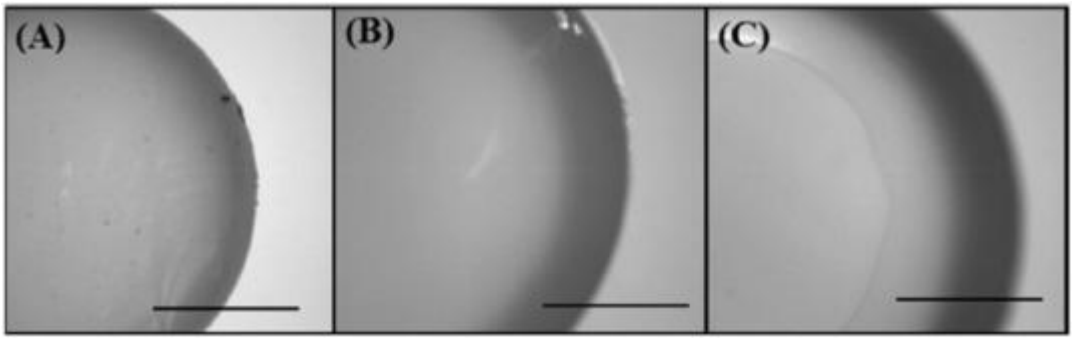


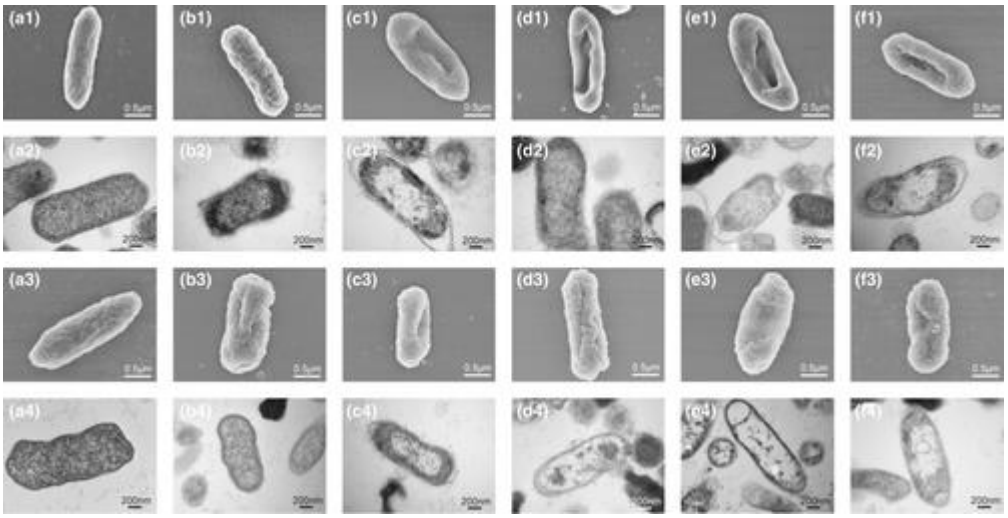
Fig. 5 Nanoliposome loading peptides with MW of 5–10 kDa: **a** optical microscope image at 400× magnification; **b**, **c** SEM images at 2200 and 6000× magnifications; **d**, **e** TEM images at 40,000× and 400,000× magnifications

Below are a number of articles from the year 2022 featuring TEM images, only including the reference in the image.

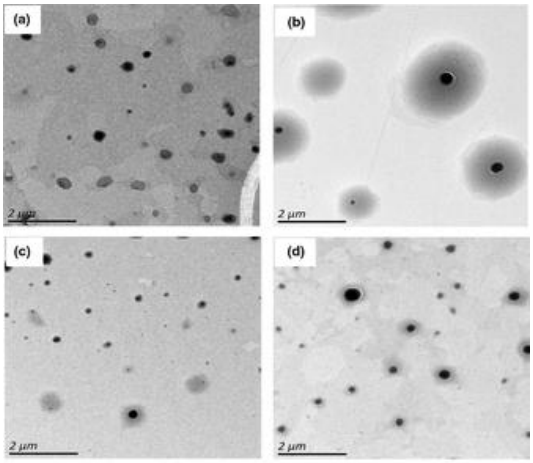
1- Maleki, G., Woltering, E. J., & Mozafari, M. R. (**2022**). Applications of chitosan-based carrier as an encapsulating agent in food industry. ***Trends in Food Science & Technology***, 120, 88-99. https://doi.org/https://doi.org/10.1016/j.tifs.2022.01.001



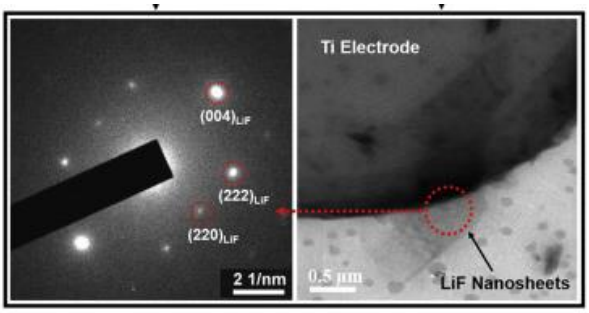
2- Zhang, R., Li, Q., Yang, L., Dwibedi, V., Ge, Y., Zhang, D., ... & Sun, T. (**2022**). The antibacterial activity and antibacterial mechanism of the tea polyphenol liposomes/lysozyme–chitosan gradual sustained release composite coating. ***International Journal of Food Science & Technology*.**



3- Huang, M., Wang, J., Tan, C., Ying, R., Wu, X., Chen, W., ... & Ahmad, M. (**2022**). Liposomal co-delivery strategy to improve stability and antioxidant activity of trans-resveratrol and naringenin. ***International Journal of Food Science & Technology***, *57*(5), 2701-2714.



4- Zhang, Q., Ma, J., Mei, L., Liu, J., Li, Z., Li, J., & Zeng, Z. (**2022**). In situ TEM visualization of LiF nanosheet formation on the cathode-electrolyte interphase (CEI) in liquid-electrolyte lithium-ion batteries. ***Matter***, *5*(4), 1235-1250.



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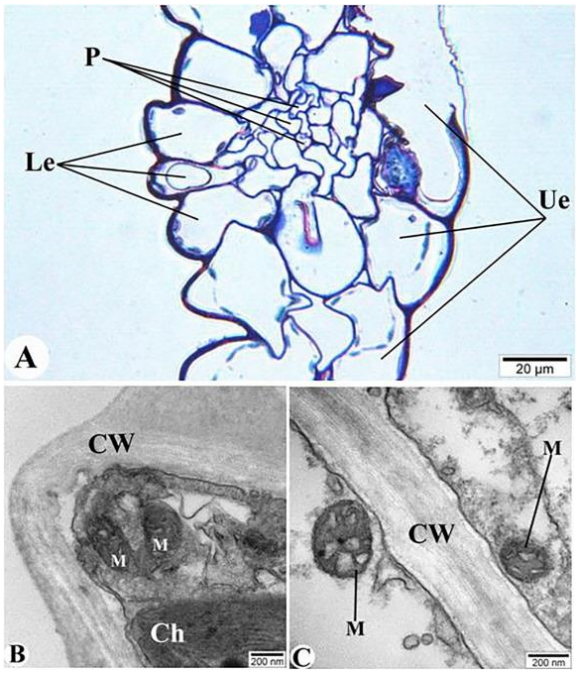
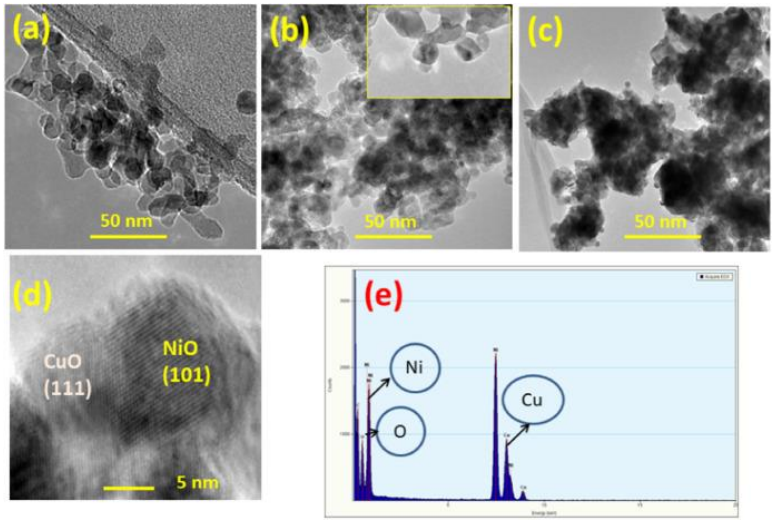
105 5- Rzayev, F. H., Gasimov, E. K., Agayeva, N. J., Manafov, A. A., Mamedov, C. A.,
106 Ahmadov, I. S., ... & Choi, K. C. (**2022**). Microscopic characterization of
107 bioaccumulated aluminium nanoparticles in simplified food chain of aquatic
108 ecosystem. ***Journal of King Saud University-Science***, *34*(1), 101666.



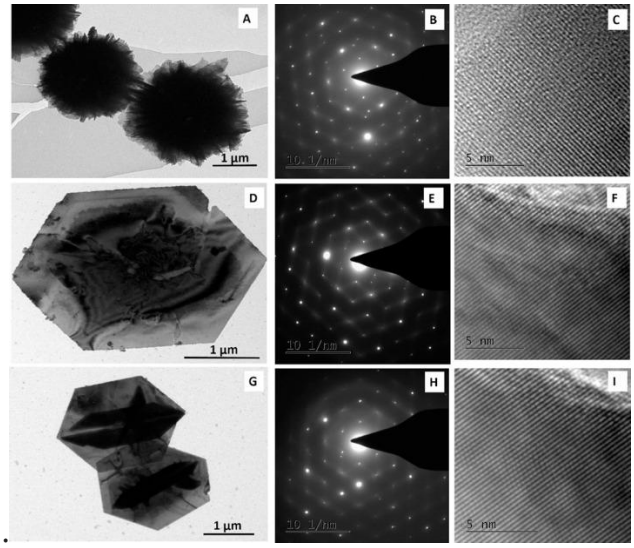
109

110 **6-** Gnanasekaran, L., Santhamoorthy, M., Naushad, M., ALOthman, Z. A., Soto-
111 Moscoso, M., Show, P. L., & Khoo, K. S. (**2022**). Photocatalytic removal of food
112 colorant using NiO/CuO heterojunction nanomaterials. ***Food and Chemical***

Toxicology, *167*, 113277.

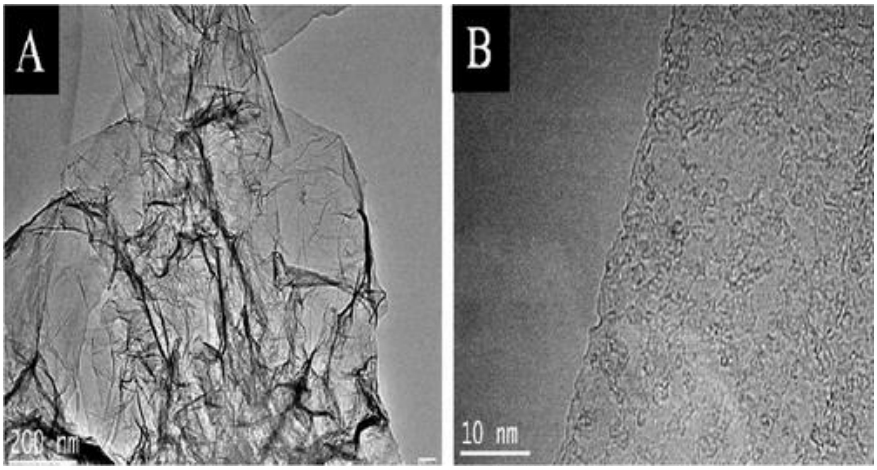


7- Radhakrishnan, S., Mathiyarasu, J., & Kim, B. S. (**2022**). Environmental-assisted shape-controlled synthesis and electrocatalytic performance of CuS nanostructures for vanillin detection in commercial food products. ***Applied Materials Today***, *27*, 101428

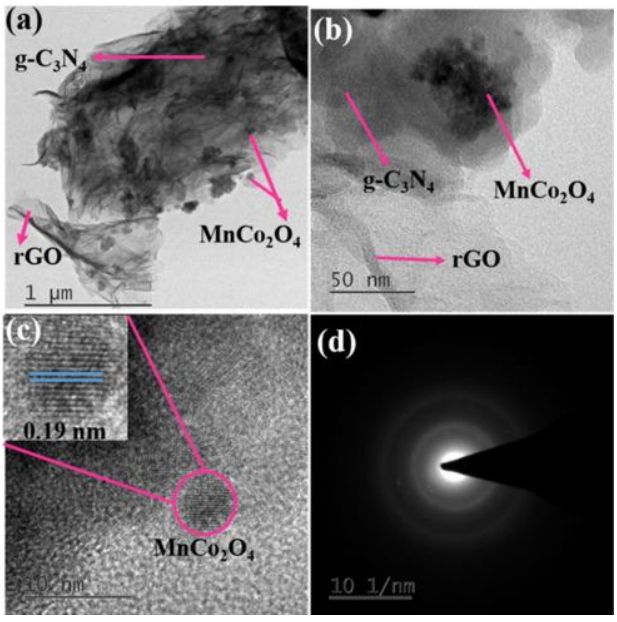


.

8- Raul, P. K., Thakuria, A., Das, B., Devi, R. R., Tiwari, G., Yellappa, C., & Kamboj, D. V. (**2022**). Carbon nanostructures as antibacterials and active food-packaging materials: A review. ***ACS omega***, *7*(14), 11555-11559.



9- Maji, B., Achary, L. S. K., Barik, B., Sahoo, S. J., Mohanty, A., & Dash, P. (**2022**). MnCo2O4 decorated (2D/2D) rGO/g-C3N4-based Non-Enzymatic sensor for highly selective and sensitive detection of Chlorpyrifos in water and food samples. ***Journal of Electroanalytical Chemistry***, *909*, 116115.



Finally, following modifications made in the article are highlighted:

The magnification value is based on the reference measure found in the image, pages 26-27 lines 608 to 612.

The applied voltage was incorporated in the methodology and in figure caption page 08 lines 168 to 170.

Reviewer #3: This study provides an electrotechnology to assist anthocyanin extraction from fresh and waste blueberries by using high voltage electrical discharge, which is interesting and of significance for the industry. The authors have made a lot of revisions according to the reviewers, and the manuscript has improved to a large degree.

After reviewing the revised manuscript, I still have some suggestions or questions for the authors to address.

1. The title includes "fresh and waste blueberries", however, I do not find any experiments or data on waste blueberries. What does the waste blueberry refer to here? Fresh and waste blueberries are different, and the obtained results may vary. Line 41: the introduction starts with "industrial agrifood waste". Again, the experiments were not involved with the waste blueberry. Maybe the waste blueberries could be discussed in future and prospects section. I think they need to be adjusted.

Answer: This recommendation has been considered in the new version of the manuscript and incorporated in page 17 lines 382 to 384, and the title was modified in the new version of the paper as follows: "Testing of a new high voltage electrical discharge generator prototype at high frequencies to assist anthocyanin extraction from blueberries"

2. Line 55: the authors do not have to mention TEM specifically here, maybe just use "microstructure observation".

Answer: This recommendation has been considered in the new version of the manuscript and “microstructure observation” paragraph was incorporated in page 03 lines 55

3. Line 56-61: these descriptions on "electrical impedance" are not necessary; at least, this should be shortened. In addition, I do not really like the sentence "including biological tissues such as food", as "food" is usually not associated with biological tissues.

Answer: This recommendation has been considered in the new version of the manuscript incorporated in page 03 lines 55 to 58. The paragraph was reduced and “including biological tissues” was eliminated

4. Line 79-82: there are too many citations here. Are they really necessary?

Answer: This recommendation has been considered in the new version of the manuscript incorporated in page 04 lines 74 to 78. Only more recent citations were considered

5. In Fig.4 (h-j), the power of each figure is E=10 kV/cm. t=10 s. what is the difference?

Answer: Effectively there are typing errors in Fig.4 i and Fig. 4.j They were modified in the new paper version

6. Line 330: It is necessary to clarify

Answer: This recommendation has been considered in the new version of the manuscript incorporated in page 15 lines 331.

172 The word “clarify” was changed to the word “highlight”, as it is more suitable.

Highlights

- A new high voltage electric discharge (HVED) prototype was developed
- The parameters of the HVED prototype were validated
- The HVED treatments were compared with traditional treatments
- The HVED is a "green" method to extract valuable compounds from blueberries

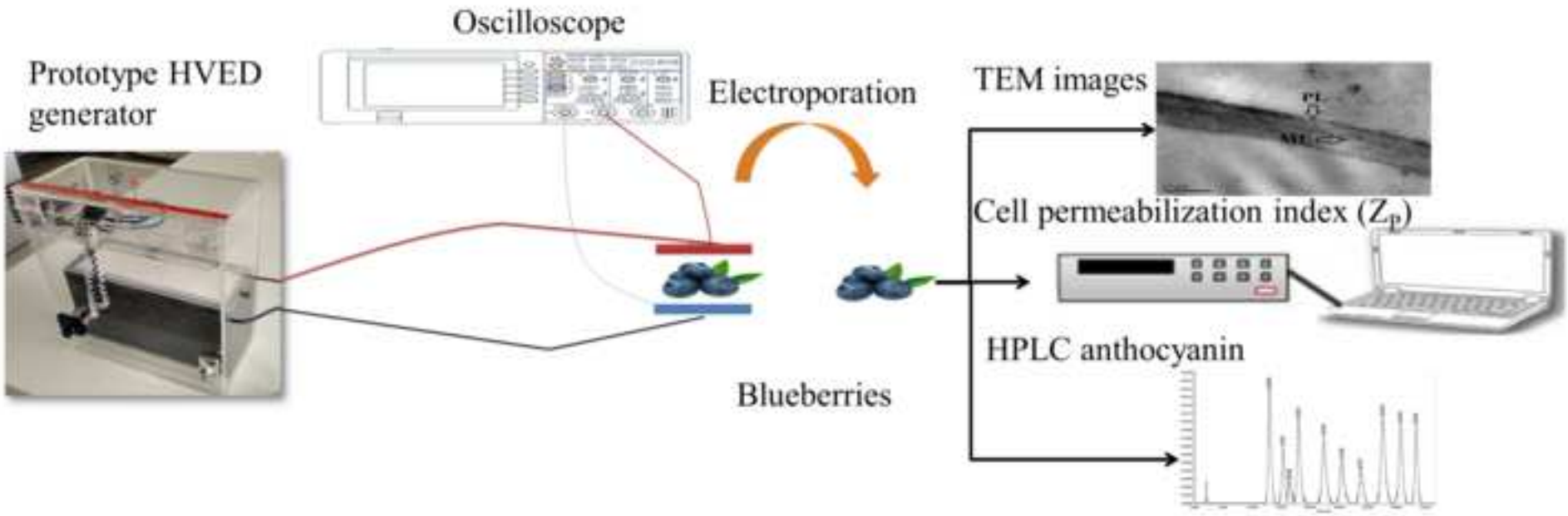


Figure 1

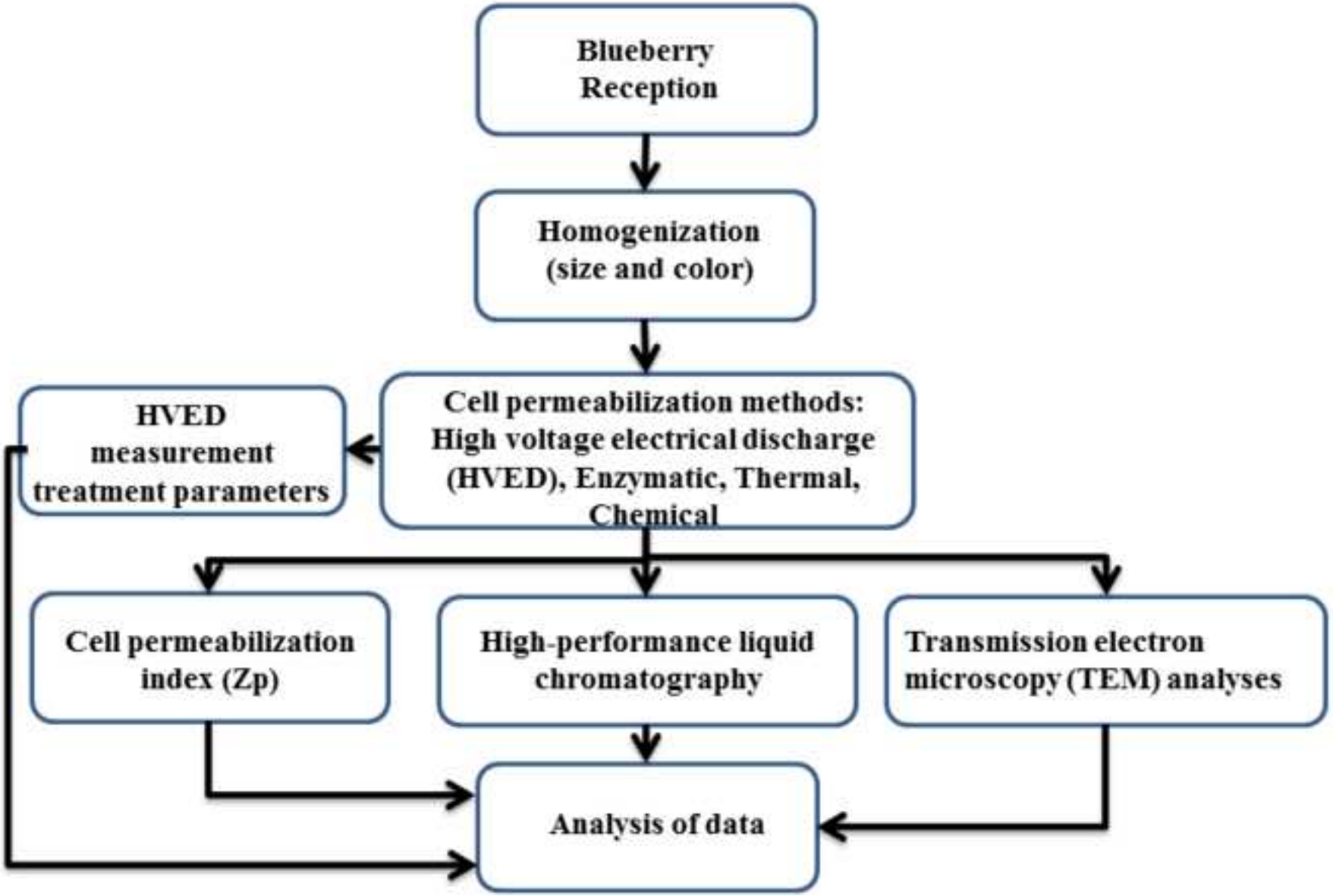


Figure 2

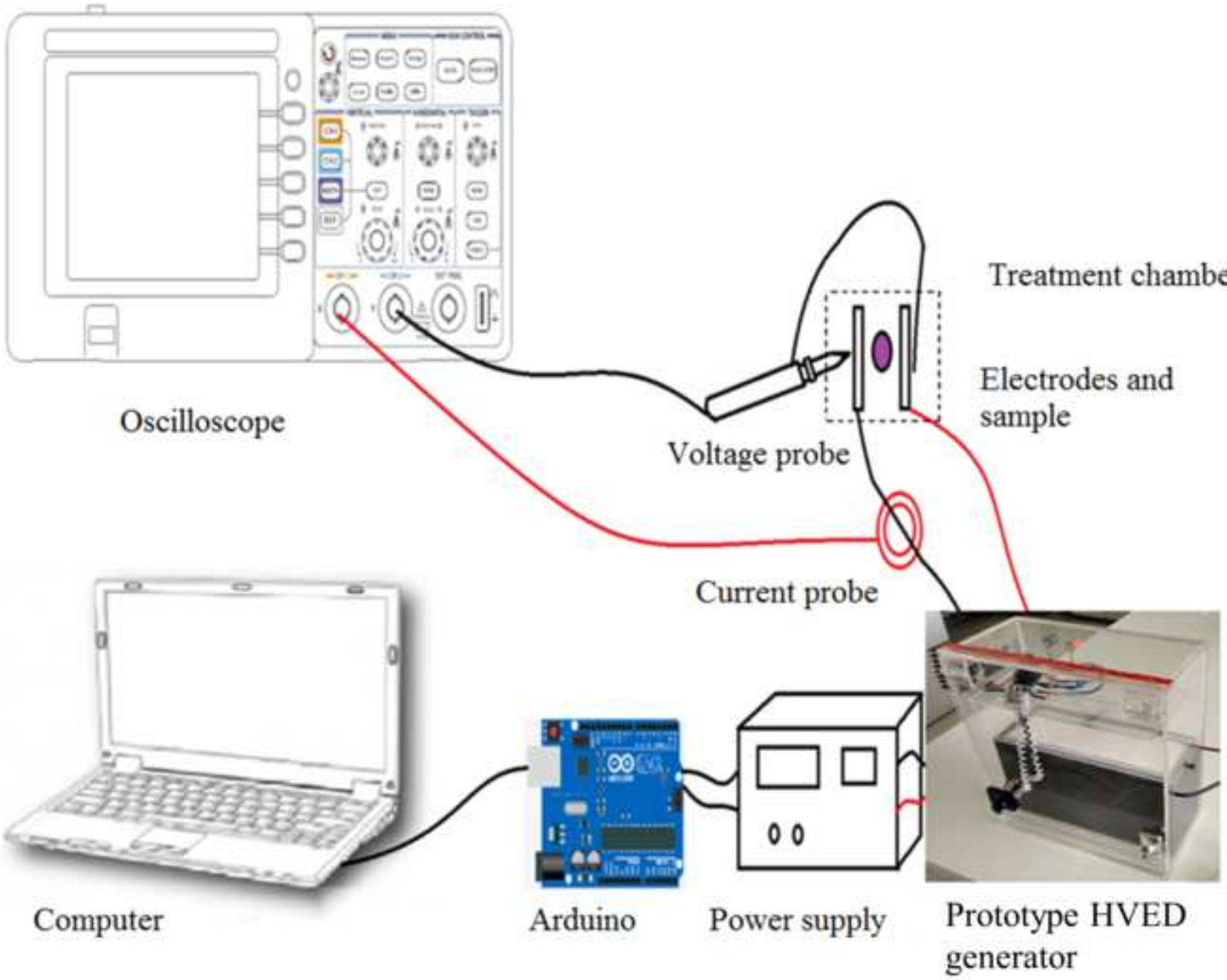


Figure 3

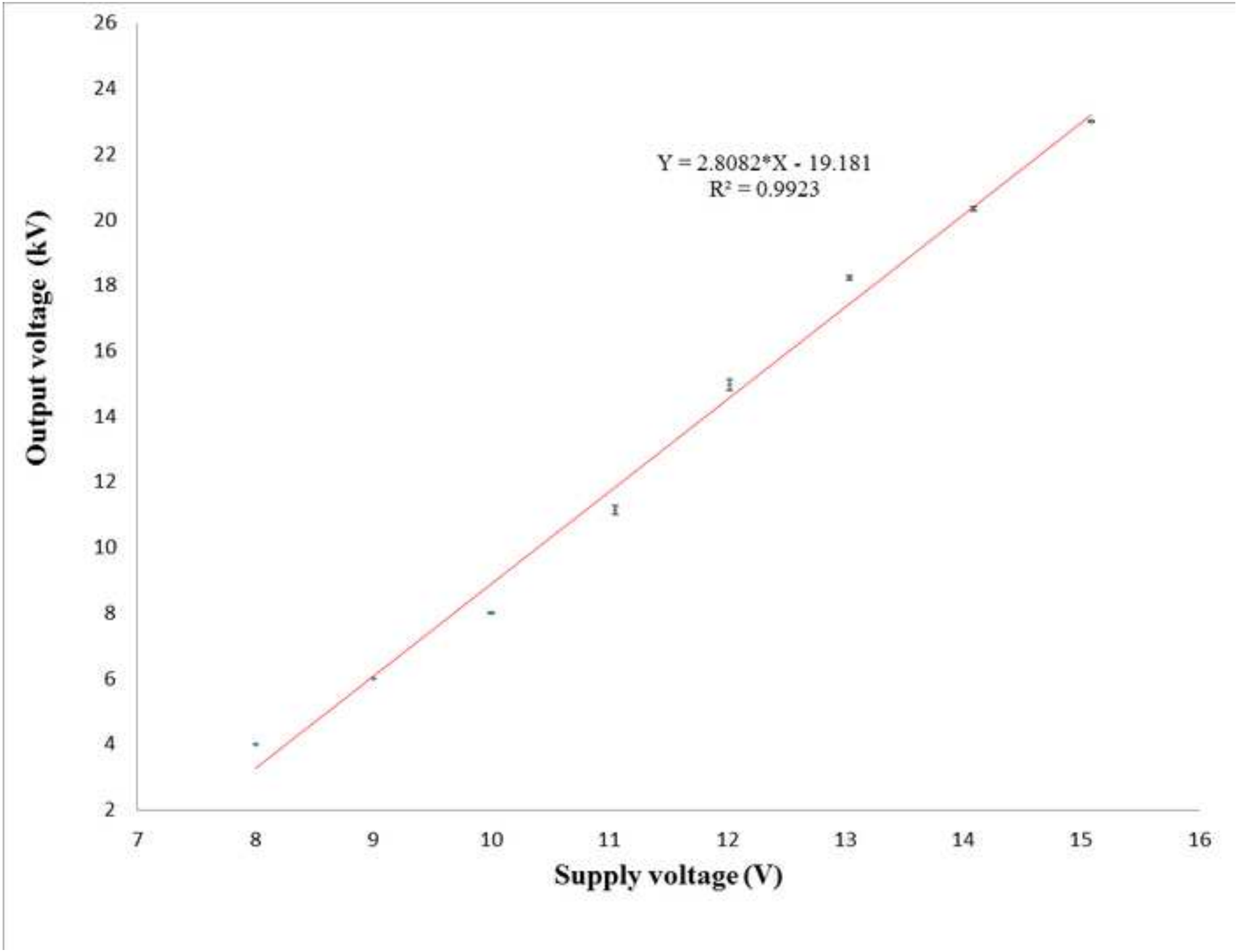


Figure 5

Click here to access/download;Figure;Figure 5.tif

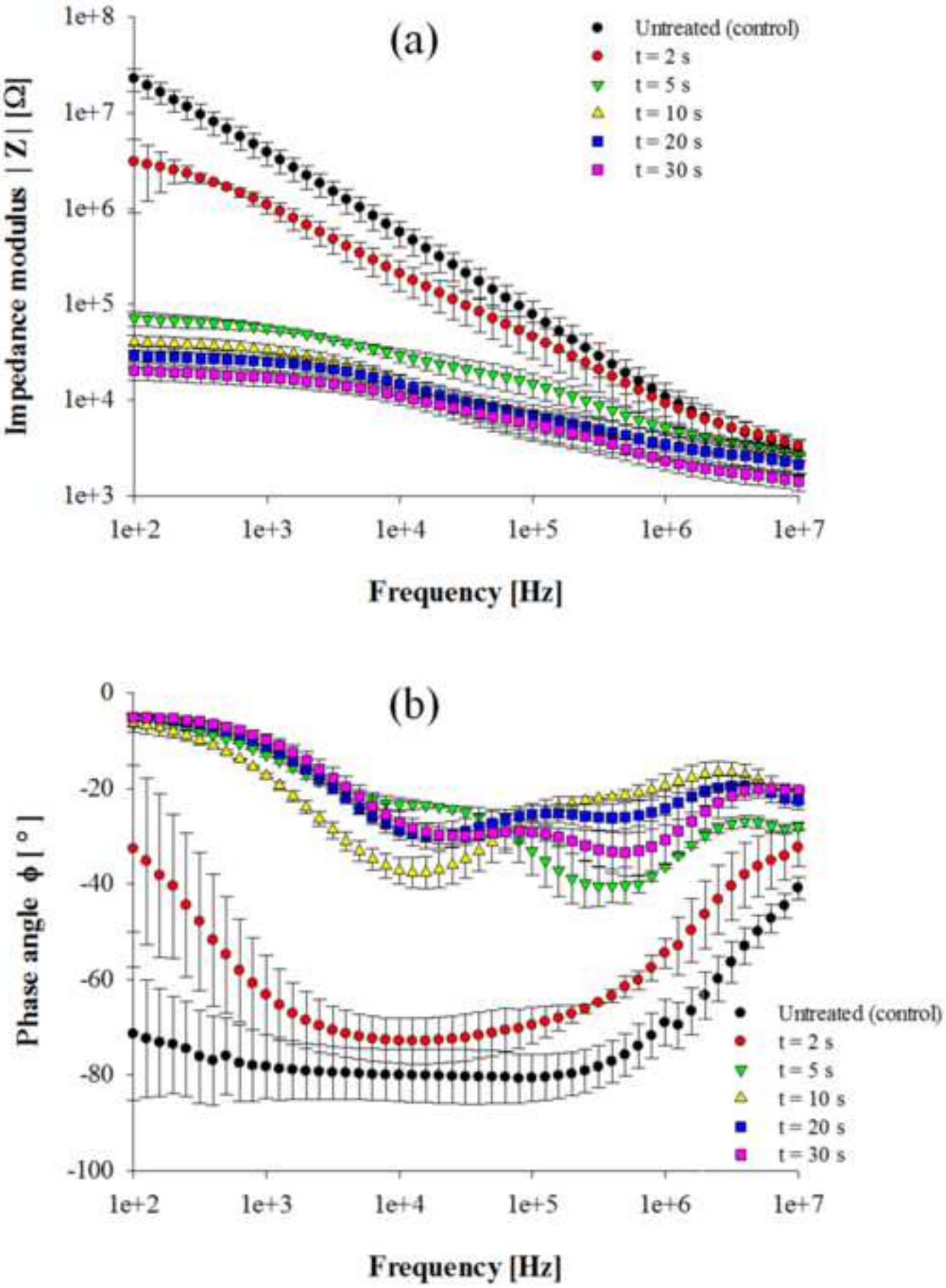


Figure 6

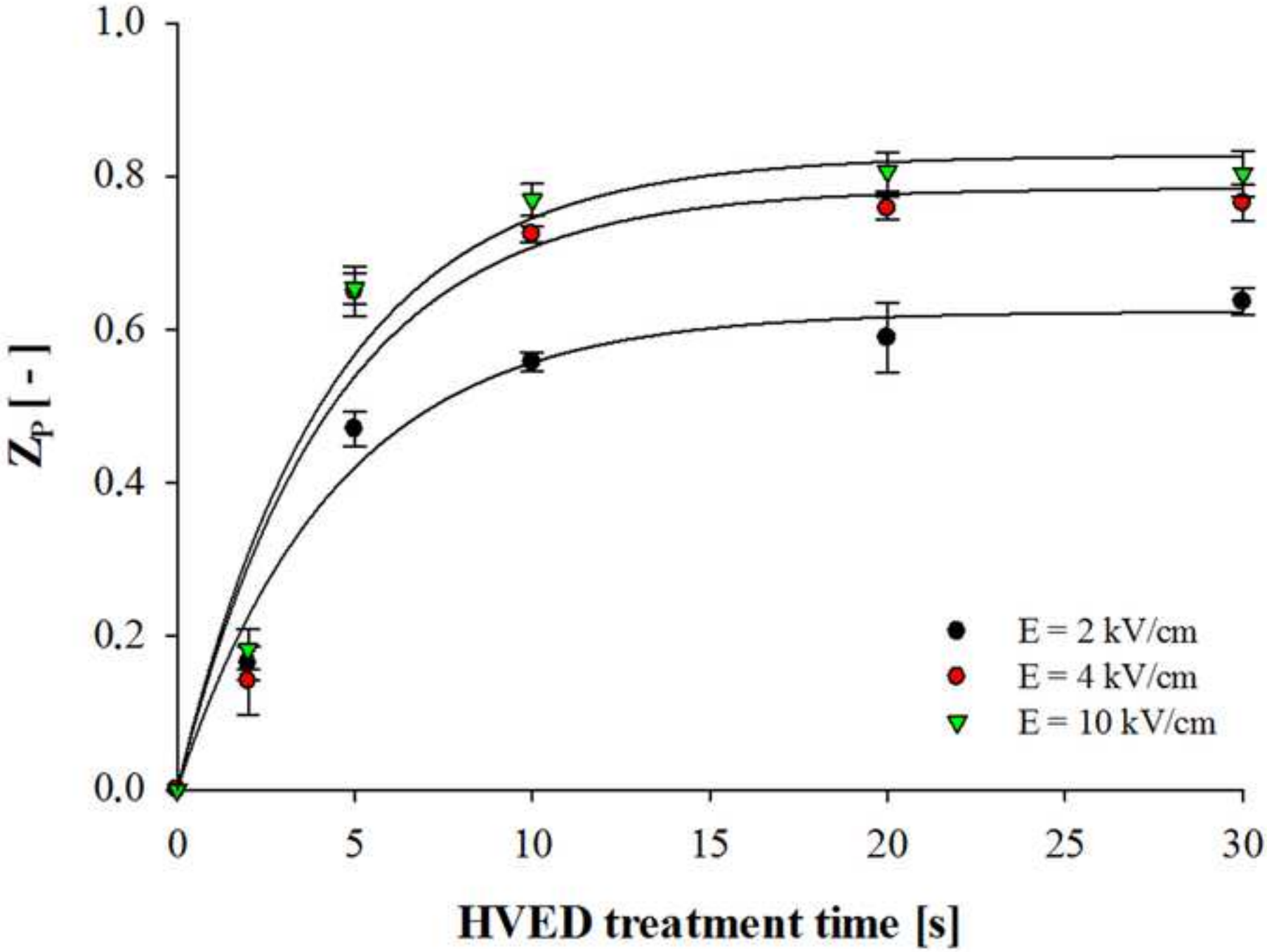


Figure 7

Click here to access/download;Figure;Figure 7.tif

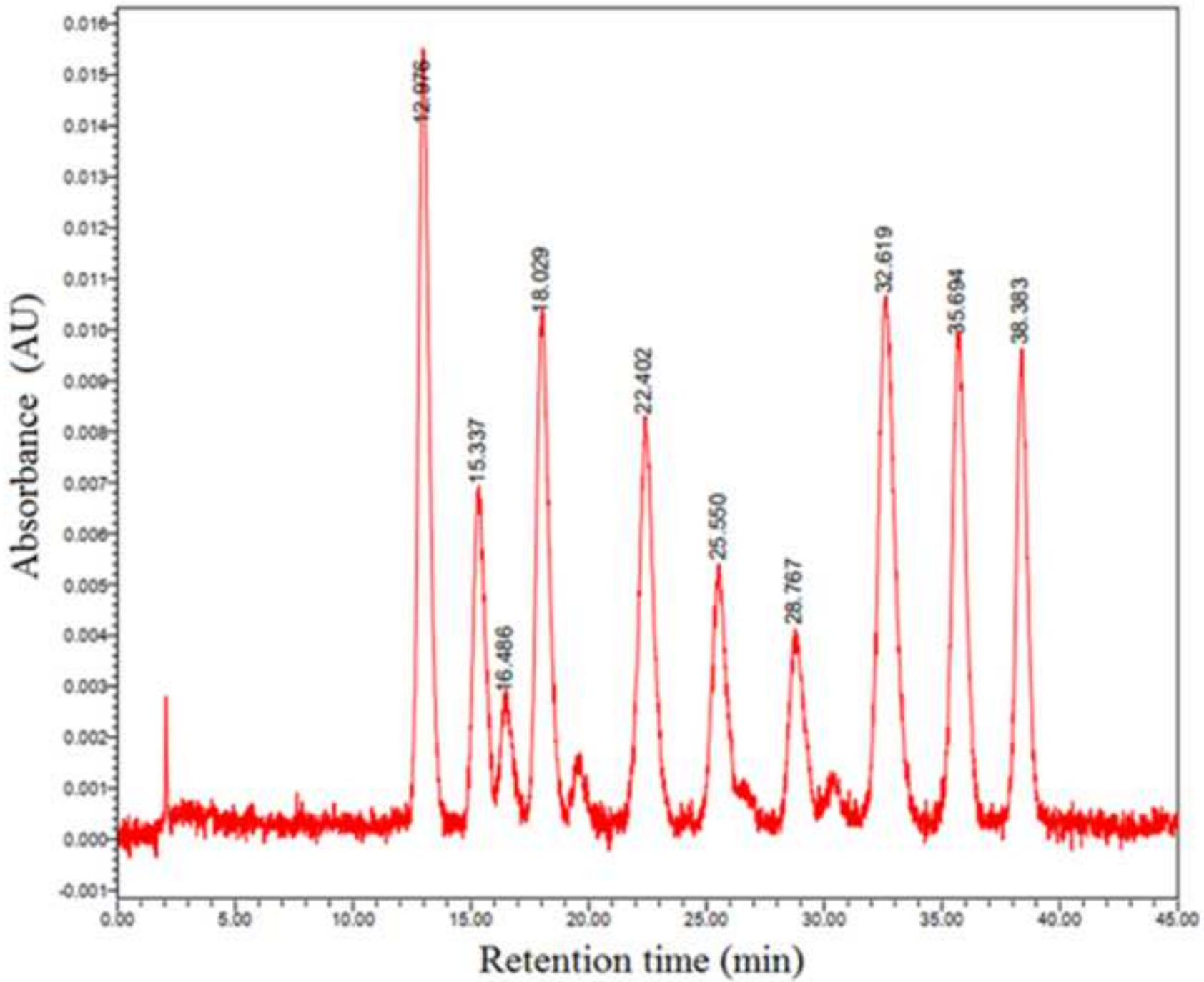
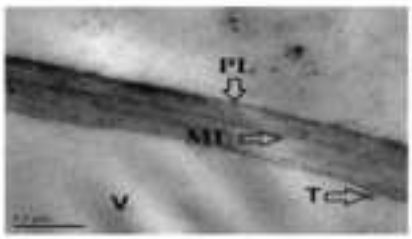


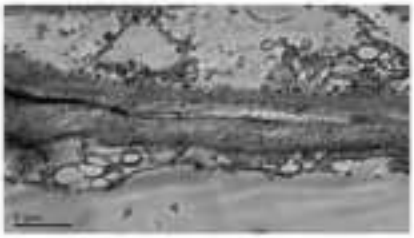
Figure 4



(a) Control

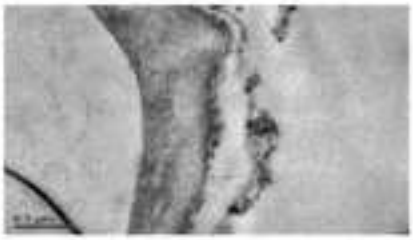
(b) E = 2 kV/cm, t = 10 s

(c) E = 2 kV/cm, t = 20 s

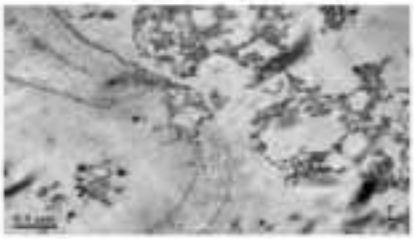


(d) E = 2 kV/cm, t = 30 s

(e) E = 4 kV/cm, t = 10 s

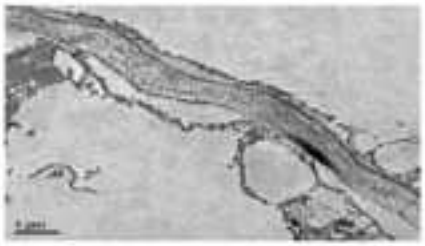


(f) E = 4 kV/cm, t = 20 s

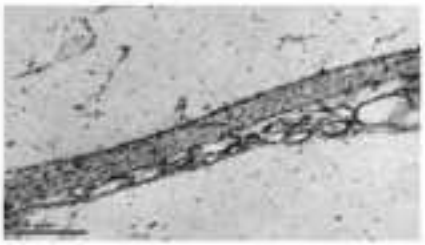


(g) T6 E = 4 kV/cm, t = 30 s

(h) T7 E = 10 kV/cm, t = 10 s



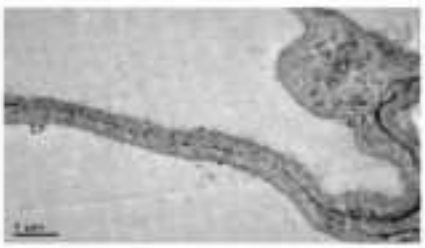
(i) T8 E = 10 kV/cm, t = 20 s



(j) T9 E = 10 kV/cm, t = 30 s

(k) Thermal

(l) Chemical



(m) Enzymatic

Table 1. Specific energy consumption (WT in kJ/kg) applied to blueberries during treatments according to the combination of applied electric field intensity (E in kV/cm) and process time (t in s). Results are expressed as mean ± SD.

HVED	Specific energy consumption [kJ/kg]		
Exposure time [s]	E = 2 kV/cm	E = 4 kV/cm	E = 10 kV/cm
2	6.00 ± 0.18	21.00 ± 0.62	41.00 ± 1.23
5	16.00 ± 0.77	53.00 ± 1.55	104.00 ± 1.7
10	31.00 ±1.82	133.36 ± 3.30	204.00 ± 1.08
20	60.00 ± 5.88	266.72 ± 3.05	401.00 ± 1.14
30	93.00 ± 8.20	396.00 ± 5.50	579.00 ± 24.4

Table 2. Equipment parameters of high voltage electric discharge (HVED) used in recent research are listed: Product, voltage, frequency, extract, energy, and authors.

Product	Voltage [kV]	Frequency [Hz]	Extract	Energy [kJ/kg]	Authors
Cocoa shells	15	40	Multi-element	--	(Barišić et al., 2022)
Fermented grape pomace	40	0.5	Bio-compounds	118	(Barba et al., 2015b)
Grapefruit peels	40	0.5	Polyphenol	7.27 to 218	(El Kantar et al., 2019)
Microalgae	40	0.5	Bio-molecules	--	(Zhang et al., 2020)
Orange peels	40	0.5	Reduce sugar	222	(El Kantar et al. 2018)
Oregano	25	100	Bioactive compounds	--	(Nutrizio et al., 2021)
Peanut shells	20	1000	Flavonoids	--	(Yan et al., 2018)
Pomegranate peel	20	1000	Phenols	--	(Xi et al., 2017)
Pomegranate fruit	40	0.5	Proteins/polyphenols	27 to 373	(Hernández-Corroto et al., 2022)
Canola straw	--	0.5	Lignin	800	(Brahim et al., 2017)
Sage	25	100	Antioxidant	--	(Nutrizio et al., 2020)
Sesame seeds	40	0.5	Oil	40 to 240	(Sarkis et al., 2015b)
Sugar beets	40	0.5	Pectin	76.2	(Almohammed et al., 2017)
Vine shoots	40	0.5	Polyphenol	101 to 609	(Rajha et al., 2015b)

Table 3. Concentration (mg/L) of individual and total anthocyanins in methanolic extracts from untreated, HVED treated (10 kV/cm, 10 s), and alternatively processed (thermal, enzymatic, chemical) blueberries.

Peak n°	Retention time [min]	Anthocyanin	Concentration [mg/L]				
			Control	HVED	Thermal	Enzymatic	NaOH
1	12.97	D-3-O-Gal	11.81 ± 0.63^{b}	25.36 ± 1.30^{c}	31.58 ± 0.85^{e}	6.58 ± 0.14^{a}	28.37 ± 1.40^{d}
2	15.33	D-3-O-Glu	5.94 ± 0.59^{b}	9.88 ± 0.91^{c}	14.33 ± 0.60^{d}	3.51 ± 0.33^{a}	12.54 ± 0.97^{d}
3	16.48	C-3-O-Gal	4.29 ± 0.91^{a}	6.72 ± 0.66^{b}	7.76 ± 0.37^{b}	3.80 ± 0.41^{a}	8.12 ± 0.92^{b}
4	18.02	D-3-O-Ara	9.31 ± 1.36^{b}	18.59 ± 1.90^{c}	25.04 ± 1.11^{e}	4.55 ± 0.66^{a}	20.48 ± 1.24^{c}
5	22.40	Pet-3-O-Gal	9.39 ± 0.95^{ab}	16.72 ± 1.80^{c}	22.51 ± 0.22^{d}	5.66 ± 0.34^{a}	13.76 ± 3.02^{bc}
6	25.55	Pet-3-O-Glu	4.73 ± 0.33^{a}	8.12 ± 0.68^{b}	11.24 ± 0.48^{c}	3.01 ± 0.45^{a}	8.29 ± 1.09^{b}
7	28.76	Peo-3-O-Glu.	1.27 ± 0.35^{b}	2.33 ± 0.07^{c}	3.26 ± 0.22^{d}	0.56 ± 0.16^{a}	1.61 ± 0.30^{b}
8	32.61	M-3-O-Gal	15.56 ± 2.44^{b}	17.59 ± 0.04^{b}	30.54 ± 1.93^{c}	9.53 ± 0.29^{a}	14.83 ± 0.01^{b}
9	35.69	M-3-O-Glu	11.39 ± 3.04^{b}	17.41 ± 0.22^{c}	24.50 ± 0.75^{d}	6.61 ± 0.35^{a}	10.74 ± 0.28^{b}
10	38.38	M-3-O-Ara	9.40 ± 1.42^{b}	14.10 ± 0.80^{c}	20.15 ± 0.73^{d}	6.90 ± 0.49^{a}	10.76 ± 0.57^{b}
		Total anthocyanins	83.09 ± 1.20 ^{b}	136.82 ± 0.84 ^{c}	190.92 ± 0.73 ^{e}	50.71 ± 0.36 ^{a}	129.50 ± 0.98 ^{d}

Different superscript letters in the same row are significantly different ($p < 0.05$).

The authors declared that there is no conflict of interest.

Author statement

R. Díaz-Álvarez: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft, D. Carullo: Methodology, Formal analysis, Writing - Original Draft, G. Pataro: Methodology, Validation, Formal analysis, Supervision Giovanna Ferrari: Validation, Resources, Supervision, Project administration Segura-Ponce, L: Conceptualization, Methodology, Validation, Resources, Formal analysis, Supervision, Writing - Review & Editing, Project administration, Funding acquisition.

Testing of a new high voltage electrical discharge generator prototype at high frequencies to assist anthocyanin extraction from blueberries

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Abstract

Traditional extraction methods are based on high-temperature maceration with organic solvents, which are dangerous for human health. A viable alternative to overcome the issues associated with conventional extraction is to increase cell tissue permeability by applying high voltage electrical discharge (HVED) treatments. The objective of this work was to validate the electroporation of blueberry plant cells using a new HVED generator prototype at a high frequency, investigate the effect, intensity, and duration of the applied voltage, and recover anthocyanins from its electroporated cells. The electroporation level of the HVED-treated blueberries was measured qualitatively by transmission electron microscopy (TEM) analysis. Meanwhile, it was quantitatively measured by the cell permeabilization index (Zp) and anthocyanin extraction level. Results of the micrographs (TEM) showed electroporation in all treatments in which Zp was 0.24 when applying a 2 kV treatment for 2 s, whereas a 3-fold increase in tissue damage was revealed with the most powerful treatment (10 kV voltage, 30 s). In addition, anthocyanin values ranged from 83.09 ± 1.20 (control) to 136.82 ± 0.84 (HVED), which was 64.66% higher. The HVED treatment can increase mass transfer rates during conventional extraction processes. It should be noted that the validated prototype required a low specific energy requirement (31 to 204 kJ/kg) for proper tissue electropermeabilization. In conclusion, we demonstrated the capability of the developed HVED prototype to boost mass transfer phenomena and thus potentially increase its adaptability to assist dissimilar industrial processes or waste (e.g., peels and seeds) such as freeze-drying operations.

Keywords: Green extraction, Cell permeability, High voltage electrical discharge, Anthocyanins, Blueberry.

1. Introduction

Increasing interest has been shown to valorize industrial agrifood waste through an efficient recovery of its major bioactive constituents, which can be exploited particularly in the food, feed, pharmaceutical, and cosmetic industries due to high consumer demand for natural products.

Such compounds are typically recovered by conventional extraction methods, which require large volumes of polluting and harmful solvents (e.g., hexane, acetone, and petroleum ether) and long maceration times (Parniakov et al., 2014). Recent efforts have explored the use of electrotechnologies as biomass pretreatments such as pulsed electric field (PEF) (Carullo et al., 2018; Genovese et al., 2021; Naliyadhara et al., 2022; Palma-Acevedo et al., 2022), high voltage electric field discharge (HVED) (Almohammed et al., 2017; El Kantar et al., 2019; Barišić et al., 2022), and ohmic heating (Kulshrestha et al., 2006; Moreno et al., 2012). All these technologies provoke mild cell disruption that weakens or ruptures cell sheaths to ultimately intensify the extractability of the targeted intracellular compounds without jeopardizing their original integrity (Barba et al., 2015a).

The efficiency of such electrotechnologies has been determined with mass transfer measurements, impedance measurements of the cellular matrix, (Genovese et al., 2021), or by microstructure observation (Carullo et al., 2018). The electrical impedance measurement is a methodology to characterize electrical properties (resistance and capacitance); the cell permeabilization index (Zp) can be calculated with impedance (Alaoui, 2019). Electrical impedance is vital to study cell electroporation because it is a non-invasive and quantitative analytical method to non-destructively. (Chemat et al., 2017; Xu et al., 2016a).

Within this framework, PEF and HVDE are distinguished among the other electrotechnologies due to their efficiency in the electroporation process (Barba et al., 2015b). The potential of pulsed electric field (PEF) technology to induce non-thermal membrane permeability in biological cells has been successfully demonstrated (Mahn et al., 2021; Palma-Acevedo et al., 2022; Tylewicz et al., 2022; Zhang et al., 2022). One application of PEF-assisted extraction is cold diffusion based on the rupture of the cell membrane when subjected to external PEF; this increases the electrical conductivity and permeability

of the intracellular material (Naliyadhara et al., 2022; Sarkis et al., 2015a). The high voltage electrical discharge (HVED) is another cell disintegration technique for wet biomass, which is based on the electrical breakdown phenomenon in water. Specifically, high energy during an HVED treatment accumulates in the aqueous suspension placed in a batch treatment chamber between a high voltage needle electrode and a plated grounded electrode through a plasma channel consisting of a rapid high current/high voltage electrical discharge (40 to 60 kV; 10 kA) (Anukiruthika et al., 2021; Wang et al., 2018).

The HVED has been effectively applied to extract different molecules of interest from products such as cocoa shell, grapefruit peels, microalgae (*Nannochloropsis oculata*), oregano, peanut shells, pomegranate fruit, and sage. The experiments were carried out by applying electric fields ranging from 15 to 40 kV, frequency from 0.5 to 1000 Hz, and energy consumption from 7.27 to 800 kJ/kg (Barišić et al., 2022; El Kantar et al., 2019; Zhang et al., 2020; Nutrizio et al., 2021; Yan et al., 2018; Hernández-Corroto et al., 2022; Nutrizio et al., 2020).

The range of applied frequencies is an interesting factor to study during the electroporation process. It has been shown that using higher frequencies in PEF allows for greater permeabilization efficiency than lower frequencies (Ruzgys et al., 2019) and energy output on each pulsed is in the order of microjoules because of the reduced exposure time of the sample; therefore, the treatment is predominantly non-thermal (Novickij et al., 2016). High frequency ranges are the least studied conditions and a deeper understanding of the process is required that will allow better control and optimization of electroporation protocols (Kohler et al., 2015). One of the main reasons why high frequencies have been poorly addressed is the lack of high-power electroporation equipment, while lower frequency ranges are adequately addressed (Novickij et al., 2016).

Blueberries have an ideal matrix for testing a high-voltage electrical discharge generator prototype to extract bioactive components due to their high content of phenols, anthocyanins, and

antioxidants (Silva et al., 2020; Nowak et al., 2019). Particularly, waste is an excellent source of various low-cost compounds such as polyphenols, polysaccharides, proteins, flavonoids, and alkaloids (Cascaes et al., 2021; Gil-Martín et al., 2022; Li et al., 2019; Madureira et al., 2020; Marić et al., 2018). Therefore, the aim of this study was to validate a new HVED generator prototype that operates at high frequency in experiments to assist anthocyanin extraction in fresh and waste bluberries.

2. Materials and methods

To validate the ability to electroporate blueberry cells through the HVED generator prototype, several step-by-step experiments were performed to evaluate electroporation and treatment conditions to obtain optimal between electroporation and energy consumption. Electroporation was evaluated using analysis of transmission electron microscopy (TEM), cell permeabilization index (Zp), and the extraction and analysis of anthocyanins by high-performance liquid chromatography with diode array detection (HPLC-DAD). Chemical, enzymatic and thermal pretreatments were carried out in order to compare the effectiveness of the HVED treatments. Figure 1 shows a flow diagram of the process carried out in this study.

2.1. Materials and reagents

Fresh blueberries (*Vaccinium myrtillus L.*) were grown in a commercial orchard in Cilento (40°18′N 15°18′E, Salerno, Italia). Following their hand-harvesting, performed at their full ripening stage, blueberries were collected in fruit boxes and immediately transported to the laboratories of ProdAl Scarl (Fisciano, Italy) by a cooled truck (maintaining temperature at 4 ± 1 ◦C). Samples were then stored under refrigerated conditions and at 95 ± 1% relative humidity (RH) until their use, within 2 days from the harvest. Upon their arrival at the laboratories, the samples' moisture content (wet basis) was determined by their oven-drying (OV-12 Lab Companion, Seoul, Korea), carried out at a temperature of 70 °C and pressure ≤ 100 mm Hg (13.3 kPa), according to the AOAC Official Method 934.06.

Fruits of similar size (15 ± 3 mm diameter) and color (hue angle = 185.2 ± 3.4) were accurately selected in an attempt to homogenize the anthocyanin content of each sample lot of blueberries before undergoing the HVED pre-treatment stage. Whole homogenized blueberries were pretreated with HVED.

HPLC grade formic acid (purity ≥ 98%), and acetonitrile (purity ≥ 99.9%), were supplied by Sigma Aldrich (Steinheim, Germany), together with Delphinidin-3-glucoside (purity ≥ 95.0%), peonidin-3-glucoside (purity ≥ 97.0%), and petunidin-3-glucoside (purity ≥ 95.0%) analytical standards. All other chemicals and solvents were of analytical grade and purchased from Merck S.A. (Santiago, Chile). The MACERASE Pectinase, *Rhizopus* sp., was supplied by Merck S.A (Concepción, Chile). It had a specific activity of ≥ 3000 units/g dw.

2.2. Experimental Design

A multilevel factorial design with the two factors of applied field intensity (E = 2, 4, and 10 kV/cm) and processing time (t = 2, 5, 10, 20, and 30 s) was used to study the electroporation and its effect and interaction in the extraction yield of anthocyanins in blueberries in the HVED experiments. Chemical, enzymatic and thermal experiments were performed in triplicate at given operations conditions. The order of the experiments was completely randomized to minimize bias.

2.3. Cell permeabilization methods

2.3.1 High voltage electrical discharge (HVED)

The HVED generator prototype was developed at the Universidad del Bío-Bío (Chillán, Chile); it is illustrated in Figure 2. The equipment had an operating range from 0 to 60 kV and a constant sinusoidal frequency of 200 kHz. The treatment chamber (Figure 2) had two parallel circular-shaped electrodes (20 mm diameter) with a fixed gap (20 mm) called the inter-electrode gap. The applied voltage and current signals in the treatment chamber were measured with a high voltage probe (P6015A, Tektronix, Wilsonville, OR, USA) and a current probe (Rogowski coil 2 - 0.1, Stangenes Inc., Palo Alto, CA, USA) connected to a 300 MHz digital oscilloscope (TDS 3034B, Tektronix, Wilsonville, OR, USA).

A computer was connected to an Arduino card to control the operating time of the high voltage generator. The power supply controlled the voltage variation and direct current of the high voltage generator.

The peak electric field intensity (E, kV/cm) was calculated by dividing the applied voltage by the inter-electrode gap distance, while the specific energy consumption (W_T, kJ/kg) was expressed in Eq.1 as follows:

$$W_T = \frac{W}{m} \quad (1)$$

where W is the amount of energy applied to the fruit samples from the different voltage and current intensity combinations and m is the mass of the fruit sample.

2.3.2. Enzymatic treatment

For the enzymatic treatment, 100 g of blueberries were processed, submerged in a pectinase enzyme solution, Rhizopus sp. dissolved in 1 mg/mL deionized water for 24 h. The temperature of the enzymatic treatment was maintained at the desired level with the help of a water bath (WB-22, Daihan Scientific, Seoul, Korea) at a constant temperature (40 ± 0.5 °C) (Takebe et al., 1968). At the end of the enzyme treatment, the enzyme in the sample was inactivated by heating the suspension at 90 °C for 5 min in a water bath. The blueberries were then washed with deionized water for 10 min. (Xu et al., 2016b).

2.3.3. Thermal treatment

The thermal treatment with hot water was performed with 100 g blueberries. These were placed in a flask, 1 L distilled water was added, the desired temperature was maintained with a water bath (WB-22, Daihan Scientific, Seoul, Korea) at a constant temperature (70 ± 0.5 °C) for 30 min, and then left to stand at room temperature for 30 min (Jiang et al., 2020).

2.3.4. Chemical treatment

The chemical treatment consisted of immersing 100 g fresh blueberries in an alkaline solution of sodium hydroxide (NaOH) with a 2% concentration at 25 °C for 10 min. Immediately after the treatment with NaOH, the blueberries were washed with a pressurized water spray for 2 min. (Zhou et al., 2022).

2.4. Transmission electron microscopy (TEM) analyses

The microstructure of untreated (control) and treated (HVED, chemical, enzymatic, thermal) samples was observed with a TEM apparatus (JEM 12000EX-II, JEOL, Welwyn Garden City, UK) operating at 100 kV in a magnification range from 18000 x to 54000x. The system was equipped with a high resolution digital imaging camera (model 782, ES500W Erlangshen Gatan Inc., Pleasanton, CA, USA), and the scanning was executed moving from the surface to the very center of the fruit.

Samples were prepared by immersing them in glutaraldehyde (2 to 4%) for 4 to 24 h in 0.1 M phosphate buffer (pH = 7.2 to 7.4) at 4ºC to enable tissue fixation. The buffer was removed, followed by a second fixation step using a 1% osmium tetroxide solution for 1 to 2 h in the same phosphate buffer. Sample osmotic dehydration occurred with increasing ethanol solution concentrations (30%, 50%, 70%, 85%, and 95%) for 15 min each. The samples were subsequently immersed in pure ethanol for 20 min and dried in a critical point dryer (Balzers-Union, Balzers, Liechtenstein), embedded in epoxy resin (60 °C for 72 h). Ultrathin sections were obtained by cutting these resin blocks into approximately 70 nm thick films. These were finally double-stained with uranyl acetate (25 to 30 min) and lead citrate (5 to 10 min) and observed (Segura-Ponce et al., 2019).

2.5. Cell permeabilization index (Z_P)

All the electrical treatments designed to measure the cell permeabilization index (Z_P) is performed with the HVED generator prototype. Comparison treatments (chemical, enzymatic, thermal) were also measured. The extent of cell damage in blueberry tissues induced by the HVED pretreatments was assessed by Z_P. The Z_P values were determined via impedance analyses according to the method described by Angersbach et al.,(1999; 1997) and Bobinaitė et al., 2015. Complex electrical impedance

measurements were performed in triplicate in frequency sweep mode, which ranges from 10^2 to 10^6 Hz. The blueberry samples (1.7 g) were located in the measurement cell connected to an impedance analyzer (1260, Solartron Analytical, Farnborough, UK) and subjected to electric fields (E) of 2, 4, and 10 kV/cm at different exposure times (t): 2, 5, 10, 20, and 30 s. The electrical impedance measurements were taken in all the blueberry samples before and after the HVED treatments.

Regardless of the applied treatment condition, the Z_P value, ranging from 0 (intact tissue) to 1 (completely electroporated tissue), was calculated (Eq. 2) based on the absolute value of the complex impedance of untreated "Z_{untr}" and treated "Z_{tr}" tissue according to the tested frequency (Donsì et al., 2010).

$$Z_P = \frac{Log|Z_{untr}\ (0.1kHz)| - Log|Z_{tr}(0.1kHz)|}{Log|Z_{untr}\ (0.1kHz)| - Log|Z_{tr}(1\ MHz)|} \quad (2)$$

2.6. Anthocyanin extraction

For each experiment, anthocyanin recovery from untreated or HVED pretreated blueberries was carried out according to the method developed by Zhang et al. (2007) with slight modifications. Therefore, 5 g samples were taken from the treatment chamber, placed in 50 mL centrifuge tubes, and 10 mL methanol containing 2% formic acid was added to each tube. This system was then placed in a vortex for 30 s (QL-861, Kylin-Bell Lab Instruments Co., Ltd., Haimen, China) to homogenize the sample. Samples were sonicated in a KQ-500DE ultrasonic cleaner (Ultrasonic instruments, Kunshan, China) at 20°C for 20 min. Extracts from untreated and electropermeabilized samples were extracted with a centrifuge (3K30, SIGMA, Osterode am Harz, Germany) at 10,000 rpm for 10 min to enhance the separation of the exhausted solid and clear supernatant. The extraction procedure was repeated three times on the remaining waste until the blueberries became colorless. Finally, all the extracts were filtered through regenerated cellulose syringe-tip filters (0.45 µm) before undergoing further characterization;

for example, high-performance liquid chromatography with diode-array detection (HPLC-DAD) analyses.

2.7. High-performance liquid chromatography with diode-array detection (HPLC-DAD) analysis of anthocyanins

Individual anthocyanin compounds were identified by liquid chromatographic analysis. Anthocyanins were separated with an HPLC Waters 2695 system coupled to a photodiode array detector (DAD) (Waters 2998, Waters Corporation, Milford, MA, USA). An ACE Excel 5 Super C18 column (5µm, 250 mm × 4.6 mm, Aberdeen, Scotland) was used for the analytical separation of anthocyanins according to the method previously optimized by Wang et al. (2014). The temperature of the HPLC column was set at 25°C. Eluent A was double distilled water, including 10% formic acid, and eluent B was 15% methanol in acetonitrile. A gradient elution program included eluents A and B, which applied 5% B for 0 min, 12% B for 30 min, 25% B for 50 min, and 5% B for 60 min. The flow rate of the mobile phase through the column and the injection volume was 1 mL/min and 10 µL, respectively, with an absorbance detection wavelength set at 520 nm.

Three commercial standards, delphinidin-3-glucoside, peonidin-3-glucoside, and petunidin-3-glucoside, were used to quantify and identify the anthocyanins in all the extracts. The standards were alternatively dissolved in a mixture of solvents A and B (10:90 v/v) in the 3.125 to 100 µg/mL concentration range to generate 5-point external standard calibration curves with acceptable linearity (R^2 = 0.999). The results were expressed as mg/L.

2.8. Statistical analysis

All experiments and analyses of collected samples were performed in triplicate, and the mean values and standard deviations of experimental data were calculated. Statistically significant differences ($p < 0.05$) among the means were evaluated with a one-way analysis of variance (ANOVA) and Tukey's

test. Data were analyzed with the Statgraphics-Centurion XVI version 16.1.03 software (Statistical Graphics Corp., Herndon, VA, USA).

3. Results and discussion

3.1. Measurement of equipment parameters

Figure 3 shows the relationship between the applied voltage from the power supply (input) (Figure 2) and the HVED prototype voltage (output). There was a linear correlation between both voltage measurements (r^2 = 0.9923) at a working frequency of 200 kHz, which was verified with an oscilloscope when measuring the voltage. Equation (3) shows the relationship between both measured voltages.

$$Y = 2.8082 * X - 19.181 \quad (3)$$

These results are necessary to calculate the amount of specific energy applied during the HVED treatment. Table 1 shows the specific energy consumption (WT expressed as kJ/kg) applied to blueberries during the HVED treatments according to the combination of applied electric field intensity (E expressed as kV/cm) and process time (t expressed in s).

When comparing the parameters of this prototype with the equipment used in other studies (Table 2), voltages that were used ranged from 15 to 40 kV with a frequency range of 0.5 to 1000 Hz (1 kHz) in different food matrices. The HVED prototype used in the present study generates voltages in the same range; however, the difference with this equipment is that it works at a high frequency of 200 KHz. As was previously mentioned, this is an important factor to consider when electroporation is generated at the cellular level.

The energy consumption of the authors cited in Table 2 ranges from 7.27 to 800 kJ/kg depending on the sample, which is due to the difference in impedance of each sample. In the treatments of blueberries in the present study with the new HVED prototype, different electric field intensities, which varied between 6 and 579 kJ/kg were used.

3.2. Effect of high voltage electric discharge (HVED) pretreatment on the structural features of blueberry cellular tissues

The stress resistance of the cell wall is due to its structural protein (5 to 10%), cellulose (30 to 40%), hemicellulose (30%), and pectin (15 to 30%) composition (Blaker & Olmstead, 2015). It is also directly related to its own thickness, which is the main contributing factor to the firmness and texture of the fruit cellular tissues (Li et al., 2017). The TEM micrographs of blueberry parenchyma tissue are shown in Figure 4 for untreated samples (Figure 4a) with a humidity of 79.07 ± 1.24%, HVED treated samples (E = 2 to 10 kV/cm, t = 10 to 30 s) (Figures 4 b to 4 j), and alternatively processed samples in a thermal (Figure 4 k), chemical (Figure 4 l), and enzymatic (Figure 4 m) process. The micrographs enabled an in-depth observation of the sample cellular structures focused on the middle lamella, cell wall, intercellular space, tonoplast, plasmalemma, and protoplast.

There was a high degree of cell compartmentalization and small intracellular spaces. The fresh samples were composed of numerous closely bound cells (Figure 4 a) using a well-limited middle lamella. A large vacuole occupied the most significant space in the cells, which is a feature in all micrographs. Moreno et al. (2012) obtained similar results in strawberries treated by Ohmic heating to those shown in Figures 4 b to 4 m show that all HVED treatments produced cell plasmolysis, cell collapse, intracellular space, cell contraction, cell deformation, protoplast contraction, and distortion of the cell wall border. The middle lamellae and walls suffered degradation in all treatments. Cell wall degradation was correlated with the deterioration of the middle lamella. These changes generated the loss of firmness, cell cohesion, and texture quality of the fruit. (Moreno et al., 2012). Blueberry samples treated with HVED (Figures 4b to 4j) showed altered and perforated cell walls, which can be explained by the electropermeabilization mechanism. Similar behavior was shown in samples treated by traditional processes (thermal, chemical, and enzymatic) displayed in (Figures 4k, 4l, and 4m). Their cellular structure was also altered, more specifically in the membrane and cell wall. When cells are permeabilized,

channels open and enable diffusion into the tissue (Kulshrestha et al., 2006) as shown in Figure 4. In treatments with higher voltages (Figures 4h, 4i, and 4j), perforations in the membrane and disintegration of the cell wall and middle lamella were easier to identify. Figure 4g illustrates that the protoplast and cell contents are retracted to the center of the cell and show signs of plasmolysis.

The electroporated cells underwent plasmolysis, which deformed the cell wall and membrane causing the collapse of the intercellular spaces (Figures 4d to 4j). The middle lamella of the cell walls was detached and damaged, causing cell deformation and cell rupture. Similar results were obtained by Faridnia et al. (2015), who applied PEF (0.2 to 1.1 kV/cm) to raw potato samples. Their micrographs showed that the higher the intensity of the applied electric field, the greater the potential to irreversibly alter the permeability of biological cells, thus affecting the appearance and properties of plant cells. Zhang et al. (2022) also applied PEF (0.5 to 20 kV/cm) to fried potatoes and reported that most of the cell walls collapsed and dense pores were observed when the electric field intensity was higher. Small porous structures of the cell wall (10 – 50 nm) were also broken down and formed larger porous structures (50 to 100 nm), thus increasing water loss and oil adsorption during frying. Han et al. (2009) found similar results in their study of cornstarch treated with PEF at 30, 40, and 50 kV/cm.

Similar modifications on the cellular structure were identified with the HVED treatments under different experimental conditions when comparing our results with those reported in the literature (Faridnia et al., 2015; Han et al., 2009; Zhang et al., 2022)., A higher degree of cellular disintegration occurred at higher applied voltages and longer exposure times. Image analysis using micrographs is a qualitative rather than a quantitative method, but it was clear from the images that electroporation occurred in the blueberry cell matrix.

3.3. Effect of high voltage electric discharge (HVED) pretreatment on the cell permeabilization index (Z_P)

Figure 5 shows the impedance spectra of the modulus (a) and phase angle (b) as a function of the investigated frequency range between 100 and 1000 kHz (10^2 to 10^6 Hz) of the impedance equipment for the untreated (control) or HVED treated samples at 4 kV/cm at different processing times (t = 2 to 30 s), according to the methodology proposed in section 2.5. Results are similar to those reported by Donsì et al. (2010), i.e., a typical sigmoidal decreasing trend for control samples has been reported as having two characteristic zones associated with an unusual electrical behavior. These authors indicated that high impedance moduli occurred at low frequencies (10^2–$5x10^3$ Hz), which promoted the ability of tissue-forming cell membranes to act as "current blocker" systems (e.g., capacitors), whereas at high frequencies (3×10^3 to 5×10^7 Hz), there was a short circuit that produced a typical pure ohmic behavior. Therefore, cellular tissue damage produced by electropermeabilization could be detected only in the low frequency range.

The application of intermediate electrical treatments (E = 4 kV/cm) to blueberries produced a significant decrease ($p < 0.05$) in the impedance modulus curves, the magnitude of which was amplified with increasing processing time and indicated electroporation phenomena. The latter effect is also clear in the phase angle curves, which are depicted in Figure 5b and show an abrupt shift toward higher values after applying electrical energy to the samples. Based on the minimum detected peak value from untreated samples ($\phi \approx -80°$), an approximate 3-fold increase in this parameter occurred when HVED treatments were longer than 10 s. The simultaneous analysis of Figures 5a and 5b suggests that the degree of cellular tissue damage increases with the intensity of the applied HVED treatments.

Figure 6 displays the Z_P vs. the HVED treatment time for different electric fields in blueberries samples. Results were the mean of triplicate samples. These results concur with those displayed in Figure 5, that is, cellular tissue damage increases with the applied electric field intensity. Regardless of the applied electric field intensity, the Z_P parameter continued to increase until it reached a saturation value at 10 s treatment time; any further increase in the degree of electroporation was barely detectable over

this value. The statistical analysis also highlighted the influence of the electric field intensity on the degree of cell permeabilization of blueberry tissues. For example, after 10 s of the applied HVED treatment, the Zp index significantly increased ($p < 0.05$) when the electric field intensity increased within the study range (E = 2 to 10 kV/cm) it reached the highest value under the most intense treatment condition. According to the results displayed in Figure 4, the optimal condition is reached in the treatment using 4 kV/cm for 10 s with a Zp value of 0.68 ± 0.03 and energy consumption of 133.36 ± 3.30 kJ/kg (Table 1). It is necessary to highlight that all the experiments were performed at a constant frequency of 200 kHz. Rajha et al. (2015a) compared ultrasound, PEF, and HVED treatments applied to grapevine shoots; for the HVED treatments developed at E = 40 kV/cm with a frequency of 0.5 Hz, they reported a Zp value of 0.71 (similar to the one in present study), but energy consumption (242 kJ/kg) and total exposure time (25 min) were higher. El Kantar et al. (2018) performed treatments in orange peels by HVED under conditions similar to the Rajha experiments (E = 40 kV/cm with a frequency of 0.5 Hz). They reached a Zp value of 0.47 with an energy consumption of 222 kJ/kg and a total exposure time of 23 min. We can conclude that with the new HVED prototype developed in the present study, it is possible to decrease exposure times with lower energy consumption and good Zp values.

It is worth mentioning that in another study with PEF applied to potato, onion, and carrot samples, Zp values of 0.83 ± 0.08, 0.72 ± 0.09, and 0.65 ± 0.1, were obtained, respectively (Shorstkii et al., 2022).

Finally, the comparison treatments (chemical, enzymatic, and thermal) displayed lower values of Zp than those treated by HVED (chemical: 0.5 ± 0.03; thermal: 0.1 ± 0.01; and enzymatic: 0.28 ± 0.03) because they all affect the outer cells of the blueberries.

3.4. Comparative effect of different cell permeabilization methods on anthocyanin recovery

Table 3 shows values for anthocyanins extracted from the blueberry samples for the different treatments, that is, untreated (control), HVED, thermal, enzymatic, and chemical (NaOH) measured by HPLC. Figure 7 displays an HPLC chromatogram of methanolic extracts obtained from untreated

blueberry samples at the 520 nm wavelength. Ten anthocyanins were identified and quantified in both the treated and control samples. Total anthocyanin values in the blueberry samples ranged from 50.71 to 190.92 mg/L. The highest anthocyanin percentage in the blueberries was in malvidin 3-O-galactoside (M-3O-Gal), followed by delphinidin 3-O-galactoside (D-3-O-Gal) and malvidin 3-O-glucoside (M-3-O-Glu).

There was a significant increase in total anthocyanin values when comparing the control with the HVED treatment at 10 kV/cm for 10 s. Anthocyanin values ranged from 83.09 ± 1.20 (control) to 136.82 ± 0.84 (HVED), which was 64.66% higher. It can be inferred that the damage caused by the HVED treatments to blueberry cells in the membrane is related to the increased extraction of anthocyanins from the blueberry samples. Bobinaitė et al. (2015) studied anthocyanin extraction from blueberries with PEF, showing a significant increase in anthocyanin and total phenol content of treated blueberries when applying a field intensity of 1 kV/cm. However, they found a slight decrease when a more intense PEF treatment (5 kV/cm) was applied.

Total extraction values for the thermal treatment in our study were 39.54 % higher than for HVED, the enzymatic treatment values were 62.3% lower than HVED, and the chemical treatment showed a slight 5.30% decrease compared with HVED. Nevertheless, the enzymatic and chemical treatments needed an additional process to inactivate the enzymes and regulate pH, respectively, whereas the thermal treatment requires more energy than HVED. It is also important to mention that thermal treatments degrade sample anthocyanins (Musilová et al., 2022; Zhou et al., 2018). The so-called electrotechnologies, such as PEF, ohmic heating, and HVED, have reduced the degradation of valuable compounds (Kostelac et al., 2020; Puértolas & Barba, 2016).

4. Conclusions

Cell membrane electroporation was observed both qualitatively (transmission electron microscopy imaging) and quantitatively (cell permeabilization index, Zp) in all the samples treated with

368 the high voltage electric discharge (HVED) generator prototype, whose extent was dependent both on
369 the applied electric field intensity and exposure time.

370 It can be stated that the new HVED prototype developed in the present study can decrease
371 exposure times with lower energy consumption and good Zp values at high operational frequency.
372 Another important conclusion is that the extraction of valuable compounds (e.g., anthocyanins) was
373 enhanced in the case of HVED-treated samples. However, the prototype has limitations related to sample
374 capacity since it has been set on a laboratory scale.

375 Therefore, further studies are required to seek for a scaling up of the technology, as well as to
376 unravel the potential of HVED to assist extraction processes of blueberry waste (e.g., skins and seeds),
377 in view of a full raw materials utilization.

378

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385 **References**

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Figure captions

Figure 1. Process flow diagram

Figure 2. Schematic representation of the high voltage electric discharge (HVED) prototype system: oscilloscope (TDS 3034B, Tektronix, Wilsonville, OR, USA), voltage probe (P6015A, Tektronix, Wilsonville, OR, USA), current probe (Rogowski coil 2 - 0.1, Stangenes Inc., Palo Alto, CA, USA), Computer, Arduino Uno Rev 3, 12 volt direct current power supply, and HVED prototype generator.

Figure 3. Linear relationship between the input voltage and the voltage produced by the high voltage electric discharge (HVED) prototype across the electrodes of the treatment chamber.

Figure 4. Transmission electron microscopy (TEM) operated at 100 kV in a magnification range from 18,000x to 54,000x. Each image has a reference line in micrometers and micrographs of parenchyma

tissue from untreated (control), high voltage electric discharge (HVED) treated (E = 2 to 10 kV/cm, t = 10 to 30 s), and alternatively processed (thermal, chemical, enzymatic) blueberries. ML: middle lamella; PL: plasmalemma; T: tonoplast; V: vacuole.

Figure 5. Impedance modulus (a) and phase angle (b) curves against frequency for untreated (control) and high voltage electric discharge (HVED) treated (E = 4 kV/cm) blueberries. Standard deviations were used as error bars ($p < 0.05$).

Figure 6. Cell permeabilization index (Z_P) of blueberries as a function of the high voltage electric discharge (HVED) treatment intensity (E = 2 to 10 kV/cm, t = 2 to 30 s). Standard deviations were used as error bars ($p < 0.05$).

Figure 7. High-performance liquid chromatography **(**HPLC) chromatogram at 520 nm wavelength of methanolic extracts from untreated blueberries. Peak identification: 1) delphinidin 3-O-galactoside (D-3-O-Gal), 2) delphinidin 3-O-glucoside (D-3-O-Glu), 3) cyanidin 3-O-galactoside (C-3-O-Gal), 4) delphinidin 3-O-arabinoside (D-3-O-Ara), 5) petunidin 3-O-galactoside (Pet-3-O-Gal), 6) petunidin 3-O-glucoside (Pet-3-O-Glu), 7) peonidin 3-O-glucoside (Peo-3-O-Glu), 8) malvidin 3-O-galactoside (M-3-O-Gal), 9) malvidin 3-O-glucoside (M-3-O-Glu), and 10) malvidin 3-O-arabinoside (M-3-O-Ara).