Master's Programme in Food Technology and Nutrition

MASTER THESIS

Shelf life of fresh-cut fruits and salad leaves treated with Pulsed Electric Field (PEF)

by

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Master's Thesis
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Abstract:

Application of Pulsed Electric Field (PEF) treatment on five fresh commodities was analysed for the changes in the metabolic and physiological effects on their shelf life. Treatment parameters were optimised in order to achieve uniform electroporation on the samples, which was analysed by Propidium Iodide staining for the leaf samples. The viability of electroporation of greenhouse (GH) rocket, commercial rocket and spinach leaves was evaluated by FDA vital staining. Bulk evaluation of electroporation of mango and kiwi fruits along with the leaf samples was done by impedance measurement, monitoring tissue leakage caused by permeabilization. The evaluation of metabolic responses of the samples was carried out by isothermal calorimeter where increase in metabolic heat production (thermal power) of all the treated samples was recorded. The increase in thermal power is the result of an increase in the internal metabolic activity of tissues, provoked by PEF induced stress. A decrease in metabolic heat rate was observed with increasing intensity of PEF parameters, due to the possible consequences of loss in cell viability.

After the optimisation of PEF parameters, the samples were treated, packed and stored at 4 °C. Measurements for the colour change was recorded using spectrophotometer and composition of O_2 and CO_2 was monitored using a gas analyser. PEF treatment showed effective results in preserving the colour of greenhouse rocket leaves. No effects of treatment were observed in commercial rocket and spinach leaves and the treatment resulted in deterioration of mango and kiwi fruits. Composition of O_2 and CO_2 did not show differences among samples.

Keywords: PEF, pulsed electric field, reversible electroporation, calorimeter, metabolic rate, shelf life, fresh commodities

Contents

1. Introduction

There has been a recent surge on minimal food processing due to increasing consumer demands. The demands to develop affordable and sustainable methods to increase the shelf life of food products have led the food processing industries to face several challenges. Hurdle technology such as freezing, chilling, packaging, heat treatment etc. is the most common approach by food industries for extending the shelf life of fresh commodities. Over the past decade, processes known as 'novel food processing' technologies have started to come into the picture of global food production where the main purpose is to preserve the nutritional profile and enhance the functional properties of food products, apart from the main aim of preserving and extending the shelf life (Koutchma, 2014).

Pulsed electric field (PEF) treatment is one of the novel processing technologies, where short pulses of high voltage electric field are utilized for applications such as mass transfer and inactivation of pathogens, offering energy effective processing for the industry without significant increase of heat, thereby preserving the nutritional composition of fresh produce. Most of the previous work done by electrical treatment are based on irreversible electroporation which results in cell degradation, mainly aimed for microbial inactivation and applications of mass transfer from plant tissues. This project deals with the reversible form of electroporation and its effect on the metabolic heat rate and shelf life of fresh products.

Five fresh products were chosen for this project, three of which were salad leaves and two were fruits. PEF was tested as a possible method for increased shelf life of the commodities. The possibility of PEF treatment and its effects on the quality of fresh produce may present an interesting opportunity for the fresh-cut sector and minimal processing. Establishing the parameters of PEF which could influence the metabolic response of fresh products is necessary to maintain the quality of final products during storage and post processing. The project also uses analytical tools such as isothermal calorimeter, impedance measurement and fluorescence microscopy for the optimisation of PEF parameters.

2. Aims

General aim

- To study the effect of PEF treatment on the shelf life of selected packed fruits and vegetables.

Specific aims

- To optimise pulsed electric field parameters to achieve reversible electroporation on fresh-cut fruits and green vegetables.
- To study the effects of PEF treatment on the heat production rate and impedance of fresh products.

3. Background

The trend of minimally processed foods is increasing due to lifestyle changes, and the short shelf life of fruits and vegetables presents practical challenges to food industries in order to maximise the storage life and to prevent losses. Fresh cut fruits and vegetables are prepared by peeling, dicing and slicing in order to provide convenience to the consumers. The desirability of fresh-cut products is characterised by its freshness, nutritional composition, convenience, safety and sensory attributes added by its extended shelf-life (González *et al.*, 2004). Due to similar nutritional content as the whole products, fresh-cut products come with added advantage of convenience as well as lower price compared to other processing techniques (Artés *et al.*, 2009).

The ready-to-eat fresh products are manufactured by minimal processing, which makes them susceptible to degradation in quality during processing and storage due to physiological deterioration, biochemical changes and microbial spoilage (O'Beirne *et al.*, 2003). The absence of epidermis and damaged cells during cutting of fruits exposes them to the atmosphere, making them highly perishable due to microbial growth and oxidation (Watada, 1999). This consequence of wounding related to minimal processing results in a variety of physiological changes affecting the overall viability of the produce (Saltveit, 1996). Along with unit operations such as peeling, washing, cutting, and packaging of fresh-cut products, the shelf life of cut products also depends upon optimum storage temperature, use of antioxidants as antibrowning agents, good manufacturing practices, atmosphere composition and suitable packaging techniques (Artés *et al.*, 2009).

3.1 Factors affecting the shelf life of fresh products

Wounding of the vegetable and fruit tissues during post-harvest and pre-processing treatments directly influences their shelf life. The physiological changes caused due to the processing of fresh produce may result in various post-processing consequences such as discolouration on

the cut-surface due to enzymatic browning, production in off-flavour compounds, shrinkage, accelerated softening, water loss, loss of chlorophyll, pigment formation, lipid oxidation and decay which lead to a shorter shelf life (Jideani *et al.,* 2017; Toivonen & De-Ell, 2002). Cell breakdown during cutting of fresh products also releases intracellular constituents such as oxidizing enzymes which causes rapid decay of the product (Pradas-Baena *et al.,* 2015). Responses on phenolic metabolism is triggered when the wounding stress occurs while cutting the produce (Rhodes and Wooltorton, 1978). Wounding also results in an increased respiration rate, high levels of ethylene production, oxidative browning and degradation of membrane lipids (Oliveira *et al.,* 1998; Smetanska *et al.,* 2013). Intrinsic factors of the product such as pH, water activity, available oxygen, microbial flora and formulation of the product determines its quality and shelf life after the packaging (Brown & Williams, 2003).

Among various methods used in order to monitor the quality of fresh products, the deterioration is visually characterised by changes in colour due to oxidative browning, flaccidity due to water loss and also microbial contamination (Varoquaux & Wiley, 1994). Physiology of the product, pre and post-processing treatments and packaging determines the microbial growth on the minimally processed product (Watada, 1997). The most common properties of fresh cut products such as colour, taste and firmness change over storage time and determines the quality of the commodity.

3.2 Quality assessment of packed fruits and vegetables

3.2.1 Factors affecting respiration rate and internal composition of packed products

The mixture of gases in the packaging depends on the type of product, the type of packaging material as well as the storage temperature (Oliveira *et al.,* 2015). The type and maturity stage are the major factors that affect the respiration rate of the commodity, with varying metabolic activity resulting in distinctive respiration rates (Fonseca Oliveira & Brecht, 2002). According to Kader & Saltveit, (2003), a sufficient amount of oxygen concentration is necessary to maintain aerobic respiration, and senescence can be slowed if the concentration of O_2 is less than 10%. On the other hand, depending on the tolerance of the commodity, the accumulation of CO_2 can either be beneficial or harmful (Kader & Saltveit, 2003). Atmosphere of 3-6% O_2 and 2-10% CO_2 is considered ideal for microbial control that helps in the extension of shelf life of variety of fresh-cut products (Oliveira *et al.,* 2015) (see Table 1 in the Appendix for MAP storage recommendations- (Gorny, 1997)). Wounding or cutting results in higher ethylene production rate that could influence the respiration rate and consequently advance the deterioration and stimulate the ripening of climacteric fruits (Brecht, 1995). Proper temperature management helps to control the respiration rate of the commodities. Respiration rate is directly influenced by the storage temperature of the commodity. According to Zagory & Kader, (1988) metabolic reactions generally increase two or three fold for every 10 °C rise in temperature. Lower temperature is considered ideal to store fresh products for a longer shelf life as it slows down the ripening due to decreased metabolic activity. Low relative humidity also affects the respiration rate as it results in loss in moisture, transpirational damage and increased respiration (Kader, 1987).

Pre-treatments such as handling operations, application of heat, packaging material and type of packaging of food products could also influence their shelf life. Molnar & Friedman, (1990) observed that enzymatic browning of the commodities caused by oxidation can be inhibited by the exclusion of oxygen from the packaging. On the other hand, Bolin and Huxsoll (1989), in their work for the storage stability of minimally processed peaches, apricots and pears stated that deterioration of plant cells can only be slightly altered by modified atmosphere packaging. Packaging film permeability plays a major role when fresh products are packed; the permeability of the packaging material could be modified in order to match with the respiration rate of the product to avoid anaerobic conditions that may create appropriate environment for the growth of certain microorganisms (Laurila & Ahvenainen, 2002). Mild heat pre-treatments have also been studied showing effective quality attributes such as firmness and colour of peaches (Steiner *et al*., 2006), improvement in firmness of vegetables such as carrots and green beans (Bourne, 1987), potatoes (Andersson *et al*., 1994) and fruits such as cantaloupe (Luna-Guzmán, Cantwell & Barrett, 1999) and rocha pear (Abreu *et al*., 2003).

3.2.2 Colour change

Measuring the colour of the food product is important for monitoring its shelf life because various factors such as enzymatic browning, pigmentation and loss in chlorophyll could lead to change in the colour during the storage life of the commodity. Major cause for chlorophyll or colour loss during the storage of green vegetables can be attributed to factors such as light, humidity, temperature, internal gas composition of the packages including ethylene and enzymes present in the leaves (Yamauchi & Watada, 1991). Colour change can also be caused due to senescence as it results in yellowing of the leaves, observed by Able *et al*., (2003) for Asian green leafy vegetables.

There have been several colour measuring instruments used but CIE L*a*b* system described by Commission Internationale de l'Eclairage (CIE) has been widely implemented by food industries to measure colour change of food products. It is an effective method of measuring colour differences and tracking colour changes during processing and storage (Wrolstad, Durst & Lee, 2005). There are three colorimetric coordinates or variables based on which the instrument measures the colour, and the specification of the colours are described with those variables that correspond to the parameters of hue, saturation and lightness (Frausto-Reyes *et al*., 2009). L* is the first value that measures the lightness of the product where positive values represent lightness and negative values represent darkness. Positive values of a* represent ‘redness’ and negative values show ‘greenness’, whereas, positive b* value is the measure of ‘yellowness’ and negative value is the measure of ‘blueness’ (Wrolstad, Durst & Lee, 2005). These parameters can be further utilized to calculate total colour change ΔE* (Frausto-Reyes *et al*., 2009).

3.3 Pulsed Electric Field

Pulsed Electric Field (PEF) can be applied on biological cells to induce permeabilization of cell membranes (Zimmermann *et al.*, 1974), technically it is known as electropermeabilization or electroporation. PEF treatment is a novel food processing technology, a non-thermal treatment of food products which involves the application of short, high intensity electric pulses in order to alter the cells of food commodities such as fruits and vegetables by creating pores in the cell membrane. Depending on the number of pulses and strength of the electrical field, permeabilization could occur in either reversible or irreversible forms (Knorr *et al.*, 2001). From the past decade, PEF treatment in its irreversible form has found its way in a large number of commercial applications (Toepfl *et al.*, 2006), such as drying, cutting, freezing, extraction, pasteurisation and more. There are two main applications of irreversible PEF treatment in food processing, i) non-thermal inactivation of microorganisms, used for pasteurisation of liquid foods, and ii) enhancement of mass transfer from cells induced by cell damage (Jäger, 2013).

During the application of the electric field, the membrane breaks down when the transmembrane potential reaches a critical value of approximately 1 V for a bimolecular lipid membrane (Kinosita & Tsong 1977), known as critical electric field strength (E_c) (Aronsson *et al.*, 2001). If the electric field strength (E) is lower than the critical electric field strength (E_c), no permeabilization is achieved. The application of relative low electric field results in reversible permeabilization where cell membrane recovers its structure and functionality (Donsi *et al.*, 2010). Reversible electroporation allows the pores in cell membrane to recover and continue with normal cell functions. Electric field strength applied at a stronger intensity results in irreversible permeabilization causing cell disintegration and loss in cell viability (Zimmermann, 1986).. It should be noted that in reversible electroporation, molecular transport between the porous cell membrane is observed from pore formation till the membrane resealing is completed (Kandušer & Miklavčič, 2009).

Overall, electroporation can be explained in different phases (Donsi *et al.*, 2010, Vorobiev & Lebovka, 2009; Kandušer & Miklavčič, 2009):

- charging and polarization of the membranes (charging time of ≈1 μs)
- temporal destabilization and creation of pores (reported as occurring on time scales of 10 ns depending on the length of the pulse)
- expansion of pore radii and aggregation of different pores (in a time range of hundred microseconds to milliseconds, depending on the length and duration of pulses)
- resealing of pores taking place after the application of electric pulses (the time scale of seconds).

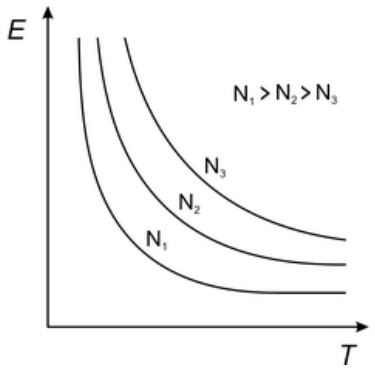


Figure 1. Electropermeabilization of cells increasing with increasing number of PEF parameters such as applied pulses, electric field strength (E), pulse duration (T), and number of pulses (N) (from Kandušer & Miklavčič, 2009).

The extent of electroporation of cell membrane is dependent on the parameters of applied PEF (Canatella *et al.*, 2001). As the parameters such as number of pulses, electric field strength, total duration of pulses increases, the permeabilization of cell membrane also increases which sets up a direct relation between the two (Figure 1). Depending on these parameters, reversible and irreversible electroporation can be achieved by decreasing and increasing the values of the parameters (Pavlin *et al.*, 2008).

Electroporation results in increased extracellular conductivity due to the release of intracellular constituents through the permeabilized cell membrane, which increases the conductivity of the solution where the product is treated in (Kinosita & Tsong 1979). The overall bulk-assessment of PEF treatment can be done by measuring the impedance of the solution containing the treated sample. The extent of damaged cells can be identified by measuring the changes in the impedance of treated and untreated samples (Angersbach, Heinz & Knorr, 2002). Damaged cells are characterised by the leakage of their cell constituents, which increases the conductivity of the solution resulting in lower impedance. Similar results are obtained when irreversible electroporation is achieved; permeabilization results in the leakage of cell constituents due to membrane disintegration and loss in cell viability (Zimmermann, 1986). Reversible electroporation, on the other hand, allows the cells to recover their cell membrane after permeabilization which causes lesser leakage of cell constituents. This treatment provokes less influence on the conductivity of the solution resulting in higher impedance (conductivity is the reciprocal of impedance or resistance) values which makes the difference between damaged cells and viable cells distinguishable by impedance measurements.

The electrical model by Fricke (1925) is employed in order to estimate the level of electroporation by measuring the electrical resistance of the cells over a range of frequencies, where intracellular and extracellular medium is considered as resistance due to the presence of ions such as Na+, Cl- and K+, while, the cell membrane acts as a capacitance as it consists of a thin lipid bilayer. When the electric field is passed through the cells, low-frequency currents will not penetrate through the cell membrane as it acts as a capacitor, therefore the impedance or resistance measurements will show higher values at low frequencies than at high frequencies

(Fricke, 1925). Cell membrane permeability increases after electroporation, so the PEF treated cells will have lower resistance at lower frequency because of the increase in conductivity of cell membrane and leaching of intracellular constituents and ions into the extracellular medium (Fricke, 1925).

In the reversible form of the PEF technology, cell properties such as size, conductivity and orientation, could directly influence the effect of PEF on the cells, provoking stress responses. The physiological responses to PEF induced stress are still largely unknown (Gomez Galindo *et al.*, 2009), however it is well known that PEF alters the metabolism of the cells through several physiological and chemical alterations including oxidative stress (Gabriel and Teissié, 1994, Gómez Galindo, 2016).

3.4 Isothermal calorimetry

The isothermal calorimeter is an instrument that measures the heat produced by the sample. The sample is kept in an ampoule and the ampoule holder is connected with a heat flow sensor on the thermostated heat sink, which measures the heat generated by the heat flow sensor as a voltage (Wadsö, 2010). It is a measurement technique in which thermal power or heat production rate of a sample is measured over time at a constant temperature, as almost all physical, chemical and biological processes produce heat (Wadsö & Gómez Galindo, 2009). Several food science-related phenomena can be studied using isothermal calorimeters such as microbiological studies, vegetable tissue respiration, effects of thermal treatment on the food samples, and physical processes such as crystallisation (Wadsö & Gómez Galindo, 2009). The use of isothermal calorimetry nowadays is common in fundamental biology, microbiology and food sciences. (Wadsö & Gómez Galindo, 2009). Based on these usage of calorimetry for food related commodities, the overall metabolic activity of fresh-cut fruits, vegetables and leaves can be measured in the form of thermal power. The heat produced by the sample and total thermal power measured by the calorimeter can be utilised to model kinetics such as degradation processes (Hansen, 2000). This degradation process can be correlated with the approximate shelf life of fresh commodities.

Prior works have been done to study the shelf life of food products using isothermal calorimetry. Riva, Fessas & Schiraldi, (2001) studied the shelf life of whole eggs, milk and fresh carrots using isothermal calorimetry and explained the effects of temperature on the shelf life of the commodities. Their stability times were also evaluated by using parameters such as microbial plate count and pH. Similar work on the shelf life was done for fresh cut pineapples by (Iversen, Wilhelmsen & Criddle, 1989) using isothermal calorimeter where suitable storage conditions, effects of inhibitor and antibiotics were examined for the pineapples in the study. Prediction of the shelf life of carrot juice and microbial effects on ground meat were studied by Alklint, Wadsö & Sjöholm, (2005) and Gram & Sögaard, (1985) using calorimetry respectively. Processing consequences such as wounding stress resulting in the changes in metabolic activity can also be detected by calorimetric responses. Increase in the metabolic activity of wounded or cut fresh products is caused due to the increased biosynthetic processes that take place in wound healing (Laties, 1978, Wadsö *et al*, (2004).

3.4.1 Calorimetry for measuring metabolic stress induced by PEF treatment

The stress response, respiration and viability of plant cells can be monitored by calorimetric measurements as a direct indication of their metabolic responses (Criddle *et al.,* 1991, Gómez Galindo *et al*, 2008). In a work by Dellarosa *et al,* (2016), metabolic response of fresh-cut apples treated with pulsed electric field was studied using isothermal calorimeter where reversible and irreversible electroporation were distinguished by the differences in the metabolic heat responses of the sample. Drop in the metabolic heat along with the respiration rate was noted as a result of irreversible electroporation, due to probable consequence of loss in cell viability. Metabolic response of potato tissue induced by PEF treatment was also examined by Gómez Galindo *et al*, (2008), where calorimetry is suggested to be a reliable method to provide information on metabolic responses of fresh products that cannot be achieved from electrical measurements such as impedance measurement. It was observed in the study that metabolic responses are strongly dependent on pulse parameters and intensity. Isothermal calorimetry is utilized in this project to measure the thermal power as a reaction to metabolic stress induced by electrical treatment.

4. Materials and methods

4.1 Raw Materials

Five different fruits and vegetables were chosen for this project. Green-leafy vegetables such as commercial rocket, greenhouse rocket and baby spinach leaves were chosen as vegetables. Mangoes and kiwi fruits were also chosen for the study. Both mango and kiwi fruits are climacteric fruits with higher rate of ethylene production and respiration rate during the ripening process (Singh *et al.*, 2013; Antunes and Sfakiotakis, 2002). The fresh leaves were trimmed and washed, and the fruits were cut according to the measurements stated below.

4.1.1 Fruits

Mango:
Mango fruits, imported from Peru, were purchased from the local supermarket, which is imported from Peru. The Brix content was analysed with a refractometer (Hanna HI96801, Hanna Instruments Inc., USA) and fruits within the range of 14-16% brix were used for the tests in order to assure the same level of maturity of the fruits used for the experiment. Fruits that had lesser or more brix content were not used. The instrument was calibrated using deionized water every time before the analysis of samples.

The fruits were purchased within 3 days of their arrival at the store. They were washed and sliced in two sections along with the stone, peeled and cut into 2 x 2 x 0.4 cm cubes for all the treatments.

Kiwi fruits:
Kiwi fruits were purchased from the local supermarket, which were imported from Italy. Their Brix content was analysed a refractometer and fruits within the range of 13-15% brix were used for the tests. The fruits were bought within 3 days of their arrival at the store. They were peeled and cut into two halves lengthwise, which was further cut into 0.4 cm slices for all the treatments.

4.1.2 Vegetables

Greenhouse (GH) Rocket leaves:
Rocket leaves were grown in a greenhouse with controlled temperature of 16-20 °C and 45-50% RH. The seeds were planted 1.5 cm deep in the soil, with planting point holes of 3 columns and 7 rows in 56 (L) x 25 (W) x 6 (H) cm trays. Leaves 7.0 ± 0.5 cm long 2.5 ± 0.4 cm wide were harvested after 5 weeks of sowing time, around 10-11 am every time and stored in a plastic box lined with a wet paper towel until used. They were transported to the laboratory, rinsed with tap water and treated within an hour from the harvest.

Commercial Rocket leaves:
Rocket leaves were purchased from the local supermarket on the first day of the arrival from the distributor. The leaves were imported from Spain and transferred within 4-5 days to the central warehouse in Sweden. Just like the greenhouse rocket, these store-purchased leaves were also chosen with same dimensions of length and width. The leaves were transported to the laboratory, stored in a box lined with wet tissue paper to maintain humid conditions, refrigerated at 4 °C and used within 4 hours of purchase.

Baby Spinach leaves:
Spinach leaves were purchased from the local supplier Cater Grönt, Malmö, Sweden. They were stored at refrigerated temperature of 4-6 °C after the arrival from the distributor. The leaves were washed and packed in 500 g packages by the supplier with shelf life of 9 days from the day of packaging. They were purchased from the supplier on the same day of arrival, transported to the laboratory, rinsed with tap water, stored in a plastic container lined with wet tissue paper and used within the same day.

4.2 Treatments

4.2.1 Electrical treatment

Samples of the fruits and vegetables were transferred to a PEF chamber having 0.5 cm distance between both electrodes. An electrolyte solution was prepared by mixing deionized water and sodium chloride (NaCl) and the conductivity was adjusted to 130 μS. The conductivity was measured with a conductivity meter (Eutech Instruments, USA). This electrolyte solution was added to the PEF chamber along with the sample just enough to cover both the electrodes of the chamber. The PEF chamber was connected to ARC Aroma Pure AB CEPT pulse generator (Lund, Sweden). The input of treatment parameters was given by the computer software ARC CEPT HM13 (Lund, Sweden).

Different optimised PEF parameters are stated in Table 1. The optimisation and steps of achieving reversible electroporation are explained in the Results and Discussion section.

Table 1: PEF treatment parameters for the samples used in the investigation

Sample	Type of pulses	Amplitude (V/cm)	Pulse width (µs)	Pulse space (µs)	No of pulses	Other parameters
GH Rocket	Bipolar	1100	20	1000	500	-
Commercial rocket leaves	Bipolar	1300	20	1000	500	-
Spinach leaves	Bipolar	800	200	1600	500	2 trains 10 s interval
Kiwi fruit	Monopolar	200	100	1200	100	-
Mango	Monopolar	200	100	1200	100	-

A simplified schematic representation of monopolar and bipolar pulses is shown in Figure 2. Bipolar pulses were aimed for the leaf samples, while monopolar pulses were used for the fruit samples with lower amplitude to achieve permeabilization.

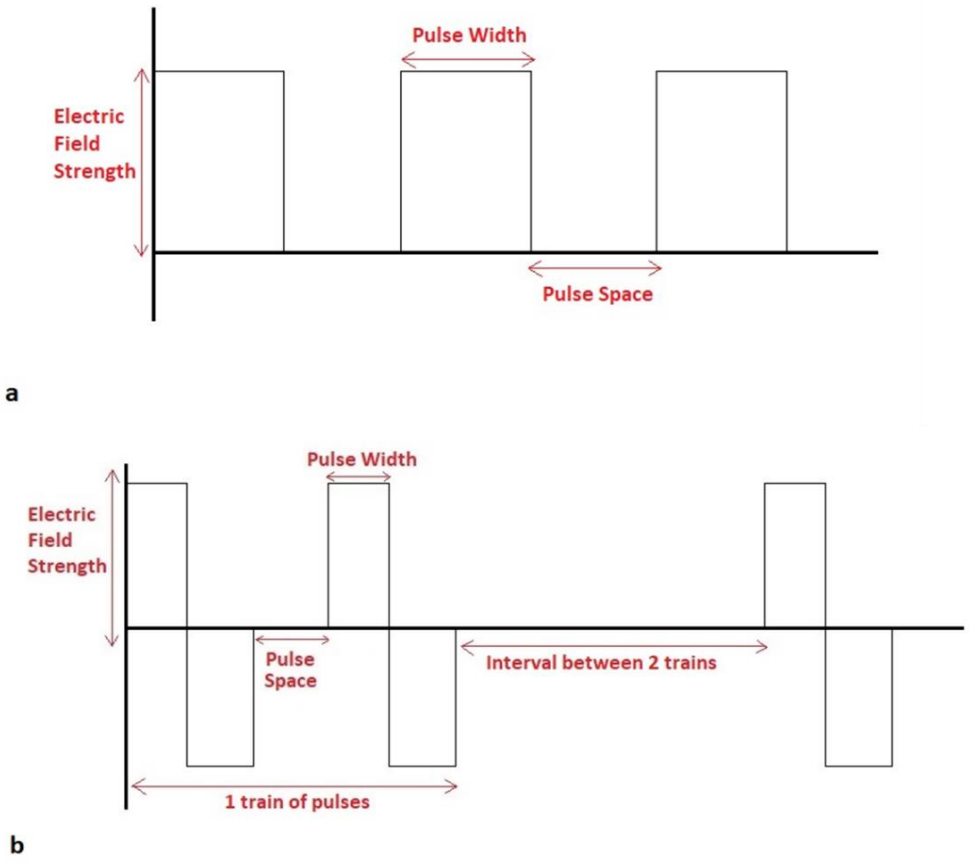


Figure 2. Schematic representation of, a) monopolar electric pulses applied to fruit samples, and b) bipolar electric pulses applied to leaf samples.

4.2.2 Packing of samples

Treated and untreated samples were packed in commercial salad leaves plastic film with dimensions of 15 (L) x 10 (W) cm and were heat-sealed and taped on all sides.

The packaging was done for 10 g sample of leaves and 20 g cut fruits after the treatment. The bags were stored in a cold room at 4 °C for further readings and analysis every day. Two bags were used every day for the measurements. Readings were taken for 22 days for greenhouse rocket leaves, 21 days for commercial rocket and spinach leaves and 8 days for both mango and kiwi samples.

4.3 Analysis

4.3.1 Fluorescence microscopy for the evaluation of PEF treatment

The uniform electroporation of the whole cross-section of a leaf is characterised and indicated by homogeneous electroporation of the leaves' surface (Dymek *et al.*, 2015), which was observed under the microscope using 250 µM Propidium Iodide (PI) solution as electroporation indicator. PI has the ability to enter only electroporated cells and later binding to the nucleus, producing fluorescence. The leaves were PEF treated with the PI solution of conductivity 130 µS in the PEF chamber, and observed under the microscope (Nikon Eclipse Ti-U, Tokyo, Japan) with red fluorescence filter at 10 X magnification.

Evaluation of the viability of electroporated leaves was determined by treating the leaves with Fluorescein Diacetate (FDA) vital staining. The FDA stock solution was prepared by mixing 0.125 g of FDA in 25 ml acetone. 1 ml of this FDA stock solution was further mixed with 100 ml deionised water to get 0.12 mM FDA solution. After PEF treatment, the leaves were kept in a sealed container lined with wet tissue paper and stored in the refrigerator for 24 hours at 4 °C. After 24 hours, the leaves were dipped for 15 min in the prepared FDA solution and then observed under the microscope with green fluorescence filter at magnifications of 10 X. The viability of the cells was checked by microscopic observations. The viable or living cells were bright green in colour, whereas, the dead cells stayed dark.

4.3.2 Impedance measurements

A conductive solution was made by mixing NaCl in deionized water to achieve final conductivity of 130 µS. The samples were first PEF treated with the conductive solution in the treatment chamber and then the chamber was connected to the impedance analyser for resistance measurement at frequencies between 0.5 to 10 kHz. Resistance of the samples were measured by 4192A LF Impedance Analyser (Hewlett Packard, USA). Fresh untreated samples were chosen as positive control, whereas frozen and thawed samples were used as negative control for the measurement of dead cells.

4.3.3 Isothermal Calorimetry for the evaluation of PEF treatment and metabolic activity

An isothermal calorimeter device (Calmetrix ICAL-2000 HPC, Calmetrix Inc., USA) was used to measure metabolic activity of treated and untreated samples. It contains two twin calorimeter chambers where the heat of the sample flows towards the heat sink and the heat flow sensor in the middle records the heat transfer. The temperature of the instrument is kept constant. The calibrated instrument was run for 20 hours at 20 °C with water as a reference. No fluctuations of the internal temperature were recorded in that period. Just beside the sample holder inside the chamber, the instrument takes the output from the reference. A reference of 75 g stainless steel (heat capacity 0.5 kJ/kg-K) was used for 10 g samples of leaves. For 20 g of fruit samples aluminium reference of 90 g (heat capacity of 0.91 kJ/kg-K) was used. In order to get accurate results with fewer disturbances, the reference should have the same heat capacity as the sample. Two different baselines were recorded before running the experiment, each with stainless steel and aluminium reference. The reason behind using two different baselines was the different weight of the samples used in the experiment. After the ampules were placed in the device, it was closed and left for 15-30 minutes until the calorimeter stabilizes and reaches the working temperature of 20 °C. The experiment was run for 20 hours and the device was connected to the computer which recorded the output of heat flow every minute.

The treated samples were kept in ampoules and sealed airtight with the help of a tape. They were left for 15 minutes at room temperature before placing them in the calorimeter. Three different experiments were performed where 10 g of spinach leaves, commercial and greenhouse rocket leaves were treated and kept in the calorimeter for the measurements. Similarly, kiwi fruits and mangoes were treated and 20 g of the sample were placed in the calorimeter. Measurements were also performed with untreated samples as control.

The heat production rate (thermal power) of the samples was calculated using equation 1

$$P = [\varepsilon(V_s - V_b) \div m] \times 1000 \quad (1)$$

Where P is the thermal power of the sample (mW/g), ε is the calibration coefficient of the calorimeter (W/V), V_s is the voltage of sample recorded from the calorimeter (V), V_b is the voltage recorded of the baseline (V), and m is the mass of the sample (g).

4.3.4 Measurements of atmospheric composition

To measure the composition of CO_2 and O_2 inside the sealed bags, a gas analyser (Dansensor Checkmate 3) was used. The device is provided with a needle detector that sucks the air inside the bags when it is punctured. One measurement was taken per bag.

4.3.5 Colour measurements

The colour measurement of the samples was done using a spectrophotometer CM-700d Konica Minolta Sensing, Inc (Japan).
Both treated and untreated samples were measured to evaluate the colour change on their surfaces. Three individual samples were taken for the measurement. Three readings were taken from the instrument: L* (represents the brightness), a* (represents how green is the sample) and b* (represents yellowness). ΔE* or the colour change was calculated from the equation 2

$$\Delta E^* = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2} \quad (2)$$

4.3.6 Visual observations

Pictures were taken each day and compiled together for visual representation of the samples. Each group of pictures includes images of treated and untreated samples, along with pictures of fresh samples as a control.

5. Results

5.1 Unpacked vegetables

5.1.1 Reversible electroporation indicated by Propidium Iodide and Fluorescein Diacetate

PEF treatment with low electric strength that were below 900 V/cm for rocket leaves and 600 V/cm for spinach leaves were found ineffective as the PI solution was not able to stain the cells uniformly. The electric field was adjusted to get the optimum level of electroporation. After several trials and optimisation of the parameters, uniform electroporation was achieved for the leaves with bipolar pulses (see Table 1 for optimized parameters).

Observation of the leaves was also done by Fluorescein Diacetate (FDA) to check their survival 24 hours after the treatment. Figure 3a, 3b and 3c shows stained nuclei, shown as bright spots, of the greenhouse rocket, commercial rocket and spinach leaves respectively, stained by Propidium Iodide solution. Figure 3d, 3e and 3f shows typical observation of leaves stained with FDA in similar order. Viable cells are stained with bright green colour as shown in the figure, meanwhile dead cells remain dark.

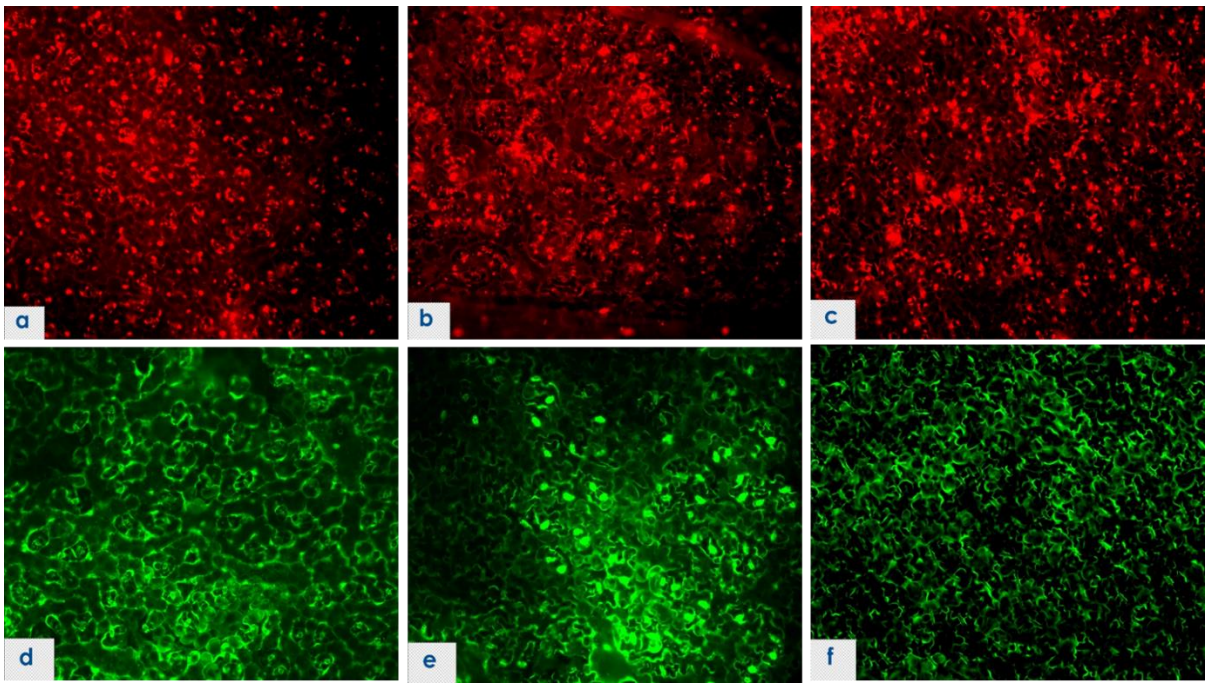


Figure 3 Representative microscopic observation of PI and FDA staining of leaf cells taken after the treatment with PEF. Figures a, b and c show PI staining of greenhouse rocket, commercial rocket leaves and spinach leaves respectively. Figure d, e and f shows FDA staining in similar order. Staining procedures were performed as indicated in the materials and methods section.

Unlike the leaves, where it was comparatively easier to monitor the electroporation of the cells using staining techniques, the microscopy techniques presented difficulties with the fruits. PI staining could not clearly identify cell nucleus under the microscope.

5.1.2 Resistance measurement

Fruits

Mango:
The measured resistance is plotted over a range of frequencies for treated and untreated samples in (Figure 4a). Resistance values were obtained for different electric field strengths which resulted in considerable differences as the frequency changed. This frequency range was chosen as the optimum because the difference between viable and non-viable cells was more significant in this range. Higher frequency would provoke an electric current to pass through the cells which is not desirable. It can be seen from Figure 4a that the resistance of the dead sample is the lowest of all other treatments, most probably due to the damage and leakage of cells which makes the solution more conductive (less resistant). On the other hand, the resistance of the samples treated with 200 V/cm and 500 V/cm lies between the curves of the untreated and dead samples. These two treatments were further analysed for their metabolic response in the calorimeter.

Kiwi fruit:
Figure 4b shows that the samples treated with 200 V, 300 V and 400 V/cm amplitude electric field lie below under the curve of the untreated sample. The lowest resistance showed by dead sample might correspond to the larger extent of cell damage and leakage into the solution, causing a decrease in conductivity (increased resistance). Although the difference between 200 V, 300 V and 400 V/cm treatments was distinguishable, they were further analysed for their metabolic response in the calorimeter.

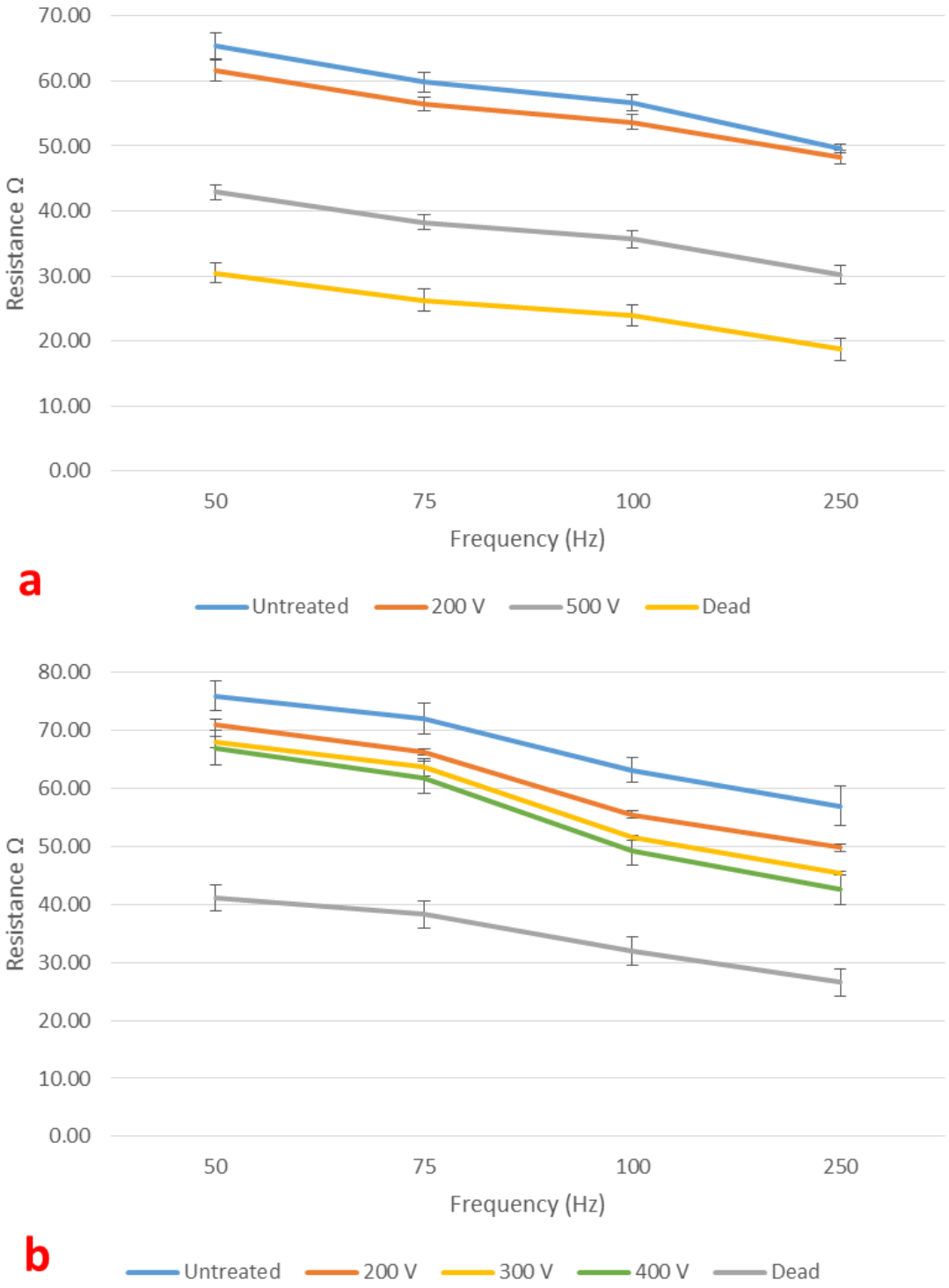
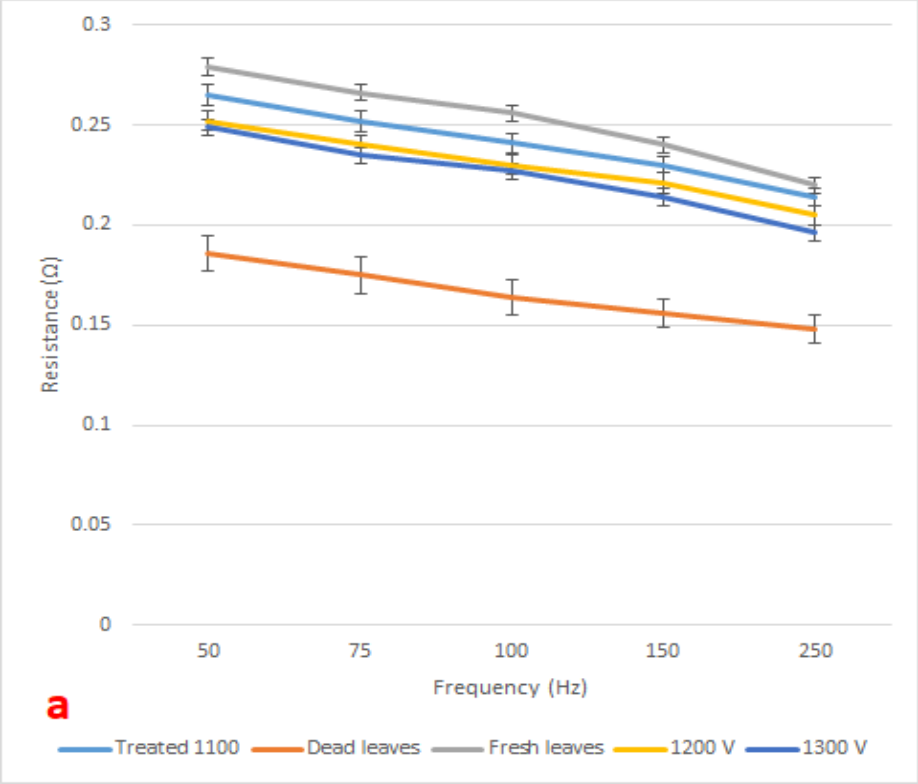


Figure 4 a) Resistance of mango samples measured after different PEF treatments. Reported are measurements taken after 200 V/cm and 500 V/cm PEF treatment, fresh untreated sample and dead sample. b) Resistance of kiwi samples measured after different PEF treatments. Reported are measurements taken after 200 V/cm, 300 V/cm and 400 V/cm PEF treatment, fresh untreated sample and dead sample. Dead sample refers to frozen and thawed untreated samples of fruits. Error bars represent the standard deviation of 3 measurements.

Vegetables

Like the fruit samples above, the frequency range of 50-250 Hz was also chosen for the leaves for the impedance measurement results. Less tissue resistance was observed as the electric field strength was increased. In Figures 5a, b and c, drastic significant difference between fresh and dead leaves was seen which can be accounted to increased leakage of cell constituents in the solution. As the treatment strength is increasing, the corresponding resistance decreased. The following treatments: 1100 V/cm for GH rocket leaves (Figure 5a), 1300 V/cm for commercial rocket leaves (Figure 5b) and 800 V/cm for baby spinach leaves (Figure 5c) were chosen ideal parameters for electrical field strength.



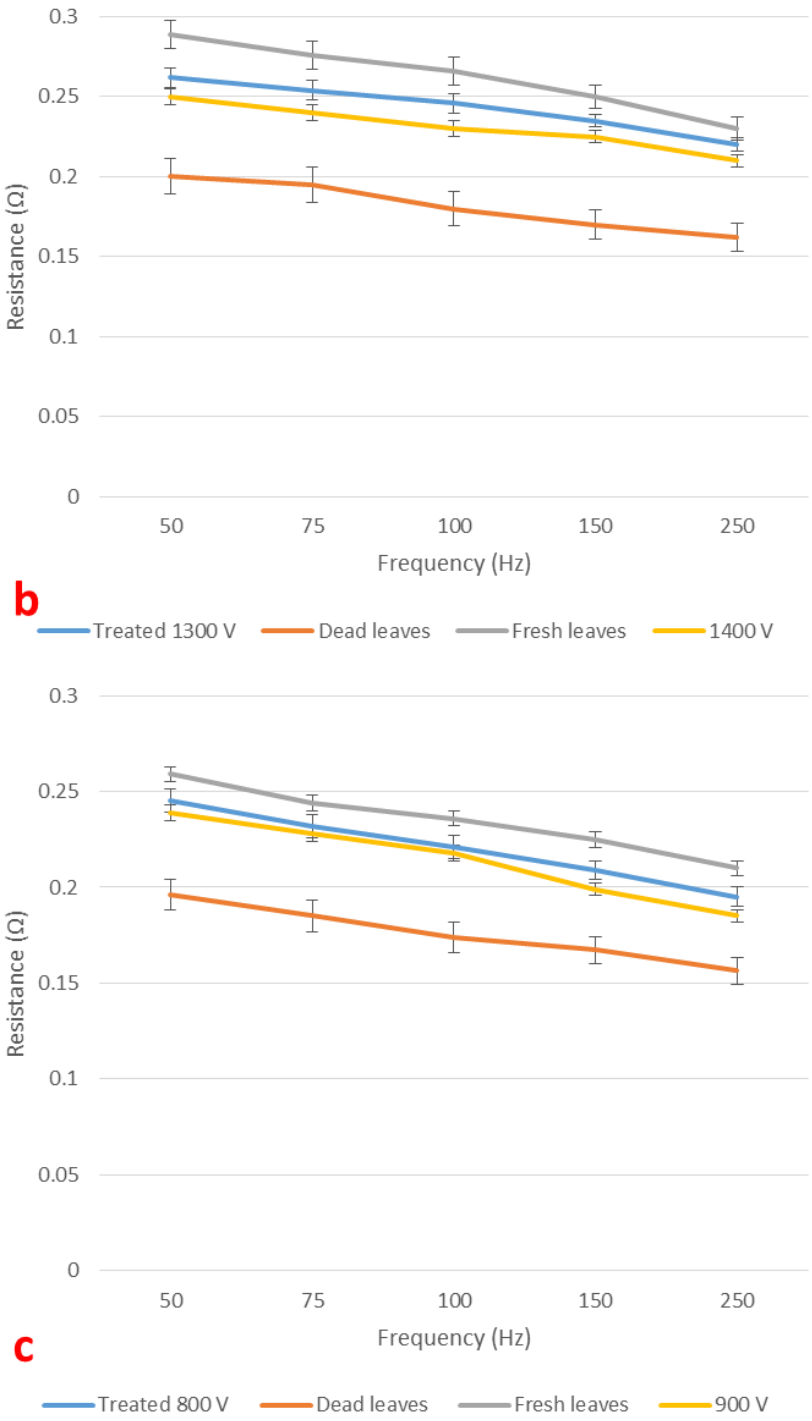


Figure 5 a) Resistance measured for GH rocket leaves before the treatment, after PEF treatment at 1100 V/cm and dead leaves, b) measured resistance for commercial rocket leaves before the treatment, after PEF treatment at 1300 V/cm and dead leaves, and c) resistance measured for baby spinach leaves before the treatment, after PEF treatment at 800 V/cm and dead leaves. The resistance measurements are plotted against a range of frequencies that include readings of fresh and dead samples where dead refers to frozen and thawed untreated leaves. Error bars represent the standard deviation

5.2 Calorimetric measurements

Fruits

Mango:

It was observed from Figure 6a that the metabolic response induced by the 300 V/cm, 400 V/cm and 500 V/cm treatments showed significantly lower values than the treated sample with 200 V/cm electric strength and control samples with initial thermal power between 0.11, 0.12 and 0.08 mW/g respectively. The sample treated with 200 V/cm exhibited 0.31 mW/g thermal power initially in the calorimeter, which was 0.09 mW/g more than the control sample.

Kiwi

Initially, the calorimetric signal was disturbed when the ampoule was placed inside the calorimeter. A further 1.5 hours (5400 s) was required for the signal to stabilize. The signal received by the calorimeter was recorded and the curve of thermal power was plotted against the time. From Figure 6b, it can be seen that the initial values of the thermal power of the treated sample with 200 V/cm was 0.43 mW/g compared to 0.17 mW/g of the control sample. Just like the mango samples, kiwi fruits after the treatment show higher initial values of thermal power which corresponds to higher metabolic activity due to the treatment. Other parameters with higher treatment intensities exhibited lower values than the untreated sample.

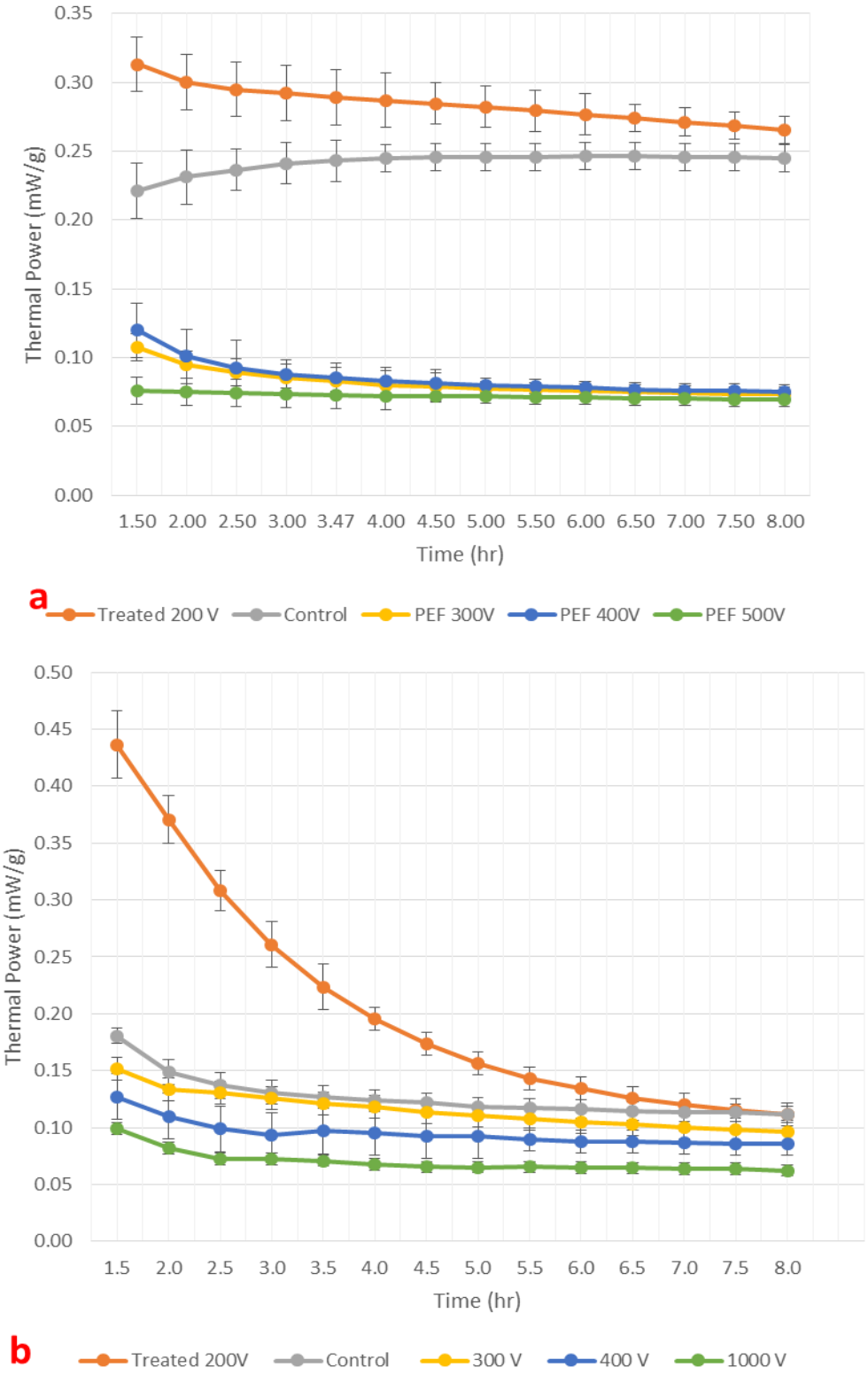
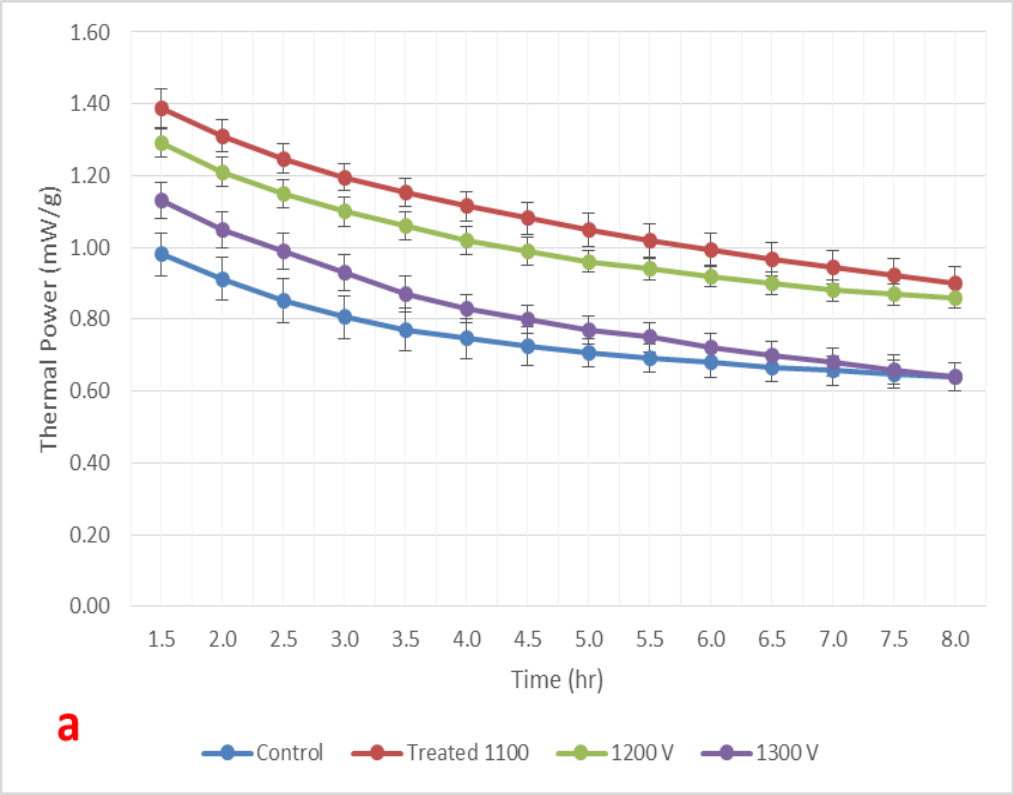


Figure 6 Thermal power of mango samples (a) and kiwi (b) as a function of time, measured by isothermal calorimetry. Mango was treated with 200 V/cm, 300 V/cm , 400 V/cm and 500 V/cm and Kiwi with 200 V, 300 V , 400 V and 1000 V/cm. Reported are average and STD of 3 measurements.

Vegetables

It can be observed from Figures 7a, 7b and 7c for GH rocket, commercial rocket and spinach leaves that the response of all the samples treated with PEF showed increase in initial metabolic heat compared to the control samples which may correspond to the stress provoked by the treatment. The readings are taken from 1.5 hours after the signal was stabilized. From the figures 7a and 7b of GH rocket and commercial rocket, the difference of 0.21 mW/g and 0.41 mW/g was recorded respectively between treated and untreated samples after 1.5 hours.
Spinach shows lower metabolic thermal power than rocket leaves with initial value of 0.5 mW/g for untreated leaves. The difference of 0.28 mW/g was recorded between treated and control spinach leaves after 1 hour of stabilization of the readings.

Treatment parameters with 1200 and 1300 V/cm also showed an increase in the initial metabolic heat (Figure 7a). However, considering the impedance measurement and fluorescence microscopy observations, the electric strength of 1100 V/cm was chosen for GH rocket leaves due to uniform electroporation on the surface (see Figure 3-a and d) and highest impedance measurement, but still below the impedance of the control (Figure 5a). The comparison of the results of impedance measurement and fluorescence microscopy with high metabolic heat (Figure 7a) of the treatment at 1100 V/cm strength suggested this treatment to be chosen for the packing experiment. Similarly, 1300 V/cm was chosen for commercial rocket leaves (Figure 7b) and 800 V/cm for spinach leaves (Figure 7c).



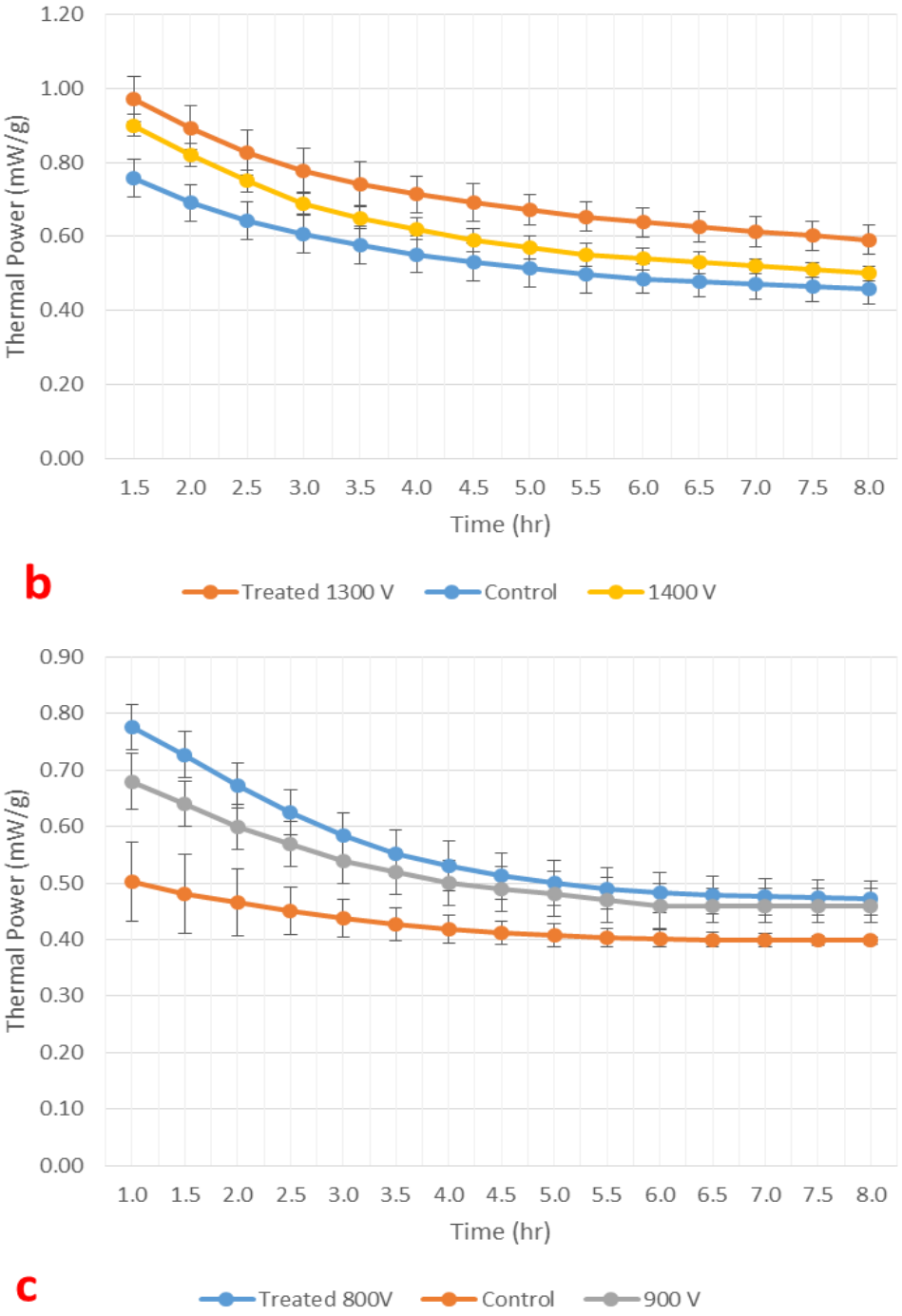


Figure 7 a) Calorimetric measurement of metabolic thermal power of treated and untreated GH rocket leaves, b) commercial rocket leaves, and c) spinach leaves. Reported are average and STD of 3 replications

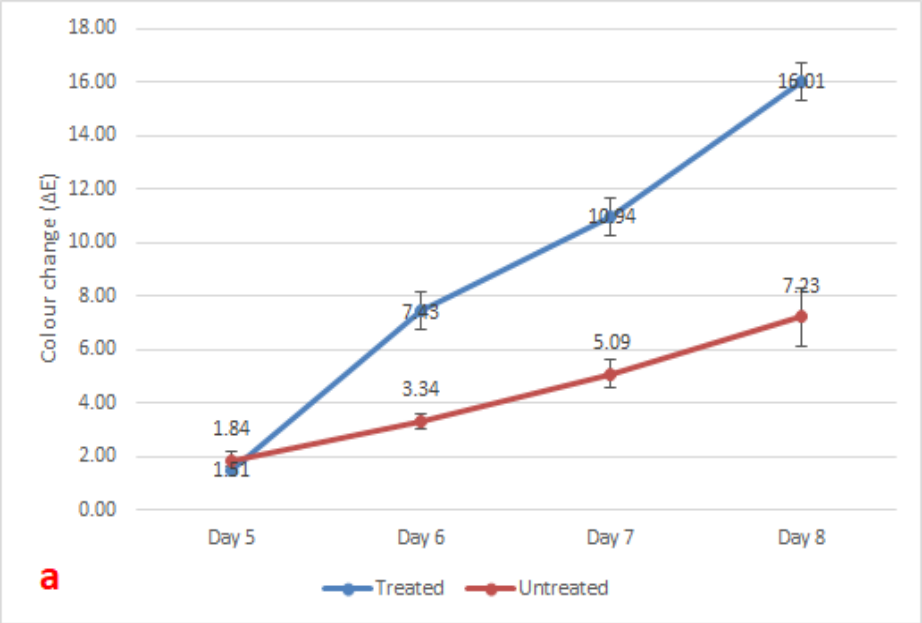
5.3 Packed samples

5.3.1 Colour and visual changes

5.3.1.1 Packed fruits

Mango

In the mango samples, the colour of the treated slices showed significant difference from day 6 (Figure 8a). Although darkness was observed in both treated and control, from day 6 treated samples showed changes in both consistency as well as colour. On day 7, some leakage in the packages was observed but softness and disintegration of the slices was noted for treated slices on day 8.
Darkness in the treated samples is reported in Figure 8b which can be accounted to increased oxidation and enzymatic reactions. The control samples appeared dry with no leakage observed till day 8. Therefore, deterioration was more pronounced in the PEF treated samples.



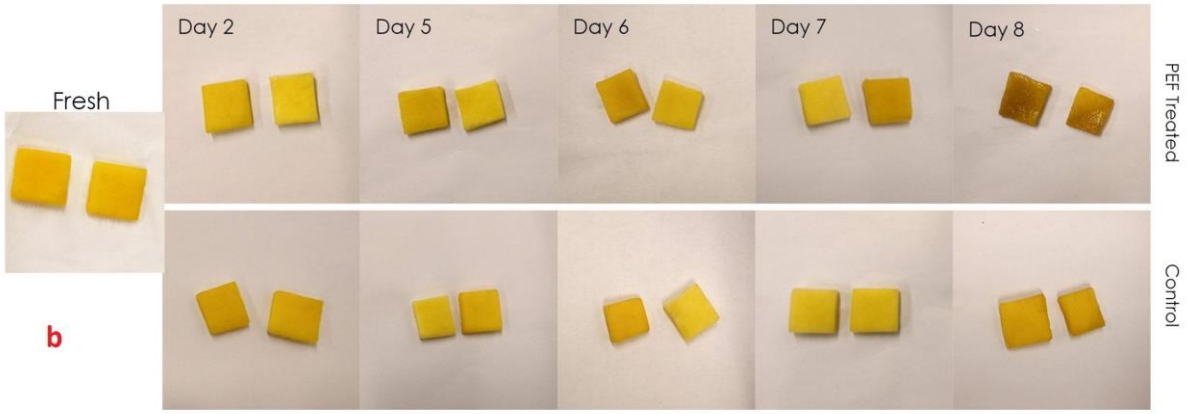
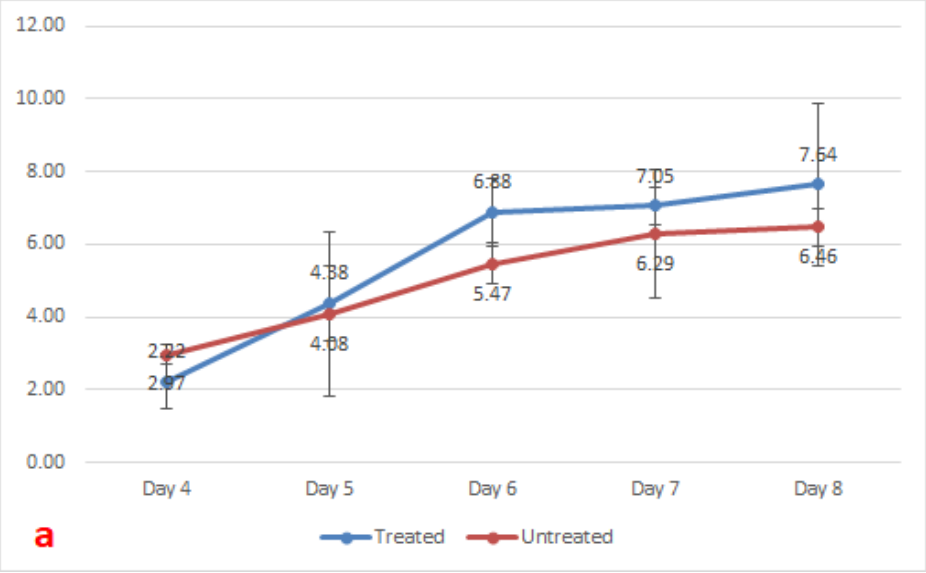


Figure 8 (a) Colour change (ΔE^*) of packed treated and untreated mango fruit slices taken by spectrophotometer. The packages were stored at 4 °C and the readings were taken for 8 days. (b) Comparison of changes in visual appearance of packed control and PEF treated mango slices stored at 4 °C for 8 days. Reported are average and STD of 3 measurements.

Kiwi fruit

The colour measurement (Figure 9a) showed no significant differences between the samples. Increase in the colour change was observed in both the samples from day 5, with values increasing until day 8. Some darkness in the fruit sample was noticed from day 5 in both samples which could be the result of oxidation and enzymatic reactions.
Leakage in the treated samples was observed from day 6 of storage. Slices were found to be soft and mushy on day 7 and disintegration was noted down on day 8 (Figure 9b). Therefore, the treated sample showed faster deterioration than the control samples.



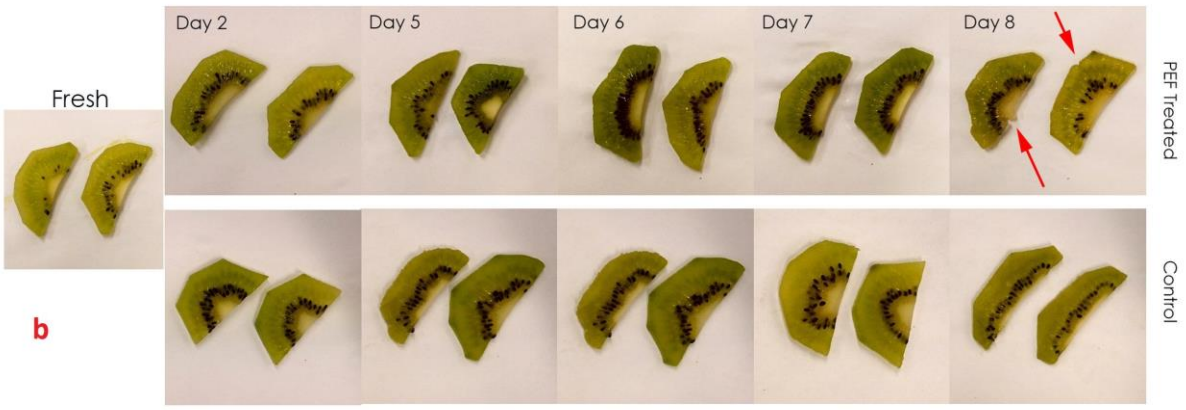


Figure 9 (a) Colour change (ΔE^*) of packed treated and untreated kiwi fruit slices. The packages were stored at 4 °C and the readings were taken for 8 days. (b) Comparison of changes in visual appearance of packed control and PEF treated kiwi fruit slices stored at 4 °C for 8 days. Red arrows represent disintegration of the slices. Reported are average and STD of 3 measurements.

5.3.1.2 Packed Vegetables

Greenhouse Rocket leaves

Greenhouse rocket leaves showed changes in the colour in the span of 22 days of storage, but visible changes were observed in the last three days of their storage. The colour change showed significant differences between treated and control samples from day 20. ΔE^* value (Figure 10a) for control samples increased from 4.94 to 11.81 and continued to increase till 22.8 on day 22, compared to that of treated leaves which has ΔE of 10.66 on day 22. It can be noted that colour change in both the samples are increasing from day 19 to day 22 which may be accounted to the loss in chlorophyll content.

Apart from the colour change, control samples also showed wilting and softness in the leaves. From day 20 the colour of control leaves changed to bright green with yellow hue all over (Figure 10b), thus showing loss in green colour much more than the treated leaves. Some brown spots were also observed on the untreated leaves which is believed to be the result of the loss in chlorophyll content of the leaves. Some changes in the turgidity of the treated leaves were also observed as they became softer with the passing days.

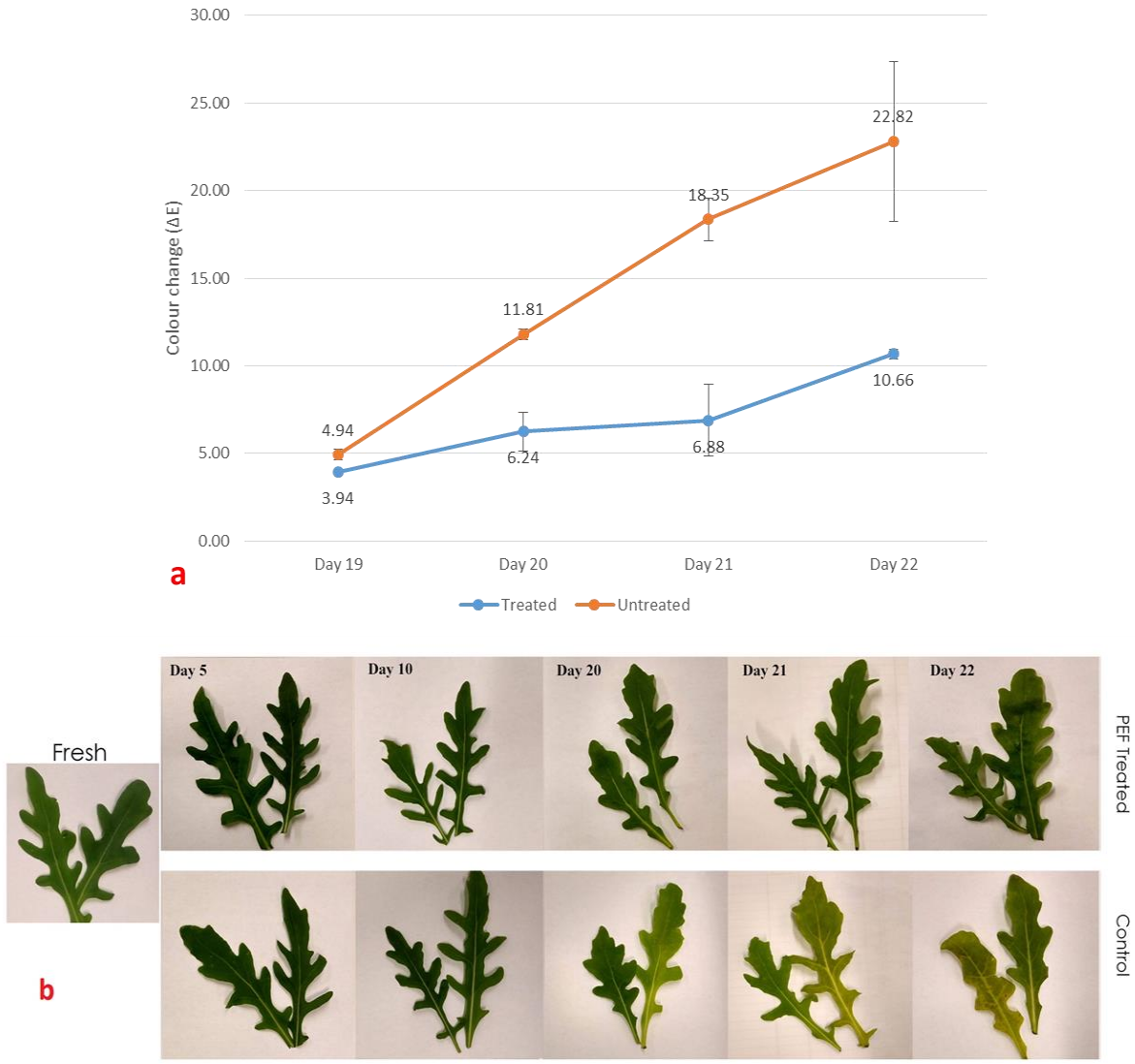


Figure 10 (a) Colour change (ΔE^*) of packed treated and untreated greenhouse rocket leaves. The packages were stored at 4 °C and the readings were taken from day 19 to day 22. (b) Comparison of changes in visual appearance of packed control and PEF treated greenhouse rocket leaves stored at 4 °C for 22 days. Reported are average and STD of 3 measurements.

Commercial Rocket leaves

It can be noted from Figure 11a that there was no significant difference between the colour change of the control and treated sample compared with that of the fresh sample. It can be noted that ΔE values increased from day 15 but no significant change in the colour was noted between the treated and control leaves. It can be concluded that there was no impact of PEF treatment on the colour change of the commercial rocket leaves.

Figure 11b shows that no visual colour changes were observed, although there were some textural changes observed in the leaves. Both samples have lost their turgidity after day 14 and accumulation of liquid in the package was observed from day 16. Both treated and control leaves were wilted and lost their texture by day 21.

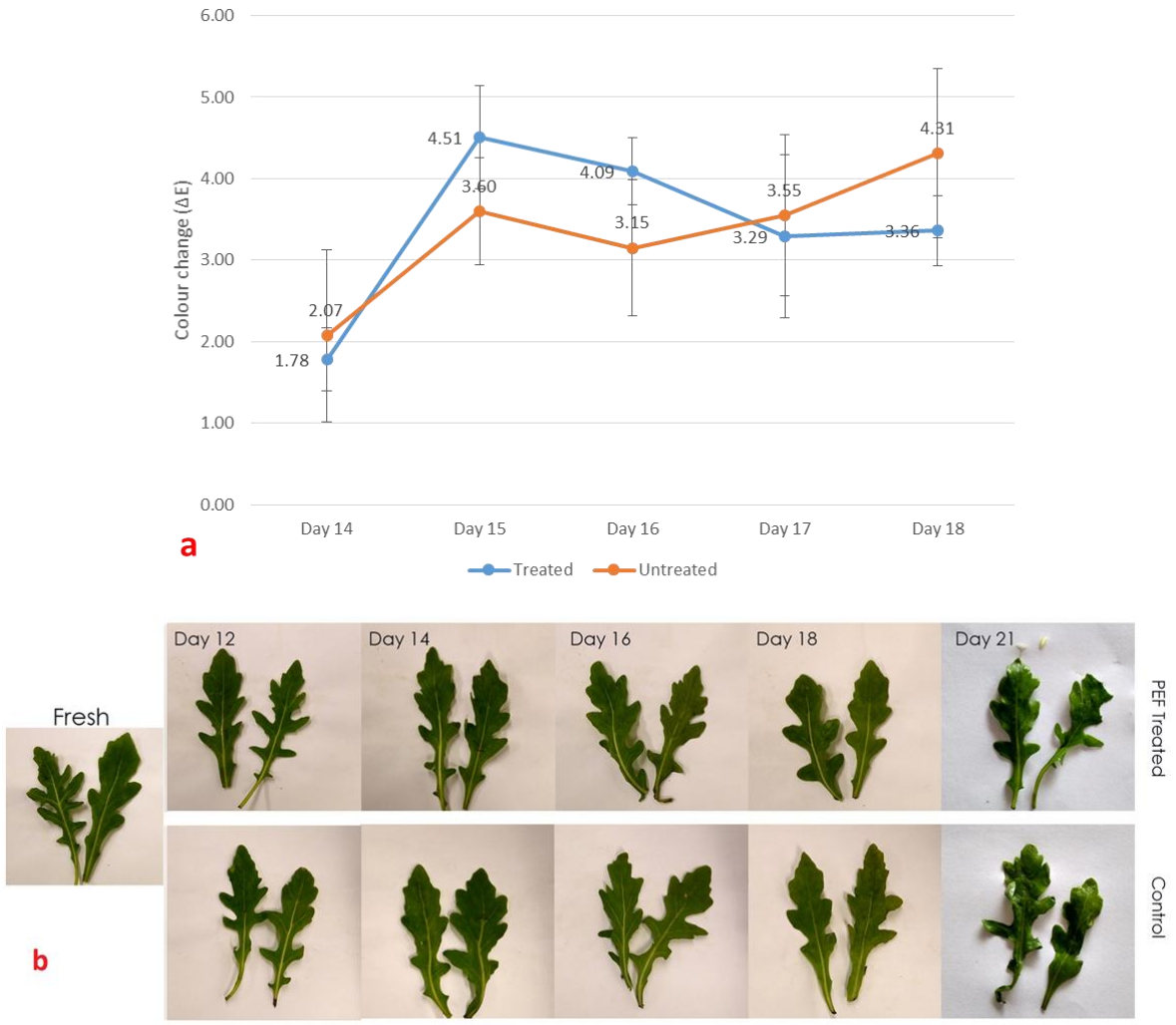


Figure 11 (a) Colour change (ΔE^*) of packed treated and untreated commercial rocket leaves taken by spectrophotometer. The packages were stored at 4 °C and the readings were taken for 18 days. (b) Comparison of changes in the visual appearance of packed control and PEF treated commercial rocket leaves stored at 4 °C for 21 days. Reported are average and STD of 3 measurements.

Spinach

Changes in the green and yellow hues were observed for control samples of baby spinach leaves as they started to show differences in their colour intensities from day 16 (Figure 12a). A significant difference for ΔE values between the control and treated samples were reported from day 16 with the difference of 2.78 in colour change, indicating the loss of green colour of the control leaves, a change that was less pronounced in the treated samples.

However, loss in turgidity was reported in the treated leaves from day 14 and leakage was also observed in the packages from day 15. Treated leaves were found to be more wrinkled than the untreated ones, Red arrows in figure 12b on day 18 and 21 indicate their visible quality deterioration. Hence, the above observations for baby spinach leaves concluded that PEF treatment had an effect on colour preservation (as it did in the case of GH rocket leaves) but did not improve the shelf life of the packed leaves, as they deteriorated faster than the control samples.

Figure 12 (a) Colour change (ΔE^*) of packed treated and untreated baby spinach leaves taken by spectrophotometer. The packages were stored at 4 °C and the readings were taken for 18 days. (b) Comparison of changes in visual appearance of packed control and PEF treated baby spinach leaves stored at 4 °C for 21 days. Red arrows represent damaged area in the leaves. Reported are average and STD of 3 measurements

In the results of colour changes obtained for GH rocket, commercial rocket leaves and spinach leaves from figures 10, 11 and 12 respectively, no change in the colour was observed before the time they are reported in the figures. Only the readings for the last days of their storage when changes in the colour were observable by colorimeter are reported. Green vegetables undergo colour change due to the loss of chlorophyll content during their storage (Agüero *et al.,* 2008), and changes in the colour of the leaves were recognized after extended storage time in this case, which was 6-7 days later than their actual shelf life stated in the packages by the supplier. For both treated and untreated samples, colour change readings for the last 4-5 days of their storage is shown in order to examine the effects of PEF treatment in preserving the colour of the treated samples. No differences in the colour change were recorded for GH rocket leaves before day 19 and for commercial rocket and spinach leaves before day 14.

5.3.2 Gas composition in the bags (O_2 and CO_2)

Gas readings were taken to observe possible changes in the composition of O_2 and CO_2 inside the packages of mango and kiwi (Figure 13a and 13b) and commercial rocket, spinach leaves (Figure 14a and 14b) stored at refrigerated temperature of 4 °C. Both commercial rocket leaves and spinach leaves showed similar trend of the oxygen content which is decreasing from the initial value, taken on the day of packing.

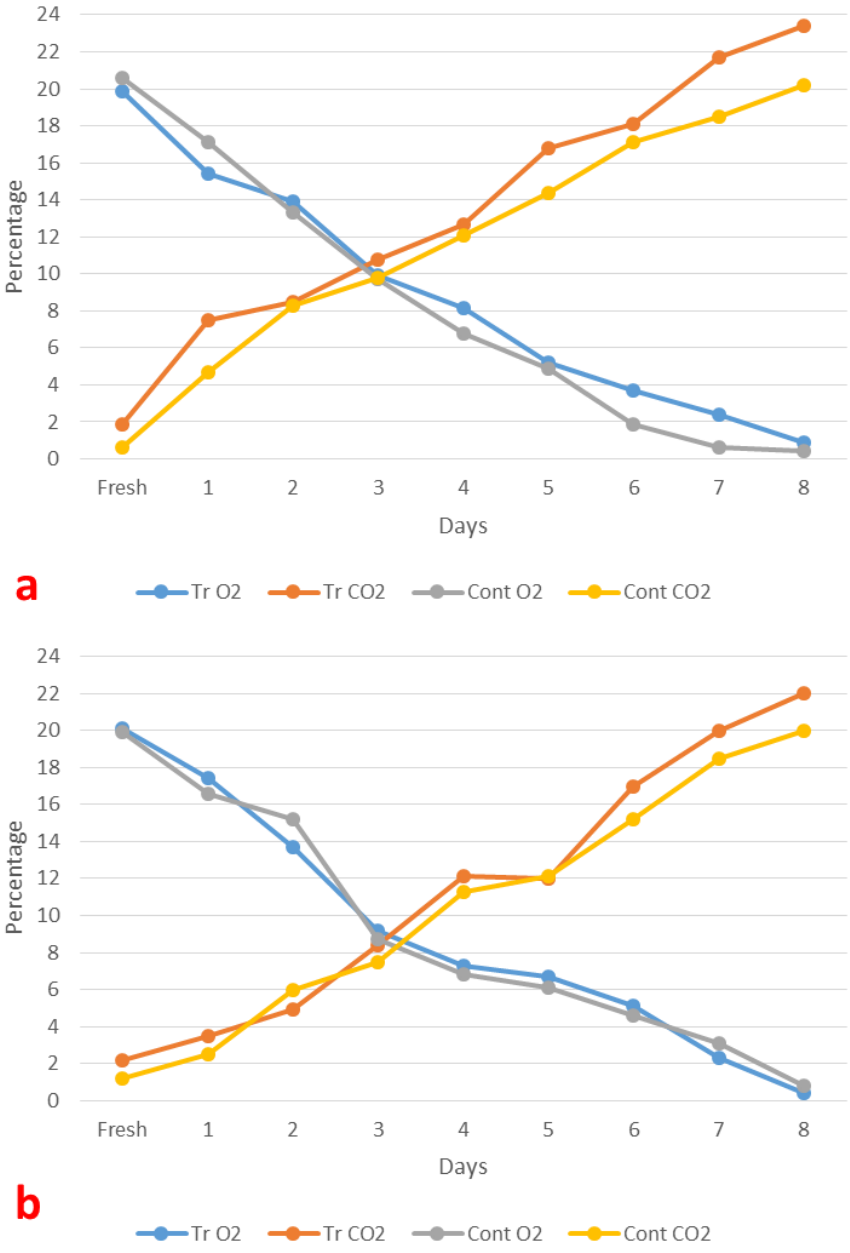


Figure 13 a) Changes in the internal gas composition (O_2 and CO_2) of packed mango, and b) kiwi fruits taken by gas analyser for both treated and control samples over 18 days

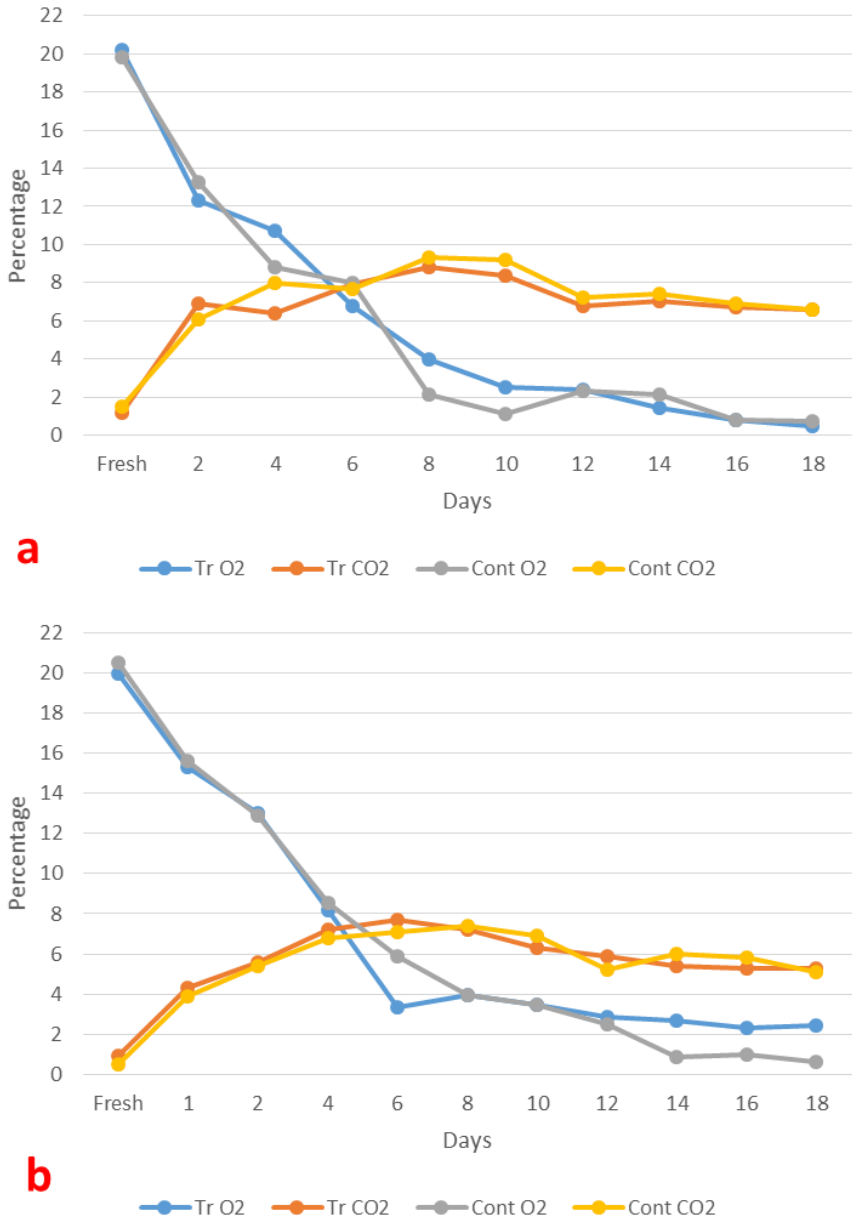


Figure 13 a) Changes in the internal gas composition (O_2 and CO_2) of packed commercial rocket leaves, and b) spinach leaves taken by gas analyser for both treated and control samples over 18 days

From the Figures 14 a and b, for commercial rocket and spinach leaves, the level of carbon dioxide increases from the day of packing and reaches a plateau from day 6 for both the treated and control samples, without further increase. Continuous decrease in the oxygen level was observed from the first day, and remained between 0-3% from day 12 to 18 in both treated and control samples of spinach and rocket leaves. No difference in the respiration rate between the treated and untreated leaves of rocket and spinach leaves was noticed as the trend showed rather similar responses of the gases produced and consumed in the package.

It could be observed from the Figure 13a, that the trend in the changes of both oxygen and carbon dioxide for kiwi fruit are different from that of the leaves. The kiwi samples show high O_2 consumption and CO_2 production.

Similar trend of increasing levels of CO_2 and decreasing O_2 in the untreated sample was observed in both kiwis and mango, which presents deterioration. However, the increase in CO_2 in the treated sample, leakage, fermented odour and disintegration in the texture concludes that the treated kiwi fruit was deteriorated and showed poorer results than the control sample.

6. Discussion

6.1 Changes in electrical resistance

The resistance is reported to decrease with the increase in frequency (Figure 4 and 5). At higher frequency the cell membrane allows the electric current to pass through, which represents the combined impedance of both extracellular and intracellular medium. Therefore, electroporation is best detectable in low range of frequencies as the cell membrane acts like a capacitor, giving resistant to the flow of electric current through it (Donsì, Ferrari & Pataro, 2010). At low frequencies, the electric current passes around the cell and the measured resistance represents the resistance of the extracellular space, picking up the leakage from the cells. Freezing and thawing (for dead samples) results in the leakage of cell constituents in the PEF solution giving the lowest resistance in Figures 4 and 5. Bulk electroporation of treated samples is distinguishable in the frequency range 50-250 Hz where it lies below the resistance curve of untreated fresh samples.

The resistance is also highly dependent on the electrical field strength. As the treatment parameters are increasing, the leakage also increases due to increasing permeability which results in the lower resistance of the solution containing sample. In a work by Dellarosa *et al.,* (2016), of metabolic response of fresh-cut apples treated with pulsed electric field, it was observed that the highest electric field strength used for the PEF treatment showed lowest resistivity in impedance measurement. This result was related to the irreversibility in permeabilization of the tissue as higher electric field alters the structure of tissues the most. Similar results were obtained in the resistance readings in figures 4 and 5 where highest electrical strength results in the lowest drop in the resistance.

6.2 Heat production rate- metabolic response of samples

Metabolic responses of cells subjected to external electric field are not well understood (Gómez Galindo *et al.* 2008), however, increased bulk heat production of the samples detected in calorimetry measurements indicate that the stress induced to the samples subjected to electric field can be directly related to their increased metabolic thermal power (Gómez Galindo *et al.* 2008), as reported in Figure 7.

From the different parameters used for the treatment, the lowest field strength induced stress responses which could be attributed to the process of recovery of cell membrane (Vorobiev & Lebovka, 2009) in reversible electroporation (Dellarosa *et al.*, 2016). Parameters with higher treatment intensities exhibited lower thermal power than the control sample which could be the consequence of loss in cell viability.

6.3 Colour and texture changes during storage

Changes in turgidity and colour were observed for all the samples during their storage. Due to altered biochemical changes after the recovery of cells from reversible PEF treatments, changes in enzymatic activity, chlorophyll content and turgidity may be expected over time. This change in the turgidity of treated samples can be caused by the effects and intensity of the treatment. Giner *et al,* (2001) concluded that the degree of degradation of tissues related to pulsed electric field depends on PEF parameters such as electric field intensity, pulse width, number of pulses as well as temperature and enzymes present in the product.

The colour change in the control samples of GH rocket leaves may have been the result of senescence. Although change in colour was also observed in the treated leaves, it was not as drastic and took place a few days later than in the untreated leaves (Figure 10). Increased colour change of treated fruit samples along with their softness was associated with the deteriorating effects of PEF treatment over time which may have triggered enzymatic activity and oxidative stress of the samples.
The lower turgidity and softness of treated spinach leaves may have been the result of the inability of tissues to entirely recover from the electroporation over time. Just like GH rocket, the PEF treatment of spinach leaves may have resulted in the preservation of green colour.

6.4 Metabolic responses in calorimeter and gas composition

Measuring heat production, consumption of oxygen and production of carbon dioxide provides an effective way of observing the metabolic responses of living plant tissues (Criddle, Breidenbach & Hansen, 1991). The changes in the ratio of O_2 and CO_2 content during the storage provides direct response of the changes in the metabolic activity of tissues (Criddle *et al.*, 1990).

Although the treated samples did show effects in heat production rates, the electrical treatments did not present any effects on the respiration rate during storage of samples as there was no difference in the consumption of O_2 and production of CO_2 (Figures 13 and 14) in the gas analysis of both treated and untreated samples. These two measurements represent tissue responses in two different time scales. Short term (hours) responses to the application of PEF were picked up by the calorimeter while long term responses (days) were measured with the gas composition in the packages. Clearly, PEF affects the metabolism of the measured commodities only for few hours. The treated tissues may recover from the electric field and their respiration in the package is the same as the control samples. Interestingly, the colour of the leaves was kept better in PEF-treated samples, which suggests that some PEF-induced responses may have long term consequences.

7. Conclusions

- Out of all the five commodities used, PEF treatment worked in extending the shelf life of GH rocket leaves by preserving their colour during storage. The treatment did not work for commercial rocket, spinach leaves, kiwi fruit and mango as they showed deterioration in the colour and texture.

- No changes in the gas composition were observed during storage of the tested fruits and vegetables, which may be related to the recovery of the tissues from electrical treatment over time and their normal functionality and respiration during their shelf life in the package.

- PEF treatment with 1100 V/cm amplitude showed effects on retaining the colour of the packed greenhouse rocket leaves. This was suggested to be a long-term consequence of PEF treatment on GH leaves, which may show potential for the applicability of reversible PEF treatments in green vegetables.

- Lower electric field resulted in higher impedance of the solution which decreased with the increase in the intensity of electric field. This represented more leakage and damage of cells due to irreversibility with the increase in electric field parameters.

- Higher metabolic heat was recorded for the samples treated with lower electric field and vice versa. Reversible PEF treatment lead to an increase in the metabolic heat which corresponds to the stress provoked by PEF treatment.

8. Future work

Further studies on the chlorophyll measurement could be done during shelf life study of the treated green vegetables, this could provide information on possible effects of reversible PEF treatment on the changes in chlorophyll content of the leaves.

This study has not looked upon the effects of microbial growth and enzymatic reactions, more studies could be done to get the insight in this field.

Bigger packing (200 g to 500 g) of treated leaves could be done for further shelf life trials to get the exact picture of supermarket storage. There are possibilities of obtaining different results by packing the green vegetables in bigger amounts due to possible changes in their respiration and metabolic heat in bulk amount

Effect of reversible PEF treatment can be studied for different varieties of climacteric and non-climacteric fruits in the future.

9. Appendix

Product	Temperature (°C)	Atmosphere	
		O2 (%)	CO2 (%)
Fresh-cut Vegetables			
Broccoli	0-5	2-3	6-7
Shredded cabbage	0-5	5-7.5	15
Shredded, sticks or sliced carrots	0-5	2-5	15-20
Sliced leek	0-5	5	5
Chopped butterhead lettuce	0-5	1-3	5-10
Chopped green leaf lettuce	0-5	0.5-3	5-10
Chopped or shredded iceberg lettuce	0-5	0.5-3	10-15
Chopped red leaf lettuce	0-5	0.5-3	10-15
Chopped romaine lettuce	0-5	0.5-3	5-10
Sliced mushrooms	0-5	3	10
Sliced or diced onion	0-5	2-5	10-15
Diced peppers	0-5	3	5-10
Sliced or whole-peeled potato	0-5	1-3	6-9
Sliced rutabaga	0-5	5	5
Cleaned spinach	0-5	0.8-3	8-10
Sliced tomato	0-5	3	3
Sliced zucchini	5	0.25-1	-
Fresh-cut fruits			
Sliced apples	0-5	<1	-
Cubed cantaloupe	0-5	3-5	6-15
Cubed honeydew	0-5	2	10
Sliced kiwifruit	0-5	2-4	5-10
Sliced orange	0-5	14-21	7-10
Sliced peach	0-5	1-2	5-12
Sliced pear	0-5	0.5	<10
Sliced persimmon	0-5	2	12
Arils (seed coating) pomegranate	0-5	-	15-20
Sliced strawberry	0-5	1-2	5-10

Table 2 Modified atmosphere storage recommendations for selected fresh-cut fruits and vegetables (Gorny, 1997)

10. Acknowledgment

I would like to express my sincere gratitude and admiration to my supervisor Federico Gomez for his support, guidance, and valuable inputs throughout my thesis project. Additionally I thank my examiner, Juscelino Tovar for taking the time to review my work and give his thoughtful feedback.

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I hope my mother and brother are always watching me and motivating me to live life positively and keep on going no matter what happens. No words to thank my dear father for being an inspiration. And last but never the least, my friends for pushing me forward. Words cannot describe how grateful I am to have you all.

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Lund University

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